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Application Number: 15526162 Document Date: 05/11/2017

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Form Revision Date: August 26, 2013

CSIRO Exhibit 1007

PTO-1390 (01-17)
Approved for use through 8/31/2019. OMB 0651-0021
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TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A SUBMISSION UNDER 35 U.S.C. 371		Attorney Docket No. 32471/H150220
		U.S. Application No. (if known, see 37 CFR 1.5)
International Application No. PCT/EP2015/076630	International Filing Date 13 November 2015	Priority Date Claimed 14 November 2014
Title of Invention MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS		
First Named Inventor Toralf Senger et al.		

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). NOTE: The express request under 35 U.S.C. 371(f) will not be effective unless the requirements under 35 U.S.C. 371(c)(1), (2), and (4) for payment of the basic national fee, copy of the International Application and English translation thereof (if required), and the oath or declaration of the inventor(s) have been received.

2. ☐ A copy of the International Application (35 U.S.C. 371(c)(2)) is attached hereto (not required if the International Application was previously communicated by the International Bureau or was filed in the United States Receiving Office (RO/US)).

3. An English language translation of the International Application (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).

4. An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4))
   a. ☐ is attached.
   b. ☐ was previously filed in the international phase under PCT Rule 4.17(iv).

Items 5 to 8 below concern amendments made in the international phase.

PCT Article 19 and 34 amendments

5. ☒ Amendments to the claims under PCT Article 19 are attached (not required if communicated by the International Bureau) (35 U.S.C. 371(c)(3)).

6. ☐ English translation of the PCT Article 19 amendment is attached (35 U.S.C. 371(c)(3)).

7. ☐ English translation of annexes (Article 19 and/or 34 amendments only) of the International Preliminary Examination Report is attached (35 U.S.C. 371(c)(5)).

Cancellation of amendments made in the international phase

8a. ☐ Do not enter the amendment made in the international phase under PCT Article 19.

8b. ☐ Do not enter the amendment made in the international phase under PCT Article 34.

NOTE: A proper amendment made in English under Article 19 or 34 will be entered in the U.S. national phase application absent a clear instruction from applicant not to enter the amendment(s).

The following items 9 to 17 concern a document(s) or information included.

9. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

10. ☒ A preliminary amendment.

11. ☒ An Application Data Sheet under 37 CFR 1.76.

12. ☐ A substitute specification. NOTE: A substitute specification cannot include claims. See 37 CFR 1.125(b).

13. ☐ A power of attorney and/or change of address letter.

14. ☒ A computer-readable form of the sequence listing in accordance with PCT Rule 13*ter*.3 and 37 CFR 1.821-1.825.

15. ☐ Assignment papers *(cover sheet and document(s))*. Name of Assignee: _____

16. ☐ 37 CFR 3.73(c) Statement *(when there is an Assignee)*.

U.S. APPLN. No. (if known – see 37 CFR 1.5)	INTERNATIONAL APPLICATION No.	ATTORNEY DOCKET No.
	PCT/EP2015/076630	32471/H150220

17. [x] Other items or information: WO 2016/075325; International Search Report; Written Opinion; PCT IB304 Form; PCT IB308 (second) Form; ATCC Receipt from Deposit of Biological Materials; Biological Materials Submission Receipt; IB306 Form

The following fees have been submitted.	CALCULATIONS	PTO USE ONLY
18. [x] Basic national fee (37 CFR 1.492(a)) **$280**	$ 280.00	
19. [x] Examination fee (37 CFR 1.492(c)) If the written opinion prepared by ISA/US or the international preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4) ..... **$0** All other situations **$720**	$ 720.00	
20. [x] Search fee (37 CFR 1.492(b)) If the written opinion prepared by ISA/US or the international preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4) ..... **$0** Search fee (37 CFR 1.445(a)(2)) has been paid on the international application to the USPTO as an International Searching Authority ..... **$120** International Search Report prepared by an ISA other than the US and provided to the Office or previously communicated to the US by the IB ..... **$480** All other situations **$600**	$ 480.00	
TOTAL OF 18, 19, and 20 =	$ 1,480.00	
[ ] Additional fee for specification and drawings filed in paper over 100 sheets (excluding sequence listing in compliance with 37 CFR 1.821(c) or (e) in an electronic medium or computer program listing in an electronic medium) (37 CFR 1.492(j)). Fee for each additional 50 sheets of paper or fraction thereof **$400**		

Total Sheets	Extra Sheets	Number of each addition 50 or fraction thereof (round **up** to a whole number)	RATE	
24 - 100 =	/ 50 =		x **$400**	$

Surcharge of **$140.00** for furnishing any of the search fee, examination fee, or the oath or declaration after the date of commencement of the national stage (37 CFR 1.492(h)).				$ 140.00
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	- 20 =	0	x **$80**	$
Independent claims	- 3 =	0	x **$420**	$
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$780**	$
Processing fee of **$140.00** for furnishing the English translation later than 30 months from the earliest claimed priority date (37 CFR 1.492(i)). +				$
TOTAL OF ABOVE CALCULATIONS =				$ 1,620.00
[ ] **Applicant asserts small entity status.** See 37 CFR 1.27. Fees above are reduced by ½.				
[ ] **Applicant certifies micro entity status.** See 37 CFR 1.29. Fees above are reduced by ¾. Applicant must attach form PTO/SB/15A or B or equivalent.				
TOTAL NATIONAL FEE =				$ 1,620.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property. +				$
TOTAL FEES ENCLOSED =				$ 1,620.00
			Amount to be refunded:	$
			Amount to be charged:	$

a. [ ] A check in the amount of $ _____ to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees.

c. [X] The Director is hereby authorized to charge additional fees which may be required, or credit any overpayment, to Deposit Account No. 13-2855 as follows:

i. [X] any required fee.

ii. [ ] any required fee except for excess claims fees required under 37 CFR 1.492(d) and (e) and multiple dependent claim fee required under 37 CFR 1.492(f).

d. [X] Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038. The PTO-2038 should only be mailed or faxed to the USPTO. However, when paying the basic national fee, the PTO-2038 may NOT be faxed to the USPTO.

ADVISORY: If filing by EFS-Web, do **NOT** attach the PTO-2038 form as a PDF along with your EFS-Web submission. Please be advised that this is **not** recommended and by doing so your **credit card information may be displayed via PAIR**. To protect your information, it is recommended to pay fees online by using the electronic payment method.

NOTE: Where an appropriate time limit under 37 CFR 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the International Application to pending status.

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File)Transition Applications

[ ] This application (1) claims priority to or the benefit of an application filed before March 16, 2013, and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.

NOTE 1: By providing this statement under 37 CFR 1.55 or 1.78, **this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA**.

NOTE 2: A U.S. national stage application may not claim priority to the international application of which it is the national phase. The filing date of a U.S. national stage application is the international filing date. See 35 U.S.C. 363.

Correspondence Address

[X] The address associated with Customer Number: 04743 **OR** [ ] Correspondence address below

Name					
Address					
City		State		Zip Code	
Country			Telephone		
Email					

Signature	/Joshua T. Elliott/	Date	May 11, 2017
Name (Print/Type)	Joshua T. Elliott	Registration No. (Attorney/Agent)	43,603

ADVANCE E-MAIL

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92*bis*.1 and Administrative Instructions, Section 422)

To:

HERZOG FIESSER & PARTNER
PATENTANWÄLTE PARTG MBB
Dudenstraße 46
68167 Mannheim
ALLEMAGNE

Date of mailing *(day/month/year)*
20 March 2017 (20.03.2017)

Applicant's or agent's file reference
BPS150220PC

IMPORTANT NOTIFICATION

International application No.
PCT/EP2015/076630

International filing date *(day/month/year)*
13 November 2015 (13.11.2015)

1. The following indications appeared on record concerning:

☐ the applicant ☒ the inventor ☐ the agent ☐ the common representative

Name and Address

MARTY, Laurent
KUNZE, Irene
HAERTEL, Heiko A.
BREMMER, Steven
BREAZEALE, Steven
BAUER, Jörg
VRINTEN, Patricia
STYMNE, Sten
LINDBERG YILMAZ, Jenny
MCELVER, John
REIN, Dietrich

State of Nationality | State of Residence

Telephone No.

Facsimile No.

E-mail address

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☐ the address ☐ the nationality ☐ the residence

Name and Address

State of Nationality | State of Residence

Telephone No.

Facsimile No.

E-mail address
☐ Notifications by e-mail authorized

3. Further observations, if necessary:
The persons identified in Box 1 have been deleted from the records.

4. A copy of this notification has been sent to:

☒ the receiving Office
☒ the International Searching Authority
☐ the Authority(ies) specified for supplementary search
☐ the International Preliminary Examining Authority
☒ the designated Offices concerned
☐ the elected Offices concerned
☐ other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. +41 22 338 89 75

Authorized officer
Suaton Sophie
e-mail pct.team5@wipo.int
Telephone No. +41 22 338 74 05

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 May 2016 (19.05.2016)

WIPO | PCT

(10) International Publication Number
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C12N 15/82 (2006.01)

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(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/079,622 14 November 2014 (14.11.2014) US
62/234,373 29 September 2015 (29.09.2015) US

(71) Applicant: **BASF PLANT SCIENCE COMPANY GMBH** [DE/DE]; 67056 Ludwigshafen (DE).

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(74) Agent: **HERZOG FIESSER & PARTNER PATENTANWÄLTE PARTG MBB**; Dudenstraße 46, 68167 Mannheim (DE).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *with amended claims (Art. 19(1))*

— *with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description (Rules 13bis.4(d)(i) and 48.2(a)(viii))*

Date of publication of the amended claims: 21 July 2016

WO 2016/075325 A4

(54) **Title**: MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS

(57) **Abstract**: The present invention generally is concerned with the modification of plant lipids containing PUFAs. In this context, the invention is particularly concerned with plants and plant materials for such modifications, wherein the plants preferably are oilseed plants. Regarding plant parts, the invention is particularly concerned with seeds of such plants and preferably seeds of oilseed plants. The invention is also concerned with plant positions obtainable or obtained by the modification method of the invention, and with full stuff of feedstuff comprising such liquid compositions.

CSIRO Exhibit 1007

AMENDED CLAIMS
received by the International Bureau on 09 May 2016 (09.05.2016)

1. Extracted *Brassica napus* plant lipid composition comprising eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and optionally arachidonic acid (ARA), wherein
   a) the content of EPA is at least 5% higher than of ARA, and/or
   b) the sum of contents of EPA+DHA is at least 7% higher than ARA and/or
   c) the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%.

2. *Brassica napus* plant or part therof, comprising lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and optionally arachidonic acid (ARA), wherein
   a) the content of EPA is at least 5% higher than of ARA, and/or
   b) the sum of contents of EPA+DHA is at least 7% higher than ARA and/or
   c) the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%.

3. *Brassica napus* plant or part thereof, comprising lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), wherein, when the *Brassica napus* plant or part thereof is grown, the content of ARA decreases at least 0.5% by weight of total lipids while preferably the content of EPA and/or DHA increases.

4. *Brassica napus* plant or part thereof according to claim 3, wherein the content of EPA increases at least 1.0% by weight of total lipids.

5. *Brassica napus* plant according to any of claims 2 to 4, comprising a nucleic acid comprising
   a) a Delta-5 elongase gene under the control of a promoter such that expression of the Delta-5 elongase gene is maintained or increased in late stage seed development, and/or
   b) a Delta-5 desaturase gene under the control of a promoter such that expression of the Delta-5 desaturase gene is reduced or prevented in late stage seed development.

6. Seed of a *Brassica napus* plant according to any of claims 2 to 5.

7. *Brassica napus* plant lipid composition obtainable or obtained by a process comprising the steps of

a) growing a *Brassica napus* plant according to any of claims 2 to 5 at least until the lipids content of ARA has decreased and preferably the lipids content of EPA and/or DHA has increased, and
b) harvesting the plant or a part thereof and
c) extracting lipids composition from the harvested material to obtain said lipid composition.

8. *Brassica napus* plant lipid composition according to claim 7, wherein the process further comprises degumming, deodorising, bleaching, decolourising, drying, winterizing and/or fractionating of the extracted lipids to obtain said lipid composition.

9. Foodstuff or feedstuff comprising a *Brassica napus* plant lipid composition according to any of claims 1, 7 or 8.

10. Method of altering plant lipids composition, comprising the step of growing a *Brassica napus* plant according to any of claims 2 to 5 to produce lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), wherein the step of growing and lipids production is continued until the content of ARA has decreased while preferably the content of EPA and/or DHA has increased.

11. Method of producing a *Brassica napus* plant lipid composition, comprising the steps of
a) growing *Brassica napus* plants according to any of claims 2 to 5,
b) harvesting the plants or a part thereof when the lipids content of ARA has decreased and preferably the lipids content of EPA and/or DHA has increased.

12. Method according to claim 10 or 11, wherein ARA decreases by at least 0.5 wt.-%, preferably from at most 4 wt.-% to at most 3.5 wt.-%, while preferably EPA increases by at least 1 wt.-%, preferably from at least 6 wt.-% to at least 7 wt.-%.

WO 2016/075325
PCT/EP2015/076630

PATENT COOPERATION TREATY

ADVANCE E-MAIL

From the INTERNATIONAL BUREAU

PCT

SECOND AND SUPPLEMENTARY NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION (TO DESIGNATED OFFICES WHICH APPLY THE 30 MONTH TIME LIMIT UNDER ARTICLE 22(1))

(PCT Rule 47.1(c))

To:

HERZOG FIESSER & PARTNER PATENTANWÄLTE
PARTG MBB
Dudenstraße 46
68167 Mannheim
ALLEMAGNE

Date of mailing *(day/month/year)*
16 March 2017 (16.03.2017)

Applicant's or agent's file reference
BPS150220PC

IMPORTANT NOTICE

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2015/076630	13 November 2015 (13.11.2015)	14 November 2014 (14.11.2014)

Applicant
BASF PLANT SCIENCE COMPANY GMBH

1. **ATTENTION**: For any designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002 (30 months from the priority date), **does not apply**, please see Form PCT/IB/308(First Notice) issued previously.

2. Notice is hereby given that the following designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002, **does apply**, has/have requested that the communication of the international application, as provided for in Article 20, be effected under Rule 93*bis*.1. The International Bureau has effected that communication on the date indicated below:
19 May 2016 (19.05.2016)

   AZ, BY, CN, EP, HU, KG, KP, KR, MD, MK, MZ, NA, NG, PG, RU, SY, TM

   In accordance with Rule 47.1(c-*bis*)(i), those Offices will accept the present notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

3. The following designated Offices, for which the time limit under Article 22(1), as in force from 1 April 2002, **does apply**, have not requested, as at the time of mailing of the present notice, that the communication of the international application be effected under Rule 93*bis*.1 :

   AE, AG, AL, AM, AO, AP, AT, AU, BA, BB, BG, BH, BN, BR, BW, BZ, CA, CH, CL, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EA, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, ID, IL, IN, IR, IS, JP, KE, KN, KZ, LA, LC, LK, LR, LS, LY, MA, ME, MG, MN, MW, MX, MY, NI, NO, NZ, OA, OM, PA, PE, PH, PL, PT, QA, RO, RS, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, TH, TJ, TN, TR, TT, UA, US, UZ, VC, VN, ZA, ZM, ZW

   In accordance with Rule 47.1(c-*bis*)(ii), those Offices accept the present notice as conclusive evidence that the Contracting State for which that Office acts as a designated Office does not require the furnishing, under Article 22, by the applicant of a copy of the international application.

4. **TIME LIMITS for entry into the national phase**

   For the designated or elected Office(s) listed above, the applicable time limit for entering the national phase will, **subject to what is said in the following paragraph,** be **30 MONTHS** from the priority date.

   In practice, **time limits other than the 30-month time limit** will continue to apply, for various periods of time, in respect of certain of the designated or elected Office(s) listed above. For **regular updates on the applicable time limits** (30 or 31 months, or other time limit), Office by Office, refer to the *PCT Gazette*, the *PCT Newsletter* and the *PCT Applicant's Guide*, Volume II, National Chapters, all available from WIPO's Internet site, at http://www.wipo.int/pct/en/index.html.

   It is the applicant's **sole responsibility** to monitor all these time limits.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. +41 22 338 82 70

Authorized officer
Nora Lindner

e-mail: pct.team5@wipo.int

ADVANCE E-MAIL

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION, OBTENTION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

HERZOG FIESSER & PARTNER
PATENTANWÄLTE PARTG MBB
Dudenstraße 46
68167 Mannheim
ALLEMAGNE

Date of mailing *(day/month/year)* 01 March 2016 (01.03.2016)	
Applicant's or agent's file reference BPS150220PC	**IMPORTANT NOTIFICATION**
International application No. PCT/EP2015/076630	International filing date *(day/month/year)* 13 November 2015 (13.11.2015)
International publication date *(day/month/year)* **Not yet published**	Priority date *(day/month/year)* 14 November 2014 (14.11.2014)
Applicant BASF PLANT SCIENCE COMPANY GMBH	

The applicant is hereby notified of the date of receipt (or of obtaining by the International Bureau) of the priority document(s) relating to all earlier application(s) whose priority is claimed. Unless otherwise indicated by the letters "NR", in the right-hand column or by an asterisk appearing next to the date of receipt, **the priority document concerned was submitted or transmitted to or obtained by the International Bureau in compliance with Rule 17.1(a), (b) or (b-*bis*)**. This Form replaces any previously issued notification concerning submission, transmittal or obtaining of priority documents.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
14 November 2014 (14.11.2014)	62/079,622	US	24 February 2016 (24.02.2016)
29 September 2015 (29.09.2015)	62/234,373	US	24 February 2016 (24.02.2016)

The letters "NR" denote a priority document which, on the date of mailing of this Form, had not yet been received or obtained by the International Bureau in compliance with Rule 17.1(a), (b) or (b-*bis*). Where the applicant has failed to either submit, request to prepare and transmit, or to request the International Bureau to obtain the priority document within the applicable time limit under that Rule, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

An asterisk "*" next to a date of receipt, denotes a priority document submitted or transmitted to or obtained by the International Bureau but not in compliance with Rule 17.1(a), (b) or (b-*bis*) (the priority document was received after the time limit prescribed in Rule 17.1(a); the request to prepare and transmit the priority document was submitted to the receiving Office after the applicable time limit under Rule 17.1(b) or the request to the International Bureau to obtain the priority document was made after the applicable time limit under Rule 17.1(b-*bis*)). Even though the priority document was not furnished in compliance with Rule 17.1(a), (b) or (b-*bis*), the International Bureau will nevertheless transmit a copy of the document to the designated Offices, for their consideration. In case such a copy is not accepted by the designated Office as the priority document, Rule 17.1(c) provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. +41 22 338 89 75

Authorized officer
Nora Lindner
e-mail pt05.pct@wipo.int
Telephone No. +41 22 338 74 05

The undersigned BASF Plant Science LP, 26 Davis Drive, 27709-3528 Research Triangle Park, United States, has deposited with ATCC Corporate Development, 10801 University Boulevard Manassas, Virginia 20110-2209 USA under accession numbers
PTA-121703
PTA-122340
biological material on the same terms as those laid down in the Budapest Treaty. The undersigned depositor has authorised BASF Plant Science Company GmbH, Limburgerhof, Germany to refer to the aforementioned deposited biological material in International patent application No. PCT/EP2015/076630 and has given his unreserved and irrevocable consent to the deposited material being made available to the public in accordance with Rule 33 EPC as from the date of filing of the aforementioned International patent application.

BASF Plant Science LP, Research Triangle Park, United States

Signature:

Name: RICK VAN GENDEREN

Date: 3/3/16

CSIRO Exhibit 1007

BUDAPEST RESTRICTED CERTIFICATE OF DEPOSIT
BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE
INTERNATIONAL FORM
RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3 AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

The American Type Culture Collection (ATCC®) has received your deposit of seeds/strain(s)/strain(s) in connection with the filing of an application for patent. The following information is provided to fulfill Patent Office requirements.

To: **Cynthia Stokley**
BASF Plant Science LP
26 Davis Drive
Research Triangle, NC 27709

Deposited on Behalf of: **BASF Plant Science LP**

Date of Receipt of seeds/strain(s) by the ATCC®: **11/04/2014**

Depositor Reference: **Brassica napus LBFLFK**

Strain: **LBFLFK**

Quantity: **100 Packets/25 seeds in each packet**

ATCC Designation: **PTA-121703**

The ATCC® understands that:

1 The deposit of these seeds/strain(s) does not grant ATCC® a license, either express or implied, to infringe the patent, and our release of these seeds/strain(s) to others does not grant them a license, either express or implied, to infringe the patent.

2 If the deposit should die or be destroyed during the effective term of the patent, it shall be your responsibility to replace it with viable material. It is also your responsibility to supply a sufficient quantity for distribution for the deposit term. ATCC® will distribute and maintain the material for 30 years or 5 years following the most recent request for the deposit, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

Prior to the issuance of a U.S. Patent, the ATCC® agrees in consideration for a one-time service charge, not to distribute these seeds/strain(s) or any information relating thereto or to their deposit except as instructed by the depositor or relevant patent office. After relevant patent issues we are responsible to release the seeds/strain(s) and they will be made available for distribution to the public without any restrictions. We will inform you of requests for the seeds/strain(s) for 30 years from date of deposit.

The deposit was tested **11/10/2014** and on that date, the seeds/strain(s) were viable
International Depository Authority: American Type Culture Collection (ATCC®), Manassas, VA, USA

Signature of person having authority to represent ATCC®:

Rochelle Harrington

Rochelle Harrington

11/11/2014
Date

cc: **Patricia A. McDaniels**

Ref: Docket or Case No: **INV0077877**

BUDAPEST RESTRICTED CERTIFICATE OF DEPOSIT
BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE
INTERNATIONAL FORM
RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

The American Type Culture Collection (ATCC®) has received your deposit of seeds/strain(s)/strain(s) in connection with the filing of an application for patent. The following information is provided to fulfill Patent Office requirements.

To: **Cynthia Stokley**
BASF Plant Science LP
26 Davis Drive
Research Triangle, NC 27709

Deposited on Behalf of: **BASF Plant Science LP**

Date of Receipt of seeds/strain(s) by the ATCC®: **07/31/2015**

Depositor Reference: **Brassica napus LBFDAU**

Strain: **LBFDAU**

Quantity: **100 Packets/25 seeds in each packet**

ATCC Designation: **PTA-122340**

The ATCC® understands that:

1 The deposit of these seeds/strain(s) does not grant ATCC® a license, either express or implied, to infringe the patent, and our release of these seeds/strain(s) to others does not grant them a license, either express or implied, to infringe the patent.

2 If the deposit should die or be destroyed during the effective term of the patent, it shall be your responsibility to replace it with viable material. It is also your responsibility to supply a sufficient quantity for distribution for the deposit term. ATCC® will distribute and maintain the material for 30 years or 5 years following the most recent request for the deposit, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

Prior to the issuance of a U.S. Patent, the ATCC® agrees in consideration for a one-time service charge, not to distribute these seeds/strain(s) or any information relating thereto or to their deposit except as instructed by the depositor or relevant patent office. After relevant patent issues we are responsible to release the seeds/strain(s) and they will be made available for distribution to the public without any restrictions. We will inform you of requests for the seeds/strain(s) for 30 years from date of deposit.

The deposit was tested **08/24/2015** and on that date, the seeds/strain(s) were viable
International Depository Authority: American Type Culture Collection (ATCC®), Manassas, VA, USA

Signature of person having authority to represent ATCC®:

Rochelle Harrington

Rochelle Harrington

08/25/2015
Date

cc: **Patricia A. McDaniels**

Ref: Docket or Case No: **INV0077877**

BUDAPEST RESTRICTED CERTIFICATE OF DEPOSIT
BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE
INTERNATIONAL FORM
RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

The American Type Culture Collection (ATCC®) has received your deposit of seeds/strain(s)/strain(s) in connection with the filing of an application for patent. The following information is provided to fulfill Patent Office requirements.

To: **Cynthia Stokley**
BASF Plant Science LP
26 Davis Drive
Research Triangle, NC 27709

Deposited on Behalf of: **BASF Plant Science LP**

Date of Receipt of seeds/strain(s) by the ATCC®: **11/04/2014**

Depositor Reference: **Brassica napus LBFLFK**

Strain: **LBFLFK**

Quantity: **100 Packets/25 seeds in each packet**

ATCC Designation: **PTA-121703**

The ATCC® understands that:

1 The deposit of these seeds/strain(s) does not grant ATCC® a license, either express or implied, to infringe the patent, and our release of these seeds/strain(s) to others does not grant them a license, either express or implied, to infringe the patent.

2 If the deposit should die or be destroyed during the effective term of the patent, it shall be your responsibility to replace it with viable material. It is also your responsibility to supply a sufficient quantity for distribution for the deposit term. ATCC® will distribute and maintain the material for 30 years or 5 years following the most recent request for the deposit, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

Prior to the issuance of a U.S. Patent, the ATCC® agrees in consideration for a one-time service charge, not to distribute these seeds/strain(s) or any information relating thereto or to their deposit except as instructed by the depositor or relevant patent office. After relevant patent issues we are responsible to release the seeds/strain(s) and they will be made available for distribution to the public without any restrictions. We will inform you of requests for the seeds/strain(s) for 30 years from date of deposit.

The deposit was tested **11/10/2014** and on that date, the seeds/strain(s) were viable
International Depository Authority: American Type Culture Collection (ATCC®), Manassas, VA, USA

Signature of person having authority to represent ATCC®:

Rochelle Harrington

Rochelle Harrington

11/11/2014
Date

cc: **Patricia A. McDaniels**

Ref: Docket or Case No: **INV0077877**

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2015/076630	13.11.2015	14.11.2014

International Patent Classification (IPC) or both national classification and IPC
INV. C12N15/82

Applicant
BASF PLANT SCIENCE COMPANY GMBH

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Fax: +31 70 340 - 3016	see form PCT/ISA/210	Blanco Urgoiti, B Telephone No. +31 70 340-0

Form PCT/ISA/237 (Cover Sheet) (January 2015)

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	10
	No:	Claims	1-9, 11, 12
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-12
Industrial applicability (IA)	Yes:	Claims	1-12
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Reference is made to the following documents:

D1 WO 2011/006948 A1 (BASF PLANT SCIENCE CO GMBH [DE]; BAUER JOERG [DE]; NAPIER JOHNATHAN A) 20 January 2011 (2011-01-20)

D2 WO 2010/023202 A2 (BASF PLANT SCIENCE GMBH [DE]; BIORIGINAL FOOD & SCIENCE CORP [CA]; BAU) 4 March 2010 (2010-03-04)

D3 WO 2013/153404 A1 (ROTHAMSTED RES LTD [GB]) 17 October 2013 (2013-10-17)

2 NOVELTY AND INVENTIVE STEP (Arts. 33(2) and (3) PCT)

2.1 D1 discloses transgenic Arabidopsis plants transformed with different constructs that express genes of the very long chain polyunsaturated fatty acid (VLC-PUFA) pathway. Plants transformed with the so called OstELO5EmD4, expressed, among others Δ5-elongase from *Ostreococcus tauri* under conlinin promoter (Lu_Cnl) and show DHA levels up to 4,7%, compared to 3% EPA and 1% ARA (see fig. 10 and p.33, l.26-35). Mature seeds of transgenic Arabidopsis transformed with the so called OstELO5TcD4, comprising Δ5-desaturase from *Thraustochytrium* sp. under conlinin promoter (Lu_Cnl), among others, show 8,7% EPA compared to 0,3% DHA and 1,3% ARA (see Fig 12, and p. 33, l 36 onwards; p. 32, 7-11, p.33, l.26-p.34, l.14).

2.2 D2 discloses *Brassica carinata* plants transformed with a so called Napin-4 construct comprising genes of the VLC-PUFA biosynthesis pathway, Δ5-desaturase from *Thraustochytrium* sp. among others. The amount of ARA in these plants ranged from 0.4-4.3% (average: 2.8%), while EPA reached an average level of 9.3%, with the highest value observed being 13.7% (see table 7 and l.13-21 both on p. 50). Lipid extraction is achieved following protocols described in standard literature (see example 4.

2.3 D3 discloses Camelina plants transformed with a construct (DHA-1) comprising Δ5-desaturase from *Thraustochytrium* sp. and Δ5-elongase from *Ostreococcus tauri* among all the genes needed in the biosynthesis of VLC-PUFAs that produce up to 8,8% DHA and 7,5% EPA whereas ARA content is 1,4%.

2.4 In view of any of D1, D2 or D3 the subject-matter of claims 1 and 2 is not novel in the sense of Article 33(2) PCT. The same holds for related claims 5-9.

2.5 The subject-matter of claim 3 relates to a plant that encompasses any plant that produces EPA, DHA and ARA as for example the plants of D1, D2 or D3, wherein during growing, from the germination of the seed to the production of its own seeds, there is a natural decrease/increase in the amount of fatty acids in the plant. Novelty of claim 3 cannot therefore be acknowledged. The same holds true for the methods of claims 11 and 12.

2.6 The description in the present application (see example 1, p. 12) teaches that all the plants tested disclose Δ5-elongase from *Ostreococcus tauri* under the conlinin promoter. In view of D1, and due to the fact that the both genes are undistinguishable, the subject-matter of claim 4 is not novel.

2.7 In view of D1, the provision of a method of altering plant lipid composition that merely comprised growing a plant not novel over D1, cannot be considered to involve an inventive step in the sense of Art. 33(3) PCT.

3 INDUSTRIAL APPLICABILITY

The subject-matter of claims 1-12 seem to be susceptible of Industrial Applicability as defined in Article 33(4) PCT

Re Item VIII

4 ARTICLE 6 PCT

4.1 The relative terms "the content of ARA decreases" or "has decreased"; "the content of EPA and/or DHA increases" or "has increased"; " Delta-5 elongase gene is maintained or increased in late stage seed development" and "Delta-5 desaturase gene is reduced or prevented in late stage seed development" used in claims 3, 4, 7, and 10 to 12 do not allow to unambiguously determine the scope of protection sought, because it is not clear from the

wording of the above mentioned claims to what they are compared to, and leave the reader in doubt as to the meaning of the technical features to which they refer, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
BPS150220PC		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP2015/076630	13 November 2015 (13-11-2015)	14 November 2014 (14-11-2014)
Applicant		
BASF PLANT SCIENCE COMPANY GMBH		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language,** the international search was carried out on the basis of:
      [X] the international application in the language in which it was filed
      [ ] a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))
   b. [ ] This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. [X] With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. [ ] **Certain claims were found unsearchable** (See Box No. II)

3. [ ] **Unity of invention is lacking** (see Box No III)

4. With regard to the **title,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6. With regard to the **drawings,**
   a. the figure of the **drawings** to be published with the abstract is Figure No. _____
      [ ] as suggested by the applicant
      [ ] as selected by this Authority, because the applicant failed to suggest a figure
      [ ] as selected by this Authority, because this figure better characterizes the invention
   b. [ ] none of the figures is to be published with the abstract

Form PCT/ISA/210 (first sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2015/076630

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/076630

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/82
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/006948 A1 (BASF PLANT SCIENCE CO GMBH [DE]; BAUER JOERG [DE]; NAPIER JOHNATHAN A) 20 January 2011 (2011-01-20) the whole document figures 10,12 page 32 - page 34	1-12
X	WO 2010/023202 A2 (BASF PLANT SCIENCE GMBH [DE]; BIORIGINAL FOOD & SCIENCE CORP [CA]; BAU) 4 March 2010 (2010-03-04) the whole document page 50, line 13 - line 21; table 7	1-3,5-9, 11,12
X	WO 2013/153404 A1 (ROTHAMSTED RES LTD [GB]) 17 October 2013 (2013-10-17) page 45 - page 46; example 3	1-3,5-9, 11,12

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 February 2016	07/03/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Blanco Urgoiti, B

1

Form PCT/ISA/210 (second sheet) (April 2005)

CSIRO Exhibit 1007

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2015/076630

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2011006948 A1	20-01-2011	AU 2010272536 A1	02-02-2012
		CA 2768082 A1	20-01-2011
		DE 112010002967 T5	11-10-2012
		EP 2454278 A1	23-05-2012
		US 2012124705 A1	17-05-2012
		WO 2011006948 A1	20-01-2011
WO 2010023202 A2	04-03-2010	AU 2009286755 A1	04-03-2010
		AU 2016200386 A1	11-02-2016
		CA 2734810 A1	04-03-2010
		DE 112009002048 T5	26-01-2012
		EP 2331695 A2	15-06-2011
		JP 5757870 B2	05-08-2015
		JP 2012500640 A	12-01-2012
		US 2011162105 A1	30-06-2011
		WO 2010023202 A2	04-03-2010
WO 2013153404 A1	17-10-2013	AU 2013246661 A1	16-10-2014
		CA 2869738 A1	17-10-2013
		CL 2014002723 A1	15-05-2015
		EP 2836599 A1	18-02-2015
		US 2015216828 A1	06-08-2015
		WO 2013153404 A1	17-10-2013

Form PCT/ISA/210 (patent family annex) (April 2005)

CSIRO Exhibit 1007

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 May 2016 (19.05.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/075325 A1

(51) International Patent Classification:
C12N 15/82 (2006.01)

(21) International Application Number:
PCT/EP2015/076630

(22) International Filing Date:
13 November 2015 (13.11.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/079,622 14 November 2014 (14.11.2014) US
62/234,373 29 September 2015 (29.09.2015) US

(71) Applicant: **BASF PLANT SCIENCE COMPANY GMBH** [DE/DE]; 67056 Ludwigshafen (DE).

(72) Inventors: **SENGER, Toralf**; 609 Cheselden Drive, Durham, North Carolina 27713 (US). **MARTY, Laurent**; Neue Stuecker 21, 69118 Heidelberg (DE). **KUNZE, Irene**; Mühlenweg 11, 06466 Gatersleben (DE). **HAERTEL, Heiko A.**; Rudolf-Reusch-Str. 23D, 10367 Berlin (DE). **BREMMER, Steven**; 1604 Kelvington Place, Apex, North Carolina 27502 (US). **BREAZEALE, Steven**; 462 Neodak Rd., Apex, North Carolina 27523 (US). **BAUER, Jörg**; 1004 Scholastic Circle, Durham, North Carolina 27713 (US). **VRINTEN, Patricia**; 725 310 Stillwater Drive, Saskatoon, Saskatchewan S7J 4H5 (CA). **STYMNE, Sten**; Radmansgatan 1, S-26132 Landskrona (SE). **LINDBERG YILMAZ, Jenny**; Per Ols väg 9, S-23737 Bfärred (SE). **MCELVER, John**; 5112 Gatewood Dr., Durham, North Carolina 27712 (US). **REIN, Dietrich**; Flottwellstrasse 3a, 10785 Berlin (DE). **ANDRE, Carl**; 1012 Winona Rd, Raleigh, North Carolina 27609 (US).

(74) Agent: **HERZOG FIESSER & PARTNER PATENTANWÄLTE PARTG MBB**; Dudenstraße 46, 68167 Mannheim (DE).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description (Rules 13bis.4(d)(i) and 48.2(a)(viii))*

(54) **Title**: MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS

(57) **Abstract**: The present invention generally is concerned with the modification of plant lipids containing PUFAs. In this context, the invention is particularly concerned with plants and plant materials for such modifications, wherein the plants preferably are oil-seed plants. Regarding plant parts, the invention is particularly concerned with seeds of such plants and preferably seeds of oilseed plants. The invention is also concerned with plant positions obtainable or obtained by the modification method of the invention, and with full stuff of feedstuff comprising such liquid compositions.

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CSIRO Exhibit 1007

Modification of plant lipids containing PUFAs

This application claims priority to U.S. Provisional Patent Application Serial Number 62/079622 application number filed November 14, 2014 and to U.S. Provisional Patent Application Serial Number 62/234373 filed September 29, 2015, which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

The present invention generally is concerned with the modification of plant lipids containing PUFAs. In this context, the invention is particularly concerned with plants and plant materials for such modifications, wherein the plants preferably are oilseed plants. Regarding plant parts, the invention is particularly concerned with seeds of such plants and preferably seeds of oilseed plants. The invention is also concerned with plant compositions obtainable or obtained by the modification method of the invention, and with foodstuff of feedstuff comprising such liquid compositions.

BACKGROUND OF THE INVENTION

It is generally recognised that polyunsaturated fatty acids ("PUFAs") convey health benefits. In this context, EPA and DHA are particularly coveted; they are used as dietary supplements for example to alleviate cardiovascular or neurological pathological conditions or ailments. Polyunsaturated fatty acids are currently predominantly obtained from fish oils, because wild-type plants lack the required enzymes to produce polyunsaturated fatty acids, particularly EPA and DHA, in sufficient quantities. Efforts have been made to produce polyunsaturated fatty acids in plants and particularly in oilseed plants.

The production of EPA and DHA is a metabolic pathway wherein fatty acids are treated by desaturases and elongases to produce ever longer and more unsaturated fatty acids. A depiction of the pathway can be found in WO 2006/012325, figure 9, and WO 2005/083093, figure 1. The desaturases and elongases involved in the pathway generally react both on omega-3 and omega-6 polyunsaturated fatty acids. One intermediate in the production of EPA and DHA generally is arachidonic acid. This polyunsaturated fatty acid is generally undesirable in dietary compositions, foodstuff and feedstuff due to its involvement in inflammatory processes. Thus, it is generally desired to obtain compositions with a high content of EPA and/or DHA and a low content of arachidonic acid. However, as arachidonic acid is a metabolit in the production of DHA and because arachidonic acid can be converted by omega-3 desaturases to and from EPA, it is generally not possible to avoid concomitant production of arachidonic acid in transgenic plant metabolism.

It is thus an object of the present invention to provide materials and methods for reducing the content of arachidonic acid in lipid compositions containing EPA and/or DHA. In particular, it is an object of the invention to provide materials and methods for reducing the content of arachidonic acid in plant lipid compositions, preferably in lipid compositions obtainable or obtained from oilseed plants.

BRIEF SUMMARY OF THE INVENTION

The invention therefore provides extracted plant lipid compositions comprising eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and optionally arachidonic acid (ARA), wherein

a) the content of EPA is at least 5% higher than of ARA, and/or

b) the sum of contents of EPA+DHA is at least 7% higher than ARA and/or

c) the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%.

The invention also provides plants or parts therof, comprising lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and optionally arachidonic acid (ARA), wherein

a) the content of EPA is at least 5% higher than of ARA, and/or

b) the sum of contents of EPA+DHA is at least 7% higher than ARA and/or

c) the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%.

Also, the invention provides plants or parts thereof, comprising lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), wherein, when the plant or part thereof is grown, the content of ARA decreases while preferably the content of EPA and/or DHA increases.

According to the invention is also provided a plant comprising a nucleic acid comprising

a) a Delta-5 elongase gene under the control of a promoter such that expression of the Delta-5 elongase gene is maintained or increased in late stage seed development, and/or

b) a Delta-5 desaturase gene under the control of a promoter such that expression of the Delta-5 desaturase gene is reduced or prevented in late stage seed development.

The invention also provides seeds of a plant of the present invention.

Further, the invention provides plant lipid compositions obtainable or obtained by a process comprising the steps of

a) growing a plant of the present invention at least until the lipids content of ARA has decreased and preferably the lipids content of EPA and/or DHA has increased, and

b) harvesting the plant or a part thereof and
c) extracting lipids composition from the harvested material to obtain said lipid composition.

The invention also provides foodstuff or feedstuff comprising a lipid composition of the present invention.

Furthermore, the invention provides methods of altering plant lipids composition, comprising the step of growing a plant of the invention to produce lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), wherein the step of growing and lipids production is continued until the content of ARA has decreased while preferably the content of EPA and/or DHA has increased.

And the invention provides methods of producing a plant lipid composition, comprising the steps of
a) growing plants of the invention,
b) harvesting the plants or a part thereof when the lipids content of ARA has decreased and preferably the lipids content of EPA and/or DHA has increased.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides extracted plant lipid compositions. The lipid compositions comprise EPA and DHA. The extracted plant lipid compositions will generally also comprise ARA, even though this compound is generally not desired as a component but is normally unavoidable due to its function as intermediate metabolite in the production of EPA and/or DHA.

According to the invention, the content of EPA is at least 5% higher than the content of ARA. Unless indicated otherwise, in the context of the present invention a comparison of content numbers is to mean the difference between the respective percentage numbers; such difference is sometimes also called a difference in points percentage. Thus, when the content of EPA in an exemplary composition is for example 7 wt.-%, the content of ARA in the composition is at most 2 wt.-% of total fatty acids of the lipids composition.

It is a particular advantage of the present invention to provide means for the production of lipid compositions exhibiting such strong difference in contents between EPA and ARA. It was particularly surprising that such a marked difference could be maintained in plant lipid compositions, that is in lipid compositions produced in plant material or extracted therefrom as described herein, because both ARA and EPA are produced by the same class of enzymes, that is Delta-5 desaturases, and typically a Delta-5 desaturase producing EPA will also produce ARA. Also, ARA and EPA are converted into each other by action of omega 3 desaturases naturally

present in material or introduced into the plant genome for the purposes of polyunsaturated fatty acid production. It was therefore expected that the composition of plant lipids could not be tilted in favour of high EPA contents without also increasing ARA content. However, the inventors have surprisingly found and provide herein a way not only to increase the EPA content without correspondingly also increasing the content of unwanted ARA; instead, the invention surprisingly provides means for actually decreasing the lipid content of ARA during lipid production already in the plant. Such decrease in ARA content at a time of continuing synthesis of EPA had not been observed or been expected to be possible at all before.

The invention therefore also advantageously provides extracted plant lipid compositions wherein the sum of contents of EPA plus DHA is at least 7% higher than the content of ARA. Providing such a marked difference in contents is even more surprising as EPA is converted into DHA by the action of Delta-5 elongase and Delta 4 desaturase. Thus, EPA is effectively consumed in the production of DHA; it is therefore a particular advantage of the present invention to maintain a high difference in the contents of EPA, DHA and ARA. As described hereinafter, achieving such high difference in contents is possible by the unexpected depletion of ARA in plant lipids during ongoing synthesis of EPA and DHA.

The invention also provides extracted plant lipid compositions wherein the content of ARA is less than 4% and the content of EPA is more than 7%. Even more preferably, the invention provides extracted plant lipid compositions wherein the content of ARA is less than 4%, the content of EPA is more than 7% and the content of DHA is more than 2%. Such compositions are particularly advantageous results of the unexpected mechanism of plant lipid production provided by the invention and simultaneously attain a high EPA/DHA content and a low ARA content.

Polyunsaturated fatty acids (PUFAs) are generally known to the skilled person, important polyunsaturated fatty acids are categorised into an omega-3, omega-6 and omega-9 series, without any limitation intended. Polyunsaturated fatty acids of the omega-6 series include, for example, and without limitation, linoleic acid (18:2 n-6; LA), gamma-linolenic acid (18:3 n-6; GLA), di-homo-gamma-linolenic acid (C20:3 n-6; DGLA), arachidonic acid (C20:4 n-6; ARA), adrenic acid (also called docosatetraenoic acid or DTA; C22:4 n-6) and docosapentaenoic acid (C22:5 n-6). Polyunsaturated fatty acids of the omega-3 series include, for example and without limitation, alpha-linolenic acid (18:3 n-3, ALA), stearidonic acid (18:4 n-3; STA or SDA), eicosatrienoic acid (C20:3 n-3; ETA), eicosatetraenoic acid (C20:4 n-3; ETA), eicosapentaenoic acid (C20:5 n-3; EPA), docosapentaenoic acid (C22:5 n-3; DPA) and docosahexaenoic acid (C22:6 n-3; DHA). Polyunsaturated fatty acids also include fatty acids with greater than 22 carbons and 4 or more double bonds, for example and without limitation, C28:8 (n-3). Polynsaturated fatty acids of the omega-9 series include, for example, and without limitation, mead acid (20:3 n-9; 5,8,11-

eicosatrienoic acid), erucic acid (22:1 n-9; 13-docosenoic acid) and nervonic acid (24:1 n-9; 15-tetracosenoic acid). Further polyunsaturated fatty acids are eicosadienoic acid (C20:2d11,14; EDA) and eicosatrienoic acid (20:3d11,14,17; ETrA).

Within the context of the present invention, lipids content is expressed as weight percentage of a specific fatty acid relative to total fatty acids determined in the respective lipids composition. Preferably, the total fatty acids tested for are: 14:0, 16:0, 16:1n-7, 16:1n-9, 16:3n-3, 17:0, 18:0, 18:1n-7, 18:1n-9, 18:2n-6 (LA), 18:2n-9, 18:3n-3 (ALA), 18:3n-6 (GLA), 18:4n-3 (SDA), 20:0, 20:1n-9, 20:2n-6, 20:2n-9, 20:3n-3, 20:3n-6 (DGLA), 20:3n-9, 20:4n-3 (ETA), 20:4n-6 (ARA), 20:5n-3 (EPA), 22:0, 22:1n-9, 22:2n-6, 22:4n-3, 22:4n-6, 22:5n-3 (DPA), 22:5n-6, 22:6n-3 (DHA), 24:0 and 24:1n-9.

It is a particular advantage of the present invention that the lipids contents described herein, unless explicitly noted otherwise, are determined without artificial enrichment or depletion of one or more fatty acids; the lipid content of a fatty acid is thus substantially the same as in the plant or part thereof prior to extraction.

The extracted lipid preferably is in the form of an oil, wherein at least 90%, more preferably least 95% and even more preferably at least about 98%, or between 95% and 98%, by weight of the oil is the lipid. Such oils can be obtained from plant material by methods known to the skilled person and/or as described herein.

According to the invention, the extracted plant lipid composition is a composition produced by a plant or plant material - preferred ways of producing such lipid compositions in plants and plant materials are also described herein-, and extracted from such lipids and optionally purified. Preferably, the extracted plant lipid composition is a composition to which no additional fatty acids have been added. It is a particular advantage of the present invention that the high difference between the contents of EPA and ARA can be achieved without adding "foreign" EPA to the composition, that is without addition of EPA that has not been produced by the plant or plant material the extract is obtained from. In particular, the contents of EPA and DHA can be achieved according to the invention without addition of fish oil or of corresponding polyunsaturated fatty acids obtained from fish oil.

Within the context of the present invention, reference is made to plants and to corresponding plant material. The plants (and correspondingly the plant material) refer to preferably is of family Brassicaceae. It is a particular advantage of the present invention that the lipid compositions of the present invention can be produced in and extracted from plants of this family, because such plants allow for the production of high amounts of fatty acids particularly in their seed oil. Also,

many species belonging to this family have a long tradition as crop plants, the contents of their oil is thus generally considered useful for consumption and/or easy to obtain and purify for technical purposes or for purposes of consumption.

Plants according to the invention and corresponding plant material preferably belong to the tribus Aethionemeae, Alysseae, Alyssopsideae, Anastaticeae, Anchonieae, Aphragmeae, Arabideae, Asteae, Biscutelleae, Bivonaeeae, Boechereae, Brassiceae, Buniadeae, Calepineae, Camelineae, Cardamineae, Chorisporeae, Cochlearieae, Coluteocarpeae, Conringieae, Cremolobeae, Crucihimalayeae, Descurainieae, Dontostemoneae, Erysimeae, Euclidieae, Eudemeae, Eutremeae, Halimolobeae, Heliophileae, Hesperideae, Iberideae, Isatideae, Kernereae, Lepidieae, Malcolmieae, Megacarpaeeae, Microlepidieae, Noccaeeae, Notothlaspideae, Oreophytoneae, Physarieae, Schizopetaleae, Scoliaxoneae, Sisymbrieae, Smelowskieae, Stevenieae, Thelypodieae, Thlaspideae, Turritideae or Yinshanieae, and even more preferably belong genus Ammosperma, Brassica, Brassica x Raphanus, Cakile, Carrichtera, Ceratocnemum, Coincya, Cordylocarpus, Crambe, Crambella, Didesmus, Diplotaxis, Douepea, Enarthrocarpus, Eremophyton, Eruca, Erucaria, Erucastrum, Euzomodendron, Fezia, Foleyola, Fortuynia, Guiraoa, Hemicrambe, Henophyton, Hirschfeldia, Kremeriella, Moricandia, Morisia, Muricaria, Nasturtiopsis, Orychophragmus, Otocarpus, Physorhynchus, Pseuderucaria, Psychine, Raffenaldia, Raphanus, Rapistrum, Rytidocarpus, Savignya, Schouwia, Sinapidendron, Sinapis, Succowia, Trachystoma, Vella or Zilla. Plants of the aforementioned taxa belong to the family of Brassicaceae and thus can allow for the easy manifestation of the advantages described above in view of said taxonomic family.

Even more preferably the plant or plant material according to the invention belongs to a crop plant of genus Camelina or Brassica. Plants of these genera have traditionally been used in agriculture, their oils have been used for human or animal consumption for a long time. Also, agricultural practices in view of these genera have long been established, for example materials and methods for defense against fungi, insects and weeds. Thus, the production of plant lipids according to the invention in such genera is made particularly easy for the person skilled in agriculture.

Even more preferably a plant and correspondingly plant material according to the invention belongs to any of the species Camelina sativa, Brassica aucheri, Brassica balearica, Brassica barrelieri, Brassica carinata, Brassica carinata x Brassica napus, Brassica carinata x Brassica rapa, Brassica cretica, Brassica deflexa, Brassica desnottesii, Brassica drepanensis, Brassica elongata, Brassica fruticulosa, Brassica gravinae, Brassica hilarionis, Brassica hybrid cultivar, Brassica incana, Brassica insularis, Brassica juncea, Brassica macrocarpa, Brassica maurorum, Brassica montana, Brassica napus (rape, canola), Brassica napus x Brassica rapa, Brassica nigra, Brassica oleracea, Brassica oleracea x Brassica rapa subsp. pekinensis, Brassica

oxyrrhina, Brassica procumbens, Brassica rapa, Brassica rapa x Brassica nigra, Brassica repanda, Brassica rupestris, Brassica ruvo, Brassica souliei, Brassica spinescens, Brassica tournefortii or Brassica villosa, even more preferably to any of the species Brassica carinata, Brassica carinata x Brassica napus or Brassica napus, most preferably of species Brassica napus. Plants of genus Brassica napus are also known as rape seed or canola and have a long tradition as a cheap and readily available source of plant oils and lipids fit for human or animal consumption.

Particularly preferred plants and plant materials are derived from transgenic Brassica event LBFLFK deposited as ATCC Designation "PTA-121703" as described herein, Brassica event LBFLFK contains two insertions of the binary T-plasmid VC-LTM593-1qcz rc as described in the examples section, or from transgenic Brassica event LBFDAU deposited as ATCC Designation "PTA-122340" as also described herein. For these events, particularly high contents of EPA and DHA can be achieved together with low contents of ARA.

Plants and plant materials also preferred according to the invention can be obtained by propagation of these events into other germplasms of plants of genus Camelina and even more preferably of genus Brassica. It is particularly preferred to use as plants and plant materials according to the invention plants resulting from a crossing of a transgenic event according to the invention, particularly of the event LBLFK, with plants of the species to Brassica carinata, even more preferably after backcrossing into Brassica napus. For such plants particularly high contents of EPA and/or DHA and low contents of ARA in the plant lipids according to the invention can be achieved.

According to the invention, the content of ARA preferably decreases by at least .5% during growth of the plant or plant material, preferably during seed development. Thus, by analysing the composition of plant lipids in said plant or plant material, a peak of ARA content can be observed. For example, when a peak content of ARA of 4% is observed, the plant or plant material is harvested only after the content of ARA has decreased to at most 3.5%. It is an advantage of the present invention that a reduction in lipids content of ARA by 0.5 percentage points can be achieved without compromising total lipids production and particularly without compromising the amount and content of EPA and/or DHA obtainable from such plant or plant material.

Preferably, when the plant or plant material of the invention is grown, the lipids content of EPA is maintained even during the reduction of ARA content. Even more preferably the lipids content of EPA increases by at least 1% during the period in which the content of ARA is reduced. Thus, for example the lipids content of EPA in plant seeds increases from 6% to 7% while simultaneously the lipids content of ARA in said plant material decreases from 4% to at most 3.5%. Even more

preferably, the lipids content of EPA and DHA increase during the period of reduction of ARA lipids content when the plant or plant material of the present invention is grown. As noted herein before, it is a particular advantage that the present invention allows for such ongoing synthesis of EPA and DHA even though the content of the metabolic intermediate ARA is reduced.

As described above, the plants and plant material of the present invention preferably are oilseed plants. When the plants of the present invention are grown, it is preferred that they reach their maximum ARA lipids content before late stage seed development. Thus, sufficient time remains for the plant of the present invention to produce in its seed the desired quantities and contents of EPA and/or DHA while reducing the lipids content of ARA. According to the invention, the maximum of ARA lipids content is preferably reached in the developing seeds within 25 to 35 days after flowering where the plants of the present invention belong to species Brassica napus. Correspondingly late stage seed development preferably starts 38 days after flowering in Brassica napus, even more preferably 36 days and even more preferably 35 days after flowering. The skilled person understands that oilseed plants develop many flowers and that individual flowers start to bloom at different days. Thus, the term "days after flowering" refers to the days after flowering of the individual flower and not to the first flower detected on for example a field of plants of the present invention.

A plant or plant material according to the present invention preferably comprises a nucleic acid comprising

a) a Delta-5 elongase gene under the control of a promoter such that expression of the Delta-5 elongase gene is maintained or increased in late stage seed development, and/or

b) a Delta-5 desaturase gene under the control of a promoter such that expression of the Delta-5 desaturase gene is reduced or prevented in late stage seed development.

The inventors have found that by carefully regulating the expression particularly of Delta-5 elongase activity it is possible to achieve the desired reduction in ARA lipids content while maintaining ongoing synthesis of EPA and/or DHA.

The promoters according to the present invention preferably are seed specific promoters. Gene expression can be regulated by any means available to the skilled person. For example, gene expression can be achieved by creating the appropriate construct topology such that transformed nucleic acids (also called "T-DNA" in the art) will, by their very own arrangement of promoters, genes and terminators (collectively also called "expression cassette") achieve the desired regulation pattern. For example, an expression cassette comprising a promoter and operably linked thereto a Delta-5 elongase gene located in the vicinity of another promoter exhibiting strong late stage seed development gene expression can allow for maintained or increased expression

of the Delta-5 elongase gene in late stage seed development. This is particularly so where the expression cassette comprising the Delta-5 elongase gene is separated from one border of integrated T-DNA by at most one expression cassette and from the other border of the T-DNA by at least 5 expression cassettes. This way the T-DNA is long enough to effectively insulate the expression cassettes of the T-DNA from, teen effects of the plant chromosome the T-DNA has integrated into. Preferably, the expression cassette comprising the Delta- 5 the gene is separated from one border of the T-DNA by at most 3, more preferably 1 or 2 and even more preferably by 1 other expression cassette. For the purposes of the present invention, the expression cassette is preferred to in this paragraph contain genes required for the synthesis of polyunsaturated fatty acids and particularly genes coding for desaturases and elongases.

Increased Delta-5 elongase gene expression can also be achieved by the action of an inductor, such that at least one Delta-5 elongase gene is under the control of an inducible promoter; increase can also be achieved by removal of a repressor, such that the repressor is only being produced during early stages of seed development. Preferably, at least one Delta-5 elongase gene is additionally present and expressed under the control of a constitutively and strongly active promotor to achieve a high Delta-5 elongase gene expression also in early and mid seed development stages.

Decreased Delta-5 desaturase expression can be correspondingly achieved by T-DNA topology and/or by placing a Delta-5 desaturase gene under the control of an inducible promoter, wherein the inductor is not or to a lesser extent produced during late seed development, and/or by placing a Delta-5 desaturase gene under the control of a repressible promoter wherein the repressor is produced predominantly or only during late stage seed development.

Examples of corresponding promoters, inductors and repressors and their interaction are described in Hull et al., Plant Science 114, 181-192, Fujiwara et al., Plant Molecular Biology 20, 1059-1069 and Vilardell et al., Plant Molecular Biology 24, 561-569, all incorporated herein by reference.

The invention also provides seeds of a plant of the present invention. Such seeds are useful for planting of new plants of the present invention to produce polyunsaturated fatty acids. Seeds of the present invention are also useful for extraction purposes to obtain and extracted plant lipid composition of the present invention. In each case, the benefits described above can be achieved by the seeds of the present invention.

The invention also provides a plant lipid composition obtainable or obtained by a process comprising the steps of

a) growing a plant of the present invention at least until the lipids content of ARA has decreased and preferably the lipids content of EPA and/or DHA has increased, and
b) harvesting the plant or a part thereof and
c) extracting lipids composition from the harvested material to obtain said lipid composition.

In such process, the beneficial reduction of ARA content in plant lipids provided for by the present invention can be achieved and the corresponding benefits for plant lipid compositions can be materialised.

The process optionally also comprises the step of storing of harvested material, preferably of plant seeds. It is a particular advantage of the present invention that the plant seeds can be stored without compromising the amount and composition of plant seed oils and lipids. This was particularly surprising because polyunsaturated fatty acids are particularly prone to oxidation. Thus, it is advantageous that the plant seeds according to the present invention obtained as harvested material in said process can be stored for example for a month or at least for 7 days at ambient temperatures without loss of seed oil content and particularly without decrease of EPA and/or DHA in seed lipids and seed oil.

The process preferably also comprises the steps of threshing and collecting of seats. Particularly for plants of genus Brassica that a seeds are produced in house gutter and thus need to be separated from unwanted plant material. It is an advantage it is an advantage of the present invention that the seeds can be separated from unwanted plant material for example by threshing without compromising polyunsaturated fatty acid amount and composition in seed lipids and seed oil.

In the process, extraction preferably is performed using pressure and most preferably under an atmosphere with reduced oxygen content compared to ambient temperature; preferably, extraction is performed in the absence of oxygen, for example under a protective atmosphere. Corresponding extraction procedures are known to the skilled person, some extraction procedures are also described herein.

In the process harvesting of plant materials and preferably harvesting of seeds is preferably effected on ripe seeds, that is in late stage seed development. In ripe seeds the lipids content of ARA has had enough time to decrease and the contents of EPA and/or DHA could be increased. When such process is applied on plants of the invention of genus Brassica, harvesting is done preferably after 30 days after first flowering, preferably after 35 days, even more preferably after 40 days, even more preferably after 42 days, even more preferably on or after 43 days and even

more preferably after or on 44 days and even more preferably on or after 45 and even more preferably on or after 46 days after first flowering of the plants.

The process preferably further comprises degumming, deodorising, bleaching, decolourising, drying, winterizing and/or fractionating of the extracted lipids to obtain said lipid composition. This way unwanted impurities of the lipids and/or oil can be removed. Corresponding processes and techniques are known to the skilled person.

The invention also provides foodstuff or feedstuff comprising a lipid composition of the invention. Such food- and feedstuff benefit from the high EPA and/or DHA lipids content and low ARA lipids content achieved by the present invention.

Correspondingly, the invention also provides a method of altering plant lipids composition, comprising the step of growing a plant pf the present invention to produce lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), wherein the step of growing and lipids production is continued until the content of ARA has decreased while preferably the content of EPA and/or DHA has increased. As described above, the content of ARA is preferably decreased by at least 0.5% and preferably is finally at most 4%, preferably is at most 3% and even more preferably at most 2.6% by weight of total lipids. Also as described above, the content of EPA is preferably increased by at least 1% and preferably is finally at least 7%, even more preferably at least 7.5% of total lipids.

The invention also provides a method of producing a plant lipid composition, comprising the steps of

a) growing plants of the present invention,
b) harvesting the plants or a part thereof when the lipids content of ARA has decreased and preferably the lipids content of EPA and/or DHA has increased.

The method allows to materialize the advantages and benefits described herein.

Preferably, Brassica plants of the present invention are grown on a field of commercial scale, preferably at least one acre of size. After 25 days after first appearance of flowers, samples of developing seeds and their lipids are analysed as described herein. Over the next 15 days, preferably over the next 10 days, at least two additional samples of developing seeds are taken and their lipids are also analysed. This way the peak of ARA lipids content can be detected and harvesting can be appropriately delayed to allow the plants of the invention to decrease ARA content and increase EPA and/or DHA content in the lipids of developing seeds.

Also, the invention provides a method of producing seed, comprising the steps of

a) growing plants of the present invention, and

b) harvesting seeds of the plants when the lipids content of ARA has decreased and preferably the lipids content of EPA and/or DHA has increased.

The method allows to materialize the advantages and benefits described herein.

The invention is hereinafter further described by way of examples; the examples are provided for illustrative purposes only and are not intended to limit the invention or the scope of the claims.

EXAMPLES

Example 1: Plant Growth and Sampling

Homozygous T3 plants of event LBFLFK (containing a two copies VC-LTM593-1qcz rc), homozygous T3 plants of event LBFGKN (containing one copy of VC-LTM593-1qcz rc), homozygous T4 plants of event LANPMZ (containing one copy each of VC-LJB2197-1qcz and VC-LLM337-1qcz rc) and homozygous T4 plants of event LAODDN (containing one copy each of VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc) were sown in the field. Plants of the events were obtained and propagated as described in the examples of the priority documents; these are included herein by reference. All events comprise one gene coding for a Delta-5 elongase based on that obtained from Ostreococcus tauri ("d5Elo OT_GA3"). All events further contain one gene coding for a Delta-5 desaturase based on that obtained from Thraustochytrium sp. ("d5Des Tc_GA2"). Events LBFLFK and LBFGKN contain a further copy of the Delta-5 desaturase gene under the control of another promoter (SETL instead of Conlinin). In the week following the date of first flower, individual racemes were visibly marked on the stem just above the most recently opened flower. For every raceme, the three pods immediately below the mark were considered to be the same age (i.e. flowered or were pollinated on the same day). Starting at 14 days after marking and until 46 days after marking, the three pods below the mark on each raceme were collected at various time points. At each time point, approximately 150 pods from 50 individual plants were sampled. Each individual plant was sampled only once in its lifespan. Immature seeds were dissected from the pods immediately after removal from the raceme and were promptly frozen on dry ice. The age of the seeds was determined by the age of the mark on the raceme, meaning that the three pods (and the seeds inside) taken from immediately below a 15 day-old mark were assumed to be 15 days after flowering. For each event, at each time point, seeds from about 150 pods were pooled into a single sample. For analysis, each seed sample was pulverized to powder while still frozen, and the powder was dispensed into aliquot amounts to be used as technical replicates for lipid analysis and gene expression analysis by quantitative real time PCR.

Example 2: Lipid extraction and lipid analysis of plant oils

Lipids were extracted as described in the standard literature including Ullman, Encyclopedia of Industrial Chemistry, Bd. A2, S. 89-90 und S. 443-613, VCH: Weinheim (1985); Fallon, A., et al., (1987) "Applications of HPLC in Biochemistry" in: Laboratory Techniques in Biochemistry and Molecular Biology, Bd. 17; Rehm et al. (1993) Biotechnology, Bd. 3, Kapitel III: "Product recovery and purification", S. 469-714, VCH: Weinheim; Belter, P.A., et al. (1988) Bioseparations: downstream processing for Biotechnology, John Wiley and Sons; Kennedy, J.F., und Cabral, J.M.S. (1992) Recovery processes for biological Materials, John Wiley and Sons; Shaeiwitz, J.A., und Henry, J.D. (1988) Biochemical Separations, in: Ullmann's Encyclopedia of Industrial Chemistry, Bd. B3; Kapitel 11, S. 1-27, VCH: Weinheim; and Dechow, F.J. (1989) Separation and purification techniques in biotechnology, Noyes Publications.

It is acknowledged that extraction of lipids and fatty acids can be carried out using other protocols than those cited above, such as described in Cahoon et al. (1999) Proc. Natl. Acad. Sci. USA 96 (22):12935-12940, and Browse et al. (1986) Analytic Biochemistry 152:141-145. The protocols used for quantitative and qualitative analysis of lipids or fatty acids are described in Christie, William W., Advances in Lipid Methodology, Ayr/Scotland: Oily Press (Oily Press Lipid Library; 2); Christie, William W., Gas Chromatography and Lipids. A Practical Guide - Ayr, Scotland: Oily Press, 1989, Repr. 1992, IX, 307 S. (Oily Press Lipid Library; 1); "Progress in Lipid Research, Oxford: Pergamon Press, 1 (1952) - 16 (1977) u.d.T.: Progress in the Chemistry of Fats and Other Lipids CODEN.

The fatty acids analysed were: 14:0, 16:0, 16:1n-7, 16:1n-9, 16:3n-3, 17:0, 18:0, 18:1n-7, 18:1n-9, 18:2n-6 (LA), 18:2n-9, 18:3n-3 (ALA), 18:3n-6 (GLA), 18:4n-3 (SDA), 20:0, 20:1n-9, 20:2n-6, 20:2n-9, 20:3n-3, 20:3n-6 (DGLA), 20:3n-9, 20:4n-3 (ETA), 20:4n-6 (ARA), 20:5n-3 (EPA), 22:0, 22:1n-9, 22:2n-6, 22:4n-3, 22:4n-6, 22:5n-3 (DPA), 22:5n-6, 22:6n-3 (DHA), 24:0, 24:1n-9.

The content (levels) of fatty acids is expressed throughout the present invention as percentage (weight of a particular fatty acid) of the (total weight of all fatty acids).

Example 3: Quantitative real time PCR protocol

RNA was extracted according to the protocol "SG-MA_0007-2009 RNA isolation" using Spectrum Plant Total RNA-KIT part number STRN50 (SIGMA-ALDRICH GmbH, Munich, Germany). In average the concentration of total RNA was about 450ng/µl. The 260/280 ratio was at 2.2 and the 260/230 ratio at 2.3.

For cDNA synthesis for qPCR 1µg of total RNA was treated with DNAseI (DEOXYRIBUNUCLEASE I (AMP-D1, Amplification Grade from SIGMA-Aldrich, GmbH) according to the supplier's protocol. After DNAseI treatment, the reverse transcription reaction was performed with the SuperScript™ III First-Strand Synthesis SuperMix for qRT-PCR

(Invitrogen, Cat. No. 11752-250) and with a combination of oligo dT and random hexamers to ensure thorough and even representation of all transcripts, regardless of length.

Transcript measurement by quantitative real time PCR was carried out using procedures considered standard to those skilled in the art; see Livak and Schmittgen (2001). The qPCR reactions were done as simplex TaqMan reactions. The endogenous reference gene was isolated in house and used due to predicted stability of the transcript based on the observed stability of the transcript corresponding to the orthologue in *Arabidopsis thaliana* during development. The brassica napus ortholog was isolated and the gene, SEQ ID, was part of the glycosyl-phosphatidylinositol aminotransferase pathway (GPI). The cDNA reactions, described above, were diluted 1:4. 2µl cDNA, which corresponded to 25ng of total RNA, was used per 10µl qPCR reaction with JumpStart TAQ ReadyMix (P2893-400RXN Sigma-Aldrich, GmbH). Primer/probe concentrations were 900 nmol for forward and reverse primer and 100nmol TaqMan probe. The TaqMan probes for targets of interest were labeled with FAM/BHQ1, and the reference gene was labeled with Yakima Yellow/BHQ1.

Each qPCR assay included a 1:1 dilution curve (5 dilution steps) with cDNA from the pool VC-RTP10690-1qcz_F, a no template control, three -RT controls (VC-RTP10690-1qcz_F, VC-LTM593-1qcz rc (~4w) and co-transformation VC-LJB2197-1qcz + VC-LLM337-1qcz rc). From each pool three independent aliquots of cDNA were measured as technical repeats. The ABI PRISM® 7900 sequence detection system (Applied Biosystem) was used with the following PCR Conditions:

Initial denaturation	95°C for 300 seconds	1 cycle
Amplification	95°C for 15 seconds/60°C for 60 seconds	repete for 40 cycles

The raw data were the Ct values for the target and the endogenous reference gene, respectively. The dCt values were calculated by subtraction: Ct(GOI)-Ct(Ref). The Reference dCt value was set to equal zero, which was interpreted as meaning that if there was no difference between GPI and the gene of interest (dCt = 0) the expression was = 1. The fold expression was equal to 2^{-dCt} (where the dCt = (Ct(GOI)-Ct(Ref)-0)). Three samples from each pool were taken and the geometric mean was calculated. The slopes of dilution curves were calculated for each gene of interest and the endogenous reference gene (GPI) as a measure for the amplification efficiency. Table PCR1, Table PCR2 and Table PCR3 indicate the probes and primers used to amplify the genes for qPCR assays.

Table PCR1: Probes used in the qPCR reactions

Target of Interest	Probe Name	Probe Oligo

D12Des(PS-GA)	D12DESPS-138Fam	TGCCTGGATACCTCTTCTTCAACGCTACTG
d6-Des(Otfebit)	D6DES-653FAM	ACTCCATGCACAACAAGCACCACGC
d6Elo (Pp GA)	D6Elo-296-FAM	TGTGCGTGGGTATCGCTTACCAAGC
d6Elo(Tp GA)	D6Elo-280-FAM	AGGAACGGATACACCGTTATGCCATGC
d5DES(Tc_GA)	D5DES-652-FAM	TTGGAGCACGATGTGGATTTGA
d5DES(Tc_GA)3'	D5DES-1147-Fam	CAACCGCTCCACAATTCAGGTTCAAGG
o3DES(Pi_GA2)	o3DES-594FAM	CGCTCACTTCTTCGTTGCTGGACTCTC
o3DES(PIR_GA)	o3DESPIR-198FAM	ATCATCTCTCTCGGAGTTC
d5Elo(Ot_GA3)	E011	TGACAAACAAGCCACCAAGCCCAA
d4DES(TC_GA)	D4DES-Tc-FAM	TGCTTCCCCAATGTACGTTGCTAGGTTCT
d4Des(Eg_GA)	D4DES-Eg-FAM	AAGGCACATCCTCC
d4Des(PI_GA2)	D4DES-PI-770FAM	AGCTTCTTTTCTTGGACGCCCTTGAGC
GPI	Exp3-78-YAK	GGATTCGACATTCCATCGGCTTTGA

Table PCR2: Forward primers used in qPCR

Target of Interest	Forward Primer Name	Forward Primer Oligo
D12Des(PS-GA)	D12DESPS-112F	CGTGTACATGTTGGTTGTTGGAT
d6-Des(Otfebit)	D6DES-629F	TGGCTGGATCTGGAGATATGTG
d6Elo (Pp GA)	D6Elo-271F	TTCTGCTTCGCTTTGTCTCTTTAC
d6Elo(Tp GA)	D6Elo-259F	GAGGCTGGATTCCTCGCTTA
d5DES(Tc_GA)	D5DES-631Fa	CACCACGCTGCTCCAAACAG
d5DES(Tc_GA)3'	D5DES-1120F	ACTTCCAAATCGAGCACCACTT
o3DES(Pi_GA2)	o3DES-572F	CCGCTGTGGTTATCTCTTTGC
o3DES(PIR_GA)	o3DESPIR-160F	CTTGGGAGGCTATGTATGTTAGAAGA
d5Elo(Ot_GA3)	MA54	GCAATCGTTGGTAGCCATGA
d4DES(TC_GA)	D4DES-Tc-F	CAAATCGATGCTGAGTGCAGAT
d4Des(Eg_GA)	D4DES-EG-F	TGACAAGTAAGCCATCCGTCAGT
d4Des(PI_GA2)	D4DES-PI-746-F	CTGGTGAGGCTATGTACGCTTTT
GPI	Exp 3-52F	GATGAATATCCTCCTGATGCTAACC

Table PCR3: Reverse primers used for qPCR

Target of Interest	Reverse Primer Name	Reverse Primer Oligo
D12Des(PS-GA)	D12DESPS-201R	TGAGACCTAGACTTTCCCCAGTACTT
d6-Des(Otfebit)	D6DES-706R	CCATATCGTGCCTCACTTTTTG
d6Elo (Pp GA)	D6Elo-345R	CCACAAGGAATATCTCCAGGTGAT
d6Elo(Tp GA)	D6Elo-330R	TGGATCGTTCACGTTGAAGTG
d5DES(Tc_GA)	D5DES-695R	AAAGCAACGAGTGGCAAGGT
d5DES(Tc_GA)3'	D5DES-1200R	AGAGAGCCTCAACTCTTGGAGAGA
o3DES(Pi_GA2)	o3DES-652R	TCTTAAGTCCCAACTGGAGAGACA
o3DES(PIR_GA)	o3DESPIR-262R	AAACCAAGGAGCGTCAAGTCTAGA
d5Elo(Ot_GA3)	MA55	CGTGTACCACCACGCTTTGT
d4DES(TC_GA)	D4DES-Tc-988R	AACACGGTCAAAGCCTTCATAATC
d4Des(Eg_GA)	D4DES-Eg-R	ACTTTTCACCACCGACGAAGTT
d4Des(PI_GA2)	D4DES-PI-817R	CCTCCCACCTCCAAGCAA
GPI	Exp 3-128R	CTTGCATGATGATCAGGAAAGC

Example 4:

According to the procedures in example 3 mRNA concentrations in seed were determined for each event at various times after flowering. Tables QPCR1 and QPCR2 describe the amounts of mRNA coding for Delta-5 elongase and Delta-5 desaturase genes, respectively. Missing values indicated that no measurements were taken at the respective day for the plants of the respective event. The mRNA concentrations are given in arbitrary units; within each table QPCR1 and QPCR2 the values are thus commensurate; absolute values cannot be compared within tables but comparisons can be validly made for tendencies and trends.

Table QPCR1 shows that expression of the only Delta-5 elongase gene of the events LBFGKN and LBFLFK continued even after 30 days after flowering, whereas expression of the Delta-5 elongase gene of the events LANPMZ and LAODDN was severely reduced or only marginally detectable after 30 days of flowering. Table QPCR2 shows that for all events clearly detectable Delta-5 desaturase mRNA could be detected at all assay dates.

Table QPCR1: Total Delta-5 elongase (d5Elo(Ot_GA3)) mRNA quantity, assay-specific units

	event			
Days after flowering	LANPMZ	LAODDN	LBFGKN	LBFLFK
14	13,3	14,5	11,0	20,7
17		55,7	10,1	6,0
18	15,7			

21	38,8	66,8	29,4	55,5
24		53,6	9,5	40,1
25	19,7			
28	15,7	26,6	10,0	45,1
31		10,6	13,4	23,6
32	0,9			
35	0,9	0,8	10,5	17,9
38		9,0	10,2	13,3
39	0,5			
42	1,3	1,7	19,1	
45		0,9	10,4	30,8
46	1,5			35,7

Table QPCR2: Total Delta-5 desaturase mRNA quantity, assay-specific units

	Event			
Days after flowering	LANPMZ	LAODDN	LBFGKN	LBFLFK
14	55,0	72,7	80,9	124,4
17		168,0	98,1	45,0
18	70,5			
21	199,2	364,7	302,5	292,6
24		308,6	453,4	722,4
25	388,1			
28	615,8	864,2	440,8	1767,1
31		2072,5	763,8	1076,8
32	996,8			
35	452,9		578,6	558,3
38		2987,3	391,5	302,6
39	369,1			
42	497,4	914,4	602,8	
45		679,0	472,5	762,9
46	385,7			1396,4

Example 5: Lipids composition data

The composition of seed lipids of the events was analysed as described above in example 2. As can be seen in Table FA1, the content of ARA in total extracted seed lipids of events LANPMZ

and LAODDN did not significantly decrease over time, whereas the content of ARA ARA in total extracted seed lipids of events LBFGKN and LBFLFK decreases by 0.53 % and 0.72%, respectively. Table FA2 shows that EPA content continued to increase in total extracted seed lipids for all events; Table FA3 shows that also DHA content increased in total extracted seed lipids for all events.

Table FA4 summarizes the seed lipids compositions in the last extracts obtained for each event. The table shows that only for events LBFGKN and LBFLFK a difference in EPA and ARA content of more than 5% could be achieved and a difference in (EPA+DHA) and ARA content of more than 7% could be achieved.

Table FA1: ARA content of seed lipids

	event			
Days after flowering	LANPMZ	LAODDN	LBFGKN	LBFLFK
14	0,1	0,1	0,2	0,2
17		0,3	0,3	0,6
18	0,9			
21	2,0	0,9	1,2	1,9
24		1,2	1,8	2,5
25	2,8			
28	3,1	1,5	2,6	3,0
31		1,5	3,0	3,3
32	3,3			
35	3,6	1,48	2,8	3,1
38		1,4	2,6	2,8
39	3,6			
42	3,6	1,4	2,6	
45		1,3	2,5	2,6
46	3,6			2,5

Table FA2: EPA contents of seed lipids

	event			
Days after flowering	LANPMZ	LAODDN	LBFGKN	LBFLFK
14	0,1	0,2	0,1	0,0
17		0,4	0,4	1,0

18	0,7			
21	1,7	1,6	2,0	3,3
24		2,4	3,2	4,8
25	3,2			
28	3,9	3,2	4,7	6,2
31		4,0	6,1	7,5
32	4,7			
35	5,2	4,53	6,7	7,8
38		4,6	6,8	8,1
39	5,3			
42	5,5	4,9	7,3	
45		4,5	7,6	8,3
46	5,6			8,6

Table FA3: DHA seeds lipid content

	event			
Days after flowering	LANPMZ	LAODDN	LBFGKN	LBFLFK
14	0,0	0,0	0,0	0,0
17		0,0	0,1	0,2
18	0,1			
21	0,3	0,2	0,4	0,4
24		0,3	0,5	0,6
25	0,5			
28	0,7	0,5	0,8	0,8
31		0,7	1,1	1,0
32	0,9			
35	1,1	0,87	1,4	1,1
38		1,0	1,4	1,2
39	1,2			
42	1,3	1,0	1,6	
45		0,9	1,7	1,3
46	1,3			1,4

Table FA4: composition of last lipids extract obtained for each event

	Event			
Content of:	LANPMZ	LAODDN	LFGKN	LBLFK

EPA	5,55	4,54	7,64	8,57
DHA	3	2,46	2,39	3,61
ARA	3,59	1,26	2,47	2,54
EPA-ARA	1,96	3,28	5,17	6,03
(EPA+DHA)-ARA	4,96	5,74	7,56	9,64

0-1	**Form PCT/RO/134 Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)**	
0-1-1	Prepared Using	PCT Online Filing Version 3.5.000.244e MT/FOP 20141031/0.20.5.20
0-2	**International Application No.**	PCT/EP2015/076630
0-3	**Applicant's or agent's file reference**	BPS150220PC

1	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
1-1	**page**	7
1-2	**line**	10
1-3	**Identification of deposit**	
1-3-1	Name of depositary institution	ATCC American Type Culture Collection (ATCC)
1-3-2	Address of depositary institution	10801 University Boulevard, Manassas, Virginia 20110-2209, United States of America
1-3-3	Date of deposit	04 November 2014 (04.11.2014)
1-3-4	Accession Number	ATCC PTA-121703
1-5	**Designated States for Which Indications are Made**	All designations
2	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
2-1	**page**	7
2-2	**line**	13
2-3	**Identification of deposit**	
2-3-1	Name of depositary institution	ATCC American Type Culture Collection (ATCC)
2-3-2	Address of depositary institution	10801 University Boulevard, Manassas, Virginia 20110-2209, United States of America
2-3-3	Date of deposit	31 July 2015 (31.07.2015)
2-3-4	Accession Number	ATCC PTA-122340
2-5	**Designated States for Which Indications are Made**	All designations

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0-4	**This form was received with the international application:** (yes or no)	YES
0-4-1	Authorized officer	Wilson, Patrick

Claims

1. Extracted plant lipid composition comprising eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and optionally arachidonic acid (ARA), wherein
   a) the content of EPA is at least 5% higher than of ARA, and/or
   b) the sum of contents of EPA+DHA is at least 7% higher than ARA and/or
   c) the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%.

2. Plant or part therof, comprising lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and optionally arachidonic acid (ARA), wherein
   a) the content of EPA is at least 5% higher than of ARA, and/or
   b) the sum of contents of EPA+DHA is at least 7% higher than ARA and/or
   c) the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%.

3. Plant or part thereof, comprising lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), wherein, when the plant or part thereof is grown, the content of ARA decreases while preferably the content of EPA and/or DHA increases.

4. Plant according to any of claims 2 or 3, comprising a nucleic acid comprising
   a) a Delta-5 elongase gene under the control of a promoter such that expression of the Delta-5 elongase gene is maintained or increased in late stage seed development, and/or
   b) a Delta-5 desaturase gene under the control of a promoter such that expression of the Delta-5 desaturase gene is reduced or prevented in late stage seed development.

5. Plant according to any of claims 2 to 4, wherein the plant belongs to genus Camelina or Brassica.

6. Seed of a plant according to any of claims 2 to 5.

7. Plant lipid composition obtainable or obtained by a process comprising the steps of
   a) growing a plant according to any of claims 2 to 5 at least until the lipids content of ARA has decreased and preferably the lipids content of EPA and/or DHA has increased, and

b) harvesting the plant or a part thereof and
c) extracting lipids composition from the harvested material to obtain said lipid composition.

8. Plant lipid composition according to claim 7, wherein the process further comprises degumming, deodorising, bleaching, decolourising, drying, winterizing and/or fractionating of the extracted lipids to obtain said lipid composition.

9. Foodstuff or feedstuff comprising a lipid composition according to any of claims 1 or 8.

10. Method of altering plant lipids composition, comprising the step of growing a plant according to any of claims 2 to 5 to produce lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), wherein the step of growing and lipids production is continued until the content of ARA has decreased while preferably the content of EPA and/or DHA has increased.

11. Method of producing a plant lipid composition, comprising the steps of
a) growing plants according to any of claims 2 to 5,
b) harvesting the plants or a part thereof when the lipids content of ARA has decreased and preferably the lipids content of EPA and/or DHA has increased.

12. Method according to claim 10 or 11, wherein ARA decreases by at least 0.5 wt.-%, preferably from at most 4 wt.-% to at most 3.5 wt.-%, while preferably EPA increases by at least 1 wt.-%, preferably from at least 6 wt.-% to at least 7 wt.-%.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/076630

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/82
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/006948 A1 (BASF PLANT SCIENCE CO GMBH [DE]; BAUER JOERG [DE]; NAPIER JOHNATHAN A) 20 January 2011 (2011-01-20) the whole document figures 10,12 page 32 - page 34 -----	1-12
X	WO 2010/023202 A2 (BASF PLANT SCIENCE GMBH [DE]; BIORIGINAL FOOD & SCIENCE CORP [CA]; BAU) 4 March 2010 (2010-03-04) the whole document page 50, line 13 - line 21; table 7 -----	1-3,5-9, 11,12
X	WO 2013/153404 A1 (ROTHAMSTED RES LTD [GB]) 17 October 2013 (2013-10-17) page 45 - page 46; example 3 -----	1-3,5-9, 11,12

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 February 2016	07/03/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Blanco Urgoiti, B

Form PCT/ISA/210 (second sheet) (April 2005)

CSIRO Exhibit 1007

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2015/076630

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2011006948 A1	20-01-2011	AU 2010272536 A1	02-02-2012
		CA 2768082 A1	20-01-2011
		DE 112010002967 T5	11-10-2012
		EP 2454278 A1	23-05-2012
		US 2012124705 A1	17-05-2012
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WO 2010023202 A2	04-03-2010	AU 2009286755 A1	04-03-2010
		AU 2016200386 A1	11-02-2016
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		DE 112009002048 T5	26-01-2012
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		JP 2012500640 A	12-01-2012
		US 2011162105 A1	30-06-2011
		WO 2010023202 A2	04-03-2010
WO 2013153404 A1	17-10-2013	AU 2013246661 A1	16-10-2014
		CA 2869738 A1	17-10-2013
		CL 2014002723 A1	15-05-2015
		EP 2836599 A1	18-02-2015
		US 2015216828 A1	06-08-2015
		WO 2013153404 A1	17-10-2013

Form PCT/ISA/210 (patent family annex) (April 2005)

CSIRO Exhibit 1007

Electronic Patent Application Fee Transmittal	
Application Number:	
Filing Date:	
Title of Invention:	MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS
First Named Inventor/Applicant Name:	Toralf Senger
Filer:	Joshua Thomas Elliott/Audrey Nagelberg
Attorney Docket Number:	32471/H150220

Filed as Large Entity

Filing Fees for U.S. National Stage under 35 USC 371

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
NATIONAL STAGE FEE	1631	1	280	280
NATL STAGE SEARCH FEE - REPORT PROVIDED	1642	1	480	480
NATIONAL STAGE EXAM - ALL OTHER CASES	1633	1	720	720
Pages:				
Claims:				
Miscellaneous-Filing:				
OATH/DECL > 30 MOS FROM 371 COMMENCEMENT	1617	1	140	140
Petition:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		1620

Electronic Acknowledgement Receipt	
EFS ID:	29185127
Application Number:	15526162
International Application Number:	PCT/EP2015/076630
Confirmation Number:	1033
Title of Invention:	MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS
First Named Inventor/Applicant Name:	Toralf Senger
Customer Number:	04743
Filer:	Joshua Thomas Elliott
Filer Authorized By:	
Attorney Docket Number:	32471/H150220
Receipt Date:	11-MAY-2017
Filing Date:	
Time Stamp:	15:58:46
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$1620
RAM confirmation Number	051217INTEFSW15592500
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Sequence Listing (Text File)	H150220_Seqlisting.TXT	14416	no	-
Warnings:					
Information:					
2	Preliminary Amendment	Preliminary_Amendment_Under_37_CFR1.PDF	34176 a70821f36679352d4b3252d98f77b41b80de57ad	no	6
Warnings:					
Information:					
3	Application Data Sheet	Application_Data_Sheet.PDF	28581 2758db31d34370da8918135e6e205983f0736d07	no	5
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
4	Transmittal of New Application	Transmittal_Letter_to_the_United_States_Designated-Elected_Office.PDF	46770 2a87b576c045afd458999f30a30abb1783aae6ab	no	3
Warnings:					
Information:					
5	Documents submitted with 371 Applications	IB306.PDF	39196 ced83d5fb5c662026984d74b5bb31a972c8d44e0	no	1
Warnings:					
Information:					
6	Documents submitted with 371 Applications	Article_19_Amendment.PDF	146464 b4214053611a33aaa351e1f602ba20958a968d0b	no	3
Warnings:					

Information:					
7	Documents submitted with 371 Applications	IB308_Form.PDF	62885 416a12d69467d73c1bf03edc1422bccc5e7f1717	no	1
Warnings:					
Information:					
8	Documents submitted with 371 Applications	IB304_Form.PDF	61792 3891be89eb2208060fa014f2162ef8b6356d1835	no	1
Warnings:					
Information:					
9	Documents submitted with 371 Applications	Biological_Materials_Deposit.PDF	21043 cdff714cf638b5086718a6741e5becbfda85cfed	no	1
Warnings:					
Information:					
10	Documents submitted with 371 Applications	ATCC_Receipt_Biological_Materials.PDF	281096 b89248a92f0414f473b33e233cc3c8593150951f	no	6
Warnings:					
Information:					
11	Documents submitted with 371 Applications	WrittenOpinion.PDF	195366 8080439d3dc038b7cbe870fdef9db8f777c4d253	no	6
Warnings:					
Information:					
12	Documents submitted with 371 Applications	ISR.PDF	128438 4923e4baaaff3707f43fae61973ef544570f7951	no	4
Warnings:					
Information:					
13	Documents submitted with 371 Applications	WIPOPublication.PDF	1195704 d0497744f7a022f7412cd86b0b5df363fb39a6a6	no	26
Warnings:					
Information:					

14	Fee Worksheet (SB06)	fee-info.pdf	36914 92ec6243704dd24d14d78c181653e0bfe60eaa99	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	2292841	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: May 11, 2017
Electronic Signature for Joshua T. Elliott: /Joshua T. Elliott/

Docket No.: 32471/H150220
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Toralf Senger et al.

Application No.: National Stage of PCT/EP2015/076630

Confirmation No.: N/A

Filed: Concurrently Herewith

Art Unit: N/A

For: MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS

Examiner: Not Yet Assigned

PRELIMINARY AMENDMENT UNDER 37 C.F.R. § 1.115

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

INTRODUCTORY COMMENTS

Prior to examination on the merits, please amend the above-identified U.S. patent application as follows:

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims are reflected in the listing of claims which begins on page 3 of this paper.

Remarks/Arguments begin on page 6 of this paper.

AMENDMENTS TO THE SPECIFICATION

Please amend the first paragraph of page 1 of the Specification as follows:

This application is a National Stage application of International Application No. PCT/EP2015/076630, filed November 13, 2015, which claims the benefit of ~~priority to~~ U.S. Provisional Patent Application ~~Serial~~ Number 62/079,622, ~~application number~~ filed November 14, 2014 and ~~to~~ U.S. Provisional Patent Application ~~Serial~~ Number 62/234,373, filed September 29, 2015, which are incorporated herein by reference in their entirety.

Please add the following paragraph after the above-referenced paragraph to page 1 of the Specification:

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

This application incorporates by reference in its entirety a computer-readable nucleotide/amino acid sequence listing identified as one 14,416 byte ASCII (text) file named "H150220_Seqlisting.txt", created May 9, 2017.

AMENDMENTS TO THE CLAIMS

1. (Original) Extracted *Brassica napus* plant lipid composition comprising eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and optionally arachidonic acid (ARA), wherein

a) the content of EPA is at least 5% higher than of ARA, and/or

b) the sum of contents of EPA+DHA is at least 7% higher than ARA and/or

c) the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%.

2. (Original) *Brassica napus* plant or part therof, comprising lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and optionally arachidonic acid (ARA), wherein

a) the content of EPA is at least 5% higher than of ARA, and/or

b) the sum of contents of EPA+DHA is at least 7% higher than ARA and/or

c) the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%.

3. (Currently amended) *Brassica napus* plant or part thereof, comprising lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), wherein, when the Brassica napus plant or part thereof is grown, the content of ARA decreases at least 0.5% by weight of total lipids ~~while preferably the content of EPA and/or DHA increases~~.

4. (Original) *Brassica napus* plant or part thereof according to claim 3, wherein the content of EPA increases at least 1.0% by weight of total lipids.

5. (Currently amended) *Brassica napus* plant according to ~~any of~~ claim[[s]] 2 ~~to 4~~, comprising a nucleic acid comprising

a) a Delta-5 elongase gene under the control of a promoter such that expression of the Delta-5 elongase gene is maintained or increased in late stage seed development, and/or

b) a Delta-5 desaturase gene under the control of a promoter such that expression of the Delta-5 desaturase gene is reduced or prevented in late stage seed development.

6. (Currently amended) Seed of a *Brassica napus* plant according to ~~any of~~ claim[[s]] 2 ~~to 5~~.

7. (Currently amended) *Brassica napus* plant lipid composition obtainable or obtained by a process comprising the steps of

a) growing a Brassica napus plant according to ~~any of~~ claim[[s]] 2 ~~to 5~~ at least until the lipids content of ARA has decreased ~~and preferably the lipids content of EPA and/or DHA has increased~~, and

b) harvesting the plant or a part thereof and

c) extracting lipids composition from the harvested material to obtain said lipid composition.

8. (Original) *Brassica napus* plant lipid composition according to claim 7, wherein the process further comprises degumming, deodorising, bleaching, decolourising, drying, winterizing and/or fractionating of the extracted lipids to obtain said lipid composition.

9. (Currently amended) Foodstuff or feedstuff comprising a *Brassica napus* plant lipid composition according to ~~any of~~ claim[[s]] 1~~, 7 or 8~~.

10. (Currently amended) Method of altering plant lipids composition, comprising the step of growing a *Brassica napus* plant according to ~~any of~~ claim[[s]] 2 ~~to 5~~ to

produce lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), wherein the step of growing and lipids production is continued until the content of ARA has decreased ~~while preferably the content of EPA and/or DHA has increased~~.

11. (Currently amended) Method of producing a *Brassica napus* plant lipid composition, comprising the steps of

a) growing Brassica napus plants according to ~~any of~~ claim[[s]] 2 ~~to 5~~,

b) harvesting the plants or a part thereof when the lipids content of ARA has decreased ~~and preferably the lipids content of EPA and/or DHA has increased~~.

12. (Currently amended) Method according to claim 10 ~~or 11~~, wherein ARA decreases by at least 0.5 wt.-%~~, preferably from at most 4 wt.-% to at most 3.5 wt.-%, while preferably EPA increases by at least 1 wt.-%, preferably from at least 6 wt.-% to at least 7 wt.-%.~~

REMARKS

The specification has been amended to update the cross-reference to related applications and to add a reference to the sequence listing, which do not add new matter.

Upon entry of the amendment, claims 1-12 will be pending. Claims 3, 5-7 and 9-12 have been amended to place the claims in better form for examination. No new matter has been added by way of the amendments set forth herein.

In view of the foregoing amendment, Applicant believes the pending application is in condition for allowance, and such action is solicited. Should the examiner wish to discuss the foregoing, or any matter of form or substance in an effort to advance this application toward allowance, the examiner is invited to telephone the undersigned attorney at the telephone number listed below.

Dated: May 11, 2017

Respectfully submitted,

Electronic signature: /Joshua T. Elliott/
Joshua T. Elliott
Registration No.: 43,603
MARSHALL, GERSTEIN & BORUN LLP
233 S. Wacker Drive
6300 Willis Tower
Chicago, Illinois 60606-6357
(312) 474-6300
Attorney for Applicant

Application Data Sheet

Inventor Information

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State or Province of mailing address::	NC

Country of mailing address::	US
Postal or Zip Code of mailing address::	27609

Correspondence Information

Correspondence Customer Number::	04743

Application Information

Application Type::	Regular
Subject Matter::	Utility
CD-ROM or CD-R?::	None
Sequence submission?::	Yes
Computer Readable Form (CRF)?::	Text File
Title::	MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS
Attorney Docket Number::	32471/H150220
Request for Early Publication?::	No
Request for Non-Publication?::	No
Small Entity?::	No
Petition included?::	No
Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2::	No

Representative Information

Representative Customer Number::	04743

Domestic Priority Information

Application::	Continuity Type::	Parent Application::	Parent Filing Date::	Prior Appl Status::
This Application	a 371 of international	PCT/EP2015/076630	11/13/15	
PCT/EP2015/076630	Claims benefit of provisional	62/234,373	09/29/15	
PCT/EP2015/076630	Claims benefit of provisional	62/079,622	11/14/14	

Foreign Priority Information

Applicant Information

Applicant Number::	1
Applicant Type::	Assignee
Organization Name::	BASF PLANT SCIENCE COMPANY GMBH
Street of mailing address::	Carl-Bosch Strasse 38
City of mailing address::	Ludwigshafen
Country of mailing address::	Germany
Postal or Zip Code of mailing address::	D-67056

Assignee Information Including Non-Applicant Assignee Information

Authorization or Opt-Out of Authorization to Permit Access

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Joshua T. Elliott/	Date (YYYY-MM-DD)	2017-05-11
Name	Joshua T. Elliott	Registration Number	43,603

ADVANCE E-MAIL

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92*bis*.1 and Administrative Instructions, Section 422)

To:

HERZOG FIESSER & PARTNER
PATENTANWÄLTE PARTG MBB
Dudenstraße 46
68167 Mannheim
ALLEMAGNE

Date of mailing *(day/month/year)*
20 March 2017 (20.03.2017)

Applicant's or agent's file reference
BPS150220PC

IMPORTANT NOTIFICATION

International application No.
PCT/EP2015/076630

International filing date *(day/month/year)*
13 November 2015 (13.11.2015)

1. The following indications appeared on record concerning:

☐ the applicant ☒ the inventor ☐ the agent ☐ the common representative

Name and Address

MARTY, Laurent
KUNZE, Irene
HAERTEL, Heiko A.
BREMMER, Steven
BREAZEALE, Steven
BAUER, Jörg
VRINTEN, Patricia
STYMNE, Sten
LINDBERG YILMAZ, Jenny
MCELVER, John
REIN, Dietrich

State of Nationality | State of Residence

Telephone No.

Facsimile No.

E-mail address

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☐ the address ☐ the nationality ☐ the residence

Name and Address

State of Nationality | State of Residence

Telephone No.

Facsimile No.

E-mail address
☐ Notifications by e-mail authorized

3. Further observations, if necessary:
The persons identified in Box 1 have been deleted from the records.

4. A copy of this notification has been sent to:

☒ the receiving Office
☒ the International Searching Authority
☐ the Authority(ies) specified for supplementary search
☐ the International Preliminary Examining Authority
☒ the designated Offices concerned
☐ the elected Offices concerned
☐ other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. +41 22 338 89 75

Authorized officer
Suaton Sophie
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Telephone No. +41 22 338 74 05

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference BPS150220PC	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/EP2015/076630	International filing date *(day/month/year)* 13 November 2015 (13.11.2015)	Priority date *(day/month/year)* 14 November 2014 (14.11.2014)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant BASF PLANT SCIENCE COMPANY GMBH		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 16 May 2017 (16.05.2017)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Nora Lindner e-mail: pct.team5@wipo.int

Form PCT/IB/373 (January 2004)

CSIRO Exhibit 1007

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2015/076630	13.11.2015	14.11.2014

International Patent Classification (IPC) or both national classification and IPC
INV. C12N15/82

Applicant
BASF PLANT SCIENCE COMPANY GMBH

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Fax: +31 70 340 - 3016	see form PCT/ISA/210	Blanco Urgoiti, B Telephone No. +31 70 340-0

Form PCT/ISA/237 (Cover Sheet) (January 2015)

CSIRO Exhibit 1007

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	10
	No:	Claims	1-9, 11, 12
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-12
Industrial applicability (IA)	Yes:	Claims	1-12
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Reference is made to the following documents:

D1 WO 2011/006948 A1 (BASF PLANT SCIENCE CO GMBH [DE]; BAUER JOERG [DE]; NAPIER JOHNATHAN A) 20 January 2011 (2011-01-20)

D2 WO 2010/023202 A2 (BASF PLANT SCIENCE GMBH [DE]; BIORIGINAL FOOD & SCIENCE CORP [CA]; BAU) 4 March 2010 (2010-03-04)

D3 WO 2013/153404 A1 (ROTHAMSTED RES LTD [GB]) 17 October 2013 (2013-10-17)

2 NOVELTY AND INVENTIVE STEP (Arts. 33(2) and (3) PCT)

2.1 D1 discloses transgenic Arabidopsis plants transformed with different constructs that express genes of the very long chain polyunsaturated fatty acid (VLC-PUFA) pathway. Plants transformed with the so called OstELO5EmD4, expressed, among others Δ5-elongase from *Ostreococcus tauri* under conlinin promoter (Lu_Cnl) and show DHA levels up to 4,7%, compared to 3% EPA and 1% ARA (see fig. 10 and p.33, l.26-35). Mature seeds of transgenic Arabidopsis transformed with the so called OstELO5TcD4, comprising Δ5-desaturase from *Thraustochytrium* sp. under conlinin promoter (Lu_Cnl), among others, show 8,7% EPA compared to 0,3% DHA and 1,3% ARA (see Fig 12, and p. 33, l 36 onwards; p. 32, 7-11, p.33, l.26-p.34, l.14).

2.2 D2 discloses *Brassica carinata* plants transformed with a so called Napin-4 construct comprising genes of the VLC-PUFA biosynthesis pathway, Δ5-desaturase from *Thraustochytrium* sp. among others. The amount of ARA in these plants ranged from 0.4-4.3% (average: 2.8%), while EPA reached an average level of 9.3%, with the highest value observed being 13.7% (see table 7 and l.13-21 both on p. 50). Lipid extraction is achieved following protocols described in standard literature (see example 4.

2.3 D3 discloses Camelina plants transformed with a construct (DHA-1) comprising Δ5-desaturase from *Thraustochytrium* sp. and Δ5-elongase from *Ostreococcus tauri* among all the genes needed in the biosynthesis of VLC-PUFAs that produce up to 8,8% DHA and 7,5% EPA whereas ARA content is 1,4%.

2.4 In view of any of D1, D2 or D3 the subject-matter of claims 1 and 2 is not novel in the sense of Article 33(2) PCT. The same holds for related claims 5-9.

2.5 The subject-matter of claim 3 relates to a plant that encompasses any plant that produces EPA, DHA and ARA as for example the plants of D1, D2 or D3, wherein during growing, from the germination of the seed to the production of its own seeds, there is a natural decrease/increase in the amount of fatty acids in the plant. Novelty of claim 3 cannot therefore be acknowledged. The same holds true for the methods of claims 11 and 12.

2.6 The description in the present application (see example 1, p. 12) teaches that all the plants tested disclose Δ5-elongase from *Ostreococcus tauri* under the conlinin promoter. In view of D1, and due to the fact that the both genes are undistinguishable, the subject-matter of claim 4 is not novel.

2.7 In view of D1, the provision of a method of altering plant lipid composition that merely comprised growing a plant not novel over D1, cannot be considered to involve an inventive step in the sense of Art. 33(3) PCT.

3 INDUSTRIAL APPLICABILITY

The subject-matter of claims 1-12 seem to be susceptible of Industrial Applicability as defined in Article 33(4) PCT

Re Item VIII

4 ARTICLE 6 PCT

4.1 The relative terms "the content of ARA decreases" or "has decreased"; "the content of EPA and/or DHA increases" or "has increased"; " Delta-5 elongase gene is maintained or increased in late stage seed development" and "Delta-5 desaturase gene is reduced or prevented in late stage seed development" used in claims 3, 4, 7, and 10 to 12 do not allow to unambiguously determine the scope of protection sought, because it is not clear from the

wording of the above mentioned claims to what they are compared to, and leave the reader in doubt as to the meaning of the technical features to which they refer, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).

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December 31, 2015

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APPLICATION NUMBER: *62/234,373*
FILING DATE: *September 29, 2015*

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MATERIALS AND METHODS FOR PUFA PRODUCTION, AND PUFA-CONTAINING COMPOSITIONS

FIELD OF THE INVENTION

The invention generally pertains to the field of manufacture of fatty acids, particularly for large-scale production of very long chain polyunsaturated fatty acids (VLC-PUFAs, also called polyunsaturated fatty acids or PUFAs), e.g. eicosapentaenoic acid (EPA), omega-3 docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA). The invention particularly is concerned with the production of VLC-PUFAs in plants and thus inter alia provides nucleic acids for transformation of plants to enable such transformed plants to produce VLC-PUFAs. To this end, the invention also provides transgenic constructs and expression vectors containing desaturase and elongase genes and host cells into which the constructs and expression vectors have been introduced. The present invention also relates to methods for the manufacture of oil, fatty acid- or lipids-containing compositions, and to such oils and lipids as such. In addition, the invention is concerned with methods for further improving the production of VLC-PUFAs in plants.

BACKGROUND OF THE INVENTION

Fatty acids are carboxylic acids with long-chain hydrocarbon side groups that play a fundamental role in many biological processes. Fatty acids are rarely found free in nature but, rather, occur in esterified form as the major component of lipids. As such, lipids/fatty acids are sources of energy (e.g., beta-oxidation). In addition, lipids/fatty acids are an integral part of cell membranes and, therefore, are indispensable for processing biological or biochemical information.

Long chain polyunsaturated fatty acids (VLC-PUFAs) such as docosahexaenoic acid (DHA, 22:6(4,7,10,13,16,19)) are essential components of cell membranes of various tissues and organelles in mammals (nerve, retina, brain and immune cells). Clinical studies have shown that DHA is essential for the growth and development of the brain in infants, and for maintenance of normal brain function in adults (Martinetz, M. (1992) J. Pediatr. 120:S129 S138). DHA also has significant effects on photoreceptor function involved in the signal transduction process, rhodopsin activation, and rod and cone development (Giusto, N.M., et al. (2000) Prog. Lipid Res. 39:315-391). In addition, some positive effects of DHA were also found on diseases such as hypertension, arthritis, atherosclerosis, depression, thrombosis and cancers (Horrocks, L.A. and Yeo, Y.K. (1999) Pharmacol. Res. 40:211-215). Therefore, appropriate dietary supply of the fatty acid is important for human health. Because such fatty acids cannot be efficiently synthesized by infants, young children and senior citizens, it is particularly important for these individuals to adequately intake these fatty acids from the diet (Spector, A.A. (1999) Lipids 34:S1 S3).

EPA and also ARA (arachidonic acid, 20:4n-6 (5,8,11,14)) are both delta 5 (d5) essential fatty acids. They form a unique class of food and feed constituents for humans and animals. EPA belongs to the n-3 series with five double bonds in the acyl chain. EPA is found in marine food and is abundant in oily fish from North Atlantic. ARA belongs to the n-6 series with four double bonds. The lack of a double bond in the delta-3 position confers on ARA different properties than those found in EPA. The eicosanoids produced from ARA (sometimes abbreviated "AA") have strong inflammatory and platelet aggregating properties, whereas those derived from EPA have anti-inflammatory and anti-platelet aggregating properties. ARA can be obtained from some foods such as meat, fish and eggs, but the concentration is low.

Gamma-linolenic acid (GLA, C18:3n-6 (6,9,12)) is another essential fatty acid found in mammals. GLA is the metabolic intermediate for very long chain n-6 fatty acids and for various active molecules. In mammals, formation of long chain polyunsaturated fatty acids is rate-limited by delta-6 desaturation. Many physiological and pathological conditions such as aging, stress, diabetes, eczema, and some infections have been shown to depress the delta-6 desaturation step. In addition, GLA is readily catabolized from the oxidation and rapid cell division associated with certain disorders, e.g., cancer or inflammation. Therefore, dietary supplementation with GLA can reduce the risks of these disorders. Clinical studies have shown that dietary supplementation with GLA is effective in treating some pathological conditions such as atopic eczema, premenstrual syndrome, diabetes, hypercholesterolemia, and inflammatory and cardiovascular disorders.

A large number of benefitial health effects have been shown for DHA or mixtures of EPA and DHA.

Although biotechnology offers an attractive route for the production of specialty fatty acids, current techniques fail to provide an efficient means for the large scale production of unsaturated fatty acids. Accordingly, there exists a need for an improved and efficient method of producing very long chain poly unsaturated fatty acids (VLC-PUFAs), such as EPA and DHA.

To make possible the fortification of food and/or of feed with polyunsaturated omega-3-fatty acids, there is still a great need for a simple, inexpensive process for the production of each of the aforementioned long chain polyunsaturated fatty acids, especially in eukaryotic systems.

SUMMARY OF THE INVENTION

The invention is thus concerned with providing a reliable source for easy manufacture of VLC-PUFAs. To this end the invention is also concerned with providing plants reliably

producing VLC-PUFAS, preferably EPA and/or DHA. The invention is also concerned with providing means and methods for obtaining, improving and farming such plants, and also with VLC-PUFA containing oil obtainable from such plants, particularly from the seeds thereof. Also, the invention provides uses for such plants and parts thereof.

According to the invention there is thus provided a T-DNA for expression of a target gene in a plant. The invention beneficially provides a system for transformation of plant tissue and for generation of recombinant plants, wherein the recombinant plant differs from the respective parental plant (for the purposes of the present invention the parental plant is termed a wild-type plant regardless of whether or not such parental plant is as such found in nature) by the introduction of T-DNA. The T-DNA introduced into the parental plant beneficially has a length of at least 30000 nucleotides.

The invention also provides plants with a genotype that confers a heritable phenotype of high seed oil VLC-PUFA content in one or more of their tissues or components, preferably a high content of EPA and/or DHA in seed oil. The invention further provides material comprising a high VLC-PUFA content relative to their total oil content, preferably a high content of EPA and/or DHA. Also, the invention provides exemplary events of Brassica plants. Most beneficially the invention provides oil comprising a high VLC-PUFA content, preferably a high content of EPA and/or DHA.

The invention also provides methods of producing an oil, wherein the oil has a high VLC-PUFA content, a high content of EPA and/or DHA. In particularly preferred aspects these methods are for producing a corresponding plant oil.

The invention also provides methods for creating a plant, such that the plant or progeny thereof can be used as a source of an oil, wherein the oil has a high VLC-PUFA content, a high content of EPA and/or DHA. Thus, the invention beneficially also provides methods for the production of plants having a heritable phenotype of high seed oil VLC-PUFA content in one or more of their tissues or components, preferably a high content of EPA and/or DHA in seed oil.

The invention also provides means for optimizing a method for creating plants according to the invention. In this respect, the invention provides a system of methods for analyzing enzyme specificities, particularly for analyzing desaturase reaction specificity and for analyzing elongase specificities, for optimization of a metabolic pathway and for determining CoA-dependence of a target desaturase.

DETAILED DESCRIPTION OF THE INVENTION

Various aspects of the invention are hereinafter described in more detail. It is to be understood that the detailed description is not intended to limit the scope of the claims.

The term "polyunsaturated fatty acids (PUFA)" as used herein refers to fatty acids comprising at least two, preferably, three, four, five or six, double bonds. Moreover, it is to be understood that such fatty acids comprise, preferably from 18 to 24 carbon atoms in the fatty acid chain. More preferably, the term relates to long chain PUFA (VLC-PUFA) having from 20 to 24 carbon atoms in the fatty acid chain. Particularly, polyunsaturated fatty acids in the sense of the present invention are DHGLA 20:3 (8,11,14), ARA 20:4 (5,8,11,14), iARA 20:4(8,11,14,17), EPA 20:5 (5,8,11,14,17), DPA 22:5 (4,7,10,13,16), DHA 22:6 (4,7,10,13,16,19), 20:4 (8,11,14,17), more preferably, arachidonic acid (ARA) 20:4 (5,8,11,14), eicosapentaenoic acid (EPA) 20:5 (5,8,11,14,17), and docosahexaenoic acid (DHA) 22:6 (4,7,10,13,16,19). Thus, it will be understood that most preferably, the methods provided by the present invention pertaining to the manufacture of EPA and/or DHA. Moreover, also encompassed are the intermediates of VLC-PUFA which occur during synthesis. Such intermediates are, preferably, formed from substrates by the desaturase, keto-acyl-CoA-synthase, keto-acyl-CoA-reductase, dehydratase and enoyl-CoA-reductase activity of the polypeptide of the present invention. Preferably, substrates encompass LA 18:2 (9,12), GLA 18:3 (6,9,12), DHGLA 20:3 (8,11,14), ARA 20:4 (5,8,11,14), eicosadienoic acid 20:2 (11,14), eicosatetraenoic acid 20:4 (8,11,14,17), eicosapentaenoic acid 20:5 (5,8,11,14,17). Systematic names of fatty acids including polyunsaturated fatty acids, their corresponding trivial names and shorthand notations used according to the present invention are given in table Table 18 (and in table 181).

The term "cultivating" as used herein refers to maintaining and growing the transgenic plant under culture conditions which allow the cells to produce the said polyunsaturated fatty acid, i.e. the PUFA and/or VLC-PUFA referred to above. This implies that the polynucleotide of the present invention is expressed in the transgenic plant so that the desaturase, elongase as also the keto-acyl-CoA-synthase, keto-acyl-CoA-reductase, dehydratase and enoyl-CoA-reductase activity is present. Suitable culture conditions for cultivating the host cell are described in more detail below.

The term "obtaining" as used herein encompasses the provision of the cell culture including the host cells and the culture medium or the plant or plant part, particularly the seed, of the current invention, as well as the provision of purified or partially purified preparations thereof comprising the polyunsaturated fatty acids, preferably, ARA, EPA, DHA, in free or in CoA bound form, as membrane phospholipids or as triacylglyceride estres. More preferably, the PUFA and VLC-PUFA are to be obtained as triglyceride esters, e.g., in form of an oil. More details on purification techniques can be found elsewhere herein below.

The term "polynucleotide" according to the present invention refers to a desoxyribonucleic acid or ribonucleic acid. Unless stated otherwise, "polynucleotide" herein refers to a single strand of a DNA polynucleotide or to a double stranded DNA polynucleotide. The length of a polynucleotide is designated according to the invention by the specification of a number of basebairs ("bp") or nucleotides ("nt"). According to the invention, both specifications are used interchangeably, regardless whether or not the respective nucleic acid is a single or double stranded nucleic acid. Also, as polynucleotides are defined by their respective nucleotide sequence, the terms nucleotide/polynucleotide and nucleotide sequence/polynucleotide sequence are used interchangeably, thus that a reference to a nucleic acid sequence also is meant to define a nucleic acid comprising or consisting of a nucleic acid stretch the sequence of which is identical to the nucleic acid sequence.

In particular, the term "polynucleotide" as used in accordance with the present invention as far as it relates to a desaturase or elongase gene relates to a polynucleotide comprising a nucleic acid sequence which encodes a polypeptide having desaturase or elongase activity. Preferably, the polypeptide encoded by the polynucleotide of the present invention having desaturase, or elongase activity upon expression in a plant shall be capable of increasing the amount of PUFA and, in particular, VLC-PUFA in, e.g., seed oils or an entire plant or parts thereof. Whether an increase is statistically significant can be determined by statistical tests well known in the art including, e.g., Student's t-test with a confidentiality level of at least 90%, preferably of at least 95% and even more preferably of at least 98%. More preferably, the increase is an increase of the amount of triglycerides containing VLC-PUFA of at least 5%, at least 10%, at least 15%, at least 20% or at least 30% compared to wildtype control. Preferably, the VLC-PUFA referred to before is a polyunsaturated fatty acid having a C20, C22 or C24 fatty acid body, more preferably EPA or DHA. Lipid analysis of oil samples are shown in the accompanying Examples.

The fatty acid esters with polyunsaturated C20- and/or C22-fatty acid molecules can be isolated in the form of an oil or lipid, for example, in the form of compounds such as sphingolipids, phosphoglycerides, lipids, glycolipids such as glycosphingolipids, phospholipids such as phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol or diphosphatidylglycerol, monoacylglycerides, diacylglycerides, triacylglycerides or other fatty acid esters such as the acetylcoenzyme A esters which comprise the polyunsaturated fatty acids with at least two, three, four, five or six, preferably five or six, double bonds, from the organisms which were used for the preparation of the fatty acid esters. Preferably, they are isolated in the form of their diacylglycerides, triacylglycerides and/or in the form of phosphatidylcholine, especially preferably in the form of the triacylglycerides. In addition to these esters, the polyunsaturated fatty acids are also present in the non-human transgenic organisms or host cells, preferably in the plants, as free fatty acids or bound in other compounds. As a rule, the various abovementioned compounds (fatty acid esters and free fatty acids) are present

in the organisms with an approximate distribution of 80 to 90% by weight of triglycerides, 2 to 5% by weight of diglycerides, 5 to 10% by weight of monoglycerides, 1 to 5% by weight of free fatty acids, 2 to 8% by weight of phospholipids, the total of the various compounds amounting to 100% by weight. In the process of the invention, the VLC-PUFAs which have been produced are produced in a content as for DHA of at least 5,5% by weight, at least 6% by weight, at least 7% by weight, advantageously at least 8% by weight, preferably at least 9% by weight, especially preferably at least 10,5% by weight, very especially preferably at least 20% by weight, as for EPA of at least 9,5% by weight, at least 10% by weight, at least 11% by weight, advantageously at least 12% by weight, preferably at least 13% by weight, especially preferably at least 14,5% by weight, very especially preferably at least 30% by weight based on the total fatty acids in the non-human transgenic organisms or the host cell referred to above. The fatty acids are, preferably, produced in bound form. It is possible, with the aid of the polynucleotides and polypeptides of the present invention, for these unsaturated fatty acids to be positioned at the sn1, sn2 and/or sn3 position of the triglycerides which are, preferably, to be produced.

In a method or manufacturing process of the present invention the polynucleotides and polypeptides of the present invention may be used with at least one further polynucleotide encoding an enzyme of the fatty acid or lipid biosynthesis. Preferred enzymes are in this context the desaturases and elongases as mentioned above, but also polynucleotide encoding an enzyme having delta-8-desaturase and/or ddelta-9-elongase activity. All these enzymes reflect the individual steps according to which the end products of the method of the present invention, for example EPA or DHA are produced from the starting compounds linoleic acid (C18:2) or linolenic acid (C18:3). As a rule, these compounds are not generated as essentially pure products. Rather, small traces of the precursors may be also present in the end product. If, for example, both linoleic acid and linolenic acid are present in the starting host cell, organism, or the starting plant, the end products, such as EPA or DHA, are present as mixtures. The precursors should advantageously not amount to more than 20% by weight, preferably not to more than 15% by weight, more preferably, not to more than 10% by weight, most preferably not to more than 5% by weight, based on the amount of the end product in question. Advantageously, only EPA or more preferably only DHA, bound or as free acids, is/are produced as end product(s) in the process of the invention in a host cell. If the compounds EPA and DHA are produced simultaneously, they are, preferably, produced in a ratio of at least 1:2 (DHA:EPA), more preferably, the ratios are at least 1:5 and, most preferably, 1:8. Fatty acid esters or fatty acid mixtures produced by the invention, preferably, comprise 6 to 15% of palmitic acid, 1 to 6% of stearic acid, 7-85% of oleic acid, 0.5 to 8% of vaccenic acid, 0.1 to 1% of arachic acid, 7 to 25% of saturated fatty acids, 8 to 85% of monounsaturated fatty acids and 60 to 85% of polyunsaturated fatty acids, in each case based on 100% and on the total fatty acid content of the organisms. DHA as a preferred long chain polyunsaturated fatty acid is present in the fatty acid esters or fatty acid mixtures in a concentration of, preferably, at least 0.1; 0.2; 0.3;

0.4; 0.5; 0.6; 0.7; 0.8; 0.9 or 1%, based on the total fatty acid content. Moreover, the fatty acid esters or fatty acid mixtures which have been produced by the method of the invention, preferably, comprise further fatty acids selected from the group of the fatty acids erucic acid (13-docosaenoic acid), sterculic acid (9,10-methyleneoctadec-9-enoic acid), malvalic acid (8,9-methyleneheptadec-8-enoic acid), chaulmoogric acid (cyclopentenedodecanoic acid), furan fatty acid (9, 12-epoxyoctadeca-9, 11-dienoic acid), vernolic acid (9,10-epoxyoctadec-12-enoic acid), tariric acid (6-octadecynoic acid), 6-nonadecynoic acid, santalbic acid (t11-octadecen-9-ynoic acid), 6,9-octadecenynoic acid, pyrulic acid (t10-heptadecen-8-ynoic acid), crepenyninic acid (9-octadecen-12-ynoic acid), 13,14-dihydrooropheic acid, octadecen-13-ene-9,11-diynoic acid, petroselenic acid (cis-6-octadecenoic acid), 9c,12t-octadecadienoic acid, calendulic acid (8t10t12coctadecatrienoicacid), catalpic acid (9t11 t13c-octadecatrienoic acid), eleostearic acid (9c11 t13t-octadecatrienoic acid), jacaric acid (8c1 Ot12c-octadecatrienoic acid), punicic acid (9c11 t13c-octadecatrienoic acid), parinaric acid (9c11 t13t15c-octadecatetraenoic acid), pinolenic acid (all-cis-5,9,12-octadecatrienoic acid), laballenic acid (5,6-octadecadienallenic acid), ricinoleic acid (12-hydroxyoleic acid) and/or coriolic acid (13-hydroxy-9c, 11t-octadecadienoic acid). The fatty acid esters or fatty acid mixtures produced by the process of the present invention, preferably, comprise less than 0.1 %, based on the total fatty acids, or no butyric acid, no cholesterol, no clupanodonic acid (= docosapentaenoic acid, C22:5M,8,12,15,21) and no nisinic acid (tetracosahexaenoic acid, C23:6L13,8,12,15,18,21).

Chemically pure VLC-PUFAs or fatty acid compositions can also be synthesized by the methods described herein. To this end, the fatty acids or the fatty acid compositions are isolated from a corresponding sample via extraction, distillation, crystallization, chromatography or a combination of these methods. These chemically pure fatty acids or fatty acid compositions are advantageous for applications in the food industry sector, the cosmetic sector and especially the pharmacological industry sector.

The term "desaturase" encompasses all enzymatic activities and enzymes catalyzing the desaturation of fatty acids with different lengths and numbers of unsaturated carbon atom double bonds. Specifically this includes delta 4 (d4)-desaturase, catalyzing the dehydrogenation of the 4th and 5th carbon atom; Delta 5 (d5)-desaturase catalyzing the dehydrogenation of the 5th and 6th carbon atom; Delta 6 (d6)-desaturase catalyzing the dehydrogenation of the 6th and 7th carbon atom; Delta 8 (d8)-desaturase catalyzing the dehydrogenation of the 8th and 9th carbon atom; Delta 9 (d9)-desaturase catalyzing the dehydrogenation of the 9th and 10th carbon atom; Delta 12 (d12)-desaturase catalyzing the dehydrogenation of the 12th and 13th carbon atom; Delta 15 (d15)-desaturase catalyzing the dehydrogenation of the 15th and 16th carbon atom.

The terms "elongase" encompasses all enzymatic activities and enzymes catalyzing the elongation of fatty acids with different lengths and numbers of unsaturated carbon atom

double bonds. Especially the term "elongase" as used herein refers to the activity of an elongase, introducing two carbon molecules into the carbon chain of a fatty acid, preferably in the positions 5, 6, 9, 12 and/or 15 of fatty acids.

In the studies underlying this invention, enzymes with superior desaturase and elongase catalytic activities for the production of VLC-PUFA has been provided.

Polynucleotides encoding polypeptides which exhibit delta-6-elongase activity have been described in WO2001/059128, WO2004/087902 and WO2005/012316, said documents, describing this enzyme from Physcomitrella patens, are incorporated herein in their entirety.

Polynucleotides encoding polypeptides which exhibit delta-5-desaturase activity have been described in WO2002026946 and WO2003/093482, said documents, describing this enzyme from Thraustochytrium sp., are incorporated herein in their entirety.

Polynucleotides encoding polypeptides which exhibit delta-6-desaturase activity have been described in WO2005/012316, WO2005/083093, WO2006/008099 and WO2006/069710, said documents, describing this enzyme from Ostreococcus tauri, are incorporated herein in their entirety.

Polynucleotides encoding polypeptides which exhibit delta-6-elongase activity have been described in WO2005/012316, WO2005/007845 and WO2006/069710, said documents, describing this enzyme from Thalassiosira pseudonana, are incorporated herein in their entirety.

Polynucleotides encoding polypeptides which exhibit delta-12-desaturase activity have been described for example in WO2006100241, said documents, describing this enzyme from Phytophthora sojae, are incorporated herein in their entirety.

Polynucleotides encoding polypeptides which exhibit delta-4-desaturase activity have been described for example in WO2004/090123, said documents, describing this enzyme from Euglena gracilis, are incorporated herein in their entirety.

Polynucleotides encoding polypeptides which exhibit delta-5-elongase activity have been described for example in WO2005/012316 and WO2007/096387, said documents, describing this enzyme from Ostreococcus tauri, are incorporated herein in their entirety.

Polynucleotides encoding polypeptides which exhibit omega 3-desaturase activity have been described for example in WO2008/022963, said documents, describing this enzyme from Phytium irregulare, are incorporated herein in their entirety.

Polynucleotides encoding polypeptides which exhibit omega 3-desaturase activity have been described for example in WO2005012316 and WO2005083053, said documents, describing this enzyme from Phytophthora infestans, are incorporated herein in their entirety.

Polynucleotides encoding polypeptides which exhibit delta-4-desaturase activity have been described for example in WO2002026946, said documents, describing this enzyme from Thraustochytrium sp., are incorporated herein in their entirety.

Polynucleotides coding for a delta-4 desaturase from Pavlova lutheri are described in WO2003078639 and WO2005007845. These documents are incorporated herein in their entirety, particularly in sofar as the documents relate to the delta-4 desaturase "PIDES 1"and figures 3a-3d of WO2003078639 and figures 3a, 3b of WO2005007845, respectively.

The polynucleotides encoding the aforementioned polypeptides are herein also referred to as "target genes".The polynucleotides are well known in the art. The sequences of said polynucleotides can be found in the sequence of the T-DNAs disclosed in the Examples section. E.g., the vector VC-LTM593-1qcz comprises the sequences of the said polynucleotides. The sequence of this vector is shown in SEQ ID NO: 3 (see table 193). The positions of the respective polynucleotides in this vector are indicted in table 11. E.g., the sequence of the delta-6 elongase from Physcomitrella patens comprises the sequence of nucleotides 1267 tp 2139 of SEQ ID NO: 3.

Polynucleotides encoding polypeptides which exhibit delta-15-desaturase activity have been described for example in WO2010/066703, said documents, describing this enzyme from Cochliobolus heterostrophus C5, are incorporated herein in their entirety. For the sequence of the polynucleotide encoding a polypeptides which exhibits delta-15-desaturase activity from Cochliobolus heterostrophus C5 see, e.g. nucleotides 2151 to 3654 of VC-LLM338-3qcz rc. The sequence of this vector is shown in SEQ ID NO: 9.

A polynucleotide encoding a polypeptide having a desaturase or elongase activity as specified above is obtainable or obtained in accordance with the present invention for example from an organism of genus Ostreococcus, Thraustochytrium, Euglena, Thalassiosira, Phytophthora, Phytium, Cochliobolus, Physcomitrella. However, orthologs, paralogs or other homologs may be identified from other species. Preferably, they are obtained from plants such as algae, for example Isochrysis, Mantoniella, Crypthecodinium, algae/diatoms such as Phaeodactylum, mosses such as Ceratodon, or higher plants such as the Primulaceae such as Aleuritia, Calendula stellata, Osteospermum spinescens or Osteospermum hyoseroides, microorganisms such as fungi, such as Aspergillus, Entomophthora, Mucor or Mortierella, bacteria such as Shewanella, yeasts or animals. Preferred animals are nematodes such as Caenorhabditis, insects or vertebrates. Among

the vertebrates, the nucleic acid molecules may, preferably, be derived from Euteleostomi, Actinopterygii; Neopterygii; Teleostei; Euteleostei, Protacanthopterygii, Salmoniformes; Salmonidae or Oncorhynchus, more preferably, from the order of the Salmoniformes, most preferably, the family of the Salmonidae, such as the genus Salmo, for example from the genera and species Oncorhynchus mykiss, Trutta trutta or Salmo trutta fario. Moreover, the nucleic acid molecules may be obtained from the diatoms such as the genera Thalassiosira or Phaeodactylum.

Thus, the term "polynucleotide" as used in accordance with the present invention further encompasses variants or derivatives of the aforementioned specific polynucleotides representing orthologs, paralogs or other homologs of the polynucleotide of the present invention. Moreover, variants or derivatives of the polynucleotide of the present invention also include artificially generated muteins. Said muteins include, e.g., enzymes which are generated by mutagenesis techniques and which exhibit improved or altered substrate specificity, or codon optimized polynucleotides.

Nucleic acid variants or derivatives according to the invention are polynucleotides which differ from a given reference polynucleotide by at least one nucleotide substitution, addition and/or deletion. If the reference polynucleotide codes for a protein, the function of this protein is conserved in the variant or derivative polynucleotide, such that a variant nucleic acid sequence shall still encode a polypeptide having a desaturase or elongase activity as specified above. Variants or derivatives also encompass polynucleotides comprising a nucleic acid sequence which is capable of hybridizing to the aforementioned specific nucleic acid sequences, preferably, under stringent hybridization conditions. These stringent conditions are known to the skilled in the art and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N. Y. (1989), 6.3.1-6.3.6. A preferred example for stringent hybridization conditions are hybridization conditions in 6 ´ sodium chloride/sodium citrate (= SSC) at approximately 45°C, followed by one or more wash steps in 0.2 ´ SSC, 0.1% SDS at 50 to 65°C. The skilled worker knows that these hybridization conditions differ depending on the type of nucleic acid and, for example when organic solvents are present, with regard to the temperature and concentration of the buffer. For example, under "standard hybridization conditions" the temperature differs depending on the type of nucleic acid between 42°C and 58°C in aqueous buffer with a concentration of 0.1 to 5 ´ SSC (pH 7.2). If organic solvent is present in the abovementioned buffer, for example 50% formamide, the temperature under standard conditions is approximately 42°C. The hybridization conditions for DNA: DNA hybrids are, preferably, 0.1 ´ SSC and 20°C to 45°C, preferably between 30°C and 45°C. The hybridization conditions for DNA:RNA hybrids are, preferably, 0.1 ´ SSC and 30°C to 55°C, preferably between 45°C and 55°C. The abovementioned hybridization temperatures are determined for example for a nucleic acid with approximately 100 bp (= base pairs) in length and a G + C content of 50% in the absence of formamide. The skilled worker knows how to determine the hybridization

conditions required by referring to textbooks such as the textbook mentioned above, or the following textbooks: Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989; Hames and Higgins (Ed.) 1985, "Nucleic Acids Hybridization: A Practical Approach", IRL Press at Oxford University Press, Oxford; Brown (Ed.) 1991, "Essential Molecular Biology: A Practical Approach", IRL Press at Oxford University Press, Oxford. Alternatively, polynucleotide variants are obtainable by PCR-based techniques such as mixed oligonucleotide primer based amplification of DNA, i.e. using degenerated primers against conserved domains of the polypeptides of the present invention. Conserved domains of the polypeptide of the present invention may be identified by a sequence comparison of the nucleic acid sequences of the polynucleotides or the amino acid sequences of the polypeptides of the present invention. Oligonucleotides suitable as PCR primers as well as suitable PCR conditions are described in the accompanying Examples. As a template, DNA or cDNA from bacteria, fungi, plants or animals may be used. Further, variants include polynucleotides comprising nucleic acid sequences which are at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the nucleic acid coding sequences shown in any one of the T-DNA sequences given in the corresponding tables of the examples. Of course, the variants must retain the function of the respective enzyme, i.e. a variant of a delta-4-desaturase must retain delta-4-desaturase activity.

The percent identity values are, preferably, calculated over the entire amino acid or nucleic acid sequence region. A series of programs based on a variety of algorithms is available to the skilled worker for comparing different sequences. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch algorithm (Needleman 1970, J. Mol. Biol. (48):444-453) which has been incorporated into the needle program in the EMBOSS software package (EMBOSS: The European Molecular Biology Open Software Suite, Rice,P., Longden,I., and Bleasby,A, Trends in Genetics 16(6), 276-277, 2000), a BLOSUM62 scoring matrix, and a gap opening penalty of 10 and a gap entension pentalty of 0.5. Guides for local installation of the EMBOSS package as well as links to WEB-Services can be found at http://emboss.sourceforge.net. A preferred, non-limiting example of parameters to be used for aligning two amino acid sequences using the needle program are the default parameters, including the EBLOSUM62 scoring matrix, a gap opening penalty of 10 and a gap extension penalty of 0.5. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the needle program in the EMBOSS software package (EMBOSS: The European Molecular Biology Open Software Suite, Rice,P., Longden,I., and Bleasby,A, Trends in Genetics 16(6), 276-277, 2000), using the EDNAFULL scoring matrix and a gap opening penalty of 10 and a gap extension penalty of 0.5. A preferred, non-limiting example of parameters to be used in conjunction for aligning two nucleic acid sequences using the needle program are the default parameters, including the EDNAFULL scoring matrix, a gap opening penalty of 10 and a gap extension

penalty of 0.5. The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the BLAST series of programs (version 2.2) of Altschul et al. (Altschul 1990, J. Mol. Biol. 215:403-10). BLAST using desaturase and elongase nucleic acid sequences of the invention as query sequence can be performed with the BLASTn, BLASTx or the tBLASTx program using default parameters to obtain either nucleotide sequences (BLASTn, tBLASTx) or amino acid sequences (BLASTx) homologous to desaturase and elongase sequences of the invention. BLAST using desaturase and elongase protein sequences of the invention as query sequence can be performed with the BLASTp or the tBLASTn program using default parameters to obtain either amino acid sequences (BLASTp) or nucleic acid sequences (tBLASTn) homologous to desaturase and elongase sequences of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST using default parameters can be utilized as described in Altschul et al. (Altschul 1997, Nucleic Acids Res. 25(17):3389-3402).

A polynucleotide comprising a fragment of any nucleic acid, particularly of any of the aforementioned nucleic acid sequences, is also encompassed as a polynucleotide of the present invention. The fragments shall encode polypeptides which still have desaturase or elongase activity as specified above. Accordingly, the polypeptide may comprise or consist of the domains of the polypeptide of the present invention conferring the said biological activity. A fragment as meant herein, preferably, comprises at least 50, at least 100, at least 250 or at least 500 consecutive nucleotides of any one of the aforementioned nucleic acid sequences or encodes an amino acid sequence comprising at least 20, at least 30, at least 50, at least 80, at least 100 or at least 150 consecutive amino acids of any one of the aforementioned amino acid sequences.

The variant polynucleotides or fragments referred to above, preferably, encode polypeptides retaining desaturase or elongase activity to a significant extent, preferably, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% or at least 90% of the desaturase or elongase activity exhibited by any of the polypeptide comprised in any of the T-DNAs given in the accompanying Examples.

Further enzymes beneficial for the present invention as described in detail in the examples are aclytransferases and transacylases (cf. WO 2011161093 A1). One group of acyltransferases having three distinct enzymatic activities are enzymes of the "Kennedy pathway", which are located on the cytoplasmic side of the membrane system of the endoplasmic reticulum (ER). The ER-bound acyltransferases in the microsomal fraction use acyl-CoA as the activated form of fatty acids. Glycerol-3-phosphate acyltransferase (GPAT) catalyzes the incorporation of acyl groups at the sn-1 position of glycerol-3-phosphate. 1-Acylglycerol-3-phosphate acyltransferase, also known as lysophosphatidic acid

acyltransferase (LPAAT), catalyze the incorporation of acyl groups at the sn-2 position of lysophosphatidic acid (LPA). After dephosphorylation of phosphatidic acid by phosphatidic acid phosphatase (PAP), diacylglycerol acyltransferase (DGAT) catalyzes the incorporation of acyl groups at the sn-3 position of diacylglycerols. Further enzymes directly involved in TAG biosynthesis - apart from the said Kennedy pathway enzymes - are the phospholipid diacylglycerol acyltransferase (PDAT), an enzyme that transfers acyl groups from the sn-2 position of membrane lipids to the sn-3 position of diacylglycerols, and diacylglyceroldiacylglycerol transacylase (DDAT), an enzyme that transfers acylgroups from the sn-2 position of one diacylglycerol-molecule to the sn-3 position of another diacylglycerol-molecule. Lysophospholipid acyltransferase (LPLAT) represents a class of acyltransferases that are capable of incorporating activated acyl groups from acyl-CoA to membrane lipids, and possibly catalyze also the reverse reaction. More specifically, LPLATs can have activity as lysophosphophatidylethanolamine acyltransferase (LPEAT) and lysophosphatidylcholine acyltransferase (LPCAT). Further enzymes, such as lecithin cholesterol acyltransferase (LCAT) can be involved in the transfer of acyl groups from membrane lipids into triacylglycerides, as well. The documents WO 98/54302 and WO 98/54303 disclose a human LPAAT and its potential use for the therapy of diseases, as a diagnostic, and a method for identifying modulators of the human LPAAT. Moreover, a variety of acyltransferases with a wide range of enzymatic functions have been described in the documents WO 98/55632, WO 98/55631 , WO 94/13814, WO 96/24674, WO 95/27791, WO 00/18889, WO 00/18889, WO 93/10241, Akermoun 2000, Biochemical Society Transactions 28: 713-715, Tumaney 1999, Biochimica et Biophysica Acta 1439: 47-56, Fraser 2000, Biochemical Society Transactions 28: 715- 7718, Stymne 1984, Biochem. J. 223: 305-314, Yamashita 2001, Journal of Biological Chemistry 276: 26745-26752, and WO 00/18889.

The term "expression control sequence" as used herein refers to a nucleic acid sequence which is capable of governing, i.e. initiating and controlling, transcription of a nucleic acid sequence of interest, in the present case the nucleic sequences recited above. Such a sequence usually comprises or consists of a promoter or a combination of a promoter and enhancer sequences. Expression of a polynucleotide comprises transcription of the nucleic acid molecule, preferably, into a translatable mRNA. Additional regulatory elements may include transcriptional as well as translational enhancers. The following promoters and expression control sequences may be, preferably, used in an expression vector according to the present invention. The cos, tac, trp, tet, trp-tet, lpp, lac, lpp-lac, laclq, T7, T5, T3, gal, trc, ara, SP6, λ-PR or λ-PL promoters are, preferably, used in Gram-negative bacteria. For Gram-positive bacteria, promoters amy and SPO2 may be used. From yeast or fungal promoters ADC1, AOX1r, GAL1, MFα, AC, P-60, CYC1, GAPDH, TEF, rp28, ADH are, preferably, used. For animal cell or organism expression, the promoters CMV-, SV40-, RSV-promoter (Rous sarcoma virus), CMV-enhancer, SV40-enhancer are preferably used. From plants the promoters CaMV/35S (Franck 1980, Cell 21: 285-294], PRP1 (Ward 1993,

Plant. Mol. Biol. 22), SSU, OCS, lib4, usp, STLS1, B33, nos or the ubiquitin or phaseolin promoter. Also preferred in this context are inducible promoters, such as the promoters described in EP 0388186 A1 (i.e. a benzylsulfonamide-inducible promoter), Gatz 1992, Plant J. 2:397-404 (i.e. a tetracyclin-inducible promoter), EP 0335528 A1 (i.e. a abscisic-acid-inducible promoter) or WO 93/21334 (i.e. a ethanol- or cyclohexenol-inducible promoter). Further suitable plant promoters are the promoter of cytosolic FBPase or the ST-LSI promoter from potato (Stockhaus 1989, EMBO J. 8, 2445), the phosphoribosyl-pyrophosphate amidotransferase promoter from Glycine max (Genbank accession No. U87999) or the node-specific promoter described in EP 0249676 A1. Particularly preferred are promoters which enable the expression in tissues which are involved in the biosynthesis of fatty acids. Also particularly preferred are seed-specific promoters such as the USP promoter in accordance with the practice, but also other promoters such as the LeB4, DC3, phaseolin or napin promoters. Further especially preferred promoters are seed-specific promoters which can be used for monocotyledonous or dicotyledonous plants and which are described in US 5,608,152 (napin promoter from oilseed rape), WO 98/45461 (oleosin promoter from Arobidopsis, US 5,504,200 (phaseolin promoter from Phaseolus vulgaris), WO 91/13980 (Bce4 promoter from Brassica), by Baeumlein et al., Plant J., 2, 2, 1992:233-239 (LeB4 promoter from a legume), these promoters being suitable for dicots. The following promoters are suitable for monocots: lpt-2 or lpt-1 promoter from barley (WO 95/15389 and WO 95/23230), hordein promoter from barley and other promoters which are suitable and which are described in WO 99/16890. In principle, it is possible to use all natural promoters together with their regulatory sequences, such as those mentioned above, for the novel process. Likewise, it is possible and advantageous to use synthetic promoters, either additionally or alone, especially when they mediate a seed-specific expression, such as, for example, as described in WO 99/16890. In a particular embodiment, seed-specific promoters are utilized to enhance the production of the desired PUFA or VLC-PUFA.

The term "operatively linked" as used herein means that the expression control sequence and the nucleic acid of interest are linked so that the expression of the said nucleic acid of interest can be governed by the said expression control sequence, i.e. the expression control sequence shall be functionally linked to the said nucleic acid sequence to be expressed. Accordingly, the expression control sequence and, the nucleic acid sequence to be expressed may be physically linked to each other, e.g., by inserting the expression control sequence at the 5´end of the nucleic acid sequence to be expressed. Alternatively, the expression control sequence and the nucleic acid to be expressed may be merely in physical proximity so that the expression control sequence is capable of governing the expression of at least one nucleic acid sequence of interest. The expression control sequence and the nucleic acid to be expressed are, preferably, separated by not more than 500 bp, 300 bp, 100 bp, 80 bp, 60 bp, 40 bp, 20 bp, 10 bp or 5 bp.

Preferred polynucleotides of the present invention comprise, in addition to a promotor, a terminator sequence operatively linked to the nucleic acid sequence of interest.

The term "terminator" as used herein refers to a nucleic acid sequence which is capable of terminating transcription. These sequences will cause dissociation of the transcription machinery from the nucleic acid sequence to be transcribed. Preferably, the terminator shall be active in plants and, in particular, in plant seeds. Suitable terminators are known in the art and, preferably, include polyadenylation signals such as the SV40-poly-A site or the tk-poly-A site or one of the plant specific signals indicated in Loke et al. (Loke 2005, Plant Physiol 138, pp. 1457-1468), downstream of the nucleic acid sequence to be expressed.

The invention furthermore relates to recombinant nucleic acid molecules comprising at least one nucleic acid sequence which codes for a polypeptide having desaturase and/or elongase activity which is modified by comparison with the nucleic acid sequence in the organism from which the sequence originates in that it is adapted to the codon usage in one or more plant species.

For the purposes of the invention "recombinant" means with regard to, for example, a nucleic acid sequence, an expression cassette (=gene construct) or a vector comprising the nucleic acid sequences used in the process according to the invention or a host cell transformed with the nucleic acid sequences, expression cassette or vector used in the process according to the invention, all those constructions brought about by recombinant methods in which either the nucleic acid sequence, or a genetic control sequence which is operably linked with the nucleic acid sequence, for example a promoter, or are not located in their natural genetic environment or have been modified by recombinant methods.

Preferably, the plant cell of the present invention is an oilseed crop plant cell. More preferably, said oilseed crop is selected from the group consisting of flax (Linum sp.), rapeseed (Brassica sp.), soybean (Glycine and Soja sp.), sunflower (Helianthus sp.), cotton (Gossypium sp.), corn (Zea mays), olive (Olea sp.), safflower (Carthamus sp.), cocoa (Theobroma cacoa), peanut (Arachis sp.), hemp, camelina, crambe, oil palm, coconuts, groundnuts, sesame seed, castor bean, lesquerella, tallow tree, sheanuts, tungnuts, kapok fruit, poppy seed, jojoba seeds and perilla. Preferred plants to be used for introducing the polynucleotide or T-DNA of the invention are plants which are capable of synthesizing fatty acids, such as all dicotyledonous or monocotyledonous plants, algae or mosses. It is to be understood that host cells derived from a plant may also be used for producing a plant according to the present invention. Preferred plants are selected from the group of the plant families Adelotheciaceae, Anacardiaceae, Arecaceae, Asteraceae, Apiaceae, Betulaceae, Boraginaceae, Brassicaceae, Bromeliaceae, Caricaceae, Cannabaceae, Convolvulaceae, Chenopodiaceae, Compositae, Crypthecodiniaceae, Cruciferae, Cucurbitaceae, Ditrichaceae, Elaeagnaceae, Ericaceae, Euphorbiaceae, Fabaceae, Geraniaceae,

Gramineae, Juglandaceae, Lauraceae, Leguminosae, Linaceae, Malvaceae, Moringaceae, Marchantiaceae, Onagraceae, Olacaceae, Oleaceae, Papaveraceae, Piperaceae, Pedaliaceae, Poaceae, Solanaceae, Prasinophyceae or vegetable plants or ornamentals such as Tagetes. Examples which may be mentioned are the following plants selected from the group consisting of: Adelotheciaceae such as the genera Physcomitrella, such as the genus and species Physcomitrella patens, Anacardiaceae such as the genera Pistacia, Mangifera, Anacardium, for example the genus and species Pistacia vera [pistachio], Mangifer indica [mango] or Anacardium occidentale [cashew], Asteraceae, such as the genera Calendula, Carthamus, Centaurea, Cichorium, Cynara, Helianthus, Lactuca, Locusta, Tagetes, Valeriana, for example the genus and species Calendula officinalis [common marigold], Carthamus tinctorius [safflower], Centaurea cyanus [cornflower], Cichorium intybus [chicory], Cynara scolymus [artichoke], Helianthus annus [sunflower], Lactuca sativa, Lactuca crispa, Lactuca esculenta, Lactuca scariola L. ssp. sativa, Lactuca scariola L. var. integrata, Lactuca scariola L. var. integrifolia, Lactuca sativa subsp. romana, Locusta communis, Valeriana locusta [salad vegetables], Tagetes lucida, Tagetes erecta or Tagetes tenuifolia [african or french marigold], Apiaceae, such as the genus Daucus, for example the genus and species Daucus carota [carrot], Betulaceae, such as the genus Corylus, for example the genera and species Corylus avellana or Corylus colurna [hazelnut], Boraginaceae, such as the genus Borago, for example the genus and species Borago officinalis [borage], Brassicaceae, such as the genera Brassica, Melanosinapis, Sinapis, Arabadopsis, for example the genera and species Brassica napus, Brassica rapa ssp. [oilseed rape], Sinapis arvensis Brassica juncea, Brassica juncea var. juncea, Brassica juncea var. crispifolia, Brassica juncea var. foliosa, Brassica nigra, Brassica sinapioides, Melanosinapis communis [mustard], Brassica oleracea [fodder beet] or Arabidopsis thaliana, Bromeliaceae, such as the genera Anana, Bromelia (pineapple), for example the genera and species Anana comosus, Ananas ananas or Bromelia comosa [pineapple], Caricaceae, such as the genus Carica, such as the genus and species Carica papaya [pawpaw], Cannabaceae, such as the genus Cannabis, such as the genus and species Cannabis sativa [hemp], Convolvulaceae, such as the genera Ipomea, Convolvulus, for example the genera and species Ipomoea batatus, Ipomoea pandurata, Convolvulus batatas, Convolvulus tiliaceus, Ipomoea fastigiata, Ipomoea tiliacea, Ipomoea triloba or Convolvulus panduratus [sweet potato, batate], Chenopodiaceae, such as the genus Beta, such as the genera and species Beta vulgaris, Beta vulgaris var. altissima, Beta vulgaris var.Vulgaris, Beta maritima, Beta vulgaris var. perennis, Beta vulgaris var. conditiva or Beta vulgaris var. esculenta [sugarbeet], Crypthecodiniaceae, such as the genus Crypthecodinium, for example the genus and species Cryptecodinium cohnii, Cucurbitaceae, such as the genus Cucurbita, for example the genera and species Cucurbita maxima, Cucurbita mixta, Cucurbita pepo or Cucurbita moschata [pumpkin/squash], Cymbellaceae such as the genera Amphora, Cymbella, Okedenia, Phaeodactylum, Reimeria, for example the genus and species Phaeodactylum tricornutum, Ditrichaceae such as the genera Ditrichaceae, Astomiopsis, Ceratodon, Chrysoblastella,

Ditrichum, Distichium, Eccremidium, Lophidion, Philibertiella, Pleuridium, Saelania, Trichodon, Skottsbergia, for example the genera and species Ceratodon antarcticus, Ceratodon columbiae, Ceratodon heterophyllus, Ceratodon purpureus, Ceratodon purpureus, Ceratodon purpureus ssp. convolutus, Ceratodon, purpureus spp. stenocarpus, Ceratodon purpureus var. rotundifolius, Ceratodon ratodon, Ceratodon stenocarpus, Chrysoblastella chilensis, Ditrichum ambiguum, Ditrichum brevisetum, Ditrichum crispatissimum, Ditrichum difficile, Ditrichum falcifolium, Ditrichum flexicaule, Ditrichum giganteum, Ditrichum heteromallum, Ditrichum lineare, Ditrichum lineare, Ditrichum montanum, Ditrichum montanum, Ditrichum pallidum, Ditrichum punctulatum, Ditrichum pusillum, Ditrichum pusillum var. tortile, Ditrichum rhynchostegium, Ditrichum schimperi, Ditrichum tortile, Distichium capillaceum, Distichium hagenii, Distichium inclinatum, Distichium macounii, Eccremidium floridanum, Eccremidium whiteleggei, Lophidion strictus, Pleuridium acuminatum, Pleuridium alternifolium, Pleuridium holdridgei, Pleuridium mexicanum, Pleuridium ravenelii, Pleuridium subulatum, Saelania glaucescens, Trichodon borealis, Trichodon cylindricus or Trichodon cylindricus var. oblongus, Elaeagnaceae such as the genus Elaeagnus, for example the genus and species Olea europaea [olive], Ericaceae such as the genus Kalmia, for example the genera and species Kalmia latifolia, Kalmia angustifolia, Kalmia microphylla, Kalmia polifolia, Kalmia occidentalis, Cistus chamaerhodendros or Kalmia lucida [mountain laurel], Euphorbiaceae such as the genera Manihot, Janipha, Jatropha, Ricinus, for example the genera and species Manihot utilissima, Janipha manihot, Jatropha manihot, Manihot aipil, Manihot dulcis, Manihot manihot, Manihot melanobasis, Manihot esculenta [manihot] or Ricinus communis [castor-oil plant], Fabaceae such as the genera Pisum, Albizia, Cathormion, Feuillea, Inga, Pithecolobium, Acacia, Mimosa, Medicajo, Glycine, Dolichos, Phaseolus, Soja, for example the genera and species Pisum sativum, Pisum arvense, Pisum humile [pea], Albizia berteriana, Albizia julibrissin, Albizia lebbeck, Acacia berteriana, Acacia littoralis, Albizia berteriana, Albizzia berteriana, Cathormion berteriana, Feuillea berteriana, Inga fragrans, Pithecellobium berterianum, Pithecellobium fragrans, Pithecolobium berterianum, Pseudalbizzia berteriana, Acacia julibrissin, Acacia nemu, Albizia nemu, Feuilleea julibrissin, Mimosa julibrissin, Mimosa speciosa, Sericanrda julibrissin, Acacia lebbeck, Acacia macrophylla, Albizia lebbek, Feuilleea lebbeck, Mimosa lebbeck, Mimosa speciosa [silk tree], Medicago sativa, Medicago falcata, Medicago varia [alfalfa], Glycine max Dolichos soja, Glycine gracilis, Glycine hispida, Phaseolus max, Soja hispida or Soja max [soybean], Funariaceae such as the genera Aphanorrhegma, Entosthodon, Funaria, Physcomitrella, Physcomitrium, for example the genera and species Aphanorrhegma serratum, Entosthodon attenuatus, Entosthodon bolanderi, Entosthodon bonplandii, Entosthodon californicus, Entosthodon drummondii, Entosthodon jamesonii, Entosthodon leibergii, Entosthodon neoscoticus, Entosthodon rubrisetus, Entosthodon spathulifolius, Entosthodon tucsoni, Funaria americana, Funaria bolanderi, Funaria calcarea, Funaria californica, Funaria calvescens, Funaria convoluta, Funaria flavicans, Funaria groutiana, Funaria hygrometrica, Funaria hygrometrica var. arctica, Funaria hygrometrica var.

calvescens, Funaria hygrometrica var. convoluta, Funaria hygrometrica var. muralis, Funaria hygrometrica var. utahensis, Funaria microstoma, Funaria microstoma var. obtusifolia, Funaria muhlenbergii, Funaria orcuttii, Funaria plano-convexa, Funaria polaris, Funaria ravenelii, Funaria rubriseta, Funaria serrata, Funaria sonorae, Funaria sublimbatus, Funaria tucsoni, Physcomitrella californica, Physcomitrella patens, Physcomitrella readeri, Physco¬mitrium australe, Physcomitrium californicum, Physcomitrium collenchymatum, Physcomitrium coloradense, Physcomitrium cupuliferum, Physcomitrium drummondii, Physcomitrium eurystomum, Physcomitrium flexifolium, Physcomitrium hookeri, Physcomitrium hookeri var. serratum, Physcomitrium immersum, Physcomitrium kellermanii, Physcomitrium megalocarpum, Physcomitrium pyriforme, Physcomitrium pyriforme var. serratum, Physcomitrium rufipes, Physcomitrium sandbergii, Physcomitrium subsphaericum, Physcomitrium washingtoniense, Geraniaceae, such as the genera Pelargonium, Cocos, Oleum, for example the genera and species Cocos nucifera, Pelargonium grossularioides or Oleum cocois [coconut], Gramineae, such as the genus Saccharum, for example the genus and species Saccharum officinarum, Juglandaceae, such as the genera Juglans, Wallia, for example the genera and species Juglans regia, Juglans ailanthifolia, Juglans sieboldiana, Juglans cinerea, Wallia cinerea, Juglans bixbyi, Juglans californica, Juglans hindsii, Juglans intermedia, Juglans jamaicensis, Juglans major, Juglans microcarpa, Juglans nigra or Wallia nigra [walnut], Lauraceae, such as the genera Persea, Laurus, for example the genera and species Laurus nobilis [bay], Persea americana, Persea gratissima or Persea persea [avocado], Leguminosae, such as the genus Arachis, for example the genus and species Arachis hypogaea [peanut], Linaceae, such as the genera Linum, Adenolinum, for example the genera and species Linum usitatissimum, Linum humile, Linum austriacum, Linum bienne, Linum angustifolium, Linum catharticum, Linum flavum, Linum grandiflorum, Adenolinum grandiflorum, Linum lewisii, Linum narbonense, Linum perenne, Linum perenne var. lewisii, Linum pratense or Linum trigynum [linseed], Lythrarieae, such as the genus Punica, for example the genus and species Punica granatum [pomegranate], Malvaceae, such as the genus Gossypium, for example the genera and species Gossypium hirsutum, Gossypium arboreum, Gossypium barbadense, Gossypium herbaceum or Gossypium thurberi [cotton], Marchantiaceae, such as the genus Marchantia, for example the genera and species Marchantia berteroana, Marchantia foliacea, Marchantia macropora, Musaceae, such as the genus Musa, for example the genera and species Musa nana, Musa acuminata, Musa paradisiaca, Musa spp. [banana], Onagraceae, such as the genera Camissonia, Oenothera, for example the genera and species Oenothera biennis or Camissonia brevipes [evening primrose], Palmae, such as the genus Elacis, for example the genus and species Elaeis guineensis [oil palm], Papaveraceae, such as the genus Papaver, for example the genera and species Papaver orientale, Papaver rhoeas, Papaver dubium [poppy], Pedaliaceae, such as the genus Sesamum, for example the genus and species Sesamum indicum [sesame], Piperaceae, such as the genera Piper, Artanthe, Peperomia, Steffensia, for example the genera and species Piper aduncum, Piper amalago, Piper angustifolium, Piper auritum, Piper betel,

Piper cubeba, Piper longum, Piper nigrum, Piper retrofractum, Artanthe adunca, Artanthe elongata, Peperomia elongata, Piper elongatum, Steffensia elongata [cayenne pepper], Poaceae, such as the genera Hordeum, Secale, Avena, Sorghum, Andropogon, Holcus, Panicum, Oryza, Zea (maize), Triticum, for example the genera and species Hordeum vulgare, Hordeum jubatum, Hordeum murinum, Hordeum secalinum, Hordeum distichon, Hordeum aegiceras, Hordeum hexastichon, Hordeum hexastichum, Hordeum irregulare, Hordeum sativum, Hordeum secalinum [barley], Secale cereale [rye], Avena sativa, Avena fatua, Avena byzantina, Avena fatua var. sativa, Avena hybrida [oats], Sorghum bicolor, Sorghum halepense, Sorghum saccharatum, Sorghum vulgare, Andropogon drummondii, Holcus bicolor, Holcus sorghum, Sorghum aethiopicum, Sorghum arundinaceum, Sorghum caffrorum, Sorghum cernuum, Sorghum dochna, Sorghum drummondii, Sorghum durra, Sorghum guineense, Sorghum lanceolatum, Sorghum nervosum, Sorghum saccharatum, Sorghum subglabrescens, Sorghum verticilliflorum, Sorghum vulgare, Holcus halepensis, Sorghum miliaceum, Panicum militaceum [millet], Oryza sativa, Oryza latifolia [rice], Zea mays [maize], Triticum aestivum, Triticum durum, Triticum turgidum, Triticum hybernum, Triticum macha, Triticum sativum or Triticum vulgare [wheat], Porphyridiaceae, such as the genera Chroothece, Flintiella, Petrovanella, Porphyridium, Rhodella, Rhodosorus, Vanhoeffenia, for example the genus and species Porphyridium cruentum, Proteaceae, such as the genus Macadamia, for example the genus and species Macadamia intergrifolia [macadamia], Prasinophyceae such as the genera Nephroselmis, Prasinococcus, Scherffelia, Tetraselmis, Mantoniella, Ostreococcus, for example the genera and species Nephroselmis olivacea, Prasinococcus capsulatus, Scherffelia dubia, Tetraselmis chui, Tetraselmis suecica, Mantoniella squamata, Ostreococcus tauri, Rubiaceae such as the genus Cofea, for example the genera and species Cofea spp., Coffea arabica, Coffea canephora or Coffea liberica [coffee], Scrophulariaceae such as the genus Verbascum, for example the genera and species Verbascum blattaria, Verbascum chaixii, Verbascum densiflorum, Verbascum lagurus, Verbascum longifolium, Verbascum lychnitis, Verbascum nigrum, Verbascum olympicum, Verbascum phlomoides, Verbascum phoenicum, Verbascum pulverulentum or Verbascum thapsus [mullein], Solanaceae such as the genera Capsicum, Nicotiana, Solanum, Lycopersicon, for example the genera and species Capsicum annuum, Capsicum annuum var. glabriusculum, Capsicum frutescens [pepper], Capsicum annuum [paprika], Nicotiana tabacum, Nicotiana alata, Nicotiana attenuata, Nicotiana glauca, Nicotiana langsdorffii, Nicotiana obtusifolia, Nicotiana quadrivalvis, Nicotiana repanda, Nicotiana rustica, Nicotiana sylvestris [tobacco], Solanum tuberosum [potato], Solanum melongena [eggplant], Lycopersicon esculentum, Lycopersicon lycopersicum, Lycopersicon pyriforme, Solanum integrifolium or Solanum lycopersicum [tomato], Sterculiaceae, such as the genus Theobroma, for example the genus and species Theobroma cacao [cacao] or Theaceae, such as the genus Camellia, for example the genus and species Camellia sinensis [tea]. In particular preferred plants to be used as transgenic plants in accordance with the present invention are oil fruit crops which comprise large amounts of lipid compounds, such as peanut, oilseed rape, canola, sunflower,

safflower, poppy, mustard, hemp, castor-oil plant, olive, sesame, Calendula, Punica, evening primrose, mullein, thistle, wild roses, hazelnut, almond, macadamia, avocado, bay, pumpkin/squash, linseed, soybean, pistachios, borage, trees (oil palm, coconut, walnut) or crops such as maize, wheat, rye, oats, triticale, rice, barley, cotton, cassava, pepper, Tagetes, Solanaceae plants such as potato, tobacco, eggplant and tomato, Vicia species, pea, alfalfa or bushy plants (coffee, cacao, tea), Salix species, and perennial grasses and fodder crops. Preferred plants according to the invention are oil crop plants such as peanut, oilseed rape, canola, sunflower, safflower, poppy, mustard, hemp, castor-oil plant, olive, Calendula, Punica, evening primrose, pumpkin/squash, linseed, soybean, borage, trees (oil palm, coconut). Especially preferred are sunflower, safflower, tobacco, mullein, sesame, cotton, pumpkin/squash, poppy, evening primrose, walnut, linseed, hemp, thistle or safflower. Very especially preferred plants are plants such as safflower, sunflower, poppy, evening primrose, walnut, linseed, or hemp, or. most preferred, plants of family Brassicaceae.

The invention is also concerned with providing constructs for establishing high content of VLC-PUFAs in plants or parts thereof, particularly in plant oils.

As such, the invention provides a T-DNA for expression of a target gene in a plant, wherein the T-DNA comprises a left and a right border element and at least one expression cassette comprising a promotor, operatively linked thereto a target gene, and downstream thereof a terminator, wherein the length of the T-DNA, measured from left to right border element and comprising the target gene, has a length of at least 30000 bp. In an embodiment, the expression cassette is separated from the closest border of the T-DNA by a separator of at least 500 bp length.

As described elsewhere herein, the the T-DNA of the present invention may comprise multiple expression cassettes encoding various, i.e. multiple proteins. In an embodiment, expression cassettes are separated from each other by a separator of at least 100 base pairs, preferably of 100-200 base pairs. Thus, there is a separator between each expression cassette.

The invention thus provides nucleic acids, i.e. polynucleotides. A polynucleotide according to the present invention is or comprises a T-DNA according to the present invention. Thus, a T-DNA according to the present invention is a polynucleotide, preferably a DNA, and most preferably a double stranded DNA. A "T-DNA" according to the invention is a nucleic acid capable of eventual integration into the genetic material (genome) of a plant. The skilled person understands that for such integration a transformation of respective plant material is required, prefered transformation methods and plant generation methods are described herein.

According to the invention are also provided nucleic acids comprising a T-DNA as defined according to the present invention. For example, a T-DNA of the present invention may be comprised in a circular nucleic acid, e.g. a plasmid, such that an additional nucleic acid section is present between the left and right border elements, i.e. "opposite" of the expression cassette(s) according to the present invention. Such circular nucleic acid may be mapped into a linear form using an arbitrary starting point, e.g. such that the definition "left border element – expression cassette – right border element – additional nucleic acid section opposite of the expression cassette" defines the same circular nucleic acid as the definition "expression cassette – right border element – additional nucleic acid section opposite of the expression cassette – left border element". The additional nucleic acid section preferably comprises one or more genetic elements for replication of the total nucleic acid, i.e. the nucleic acid molecule comprising the T-DNA and the additional nucleic acid section, in one or more host microorganisms, preferably in a microorganism of genus Escherichia, preferably E. coli, and/or Agrobacterium. Preferable host microorganisms are described below in more detail. Such circular nucleic acids comprising a T-DNA of the present invention are particularly useful as transformation vectors; such vectors and are described below in more detail.

The T-DNA length is preferably large, i.e. it has a minimum length of at least 30000 bp, preferably more than 30000 bp, more preferably at least 40000 bp, even more preferably at least 50000 bp and most preferably at least 60000 bp. Preferably, the length of the T-DNA is in a range of any of the aforementioned minimum lengths to 120000bp, more preferably in a range of any of the aforementioned minimum lengths to 100000bp, even more preferably in a range of any of the aforementioned minimum lengths to 90000 bp, even more preferably in a range of any of the aforementioned minimum lengths to 80000 bp. With such minimum lengths it is possible to introduce a number of genes in the form of expression cassettes such that each individual gene is operably liked to at least one promotor and at least one terminator. As is shown hereinafter in the examples section, the invention makes use of such minimum length T-DNA for introducing the genes required for the methabolic pathway of VLC-PUFA production in plants, e.g. oil seed plants, preferably plants of genus Brassica. Also, the length of the T-DNA is preferably limited as described before to allow for easy handling.

In 3' direction of the T-DNA left border element or in 5' direction of the T-DNA right border element, a separator is present setting the respective border element apart from the expression cassette comprising the target gene. The separator in 3' direction of the T-DNA left border element does not necessarily have the same length and/or sequence as the separator in 5' direction of the T-DNA right border element, as long as both separators suffice to the further requirements given below.

The separator or spacer is a section of DNA predominantly defined by its length. Its function is to separate a target gene from the T-DNA's left or right border, respectively. As will be shown in the examples, introducing a separator effectively separates the gene of interest from major influences exerted by the neighbouring genomic locations after insertion of the T-DNA into a genomic DNA. For example it is commonly believed that not all genomic loci are equally suitable for expression of a target gene, and that the same gene under the control of the same promotor and terminator may be expressed in different inensity in plants depending on the region of integration of the target gene (and its corresponding promotor and terminator) the plant genome. It is generally believed that different regions of a plant genome are accessible with differing ease for transcription factors and/or polymerase enzymes, for example due to these regions being tightly wound around histones and/or attached to the chromosomal backbone (cf. for example Deal et al., Curr Opin Plant Biol. Apr 2011; 14(2): 116–122) or other scaffold material (cf. e.g. Fukuda Y. , Plant Mol Biol. 1999 Mar; 39(5): 1051-62). The mechanism of achieving the above mentioned benefits by the T-DNA of the present invention is not understood it is convenient to think of the spacer as a means for physically providing a buffer to compensate for strain exerted by DNA winding by neighbouring histones or chromosomal backbone or other scaffold attached regions. As a model it can be thought that to transcribe a target gene, the DNA has to be partially unwound. If neighbouring regions of the target gene resist such unwinding, for example because they are tightly wound around histones or otherwise attached to a scaffold or backbone such that rotation of nucleic acid strands is limited, the spacer allows to distribute the strain created by the unwinding attempt over a longer stretch of nucleic acid, thereby reducing the force required for unwinding at the target gene.

In an embodiment, the separator has a length of at least 500 bp. The separator, thus, can be longer than 500 bp, and preferably is at least 800bp in length, more preferably at least 1000 bp. Longer spacers allow for even more physical separation between the target gene and the nearest genomic flanking region.

In another embodiment, the spacer has a length of at least 100 bp. Preferably, the spacer has a length of 100 to 200 base pairs.

The separator preferably has a sequence devoid of matrix or scaffold attachment signals. Preferably, the separator or spacer is does not comprise more than once for a length of 500 bp, preferably not more than once for a length of 1000 bp, a 5-tuple which occurs in the spacers described below in the examples for 20 or more times, summarized over all spacers given in the examples. Those tuples are, in increasing frequency in the spacers given in the examples: AGCCT, CGTAA, CTAAC, CTAGG, GTGAC, TAGGC, TAGGT, AAAAA, AACGC, TTAGC, ACGCT, GCTGA, ACGTT, AGGCT, CGTAG, CTACG, GACGT, GCTTA, AGCTT, CGCTA, TGACG, ACGTG, AGCTG, CACGT, CGTGA, CGTTA, AGCGT, TCACG, CAGCT, CGTCA, CTAGC, GCGTC, TTACG, GTAGC, TAGCG, TCAGC, TAGCT,

AGCTA, GCTAG, ACGTA, TACGT. By reducing the frequency of occurrence of one or more of the aforelisted tuples compared to the separators or spacers as given in the examples, a further increase in expression of a target gene in the T-DNA can be achieved.

The separator may contain a selectable marker. A selectable marker is a nucleic acid section whose presence preferably can be verified in seed without having to wait for the sprouting or full growth of the plant. Preferably the selectable marker conveys a phenotypical property to seed or to a growing plant, for example herbicide tolerance, coloration, seed surface properties (e.g. wrinkling), luminescence or fluorescence proteins, for example green fluorecent protein or luciferase. If for exhibiting the phenotypical feature an expression of a marker gene is required, then the separator correspondingly comprises the marker gene as selectable marker, preferably in the form of an expression cassette. Inclusion of a selectable marker in the separator is particularly advantageous since the marker allows to easily discard non-transformant plant material. Also, in such unexpected case where the T-DNA integrates in a location of the plant genome where the length and/or nucleobase composition of the spacer is insufficient to overcome gene silencing effects caused by the neighbouring genomic DNA, the selectable marker allows to easily discard such unfortunately badly performing exceptional transformants. Thus, preferably the separator comprises an expression cassette for expression of a herbicide tolerance gene. Such separator greatly reduces the chance of having to cultivate a transformant where silencing effects are so strong that even the expression of the selectable marker gene is greatly reduced or fully inhibited. According to the invention, the separator preferably does not comprise a desaturase or elongase gene, and also preferably does not comprise a promotor or operatively linked to a desaturase or elongase gene. Thus, the T-DNA of the present invention in preferred embodiments is useful for effective separation of the desaturase and elongase genes essential for the production of VLC-PUFAs from any influence of effects caused by neighbouring genomic plant DNA.

For the production of VLC-PUFAs in plants, the invention also provides a T-DNA comprising the coding sequences of any single of the tables given in the examples, preferably comprising the coding sequences and and promotors of any single of the tables given in the examples, more preferably the coding sequences and promotors and terminators of any single of the tables given in the examples, and most preferably the expression cassettes of any single of the tables of the examples.

Also, the invention provides a T-DNA for production of VLC-PUFAs in a plant, wherein the T-DNA comprises a left and a right border element and in between one or more expression cassette(s), wherein the length of the T-DNA, measured from left to right border element and comprising the one ore more expression cassettes, has a length of at least 30000 bp. In an embodiment, the expression cassette(s) closest to a left or right border element, respectively, is (are) separated from said closest border element of the T-DNA by a

separator of at least 500 bp length. It is to be understood that the one or more expression cassette(s) each comprise a promotor, operatively linked to a target gene, and downstream thereof a terminator, wherein the target gene of a respective expression cassette is a desaturase or elongase gene as required for production of a VLC-PUFA. Preferably at least one and most preferably all of the target genes codes/code for a desaturase or elongase as given in any of the tables in the examples section, and further preferably at least one and most preferably all expression cassettes consist of a combination of promotor, desaturase/elongase gene and terminator as given in any of the examples below.

In one embodiment a plant of the invention comprises one or more T-DNA of the present invention comprising one or more expression cassetts encoding for one or more d6Des, one or more d6Elo, one or more d5Des, one or more o3Des, one or more d5Elo and one or more D4Des, preferably for at least one CoA-dependent D4Des and one Phospholipid-dependent d4Des. In one embodiment, the T-DNA encodes also one or more d12Des.

In one embodiment, the plant of the invention or a part thereof as described herein comprises one or more T-DNAs of the invention which encode for at least two d6Des, at least two d6Elo and/or, at least two o3Des. In one embodiment, the present plant of the invention or a part thereof comprise a T-DNA comprising one or more expression cassets encoding for at least one CoA-depenent d4Des and at least one phopho-lipid dependent d4Des. In one embodiment the activities of the enzymes expressed by the one or more T-DNAs in the plant of the invention or a prat thereof are encode and express polpeptides having the activities shown in column 1 of Table 19. In one embodiment the plant of the invention comprises at least one T-DNA as shown in columns 1 to 9 in Table 13. In one embodiment, one T-DNA of the present invention comprises one or more gene expression cassetts encoding for the activities or enzymes listed in Table 13 flanked by the R-regions and one flanked by the R2 regions.

In one embodiment, the at least one T-DNA further comprises an expression cassette which encodes for at least one d12Des. In one embodiemt, the T-DNA or T-DNAs comprise one or more expression cassettes encoding one or more d5Des(Tc_GA), o3Des(Pir_GA), d6Elo(Tp_GA) and/or d6Elo(Pp_GA). Such plants of the present invention have shown particularly high amounts and concentrations of VLC-PUFAs over three or more generations and under different growth conditions

In one embodiment the T-DNA of the invention encodes for the activites of column 1 as disclosed in Table 19, preferably of gene combinations disclosed in Table 13, even more preferred for promoter-gene combinations as described in Table 13

As described in detail in the examples, with regards to the production of VLC-PUFAs three desaturase genes are particularly prone to gene dosage effects (also called "copy number effects"), such that increasing the number of expression cassettes comprising these respective genes leads to a stronger increase in VLC-PUFA levels in plant oils than

increasing the number of expression cassettes of other genes. These genes are the genes coding for delta-12-desaturase activity, for delta-6-desaturase activity and omega-3-desaturase activity. Thus, according to the invention each expression cassette comprising a gene coding for a delta-12-desaturase, delta-6-desaturase or omega-3-desaturase is separated from the respective closest left or right border element by a separator and optionally one or more expression cassettes. It is to be understood that where the T-DNA of the present invention comprises more than one expression cassette comprising a gene of the same function, these genes do not need to be identical concerning their nucleic acid sequence or the polypeptide sequence encoded thereby, but should be functional homologs. Thus, for example, to make use of the gene dosage effect described herein a T-DNA according to the present invention may comprise, in addition to optionally a multiplicity of genes coding for delta-6-desaturases and/or omega-3-desaturases, two, three, four or more expression cassettes each comprising a gene coding for a delta-12-desaturase, wherein the delta-12-desaturase polypeptides coded by the respective genes differ in their amino acid sequence. Likewise, a T-DNA of the present invention may comprise, in addition to optionally a multiplicity of genes coding for delta-12-desaturases and/or omega-3-desaturases, two, three, four or more expression cassettes each comprising a gene coding for a delta-6-desaturase, wherein the delta-6-desaturase polypeptides coded by the respective genes differ in their amino acid sequence, or a T-DNA of the present invention may comprise, in addition to optionally a multiplicity of genes coding for delta-12-desaturases and/or delta-6-desaturases, two, three, four or more expression cassettes each comprising a gene coding for a omega-3-desaturase, wherein the omega-3-desaturase polypeptides coded by the respective genes differ in their amino acid sequence.

According to the invention, the T-DNA may also comprise, instead of one or more of the afore mentioned coding sequences, a functional homolog thereof. A functional homolog of a coding sequence is a sequence coding for a polypeptide having the same metabolic function as the replaced coding sequence. For example, a functional homolog of a delta-5-desaturase would be another delta-5-desaturase, and a functional homolog of a delta-5-elongase would be another delta-5-elongase. The functional homolog of a coding sequence preferably codes for a polypeptide having at least 40% sequence identity to the polypeptide coded for by the corresponding coding sequence given in the corresponding table of the examples, more preferably at least 41%, more preferably at least 46%, more preferably at least 48%, more preferably at least 56%, more preferably at least 58%, more preferably at least 59%, more preferably at least 62%, more preferably at least 66%, more preferably at least 69%, more preferably at least 73%, more preferably at least 75%, more preferably at least 77%, more preferably at least 81%, more preferably at least 84%, more preferably at least 87%, more preferably at least 90%, more preferably at least 92%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98% and even more preferably at least 99%. Likewise, a functional homolog of a promotor is a sequence for starting transcription of a coding sequence located within 500 bp for a proximal promotor or, for a distal promotor, within 3000 bp distant from the promotor

TATA box closest to the coding sequence. Again, a functional homolog of a plant seed specific promotor is another plant seed specific promotor. The functional homolog of a terminator, correspondingly, is a sequence for ending transcription of a nucleic acid sequence.

The examples describe particularly preferred T-DNA sequences. As described above, the skilled person understands that the coding sequences, promotors and terminators described therein can be replaced by their functional homologs. However, the examples also describe that according to the invention, certain combinations of promotors and coding sequences, or certain combinations of promotors driving the expression of their corresponding coding sequences, or certain coding sequences or combinations thereof are particularly advantageous; such combinations or individual coding sequences should according to the invention not be replaced by functional homologs of the respective element (here: coding sequence or promotor).

A T-DNA of the present invention preferably comprises two or more genes, preferably all genes, susceptible to a gene dosage effect. As described herein, it is advantageous for achieving high conversion efficiencies of certain enzymatic acitvities, e.g. delta-12-desaturase, delta-6-desaturase and/or omega-3-desaturase activity, to introduce more than one gene coding for an enzyme having the desired activity into a plant cell. When introducing T-DNA into plant cells, generally transformation methods involving exposition of plant cells to microorganisms are employed, e.g. as described herein. As each microorganism may comprise more than one nucleic acid comprising a T-DNA of the present invention, recombinant plant cells are frequently obtained comprising two or more T-DNAs of the present invention independently integrated into the cell's genetic material. Thus, by combining genes susceptible to a gene dosage effect on one construct for transformation allows to easily exploit the independence of transformations to achieve a higher frequency of multiple insertions of such T-DNAs. This is particularly useful for transformation methods relying on co-transformation to keep the size of each construct to be transformed low. Also, where a construct comprises all genes susceptible to a gene dosage effect it is beneficially easy to increase the the number of such genes in a plant cell by over-transformation, e.g. transforming a plant cell with the T-DNA of the present invention, wherein the plant cell had previously been transformed with the same T-DNA or other genetic material.

The invention accordingly also provides a construct comprising a T-DNA according to the present invention, wherein the construct preferably is a vector for transformation of a plant cell by microorganism-mediated transformation, preferably by Agrobacterium-mediated transformation. Correspondingly, the invention also provides a transforming microorganism comprising one T-DNA according to the present invention, preferably as a construct comprising said T-DNA. Preferably the microorganism is of genus Agrobacterium,

preferably a disarmed strain thereof, and preferably of species Agrobacterium tumefaciens or, even more preferably, of species Agrobacterium rhizogenes. Corresponding strains are for example described in WO06024509A2, and methods for plant transformation using such microorganisms are for example described in WO13014585A1. These WO publications are incorporated herein in their entirety, because they contain valuable information about the creation, selection and use of such microorganisms.

The term "vector", preferably, encompasses phage, plasmid, viral vectors as well as artificial chromosomes, such as bacterial or yeast artificial chromosomes. Moreover, the term also relates to targeting constructs which allow for random or site-directed integration of the targeting construct into genomic DNA. Such target constructs, preferably, comprise DNA of sufficient length for either homolgous or heterologous recombination as described in detail below. The vector encompassing the polynucleotide of the present invention, preferably, further comprises selectable markers for propagation and/or selection in a host. The vector may be incorporated into a host cell by various techniques well known in the art. If introduced into a host cell, the vector may reside in the cytoplasm or may be incorporated into the genome. In the latter case, it is to be understood that the vector may further comprise nucleic acid sequences which allow for homologous recombination or heterologous insertion. Vectors can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. The terms "transformation" and "transfection", conjugation and transduction, as used in the present context, are intended to comprise a multiplicity of prior-art processes for introducing foreign nucleic acid (for example DNA) into a host cell, including calcium phosphate, rubidium chloride or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, natural competence, carbon-based clusters, chemically mediated transfer, electroporation or particle bombardment. Suitable methods for the transformation or transfection of host cells, including plant cells, can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) and other laboratory manuals, such as Methods in Molecular Biology, 1995, Vol. 44, Agrobacterium protocols, Ed.: Gartland and Davey, Humana Press, Totowa, New Jersey. Alternatively, a plasmid vector may be introduced by heat shock or electroporation techniques. Should the vector be a virus, it may be packaged in vitro using an appropriate packaging cell line prior to application to host cells.

Preferably, the vector referred to herein is suitable as a cloning vector, i.e. replicable in microbial systems. Such vectors ensure efficient cloning in bacteria and, preferably, yeasts or fungi and make possible the stable transformation of plants. Those which must be mentioned are, in particular, various binary and co-integrated vector systems which are suitable for the T DNA-mediated transformation. Such vector systems are, as a rule, characterized in that they contain at least the vir genes, which are required for the Agrobacterium-mediated transformation, and the sequences which delimit the T-DNA (T-

DNA border). These vector systems, preferably, also comprise further cis-regulatory regions such as promoters and terminators and/or selection markers with which suitable transformed host cells or organisms can be identified. While co-integrated vector systems have vir genes and T-DNA sequences arranged on the same vector, binary systems are based on at least two vectors, one of which bears vir genes, but no T-DNA, while a second one bears T-DNA, but no vir gene. As a consequence, the last-mentioned vectors are relatively small, easy to manipulate and can be replicated both in E. coli and in Agrobacterium. These binary vectors include vectors from the pBIB-HYG, pPZP, pBecks, pGreen series. Preferably used in accordance with the invention are Bin19, pBI101, pBinAR, pGPTV and pCAMBIA. An overview of binary vectors and their use can be found in Hellens et al, Trends in Plant Science (2000) 5, 446–451. Furthermore, by using appropriate cloning vectors, the polynucleotides can be introduced into host cells or organisms such as plants or animals and, thus, be used in the transformation of plants, such as those which are published, and cited, in: Plant Molecular Biology and Biotechnology (CRC Press, Boca Raton, Florida), chapter 6/7, pp. 71-119 (1993); F.F. White, Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, 15-38; B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press (1993), 128-143; Potrykus 1991, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42, 205-225.

More preferably, the vector of the present invention is an expression vector. In such an expression vector, i.e. a vector which comprises the polynucleotide of the invention having the nucleic acid sequence operatively linked to an expression control sequence (also called "expression cassette") allowing expression in prokaryotic plant cells or isolated fractions thereof.

Most important, the invention also provides a plant or seed thereof, comprising, integrated in its genome, a T-DNA of the present invention.

Such T-DNA preferably allows for the expression of all genes required for production of VLC-PUFAs in plants and particularly in the sees thereof, particularly in oilseed plants, and most beneficially in plants or seeds of family Brassicaceae, preferably of genus Brassica and most preferably of a species comprising a genome of one or two members of the species Brassica oleracea, Brassica nigra and Brassica rapa, thus preferably of the species Brassica napus, Brassica carinata, Brassica juncea, Brassica oleracea, Brassica nigra or Brassica rapa. Particularly preferred according to the invention are plants and seeds of the species Brassica napus and Brassica carinata.

The plants of the present invention are necessarily transgenic, i.e. they comprise genetic material not present in corresponding wild type plant or arranged differently in

corresponding wild type plant, for example differring in the number of genetic elements. For example, the plants of the present invention comprise promotors also found in wild type plants, but the plants of the present invention comprise such promotor operatively linked to a coding sequence sucht that this combination of promotor and coding sequence is not found in the corresponding wild type plant.

The plants and seeds of the present invention differ from hitherto produced plants for production of VLC-PUFAS in a number of advantageous features, some of which are described in detail in the examples. In particular, T-DNAs of the present invention allow for the generation of transformant plants (also called "recombinant plants" and seeds thereof with a high transformation frequency, with a high stability of T-DNA insertions over multiple generations of self-fertilized plants, unchanged or unimpaired phenotypical and agronomic characteristics other than VLC-PUFA production, and with high amounts and concentration of VLC-PUFAs, particularly EPA and/or DHA, in the oil of populations of such transformed plants and their corresponding progeny. The latter feature is particularly striking considering that even one full integration of the T-DNA required for establishing the metabolic pathway for VLC-PUFA production, optionally with an additional integration of a partial T-DNA of the present invention, is enough for reliable production of VLC-PUFAs and particularly of EPA and/or DHA in the combined seeds of corresponding populations of plant clones.

While previously reported expression of VLC-PUFAs either focus on the PUFA concentrations or amounts found in individual seeds, such reported amounts or concentrations are of limited value for deciding on agronomical usefulness, because seed-to-seed variability and plant-to-plant variability in VLC-PUFA amounts per seed are high even for identical clones cultivated side by side under identical greenhouse conditions. Also, it has now been found and is reported below in the examples that the contentration of VLC-PUFAs is negatively correlated with seed oil content in Brassica napus, such that the mere statement of VLC-PUFA concentration in %(w/w) of all fatty acids of a plant oil is not indicative of the VLC-PUFA amount achievable by agricultural (i.e. large scale) growing of corresponding clones. In summary, it has been notoriously difficult to distil from reported VLC-PUFA amounts or concentrations which genes, promotors, gene-promotor combinations, gene-gene combinations etc. are beneficial for VLC-PUFA synthesis in oilseed plants.

Also, it is important to note that VLC-PUFA amounts or concentrations can indeed be increased by increasing the number of expression cassettes of functionally identical genes. And it is also important to note that even though many prior art documents purport to comprise applicable technical teachings, e.g. to combine desaturases having a particular property, e.g. CoA-dependence, it is not known how to reduce such alleged technical teachings to practice, particularly as such documents only teach requirements (i.e. functional claim features) but not solutions for these requirements (i.e. structural features).

For example, such prior art documents frequently comprise only one example of a single gene and leave the reader with the instruction to start a research program to find other enzymes satisfying the functional definition given therein, provided that any other enzyme exists that satisfies those functional requirements.

Unless stated otherwise, a plant of the present invention comprising a T-DNA of the present invention can also be a plant comprising a part of a T-DNA of the present invention, where such part is sufficient for the production of a desaturase and/or elongase coded for in the corresponding full T-DNA of the present invention. Such plants most preferably comprise at least one full T-DNA of the present invention in addition to the part of a T-DNA of the present invention as defined in the previous sentence. Such plants are hereinafter also termed "partial double copy" plants. Event LBFDAU is an example of a plant comprising a part of a T-DNA of the present invention, and still being a plant of the present invention. In one embodiment the T_DNA is a full T-DNA.

Preferred plants of the present invention comprise one or more T-DNA(s) of the present invention comprising expression cassettes comprising, one or more genes encoding for one or more d5Des, one or more d6Elo, one or more d6Des, one or more o3Des, one or more d5Elo and one or more D4Des, preferably for at least one CoA-dependent D4Des and one Phospholipid-dependent d4Des. In one embodiment, at least one T-DNA further comprises a expression cassette which encodes for at least one d12Des. In one embodiemt, the T-DNA or T-DNAs further comprise one or more expression cassettes encoding one or more d5Des(Tc_GA), o3Des(Pir_GA), d6Elo(Tp_GA) and/or d6Elo(Pp_GA). Such plants of the present invention have shown particularly high amounts and concentrations of VLC-PUFAs over three or more generations and under different growth conditions.

Preferably, the plant cell of the present invention is an oilseed crop plant cell. More preferably, said oilseed crop is selected from the group consisting of flax (Linum sp.), rapeseed (Brassica sp.), soybean (Glycine and Soja sp.), sunflower (Helianthus sp.), cotton (Gossypium sp.), corn (Zea mays), olive (Olea sp.), safflower (Carthamus sp.), cocoa (Theobroma cacoa), peanut (Arachis sp.), hemp, camelina, crambe, oil palm, coconuts, groundnuts, sesame seed, castor bean, lesquerella, tallow tree, sheanuts, tungnuts, kapok fruit, poppy seed, jojoba seeds and perilla. Preferred plants to be used for introducing the polynucleotide or T-DNA of the invention are plants which are capable of synthesizing fatty acids, such as all dicotyledonous or monocotyledonous plants, algae or mosses. Preferred plants are selected from the group of the plant families Adelotheciaceae, Anacardiaceae, Arecaceae, Asteraceae, Apiaceae, Betulaceae, Boraginaceae, Brassicaceae, Bromeliaceae, Caricaceae, Cannabaceae, Convolvulaceae, Chenopodiaceae, Compositae, Crypthecodiniaceae, Cruciferae, Cucurbitaceae, Ditrichaceae, Elaeagnaceae, Ericaceae, Euphorbiaceae, Fabaceae, Geraniaceae, Gramineae, Juglandaceae, Lauraceae, Leguminosae, Linaceae, Malvaceae, Moringaceae, Marchantiaceae, Onagraceae,

Olacaceae, Oleaceae, Papaveraceae, Piperaceae, Pedaliaceae, Poaceae, Solanaceae, Prasinophyceae or vegetable plants or ornamentals such as Tagetes. Examples which may be mentioned are the following plants selected from the group consisting of: Adelotheciaceae such as the genera Physcomitrella, such as the genus and species Physcomitrella patens, Anacardiaceae such as the genera Pistacia, Mangifera, Anacardium, for example the genus and species Pistacia vera [pistachio], Mangifer indica [mango] or Anacardium occidentale [cashew], Asteraceae, such as the genera Calendula, Carthamus, Centaurea, Cichorium, Cynara, Helianthus, Lactuca, Locusta, Tagetes, Valeriana, for example the genus and species Calendula officinalis [common marigold], Carthamus tinctorius [safflower], Centaurea cyanus [cornflower], Cichorium intybus [chicory], Cynara scolymus [artichoke], Helianthus annus [sunflower], Lactuca sativa, Lactuca crispa, Lactuca esculenta, Lactuca scariola L. ssp. sativa, Lactuca scariola L. var. integrata, Lactuca scariola L. var. integrifolia, Lactuca sativa subsp. romana, Locusta communis, Valeriana locusta [salad vegetables], Tagetes lucida, Tagetes erecta or Tagetes tenuifolia [african or french marigold], Apiaceae, such as the genus Daucus, for example the genus and species Daucus carota [carrot], Betulaceae, such as the genus Corylus, for example the genera and species Corylus avellana or Corylus colurna [hazelnut], Boraginaceae, such as the genus Borago, for example the genus and species Borago officinalis [borage], Brassicaceae, such as the genera Brassica, Melanosinapis, Sinapis, Arabadopsis, for example the genera and species Brassica napus, Brassica rapa ssp. [oilseed rape], Sinapis arvensis Brassica juncea, Brassica juncea var. juncea, Brassica juncea var. crispifolia, Brassica juncea var. foliosa, Brassica nigra, Brassica sinapioides, Melanosinapis communis [mustard], Brassica oleracea [fodder beet] or Arabidopsis thaliana, Bromeliaceae, such as the genera Anana, Bromelia (pineapple), for example the genera and species Anana comosus, Ananas ananas or Bromelia comosa [pineapple], Caricaceae, such as the genus Carica, such as the genus and species Carica papaya [pawpaw], Cannabaceae, such as the genus Cannabis, such as the genus and species Cannabis sativa [hemp], Convolvulaceae, such as the genera Ipomea, Convolvulus, for example the genera and species Ipomoea batatus, Ipomoea pandurata, Convolvulus batatas, Convolvulus tiliaceus, Ipomoea fastigiata, Ipomoea tiliacea, Ipomoea triloba or Convolvulus panduratus [sweet potato, batate], Chenopodiaceae, such as the genus Beta, such as the genera and species Beta vulgaris, Beta vulgaris var. altissima, Beta vulgaris var.Vulgaris, Beta maritima, Beta vulgaris var. perennis, Beta vulgaris var. conditiva or Beta vulgaris var. esculenta [sugarbeet], Crypthecodiniaceae, such as the genus Crypthecodinium, for example the genus and species Cryptecodinium cohnii, Cucurbitaceae, such as the genus Cucurbita, for example the genera and species Cucurbita maxima, Cucurbita mixta, Cucurbita pepo or Cucurbita moschata [pumpkin/squash], Cymbellaceae such as the genera Amphora, Cymbella, Okedenia, Phaeodactylum, Reimeria, for example the genus and species Phaeodactylum tricornutum, Ditrichaceae such as the genera Ditrichaceae, Astomiopsis, Ceratodon, Chrysoblastella, Ditrichum, Distichium, Eccremidium, Lophidion, Philibertiella, Pleuridium, Saelania,

Trichodon, Skottsbergia, for example the genera and species Ceratodon antarcticus, Ceratodon columbiae, Ceratodon heterophyllus, Ceratodon purpureus, Ceratodon purpureus, Ceratodon purpureus ssp. convolutus, Ceratodon, purpureus spp. stenocarpus, Ceratodon purpureus var. rotundifolius, Ceratodon ratodon, Ceratodon stenocarpus, Chrysoblastella chilensis, Ditrichum ambiguum, Ditrichum brevisetum, Ditrichum crispatissimum, Ditrichum difficile, Ditrichum falcifolium, Ditrichum flexicaule, Ditrichum giganteum, Ditrichum heteromallum, Ditrichum lineare, Ditrichum lineare, Ditrichum montanum, Ditrichum montanum, Ditrichum pallidum, Ditrichum punctulatum, Ditrichum pusillum, Ditrichum pusillum var. tortile, Ditrichum rhynchostegium, Ditrichum schimperi, Ditrichum tortile, Distichium capillaceum, Distichium hagenii, Distichium inclinatum, Distichium macounii, Eccremidium floridanum, Eccremidium whiteleggei, Lophidion strictus, Pleuridium acuminatum, Pleuridium alternifolium, Pleuridium holdridgei, Pleuridium mexicanum, Pleuridium ravenelii, Pleuridium subulatum, Saelania glaucescens, Trichodon borealis, Trichodon cylindricus or Trichodon cylindricus var. oblongus, Elaeagnaceae such as the genus Elaeagnus, for example the genus and species Olea europaea [olive], Ericaceae such as the genus Kalmia, for example the genera and species Kalmia latifolia, Kalmia angustifolia, Kalmia microphylla, Kalmia polifolia, Kalmia occidentalis, Cistus chamaerhodendros or Kalmia lucida [mountain laurel], Euphorbiaceae such as the genera Manihot, Janipha, Jatropha, Ricinus, for example the genera and species Manihot utilissima, Janipha manihot, Jatropha manihot, Manihot aipil, Manihot dulcis, Manihot manihot, Manihot melanobasis, Manihot esculenta [manihot] or Ricinus communis [castor-oil plant], Fabaceae such as the genera Pisum, Albizia, Cathormion, Feuillea, Inga, Pithecolobium, Acacia, Mimosa, Medicajo, Glycine, Dolichos, Phaseolus, Soja, for example the genera and species Pisum sativum, Pisum arvense, Pisum humile [pea], Albizia berteriana, Albizia julibrissin, Albizia lebbeck, Acacia berteriana, Acacia littoralis, Albizia berteriana, Albizzia berteriana, Cathormion berteriana, Feuillea berteriana, Inga fragrans, Pithecellobium berterianum, Pithecellobium fragrans, Pithecolobium berterianum, Pseudalbizzia berteriana, Acacia julibrissin, Acacia nemu, Albizia nemu, Feuilleea julibrissin, Mimosa julibrissin, Mimosa speciosa, Sericanrda julibrissin, Acacia lebbeck, Acacia macrophylla, Albizia lebbek, Feuilleea lebbeck, Mimosa lebbeck, Mimosa speciosa [silk tree], Medicago sativa, Medicago falcata, Medicago varia [alfalfa], Glycine max Dolichos soja, Glycine gracilis, Glycine hispida, Phaseolus max, Soja hispida or Soja max [soybean], Funariaceae such as the genera Aphanorrhegma, Entosthodon, Funaria, Physcomitrella, Physcomitrium, for example the genera and species Aphanorrhegma serratum, Entosthodon attenuatus, Entosthodon bolanderi, Entosthodon bonplandii, Entosthodon californicus, Entosthodon drummondii, Entosthodon jamesonii, Entosthodon leibergii, Entosthodon neoscoticus, Entosthodon rubrisetus, Entosthodon spathulifolius, Entosthodon tucsoni, Funaria americana, Funaria bolanderi, Funaria calcarea, Funaria californica, Funaria calvescens, Funaria con¬voluta, Funaria flavicans, Funaria groutiana, Funaria hygrometrica, Funaria hygro¬metrica var. arctica, Funaria hygrometrica var. calvescens, Funaria hygrometrica var. convoluta, Funaria hygrometrica var. muralis,

Funaria hygrometrica var. utahensis, Funaria microstoma, Funaria microstoma var. obtusifolia, Funaria muhlenbergii, Funaria orcuttii, Funaria plano-convexa, Funaria polaris, Funaria ravenelii, Funaria rubriseta, Funaria serrata, Funaria sonorae, Funaria sublimbatus, Funaria tucsoni, Physcomitrella californica, Physcomitrella patens, Physcomitrella readeri, Physco¬mitrium australe, Physcomitrium californicum, Physcomitrium collenchymatum, Physcomitrium coloradense, Physcomitrium cupuliferum, Physcomitrium drummondii, Physcomitrium eurystomum, Physcomitrium flexifolium, Physcomitrium hookeri, Physcomitrium hookeri var. serratum, Physcomitrium immersum, Physcomitrium kellermanii, Physcomitrium megalocarpum, Physcomitrium pyriforme, Physcomitrium pyriforme var. serratum, Physcomitrium rufipes, Physcomitrium sandbergii, Physcomitrium subsphaericum, Physcomitrium washingtoniense, Geraniaceae, such as the genera Pelargonium, Cocos, Oleum, for example the genera and species Cocos nucifera, Pelargonium grossularioides or Oleum cocois [coconut], Gramineae, such as the genus Saccharum, for example the genus and species Saccharum officinarum, Juglandaceae, such as the genera Juglans, Wallia, for example the genera and species Juglans regia, Juglans ailanthifolia, Juglans sieboldiana, Juglans cinerea, Wallia cinerea, Juglans bixbyi, Juglans californica, Juglans hindsii, Juglans intermedia, Juglans jamaicensis, Juglans major, Juglans microcarpa, Juglans nigra or Wallia nigra [walnut], Lauraceae, such as the genera Persea, Laurus, for example the genera and species Laurus nobilis [bay], Persea americana, Persea gratissima or Persea persea [avocado], Leguminosae, such as the genus Arachis, for example the genus and species Arachis hypogaea [peanut], Linaceae, such as the genera Linum, Adenolinum, for example the genera and species Linum usitatissimum, Linum humile, Linum austriacum, Linum bienne, Linum angustifolium, Linum catharticum, Linum flavum, Linum grandiflorum, Adenolinum grandiflorum, Linum lewisii, Linum narbonense, Linum perenne, Linum perenne var. lewisii, Linum pratense or Linum trigynum [linseed], Lythrarieae, such as the genus Punica, for example the genus and species Punica granatum [pomegranate], Malvaceae, such as the genus Gossypium, for example the genera and species Gossypium hirsutum, Gossypium arboreum, Gossypium barbadense, Gossypium herbaceum or Gossypium thurberi [cotton], Marchantiaceae, such as the genus Marchantia, for example the genera and species Marchantia berteroana, Marchantia foliacea, Marchantia macropora, Musaceae, such as the genus Musa, for example the genera and species Musa nana, Musa acuminata, Musa paradisiaca, Musa spp. [banana], Onagraceae, such as the genera Camissonia, Oenothera, for example the genera and species Oenothera biennis or Camissonia brevipes [evening primrose], Palmae, such as the genus Elacis, for example the genus and species Elaeis guineensis [oil palm], Papaveraceae, such as the genus Papaver, for example the genera and species Papaver orientale, Papaver rhoeas, Papaver dubium [poppy], Pedaliaceae, such as the genus Sesamum, for example the genus and species Sesamum indicum [sesame], Piperaceae, such as the genera Piper, Artanthe, Peperomia, Steffensia, for example the genera and species Piper aduncum, Piper amalago, Piper angustifolium, Piper auritum, Piper betel, Piper cubeba, Piper longum, Piper nigrum, Piper retrofractum, Artanthe adunca, Artanthe

elongata, Peperomia elongata, Piper elongatum, Steffensia elongata [cayenne pepper], Poaceae, such as the genera Hordeum, Secale, Avena, Sorghum, Andropogon, Holcus, Panicum, Oryza, Zea (maize), Triticum, for example the genera and species Hordeum vulgare, Hordeum jubatum, Hordeum murinum, Hordeum secalinum, Hordeum distichon, Hordeum aegiceras, Hordeum hexastichon, Hordeum hexastichum, Hordeum irregulare, Hordeum sativum, Hordeum secalinum [barley], Secale cereale [rye], Avena sativa, Avena fatua, Avena byzantina, Avena fatua var. sativa, Avena hybrida [oats], Sorghum bicolor, Sorghum halepense, Sorghum saccharatum, Sorghum vulgare, Andropogon drummondii, Holcus bicolor, Holcus sorghum, Sorghum aethiopicum, Sorghum arundinaceum, Sorghum caffrorum, Sorghum cernuum, Sorghum dochna, Sorghum drummondii, Sorghum durra, Sorghum guineense, Sorghum lanceolatum, Sorghum nervosum, Sorghum saccharatum, Sorghum subglabrescens, Sorghum verticilliflorum, Sorghum vulgare, Holcus halepensis, Sorghum miliaceum, Panicum militaceum [millet], Oryza sativa, Oryza latifolia [rice], Zea mays [maize], Triticum aestivum, Triticum durum, Triticum turgidum, Triticum hybernum, Triticum macha, Triticum sativum or Triticum vulgare [wheat], Porphyridiaceae, such as the genera Chroothece, Flintiella, Petrovanella, Porphyridium, Rhodella, Rhodosorus, Vanhoeffenia, for example the genus and species Porphyridium cruentum, Proteaceae, such as the genus Macadamia, for example the genus and species Macadamia intergrifolia [macadamia], Prasinophyceae such as the genera Nephroselmis, Prasinococcus, Scherffelia, Tetraselmis, Mantoniella, Ostreococcus, for example the genera and species Nephroselmis olivacea, Prasinococcus capsulatus, Scherffelia dubia, Tetraselmis chui, Tetraselmis suecica, Mantoniella squamata, Ostreococcus tauri, Rubiaceae such as the genus Cofea, for example the genera and species Cofea spp., Coffea arabica, Coffea canephora or Coffea liberica [coffee], Scrophulariaceae such as the genus Verbascum, for example the genera and species Verbascum blattaria, Verbascum chaixii, Verbascum densiflorum, Verbascum lagurus, Verbascum longifolium, Verbascum lychnitis, Verbascum nigrum, Verbascum olympicum, Verbascum phlomoides, Verbascum phoenicum, Verbascum pulverulentum or Verbascum thapsus [mullein], Solanaceae such as the genera Capsicum, Nicotiana, Solanum, Lycopersicon, for example the genera and species Capsicum annuum, Capsicum annuum var. glabriusculum, Capsicum frutescens [pepper], Capsicum annuum [paprika], Nicotiana tabacum, Nicotiana alata, Nicotiana attenuata, Nicotiana glauca, Nicotiana langsdorffii, Nicotiana obtusifolia, Nicotiana quadrivalvis, Nicotiana repanda, Nicotiana rustica, Nicotiana sylvestris [tobacco], Solanum tuberosum [potato], Solanum melongena [eggplant], Lycopersicon esculentum, Lycopersicon lycopersicum, Lycopersicon pyriforme, Solanum integrifolium or Solanum lycopersicum [tomato], Sterculiaceae, such as the genus Theobroma, for example the genus and species Theobroma cacao [cacao] or Theaceae, such as the genus Camellia, for example the genus and species Camellia sinensis [tea]. In particular preferred plants to be used as transgenic plants in accordance with the present invention are oil fruit crops which comprise large amounts of lipid compounds, such as peanut, oilseed rape, canola, sunflower, safflower, poppy, mustard, hemp, castor-oil plant, olive, sesame, Calendula, Punica,

evening primrose, mullein, thistle, wild roses, hazelnut, almond, macadamia, avocado, bay, pumpkin/squash, linseed, soybean, pistachios, borage, trees (oil palm, coconut, walnut) or crops such as maize, wheat, rye, oats, triticale, rice, barley, cotton, cassava, pepper, Tagetes, Solanaceae plants such as potato, tobacco, eggplant and tomato, Vicia species, pea, alfalfa or bushy plants (coffee, cacao, tea), Salix species, and perennial grasses and fodder crops. Preferred plants according to the invention are oilseed crop plants.

Most preferably, the plant of the present invention is a plant found in the "Triangle of U", i.e. a plant of genus Brassica: Brassica napus (AA CC genome; n=19) is an amphidiploid plant of the Brassica genus but is thought to have resulted from hybridization of Brassica rapa (AA genome; n=10) and Brassica oleracea (CC genome; n=9). Brassica juncea (AA BB genome; n=18) is an amphidiploid plant of the Brassica genus that is generally thought to have resulted from the hybridization of Brassica rapa and Brassica nigra (BB genome; n=8). Under some growing conditions, B. juncea may have certain superior traits to B. napus. These superior traits may include higher yield, better drought and heat tolerance and better disease resistance. Brassica carinata (BB CC genome; n=17) is an amphidiploid plant of the Brassica genus but is thought to have resulted from hybridization of Brassica nigra and Brassica oleracea. Under some growing conditions, B. carinata may have superior traits to B. napus. Particularly, B. carinata allows for an increase in VLC-PUFA concentrations by at least 20% compared to B. napus when transformed with the same T-DNA.

The plant of the present invention preferably is a "Canola" plant. Canola is a genetic variation of rapeseed developed by Canadian plant breeders specifically for its oil and meal attributes, particularly its low level of saturated fat. Canola herein generally refers to plants of Brassica species that have less than 2% erucic acid (Delta 13-22:1) by weight in seed oil and less than 30 micromoles of glucosinolates per gram of oil free meal. Typically, canola oil may include saturated fatty acids known as palmitic acid and stearic acid, a monounsaturated fatty acid known as oleic acid, and polyunsaturated fatty acids known as linoleic acid and linolenic acid. Canola oil may contain less than about 7%(w/w) total saturated fatty acids (mostly palmitic acid and stearic acid) and greater than 40%(w/w) oleic acid (as percentages of total fatty acids). Traditionally, canola crops include varieties of Brassica napus and Brassica rapa. Preferred plants of the present invention are spring canola (Brassica napus subsp. oleifera var. annua) and winter canola (Brassica napus subsp. oleifera var. biennis). Furthermore a canola quality Brassica juncea variety, which has oil and meal qualities similar to other canola types, has been added to the canola crop family (U.S. Pat. No. 6,303,849, to Potts et al., issued on Oct. 16, 2001; U.S. Pat. No. 7,423,198, to Yao et al.; Potts and Males, 1999; all of which are incorporated herein by reference). Likewise it is possible to establish canola quality B. carinata varieties by crossing canola quality variants of Brassica napus with Brassica nigra and appropriately selecting progeny thereof, optionally after further back-crossing with B. carinata, B. napus and/or B. nigra.

The invention also provides a plant or seed thereof of family Brassicaceae, preferably of genus Brassica, with a genotype that confers a heritable phenotype of seed oil VLC-PUFA content, obtainable or obtained from progeny lines prepared by a method comprising the steps of

i) crossing a plant of family Brassicaceae, preferably of genus Brassica, most preferably of genus Brassica napus, Brassica oleracea, Brassica nigra or Brassica carinata, said plant comprising a T-DNA of the present invention and/or part of such T-DNA, with a parent plant of family Brassicaceae, preferably of genus Brassica, most preferably of genus Brassica napus, Brassica oleracea, Brassica nigra or Brassica carinata, said plant not comprising said T-DNA and/or part thereof, to yield a F1 hybrid,

ii) selfing the F1 hybrid for at least one generation, and

iii) identifying the progeny of step (ii) comprising the T-DNA of the present invention capable of producing seed comprising VLC-PUFA such that the content of all VLC-PUFA downstream of 18:1n-9 is at least 40% (w/w) of the total seed fatty acid content at an oil content of 40% (w/w), or preferably the content of EPA is at least 12% (w/w) and/or the content of DHA is at least 2% (w/w) of the total seed fatty acid content at an oil content of 40% (w/w).

This method allows to effectively incorporate genetic material of other members of family Brassicaceae, preferably of genus Brassica, into the genome of a plant comprising a T-DNA of the present invention. The method is particularly useful for combining a T-DNA of the present invention with genetic material responsible for beneficial traits exhibited in other members of family Brassicaceae. Beneficial traits of other members of family Brassicaceae are exemplarily described herein, other beneficial traits or genes and/or regulatory elements involved in the manifestation of a beneficial trait may be described elsewhere.

The parent plant not comprising the T-DNA of the present invention or part thereof preferably is an agronomically elite parent. In particular, the present invention teaches to transfer heterologous material from a plant or seed of the present invention to a different genomic background, for example a different variety or species.

In particular, the invention teaches to transfer the T-DNA or part thereof (the latter is particularly relevant for those plants of the present invention which comprise, in addition to a full T-DNA of the present invention, also a part of a T-DNA of the present invention, said part preferably comprising at least one expression cassette, the expression cassette preferably comprising a gene coding for a desaturase or elongase, preferably a delta-12-desaturase, delta-6-desaturase and/or omega-3-desaturase) into a species of genus Brassica carinata, or to introduce genetic material from Brassica carinata or Brassica nigra into the plants of the present invention comprising the T-DNA of the present invention and/or a part or two or more parts thereof. According to the invention, genes of Brassica

nigra replacing their homolog found in Brassica napus or added in addition to the homolog found in Brassica napus are particularly helpful in further increasing the amount of VLC-PUFAs in plant seeds and oils thereof.

Also, the invention teaches novel plant varieties comprising the T-DNA and/or part thereof of the present invention. Such varieties can, by selecting appropriate mating partners, be particularly adapted e.g. to selected climatic growth conditions, herbicide tolerance, stress resistance, fungal resistance, herbivore resistance, increased or reduced oil content or other beneficial features. As shown hereinafter in the examples it is particularly beneficial to provide plants of the present invention wherein the oil content thereof at harvest is lower than that of corresponding wild type plants of the same variety, such as to improve VLC-PUFA amounts in the oil of said plants of the present invention and/or VLC-PUFA concentrations in said oil.

Also, the invention provides a method for creating a plant with a genotype that confers a heritable phenotype of seed oil VLC-PUFA content, obtainable or obtained from progeny lines prepared by a method comprising the steps of

i) crossing a transgenic plant of the invention with a parent plant not comprising a T-DNA of the present invention or part thereof, said parent plant being of family Brassicaceae, preferably of genus Brassica, most preferably of genus Brassica napus, Brassica oleracea, Brassica nigra or Brassica carinata, to yield a F1 hybrid,

ii) selfing the F1 hybrid for at least one generation, and

iii) identifying the progeny of step (ii) comprising the T-DNA of the present invention capable of producing seed comprising VLC-PUFA such that the content of all VLC-PUFA downstream of 18:1n-9 is at least 40% (w/w) of the total seed fatty acid content at an oil content of 40% (w/w), or preferably the content of EPA is at least 8% (w/w) and/or the content of DHA is at least 1% (w/w) of the total seed fatty acid content at an oil content of 30% (w/w), preferably at an oil content of 35% (w/w), and more preferably at an oil content of 40% (w/w).

The method allows the creation of novel variants and transgenic species of plants of the present invention, and the seeds thereof. Such plants and seeds exhibit the aforementioned benefits of the present invention. Preferably, the content of EPA is at least 10% by weight, even more preferably at least 13% (w/w), of the total lipid content of the oil. Also preferably, the content of DHA is at least 1.5% by weight, even more preferably at least 2% (w/w), of the total lipid content of the oil. The present invention for the first time allows to achieve such high levels of VLC-PUFA in seed reliably under agronomic conditions, i.e. representative for the real yield obtained from seeds of a commercial field of at least 1 ha planted with plants of the present invention, wherein the plants have a defined copy number of genes for implementing the pathway for production of EPA and/or DHA in said plants, and the copy number being low, i.e. single-copy or partial double copy.

A plant of the present invention also includes plants obtainable or obtained by backcrossing (cross into the non-transgenic, isogenic parent line), and by crossing with other germplasms of the Triangle of U. Accordingly, the invention provides a method for creating a plant with a genotype that confers a heritable phenotype of seed oil VLC-PUFA content, obtainable or obtained from a progeny line prepared by a method comprising the steps of

i) crossing a transgenic plant of the invention (also called "non-recurring parent") with a parent plant not expressing a gene comprised in the T-DNA of the present invention, said parent plant being of family Brassicaceae, preferably of genus Brassica, most preferably of genus Brassica napus, Brassica oleracea, Brassica nigra or Brassica carinata, to yield a hybrid progeny,

ii) crossing the hybrid progeny again with the parent to obtain another hybrid progeny,

iii) optionally repeating step ii) and

iv) selecting a hybrid progeny comprising the T-DNA of the present invention.

Backcrossing methods, e.g. as described above, can be used with the present invention to improve or introduce a characteristic into the plant line comprising the T-DNA of the present invention. Such hybrid progeny is selected in step iv) which suffices predetermined parameters. The backcrossing method of the present invention thereby beneficially facilitates a modification of the genetic material of the recurrent parent with the desired gene, or preferably the T-DNA of the present invention, from the non-recurrent parent, while retaining essentially all of the rest of the desired genetic material of the recurrent parent, and therefore the desired physiological and morphological, constitution of the parent line. The selected hybrid progeny is then preferably multiplied and constitutes a line as described herein. Selection of useful progeny for repetition of step ii) can be further facilitated by the use of genomic markers. For example, such progeny is selected for the repetition of step ii) which comprises, compared to other progeny obtained in the previous crossing step, most markers also found in the parent and/or least markers also found in the non-recurring parent except the desired T-DNA of the present invention or part thereof.

Preferably, a hybrid progeny is selected which comprises the T-DNA of the present invention, and even more preferably also comprises at least one further expression cassette from the non-recurring parent of the present invention, e.g. by incorporation of an additional part of the T-DNA of the present invention into the hybrid plant genetic material.

Further preferably a hybrid progeny is obtained wherein essentially all of the desired morphological and physiological characteristics of the parent are recovered in the converted plant, in addition to genetic material from the non-recurrent parent as determined at the 5% significance level when grown under the same environmental conditions.

Further preferably, a hybrid progeny is selected which produces seed comprising VLC-PUFA such that the content of all VLC-PUFA downstream of 18:1n-9 is at least 40% (w/w) of the total seed fatty acid content at an oil content of 40% (w/w), or preferably the content of EPA is at least 8% (w/w) and/or the content of DHA is at least 1% (w/w) of the total seed fatty acid content at an oil content of 30% (w/w), preferably at an oil content of 35% (w/w), and more preferably at an oil content of 40% (w/w).

It is to be understood that such seed VLC-PUFA content is to be measured not from a single seed or from the seeds of an individual plant, but refer to the numeric average of seed VLC-PUFA content of at least 100 plants, even more preferably of at least 200 plants, even more preferably of at least 200 plants half of which have been grown in field trials in different years.

The choice of the particular non-recurrent parent will depend on the purpose of the backcross. One of the major purposes is to add some commercially desirable, agronomically important trait to the line.

The term "line" refers to a group of plants that displays very little overall variation among individuals sharing that designation. A "line" generally refers to a group of plants that display little or no genetic variation between individuals for at least one trait. A "DH (doubled haploid) line," as used in this application refers to a group of plants generated by culturing a haploid tissue and then doubling the chromosome content without accompanying cell division, to yield a plant with the diploid number of chromosomes where each chromosome pair is comprised of two duplicated chromosomes. Therefore, a DH line normally displays little or no genetic variation between individuals for traits. Lines comprising one or more genes originally comprised in a T-DNA of the present invention in the non-recurring parent also constitute plants of the present invention.

The invention is also concerned with a method of plant oil production, comprising the steps of

i) growing a plant of the present invention such as to obtain oil-containing seeds thereof,

ii) harvesting said seeds, and

iii) extracting oil from said seeds harvested in step ii), wherein the oil has a DHA content of at least 1% by weight based on the total lipid content and/or a EPA content of at least 8% by weight based on the total lipid content.

Again preferably, the content of EPA is at least 10% by weight, even more preferably at least 13% (w/w), of the total lipid content of the oil. Also preferably, the content of DHA is at least 1.5% by weight, even more preferably at least 2% (w/w), of the total lipid content of the oil. As described herein, the plant of the present invention comprises, for the purposes

of such method of plant oil production, preferably comprises a T-DNA of the present invention and optionally also one or more additional parts thereof, wherein the part or parts, respectively, comprise at least one expression cassette of the T-DNA of the present invention.

The invention is also concerned with parts of plants of the present invention. The term "parts of plants" includes anything derived from a plant of the invention, including plant parts such as cells, tissues, roots, stems, leaves, non-living harvest material, silage, seeds, seed meals and pollen. Preferably such plant part comprises a T-DNA of the present invention and/or comprises a content of EPA of at least 10% by weight, even more preferably at least 13% (w/w), of the total lipid content. Also preferably, the content of DHA is at least 1.5% by weight, even more preferably at least 2% (w/w), of the total lipid content of the plant part. Parts of plants of the present invention comprising, compared to wild-type plants, elevated content of EPA and/or DHA are particularly useful also for feed purposes, e.g. for aquaculture feed, e.g. as described in AU2011289381A and members of the patent family thereof.

As described in more detail in the examples, selection of the time of seed harvest is particularly beneficial for obtaining optimal amounts of VLC-PUFAs. As the PUFA content is inversely correlated with seed oil content, it is beneficial to harvest the seeds of plants of the present invention before maximum oil content is reached. Also, as described in the examples, the content of VLC-PUFAs changes during seed development. The invention thus provides an easy method of obtaining an oil containing a desired amount of a selected VLC-PUFA by harvesting seeds of plants of the present invention when their oil content corresponds to the desired amount of the selected VLC-PUFA or the desired composition of the oil. Thus, it is also possible to create blends of VLC-PUFA containing plant oils by mixing oils obtained by harvesting at different points in time or different degrees of seed ripening.

The plants of the present invention do not necessarily have to comprise a complete T-DNA of the present invention. As described above, by crossing (or back-crossing) methods it is possible to transfer arbitrary genetic material of one line to another line. Thus, by applying such crossing or back-crossing it is possible to transfer one or more, even all, expression cassettes comprised in a plant of the present invention (such plant comprising a T-DNA of the present invention) to another plant line, thereby losing e.g. a left or right border element (or both) and/or a spacer.

Thus, the invention also provides plants comprising genetic material hybridizing to a primer as given in Example 24.

Also, the invention provides plants comprising a heterologous nucleic acid segment inserted in its genetic material. The insertion is according to the invention in one of the below listed flanking regions or between a pair of flanking regions. Due to plant-to-plant variability, each flanking region may differ from the below indicated flanking regions by at most 10% calculated over a consecutive stretch of at least 100nt, preferably by at most 5% calculated over a consecutive stretch of at least – with increasing percentage identity more preferred - 90. 91. 92, 93, 94, 95, 96, 97, 98 or 99, preferred 100% identity for 100 nt. Even more preferably, the flanking region(s) comprise at least – with increasing percentage identity more preferred - 90. 91. 92, 93, 94, 95, 96, 97, 98 or 99, preferred 100% identity for 50 consecutive nucleotides identical to a fragment of a flanking region as given below, even more preferable the length of consecutive identical nucleotides is at least 100.

Table 176 provides an overview of all the flanking sequences of all loci obtained for each event listed in that table. In addition, event specific primers and probes are disclosed for event specific detection. The method for using those primers and probes is described in example 24.

As shown in the examples, insertions in these flanking regions have now been proven to lead to a surprisingly high production of VLC-PUFAs in seed, wherein such production is stable over many generations and under different growth conditions. Thus, insertion of other genetic material at these insertion locations also leads to a stable, high expression of inserted genes compared to insertions at other positions of the plant genome.

The invention is also concerned with establishing and optimizing efficient metabolic VLC-PUFA synthesis pathways. To this end, the invention provides a method for analysing desaturase reaction specificity, comprising the steps of

i) providing, to a desaturase, a detectably labelled molecule comprising a fatty acid moiety and a headgroup,

ii) allowing the desaturase to react on the labelled molecule, and

iii) detecting desaturation products.

The labelled molecule preferably is a fatty acid-coenzyme A or a fatty acid-phospholipid, the latter preferably being a lysophosphatidylcholine bound fatty acid. The method advantageously allows to determining desaturase headgroup preference or even headgroup specificity by detecting whether coenzyme A bound fatty acids are desaturated and/or whether phospholipid-bound fatty acids are desaturated.

Preferably the desaturase is provided as a microsomal fraction of an organism, preferably of yeast. Transgenic yeasts expressing the desaturase in question are easy to prepare and handle, and microsomal fractions comprising functional desaturases can be reliably and reproducibly prepared thereof without major burden. Microsomal fractions, particularly of

yeast, most preferably of Saccharomyces cerevisiae, also allow to convert fatty acid-coenzyme A molecules to fatty acids bound to other headgroups by using the yeast's native LPCAT. Likewise, microsomal fractions can be prepared from other cells and organisms.

Preferably the molecule is detectably labelled by including a radioactive isotope instead of a non-radioactive isotope. The isotope preferably is [14C]. Such label is easy to detect, does not interefere in biochemical reactions and can be incorporated in virtually any carbon-containing molecule, thereby allowing sensitively detecting and characterizing any desaturation product.

The fatty acid moiety preferably is a PUFA moiety, more preferably a VLC-PUFA moiety and most preferably a VLC-PUFA moiety. This way, the headgroup preference or specificity for economically important desaturases can be determined without having to resort to error prone and laborious feeding of live organisms or living cells.

The desaturase is allowed to react on the labelled molecule. If the desaturase can accept the labelled molecule as a substrate, then the desaturation reaction is performed. Preferably, the method is repeated by including, as a positive control, a labelled molecule which had been confirmed to be a substrate for the desaturase.

Detection preferably is accomplished using chromatography, most preferably thin layer chromatography. This technique is well known to the skilled person, readily available, very sensitive and allows differentiating even between very similar molecules. Thus, even if the positive control molecule is similar to the molecule of interest, a clear detection of desaturation products (if desaturation of the molecule occurred) is still possible.

The above method allows preparing a collection of specificity data for each desaturase, type of microsomal fraction (e.g. from yeast, plant cells etc.), fatty acid moiety and headgroup. Thus, the method can be used to select a desaturase for a given need, e.g. to accept CoA-bound fatty acids in plant cells for further presentation to an elongase. The method also allows establishing substrate specificty of the desaturase in an organism or organ of interest, e.g. a yeast, a plant leaf cell or a plant seed cell.

The invention also provides a method for analysing elongase reaction specificity, comprising the steps of

i) providing, to an elongase, a detectably labelled elongation substrate and a molecule to be elongated,

ii) allowing the elongase to elongate the molecule to be elongated using the labelled elongation substrate, and

iii) detecting elongation products.

Unless stated otherwise, the method for analysing elongase reaction specificity is performed corresponding to the method for analysing desaturase reaction specificity for corresponding reasons. The elongation substrate preferably is malonyl-CoA. The elongation substrate is preferably labelled radioactively, most preferably [14C] malonyl-CoA. Radioactive labelling allows for an easy and sensitive detection of elongation products. Also, labelling of the elongation substrate instead of the molecule to be elongated allows presenting a mixture of molecules to be elongated to the elongase, and only the elongation products will have incorporated significant amounts of label to render them easily detectable. Thus, in a single reaction vessel a multitude of potential molecules to be elongated can be assayed to determine which of these molecules are indeed elongated, and the relative affinity of the elongase to the respective molecule.

By combining both methods, it is possible to analyse even complex sequences of desaturation and elongation reactions. The invention thus also provides a method for pathway optimization, comprising the steps of

i) providing enzymes of a metabolic pathway and one or more substrates to be used by the first enzyme or enzymes of the pathway,

ii) reacting the enzymes and the substrates to produce products, which in turn are also exposed as potential substrates to the enzymes of the pathway, and

iii) determining the accumulation of products.

The method particularly allows providing desaturases and elongases to form a pathway. This is useful to determine the yield of product(s) of the pathway and of any unwanted side products. Also, by providing two or more different enzymes which perform the same metabolic function, e.g. a particular desaturation step, e.g. a delta-5 desaturation, it is possible to analyse if the presence of more than one type of enzymes has an effect on product formation, particularly on product formation rate. For such analysis, one would compare the results with a method performed with only one of the at least two enzymes. Thus, if addition of enzymes performing the same metabolic function leads to an increased yield or produc formation rate, then this metabolic step is subject to a gene dosage effect. To optimize the pathway in an organism one would correspondingly strive to implement the pathway step using the two or more enzymes as required.

Also, the method advantageously allows determining the mode of action of an enzyme in question. To this end, a helper enzyme is provided to produce a substrate for a target enzyme. The helper enzyme's mode of action is known. The helper enzyme is then provided with a substrate to turn into a product which could be used as a substrate of the target enzyme. Generation of product by the target enzyme is determined, preferably by measuring the amount of product per time or the final amount of product divided by the amount of substrate converted by the helper enzyme. Then the method is repeated using a helper enzyme of a different mode of action, and generation of product by the target

enzyme is determined, too. By comparing the product generation by the target enzyme for each mode of action, the mode of action of the target enzyme is defined as being the mode of action of the helper enzyme giving rise to the most intense generation of product by the target enzyme.

For example, to determine the mode of action of a target desaturase, a helper elongase is provided for which it has been text book knowledge that it is utilizing acyl CoA substrate and produces acyl-CoA products. Then, product generation by the target desaturase is measured. In another step, a helper desaturase is provided for which it has been established that it produces phosphatidylcholine-bound fatty acids. Again, product generation by the target desaturase is measured. When comparing the product generation of the target desaturase, the target desaturase can be defined as being a CoA-dependent desaturase if the product generation of the target desaturase under conditions where CoA-bound fatty acids are provided by the helper enzyme is more intense (e.g. higher conversion efficiency) than under conditions where phosphatidylcholine-bound fatty acids are provided by the helper enzyme.

Thus, the invention also provides a method for determining CoA-dependence of a target desaturase, comprising the steps of

i) providing an elongase to produce a substrate for the target desaturase, and determining conversion efficiency of the target desaturase, and

ii) providing a non-CoA dependent desaturase to produce the substrate for the target desaturase, and determining conversion efficiency of the target desaturase, and

iii) comparing the target desaturase conversion efficiencies of step i) and ii).

If the conversion efficiency of the target desaturase is larger in step i) than in step ii), then the target desaturase is CoA-dependent. Of course, both steps must be performed under comparable conditions; particularly a substrate limitation of the target desaturase must be avoided.

The method can also be performed by providing an elongase which uses the substrate of the target desaturase. Thus, the invention provides a method for determining CoA-dependence of a target desaturase, comprising the steps of

i) providing an elongase to elongate the products of the target desaturase, and determining conversion efficiency of the elongase,

ii) providing the elongase to elongate the products of a comparison desaturase known to be non-CoA dependent, and determining conversion efficiency of the elongase,

iii) comparing the elongase conversion efficiencies of step i) and ii).

If the elongase conversion efficiency is higher in step i) than in step ii), the target desaturase is CoA-dependence. Without being bound by any particular theory it is presently

expected that in such case the desaturation product does not have to be converted into an elongatable CoA-bound fatty acid, thus desaturated product can be immediately utilized by the elongase without accumulation. Of course, both steps must be performed under comparable conditions; particularly a substrate limitation of the elongase must be avoided.

The present invention also relates to oil comprising a polyunsaturated fatty acid obtainable by the aforementioned methods.

The term "oil" refers to a fatty acid mixture comprising unsaturated and/or saturated fatty acids which are esterified to triglycerides. Preferably, the triglycerides in the oil of the invention comprise PUFA or VLC-PUFA moieties as referred to above. The amount of esterified PUFA and/or VLC-PUFA is, preferably, approximately 30%, a content of 50% is more preferred, a content of 60%, 70%, 80% or more is even more preferred. The oil may further comprise free fatty acids, preferably, the PUFA and VLC-PUFA referred to above. For the analysis, the fatty acid content can be, e.g., determined by GC analysis after converting the fatty acids into the methyl esters by transesterification. The content of the various fatty acids in the oil or fat can vary, in particular depending on the source. The oil, however, shall have a non-naturally occurring composition with respect to the PUFA and/or VLC-PUFA composition and content. It is known that most of the fatty acids in plant oil are esterified in triacylglycerides. Accordingly, in the oil of the invention, the PUFAs and VLC-PUFAs are, preferably, also occur in esterified form in the triacylglcerides. It will be understood that such a unique oil composition and the unique esterification pattern of PUFA and VLC-PUFA in the triglycerides of the oil shall only be obtainable by applying the methods of the present invention specified above. Moreover, the oil of the invention may comprise other molecular species as well. Specifically, it may comprise minor impurities of the polynucleotide or vector of the invention. Such impurities, however, can be detected only by highly sensitive techniques such as PCR.

As described above, these oils, lipids or fatty acids compositions, preferably, comprise 6 to 15% of palmitic acid, 1 to 6% of stearic acid, 7-85% of oleic acid, 0.5 to 8% of vaccenic acid, 0.1 to 1% of arachic acid, 7 to 25% of saturated fatty acids, 8 to 85% of monounsaturated fatty acids and 60 to 85% of polyunsaturated fatty acids, in each case based on 100% and on the total fatty acid content of the organisms. Preferred VLC-PUFAs present in the fatty acid esters or fatty acid mixtures is, preferably, at least 5,5% to 20% of DHA and/or 9,5% to 30% EPA based on the total fatty acid content.

Moreover, the fatty acid esters or fatty acid mixtures which have been produced by the process of the invention, preferably, comprise further fatty acids selected from the group of the fatty acids erucic acid (13-docosaenoic acid), sterculic acid (9,10- methyleneoctadec-9-enoic acid), malvalic acid (S,9-methyleneheptadec-S-enoic acid), chaulmoogric acid (cyclopentenedodecanoic acid), furan fatty acid (9,12- epoxyoctadeca-9,11-dienoic acid),

vernonic acid (9,10-epoxyoctadec-12-enoic acid), tariric acid (6-octadecynoic acid), 6-nonadecynoic acid, santalbic acid (t11-octadecen- 9-ynoic acid), 6,9-octadecenynoic acid, pyrulic acid (t10-heptadecen-S-ynoic acid), crepenyninic acid (9-octadecen-12-ynoic acid), 13, 14-dihydrooropheic acid, octadecen-13-ene-9,11-diynoic acid, petroselenic acid (cis-6-octadecenoic acid), 9c,12toctadecadienoic acid, calendulic acid (St10t12c-octadecatrienoic acid), catalpic acid (9t11 t13c-octadecatrienoic acid), eleostearic acid (9c11 t13t-octadecatrienoic acid), jacaric acid (Sc10t12c-octadecatrienoic acid), punicic acid (9c11 t13c-octadecatrienoic acid), parinaric acid (9c11 t13t15c-octadecatetraenoic acid), pinolenic acid (all-cis-5,9,12-octadecatrienoic acid), laballenic acid (5,6-octadecadienallenic acid), ricinoleic acid (12-hydroxyoleic acid) and/or coriolic acid (13-hydroxy-9c, 11t-octadecadienoic acid). The fatty acid esters or fatty acid mixtures produced by the process according to the invention advantageously comprise less than 0.1 %, based on the total fatty acids, or no butter butyric acid, no cholesterol, no clupanodonic acid (= dpcpsapentaenoic acid, C22:5M,8,12,15,21) and no nisinic acid (tetracosahexaenoic acid, C23:6 L13,8,12,15,18,21).

The oils, lipids or fatty acids according to the invention, preferably, comprise at least 5.5%, 6%, 7% or 7,5%, more preferably, at least 8%, 9%, 10%, 11% or 12%, and most preferably at least 13%,14%,15%,16%. 17%, 18%, 19% or 20% of DHA, or at least 9.5%, 10%, 11% or 12%, more preferably, at least 13%, 14%, 14,5%, 15% or 16%, and most preferably at least 17%,18%,19%,20%. 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29% or 30% of EPA based on the total fatty acid content of the production host cell, organism, advantageously of a plant, especially of an oil crop such as soybean, oilseed rape, coconut, oil palm, safflower, flax, hemp, castor-oil plant, Calendula, peanut, cacao bean, sunflower or the abovementioned other monocotyledonous or dicotyledonous oil crops.

A further embodiment according to the invention is the use of the oil, lipid, fatty acids and/or the fatty acid composition in feedstuffs, foodstuffs, dietary supplies, cosmetics or pharmaceutical compositions as set forth in detail below. The oils, lipids, fatty acids or fatty acid mixtures according to the invention can be used for mixing with other oils, lipids, fatty acids or fatty acid mixtures of animal origin such as, for example, fish oils.

The term "composition" refers to any composition formulated in solid, liquid or gaseous form. Said composition comprises the compound of the invention optionally together with suitable auxiliary compounds such as diluents or carriers or further ingredients. In this context, it is distinguished for the present invention between auxiliary compounds, i.e. compounds which do not contribute to the effects elicited by the compounds of the present invention upon application of the composition for its desired purpose, and further ingredients, i.e. compounds which contribute a further effect or modulate the effect of the compounds of the present invention. Suitable diluents and/or carriers depend on the purpose for which the composition is to be used and the other ingredients. The person

skilled in the art can determine such suitable diluents and/or carriers without further ado. Examples of suitable carriers and/or diluents are well known in the art and include saline solutions such as buffers, water, emulsions, such as oil/water emulsions, various types of wetting agents, etc.

In a more preferred embodiment of the oil-, fatty acid or lipid-containing composition, the said composition is further formulated as a pharmaceutical composition, a cosmetic composition, a foodstuff, a feedstuff, preferably, fish feed or a dietary supply.

The term "pharmaceutical composition" as used herein comprises the compounds of the present invention and optionally one or more pharmaceutically acceptable carrier. The compounds of the present invention can be formulated as pharmaceutically acceptable salts. Acceptable salts comprise acetate, methylester, Hel, sulfate, chloride and the like. The pharmaceutical compositions are, preferably, administered topically or systemically. Suitable routes of administration conventionally used for drug administration are oral, intravenous, or parenteral administration as well as inhalation. However, depending on the nature and mode of action of a compound, the pharmaceutical compositions may be administered by other routes as well. For example, polynucleotide compounds may be administered in a gene therapy approach by using viral vectors or viruses or liposomes.

Moreover, the compounds can be administered in combination with other drugs either in a common pharmaceutical composition or as separated pharmaceutical compositions wherein said separated pharmaceutical compositions may be provided in form of a kit of parts. The compounds are, preferably, administered in conventional dosage forms prepared by combining the drugs with standard pharmaceutical carriers according to conventional procedures. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation. It will be appreciated that the form and character of the pharmaceutically acceptable carrier or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables. The carrier(s) must be acceptable in the sense of being compatible with the other ingredients of the formulation and being not deleterious to the recipient thereof. The pharmaceutical carrier employed may be, for example, a solid, a gel or a liquid. Exemplary of solid carriers are lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary of liquid carriers are phosphate buffered saline solution, syrup, oil such as peanut oil and olive oil, water, emulsions, various types of wetting agents, sterile solutions and the like. Similarly, the carrier or diluent may include time delay material well known to the art, such as glyceryl mono-stearate or glyceryl distearate alone or with a wax. Said suitable carriers comprise those mentioned above and others well known in the art, see, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania. The diluent(s) is/are selected so as not to affect the biological activity of the

combination. Examples of such diluents are distilled water, physiological saline, Ringer's solutions, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation may also include other carriers, adjuvants, or nontoxic, nontherapeutic, nonimmunogenic stabilizers and the like. A therapeutically effective dose refers to an amount of the compounds to be used in a pharmaceutical composition of the present invention which prevents, ameliorates or treats the symptoms accompanying a disease or condition referred to in this specification. Therapeutic efficacy and toxicity of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. The dosage regimen will be determined by the attending physician and other clinical factors; preferably in accordance with anyone of the above described methods. As is well known in the medical arts, dosages for anyone patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Progress can be monitored by periodic assessment. A typical dose can be, for example, in the range of 1 to 1000 µg; however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. Generally, the regimen as a regular administration of the pharmaceutical composition should be in the range of 1 l-lgto 10 mg units per day. If the regimen is continuous infusion, it should also be in the range of 1 l-lg to 10 mg units per kilogram of body weight per minute, respectively. Progress can be monitored by periodic assessment. However, depending on the subject and the mode of administration, the quantity of substance administration may vary over a wide range. The pharmaceutical compositions and formulations referred to herein are administered at least once in order to treat or ameliorate or prevent a disease or condition recited in this specification. However, the said pharmaceutical compositions may be administered more than one time, for example from one to four times daily up to a non-limited number of days. Specific pharmaceutical compositions are prepared in a manner well known in the pharmaceutical art and comprise at least one active compound referred to herein above in admixture or otherwise associated with a pharmaceutically acceptable carrier or diluent. For making those specific pharmaceutical compositions, the active compound(s) will usually be mixed with a carrier or the diluent, or enclosed or encapsulated in a capsule, sachet, cachet, paper or other suitable containers or vehicles. The resulting formulations are to be adopted to the mode of administration, i.e. in the forms of tablets, capsules, suppositories, solutions, suspensions or the like. Dosage recommendations shall be indicated in the prescribers or users instructions in order to anticipate dose adjustments depending on the considered recipient.

The term "cosmetic composition" relates to a composition which can be formulated as described for a pharmaceutical composition above. For a cosmetic composition, likewise, it

is envisaged that the compounds of the present invention are also, preferably, used in substantially pure form. Impurities, however, may be less critical than for a pharmaceutical composition. Cosmetic compositions are, preferably, to be applied topically.

Preferred cosmetic compositions comprising the compounds of the present invention can be formulated as a hair tonic, a hair restorer composition, a shampoo, a powder, a jelly, a hair rinse, an ointment, a hair lotion, a paste, a hair cream, a hair spray and/or a hair aerosol.

Seeds of two events described in detail in the examples section below have been deposited at ATCC under the provisions of the Budapest treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure, i.e. seeds of event "LBFLFK" = ATCC Designation "PTA-121703" and seeds of event "LBFDHG" = ATCC designation "PTA-121704". Applicants have no authority to waive any restrictions imposed by law on the transfer of biological material or its transportation in commerce. Applicants do not waive any infringement of their rights granted under this patent or rights applicable to the deposited events under the Plant Variety Protection Act (7 USC sec. 2321, et seq.), Unauthorized seed multiplication prohibited. This seed may be regulated according to national law. The deposition of seeds was made only for convenience of the person skilled in the art and does not constitute or imply any confession, admission, declaration or assertion that deposited seed are required to fully describe the invention, to fully enable the invention or for carrying out the invention or any part or aspect thereof. Also, the deposition of seeds does not constitute or imply any recommendation to limit the application of any method of the present invention to the application of such seed or any material comprised in such seed, e.g. nucleic acids, proteins or any fragment of such nucleic acid or protein.

The invention also provides a commercially relevant source of plant material, preferably of seed. As such, the invention provides a heap of at least 5kg, preferably of at least 10 kg, more preferably of at least 50kg, even more preferably of at least 100 kg, even more preferably of at least 500 kg, even more preferably of at least 1t, even more preferably of at least 2t, even more preferably of at least 5t of plant material, wherein the plant material comprises VLC-PUFAs as described according to the invention. As described herein, it is a merit of the present invention to provide, for the first time, an agronomically reliable source of VLC-PUFA plant oil, and to this end such heap of plant material is provided. The plant material is preferably plant seed, even more preferably seed of VLC-PUFA producing seed, such that the content of all VLC-PUFA downstream of 18:1n-9 is at least 40% (w/w) of the total seed fatty acid content at an oil content of 40% (w/w), or preferably the content of EPA is at least 10% (w/w) and/or the content of DHA is at least 1% (w/w) of the total seed fatty acid content at an oil content of 30% (w/w), or meal of such plant seed. Also, the invention provides a container comprising such plant seed in an amount of at least 5kg, preferably of at least 10 kg, more preferably of at least 50kg, even more preferably of at least 100 kg,

even more preferably of at least 500 kg, even more preferably of at least 1t, even more preferably of at least 2t, even more preferably of at least 5t. The invention thus demonstrates that the invention has well eclipsed anecdotal findings of lab scale VLC-PUFA containing plants, and instead has overcome the additional requirements for providing, on a large scale, a reliable source for VLC-PUFA and particularly for EPA and/or DHA in plant oil and plant material.

The present invention allows for the generation of plants comprising a modified fatty acid composition (as compared to control plants). Thus, the T-DNAs, the expression cassettes, vectors, polynucleotides (in particular the combination of polynucleotides), and polypeptides ((in particular the combination of polynucleotides) as disclosed herein can be used for modifying the fatty acid composition of a plant. In an embodiment, the content of at least one fatty acid disclosed in table 18 or 181 is modified in seed oil (increased or decreased as compared to the content in seed oil of a control plant). In another embodiment, the content of at least one fatty acid disclosed in table 18 or 181 is modified in in the monoacylglycerol (MAG) fraction, the diacylglycerol (DAG) fraction, the triacylglycerol (TAG) fraction, phosphatidylcholine (PC) fraction and/or phosphatidylethanolamine (PE) fraction of seed oil (increased or decreased as compared to the content of seed oil of a control plant). In another embodiment, the content of at least one Lysophosphatidylcholin species shown in Table 189 is modified in seed oil (increased or decreased as compared to the content in seed oil of a control plant). In another embodiment, the content of at least one Phosphatidylethanolamine species shown in Table 190 is modified in seed oil (increased or decreased as compared to the content in seed oil of a control plant). In another embodiment, the content of at least one Lysophosphatidylethanolamine species shown in Table 191 is modified in seed oil (increased or decreased as compared to the content in seed oil of a control plant). In another embodiment, the content of at least one Triacylglycerol species shown in Table 192 is modified in seed oil (increased or decreased as compared to the content in seed oil of a control plant).

The invention is further described by means of accompanying examples and figures, which, however, are not intended to limit the scope of the invention described herein.

EXAMPLES

Example 1: General cloning methods

Cloning methods as e.g. use of restriction endonucleases to cut double stranded DNA at specific sites, agarose gel electrophoreses, purification of DNA fragments, transfer of nucleic acids onto nitrocellulose and nylon membranes, joining of DNA-fragments, transformation of *E. coli* cells and culture of bacteria were performed as described in Sambrook et al. (1989) (Cold Spring Harbor Laboratory Press: ISBN 0-87965-309-6). Polymerase chain reaction was performed using Phusion™ High-Fidelity DNA Polymerase

(NEB, Frankfurt, Germany) according to the manufactures instructions. In general, primers used in PCR were designed such, that at least 20 nucleotides of the 3' end of the primer anneal perfectly with the template to amplify. Restriction sites were added by attaching the corresponding nucleotides of the recognition sites to the 5' end of the primer. Fusion PCR, for example described by K. Heckman and L. R. Pease, Nature Protocols (2207) 2, 924–932 was used as an alternative method to join two fragments of interest, e.g. a promoter to a gene or a gene to a terminator. Gene Synthesis, as for example described by Czar et al. (Trends in Biotechnology, 2009, 27(2): 63-72), was performed by Life Technologies using their Geneart® service. The Geneart® technology, described in WO2013049227 allows production of genetic elements of a few basepair (bp) in length, and was used in this invention to produce entire plasmids of about 60,000bp. Chemical synthesis of nucleotides to polynucleotides was employed for short DNA fragments, which were then combined in a sequential, modular fashion to fragments of increasing size using a combination of conventional cloning techniques as described in WO2013049227.

Example 2: Different types of plant transformation plasmids suitable to transfer of multiple expression cassettes encoding multiple proteins into the plant genome.

For agrobacteria based plant transformation, DNA constructs preferably meet a number of criteria: (1) The construct carries a number of genetic elements that are intended to be inserted into the plant genome on a so called Transfer DNA (T-DNA) between a 'T-DNA Left Border' (LB) and 'T-DNA Right Border' (2) The construct replicates in *E.coli*, because most cloning steps require DNA multiplication steps in *E.coli*. (3) The construct replicates in *Agrobacterium* (e.g. *A. tumefaciens* or *A. rhizogenes*), because the plant transformation methods rely on using *Agrobacterium* to insert the genetic elements of interest into the plant genome of a cell that was infected by *Agrobacterium*. (4) The construct contains supporting genetic elements that encode proteins which are required for infection of the plant cell, and for transfer and integration of desired genetic elements into the plant genome of an plant cell infected by the *Agrobacterium*, or the construct was used in combination with a second construct containing such supporting genetic elements that was present in the same Agrobacterium cell. (5) The constructs can contain selection markers to facilitate selection or identification of bacterial cells that contain the entire construct, and of a plant cell(s) that contains the desired genetic elements. An overview of available plasmids was given in Komori et al (2007).

Agrobacteria mediated transformation results in an almost random integration (with some bias induced by a number of factors) of the desired genetic element into chromosomes of the plant cell. The goal of the transformation was to integrate the entire T-DNA from T-DNA

Left border to T-DNA Right border into a random position of a random chromosome. It can also be desirable to integrate the entire T-DNA twice or three times into the genome, for example to increase the plant expression levels of genes encoded by the T-DNA. To avoid complex Mendelian segregation of multiple integrations, it was preferred to have all T-DNA insertions at one genomic location, ('locus'). Inserting more than 25,000 bp T-DNA into plant genomes has been found to be a particular challenge in the current invention. In particular, it has been found in this invention plasmids carrying a ColE1/pVS1 origin of replication for plasmid replication in *E.coli* and/or *Agrobacterium*, are not stable above ~25,000 bp. Such plasmids of the invention are described in Example 3. Because of this limitation, not more than ~4 to 5 gene expression cassettes can be transferred on one T-DNA containing plasmid into the plant genome. However, for the current invention up to 13 gene expression cassettes having a combined size of about 44,000 bp needed to be transferred into the plant genome. In contrast to plasmids containing the ColE1/pVS1 origin of replication for high copy plasmid replication in *E.coli* and/or *Agrobacterium*, BiBAC plasmids (Hammilton 1997) containing the F factor / pRi origin of replication for single copy plasmid replication in *E.coli* and/or *Agrobacterium* where found to be stable in this invention up to a size of ~60,000 bp. Such plasmids of the invention are described in Example 4. Both approaches described above were followed in the current invention.

Example 3: Assembly of genes required for EPA and DHA synthesis within T-plasmids containing the ColE1/pVS1 origin of replication

For synthesis of VLC-PUFA in *Brassica napus* seeds, the set of genes encoding the proteins of the metabolic VLC-PUFA pathway were combined with expression elements (promoter, terminators, Introns) and transferred into binary t-plasmids that were used for agrobacteria mediated transformation of plants. Attributed to the large number of expression cassettes promoting expression of one protein each, two binary t-plasmids T-DNA where used for cloning of the complete set of proteins required for EPA and DHA synthesis. To this end, the general cloning strategy depicted in Figure 3 was employed. While Figure 3 depicts the general strategy, cloning of the final plant expression vectors described in example 10 to 14 was not restricted to this strategy; specifically a combination of all methods known to one skilled in the art, such as cloning, the use of restriction endonucleases for generation of sticky and blunt ends, synthesis and fusion PCR has been used. Following the modular cloning scheme depicted in Figure 3, genes were either synthesized by GeneArt (Regensburg) or PCR-amplified using Phusion™ High-Fidelity DNA Polymerase (NEB, Frankfurt, Germany) according to the manufactures instructions from cDNA. In both cases an *Nco* I and/or *Asc* I restriction site at the 5' terminus, and a

Pac I restriction site at the 3' terminus (Figure 3A) were introduced to enable cloning of these genes between functional elements such as promoters and terminators using these restriction sites (see below in this example). Promoter-terminator modules or promoter-intron-terminator modules were created by complete synthesis by GeneArt (Regensburg) or by joining the corresponding expression elements using fusion PCR as described in example 1 and cloning the PCR-product into the TOPO-vector pCR2.1 (Invitrogen) according to the manufactures instructions (Figure 3B). While joining terminator sequences to promoter sequences or promoter-intron sequences either via synthesis of whole cassettes or using fusion PCR, recognition sequences for the restriction endonucleases depicted in Figure 3 were added to either side of the modules, and the recognition sites for the restriction endonucleases *Nco* I, *Asc* I and *Pac* I were introduced between promoter and terminator or between introns and terminator (see Figure 3B). To obtain the final expression modules, PCR-amplified genes were cloned between promoter and terminator or intron and terminator via *Nco* I and/or *Pac* I restriction sites (Figure 3C). Employing the custom multiple cloning site (MCS) up to three of those expression modules were combined as desired to expression cassettes harbored by either one of pENTR/A, pENTR/B or pENTR/C (Figure 3D). Finally, the Multi-site Gateway™ System (Invitrogen) was used to combine three expression cassette harbored by pENTR/A, pENTR/B and pENTR/C (Figure 3E) to obtain the final binary pSUN T-plasmids for plant transformation VC-LJB2197-1qcz, VC-LJB2755-2qcz rc, VC-LLM306-1qcz rc, VC-LLM337-1qcz rc, VC-LLM338-3qcz rc and VC-LLM391-2qcz rc. An overview of binary vectors and their usage was given by Hellens *et al*, Trends in Plant Science (2000) 5: 446–451.

The structure of the plamsids VC-LJB2197-1qcz, VC-LJB2755-2qcz rc, VC-LLM306-1qcz rc, VC-LLM337-1qcz rc, VC-LLM338-3qcz rc, VC-LLM391-2qcz rc, and VC-LTM217-1qcz rc was given in the Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, and Table 7

Nomeclature of genetic elements:

- j- indicates a junction between two genetic elements
- c- coding sequence
- t- terminator
- p- promotor
- i- intron

T-DNA Transferred DNA

- RB Right Border of the T-DNA
- LB Left Border of the T-DNA

Table 1 Genetic Elements of plasmid VC-LJB2197-1qcz. Listed are the names of the elements, the position in VC-LJB2197-1qcz (note: start position was larger than stop position for elements encoded by the complementary strand of VC-LJB2197-1qcz), the function and source of the element. The T-DNA integrated into the plant genome during the transformation process was flanked by a right border (nucleotides 148 to 4 of VC-LJB2197-1qcz) and a left border (nucleotides 22232 to 22105 of VC-LJB2197-1qcz). Elements outside of that region (=vector backbone) are required for cloning and stable maintenance in *E.coli* and/or agrobacteria.

Genetic Elements of plasmid VC-LJB2197-1qcz	From	To	Description, Function and Source of Element
b-RB[lm175]	148	4	Right border of T-DNA
p-VfUSP_684bp[LLL894]	329	1012	Promoter from *UNKNOWN SEED PROTEIN* gene *USP* (accession: X56240) from *Vicia faba*
i-Atss18_252[LJK36]	1013	1264	i-Atss18_252bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g01170, + 37 to + 288 bp (numbering relative to start of transcription) (+ 72 to + 282bp 5'UTR-Intron only)
c-d6Elo(Pp_GA2)	1267	2139	*Delta-6 ELONGASE* from *Physcomitrella patens*
t-CaMV35S	2140	2355	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
p-LuCnl(1064bp)	2448	3511	Promoter from *CONLININ* gene from *Linum usitatissimum*
i-Atss14_377[LJK32]	3512	3888	i-Atss14_377bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-d5Des(Tc_GA2)	3892	5211	*Delta-5 DESATURASE* from *Thraustochytrium* sp. ATCC21685
t-AgrOCS 192bp[LED12]	5212	5403	Terminator from *OCTOPINE SYNTHASE* gene OCS from *Agrobacterium tumefaciens*
p-SBP	5539	7337	Promoter from a *SUCROSE-BINDING PROTEIN-RELATED* gene from *Vicia faba*
i-Atss2_455bp[LJK20]	7338	7792	i-Atss2_455bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g65090, +77 to +531bp (numbering relative to start of transcription) (+113 to + 508bp 5'UTR-Intron only)
c-d6Des(Ot_febit)	7802	9172	*Delta-6 DESATURASE* from *Ostreococcus tauri*
t-StCATHD-pA	9200	9434	Terminator from *CATHEPSIN D INHIBITOR* gene [*CATHD*] from *Solanum tuberosum* [Potato]
p-LuPXR 1727bp[LLL823]	9513	11239	Promoter from *PEROXIREDOXIN LIKE PROTEIN* gene *PXR* from *Linum usitatissimum*
i-Atss1_847bp[LJK19]	11240	12086	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of

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Genetic Elements of plasmid VC-LJB2197-1qcz	From	To	Description, Function and Source of Element
			transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d6Elo(Tp_GA2)	12100	12918	*Delta-6 ELONGASE* from *Thalassiosira pseudonana*
t-AtPXR 400bp[LLL823]	12974	13373	Terminator from *PEROXIREDOXIN LIKE PROTEIN* gene *PXR* (At1g48130) from *Arabidopsis thaliana*
p-Napin A/B	13543	14206	Promoter from *napA/B* gene (napin, seed storage protein) from *Brassica napus*
i-Atss14_377[LJK32]	14207	14583	i-Atss14_377bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-c12Des(Ps_GA)	14590	15786	*Delta-12 DESATURASE* from *Phythophthora sojae*
t-E9	15805	16362	Terminator from Small Subunit of RuBisCo *rbcS* gene (*E9*) from *Pisum sativum*
t-AtAHAS-3'/UTR[bb003]	16576	17790	Terminator from *AtAHASL* [*csr1-2*] of *acetohydroxyacid synthase* gene from *Arabidopsis*
p-PcUbi4-2[long]	17823	18804	Promoter from *UBIQUITIN* gene *UBI4-2* with internal intron from *Petroselinum crispum*
c-AtAHASL_A122T_S653N	18812	20824	*ACETOHYDROXYACID SYNTHASE LARGE-SUBUNIT* gene/CDS with S653N(*csr1-2*) mutation and A122T SDM mutation from *Arabidopsis*
t-AtAHAS-3'/UTR[ac321]	20849	22064	Terminator from *AtAHASL* [*csr1-2*] of *ACETOHYDROXYACID SYNTHASE* gene from *Arabidopsis*
b-JTV_LB	22232	22105	Left border of T-DNA
c-StaA[lm500]	22338	22967	PVS1 partitioning protein
c-VS1orf3[lm500]	23203	22988	*VS1orf3*
c-repA[lm500]	23294	24469	pVS1 replication protein [*repA*] gene/CDS
o-pVS1-origin	24535	24729	broad host-range replication origin of plasmid pVS1 (Genbank: AF133831, Itoh et al.1984)
o-ColE1-bom[lm500]	25032	24830	pBR322 bom site, partial, from AF234316 pCambia2301
o-Rep-ColE1	25451	25171	pBR322 origin of replication [ecoli] from AF234316 pCambia2301
c-aadAmod1	26588	25797	Codon Optimized *Adenyltransferase* [*aadA*] gene/CDS from SUN100
p-aadA[lm800]	26767	26589	*Adenyltransferase* [*aadA*] Spectinomycin Prokaryotic promoter

Table 2: Genetic Elements of plasmid VC-LJB2755-2qcz rc. Listed are the names of the elements, the position in VC-LJB2755-2qcz rc (note: start position was larger than stop position for elements encoded by the complementary strand of VC-LJB2755-2qcz rc), the function and source of the element. The T-DNA integrated into the plant genome during the transformation process was flanked by a right border (nucleotides 148 to 4 of VC-LJB2755-2qcz rc) and a left border (nucleotides 26117 to 25990 of VC-LJB2755-2qcz rc). Elements outside of that region (=vector backbone) are required for cloning and stable maintenance in *E.coli* and/or agrobacteria.

Genetic Elements of plasmid VC-LJB2755-2qcz rc	From	To	Description, Function and Source of Element
b-RB[lm175]	148	4	Right border of T-DNA
p-LuPXR 1727bp[LLL823]	342	2068	Promoter from *PEROXIREDOXIN LIKE PROTEIN* gene *PXR* from *Linum usitatissimum*
i-Atss15_758bp[LJK33]	2069	2826	i-Atss15_758bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At2g27040, +93 bp to + 850 bp (numbering relative to start of transcription) (+128 to + 847 bp 5'UTR-Intron only)
c-o3Des(Pir_GA)	2842	3933	*Omega-3 DESATURASE* from *Pythium irregulare*
t-AtPXR 400bp[LLL823]	3990	4389	Terminator from *PEROXIREDOXIN LIKE PROTEIN* gene *PXR* (At1g48130) from *Arabidopsis thaliana*
p-LuCnl(1064bp)	4468	5531	Promoter from *CONLININ* gene from *Linum usitatissimum*
i-Atss14_377[LJK32]	5532	5908	i-Atss14_377bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-d5Des(Tc_GA2)	5912	7231	*Delta-5 DESATURASE* from *Thraustochytrium* sp. ATCC21685
t-AgrOCS 192bp[LED12]	7232	7423	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
p-SBP	7559	9357	Promoter from a *SUCROSE BINDING RELATED-PROTEIN* gene from *Vicia faba*
i-Atss2_455bp[LJK20]	9358	9812	i-Atss2_455bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g65090, +77 to +531bp (numbering relative to start of transcription) (+113 to + 508bp 5'UTR-Intron only)
c-d6Des(Ot_febit)	9822	11192	*Delta-6 DESATURASE* from *Ostreococcus tauri*
t-StCATHD-pA	11220	11454	Terminator from *CATHEPSIN D INHIBITOR* gene [CATHD] from *Solanum tuberosum* [Potato]
p-BnFae1	11533	12962	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica*

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Genetic Elements of plasmid VC-LJB2755-2qcz rc	From	To	Description, Function and Source of Element
			napus
i-Atss1_847bp[LJK19]	12963	13809	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d6Elo(Tp_GA2)	13812	14630	*Delta-6 ELONGASE* from *Thalassiosira pseudonana*
t-bnFae1	14646	15045	Terminator from *FATTY ACID ELONGASE (FAE 1.1)* gene from *Brassica juncea*
p-Napin A/B	15166	15829	Promoter from *napA/B* gene (napin, seed storage protein) from *Brassica napus*
i-Atss14_377[LJK32]	15830	16206	i-Atss14_377bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-d12Des(Ps_GA)	16213	17409	*Delta-12 DESATURASE* from *Phythophthora sojae*
t-E9	17428	17985	Terminator from Small Subunit of RuBisCo *rbcS* gene (*E9*) from *Pisum sativum*
p-VfUSP_684bp[LLL894]	18064	18747	Promoter from *UNKNOWN SEED PROTEIN* gene *USP* (accession: X56240) from *Vicia faba*
i-Atss18_252[LJK36]	18748	18999	i-Atss18_252bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g01170, + 37 to + 288 bp (numbering relative to start of transcription) (+ 72 to + 282bp 5'UTR-Intron only)
c-o3Des(Pl_GA2)	19010	20095	*Omega-3-DESATURASE* from *Phythophthora infestans*
t-CaMV35S	20104	20319	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
t-AtAHAS-3'UTR[ac321]	20460	21675	Terminator from *AtAHASL* [*csr1-2*] of *ACETYOHYDROXYACID SYNTHASE* gene from *Arabidopsis*
p-PcUbi4-2[long]	21708	22689	Promoter from *UBIQUITIN* gene *Ubi4-2* with internal intron from *Petroselinum crispum*
c-AtAHASL_A122T_S653N	22697	24709	*ACETOHYDROXYACID SYNTHASE LARGE-SUBUNIT* gene/CDS with S653N(*csr1-2*) mutation and A122T SDM mutation from *Arabidopsis*
t-AtAHAS-3'UTR[ac321]	24734	25949	Terminator from *AtAHASL* [*csr1-2*] of *ACETOHYDROXYACID SYNTHASE* gene from *Arabidopsis*
b-JTV_LB	26117	25990	Left border of T-DNA
c-StaA[im500]	26223	26852	PVS1 partitioning protein

Genetic Elements of plasmid VC-LJB2755-2qcz rc	From	To	Description, Function and Source of Element
c-VS1orf3[lm500]	27088	28873	*VS1orf3*
c-repA[lm500]	27179	28354	pVS1 replication protein [*repA*] gene/CDS
o-pVS1-origin	28420	28614	broad host-range replication origin of plasmid pVS1 (Genbank: AF133831, Itoh et al.1984)
o-ColE1-bom[lm500]	28917	28715	pBR322 bom site, partial, from AF234316 pCambia2301
o-Rep-ColE1	29336	29056	pBR322 origin of replication [*E. coli*] from AF234316 pCambia2301
c-aadAmod1	30473	29682	Codon Optimized *Adenyltransferase* [*aadA*] gene/CDS from SUN100
p-aadA[lm800]	30652	30474	*Adenyltransferase* [*aadA*] Spectinomycin Prokaryotic promoter

Table 3: Genetic Elements of plasmid VC-LLM306-1qcz rc. Listed are the names of the elements, the position in VC-LLM306-1qcz rc (note: start position was larger than stop position for elements encoded by the complementary strand of VC-LLM306-1qcz rc), the function and source of the element. The T-DNA integrated into the plant genome during the transformation process was flanked by a right border (nucleotides 148 to 4 of VC-LLM306-1qcz rc) and a left border (nucleotides 20180 to 20053 of VC-LLM306-1qcz rc). Elements outside of that region (=vector backbone) are required for cloning and stable maintenance in *E.coli* and/or agrobacteria.

Genetic Elements of plasmid VC-LLM306-1qcz rc	From	To	Description, Function and Source of Element
b-RB[lm175]	148	4	Right border of T-DNA
p-LuCnl(1064bp)	342	1405	Promoter from *CONLININ* gene from *Linum usitatissimum*
c-d4Des(Eg_GA)	1416	3041	*Delta 4 DESATURASE* from *Euglena gracilis*
t-AgrOCS 192bp[LED12]	3063	3254	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
p-BnFAE1	3448	4877	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica napus*
i-Atss1_847bp[LJK19]	4878	5724	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d5Elo(Ot_GA3)	5732	6634	*Delta-5 ELONGASE* from *Ostreococcus tauri*
t-bnFAE1	6651	7050	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
p-VfSBP_perm3	7099	8897	Promoter derived from a promoter from a *SUCROSE-BINDING PROTEIN-RELATED* gene from *Vicia faba*

Genetic Elements of plasmid VC-LLM306-1qcz rc	From	To	Description, Function and Source of Element
c-o3Des(Pir_GA)	8901	9992	*Omega-3 DESATURASE* from *Pythium irregulare*
t-StCATHD-pA	10023	10257	Terminator from *CATHEPSIN D INHIBITOR* gene [*CATHD*] from *Solanum tuberosum* [Potato]
p-VfUSP_684bp[LLL894]	10331	11014	Promoter from *UNKNOWN SEED PROTEIN* gene *USP* (accession: X56240) from *Vicia faba*
i-Atss15_758bp[LJK33]	11015	11772	i-Atss15_758bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At2g27040, +93 bp to + 850 bp (numbering relative to start of transcription) (+128 to + 847 bp 5'UTR-Intron only)
c-o3Des(Pi_GA2)	11789	12874	*Omega-3-DESATURASE* from *Phythophthora infestans*
t-CaMV35S	12924	13139	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
p-ARC5_perm1	13259	14409	Promoter derived from a promoter from *ARCILINE 5* gene from *Phaseolus vulgaris*
c-d4Des(Tc_GA)	14420	15979	*Delta-4 DESATURASE* from *Thraustochytrium* sp.
t-pvarc	15993	16592	Terminator of *ARC5* gene from *Phaseolus vulgaris*
p-BnFae1	16671	18100	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica napus*
c-d6Des(Ot_febit)	18109	19479	*Delta-6 DESATURASE* from *Ostreococcus tauri*
t-bnFae1	19493	19892	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
b-JTV_LB	20180	20053	Left border of T-DNA
c-StaA[lm500]	20286	20915	PVS1 partitioning protein
c-VS1orf3[lm500]	21151	20936	*VS1orf3*
c-repA[lm500]	21242	22417	pVS1 replication protein [*repA*] gene/CDS
o-pVS1-origin	22483	22677	broad host-range replication origin of plasmid pVS1 (Genbank: AF133831, Itoh et al.1984)
o-ColE1-bom[lm500]	22980	22778	pBR322 bom site, partial, from AF234316 pCambia2301
o-Rep-ColE1	23399	23119	pBR322 origin of replication [*E. coli*] from AF234316 pCambia2301
c-aadAmod1	24536	23745	Codon Optimized *Adenylitransferase* [*aadA*] gene/CDS from SUN100
p-aadA[lm800]	24715	24537	*Adenylitransferase* [*aadA*] Spectinomycin Prokaryotic promoter

Table 4: Genetic Elements of plasmid VC-LLM337-1qcz rc. Listed are the names of the elements, the position in VC-LLM337-1qcz rc (nucleotide number, note: start position was larger than stop position for elements encoded by the complementary strand of VC-LLM337-1qcz rc), the function and source of the element.

The T-DNA integrated into the plant genome during the transformation process was flanked by a right border (nucleotides 148 to 4 of VC-LLM337-1qcz rc) and a left border (nucleotides 16953 to 16826 of VC-LLM337-1qcz rc. Elements outside of that region (=vector backbone) are required for cloning and stable maintenance in *E.coli* and/or agrobacteria.

Genetic Elements of plasmid VC-LLM337-1qcz rc	From	To	Description, Function and Source of Element
b-RB[pm175]	148	4	Right border of T-DNA
p-LuCnl(1064bp)	342	1405	Promoter from *CONLININ* gene from *Linum usitatissimum*
c-d4Des(Eg_GA)	1416	3041	*Delta-4 DESATURASE* from *Euglena gracilis*
t-AgrOCS 192bp[LED12]	3063	3254	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
p-BnFae1	3448	4877	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica napus*
i-Atss1_847bp[LJK19]	4878	5724	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-intron only); from QC1153-1/RTP6393.
c-d5Elo(Ot_GA3)	5732	6634	*Delta-5 ELONGASE* from *Ostreococcus tauri*
t-bnFae1	6651	7050	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
p-VfSBP_perm3	7099	8897	Promoter derived from a promoter from a *SUCROSE-BINDING PROTEIN-RELATED* gene from *Vicia faba*
c-c3Des(Pir_GA)	8901	9992	*Omega-3 DESATURASE* from *Pythium irregulare*
t-StCATHD-pA	10023	10257	Terminator from *CATHEPSIN D INHIBITOR* gene [*CATHD*] from *Solanum tuberosum* [Potato]
p-VfUSP_684bp[LLL894]	10331	11014	Promoter from *UNKNOWN SEED PROTEIN* gene *USP* (accession: X56240) from *Vicia faba*
i-Atss15_758bp[LJK33]	11015	11772	i-Atss15_758bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At2g27040, +93 bp to + 850 bp (numbering relative to start of transcription) (+128 to + 847 bp 5'UTR-Intron only)
c-c3Des(Pi_GA2)	11789	12874	*Omega-3-DESATURASE* from *Phythophthora infestans*
t-CaMV35S	12924	13139	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
p-ARC5_perm1	13259	14409	Promoter derived from a promoter from *ARCILINE 5* gene from *Phaseolus vulgaris*

Genetic Elements of plasmid VC-LLM337-1qcz rc	From	To	Description, Function and Source of Element
c-d4Des(Tc_GA)	14420	15979	*Delta-4 DESATURASE* from *Thraustochytrium* sp.
t-pvarc	15993	16592	Terminator of *ARC5* gene from *Phaseolus vulgaris*
b-JTV_LB	16953	16826	Left border of T-DNA
c-StaA[lm500]	17059	17688	PVS1 partitioning protein
c-VS1orf3[lm500]	17924	17709	*VS1*orf3
c-repA[lm500]	18015	19190	pVS1 replication protein [repA] gene/CDS
o-pVS1-origin	19256	19450	broad host-range replication origin of plasmid pVS1 (Genbank: AF133831, Itoh et al.1984)
o-ColE1-bom[lm500]	19753	19551	pBR322 bom site, partial, from AF234316 pCambia2301
o-Rep-ColE1	20172	19892	pBR322 origin of replication [*E. coli*] from AF234316 pCambia2301
c-aadAmod1	21309	20518	Codon Optimized *Adenyltransferase* [*aadA*] gene/CDS from SUN100
p-aadA[lm800]	21488	21310	*Adenyltransferase* [*aadA*] Spectinomycin Prokaryotic promoter

Table 5: Genetic Elements of plasmid VC-LLM338-3qcz rc. Listed are the name of the element, the position in VC-LLM338-3qcz rc (nucleotide number, note: start position was larger than stop position for elements encoded by the complementary strand VC-LLM338-3qcz rc), the function and source of the element. The T-DNA integrated into the plant genome during the transformation process was flanked by a right border (nucleotides 148 to 4 of VC-LLM338-3qcz rc) and a left border (nucleotides 17069 to 16942 of VC-LLM338-3qcz rc). Elements outside of that region (=vector backbone) are required for cloning and stable maintenance in *E.coli* and/or agrobacteria.

Genetic Elements of plasmid VC-LLM338-3qcz rc	From	To	Description, Function and Source of Element
b-RB[lm175]	148	4	Right border of T-DNA
p-VfSBP_perm3	341	2139	Promoter derived from a promoter from a *SUCROSE-BINDING RELATED-PROTEIN* gene from *Vicia faba*
c-d15Des(Ch_ERTp_GA)	2151	3389	*Delta-15 DESATURASE* from *Cochliobolus heterostrophus* C5
t-StCATHD-pA	3420	3654	Terminator from *CATHEPSIN D INHIBITOR* gene [*CATHD*] from *Solanum tuberosum* [Potato]
p-BnFae1	3848	5277	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica napus*

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Genetic Elements of plasmid VC-LLM338-3qcz rc	From	To	Description, Function and Source of Element
i-Atss1_847bp[LJK19]	5278	6124	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-intron only); from QC1153-1/RTP6393.
c-d5Elo(Ot_GA3)	6132	7034	*Delta-5 ELONGASE* from *Ostreococcus tauri*
t-bnFae1	7051	7450	Terminator from *FATTY ACID ELONGASE (FAE 1.1)* gene from *Brassica juncea*
p-VfUSP_684bp[LLL894]	7529	8212	Promoter from *UNKNOWN SEED PROTEIN* gene *USP* (accession: X56240) from *Vicia faba*
i-Atss15_758bp[LJK33]	8213	8970	i-Atss15_758bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At2g27040, +93 bp to + 850 bp (numbering relative to start of transcription) (+128 to + 847 bp 5'UTR-intron only)
c-c3Des(Pi_GA2)	8987	10072	*Omega-3-DESATURASE* from *Phythophthora infestans*
t-CaMV35S	10122	10337	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
p-ARC5_perm1	10457	11607	Promoter derived from a promoter from *ARCILINE 5* gene from *Phaseolus vulgaris*
c-d4Des(Tc_GA)	11618	13177	*Delta-4 DESATURASE* from *Thraustochytrium* sp.
t-pvarc	13191	13790	Terminator of *ARC5* gene from *Phaseolus vulgaris*
p-LuCnl(1064bp)	13869	14932	Promoter from *CONLININ* gene from *Linum usitatissimum*
c-d4Des(Eg_GA)	14943	16568	*Delta-4 DESATURASE* from *Euglena gracilis*
t-AgrOCS 192bp[LED12]	16590	16781	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
b-JTV_LB	17069	16942	Left border of T-DNA
c-StaA[lm500]	17175	17804	PVS1 partitioning protein
c-VS1orf3[lm500]	18040	17825	*VS1orf3*
c-repA[lm500]	18131	19306	pVS1 replication protein [*repA*] gene/CDS
o-pVS1-origin	19372	19566	broad host-range replication origin of plasmid pVS1 (Genbank: AF133831, Itoh et al.1984)
o-ColE1-bom[lm500]	19869	19667	pBR322 bom site, partial, from AF234316 pCambia2301
o-Rep-ColE1	20288	20008	pBR322 origin of replication [*E. Coli*] from AF234316 pCambia2301
c-aadAmod1	21425	20634	Codon Optimized *Adenyltransferase* [*aadA*] gene/CDS from SUN100

Genetic Elements of plasmid VC-LLM338-3qcz rc	From	To	Description, Function and Source of Element
p-aadA[lm800]	21604	21426	*Adenyltransferase* [*aadA*] Spectinomycin Prokaryotic promoter

Table 6: Genetic Elements of plasmid VC-LLM391-2qcz rc rc. Listed are the names of the elements, the position in VC-LLM391-2qcz rc rc (nucleotide number, note: start position was larger than stop position for elements encoded by the complementary strand of VC-LLM391-2qcz rc rc), the function and source of the element. The T-DNA integrated into the plant genome during the transformation process was flanked by a right border (nucleotides 148 to 4 of VC-LLM391-2qcz rc rc) and a left border (nucleotides 10947 to 10820 of VC-LLM391-2qcz rc rc). Elements outside of that region (=vector backbone) are required for cloning and stable maintenance in *E.coli* and/or agrobacteria.

Genetic Elements of plasmid VC-LLM391-2qcz rc	From	To	Description, Function and Source of Element
b-RB[lm175]	148	4	Right border of T-DNA
p-BnFae1	540	1969	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica napus*
i-Atss1_847bp[LJK19]	1970	2816	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d5Elo(Ot_GA3)	2824	3726	*Delta-5 ELONGASE* from *Ostreococcus tauri*
t-bnFae1	3743	4142	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
p-ARC5perm1	4335	5485	Promoter derived from a promoter from *ARCILINE 5* gene from *Phaseolus vulgaris*
c-d4Des(Tc_GA)_T564G	5496	7055	*Delta-4 DESATURASE* from *Thraustochytrium* spp.
t-pvarc	7069	7668	Terminator of *Arc5* gene from *Phaseolus vulgaris*
p-LuCnl(1064bp)	7747	8810	Promoter from *CONLININ* gene from *Linum usitatissimum*
c-d4Des(Eg_GA)	8821	10446	*Delta-4 DESATURASE* from *Euglena gracilis*
t-AgrOCS 192bp[LED12]	10468	10659	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
b-JTV_LB	10947	10820	Left border of T-DNA
c-StaA[lm500]	11053	11682	PVS1 partitioning protein

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Genetic Elements of plasmid VC-LLM391-2qcz rc	From	To	Description, Function and Source of Element
c-VS1orf3[lm500]	11918	11703	*VS1*orf3
c-repA[lm500]	12009	13184	pVS1 replication protein [*repA*] gene/CDS
o-pVS1-origin	13250	13444	broad host-range replication origin of plasmid pVS1 (Genbank: AF133831, Itoh et al.1984)
o-ColE1-bom[lm500]	13747	13545	pBR322 bom site, partial, from AF234316 pCambia2301
o-Rep-ColE1	14166	13886	pBR322 origin of replication [*E. coli*] from AF234316 pCambia2301
c-aadAmod1	15303	14512	Codon Optimized *Adenyltransferase* [*aadA*] gene/CDS from SUN100
p-aadA[lm800]	15482	15304	*Adenyltransferase* [*aadA*] Spectinomycin Prokaryotic promoter

Table 7: Genetic Elements of plasmid VC-LLTM217-1qcz rc. Listed are the names of the elements, the position in VC-LLTM217-1qcz rc (nucleotide number, note: start position was larger than stop position for elements encoded by the complementary strand of VC-LLTM217-1qcz rc), the function and source of the element. The T-DNA integrated into the plant genome during the transformation process was flanked by a right border (nucleotides 148 to 4 of VC-LLTM217-1qcz rc) and a left border (nucleotides 10659 to 10532 of VC-LLTM217-1qcz rc). Elements outside of that region (=vector backbone) are required for cloning and stable maintenance in E.coli and/or agrobacteria.

Genetic Elements of plasmid VC-LTM217-1qcz rc	From	To	Description, Function and Source of Element
b-RB[lm175]	148	4	Right border of T-DNA
p-BnFae1	540	1969	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica napus*
i-Atss1_847bp[LJK19]	1970	2816	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d5Elo(Ot_GA3)	2824	3726	*Delta-5 ELONGASE* from *Ostreococcus tauri*
t-bnFae1	3743	4142	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
p-ARC5_perm1	4335	5485	Promoter derived from a promoter from *ARCILINE 5* gene from *Phaseolus vulgaris*

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Genetic Elements of plasmid VC-LTM217-1qcz rc	From	To	Description, Function and Source of Element
c-d4Des(Tc_GA)	5496	7055	*Delta-4 DESATURASE* from *Thraustochytrium* spp.
t-pvarc	7069	7668	Terminator of *Arc5* gene from *Phaseolus vulgaris*
p-LuCni(1064bp)	7747	8810	Promoter from *CONLININ* gene from *Linum usitatissimum*
c-d4Des(Pl_GA)2	8821	10158	*Delta-4 DESATURASE* from *Pavlova lutheri*
t-AgrOCS 192bp[LED12]	10180	10371	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
b-JTV_LB	10659	10532	Left border of T-DNA
c-StaA[lm500]	10765	11394	PVS1 partitioning protein
c-VS1orf3[lm500]	11630	11415	*VS1*orf3
c-repA[lm500]	11721	12896	pVS1 replication protein [*repA*] gene/CDS
o-pVS1-origin	12962	13156	broad host-range replication origin of plasmid pVS1 (Genbank: AF133831, Itoh et al.1984)
o-ColE1-bom[lm500]	13459	13257	pBR322 bom site, partial, from AF234316 pCambia2301
o-Rep-ColE1	13878	13598	pBR322 origin of replication [*E. coli*] from AF234316 pCambia2301
c-aadAmod1	15015	14224	Codon Optimized *Adenyltransferase* [*aadA*] gene/CDS from SUN100
p-aadA[lm800]	15194	15016	*Adenyltransferase* [*aadA*] Spectinomycin Prokaryotic promoter

Example 4: Assembly of genes required for EPA and DHA synthesis within BiBAC T-plasmids containing the F factor / pRI origin of replication

For synthesis of VLC-PUFA in *Brassica napus* seeds, the set of genes encoding the proteins of the metabolic VLC-PUFA pathway were combined with expression elements (promoter, terminators and introns) and transferred into binary t-plasmids that were used for agrobacteria mediated transformation of plants. While the large number of expression cassettes promoting expression of one protein each, were distributed in example 3 onto two binary t-plasmids T-DNA, in this example all expression cassettes have been combined onto a single binary T-plasmid. The advance of DNA synthesis allows numerous companies to offer services to use a combination of chemical synthesis and molecular biological techniques to synthesize de novo, without an initial template, polynucleotides up to the size of microbial genomes. Synthesis used in the construction of the plasmids described in this example was performed by Life Technologies using their Geneart® service. The Geneart® technology, described in WO2013049227 allows production of genetic elements of a few basepair (bp) length, and was used in this invention to produce the binary T-plasmids for plant transformation VC-RTP10690-1qcz_F, VC-RTP10691-2qcz, VC-LTM595-1qcz rc and VC-LTM593-1qcz rc having a total size of ~61.000bp for each construct. The structure of the plasmids VC-RTP10690-1qcz_F, VC-RTP10691-2qcz, VC-LTM595-1qcz rc and VC-LTM593-1qcz rc is given in: Table 8, Table 9, Table 10 and Table 11.

Table 8: Genetic Elements of plasmid RTP10690-1qcz_F. Listed are the names of the elements, the position in RTP10690-1qcz_F (nucleotide number, note: start position was larger than stop position for elements encoded by the complementary strand of SEQ ID NO.6), the function and source of the element. The T-DNA integrated into the plant genome during the transformation process was flanked by a right border (nucleotides 59918 to 148 of RTP10690-1qcz_F) and a left border (nucleotides 43853 to 43718 of RTP10690-1qcz_F). Elements outside of that region (=vector backbone) are required for cloning and stable maintenance in *E.coli* and/or agrobacteria.

Genetic Elements of plasmid RTP10690-1qcz_F	From	To	Description, Function and Source of Element
p-VfUSP_684bp[LLL894]	329	1012	Promoter from *UNKNOWN SEED PROTEIN* gene *USP* (accession: X56240) from *Vicia faba*
i-Atss18_252[LJK36]	1013	1264	i-Atss18_252bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g01170, + 37 to + 288 bp (numbering relative to start of transcription) (+ 72 to + 282bp 5'UTR-Intron only)
c-d6Elo(Pp_GA2)	1267	2139	*Delta-6 ELONGASE* from *Physcomitrella patens*
t-CaMV35S	2140	2355	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus

Genetic Elements of plasmid RTP10690-1qcz_F	From	To	Description, Function and Source of Element
p-LuCnl(1064bp)	2448	3511	Promoter from *CONLININ* gene from *Linum usitatissimum*
i-Atss14_377bp[LJK32]	3512	3888	i-Atss14_377bp[LJK32] functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-d5Des(Tc_GA2)	3892	5211	*Delta-5 DESATURASE* from *Thraustochytrium* sp. ATCC21685
t-AgrOCS 192bp[LED12]	5212	5403	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
p-SBP	5539	7337	Promoter from a *SUCROSE-BINDING PROTEIN-RELATED* gene from *Vicia faba*
i-Atss2_455bp[LJK20]	7338	7792	i-Atss2_455bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g65090, +77 to +531bp (numbering relative to start of transcription) (+113 to + 508bp 5'UTR-Intron only)
c-d6Des(Ot_febit)	7802	9172	*Delta-6 DESATURASE* from *Ostreococcus tauri*
t-StCATHD-pA	9200	9434	Terminator from *CATHEPSIN D INHIBITOR* gene [CATHD] from *Solanum tuberosum* [Potato]
p-LuPXR 1727bp[LLL823]	9513	11239	Promoter from *PEROXIREDOXIN LIKE* protein gene *PXR* from *Linum usitatissimum*
i-Atss1_847bp[LJK19]	11240	12086	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d6Elo(Tp_GA2)	12100	12918	*Delta-6 ELONGASE* from *Thalassiosira pseudonana*
t-AtPXR 400bp[LLL823]	12974	13373	Terminator from *PEROXIREDOXIN LIKE* protein gene *PXR* (At1g48130) from *Arabidopsis thaliana*
p-Napin A/B	13543	14206	Promoter from *napA/B* gene (napin, seed storage protein) from *Brassica napus*
i-Atss14_377bp[LJK32]	14207	14583	i-Atss14_377bp[LJK32] functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-d12Des(Ps_GA2)	14590	15786	*Delta-12 DESATURASE* from *Phythophthora sojae*

Genetic Elements of plasmid RTP10690-1qcz_F	From	To	Description, Function and Source of Element
t-E9	15805	16362	Terminator from Small Subunit of RuBisCo *rbcS* gene (*E9*) from *Pisum sativum*
p-LuPXR 1727bp[LLL823]	16462	18188	Promoter from *PEROXIREDOXIN LIKE* protein gene *PXR* from *Linum usitatissimum*
i-Atss15_758bp[LJK33]	18189	18946	i-Atss15_758bp[LJK33] functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At2g27040, +93 bp to + 850 bp (numbering relative to start of transcription) (+128 to + 847 bp 5'UTR-Intron only)
c-o3Des(Pir_GA)	18962	20053	*Omega-3 DESATURASE* from *Pythium irregulare*
t-AtPXR 400bp[LLL823]	20110	20509	Terminator from *PEROXIREDOXIN LIKE* protein gene *PXR* (At1g48130) from *Arabidopsis thaliana*
p-LuCnl(1064bp)	20645	21708	Promoter from *CONLININ* gene from *Linum usitatissimum*
i-Atss2_455bp[LJK20]	21709	22163	i-Atss2_455bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g65090, +77 to +531bp (numbering relative to start of transcription) (+113 to + 508bp 5'UTR-Intron only)
c-d4Des(Pl_GA)2	22181	23518	*Delta-4 DESATURASE* from *Pavlova lutheri*
t-AgrOCS 192bp[LED12]	23540	23731	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
p-BnFae1	23925	25354	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica napus*
i-Atss1_847bp[LJK19]	25355	26202	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
T	25513	25513	T inserted [14-T string found verses 13-T string in original i-Atss1_847bp[LJK19]
c-d5Elo(Ot_GA3)	26210	27112	*Delta-5 ELONGASE* from *Ostreococcus tauri*
t-bnFae1	27129	27528	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
p-ARC5_perm1	27681	28831	Promoter derived from a promoter from *ARCILINE 5* gene from *Phaseolus vulgaris*
c-d4Des(Tc_GA3)	28842	30401	*Delta-4 DESATURASE* from *Thraustochytrium* sp.
t-pvarc	30415	31014	Terminator of *ARC5* gene from *Phaseolus vulgaris*

Genetic Elements of plasmid RTP10690-1qcz_F	From	To	Description, Function and Source of Element
p-VfUSP_684bp[LLL894]	31093	31776	Promoter from unknown seed protein gene USP (accession: X56240) from *Vicia faba*
i-Atss18_252[LJK36]	31777	32028	i-Atss18_252bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g01170, + 37 to + 288 bp (numbering relative to start of transcription) (+ 72 to + 282bp 5'UTR-Intron only)
c-o3Des(Pi_GA2)	32039	33124	*Omega-3-DESATURASE* from *Phythophthora infestans*
t-CaMV35S	33133	33348	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
p-BnSETL-v1[1234bp]	33484	34717	*SETL*-v1 *Brassica napus* promoter
c-d5Des(Tc_GA2)	34720	36039	*Delta-5 DESATURASE* from *Thraustochytrium* sp. ATCC21685
t-BnSETL	36061	36674	*SETL*-v1 *Brassica napus* terminator
p-BnSETL-v1[1234bp]	36767	38000	*SETL*-v1 *Brassica napus* promoter
c-o3Des(Pir_GA)	38003	39094	*Omega-3 DESATURASE* from *Pythium irregulare*
t-BnSETL	39116	39729	*SETL*-v1 *Brassica napus* terminator
p-YPC105906 PcUbi4-2[long]	39853	40829	MTX Parsley *UBI4-2* promoter with internal intron
c-AtAHASL_A122T_S653N[minusRES]	40837	42849	*ACETOHYDROXYACID SYNTHASE LARGE SUBUNIT* gene/CDS from *Arabidopsis* with S653N (*csr1-2*) mutation and A122T SDM mutation minus restriction sites
t-AtAHAS-3'UTR[rtp4820]	42850	43629	*Arabidopsis* (dicot) *AtAHASL* 3' Un-translated Region [trimmed] terminator for *ACETOHYDROXYACID SYNTHASE* gene
b-LLB	43853	43718	Left T-DNA Left border from pTi15955 [Genbank #AF242881]
c-KanR_Tn903	45800	44985	Kanamycin Resistance selection gene/CDS
p-Kan[lm500]	45921	45801	Promoter for Kanamycin resistance gene
o-ori-2	47074	47290	ori-2 origin of replication
c-repE	47384	48139	*repE* gene/CDS
c-sopA	48718	49893	*sapA* gene/CDS
c-sopB	49893	50864	*sopB* gene/CDS
c-sopC/incD	50937	51410	*incD*/*sopC* partial gene/CDS
c-traI	51913	51972	*traI* gene/CDS
mf-traI - repA intergenic region	51961	52323	regulatory region of traR dependent quorum sensing regulon - containing 2 tra-boxes (see LI AND FARRAND JOURNAL OF BACTERIOLOGY, Jan. 2000, p. 179-188)
o-repA	52324	53541	*Rep-A* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY,

Genetic Elements of plasmid RTP10690-1qcz_F	From	To	Description, Function and Source of Element
			Jan. 2000, p. 179..188)
rr-repB	53771	54781	*rep-B* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY, Jan. 2000, p. 179..188)
o-repC	54996	56315	*rep-C* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY, Jan. 2000, p. 179..188)
mf-y4cG	56794	56324	fragment of DNA invertase homolog; similar to Rhizobium sp. NGR234 pNGR234a Y4CG
tr-Tn5	58834	57273	Transposon Tn5 sequence
o-oriT	59130	59298	oriT from pRK310 genbank file
b-RB[rtp4394]	148	59918	Right T-DNA Right border

Table 9: Genetic Elements of plasmid RTP10691-2qcz. Listed are the names of the elements, the position in RTP10691-2qcz (nucleotide number, note: start position was larger than stop position for elements encoded by the complementary strand of RTP10691-2qcz), the function and source of the element. The T-DNA integrated into the plant genome during the transformation process was flanked by a right border (nucleotides 60923 to 148 of RTP10691-2qcz and a left border (nucleotides 44858 to 44723 of RTP10691-2qcz). Elements outside of that region (=vector backbone) are required for cloning and stable maintenance in *E.coli* and/or agrobacteria.

Genetic Elements of plasmid RTP10691-2qcz	From	To	Description, Function and Source of Element
p-VfUSP_684bp[LLL894]	329	1012	Promoter from *UNKNOWN SEED PROTEIN* gene *USP* (accession: X56240) from *Vicia faba*
i-Atss18_252[LJK36]	1013	1264	i-Atss18_252bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g01170, + 37 to + 288 bp (numbering relative to start of transcription) (+ 72 to + 282bp 5'UTR-Intron only)
c-d6Elo(Pp_GA2)	1267	2139	*Delta-6 ELONGASE* from *Physcomitrella patens*
t-CaMV35S	2140	2355	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
p-LuCnl(1064bp)	2448	3511	Promoter from *CONLININ* gene from *Linum usitatissimum*
i-Atss14_377bp[LJK32]	3512	3888	i-Atss14_377bp[LJK32] functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-d5Des(Tc_GA2)	3892	5211	*Delta-5 DESATURASE* from

Genetic Elements of plasmid RTP10691-2qcz	From	To	Description, Function and Source of Element
			Thraustochytrium sp. ATCC21685
t-AgrOCS 192bp[LED12]	5212	5403	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
p-SBP	5539	7337	Promoter from a *SUCROSE-BINDING RELATED-PROTEIN* gene from *Vicia faba*
i-Atss2_455bp[LJK20]	7338	7792	i-Atss2_455bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g65090, +77 to +531bp (numbering relative to start of transcription) (+113 to + 508bp 5'UTR-Intron only)
c-d6Des(Ot_febit)	7802	9172	*Delta-6 DESATURASE* from *Ostreococcus tauri*
t-StCATHD-pA	9200	9434	Terminator from *CATHEPSIS D INHIBITOR* gene [*CATHD*] from *Solanum tuberosum* [Potato]
p-LuPXR 1727bp[LLL823]	9513	11239	Promoter from *PEROXIREDOXIN LIKE* protein gene *PXR* from *Linum usitatissimum*
i-Atss1_847bp[LJK19]	11240	12086	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d6Elo(Tp_GA2)	12100	12918	*Delta-6 ELONGASE* from *Thalassiosira pseudonana*
t-AtPXR 400bp[LLL823]	12974	13373	Terminator from *PEROXIREDOXIN LIKE* protein gene *PXR* (At1g48130) from *Arabidopsis thaliana*
p-Napin A/B	13543	14206	Promoter from *napA/B* gene (napin, seed storage protein) from *Brassica napus*
i-Atss14_377bp[LJK32]	14207	14583	i-Atss14_377bp[LJK32] functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-d12Des(Ps_GA2)	14590	15786	*Delta-12 DESATURASE* from *Phythophthora sojae*
t-E9	15805	16362	Terminator from Small Subunit of RuBisCo *rbcS* gene (*E9*) from *Pisum sativum*
p-LuPXR 1727bp[LLL823]	16462	18188	Promoter from *PEROXIREDOXIN LIKE* protein gene *PXR* from *Linum usitatissimum*
i-Atss15_758bp[LJK33]	18189	18946	i-Atss15_758bp[LJK33] functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At2g27040, +93 bp to + 850 bp (numbering relative to start of

Genetic Elements of plasmid RTP10691-2qcz	From	To	Description, Function and Source of Element
			transcription) (+128 to + 847 bp 5'UTR-Intron only)
c-o3Des(Pir_GA)	18962	20053	*Omega-3 DESATURASE* from *Pythium irregulare*
t-AtPXR 400bp[LLL823]	20110	20509	Terminator from *PEROXIREDOXIN LIKE* protein gene PXR (At1g48130) from *Arabidopsis thaliana*
p-BnSETL-v1[1234bp]	20645	21878	*SETL*-v1 *Brassica napus* promoter
c-d5Des(Tc_GA2)	21881	23200	*Delta-5 DESATURASE* from *Thraustochytrium* sp. ATCC21685
t-BnSETL	23222	23835	*SETL*-v1 *Brassica napus* terminator
p-BnFae1	24029	25458	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica napus*
i-Atss1_847bp[LJK19]	25459	26302	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
delTTT	25612	25612	Three T deleted compared to original i-Atss1_847bp[LJK19]
c-d5Elo(Ot_GA3)	26310	27212	*Delta-5 ELONGASE* from *Ostreococcus tauri*
t-bnFae1	27229	27628	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
p-ARC5_perm1	27781	28931	Promoter derived from a promoter from *ARCILINE 5* gene from *Phaseolus vulgaris*
c-d4Des(Tc_GA3)	28942	30501	*Delta-4 DESATURASE* from *Thraustochytrium* sp.
t-pvarc	30515	31114	Terminator of *ARC5* gene from *Phaseolus vulgaris*
p-VfUSP_684bp[LLL894]	31193	31876	Promoter from *UNKNOWN SEED PROTEIN* gene *USP* (accession: X56240) from *Vicia faba*
i-Atss18_252[LJK36]	31877	32128	i-Atss18_252bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g01170, + 37 to + 288 bp (numbering relative to start of transcription) (+ 72 to + 282bp 5'UTR-Intron only)
c-o3Des(Pi_GA2)	32139	33224	*Omega-3-DESATURASE* from *Phythophthora infestans*
t-CaMV35S	33233	33448	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
p-BnFae1	33642	35071	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica*

Genetic Elements of plasmid RTP10691-2qcz	From	To	Description, Function and Source of Element
			napus
i-Atss1_847bp[LJK19]	35072	35918	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d4Des(Pl_GA)2	35926	37263	*Delta-4 DESATURASE* from *Pavlova lutheri*
t-bnFae1	37280	37679	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
p-BnSETL-v1[1234bp]	37772	39005	*SETL*-v1 *Brassica napus* promoter
c-o3Des(Pir_GA)	39008	40099	*Omega-3 DESATURASE* from *Pythium irregulare*
t-BnSETL	40121	40734	*SETL*-v1 *Brassica napus* terminator
p-YPC105906_PcUbi4-2[long]	40858	41834	MTX Parsley *UBI4-2* promoter with internal intron
c-AtAHASL_A122T_S653N[minusRES]	41842	43854	*ACETOHYDROXYACID SYNTHASE LARGE SUBUNIT* gene/CDS from *Arabidopsis* with S653N (*csr1-2*) mutation and A122T SDM mutation minus restriction sites
t-AtAHAS-3'UTR[rtp4820]	43855	44634	Arabidopsis (dicot) *AtAHASL* 3' Un-translated Region [trimmed] terminator for *ACETOHYDROXYACID SYNTHASE* gene
b-LLB	44858	44723	Left T-DNA Left border from pTi15955 [Genbank #AF242881]
c-KanR_Tn903	46805	45990	Kanamycin Resistance selection gene/CDS
p-Kan[lm500]	46926	46806	Promoter for Kanamycin resistance gene
o-ori-2	48079	48295	ori-2 origin of replication
c-repE	48389	49144	*repE* gene/CDS
c-sopA	49723	50898	*sapA* gene/CDS
c-sopB	50898	51869	*sopB* gene/CDS
c-sopC/incD	51942	52415	*incD*/*sopC* partial gene/CDS
c-traI	52918	52977	*traI* gene/CDS
mf-traI - repA intergenic region	52966	53328	regulatory region of traR dependent quorum sensing regulon - containing 2 tra-boxes (see LI AND FARRAND JOURNAL OF BACTERIOLOGY, Jan. 2000, p. 179-188)
o-repA	53329	54546	*Rep-A* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY, Jan. 2000, p. 179..188)
rr-repB	54776	55786	*rep-B* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY, Jan. 2000, p. 179..188)
o-repC	56001	57320	*rep-C* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY,

Genetic Elements of plasmid RTP10691-2qcz	From	To	Description, Function and Source of Element
			Jan. 2000, p. 179..188)
mf-y4cG	57799	57329	fragment of DNA invertase homolog; similar to *Rhizobium* sp. NGR234 pNGR234a Y4CG
tr-Tn5	59839	58278	Transposon Tn5 sequence
o-oriT	60135	60303	oriT from pRK310 genbank file
b-RB[rtp4394]	60923	148	Right T-DNA Right border

Table 10: Genetic Elements of plasmid VC-LTM595-1qcz rc. Listed are the names of the elements, the position in VC-LTM595-1qcz rc (nucleotide number, note: start position was larger than stop position for elements encoded by the complementary strand of VC-LTM595-1qcz rc), the function and source of the element. The T-DNA integrated into the plant genome during the transformation process was flanked by a right border (nucleotides 60913 to 148 of VC-LTM595-1qcz rc) and a left border (nucleotides 44848 to 44713 of VC-LTM595-1qcz rc). Elements outside of that region (=vector backbone) are required for cloning and stable maintenance in *E. coli* and/or agrobacteria.

Genetic Elements of plasmid VC-LTM595-1qcz rc	From	To	Description, Function and Source of Element
p-VfUSP_684bp[LLL894]	329	1012	Promoter from *UNKNOWN SEED PROTEIN* gene *USP* (accession: X56240) from *Vicia faba*
i-Atss18_252[LJK36]	1013	1264	i-Atss18_252bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g01170, + 37 to + 288 bp (numbering relative to start of transcription) (+ 72 to + 282bp 5'UTR-Intron only)
c-d6Elo(Pp_GA2)	1267	2139	*Delta-6 ELONGASE* from *Physcomitrella patens*
t-CaMV35S	2140	2355	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
p-LuCnl(1064bp)	2448	3511	Promoter from *CONLININ* gene from *Linum usitatissimum*
i-Atss14_377bp[LJK32]	3512	3888	i-Atss14_377bp[LJK32] functional intron region; intron with partial 5' UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-d5Des(Tc_GA2)	3892	5211	*Delta-5 DESATURASE* from *Thraustochytrium* sp. ATCC21685
t-AgrOCS 192bp[LED12]	5212	5403	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
p-SBP	5539	7337	Promoter from a *SUCROSE-BINDING RELATED-PROTEIN* gene from *Vicia faba*
i-Atss2_455bp[LJK20]	7338	7792	i-Atss2_455bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g65090, +77 to +531bp (numbering relative to start of transcription) (+113 to + 508bp 5'UTR-Intron only)
c-d6Des(Ot_febit)	7802	9172	*Delta-6 DESATURASE* from *Ostreococcus tauri*
t-StCATHD-pA	9200	9434	Terminator from *CATHEPSIS D INHIBITOR* gene [*CATHD*] from *Solanum tuberosum* [Potato]
p-LuPXR 1727bp[LLL823]	9513	11239	Promoter from *PEROXIREDOXIN LIKE*

Genetic Elements of plasmid VC-LTM595-1qcz rc	From	To	Description, Function and Source of Element
			protein gene *PXR* from *Linum usitatissimum*
i-Atss1_847bp[LJK19]	11240	12086	i-Atss1_847bp functional intron region; intron with partial 5' UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d6Elo(Tp_GA2)	12100	12918	*Delta-6 ELONGASE* from *Thalassiosira pseudonana*
t-AtPXR 400bp[LLL823]	12974	13373	Terminator from *PEROXIREDOXIN LIKE* protein gene *PXR* (At1g48130) from *Arabidopsis thaliana*
p-Napin A/B	13543	14206	Promoter from *napA/B* gene (napin, seed storage protein) from *Brassica napus*
i-Atss14_377bp[LJK32]	14207	14583	i-Atss14_377bp[LJK32] functional intron region; intron with partial 5' UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-d12Des(Ps_GA2)	14590	15786	*Delta-12 DESATURASE* from *Phythophthora sojae*
t-E9	15805	16362	Terminator from Small Subunit of RuBisCo *rbcS* gene (*E9*) from *Pisum sativum*
p-LuPXR 1727bp[LLL823]	16455	18181	Promoter from *PEROXIREDOXIN LIKE* protein gene *PXR* from *Linum usitatissimum*
i-Atss15_758bp[LJK33]	18182	18939	i-Atss15_758bp[LJK33] functional intron region; intron with partial 5' UTR, *Arabidopsis thaliana*, Locus At2g27040, +93 bp to + 850 bp (numbering relative to start of transcription) (+128 to + 847 bp 5'UTR-Intron only)
c-o3Des(Pir_GA)	18955	20046	*Omega-3 DESATURASE* from *Pythium irregulare*
t-AtPXR 400bp[LLL823]	20103	20502	Terminator from *PEROXIREDOXIN LIKE* protein gene PXR (At1g48130) from *Arabidopsis thaliana*
p-BnSETL-v1[1234bp]	20638	21871	*SETL*-v1 *Brassica napus* promoter
c-d5Des(Tc_GA2)	21874	23193	*Delta-5 DESATURASE* from *Thraustochytrium* sp. ATCC21685
t-BnSETL	23215	23828	*SETL*-v1 *Brassica napus* terminator
p-BnFae1	24022	25451	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica napus*
i-Atss1_847bp[LJK19]	25452	26298	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*,

Genetic Elements of plasmid VC-LTM595-1qcz rc	From	To	Description, Function and Source of Element
			Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d5Elo(Ot_GA3)	26306	27208	*Delta-5 ELONGASE* from *Ostreococcus tauri*
t-bnFae1	27225	27624	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
p-ARC5_perm1	27771	28921	Promoter derived from a promoter from *ARCILINE 5* gene from *Phaseolus vulgaris*
c-d4Des(Tc_GA3)	28932	30491	*Delta-4 DESATURASE* from *Thraustochytrium* sp.
t-pvarc	30505	31104	Terminator of *ARC5* gene from *Phaseolus vulgaris*
p-VfUSP_684bp[LLL894]	31183	31866	Promoter from *UNKNOWN SEED PROTEIN* gene USP (accession: X56240) from *Vicia faba*
i-Atss18_252[LJK36]	31867	32118	i-Atss18_252bp functional intron region; intron with partial 5' UTR, *Arabidopsis thaliana*, Locus At1g01170, + 37 to + 288 bp (numbering relative to start of transcription) (+ 72 to + 282bp 5'UTR-Intron only)
c-o3Des(Pi_GA2)	32129	33214	*Omega-3-DESATURASE* from *Phythophthora infestans*
t-CaMV35S	33223	33438	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
p-BnFae1	33632	35061	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica napus*
i-Atss1_847bp[LJK19]	35062	35908	i-Atss1 847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d4Des(Pl_GA)2	35916	37253	*Delta-4 DESATURASE* from *Pavlova lutheri*
t-bnFae1	37270	37669	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
p-BnSETL-v1[1234bp]	37762	38995	*SETL*-v1 *Brassica napus* promoter
c-o3Des(Pir_GA)	38998	40089	*Omega-3 DESATURASE* from *Pythium irregulare*
t-BnSETL	40111	40724	*SETL*-v1 *Brassica napus* terminator
p-YPC105906_PcUbi4-2[long]	40848	41824	MTX Parsley (*Petroselinum crispum*) *UBI4-2* promoter with internal intron
c-AtAHASL_A122T_S653N[minusRES]	41832	43844	*ACETOHYDROXYACID SYNTHASE LARGE-SUBUNIT* gene/CDS from

Genetic Elements of plasmid VC-LTM595-1qcz rc	From	To	Description, Function and Source of Element
			Arabidopsis with S653N (*csr1-2*) mutation and A122T SDM mutation minus restriction sites
t-AtAHAS-3'UTR[rtp4820]	43845	44624	*Arabidopsis* (dicot) *AtAHASL* 3' Un-uranslated Region [trimmed] terminator for ACETOHYDROXYACID SYNTHASE gene
b-LLB	44848	44713	Left T-DNA Left border from pTi15955 [Genbank #AF242881]
c-KanR_Tn903	46795	45980	Kanamycin Resistance selection gene/CDS
p-Kan[lm500]	46916	46796	Promoter for Kanamycin resistance gene
o-ori-2	48069	48285	ori-2 origin of replication
c-repE	48379	49134	*repE* gene/CDS
c-sopA	49713	50888	*sapA* gene/CDS
c-sopB	50888	51859	*sopB* gene/CDS
c-sopC/incD	51932	52405	*incD*/*sopC* partial gene/CDS
c-traI	52908	52967	*traI* gene/CDS
mf-traI - repA intergenic region	52956	53318	regulatory region of traR dependent quorum sensing regulon - containing 2 tra-boxes (see LI AND FARRAND JOURNAL OF BACTERIOLOGY,Jan. 2000, p. 179-188)
o-repA	53319	54536	*Rep-A* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY,Jan. 2000, p. 179..188)
rr-repB	54766	55776	*rep-B* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY,Jan. 2000, p. 179..188)
o-repC	55991	57310	*rep-C* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY,Jan. 2000, p. 179..188)
mf-y4cG	57789	57319	fragment of DNA invertase homolog; similar to *Rhizobium* sp. NGR234 pNGR234a Y4CG
tr-Tn5	59829	58268	Transposon Tn5 sequence
o-oriT	60125	60293	oriT from pRK310 genbank file
b-RB[rtp4394]	148	60913	Right T-DNA Right border

Table 11: Genetic Elements of plasmid VC-LTM593-1qcz rc. Listed are the names of the elements, the position in VC-LTM593-1qcz rc (nucleotide number, note: start position was larger than stop position for elements encoded by the complementary strand of VC-LTM593-1qcz rc), the function and source of the element. The T-DNA integrated into the plant genome during the transformation process was flanked by a right border (nucleotides 59895 to 148 of VC-LTM593-1qcz rc) and a left border (nucleotides 43830 to43695 of VC-LTM593-1qcz rc). Elements outside of that region (=vector backbone) are required for cloning and stable maintenance in *E. coli* and/or agrobacteria.

Genetic Elements of plasmid VC-LTM593-1qcz rc	From	To	Description, Function and Source of Element
p-VfUSP_684bp[LLL894]	329	1012	Promoter from *UNKNOWN SEED PROTEIN* gene *USP* (accession: X56240) from *Vicia faba*
i-Atss18_252[LJK36]	1013	1264	i-Atss18_252bp functional intron region; intron with partial 5' UTR, *Arabidopsis thaliana*, Locus At1g01170, + 37 to + 288 bp (numbering relative to start of transcription) (+ 72 to + 282bp 5'UTR-Intron only)
c-d6Elo(Pp_GA2)	1267	2139	*Delta-6 ELONGASE* from *Physcomitrella patens*
t-CaMV35S	2140	2355	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
p-LuCnl(1064bp)	2448	3511	Promoter from *CONLININ* gene from *Linum usitatissimum*
i-Atss14_377bp[LJK32]	3512	3888	i-Atss14_377bp[LJK32] functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-d5Des(Tc_GA2)	3892	5211	*Delta-5 DESATURASE* from *Thraustochytrium* sp. ATCC21685
t-AgrOCS 192bp[LED12]	5212	5403	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
p-SBP	5539	7337	Promoter from a *SUCROSE-BINDING PROTEIN-RELATED* gene from *Vicia faba*
i-Atss2_455bp[LJK20]	7338	7792	i-Atss2_455bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g65090, +77 to +531bp (numbering relative to start of transcription) (+113 to + 508bp 5'UTR-Intron only)
c-d6Des(Ot_febit)	7802	9172	*Delta-6 DESATURASE* from *Ostreococcus tauri*
t-StCATHD-pA	9200	9434	Terminator from *CATHEPSIN D INHIBITOR* gene [*CATHD*] from *Solanum tuberosum* [Potato]
p-LuPXR 1727bp[LLL823]	9513	11239	Promoter from *PEROXIREDOXIN LIKE*

Genetic Elements of plasmid VC-LTM593-1qcz rc	From	To	Description, Function and Source of Element
			protein gene *PXR* from *Linum usitatissimum*
i-Atss1_846bp[ltm593]	11240	12085	i-Atss1_847bp functional intron region; intron with partial 5' UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); 1 bp at poly T stretch shorter compared to original i-Atss1_847bp
c-d6Elo(Tp_GA2)	12099	12917	*Delta-6 ELONGASE* from *Thalassiosira pseudonana*
t-AtPXR 400bp[LLL823]	12973	13372	Terminator from peroxiredoxin like protein gene PXR (At1g48130) from *Arabidopsis thaliana*
p-Napin A/B	13542	14205	Promoter from *napA/B* gene (napin, seed storage protein) from *Brassica napus*
i-Atss14_377bp[LJK32]	14206	14582	i-Atss14_377bp[LJK32] functional intron region; intron with partial 5' UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-d12Des(Ps_GA2)	14589	15785	*Delta-12 DESATURASE* from *Phythophthora sojae*
t-E9	15804	16361	Terminator from Small Subunit of RuBisCo *rbcS* gene (*E9*) from *Pisum sativum*
p-BnSETL-v1[1234bp]	16454	17687	*SETL*-v1 *Brassica napus* promoter
c-o3Des(Pir_GA)	17690	18781	*Omega-3 DESATURASE* from *Pythium irregulare*
t-BnSETL	18803	19416	*SETL*-v1 *Brassica napus* terminator
p-VfUSP_684bp[LLL894]	19495	20178	Promoter from *UNKNOWN SEED PROTEIN* gene *USP* (accession: X56240) from *Vicia faba*
i-Atss18_252[LJK36]	20179	20430	i-Atss18_252bp functional intron region; intron with partial 5' UTR, *Arabidopsis thaliana*, Locus At1g01170, + 37 to + 288 bp (numbering relative to start of transcription) (+ 72 to + 282bp 5'UTR-Intron only)
c-o3Des(Pi_GA2)	20441	21526	*Omega-3-DESATURASE* from *Phythophthora infestans*
t-CaMV35S	21535	21750	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
p-BnSETL-v1[1234bp]	21886	23119	*SETL*-v1 *Brassica napus* promoter
c-d5Des(Tc_GA2)	23122	24441	*Delta-5 DESATURASE* from *Thraustochytrium* sp. ATCC21685
t-BnSETL	24463	25076	*SETL*-v1 *Brassica napus* terminator
p-ARC5_perm1	25223	26373	Promoter derived from a promoter from

Genetic Elements of plasmid VC-LTM593-1qcz rc	From	To	Description, Function and Source of Element
			ARCILINE 5 gene from *Phaseolus vulgaris*
c-d4Des(Tc_GA3)	26384	27943	*Delta-4 DESATURASE* from *Thraustochytrium* sp.
t-pvarc	27957	28556	Terminator of *ARC5* gene from *Phaseolus vulgaris*
p-LuPXR 1727bp[LLL823]	28649	30375	Promoter from *PEROXIREDOXIN LIKE* protein gene *PXR* from *Linum usitatissimum*
i-Atss15_758bp[LJK33]	30376	31133	i-Atss15_758bp[LJK33] functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At2g27040, +93 bp to + 850 bp (numbering relative to start of transcription) (+128 to + 847 bp 5'UTR-Intron only)
c-o3Des(Pir_GA)	31149	32240	*Omega-3 DESATURASE* from *Pythium irregulare*
t-AtPXR 400bp[LLL823]	32297	32696	Terminator from *PEROXIREDOXIN LIKE* protein gene PXR (At1g48130) from *Arabidopsis thaliana*
p-LuCnl(1064bp)	32832	33895	Promoter from *CONLININ* gene from *Linum usitatissimum*
i-Atss2_455bp[LJK20]	33896	34350	i-Atss2_455bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g65090, +77 to +531bp (numbering relative to start of transcription) (+113 to + 508bp 5'UTR-Intron only)
c-d4Des(Pl_GA)2	34360	35697	*Delta-4 DESATURASE* from *Pavlova lutheri*
t-AgrOCS 192bp[LED12]	35719	35910	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
p-BnFae1	36104	37533	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica napus*
i-Atss1_847bp[LJK19]	37534	38380	i-Atss1_847bp functional intron region; intron with partial 5' UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d5Elo(Ot_GA3)	38388	39290	*Delta-5 ELONGASE* from *Ostreococcus tauri*
t-bnFae1	39307	39706	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
p-YPC105906_PcUbi4-2[long]	39830	40806	MTX Parsley *UBI4-2* promoter with internal intron
c-AtAHASL_A122T_S653N[minusRES]	40814	42826	*ACETOHYDROXYACID SYNTHASE LARGE-SUBUNIT* gene/CDS from *Arabidopsis* with S653N (*csr1-2*) mutation

Genetic Elements of plasmid VC-LTM593-1qcz rc	From	To	Description, Function and Source of Element
			and A122T SDM mutation minus restriction sites
t-AtAHAS-3'UTR[rtp4820]	42827	43606	*Arabidopsis* (dicot) *AtAHASL* 3' Un-translated Region [trimmed] terminator for ACETOHYDROXYACID SYNTHASE gene
b-LLB	43830	43695	Left T-DNA Left border from pTi15955 [Genbank #AF242881]
c-KanR_Tn903	45777	44962	Kanamycin Resistance selection gene/CDS
p-Kan[lm500]	45898	45778	Promoter for Kanamycin resistance gene
o-ori-2	47051	47267	ori-2 origin of replication
c-repE	47361	48116	*repE* gene/CDS
c-sopA	48695	49870	*sapA* gene/CDS
c-sopB	49870	50841	*sopB* gene/CDS
c-sopC/incD	50914	51387	*incD/sopC* partial gene/CDS
c-traI	51890	51949	*traI* gene/CDS
mf-traI - repA intergenic region	51938	52300	regulatory region of traR dependent quorum sensing regulon - containing 2 tra-boxes (see LI AND FARRAND JOURNAL OF BACTERIOLOGY, Jan. 2000, p. 179-188)
o-repA	52301	53518	*Rep-A* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY, Jan. 2000, p. 179..188)
rr-repB	53748	54758	*rep-B* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY, Jan. 2000, p. 179..188)
o-repC	54973	56292	*rep-C* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY,Jan. 2000, p. 179..188)
mf-y4cG	56771	56301	fragment of DNA invertase homolog; similar to *Rhizobium* sp. NGR234 pNGR234a Y4CG
tr-Tn5	58811	57250	Transposon Tn5 sequence
o-oriT	59107	59275	oriT from pRK310 genbank file
b-RB[rtp4394]	148	59895	Right T-DNA Right border

Table 13 compares the order of the gene expression cassettes among all the different constructs and the construct combinations, using short terms for these expression cassettes, see Table 12 for definitions. The data in Examples 10 to 19 demonstrate significant differences among the different construct or construct combinations in terms of the PUFA profile measured in transgenic seed. The differences between constructs and the construct combinations were evident even when eliminating all other sources that affect PUFA levels (e.g. different environments, plant-to-plant variability, seed oil content, T-DNA

copy number). For example VC-RTP10690-1qcz_F and VC-LMT593-1qcz rc are isogenic, i.e. the two constructs contained exactly the same gene expression cassettes. Because of the similarity between RTP10690-1qcz_F and VC-LMT593-1qcz one would expect exactly the same pathway step conversion efficiencies e.g. when comparing the average conversion efficiencies of all single copy events. However, Figure 39 shows that VC-RTP10690-1qcz_F had a Delta-4 DESATURASE conversion efficiency of 32%, (average of T1 seeds of 52 single copy T0 events), wheras VC-LMT593-1qcz rc had a Delta-4 DESATURASE conversion efficiency of 47% (average of T1 seeds of 241 single copy T0 events). This was not expected, and can be explained by transcript levels, which in turn determine protein levels. The transcript levels are affected by the genetic elements that flank the *Delta-4 DESATURASE* cassettes in VC-LMT593-1qcz rc. The observations between the two constructs is an unexpected finding and indicates that not only the genome but also the T-DNA itself impacts the Delta-4 DESATURASE conversion efficiency, that was dependant on "gene dosage" as described in Example 19. Furthermore, the data in Example 10 to 19 demonstrate that it was possible to insulate expression cassettes from such effects. As can be seen in those Examples 10-19 all single copy events were capable of producing almost exactly the same VLC-PUFA levels when eliminating all other sources that affect PUFA levels (e.g. different environments, plant-to-plant variability, seed oil content). This was particularly striking when comparing all the single copy events in Example 18. Comparing the total C20+C22 VLC-PUFA content, which was largely only controlled by how much was converted by the delta-12 desaturase and by the delta-6 desaturase, it was striking to observe there was virtually no difference between e.g. the single copy event LANPMZ obtained from the contruct combination VC-LJB2197-1qcz + VC-LLM337-1qcz rc, and all single copy events listed in Example 18. To this end, it is important to note that one side of the T-DNA that encodes either the entire pathway (Example 15 to 18) or at least the first steps of the pathway up to ARA and EPA production (Example 10 to 14) always contains the AHAS gene which confers herbicide tolerance but was not involved in the VLC-PUFA pathway. The other side of the T-DNA encodes either the entire pathway (Example 15 to 18) or at least the first steps of the pathway up to ARA and EPA production (Example 10 to 14) in most cases the *Delta-6 ELONGASE* from *Physcomitralla patens* (except in Example 13 and 14). As described in Example 19, the Delta-6 ELONGASE protein encoded by the *Physcomitrella patens* gene works close to maximum conversion efficiency (>90%), thus any increase in delta-6 elongase enzyme levels due to any effect that increases transcript levels will have virtually no effect on the C20 and C22 VLC-PUFA levels. Effectively, the T-DNA determining the total level of VLC-

PUFA accumulation are flanked on both sides by genes where expression level differences will have no impact on the VLC-PUFA accumulation. As these two genes were encoded by expression cassettes that were several thousand bp in size, it appears the genes inside the T-DNA were shielded/insulated from any effects the genomic environment could have on the expression level of those genes (e.g. the delta-12-desaturase, compare with Example 19). This effect was consistent with the observation that double copy events differ considerably more in total C20 and C22 VLC-PUFA levels: As in many cases the additional T-DNA insertions are not complete (see Example 10 to 18), resulting in exporsure of T-DNA internal genes are expoed to the genome. When these genes are susceptible to gene-dosage effects (the conversion efficiency of those genes depends on the amount of transcript and the derived amount of enzyme, compare with Example 19), then in some genomic locations the genomic envorinment boosted the transcript level.

Table 12: Definition of shorthands used for plant expression cassettes of this invention

Cassette shorthand	Promoter	Intron	Coding sequence	Terminator
USP/c-d6Elo(Pp_GA2)	p-VfUSP_684bp[LLL894]	i-Atss18_252[UK36]	c-d6Elo(Pp_GA2)	t-CaMV35S
PXR/c-o3Des(Pir_GA)	p-LuPXR 1727bp[LLL823]	i-Atss15_758bp[UK33]	c-o3Des(Pir_GA)	t-AtPXR 400bp[LLL823]
Conlinin/c-d5Des(Tc_GA2)	p-LuCnl(1064bp)	i-Atss14_377[UK32]	c-d5Des(Tc_GA2)	t-AgrOCS 192bp[LED12]
SBP/c-d6Des(Ot_febit)	p-SBP	i-Atss2_455bp[UK20]	c-d6Des(Ot_febit)	t-StCATHD-pA
PXR/c-d6Elo(Tp_GA2)	p-LuPXR 1727bp[LLL823]	i-Atss1_847bp[UK19]	c-d6Elo(Tp_GA2)	t-AtPXR 400bp[LLL823]
Fae/c-d6Elo(Tp_GA2)	p-BnFae1	i-Atss1_847bp[UK19]	c-d6Elo(Tp_GA2)	t-bnFae1
Napin/c-d12Des(Ps_GA)	p-Napin A/B	i-Atss14_377[UK32]	c-d12Des(Ps_GA)	t-E9
USP/c-o3Des(Pi_GA2)	p-VfUSP_684bp[LLL894]	i-Atss18_252[UK36]	c-o3Des(Pi_GA2)	t-CaMV35S
UBI/AHAS	p-PcUbi4-2[long]	part of PcUBI promoter	c-AtAHASL_A122T_S653N	t-AtAHAS-3'/UTR[ac321]
Conlinin/d4Des(Eg)	p-LuCnl(1064bp)	N/A	c-d4Des(Eg_GA)	t-AgrOCS 192bp[LED12]
SBP/d15Des(Ch)	p-VfSBP_perm3	N/A	c-d15Des(Ch_ERTp_GA)	t-StCATHD-pA
Conlinin/c-d4Des(Pl_GA)2_var1	p-LuCnl(1064bp)	i-Atss2_455bp[UK20]	c-d4Des(Pl_GA)2	t-AgrOCS 192bp[LED12]
SETL/c-d5Des(Tc_GA2)	p-BnSETL-v1[1234bp]	N/A	c-d5Des(Tc_GA2)	t-BnSETL
SETL/c-o3Des(Pir_GA)	p-BnSETL-v1[1234bp]	N/A	c-o3Des(Pir_GA)	t-BnSETL
FAE/c-d5Elo(Ot_GA3)	p-BnFAE1	i-Atss1_847bp[UK19]	c-d5Elo(Ot_GA3)	t-bnFAE1
SETL/c-o3Des(Pir_GA)	p-VfSBP_perm3	N/A	c-o3Des(Pir_GA)	t-StCATHD-pA
ARC/c-d4Des(Tc_GA)_var1	p-ARC5_perm1	N/A	c-d4Des(Tc_GA)	t-pvarc
ARC/c-d4Des(Tc_GA)_var2	p-ARC5_perm1	N/A	c-d4Des(Tc_GA3)	t-pvarc
Conlinin/c-d4Des(Pl_GA)2_var2	p-LuCnl(1064bp)	N/A	c-d4Des(Pl_GA)2	t-AgrOCS 192bp[LED12]
FAE/c-d4Des(Pl_GA)2	p-BnFAE1	i-Atss1_847bp[UK19]	c-d4Des(Pl_GA)2	t-bnFAE1

Table 13: Order and orientation of plant expression cassettes on T-DNAs. Per column, expression cassettes are abbreviated according to Table 12 and listed from top to bottom in the following way: The top row indicated the right border of the first T-DNA introduced into the plant genome; the following proteins are all listed according to sense orientation of transcription, pointing away from the right border. The end of the first T-DNA was indicated by 'LB'. In case a second T-DNA was used to transfer all pathway genes into the plant genome according to example 7 to 11, the right border of this T-DNA was indicated as 'RB2'. The expression cassettes of the second T-DNA are listed in the following rows. Empty cells have been introduced to facilitate comparison of the different constructs.

VC-LJB2197-1qcz + VC-LLM306-1qcz rc	VC-LJB2197-1qcz + VC-LLM337-1qcz rc	VC-LJB2197-1qcz + VC-LLM338-3qcz rc	VC-LJB2755-2qcz + VC-LLM391-2qcz rc	VC-LJB2755-2qcz + VC-LLM217-1qcz rc	RTP10690-1qcz_F	RTP10691-2qcz	VC-LMT595-1qcz rc	VC-LMT593-1qcz rc
RB	RB	RB	RB	RB	RB	RB	RB	RB
USP/c-d8Elo(Pp_GA2)	USP/c-d8Elo(Pp_GA2)	USP/c-d8Elo(Pp_GA2)	PXR/c-o3Des(Pir_GA)	PXR/c-o3Des(Pir_GA)	USP/c-d6Elo(Pp_GA2)	USP/c-d6Elo(Pp_GA2)	USP/c-d6Elo(Pp_GA2)	USP/c-d6Elo(Pp_GA2)
Conlinin/c-d5Des(Tc_GA2)	Conlinin/c-d5Des(Tc_GA2)	Conlinin/c-d5Des(Tc_GA2)	Conlinin/c-d5Des(Tc_GA2)	Conlinin/c-d5Des(Tc_GA2)	Conlinin/c-d5Des(Tc_GA2)	Conlinin/c-d5Des(Tc_GA2)	Conlinin/c-d5Des(Tc_GA2)	Conlinin/c-d5Des(Tc_GA2)
SBP/c-d6Des(Ot_febit)	SBP/c-d6Des(Ot_febit)	SBP/c-d6Des(Ot_febit)	SBP/c-d6Des(Ot_febit)	SBP/c-d6Des(Ot_febit)	SBP/c-d6Des(Ot_febit)	SBP/c-d6Des(Ot_febit)	SBP/c-d6Des(Ot_febit)	SBP/c-d6Des(Ot_febit)
PXR/c-d6Elo(Tp_GA2)	PXR/c-d6Elo(Tp_GA2)	PXR/c-d6Elo(Tp_GA2)	FAE/c-d6Elo(Tp_GA2)	FAE/c-d6Elo(Tp_GA2)	PXR/c-d6Elo(Tp_GA2)	PXR/c-d6Elo(Tp_GA2)	PXR/c-d6Elo(Tp_GA2)	PXR/c-d6Elo(Tp_GA2)
Napin/c-d12Des(Ps_GA)	Napin/c-d12Des(Ps_GA)	Napin/c-d12Des(Ps_GA)	Napin/c-d12Des(Ps_GA)	Napin/c-d12Des(Ps_GA)	Napin/c-d12Des(Ps_GA)	Napin/c-d12Des(Ps_GA)	Napin/c-d12Des(Ps_GA)	Napin/c-d12Des(Ps_GA)
			USP/c-o3Des(Pi_GA2)	USP/c-o3Des(Pi_GA2)	PXR/c-o3Des(Pir_GA)	PXR/c-o3Des(Pir_GA)	PXR/c-o3Des(Pir_GA)	
UBI/AHAS	UBI/AHAS	UBI/AHAS	UBI/AHAS	UBI/AHAS				
LB	LB	LB	LB	LB				
RB2	RB2	RB2	RB2	RB2				
Conlinin/d4Des(Eg)	Conlinin/d4Des(Eg)				Conlinin/c-d4Des(Pl_GA)2_var1	SETL/c-d5Des(Tc_GA2)	SETL/c-d5Des(Tc_GA2)	SETL/c-o3Des(Pir_GA)
FAE/c-d5Elo(Ot_GA3)	FAE/c-d5Elo(Ot_GA3)	FAE/c-d5Elo(Ot_GA3)	FAE/c-d5Elo(Ot_GA3)	FAE/c-d5Elo(Ot_GA3)	FAE/c-d5Elo(Ot_GA3)	FAE/c-d5Elo(Ot_GA3)	FAE/c-d5Elo(Ot_GA3)	USP/c-o3Des(Pi_GA2)
SETL/c-o3Des(Pir_GA)	SETL/c-o3Des(Pir_GA)							SETL/c-d5Des(Tc_GA2)
					ARC/c-d4Des(Tc_GA)_var2	ARC/c-d4Des(Tc_GA)_var2	ARC/c-d4Des(Tc_GA)_var2	ARC/c-d4Des(Tc_GA)_var2
USP/c-o3Des(Pi_GA2)	USP/c-o3Des(Pi_GA2)	USP/c-o3Des(Pi_GA2)			USP/c-o3Des(Pi_GA2)	USP/c-o3Des(Pi_GA2)	USP/c-o3Des(Pi_GA2)	PXR/c-o3Des(Pir_GA)
ARC/c-d4Des(Tc_GA)_var1	ARC/c-d4Des(Tc_GA)_var1	ARC/c-d4Des(Tc_GA)_var1	ARC/c-d4Des(Tc_GA)_var1	SETL/c-d5Des(Tc_GA2)				Conlinin/c-d4Des(Pl_GA)2_var1
	Conlinin/d4Des(Eg)	Conlinin/d4Des(Eg)	Conlinin/c-d4Des(Pl_GA)2_var2		FAE/c-d4Des(Pl_GA)2	FAE/c-d4Des(Pl_GA)2	FAE/c-d4Des(Pl_GA)2	FAE/c-d5Elo(Ct_GA3)
				SETL/c-o3Des(Pir_GA)	SETL/c-o3Des(Pir_GA)	SETL/c-o3Des(Pir_GA)	SETL/c-o3Des(Pir_GA)	
				UBI/AHAS	UBI/AHAS	UBI/AHAS	UBI/AHAS	UBI/AHAS

VC-LJB2197-1qcz + VC-LLM806-1qcz rc	VC-LJB2197-1qcz + VC-LLM337-1qcz rc	VC-LJB2197-1qcz + VC-LLM338-3qcz rc	VC-LJB2755-2qcz + VC-LLM391-2qcz rc	VC-LJB2755-2qcz + VC-LLM217-1qcz rc	RTP10690-1qcz_F	RTP10691-2qcz	VC-LMT595-1qcz rc	VC-LMT593-1qcz rc
LB2	LB2	LB2	LB2	LB2	LB	LB	LB	LB

Example 5: Procedure for production of transgenic plants using a Co-Transformation approach

In general, the transgenic rapeseed plants were generated by a modified protocol according to DeBlock et al. 1989, Plant Physiology, 91:694-701). For the generation of rapeseed plants transgenic for two different T-DNAs, the binary vectors described in example 3 were transformed into *Agrobacterium rhizogenes* SHA001 (see WO2006024509 A2 for full description of the *Agrobacterium* used). For the transformation of rapeseed plants (cv. Kumily), a co-transformation strategy was used. Transformation was performed with two different agrobacteria strains harbouring one of the two different plasmids listed in Table 14 and described in detail in Example 3, Example 4, Example 6 and/or Example 7

Table 14: Overview of combinations used in Co-transformation Strategy described in Example 3 for generation of plants harboring two different T-DNAs

ID of Combination	Plasmid containing **T-DNA 1** harbored by *Agrobacterium tumefaciens* **clone** 1		Plasmid containing **T-DNA 2** harbored by *Agrobacterium tumefaciens* **clone 2**	
	Plasmid name	Selectable marker for transgenic plants	Plasmid name	Selectable marker for transgenic plants
A	VC-LJB2197-1qcz	AHAS	VC-LLM306-1qcz rc	None
B	VC-LJB2197-1qcz	AHAS	VC-LLM337-1qcz rc	None
C	VC-LJB2197-1qcz	AHAS	VC-LLM338-3qcz rc	None
D	VC-LJB2755-2qcz rc	AHAS	VC-LLM391-2qcz rc	None
E	VC-LJB2755-2qcz rc	AHAS	VC-LTM217-1qcz rc	None

Overnight cultures of the two strains intended to be co-transformed were prepared in YEB medium with antibiotics (20 mg/L chloramphenicol, 5 mg/L tetracycline, 25 mg/L Spectinomycin) and grown at 28°C. On the next day the optical density of the culture was checked at 600 nm wave length. It reached about 1.0. Cultures of lower optical density were extended in cultivation period. Cultures with an optical density of above 1.3 were diluted with YEB medium to an OD of approximately 0.2 and cultured until they reach an OD of 1.0.

Cultures were pelleted at about 4000 g and re-suspended in liquid MS medium (Murashige and Skoog 1962), pH 5.8, 3% sucrose with 100 mg/L Acetosyringone to reach an OD_{600nm} of 0.1.

The *Agrobacterium* suspensions corresponding to each of the two constructs to be co-transformed were mixed in equal parts and used for inoculation of hypocotyl segments prepared from 5 days old etiolated seedlings.

Germination took place on half concentrated MS medium, pH 5.6 – 5.8, 1% sucrose, at 23°C in the dark for 5 days. Hypocotyl segments of 4 to 7 mm length were inoculated by dipping. Infected explants were transferred to petri dishes with co-cultivation medium (MS medium, pH 5.6, 3% sucrose, 0.6 g/L MES (2-(N-Morpholino)ethanesulfonic acid), 18 g/L mannitol, 0.7% phytoagar (Duchefa Biochemie, PO Box 809 2003 RV Haarlem, Netherlands, part number SKU:P1003), 100 mg/L Acetosyringone, 200 mg/L L-Cysteine, 1 mg/L 2,4D (2,4-Dichlorophenoxyacetic acid)) carrying one layer of Whatman filter paper on its surface. Petri dishes were sealed with tape and incubated at 23 C under long day conditions (16 h light/8 h darkness) for three days. After the three days co-cultivation period explants were transferred to MS medium, pH 5.6, 3% sucrose, 0.6 g/L MES, 18 g/L mannitol, 07% Phytoagar, 1 mg/L 2,4D and 500 mg/L Carbenicillin to prevent *Agrobacterium* growth and incubated for a recovery period under the same physical conditions as for the co-cultivation for 7 days.

For selective regeneration explants were transferred after the recovery period to MS medium, pH 5.8, 3% sucrose, 0.7% Phytoagar, 2.5 mg/L $AgNO_3$, 3 mg/L BAP (6-Benzylaminopurine), 0.1 mg/L GA (Gibberellic acid), 0.1 mg/L NAA (1-Naphthaleneacetic acid), 500 mg/L Carbenicillin, 100 nM Imazethapyr (Pursuit) and cultured for two weeks under long day conditions as described above. Sub-cultivation takes place every two weeks. Hormones were stepwise reduced as follows: BAP 3 to 0.5 to 0.05 mg/L; GA (Gibberellic acid) 0.1 to 0.25 to 0.25 mg/L; NAA 0.1 to 0 to 0 mg/L.

Developing shootlets could be harvested after the second cycle of selective regeneration. Shootlets were cut and transferred to either Elongation/rooting medium (MS medium, pH 5.8, 2%sucrose, 100 mg/L myo-inositol, 40 mg/L Adenine sulphate, 500 mg/L MES, 0.4% Sigma Agar, 150 mg/L Timentin, 0.1 mg/L IBA (Indole-3-butyric acid)) or to rock wool/stone wool or foam mats (Grodan, GRODAN Group P.O. Box 1160, 6040 KD Roermond The Netherlands, or Oasis, 919 Marvin Street, Kent, OH 44240 USA) watered with 1/10 Vol. of MS medium, pH 5.8 without sucrose under ex vitro long day conditions in covered boxes.

Shoots were elongated and rooted in *in vitro* medium and were transferred directly to soil.

Either in vitro shoots or GH adapted shoots were sampled for molecular analysis.

The following modifications were successfully tested for transformation and were alternatively used to the above described protocol, once they had been worked out.

Seeds were germinated under low light conditions (< 50 µMol/m²s) using MSB5 medium from Duchefa (Duchefa Biochemie, PO Box 809 2003 RV Haarlem, Netherlands), pH 5.8, 3% sucrose and 0.8% Oxoid agar. Germination under light conditions produces explants, which are more stable and easier to handle compared to etiolated hypocotyls.

The inoculation method can vary but the method used in the invention was inoculating explants in a bath of *Agrobacterium* cells under gentle shaking up to 4 min and sieving the explants after the incubation with shaking. Under this condition the OD_{600nm} can be reduced up to 0.01.

Medium were used either autoclaved (except antibiotics, hormones, additives such as L-cysteine, Acetosyringon, imidazolinone components) or filter sterilized prepared (Agar component autoclaved, allowed to cool to 42 C and then used).

Example 6: Procedure for production of transgenic plants using BiBACs

For BiBAC transformation the same protocol as described for the co-transformation approach was used except that only one construct was used. According to the prokaryotic kanamycin resistance gene of binary plasmid 50 mg/L kanamycin was used instead of Spectinomycin for *Agrobacterium* growth. It was observed during the course of this work that *Agrobacterium* carrying BiBACs grow very slowly, often taking 18 hours to reach a liquid culture OD_{600nm} considered optimal for use in plant transformation.

The table below gives an example for some key data documented during the transformation of the construct LTM593

	VC-LTM593-1qcz rc
Explants inoculated	37 600
Shoots harvested	2 630
Shoots sampled and analyzed for gene AHAS	1 543
Transgenic events with gene AHAS	1 050
Transformation efficiency (%)	2.8
Percentage of events that grown on herbicide seelction plates but where confirmed using qPCR to lack the herbiced resitstance marker (%)	32.0

The amount of single copy events produced by the plant transformation protocol described above was 45% and 38% of vector backbone-free events selected after transformation of the constructs LTM593 and LTM595, respectively, were single copy events (see Table 15).

Table 15: Statistics of single and double copy events with and without vector backbone in transformation experiments performed with the two BiBAC strains VC-LTM593-1qcz rc and VC-LTM595-1qcz rc

	VC-LTM593-1qcz rc		VC-LTM595-1qcz rc	
	#	%	#	%
Number of transgenic events confirmed to contain at least 1 copy of gene c-AtAHAS	1050		217	
Single copy	535	50	92	42
Single copy, vector backbone-free	478	45	83	38
Double copy	320	30	49	23
Double copy vector backbone-free	227	22	41	18

One important key finding for successful transformation was the choice of *Agrobacterium* strain. While the original method (see De Block et al. (1989) Plant Physiology 91:694-701) used the *Agrobacterium tumefaciens* strain C58C1pMP90, the described method was based on the *Agrobacterium rhizogenes* strain SHA001 (see WO2006024509 A2 for SHA001 and SHA017). Even within *Agrobacterium rhizogenes* strains we have realized a clear response of transformation success to the strain and construct used (seeTable 16).

Table 16: Impact of Agrobacterium rhizogenes strains on transformation success of BiBACs

	VC-RTP10690-1qcz_f	VC-LTM593-1qcz rc
Strain used	**SHA017**	**SHA001**
Number of inoculated explants	60700	37600
Regeneration efficiency (%)	1.8	4.1
Shoots samples and analysed for gene AHAS	1084	1543
Number of transgenic plants based on the presence of gene c-AHAS	333	1050
Transformation efficiency (TE) (%)	0.6	2.8
Percentage of events that grown on herbicide seelction plates but where confirmed using qPCR to lack the herbiced resitstance marker (%)	69.3	32.0

Table 17: Transformation Efficiencies of the various plasmids and Agrobacterium strains used. With respect to the integration of the T-DNA, it was possible that multiple copies or single copies of intact or truncated or duplicated, or truncated and duplicated T-DNA's could be inserted into the genome. The terms copy or copies refer to the number of copies of a particular T-DNA or fragment of a T-DNA were inserted into the plant genome. The term locus refers to how many different locations within the plant genome the copy or copies of the T-DNA were inserted into. Locus is defined as region of disequilibrium within the genome an area which varies between plant species and even within cultivars of a given species. For the purpose of this definition this is within one genetic map unit or CentiMorgan.

Outcome	VC-LJB2197-1qcz + VC-LLM337-1qcz rc	VC-LJB2755-2qcz rc + VC-LLM391-2qcz rc	VC-RTP10690-1qcz_f	VC-LTM593-1qcz rc
Agrobacterium strain	SHA001	SHA001	SHA017	SHA001
Transformation efficiency (TE) (%) based on the presence of gene AHAS	17	19.7	0.6	2.8
Percentage of events that grown on herbicide seelction plates but where confirmed using qPCR to lack the herbiced resitstance marker (%)	1.1	0.6	69.3	32.0
Genes of both T-DNAs present (% co-transformed)	11.2	15.1	n. a.	n. a.
% of single copy events **one locus integration**	0	0	100	100
Portion of selected "more copy events" (2 to 3 copies) with **one locus integration**	0	0	n.d.	25 from 33

Example 7: Seed Germination and Plant Growth in the Greenhouse and Field

Transformed plants were cultivated for seed production and phenotypic assessment in both the greenhouse and in the field. Greenhouse growth conditions were a sixteen hour light period followed by an eight hour dark period. The temperature was 20 degrees celsius during the light period (also called the day period) with a level of light corresponding to 200-300 micromoles of photons m-2 s-1 (this is the incident of light at the top of the plant and lights were adjusted in terms of distance from the plant to

achieve this rate). During the day period the range of light in the greenhouse varied between 130 and 500 micromoles of photons m-2 s-1. Getting out of the day range just cited triggered either the use of artificial light to bring the level up to 200-300 micromoles of photons m-2 s-1 or shading and/or shut off of lights to bring the level back to 200-300 micromoles of photons m-2 s-1. The dark period (also referred to as the night period) temperature was 18 C. Four hours before the light period began the temperature was lowered to 15 C for the remainder of the dark period. Plants were irrigated and treated for insects as necessary. The soil type was 50 % Floradur B Seed + 50 % Floradur B Cutting (including sand and perlite) provided by Floragard (Oldenburg, Germany). Plant growth was enhanced by nutrient supplementation. Nutrients were combined with the daily watering. A 0.1% (w/v) fertilizer solution (Hakaphos Blue 15(N) -10 (P) - 15(K), Compo GmbH & Co KG, Münster, Germany) was used to water the plants. Water was supplied on demand (e.g. depending on plant growth stage, water consumption etc.). To avoid cross-pollination, plants were bagged at the time when the first flowers opened. Plants were checked daily in order to ensure that all open flowers were covered by the bags. Open flowers that were not covered properly were removed.

For field grown plants, the plants were grown in six locations which correspond climatically to USDA growth zones 3a-4b and 5a, and five locations corresponding climatically to USDA growth zones 8a-9b and 11. The plants grown in the regions corresponding to USDA growth zones 3a-4b and 5a were grown in the summer and the plants grown in the regions corresponding to USDA growth zones 8a-9b and 11 were grown in the winter. Standard horticultural practices for canola were followed. Netting and other measures to protect from birds and insects were used as deemed necessary by the growers, as were herbicides and fertilizer applications. The planting density for all locations was eighty seeds per square meter with germination rate of 95 or better percent.

In the case where it was necessary to determine germination rates for the purpose of seed quality assurance or control, or where it was advantageous to germinate seeds to obtain cotyledons or seedling tissues, the following protocol was used:
150 mm by 15 mm petri-plates and Whatman (no. 2) filter paper cut into 120 mm disks were used. The filter paper was pre-moistened with sterile deionized water. One

hundred seeds of the appropriate line were obtained and spread evenly across the pre-moistened filter paper.

Clean and sterile tweezers were used to spread the seeds to obtain the uniform pattern as shown above. Additional sterile water was added to ensure the seeds and paper were wetted, but not floating (see above image). The total amount of water used per petri-plate was approximately 20 milliliters. Three plates were done for each genotype tested. The plates were sealed with surgical tape, VWR (1050 Satellite Blvd. Suwanee, GA 30024 USA) catalog number 56222-110. After the plates were sealed, they were then incubated in a germination chamber set to 90% humidity, set to a sixteen hour photoperiod with 20 degrees Celsius day temperature and 15 degrees Celsius night temperature. The light intensity was 90-120 micro-moles per square meter per second. Germination was scored twice, once at four days after placing the plates into the growth chamber and again at eight days after incubation.

Example 8: Lipid extraction and lipid analysis of plant oils

The results of genetic modifications in plants or on the production of a desired molecule, e.g. a certain fatty acid, were determined by growing the plant under suitable conditions, e.g. as described below, and analyzing the growth media and/or the cellular components for enhanced production of the desired molecule, e.g. lipids or a certain fatty acid. Lipids were extracted as described in the standard literature including Ullman, Encyclopedia of Industrial Chemistry, Bd. A2, S. 89-90 und S. 443-613, VCH: Weinheim (1985); Fallon, A., et al., (1987) "Applications of HPLC in Biochemistry" in: Laboratory Techniques in Biochemistry and Molecular Biology, Bd. 17; Rehm et al. (1993) Biotechnology, Bd. 3, Kapitel III: "Product recovery and purification", S. 469-714, VCH: Weinheim; Belter, P.A., et al. (1988) Bioseparations: downstream processing for Biotechnology, John Wiley and Sons; Kennedy, J.F., und Cabral, J.M.S. (1992) Recovery processes for biological Materials, John Wiley and Sons; Shaeiwitz, J.A., und Henry, J.D. (1988) Biochemical Separations, in: Ullmann's Encyclopedia of Industrial Chemistry, Bd. B3; Kapitel 11, S. 1-27, VCH: Weinheim; and Dechow, F.J. (1989) Separation and purification techniques in biotechnology, Noyes Publications.

It is acknowledged that extraction of lipids and fatty acids can be carried out using other protocols than those cited above, such as described in Cahoon et al. (1999) Proc. Natl. Acad. Sci. USA 96 (22):12935-12940, and Browse et al. (1986) Analytic Biochemistry 152:141-145. The protocols used for quantitative and qualitative analysis of lipids or fatty acids are described in Christie, William W., Advances in Lipid Methodology, Ayr/Scotland:

Oily Press (Oily Press Lipid Library; 2); Christie, William W., Gas Chromatography and Lipids. A Practical Guide - Ayr, Scotland: Oily Press, 1989, Repr. 1992, IX, 307 S. (Oily Press Lipid Library; 1); "Progress in Lipid Research, Oxford: Pergamon Press, 1 (1952) - 16 (1977) u.d.T.: Progress in the Chemistry of Fats and Other Lipids CODEN.

To generate transgenic plants containing the genetic elements described in examples 3 and 4 for production of EPA and DHA in seeds, rapeseed (*Brassica napus*) was transformed as described in examples 5 and 6. Selected plants containing the genetic elements described in examples 3 and 4 were grown until development of mature seeds under the conditions cited in Example 7. Fatty acids from harvested seeds were extracted as described above and analyzed using gas chromatography as described above. The content (levels) of fatty acids is expressed throughout the present invention as percentage (weight of a particular fatty acid) of the (total weight of all fatty acids). Seed oil content is expressed throughout the present invention as percentage of (oil weight) of the (total oil weight of seeds).

Table 18: Fatty acids analyzed using gas chromatography

Systematic name	Trivial Name	Short hand 1	Short hand 2
Hexadecanoic acid	Palmitic acid	16:0	
(Z)-7-Hexadecenoic acid		16:1n-9	
(Z,Z,Z)-7,10,13-Hexadecatrienoic acid		16:3n-3	
Octadecanoic acid	Stearic acid	18:0	
(Z)-9-Octadecenoic acid	Oleic acid	18:1n-9	OA
(Z,Z)-9,12-Octadecadienoic acid	Linoleic acid	18:2n-6	LA
(Z,Z)-6,9-Octadecadienoic acid		18:2n-9	
(Z,Z,Z)-9,12,15-Octadecatrienoic acid	alpha-Linolenic acid	18:3n-3	ALA
(Z,Z,Z)-6,9,12-Octadecatrienoic acid	gamma-Linolenic acid	18:3n-6	GLA
(Z,Z,Z,Z)-6,9,12,15-Octadecatetraenoic acid	Stearidonic acid	18:4n-3	SDA
Eicosanoic acid	Arachidic acid	20:0	
(Z)-11-Eicosenoic acid	Gondoic acid	20:1n-9	
(Z,Z)-11,14-Eicosadienoic acid		20:2n-6	
(Z,Z,Z)-11,14,17-Eicosatrienoic acid		20:3n-3	
(Z,Z,Z)-8,11,14-Eicosatrienoic acid	Dihomo-gamma-linolenic acid	20:3n-6	DHGLA
(Z,Z,Z,Z)-8,11,14,17-Eicosatetraenoic acid		20:4n-3	
(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic acid	Arachidonic acid	20:4n-6	ARA
(Z,Z,Z,Z,Z)-5,8,11,14,17-Eicosapentaenoic acid	Timnodonic acid	20:5n-3	EPA
Docosanoic acid	Behenic acid	22:0	
(Z)-13-Docosenoic acid	Erucic acid	22:1n-9	
(Z,Z,Z,Z)-7,10,13,16-Docosatetraenoic acid	Adrenic acid	22:4n-6	DTA
(Z,Z,Z,Z,Z)-7,10,13,16,19-Docosapentaenoic acid	Clupanodonic acid	22:5n-3	DPAn-3
(Z,Z,Z,Z,Z)-4,7,10,13,16-Docosapentaenoic acid	Osbond acid	22:5n-6	DPAn-6
(Z,Z,Z,Z,Z,Z)-4,7,10,13,16,19-Docosahexaenoic acid		22:6n-3	DHA

Example 9: Non-destructive analysis of lipids in single cotyledons of seedlings

Transformation of plants according to the methods described in Example 5 and Example 6 results in a random integration of the T-DNA into the genome. It was known that such integrations can also occur in a partial manner, furthermore multiple integrations of complete and partial T-DNAs can occur. A diploid plant contains one haploid chromosome set inherited from the ovule containing progenitor (for simplicity called the mother or female parent), and one haploid chromosome set inherited from the pollen donating progenitor (for simplicity called the father or male parent). In transformation of the T0 plant the T-DNA integrates into random position(s) of random chromosomes and will result in the maternal chromosome carrying the integration(s), and the corresponding paternal chromosome will not carry this integration, resulting in a seed heterozygous for the integration(s). Growing the T0 seed up results in a plant heterozygous for the T-DNA insert(s) and subsequent gametogenesis will result in both pollen and ovules that contain the T-DNA integration and in some pollen and ovules that do not carry the T-DNA insert(s), according to random segregation, as observed by Gregor Mendel (Mendel, 1866). Self pollination of the plant will

result in seeds which will be segregating for the T-DNA insertion(s) according to the ratios observed by Gregor Mendel (Mendel, 1866) and which are now part of the basic general knowledge in the life sciences. Due to the Mendelian segregation; for each integration of the T-DNA, one quarter (~25%) of the T1 seed have lost the integration. 50% of the T1 seed will carry the T-DNA integration either on the maternal chromosome (25%), or paternal chromosome (25%); these seeds are 'heterozygous' or 'hemizygous' related to the T-DNA integration. The remaining quarter (~25%) of the T1 seed will carry the T-DNA on the maternal and paternal chromosome; these seeds are 'homozygous' related to the T-DNA integration. For plants that follow such a sexual propagation, it is essential to genetically fix the T-DNA integration(s), by selecting progenies that are homozygous for the T-DNA integration(s); otherwise the T-DNAs insertion(s) and the trait conferred by the T-DNA insertion(s) will continue to segregate and might be lost over a number of generations. In order to identify T1 seedlings where each T-DNA integration that was essential for the trait was present, ideally homozygous, one can perform quantitative PCR to measure the copy number of the T-DNA integration(s) directly. Alternatively one can analyse the trait conferred by the presence of the T-DNAs, which at least enables the identification of all seeds that do not contain all T-DNA of interest (null-segregants). For all constructs described in Example 10 to Example 14, and where indicated, a non-destructive analysis of VLC-PUFA production was performed. To this end, T1 seeds were germinated in the dark for three days on wet filter paper. After three days, one of the two cotyledons was cut off to subject it to lipid analysis as described in Example 8: Lipid extraction and lipid analysis of plant oils, the other cotyledon, including the hypocotyl and root, was planted in soil. As an example, the result from the lipid content analysis of these cotyledons from segregating T1 seedlings of event LANPMZ obtained from the construct combination described in Example 11 are shown in Figure 22; the results of event LBDIHN obtained from the construct combination described in Example 15 are shown in Figure 23. In both of these figures, it is observed that one quarter of the seed produce a significant amount of VLC-PUFA, while producing wildtype levels of Oleic acid. One can furthermore see in both figures two additional clusters of seedlings, see Figure 23. Counting the number of seed in these respective clusters, a 1:2:1 segregation ratio was observed for the clusters that produce (~0 VLC-PUFA): (medium level of VLC-PUFAs): (high level of VLC-PUFAs). The observations demonstrate a relationship between 'gene dosage', that was the number of T-DNA copies present in the genome, and VLC-PUFA levels. For all constructs described in Example 10 to Example 13, and where indicated, this relationship was exploited to identify T1 plants where at least one T-DNA locus has become homozygous, or where multiple T-DNA integration loci are at least present, or some are homozygous while others still segregate. The applicability of this method can be demonstrated for event LANPMZ, see Figure 22, all T1 seeds of event LANPMZ are capable of producing EPA and DHA. As this requires the presence of both T-DNAs, it can be concluded that at least one copy of the T-DNA of VC-LJB2197-1qcz and one copy of the T-DNA of VC-LLM337-1qcz rc have inserted into the genome, likely at the same locus. 13 T1 seedlings of those 288 seedlings of event LANPMZ

having the highest VLC-PUFA levels have been selected and have been grown to mature plants. Copy number analysis on those 13 selected plants shown in Table 40 indicates that both T-DNAs are present in a single copy, and comparison of the T0 plant copy number results against the average result of the 13 T1 plants demonstrates that these single T-DNA insertions are homozygous (duplicated copy number). All results combined provide the information that the event LANPMZ contains the T-DNAs of construct VC-LJB2197-1qcz and the T-DNA of construct VC-LLM337-1qcz rc in one copy each, whereby both T-DNAs co-segregate in a single locus.

For a single T-DNA integration into the genome, 1 out of 4 T1 seed are expected to be homozygous for that T-DNA integration. For each additional T-DNA integration, just one quarter of all seed homozygous for all other T-DNA integrations are homozygous for the additional T-DNA integration, consequently for two T-DNA integration events into the genome 1 out of 16 T1 seed are expected to be homozygous for both T-DNA integration; for three T-DNA integration into the genome 1 out of 64 T1 seed are expected to be homozygous for all three T-DNA integration; for four T-DNA integration into the genome 1 out of 256 T1 seed are expected to be homozygous for all four T-DNA integration; and so forth. All plants Example 10 to Example 14 contain a minimum of two T-DNA insertion events (one from each plasmid) in order for the plant to contain all the necessary genes to generate all the required enzymes to reconstitute the PUFA pathway sufficiently to generate the VLC-PUFAs: DHA and EPA as well as ARA.

Example 10: Plants containing the T-DNAs of plasmid VC-LJB2197-1qcz and VC-LLM306-1qcz rc (combination A in example 5) for production of EPA and DHA in seeds

In this example, the genetic elements required for EPA and DHA synthesis were transferred into the plant genome on two different T-DNAs. To this end, the two different plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc containing two different T-DNAs where cloned into agrobacteria, and plant tissue was incubated according Example 5 at the same time with these two agrobacterial cultures that are identical apart from containing either VC-LJB2197-1qcz or VC-LLM306-1qcz rc. Due to the selectable herbicide resistance marker, regenerated plants contained at least the T-DNA of VC-LJB2197-1qcz. Only those plants were kept, that also contained the T-DNA of plasmid VC-LLM306-1qcz rc as confirmed by PCR, conducted as described in Example 24, which contains a description of PCR used for gene expression and gene copy number determination. Only plants containing both the T-DNA of plasmid VC-LJB2197-1qcz as well as the T-DNA of plasmid VC-LLM306-1qcz rc contain all the genetic elements required for EPA and DHA synthesis in seeds. The genetic elements of VC-LJB2197-1qcz, and the function of each of the elements, are listed in Table 1. The genetic elements of VC-LLM306-1qcz rc, and the function of each of the elements, are listed in Table 3. For convenience, all enzymes expressed in seeds of plants carrying

both T-DNA of VC-LJB2197-1qcz and VC-LLM306-1qcz rc that are required for EPA and DHA synthesis are additionally listed in Table 19.

Table 19: Combined list of useful genes of EPA and DHA synthesis carried by the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc.

Genes encoding enzymes for EPA and DHA synthesis	Plasmid containing T-DNA with the gene	Length (bp)	Enzymatic function and source of encoded protein
c-d12Des(Ps_GA)	VC-LJB2197-1qcz	1196	Delta-12 desaturase from *Phythophthora sojae*
c-d6Des(Ot_febit)	VC-LJB2197-1qcz	1370	Delta-6 desaturase from *Ostreococcus tauri*
c-d6Des(Ot_febit)	VC-LLM306-1qcz rc	1370	Delta-6 desaturase from *Ostreococcus tauri*
c-d6Elo(Pp_GA2)	VC-LJB2197-1qcz	872	Delta-6 elongase from *Physcomitrella patens*
c-d6Elo(Tp_GA2)	VC-LJB2197-1qcz	818	Delta-6 elongase from *Thalassiosira pseudonana*
c-d5Des(Tc_GA2)	VC-LJB2197-1qcz	1319	Delta-5 desaturase from *Thraustochytrium* sp. ATCC21685
c-o3Des(Pi_GA2)	VC-LLM306-1qcz rc	1085	Omega-3-desaturase from *Phythophthora infestans*
c-o3Des(Pir_GA)	VC-LLM306-1qcz rc	1091	Omega-3 desaturase from *Pythium irregulare*
c-d5Elo(Ot_GA3)	VC-LLM306-1qcz rc	902	Delta-5 elongase from *Ostreococcus tauri*
c-d4Des(Eg_GA)	VC-LLM338-3qcz rc	1625	Delta-4 desaturase from *Euglena gracilis*
c-d4Des(Tc_GA)	VC-LLM306-1qcz rc	1559	Delta-4 desaturase from *Thraustochytrium* sp.

Fatty acid profiles, copy number measurements, and phenotypic observations of T0 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc cultivated in greenhouses during the summer.

The data in Table 20, Table 21 and Table 22 show that there was an increase in DHA and EPA content when comparing one versus two copies of each of the T-DNAs (VC-LJB2197-1qcz and VC-LLM306-1qcz rc) in the plant. The copy number of the construct VC-LJB2197-1qcz has been determined by measuring the left border of the T-DNA in the T0 generation

and not other genetic elements along the T-DNA (see Table 20). It was possible that the 88 plants representing the single copy category do in fact contain additional T-DNA insertions, and that the 86 plant representing the double copy category might in fact lack parts of the T-DNA. Therefore, due to insufficient data to correctly classify T0 plants into "single copy" and "double copy" groups, both populations overlap. Comparisons between two and three copies of the T-DNA's revealed that there was a minimal increase in DHA and EPA, suggesting that two copies of each gene was sufficient to reach maximum performance of the VLC-PUFA biosynthesis pathway (C20 and C22 PUFAs, including, but not limited to, EPA, DHA and ARA) when considering copy numbers up to three. Of note was that the majority of insertions in this example are one or two copy events. Table 23 indicates that with respect to the PUFA pathway or the copy number of the T-DNA encoding genes of the PUFA pathway, there was no significant affect on plant morphology or development when the plant carries one, two or three copies of the T-DNA's of interest.

Table 20: Copy number measurement of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column; sc: all T0 plants where the average of all copy number assays listed in this table was 0.51-1.49, dc: all T0 plants where the average of all copy number assays listed in this table was 1.51-2.49, tc: all T0 plants where the average of all copy number assays listed in this table was 2.51-3.49.

	Copy number assays targeting the T-DNA of VC-LJB2197-1qcz. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target c-d6Des(Ot_febit) near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.							Copy number assays targeting the T-DNA of VC-LLM306-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target j-p-BnFAE_t-PvARC located near the left T-DNA border and target c-d4Des(Eg_GA) near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.						
Event	c-AHAS	j-t-E9-p3-2	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	c-d6Des(Ot_febit)	j-t-CaMV_p-LuCnl-2	j-i-Atss18_c-d6Elo(Pp_GA2)	c-d6Des(Ot_febit)	j-p-BnFAE_t-PvARC	c-d4Des(Tc_GA)	c-o3Des(Pi_GA2_SNP)	c-d5Elo(Ot_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Eg_GA)
sc (n=88)	1.2										1.1	1.2		1.1
dc (n=86)	1.6										1.8	2.4		1.7
tc (n=5)	1.9										3.4	4.2		3.2

Table 21: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 20. The number of T1 plants/events fullfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T0 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=88)	4.9 ± 0.9	0.3 ± 0.1	0.1 ± 0	2.9 ± 0.6	39.3 ± 8.2	28.4 ± 5.8	0.7 ± 4.4	5.8 ± 1.3	0.4 ± 0.6	0.1 ± 0.2	0.8 ± 0.1	0.9 ± 0.2	0.9 ± 0.6	0.3 ± 0.3	1.6 ± 0.8	0.9 ± 0.6	2.2 ± 1.7	3.3 ± 1.4	0.3 ± 0.1	0 ± 0	0.3 ± 0.3	1.3 ± 0.7	0 ± 0	0.5 ± 0.3	0.2 ± 0.3	
dc (n=86)	4.8 ± 1	0.3 ± 0.1	0.1 ± 0	3 ± 0.7	37.9 ± 9.2	27.9 ± 6.4	1.1 ± 5.7	5.8 ± 1.4	0.7 ± 0.7	0.1 ± 0.1	0.8 ± 0.2	0.9 ± 0.2	0.8 ± 0.6	0.3 ± 0.3	1.6 ± 1	1 ± 0.5	2 ± 1.6	4 ± 2.1	0.3 ± 0.1	0 ± 0	0.6 ± 0.4	1.8 ± 0.9	0 ± 0	0.8 ± 0.5	0.4 ± 0.3	

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tc (n=5)	5 ± 0.9	0.3 ± 0.1	0.1 ± 0	3.1 ± 0.7	39.1 ± 8.1	28.4 ± 4.5	0.5 ± 0.3	6.1 ± 1.5	0.9 ± 0.6	0.2 ± 0.1	0.8 ± 0.2	0.9 ± 0.2	0.7 ± 0.6	0.3 ± 0.2	2.1 ± 2.1	1.2 ± 1	1.9 ± 2.4	4 ± 2.9	0.3 ± 0.1	0 ± 0	0.7 ± 0.4	1.9 ± 1.1	0 ± 0	0.8 ± 0.5	0.8 ± 0.9	

Table 22: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 20. For each category, the fatty acid profile of the plant/event having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=1)	4.9	0.2	0.0	3.5	23.1	34.2	0.2	5.4	0.4	0.2	0.8	0.9	2.5	1.1	3.0	2.4	3.5	13.5	0.3	0.0	0.0	0.0	0.0	0.0	0.0	
dc (n=1)	7.1	0.0	0.0	4.5	3.2	44.5	1.5	0.3	0.0	0.0	1.0	1.1	2.3	0.5	9.8	1.8	0.0	12.6	0.4	0.0	1.3	4.9	0.0	2.8	0.6	
tc (n=1)	3.4	0.1	0.0	2.8	29.4	27.4	0.6	6.6	1.0	0.3	0.7	0.9	1.1	0.5	1.5	1.6	2.6	10.4	0.3	0.0	1.1	5.1	0.0	1.9	0.5	

Table 23: Phenotypic rating of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events were grouped into the categories indicated in the first column as described in Table 20. The number of T1 plants/events fullfilling these criteria are displayed in parentheses. DFF: days to first flower (days), DF: deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoI: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad)

Category of T0 plants	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC
sc (n=88)	68.6 ± 9.2	1 ± 0	1.5 ± 1.4	2.2 ± 1.7	3.1 ± 3.1	3 ± 0	3 ± 0	5 ± 0	3.3 ± 2.5	3.8 ± 0.6	115.9 ± 12.6	3.6 ± 0.4	4.1 ± 1.9
dc (n=86)	69.4 ± 8.8	1 ± 0	1.5 ± 1.1	2.2 ± 1.5	2.9 ± 2.7	3 ± 0	3 ± 0.2	5 ± 0	2.9 ± 2.3	3.8 ± 0.6	115.8 ± 10.2	3.7 ± 0.4	4.5 ± 1.8
tc (n=5)	72.2 ± 8.2	1 ± 0	1 ± 0	1.6 ± 1.3	2.4 ± 0.9	3 ± 0	3 ± 0	5 ± 0	2.4 ± 1.5	4 ± 0	117 ± 9.7	4 ± 0.5	4 ± 0.8

Fatty acid profiles, copy number measurements, and phenotypic observations of T1 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc cultivated in greenhouses during winter

The copy number analysis indicates that LALTHK was homozygous for two copies of the VC-LJB2197-1qcz T-DNA and homozygous for one copy of the VC-LLM306-1qcz rc while LALJCX was homozygous for two copies of both T-DNAs (VC-LJB2197-1qcz and VC-LLM306-1qcz rc). The other events were still partially segregating for the T-DNAs but contained at least one copy of each T-DNA in all plants. Event LALTHK had no accumulation of DHA, which illustrates the effects of truncations which can occur during insertion of the T-DNA. The events, with the exception of LALTHK, are, within error, similar to one another in terms of EPA+DHA accumulation. The similarity in copy numbers of the events, see Table 23, indicates that insertion site effects that could enhance or repress gene expression are impacting all events equally. The lack of significant variation in EPA and DHA accumulation suggests that there may be a buffering effect in the construct design, such that the T-DNA's integrate into the genome in a manner that minimizes positional effects in gene expression in the T-DNA. The event with the highest VLC-PUFA accumulation, in particular EPA and DHA, was LALFWA which had a maximum accumulation of 4.2 percent DHA and 16 percent EPA with respect to total fatty acid content in the mature seed, but on the average was similar to the other events.

Table 24: Copy number measurement of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where measured per event. The T1 plants underwent a selection from 250 segregating T1 seedlings using half-kernel analysis, where the correlation of VCL-PUFA levels with copy number was employed to select for homozygous plants, or on case of multilocus events to selecect for plants where one or more loci are homozygous. A copy number of ~2 therefore was indicative for one homozygous locus, a copy number of ~4 indicative for two homozygous loci or indicative for one homozygous locus containing two copies of the target gene measured by the assay, and so forth. Odd results of 3 and 5 indicate that at least some of the selected T1 plants carry a heterozygous locus.

	Copy number assays targeting the T-DNA of VC-LJB2197-1qcz. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target c-d6Des(Ot_febit) near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.							Copy number assays targeting the T-DNA of VC-LLM306-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target j-p-BnFAE_ t-PvARC located near the left T-DNA border and target c-d4Des(Eg_GA) near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.						
Event	c-AHAS	j-t-E9-p3-2	c-d6Elo (Tp_GA)	j-t-StCAT_p2_p-LuPXR	c-d6Des(Ot_febit)	j-t-CaMV_p-LuCnl-2	j-i-Atss18_c-d6Elo(Pp_GA2)	c-d6Des(Ot_febit)	j-p-BnFAE_t-PvARC	c-d4Des(Tc_GA)	c-o3Des(Pi_GA2_SNP)	c-d5Elo(Ot_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Eg_GA)
LALJAU (n=18)	3.1 (T0: 2)									1.3	(T0: 1.1)			
LALFWA (n=16)	3.1 (T0: 1.8)									2.6	(T0: 1.9)			(T0: 1.9)
LALJDF (n=17)	3.2 (T0: 2)									3.3	(T0: 2)			
LALTHK (n=15)	3.8 (T0: 2.1)									2.1	(T0: 1.1)			
LALTLE (n=18)	3.4 (T0: 2.8)									2.4	(T0: 2.4)			
LALIVY (n=16)	3 (T0: 1.9)									3.1	(T0: 2.1)			
LALICX (n=16)	3.4 (T0: 1.9)									3.4	(T0: 1.8)			

Table 25: Fatty acid profiles of T2 seeds harvested from T1 cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc The events are indicated in the first column, along with the number of T2 seed batches that were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1n-7	16:3n-3	18:0	18:1n-9	18:2n-6	18:2n-9	18:3n-3	18:3n-6	18:4n-3	18:4n 20:0	20:1n-9	20:2n-6	20:3n-3	20:3n-6	20:4n-3	20:4n-6	20:5n-3	22:0	22:1n-9	22:4n-6	22:5n-3	22:5n-6	22:6n-3	unknown peak a	unknown peak b

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LALJAU (n=18)	4.5 ± 0.1	0.1 ± 0	0 ± 0	3.6 ± 0.4	21.5 ± 1.8	31.6 ± 0.9	0.8 ± 0.2	3.3 ± 0.3	1.9 ± 0.7	0.2 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.8 ± 0.4	0.1 ± 0.2	3.7 ± 0.6	1.6 ± 0.4	8.6 ± 2.7	9.6 ± 1.4	0.3 ± 0	0 ± 0	0.8 ± 0.2	3.3 ± 0.6	0 ± 0	1.7 ± 0.5	0.1 ± 0.2					
LALFWA (n=16)	4.5 ± 0.5	0.1 ± 0	0 ± 0	3.5 ± 0.5	20 ± 2	30 ± 2.1	1 ± 0.1	3.8 ± 0.4	2.6 ± 0.3	0.4 ± 0.1	0.8 ± 0.1	0.6 ± 0	0.2 ± 0.1	0 ± 0	3.7 ± 0.4	1.9 ± 0.4	5.6 ± 2.3	11.6 ± 2.2	0.2 ± 0.1	0 ± 0	1.4 ± 0.3	4.7 ± 1.1	0 ± 0	2.6 ± 0.8	0.5 ± 0.4	0.4 ± 0				
LALDF (n=17)	4.3 ± 0.7	0.2 ± 0.1	0 ± 0.1	3.3 ± 0.5	20.7 ± 4.1	29.3 ± 4	2.5 ± 5.9	4.1 ± 0.4	2.4 ± 0.5	0.7 ± 1.1	0.8 ± 0.1	0.7 ± 0.1	0.3 ± 0.1	0 ± 0	2.7 ± 0.4	1.7 ± 0.3	4.6 ± 0.6	13.1 ± 3	0.3 ± 0	0 ± 0	0.9 ± 0.3	4.7 ± 1.2	0.1 ± 0.3	2.2 ± 0.6	0.2 ± 0.2	0.2 ± 0				
LALTHK (n=15)	4.7 ± 0.6	0.1 ± 0.1	0 ± 0	3.7 ± 0.4	21.4 ± 1.8	32.9 ± 1.9	0 ± 0.1	4.9 ± 0.6	0.6 ± 0.1	1 ± 0 ± 0	0.1	0.9 ± 0.1	1.7 ± 0.3	0.7 ± 0.2	2.2 ± 0.3	1.6 ± 0.2	4.3 ± 0.8	18.9 ± 2.5	0.4 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0					
LALTLE (n=18)	4.6 ± 0.6	0.1 ± 0	0 ± 0	3.6 ± 0.4	24.1 ± 4.5	29.8 ± 2.2	1 ± 0.3	4.2 ± 0.5	2 ± 0.6	0.3 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.3 ± 0.3	0 ± 0.1	3 ± 0.2	2 ± 0.3	3.8 ± 0.9	10.9 ± 3	0.1 ± 0.1	0 ± 0	1.4 ± 0.6	4.7 ± 1.1	0 ± 0	2.3 ± 0.8	0.2 ± 0.3	0.6 ± 0.1				
LALIVY (n=16)	5.4 ± 1	0.2 ± 0.1	0 ± 0	3.6 ± 0.4	21.6 ± 2.9	30.9 ± 2	0.9 ± 0.2	4.4 ± 0.5	2.1 ± 0.5	0.3 ± 0.1	0.8 ± 0.1	0.6 ± 0.2	0.3 ± 0.2	0.1 ± 0.1	2.8 ± 0.2	1.9 ± 0.3	4.2 ± 1.2	10.4 ± 1.8	0.3 ± 0.1	0 ± 0	1.3 ± 0.3	4.4 ± 0.9	0 ± 0	2.4 ± 0.6	0.6 ± 0.2	0.5 ± 0.3				
LALICX (n=16)	5 ± 0.1	0.2 ± 0	0 ± 0	3.4 ± 0.2	23.9 ± 1.6	32.4 ± 1.1	1.1 ± 0.1	4.1 ± 0.4	2.5 ± 0.3	0.4 ± 0	0.8 ± 0	0.7 ± 0	0.2 ± 0	0 ± 0	2.5 ± 0.7	1.3 ± 0.3	4.2 ± 0.3	9.9 ± 0.7	0.3 ± 0	0 ± 0	1.1 ± 0.2	3.8 ± 0.3	0 ± 0	1.7 ± 0.2	0.4 ± 0.2					

Table 26: Fatty acid profiles of one T2 seed batch per event harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T2 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1n -7	16:3n -3	18:0	18:1n -9	18:2n -6	18:2n -9	18:3n -3	18:3n -6	18:4n -3	20:0	20:1n -9	20:2n -6	20:3n -3	20:3n -6	20:4n -3	20:4n -6	20:5n -3	22:0	22:1n -9	22:4n -6	22:5n -3	22:5n -6	22:5n -3	22:6n -3	unkn own peak a	unkn own peak b
LALJAU (n=1)	4.9	0.2	0.0	4.7	18.8	30.6	1.1	3.1	3.6	0.4	1.0	0.6	0.1	0.0	2.4	1.1	5.7	12.6	0.4	0.0	1.3	4.4	0.0	2.8	0.0	0.0
LALFWA (n=1)	3.8	0.1	0.0	3.2	16.1	26.2	1.2	4.4	3.0	0.5	0.7	0.7	0.3	0.1	2.9	2.4	3.6	16.0	0.1	0.0	1.9	7.4	0.0	4.2	0.7	0.4
LALIDF (n=1)	3.5	0.1	0.1	2.8	15.8	27.7	1.2	3.7	3.6	0.5	0.7	0.7	0.3	0.0	2.6	1.9	5.5	18.0	0.3	0.0	1.4	6.2	0.0	3.2	0.1	0.2
LALTHK (n=1)	4.7	0.0	0.0	4.2	18.6	30.7	0.0	4.6	0.6	0.0	1.1	0.9	2.0	1.0	2.2	1.7	4.6	22.7	0.4	0.0	0.0	0.0	0.0	0.0	0.0	
LALTLE (n=1)	3.3	0.2	0.1	2.8	15.0	24.9	1.5	3.3	3.3	0.5	0.7	0.6	0.5	0.0	3.1	2.2	5.7	17.6	0.3	0.0	2.4	6.9	0.0	4.1	0.5	0.5
LALIVY (n=1)	5.9	0.3	0.0	3.9	16.5	26.5	0.9	4.2	2.3	0.5	0.9	0.7	0.5	0.3	2.6	2.2	3.9	14.2	0.4	0.0	1.7	6.7	0.0	4.2	0.6	0.0
LALICX (n=1)	6.1	0.7	0.2	2.3	22.0	15.1	15.3	6.6	2.5	0.5	0.6	0.6	0.5	0.0	1.2	0.9	4.6	10.0	0.4	0.0	0.8	5.5	0.0	3.5	0.0	0.0

Table 27: Phenotypic rating of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where rated per event. DFF: days to first flower (days), DF: deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique

(9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoI: number of lobes(#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad),

Event	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC
LALJAU (n=18)	62.2 ± 2.3	1 ± 0	1 ± 0	1 ± 0	1.9 ± 1	3 ± 0	3 ± 0	5 ± 0	1.3 ± 0.7	4 ± 0	129.7 ± 5.5	4.7 ± 0.2	2.1 ± 1
LALFWA (n=16)	61.6 ± 3.3	1 ± 0	1.2 ± 0.8	1 ± 0	3.5 ± 2.4	3 ± 0	3 ± 0	5 ± 0	3.1 ± 2.6	3.9 ± 0.5	127.7 ± 6.2	4.9 ± 0.4	3.5 ± 1.8
LALJDF (n=17)	58.7 ± 2.6	1 ± 0	1 ± 0	1 ± 0	1.6 ± 0.8	3 ± 0	3 ± 0	5 ± 0	1.2 ± 0.6	4 ± 0	128 ± 6.2	5 ± 0.2	2.4 ± 0.6
LALTHK (n=15)	62.1 ± 5.5	1 ± 0	1 ± 0	1 ± 0	1.7 ± 1.1	3 ± 0	3 ± 0	5 ± 0	1.1 ± 0.5	4 ± 0	124.3 ± 5	4.8 ± 0.3	3.1 ± 1.2
LALTLE (n=18)	67.8 ± 6.7	1 ± 0	1 ± 0	1 ± 0	1.7 ± 0.8	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	126.7 ± 5.2	4.4 ± 0.3	4.2 ± 1.7
LALIVY (n=16)	69.5 ± 6.2	1 ± 0	2.7 ± 1.4	1.3 ± 0.6	2.4 ± 1.4	3 ± 0	3 ± 0	5 ± 0	1.6 ± 0.9	3.3 ± 1	127.1 ± 5.8	4.4 ± 0.4	4.9 ± 3.2
LALICX (n=16)	64.2 ± 2.7	1 ± 0	2.7 ± 0.7	1 ± 0	1.4 ± 0.6	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	131.4 ± 5.7	4.5 ± 0.3	2.2 ± 0.9

Fatty acid profiles, copy number measurements, and phenotypic observations of T2 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc cultivated in greenhouses during summer

Copy number analysis, see Table 28, indicates that the chosen events are largely homozygous for the genes encoded on the T-DNAs of VC-LJB2197-1qcz and VC-LLM306-1qcz rc. All constructs but LALTLE had two copies of the T-DNAs inserted into the genome. LALTLE appeared to have more than two copies with some segregation still ongoing. Based on the copy number analysis, LALJDF had integrated one copy of the d4Des(Eg_GA) gene and LALTLE was segregating for one or two copies of that gene. The EPA and DHA data in Table 29 and Table 30 indicated the events perform equally well with perhaps LALJDF accumulating less DHA, based on Table 30. Plant morphology was the same for all the events examined. As discussed in part B, the event to event variation was minimal, suggesting that the impacts of insertion site position effects (both negative and positive) were similar for all events. The lack of insertion site effects indicates that the plasmid design/T-DNA topology was exerting a normalizing effect/mitigates insertion site effects.

Table 28: Copy number measurement of T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. The events are indicated in the first column, along with the number of T2 plants that where measured per event. The T2 plants underwent a selection from 250 segregating T2 seedlings using half-kernel analysis, where the correlation of VCL-PUFA levels with copy number was employed to select for homozygous plants, or in case of multilocus events to selecect for plants where one or more loci are homozygous. A copy number of ~2 therefore was indicative for one homozygous locus, a copy number of ~4 indicative for two homozygous loci or indicative for one homozygous locus containing two copies of the target gene measured by the assay, and so forth. Odd results of 3 and 5 indicate that at least some of the selected T2 plants carry a heterozygous locus.

	Copy number assays targeting the T-DNA of VC-LJB2197-1qcz. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target c-d6Des(Ot_febit) near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.							Copy number assays targeting the T-DNA of VC-LLM306-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target j-p-BnFAE_ t-PvARC located near the left T-DNA border and target c-d4Des(Eg_GA) near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.						
Event	c-AHA5	j-t-E9-p3-2	c-d6Elo (Tp_GA)	j-t-StCAT_ p2_p-LuPXR	c-d6Des(Ot_febit)	j-t-CaMV_p-LuCnl-2	j-i-Atss18_c-d5Elo(Pp_GA2)	c-d6Des(Ot_febit)	j-p-BnFAE_ t-PvARC	c-d4Des(Tc_GA)	c-o3Des(Pi_GA2_SNP)	c-d5Elo(Ot_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Eg_GA)
LALFWA (n=30)	4.0	3.6	3.7	3.9	7.7	4.6	3.7	7.7	3.5	3.7			3.5	3.5
LALJDF (n=30)	4.2	3.9	3.9	3.8	8.5	3.3	3.5	8.5	5.1	4.1			4.0	2.2
LALTLE (n=30)	5.2	4.6	4.7	5.0	8.9	4.4	1.8	8.9	2.6	3.6			4.6	3.2
LALIVY (n=30)	4.1	3.6	3.7	3.8	8.1	4.4	5.4	8.1	3.7	4.0			3.8	3.5

Table 29: Fatty acid profiles of T3 seeds harvested from T2 cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc The events are indicated in the first column, along with the number of T3 seed batches that where measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b

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LALFWA (n=30)	4.9 ± 0.2	0.3 ± 0.1	0.2 ± 0	4.4 ± 0.2	19.9 ± 0.9	29.5 ± 0.8	1.3 ± 0.1	3.2 ± 0.2	3 ± 0.2	0.4 ± 0	0.9 ± 0.1	0.6 ± 0	0.3 ± 0	0 ± 0	3.8 ± 0.6	2.1 ± 0.3	4 ± 0.4	11.3 ± 0.6	0.3 ± 0	0 ± 0	1.7 ± 0.2	4.3 ± 0.3	0 ± 0	2.5 ± 0.2	0.7 ± 0.2	0.3 ± 0
LALJDF (n=30)	5.4 ± 0.3	0.3 ± 0	0.2 ± 0	4.4 ± 0.2	21.4 ± 0.7	30.1 ± 1.1	1.2 ± 0.1	3.8 ± 0.3	2.7 ± 0.2	0.4 ± 0	0.9 ± 0	0.6 ± 0	0.1 ± 0.1	0 ± 0	2.2 ± 0.2	1.4 ± 0.1	4.3 ± 0.2	12.4 ± 0.7	0.2 ± 0.1	0 ± 0	1 ± 0.1	4.4 ± 0.4	0 ± 0	2.2 ± 0.2	0.1 ± 0.1	0.2 ± 0.1
LALTLE (n=30)	5.2 ± 0.4	0.3 ± 0	0.2 ± 0.1	4.6 ± 0.3	20.3 ± 0.8	28.7 ± 0.8	1.3 ± 0.2	3.4 ± 0.3	2.8 ± 0.2	0.4 ± 0.1	0.8 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	2.3 ± 0.2	1.6 ± 0.2	3.9 ± 0.2	12.1 ± 0.7	0.2 ± 0.1	0 ± 0	1.9 ± 0.2	4.9 ± 0.3	0 ± 0	2.9 ± 0.4	0.5 ± 0.1	0.3 ± 0.1
LALIVY (n=30)	5.3 ± 0.6	0.3 ± 0.1	0.2 ± 0	4.8 ± 0.5	20.4 ± 1.4	29.6 ± 1	1.2 ± 0.2	3.6 ± 0.2	2.7 ± 0.3	0.4 ± 0	0.9 ± 0.1	0.6 ± 0	0.4 ± 0.1	0.1 ± 0.1	2.5 ± 0.3	1.7 ± 0.3	4 ± 0.7	11.4 ± 0.7	0.3 ± 0	0 ± 0	1.5 ± 0.1	4.5 ± 0.4	0 ± 0	2.7 ± 0.4	0.5 ± 0.2	0.3 ± 0.1
WT Kumily (n=46)	5 ± 0.1	0.4 ± 0	0.2 ± 0	2.6 ± 0.1	66.3 ± 1.7	16.8 ± 1.3	0 ± 0	6.1 ± 0.4	0 ± 0	0 ± 0	0.9 ± 0	1.2 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.5 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Table 30: Fatty acid profiles of one T3 seed batch per event harvested from T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T3 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1n-7	16:3n-3	18:0	18:1n-9	18:2n-6	18:2n-9	18:3n-3	18:3n-6	18:4n-3	20:0	20:1n-9	20:2n-6	20:3n-3	20:3n-6	20:4n-3	20:4n-6	20:5n-3	22:0	22:1n-9	22:4n-6	22:5n-3	22:5n-6	22:6n-3	unknown peak a	unknown peak b
LALFWA (n=1)	5.4	0.4	0.2	4.6	17.5	27.9	1.1	2.9	3.3	0.4	0.9	0.5	0.3	0.0	3.7	2.0	4.5	12.7	0.3	0.0	2.2	5.0	0.0	3.2	0.6	0.3
LALJDF (n=1)	5.1	0.3	0.2	4.5	19.7	28.9	1.1	3.8	2.8	0.5	0.9	0.6	0.1	0.0	2.4	1.6	4.4	13.7	0.3	0.0	1.1	5.1	0.0	2.5	0.2	0.2
LALTLE (n=1)	5.8	0.1	0.1	5.5	20.1	27.3	1.9	3.1	3.5	0.2	0.4	0.3	0.2	0.0	1.9	0.7	4.0	13.4	0.1	0.0	1.1	5.5	0.0	4.2	0.2	0.1
LALIVY (n=1)	5.5	0.4	0.2	4.4	18.4	28.6	1.1	3.4	3.3	0.4	0.9	0.5	0.3	0.0	2.2	1.4	5.3	12.8	0.3	0.0	1.8	5.0	0.0	3.4	0.2	0.2
LALHCY (n=1)	7.6	1.1	0.7	2.3	19.7	25.6	0.9	6.3	3.6	0.7	0.8	0.6	0.0	0.0	1.2	1.1	3.9	10.4	0.0	0.0	1.0	7.0	0.0	3.4	0.0	0.0

Table 31: Phenotypic rating of T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where rated per event. DFF: days to first flower (days), DF: deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoI: number of lobes(#), PH: plant height (cm), Oil: oil content (% of seed weight), Protein: Protein content (% of seed cake without oil)

Event	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	Oil	Protein

LALFWA (n=30)	57.3 ± 2.2	9 ± 0	8.9 ± 0.4	8.9 ± 0.4	7.2 ± 1	3 ± 0	3 ± 0	5 ± 0	8 ± 1.2	3.9 ± 0.4	126.8 ± 4.3				
LALIDF (n=30)	58.1 ± 2.5	9 ± 0	9 ± 0	9 ± 0	7.7 ± 0.5	3 ± 0	3 ± 0	5 ± 0	8.2 ± 0.9	4 ± 0	130.2 ± 0.9				
LALTLE (n=30)	59.9 ± 2.8	9 ± 0	8.1 ± 1.3	8.8 ± 0.5	7.2 ± 0.9	3 ± 0	3 ± 0	5 ± 0	7.7 ± 1.3	3.9 ± 0.5	128 ± 2.8				
LALIVY (n=30)	59.6 ± 2.9	9 ± 0	8.8 ± 0.9	8.6 ± 1	6.8 ± 1.3	3 ± 0	3 ± 0	5 ± 0	7.3 ± 1.7	3.8 ± 0.6	126.8 ± 3.3				
WT Kumily (n=46)												34.9 ± 1.1	32.2 ± 1		

Fatty acid profiles, copy number measurements, and phenotypic observations of T2 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc cultivated in field trials in USDA growth zones 3a-4b and 5a during summer.

The field data on Table 32 and Table 33 indicate a consistent performance across generations for T2 and T3. The data show that the PUFA (EPA and DHA) accumulation was higher for greenhouse grown plants. Besides levels in VLC-PUFA, there was also a difference in seed oil content observed compared to the greenhouse (e.g. comparing Table 34 and Table 31). Results of this analysis are described in Example 20. The field data also demonstrate that the greenhouse data accurately indicated the consistency between events with respect to EPA and DHA accumulation, though not overall levels. As remarked upon in part C, the event to event variation was low for this construct, indicating that the T-DNAs/T-DNA design seem to be exerting a buffering/mitigating effect on gene silencing and other positional effects.

Table 32: Fatty acid profiles of T3 seeds harvested from T2 cultivated in the field corresponding to USDA growth zones 3a-4b and 5a. Field trials of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc are given below. The events are indicated in the first column, along with the number of T3 seed aliquots representing a plot were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LALFWA (n=30)	5.1 ± 0.2	0.3 ± 0.1	0.1 ± 0	2.6 ± 0.2	25.1 ± 1.8	30.8 ± 1.4	1.1 ± 0.1	5.9 ± 0.9	1.8 ± 0.2	0.3 ± 0.1	0.7 ± 0	0.7 ± 0	0.4 ± 0.1	0.1 ± 0.1	2.6 ± 0.3	1.4 ± 0.2	4.4 ± 1.2	8.5 ± 1.2	0.3 ± 0	0 ± 0	1.2 ± 0.2	4.4 ± 0.5	0 ± 0	1.4 ± 0.2	0.5 ± 0.2	0.3 ± 0.1
LALJDF (n=30)	5.1 ± 0.3	0.3 ± 0	0.1 ± 0	2.7 ± 0.2	24.4 ± 1.7	29.4 ± 1.5	1.2 ± 0.1	5.6 ± 0.4	1.8 ± 0.2	0.3 ± 0	0.7 ± 0	0.7 ± 0	0.4 ± 0.1	0.1 ± 0	2.2 ± 0.1	1.3 ± 0.1	4.5 ± 0.4	11.1 ± 0.9	0.2 ± 0.1	0 ± 0	0.9 ± 0.1	5.1 ± 0.4	0 ± 0	1.4 ± 0.2	0.1 ± 0.1	0.4 ± 0.1
LALTLE (n=30)	5.4 ± 0.2	0.3 ± 0.1	0.1 ± 0	2.7 ± 0.2	26.4 ± 3.2	28.1 ± 1	1.2 ± 0.2	5.6 ± 0.6	1.7 ± 0.3	0.3 ± 0.1	0.7 ± 0	0.7 ± 0	0.5 ± 0.2	0 ± 0.1	2.1 ± 0.2	1.2 ± 0.1	3.9 ± 0.5	9.6 ± 1.2	0.2 ± 0.1	0 ± 0	1.5 ± 0.2	5.3 ± 0.6	0 ± 0	1.5 ± 0.2	0.4 ± 0.2	0.5 ± 0.1
LALIVY (n=29)	5.2 ± 0.4	0.3 ± 0.1	0.1 ± 0	2.9 ± 0.3	26.7 ± 3.1	28.3 ± 2.2	1 ± 0.2	6.3 ± 0.6	1.4 ± 0.3	0.3 ± 0	0.7 ± 0.1	0.8 ± 0.1	0.6 ± 0.2	0.2 ± 0.1	2.1 ± 0.2	1.4 ± 0.1	3.3 ± 0.5	9.2 ± 1.1	0.3 ± 0.1	0 ± 0	1.3 ± 0.1	5 ± 0.5	0 ± 0	1.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.1
WT Kumily (n=60)	5 ± 0.3	0.4 ± 0	0.1 ± 0.1	2 ± 0.1	56.2 ± 4.2	23.3 ± 1.7	0 ± 0.1	9.5 ± 0.7	0.1 ± 0.2	0 ± 0	0.7 ± 0	1.1 ± 0.1	0.1 ± 0.1	0 ± 0	0.2 ± 0.3	0.1 ± 0.3	0.2 ± 0.5	0.4 ± 1.1	0.3 ± 0.1	0 ± 0	0 ± 0.2	0.2 ± 0.6	0 ± 0	0 ± 0.2		

Table 33: Fatty acid profiles of one T3 seed batch per event harvested from T2 plants cultivated in USDA growth zones 3a-4b and 5a field trials of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T3 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1n -7	16:3n -3	18:0	18:1n -9	18:2n -6	18:2n -9	18:3n -3	18:3n -6	18:4n -3	20:0	20:1n -9	20:2n -6	20:3n -3	20:3n -6	20:4n -3	20:4n -6	20:5n -3	22:0	22:1n -9	22:4n -6	22:5n -3	22:5n -6	22:6n -3	unknown peak a	unknown peak b
LALFWA (n=1)	5.2	0.3	0.1	2.6	25.0	28.2	1.1	5.0	2.1	0.4	0.6	0.6	0.3	0.0	2.7	1.4	4.5	10.5	0.3	0.0	1.4	5.2	0.0	1.8	0.4	0.3
LALJDF (n=1)	5.7	0.3	0.1	2.7	23.1	23.6	1.3	6.0	2.3	0.4	0.8	0.8	0.4	0.0	2.3	1.3	5.4	13.5	0.3	0.0	1.0	6.3	0.0	1.8	0.0	0.3
LALTLE (n=1)	5.5	0.3	0.1	2.6	24.9	26.7	1.3	4.7	2.1	0.4	0.7	0.6	0.4	0.1	2.1	1.2	5.0	10.9	0.3	0.0	1.8	5.8	0.0	1.9	0.2	0.5
LALIVY (n=1)	5.9	0.2	0.1	3.5	24.5	23.7	1.3	5.6	2.1	0.4	0.9	0.8	0.5	0.0	2.5	1.4	4.8	11.3	0.3	0.0	1.7	5.9	0.0	1.9	0.4	0.4

Table 34: Phenotypic rating of T2 plants cultivated in the field of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. The events are indicated in the first column, along with the number of field plots that where rated per event. Oil: oil content (% of seed weight), protein: Protein content (% of seed cake without oil)

Event	Oil	protein
LALFWA (n=30)	38.2 ± 1	27.9 ± 0.6
LALJDF (n=30)	37.6 ± 1.2	27.9 ± 0.8
LALTLE (n=30)	38.2 ± 0.9	27.5 ± 0.7
LALIVY (n=29)	37.6 ± 0.6	27.8 ± 0.5
WT Kumily (n=60)	38.7 ± 1.1	

Example 11: Plants containing the T-DNAs of plasmid VC-LJB2197-1qcz and VC-LLM337-1qcz rc (combination B in example 5) for production of EPA and DHA in seeds

In this example, the genetic elements required for EPA and DHA synthesis were transferred into the plant genome on two different T-DNAs. To this end, the two different plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc containing two different T-DNAs were cloned into agrobacteria, and plant tissue was incubated according to Example 5 at the same time with these two agrobacterial cultures that were identical apart from containing either VC-LJB2197-1qcz or VC-LLM337-1qcz rc. Due to the selectable herbicide resistance marker, regenerated plants contained the T-DNA of VC-LJB2197-1qcz. Only those plants were kept, that also contained the T-DNA of plasmid VC-LLM337-1qcz rc as confirmed by PCR, conducted as described in Example 24, which contains PCR protocols for both gene expression and copy number analysis. Only plants containing the T-DNA of plasmid VC-LJB2197-1qcz as well as the T-DNA of plasmid VC-LLM337-1qcz rc combined all the genetic elements required for EPA and DHA synthesis in seeds. The genetic elements of VC-LJB2197-1qcz and the function of each element were listed in Table 1. The genetic elements of VC-LLM337-1qcz rc and the function of each element were listed in Table 4. For convenience, all enzymes expressed in seeds of plants carrying both T-DNA of VC-LJB2197-1qcz and VC-LLM337-1qcz rc that were required for EPA and DHA synthesis are additionally listed on Table 35.

Table 35: Combined list of genes essential of EPA and DHA synthesis carried by the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc.

Genes encoding enzmyes for EPA and DHA synthesis	Plasmid containing T-DNA with the gene	Length	Enzymatic function and source of encoded protein
c-d12Des(Ps_GA)	VC-LJB2197-1qcz	1196	Delta-12 desaturase from *Phythophthora sojae*
c-d6Des(Ot_febit)	VC-LJB2197-1qcz	1370	Delta-6 desaturase from *Ostreococcus tauri*
c-d6Elo(Pp_GA2)	VC-LJB2197-1qcz	872	Delta-6 elongase from *Physcomitrella patens*
c-d6Elo(Tp_GA2)	VC-LJB2197-1qcz	818	Delta-6 elongase from *Thalassiosira pseudonana*
c-d5Des(Tc_GA2)	VC-LJB2197-1qcz	1319	Delta-5 desaturase from *Thraustochytrium* sp. ATCC21685

Genes encoding enzmyes for EPA and DHA synthesis	Plasmid containing T-DNA with the gene	Length	Enzymatic function and source of encoded protein
c-o3Des(Pi_GA2)	VC-LLM337-1qcz rc	1085	Omega-3-desaturase from *Phythophthora infestans*
c-o3Des(Pir_GA)	VC-LLM337-1qcz rc	1091	Omega-3 desaturase from *Pythium irregulare*
c-d5Elo(Ot_GA3)	VC-LLM337-1qcz rc	902	Delta-5 elongase from *Ostreococcus tauri*
c-d4Des(Eg_GA)	VC-LLM337-1qcz rc	1625	Delta-4 desaturase from *Euglena gracilis*
c-d4Des(Tc_GA)	VC-LLM337-1qcz rc	1559	Delta-4 desaturase from *Thraustochytrium* sp.

Fatty acid profiles, copy number measurements, and phenotypic observations of T0 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc cultivated in greenhouses during summer

Table 34 indicates that the T-DNA integrated as predominantly single and double copies. As observed in Example 10 there was an increase in EPA and DHA between one copy and two copies of the T-DNA from the two constructs, see Table 36, but less of a difference between two and three copies. The T1 data in Table 37 reflects this as well. As noted in Example 10, there was no observed alteration of the phenotype of the plants bearing the T-DNA from both constructs, regardless of copy number (up to three).

Table 36: Copy number measurement of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column; sc: all T1 plants where the average of all copy number assays listed in this table was 0.51-1.49, dc: all T1 plants where the average of all copy number assays listed in this table was 1.51-2.49, tc: all T1 plants where the average of all copy number assays listed in this table was 2.51-3.49.

Category of T0 plants	Copy number assays targeting the T-DNA of VC-LJB2197-1ccz. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target j-i-Atss18_c-d6Elo(Pp_GA2) near the right T-DNA border.						Copy number assays targeting the T-DNA of VC-LLM337-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target j-t-PvARC-p3 located near the left T-DNA border and target c-d4Des(Eg_GA) near the right T-DNA border.					
	c-AHAS	j-t-E9-p3-2	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-t-CaMV_p-LuCnl-2	c-d6Elo(Pp_GA)	j-i-Atss18_c-d6Elo(Pp_GA2)	j-t-PvARC-p3	c-d4Des(Tc_GA)	c-o3Des(Pl_GA2_SNP)	j-i-Atss15_c-o3Des(Pl_GA2)	c-o3Des(Plr_GA)	j-p-VfSBPperm3_c-o3Des(Plr_GA)	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Eg_GA)
sc (n=563)	1.1					1.1			1.2	1.2	1.2	1.2			1.2
dc (n=423)	1.4					1.6			2.0	1.9	2.0	1.8			2.2
tc (n=17)	2.0					2.6			2.8	1.8	3.2	2.4			3.8

Table 37: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 36. The number of T1 plants/events fullfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=563)	5.1 ± 0.6	0.3 ± 0.1	0.1 ± 0	3 ± 0.5	40.6 ± 6.2	29.4 ± 4.2	0.7 ± 4.7	6 ± 1.1	0.5 ± 0.6	0.1 ± 0.2	0.8 ± 0.1	0.9 ± 0.2	0.9 ± 0.6	0.3 ± 0.3	1.6 ± 0.8	0.9 ± 0.5	2.1 ± 1.3	3.4 ± 1.2	0.3 ± 0.1	0 ± 0	0.4 ± 0.3	1.5 ± 0.5	0 ± 0	0.6 ± 0.3	0.3 ± 0.3	
dc (n=423)	5 ± 0.8	0.3 ± 0.1	0.1 ± 0	3.1 ± 0.6	38.6 ± 8.2	28.8 ± 5.3	1.1 ± 5.9	5.9 ± 1.2	0.7 ± 0.6	0.1 ± 0.1	0.8 ± 0.1	0.9 ± 0.2	0.8 ± 0.6	0.3 ± 0.3	1.7 ± 0.9	1 ± 0.5	2.1 ± 1.5	4.3 ± 2	0.3 ± 0.1	0 ± 0	0.6 ± 0.4	1.9 ± 0.9	0 ± 0	0.8 ± 0.5	0.4 ± 0.3	
tc (n=17)	5 ± 0.9	0.3 ± 0.1	0.1 ± 0	3.1 ± 0.7	39.1 ± 8.1	28.4 ± 4.5	0.5 ± 0.3	6.1 ± 1.5	0.9 ± 0.6	0.2 ± 0.1	0.8 ± 0.2	0.9 ± 0.2	0.7 ± 0.6	0.3 ± 0.2	2.1 ± 2.1	1.2 ± 1	1.9 ± 2.4	4 ± 2.9	0.3 ± 0.1	0 ± 0	0.7 ± 0.4	1.9 ± 1.1	0 ± 0	0.8 ± 0.5	0.8 ± 0.9	

Table 38: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 36. For each category, the fatty acid profile of the plant/event having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=1)	3.6	0.1	0.1	2.8	37.6	22.1	0.8	6.4	0.5	0.3	0.8	1.2	1.1	0.6	1.8	2.1	2.9	9.0	0.4	0.0	0.5	3.5	0.0	1.6	0.3	
dc (n=1)	7.1	0.0	0.0	4.5	3.2	1.5	44.5	0.3	0.0	0.0	1.0	1.1	2.3	0.5	9.8	1.8	0.0	12.6	0.4	0.0	1.3	4.9	0.0	2.8	0.6	
tc (n=1)	3.4	0.1	0.0	2.8	29.4	27.4	0.6	6.6	1.0	0.3	0.7	0.9	1.1	0.5	1.5	1.6	2.6	10.4	0.3	0.0	1.1	5.1	0.0	1.9	0.5	

Table 39: Phenotypic rating of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 36. The number of T1 plants/events fullfilling these criteria are displayed in parentheses. DFF: days to first flower (days), DF: deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed,

1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoI: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad)

Category of T1 plants	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC
sc (n=563)	66.1 ± 8.3	1 ± 0	1.4 ± 1.1	1.7 ± 1.6	3 ± 2.8	3 ± 0	3 ± 0.1	5 ± 0	3.2 ± 2.6	3.8 ± 0.6	119.5 ± 9.8	4.7 ± 0.6	4.8 ± 2.5
dc (n=423)	66 ± 7.5	1 ± 0	1.4 ± 1.2	1.7 ± 1.4	3 ± 2.9	3 ± 0	3 ± 0.1	5 ± 0.1	3.1 ± 2.6	3.7 ± 0.7	119.2 ± 9.5	4.6 ± 0.6	5 ± 2.3
tc (n=17)	64.6 ± 7.4	1 ± 0	1.5 ± 1.5	2.8 ± 2.5	4.4 ± 3.7	3 ± 0	3 ± 0	5 ± 0	4.5 ± 3.5	3.9 ± 0.5	117.1 ± 10.5	4.6 ± 0.4	6.5 ± 2.2

Fatty acid profiles, copy number measurements, and phenotypic observations of T1 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc cultivated in greenhouses during winter

The data on Table 39 indicate that the integration of these two T-DNA's (VC-LJB2197-1qcz and VC-LLM337-1qcz rc), has occurred in such a way as to introduce copy number variation of individual genes on a given T-DNA (indicating truncations and deletions along with multiple copies being inserted). For example the event LAMABL on Table 39 was segregating for a single copy of AHAS (homozygous), two copies of j-t-StCAT_p2_p-LuPXR (homozygous), possibly three copies of c-d6Elo(Pp_GA) likely homozygous, though it could be three copies which are not homozygous for all three, and three copies of j-i-Atss18_c-d6Elo(Pp_GA2) (homozygous for all three). Data on Table 42 to Table 45 for fatty acid profile indicates some variation among the events, though not large differences. The highest event average for both DHA and EPA for the events listed on Table 41 was LAMRHL which has DHA of 1.9 and EPA of 10.5 with respect to percent of the total fatty acid content of the seed and contains what was likely a single copy of the T-DNA of of VC-LJB2197-1qcz still segregating, while VC-LLM337-1qcz rc seems to be a single copy homozygous insertion. The event, LANMGC, with the lowest levels of EPA and DHA combined, contained EPA of 3.7 and 0.8 for DHA with respect to percent of the total fatty acid content of the seed. LANMGC appeared to be homozygous single copy for VC-LJB2197 and carried at least two separate integrations of VC-LLM337. For the highest single plant level of EPA and DHA, event LAMRHL had 5 percent of DHA and 13.7 percent of EPA with respect to percentage of total fatty acids in the seed, Table 43. The data indicate that the location of the insertion site is important for EPA and DHA accumulation in this combination of constructs. As seen in previous examples, comparison of single copy insertions versus double copy insertions revealed that between single copy and double copy containing plants there was an increase in VLC-PUFA levels, but between double and triple copy containing plants there was less distinction. Table 46 displays phenotypic scoring/assessment and shows some small differences in aerial phenotype among events and between the transformed plants and untransformed reference.

Table 40: Copy number measurement of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where measured per event. The T1 plants underwent a selection from 250 segregating T1 seedlings using half-kernel analysis, where the correlation of VCL-PUFA levels with copy number was employed to select for homozygous plants, or in the case of multilocus events to selecect for plants where one or more loci are homozygous. A copy number of ~2 therefore was indicative for one homozygous locus, a copy number of ~4 indicative for two homozygous loci or indicative for one homozygous locus containing two copies of the target gene measured by the assay, and so forth. Odd results of 3 and 5 indicate that at least some of the selected T1 plants carry a heterozygous locus.

Event	Copy number assays targeting the T-DNA of VC-LJB2197-1qcz. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target j-t-Atss18_c-d6Elo(Pp_GA2) near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.							Copy number assays targeting the T-DNA of VC-LLM337-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target j-t-PvARC-p3 located near the left T-DNA border and target c-d4Des(Eg_GA) near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.				
	c-AHAS	j-t-E9-p3-2	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-t-CaMV_p-LuCnl-2	c-d6Elo(Pp_GA)	j-i-Atss18_c-d6Elo(Pp_GA2)	j-t-PvARC-p3	c-d4Des(Tc_GA)	c-o3Des(Pi_GA2_SNP)	j-i-Atss15_c-o3Des(Pi_GA2)	c-o3Des(Pir_GA)	j-p-VfSBPperm3_c-o3Des(Pir_GA)	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Eg_GA)
LALHCY (n=15)	2.9 (T0: 1.8)	4.4	1.9 (T0: 1.1)	4.6	3.5	1.9 (T0: 1.1)	2.0	2.9	2.9 (T0: 2.1)		(T0: 2.6)	(T0: 2.1)	4.5		3.0
LALIAO (n=15)	3 (T0: 1.8)	2.7	2.8 (T0: 1.9)	3.3	3.6	2.8 (T0: 1.9)	3.1	3.2	2.8 (T0: 2)		(T0: 2.2)	(T0: 1.9)	2.9		1.5
LALIKA (n=15)	2 (T0: 1.1)	2.0	3.6 (T0: 1.9)	2.0	4.1	3.6 (T0: 1.9)	3.8	3.5	3.8 (T0: 2)		(T0: 1.9)		4.0		5.5
LALITL (n=11)	1.7 (T0: 1.8)	3.5	3.2 (T0: 3)	3.5	3.1	3.2 (T0: 3)	3.2	2.3	2.4 (T0: 2)		(T0: 1.9)		2.3		2.8
LALQAM (n=15)	3.4 (T0: 1.9)	3.3	3.3 (T0: 1.9)	3.3	3.1	3.3 (T0: 1.9)	3.3	2.9	3.1 (T0: 2)		(T0: 2.1)		1.6		1.6
LALQDS (n=14)	2.7 (T0: 2)	2.8	2.3 (T0: 2.2)	3.6	2.3	2.3 (T0: 2.2)	2.3	2.8	2.9 (T0: 2)		(T0: 1.9)		4.0		2.8
LALRCQ (n=14)	3.2 (T0: 2)	3.2	3.2 (T0: 2.1)	3.3	3.0	3.2 (T0: 2.1)	3.3	3.0	3.2 (T0: 2.1)		(T0: 2.2)		4.4		4.3
LALWKF (n=14)	1.9 (T0: 1.2)	1.9	4.7 (T0: 3.2)	4.8	4.8	4.7 (T0: 3.2)	4.8	1.4	1.3 (T0: 1)		(T0: 1)		1.3		1.3
LAMABL (n=8)	1.8 (T0: 1)	1.9	5.2 (T0: 3.7)	3.6	5.8	5.2 (T0: 3.7)	5.6	1.9	1.8 (T0: 1)		(T0: 1)	(T0: 1.2)	1.8		3.5
LAMCKI (n=10)	2 (T0: 1)	3.9	2.3 (T0: 1)	3.6	2.7	2.3 (T0: 1)	2.0	3.8	4 (T0: 1.9)		(T0: 2.1)	(T0: 1.8)	3.9		3.9

LAMCLE (n=14)	3 (T0: 2)	3.0	4.3	4.2	4.2 (T0: 2.9)	4.4	2.5	2.4 (T0: 2)	(T0: 2)	(T0: 2.1)	3.4		1.3
LAMCLF (n=9)	1.6 (T0: 1)	3.3	4.1	4.7	4.4 (T0: 2.8)	3.8	2.2	1.8 (T0: 2.1)	(T0: 1.9)	(T0: 1.4)	1.8		1.8
LAMEUB (n=15)	1.9 (T0: 1)	2.1	3.6	3.9	3.7 (T0: 2.2)	3.8	1.3	1.2 (T0: 1)	(T0: 1)		1.2		1.1
LAMEUU (n=13)	1.9 (T0: 0.9)	3.8	3.3	5.6	5.4 (T0: 2.8)	5.2	3.1	3.8 (T0: 1.9)	(T0: 1.9)		3.7		3.7
LAMFJO (n=15)	2 (T0: 1.1)	2.1	2.0	2.1	2 (T0: 0.9)	2.0	1.1	1.1 (T0: 0.5)	(T0: 0.8)		1.2		1.1
LAMIRY (n=10)	1.9 (T0: 1)	1.9	2.6	2.7	2.7 (T0: 2)	2.8	1.7	2 (T0: 2.3)	(T0: 2)		3.1		2.1
LAMJIC (n=9)	1.9 (T0: 1.1)	1.7	1.6	2.5	1.8 (T0: 1)	1.8	2.3	1.8 (T0: 1)	(T0: 1)		1.8		1.8
LAMPJB (n=13)	2.8 (T0: 2)	2.9	3.6	4.4	4.1 (T0: 3)	4.1	2.6	2.8 (T0: 2.1)	(T0: 1)	(T0: 1.8)	2.8		2.6
LAMQDL (n=14)	2 (T0: 1)	2.1	2.3	2.6	2.3 (T0: 1)	2.0	0.0	2.6 (T0: 2)	(T0: 1)	(T0: 1)	1.3		1.3
LAMQNE (n=14)	2.6 (T0: 1.1)	2.6	2.7	2.8	2.8 (T0: 1.3)	2.5	2.0	1.5 (T0: 1.1)	(T0: 0.9)	(T0: 1.9)	4.0		4.2
LAMQSF (n=14)	3.5 (T0: 2.1)	4.5	3.8	4.7	4.2 (T0: 3.2)	4.0	2.9	3.7 (T0: 3.9)	(T0: 4.2)	(T0: 2.2)	3.6		3.6
LAMRCO (n=14)	2.8 (T0: 1.9)	3.7	6.6	6.5	6 (T0: 4.1)	5.5	1.8	1.3 (T0: 1.1)	(T0: 1)	(T0: 2.1)	3.9		2.5
LAMRDS (n=15)	3.2 (T0: 1.9)	3.2	3.0	3.6	3 (T0: 1.8)	3.0	3.0	3 (T0: 2)	(T0: 1.9)	(T0: 1.7)	3.1		2.6
LAMRHL (n=14)	2 (T0: 1.3)	2.2	3.2	2.8	2.1 (T0: 1.5)	1.9	4.1	3.8 (T0: 2.7)	(T0: 1.3)	(T0: 1.3)	1.9		2.0
LAMRJK (n=15)	3 (T0: 2)	3.0	2.9	1.3	1.2 (T0: 0.9)	1.0	3.0	4.5 (T0: 2.9)	(T0: 1.9)	(T0: 1.7)	2.9		2.7
LAMRNQ (n=9)	2.2 (T0: 1.9)	2.1	2.2	2.3	2.3 (T0: 2.1)	2.1	2.5	2 (T0: 1.9)	(T0: 2)	(T0: 1.9)	2.0		2.7
LAMVUB (n=15)	2.1 (T0: 1.1)	2.1	2.1	2.7	2.1 (T0: 1)	2.1	0.6	0.8 (T0: 0.8)	(T0: 0.9)		1.1		0.8
LAMYDP (n=14)	3.9 (T0: 2)	4.0	3.7	4.2	3.8 (T0: 1.9)	3.9	3.2	3.4 (T0: 2.1)	(T0: 1.9)	(T0: 1.7)	3.3		3.2
LANBCH (n=14)	3.5 (T0: 1.9)	3.2	3.3	4.0	3.5 (T0: 2)	3.4	3.5	3 (T0: 1.8)	(T0: 2)		3.3		3.3
LANCEG (n=4)	4 (T0: 2.1)	4.0	3.5	5.9	5.4 (T0: 2.9)	3.6	3.6	4.1 (T0: 2)	(T0: 2)		4.1		1.9
LANCOX (n=10)	2.2 (T0: 1)	2.5	2.2	5.4	5.1 (T0: 4.3)	4.5	1.7	1.7 (T0: 1)	(T0: 1.1)		1.8		2.6
LANFEF (n=15)	2.1 (T0: 1.1)	2.0	2.3	2.6	2.1 (T0: 1)	2.1	1.0	0.7 (T0: 1.1)	(T0: 1)	(T0: 1.8)	2.9		2.9
LANMGC (n=15)	4.2 (T0: 1.9)	4.0	4.1	4.7	4.2 (T0: 2)	4.2	2.2	3.8 (T0: 1.9)	(T0: 2)		4.0		6.0
LANMOM (n=10)	1.4 (T0: 1.2)	1.4	3.0	2.7	2 (T0: 1.1)	1.5	3.5	2.9 (T0: 1.9)	(T0: 1.9)		2.9		4.8
LANPMZ (n=13)	2.1 (T0: 1.1)	2.1	2.0	2.3	2.2 (T0: 1)	2.2	2.0	2.1 (T0: 1.1)	(T0: 1.1)		2.1		1.9
LANTLE (n=15)	2.7 (T0: 2)	2.8	2.7	3.3	2.8 (T0: 2)	3.0	2.5	2.2 (T0: 2)	(T0: 1.9)		2.3		2.3
LANTSP (n=12)	1.4 (T0: 1)	1.3	2.9	3.7	3 (T0: 1.9)	3.0	0.6	1.5 (T0: 0.9)	(T0: 1.6)		2.5		3.3
LANUC3 (n=14)	2 (T0: 1.1)	2.1	4.6	4.5	3.5 (T0: 2.1)	3.1	1.1	0.9 (T0: 2.1)	(T0: 2.8)		2.3		2.3
LAOBGQ (n=15)	1.4 (T0: 1)	1.4	6.1	5.2	4 (T0: 2.8)	4.2	1.6	2.7 (T0: 1.8)	(T0: 2.1)	(T0: 1.8)	2.8		4.0
LAOHLR (n=14)	1.9 (T0: 1.2)	4.9	4.0	4.3	3.7 (T0: 2)	3.1	2.1	1.7 (T0: 1.1)	(T0: 1)		1.7		1.7
LAOJAT (n=15)	2.2 (T0: 1.9)	3.4	3.2	3.8	3.4 (T0: 3)	3.3	2.3	2.2 (T0: 1.9)	(T0: 1.9)	(T0: 1.9)	2.3		1.2

LAOKLP (n=15)	2.1 (T0: 0.8)	2.1	2.1	2.5	2.2 (T0: 1)	2.1	1.4	1.2 (T0: 0.7)	(T0: 1)	(T0: 0.6)	1.2	1.2
LAOKTE (n=15)	3 (T0: 2)	2.8	3.5	3.5	3.3 (T0: 2.1)	3.2	3.0	2.4 (T0: 2)	(T0: 1.9)	(T0: 2.1)	2.5	2.4
LAPKLS (n=12)	3.4 (T0: 2.1)	3.1	3.6	4.2	3.1 (T0: 2.1)	3.2	2.5	1.9 (T0: 2)	(T0: 2.1)		2.0	2.1
LAPKXM (n=15)	3.2 (T0: 1.8)	3.2	3.8	4.2	3.3 (T0: 2)	3.4	3.7	2.9 (T0: 1.9)	(T0: 1.9)		3.6	0.0
LAPKZJ (n=15)	3.3 (T0: 1.4)	3.2	3.6	4.3	3.2 (T0: 1.9)	3.3	2.0	1.6 (T0: 2)	(T0: 1.9)		1.7	1.6
LAPWJD (n=14)	2.1 (T0: 1.1)	2.0	2.3	2.8	2.1 (T0: 1)	2.1	2.4	2 (T0: 1)	(T0: 1)		2.2	2.1
LAPWLP (n=14)	1.3 (T0: 1.1)	1.2	3.1	3.4	2.8 (T0: 1.9)	2.9	0.0	1.3 (T0: 1)	(T0: 1.1)		2.3	2.3
LAQYTA (n=15)	4.5 (T0: 2.8)	5.1	5.0	3.2	2.6 (T0: 2.1)	2.8	2.4	2.2 (T0: 1.9)	(T0: 2)	(T0: 2.4)	3.2	3.3
LAQYUT (n=15)	4.3 (T0: 1.9)	3.9	4.6	4.3	3 (T0: 2)	2.8	3.6	2.8 (T0: 1.6)	(T0: 1.9)	(T0: 0.6)	3.0	4.3
LAQYWQ (n=15)	2.6 (T0: 1.2)	2.8	5.6	5.9	4.5 (T0: 3)	4.6	3.2	2.7 (T0: 2)	(T0: 1.9)	(T0: 2.2)	2.6	1.4
LAQZME (n=12)	1.4 (T0: 1.2)	1.3	3.7	4.4	3.8 (T0: 2.3)	4.0	1.8	1.4 (T0: 1.1)	(T0: 1.2)	(T0: 1.2)	1.4	1.3

Table 41: Copy number measurement of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column; sc: all T1 plants where the average of all copy number assays listed in this table was 1.51-2.49, dc: all T1 plants where the average of all copy number assays listed in this table was 3.51-4.49, tc: all T1 plants where the average of all copy number assays listed in this table was 5.51-6.49. The number of T1 plants fulfilling these criteria are displayed in parentheses.

Event	Copy number assays targeting the T-DNA of VC-LJB2197-1qcz. Assays are listed according to the position of the assay target along the T-DNA, with assay A1 located near the left T-DNA border and assay A13 near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.	Copy number assays targeting the T-DNA of VC-LJB2197-1qcz. Assays are listed according to the position of the assay target along the T-DNA, with assay A1 located near the left T-DNA border and assay A13 near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.										
	c-AHAS	j-t-E9-p3-2	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-t-CaMV_p LuCnI 2	c-d6Elo(Pp_GA)	j-i-Atss18_c-d6Elo(Pp_GA2)	j-t-PvARC-p3	c-d4Des(Tc_GA)	c-o3Des(Pi_GA2_SNP)	j-i-Atss15_c-o3Des(Pi_GA2)	c-o3Des(Pir_GA)	j-p-VfSBPperm3_c-o3Des(Pir_GA)	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Eg_GA)

| | | |

LAMCLF (n=9)	5.1 ± 0.1	0.3 ± 0	0 ± 0	3.6 ± 0.1	24.7 ±3.5	28.5 ± 2	1.1 ± 0.6	2.8 ± 0.5	2 ± 0.6	0.2 ± 0.1	0.8 ± 0	0.6 ± 0.1	1.4 ± 0.7	0.1 ± 0.1	2.8 ± 0.7	1 ± 0.2	9.7 ± 2.2	9.4 ± 1	0.3 ± 0	0 ± 0	1 ± 0.3	2.8 ± 0.5	0 ± 0	1.5 ± 0.3	0 ± 0.1							
LAMEUB (n=15)	5 ± 0.2	0.3 ± 0.1	0 ± 0	3.8 ± 0.5	28.4 ±5.6	29.7 ± 2.1	0.9 ± 0.3	3.4 ± 0.6	1.3 ± 0.4	0.1 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	1 ± 0.4	0.2 ± 0.1	2.7 ± 0.3	1.1 ± 0.3	8.4 ± 2.9	7.6 ± 1.9	0.3 ± 0	0 ± 0	0.5 ± 0.2	2.2 ± 0.7	0 ± 0	1.2 ± 0.5	0.1 ± 0.1							
LAMEUU (n=13)	5 ± 0.1	0.1 ± 0.1	0 ± 0	3.3 ± 0.4	25.8 ±4.8	34.3 ± 1.8	1.4 ± 0.3	4.2 ± 0.7	2.1 ± 0.4	0.2 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.2 ± 0.2	1.8 ± 1.8	1.4 ± 1.5	1.2 ± 0.3	3.1 ± 0.8	7.8 ± 1.1	0.2 ± 0.2	0 ± 0	1.4 ± 0.2	3 ± 0.4	0 ± 0	1.5 ± 0.3	0.3 ± 0.3							
LAMFJO (n=15)	5.1 ± 0.7	0.2 ± 0.1	0 ± 0	3.5 ± 0.7	26.5 ±1.9	32.9 ± 0.8	0.2 ± 0.1	4.5 ± 0.4	0.4 ± 0.1	0 ± 0	0.9 ± 0.1	1 ± 0.1	2.2 ± 0.7	1.7 ± 1.9	3.7 ± 0.6	2 ± 0.3	4.7 ± 1.8	6.2 ± 0.7	0.3 ± 0.1	0 ± 0	0.3 ± 0.2	2.2 ± 0.3	0 ± 0.1	1 ± 0.2	0.4 ± 0.3							
LAMIRY (n=10)	5 ± 0.2	0.3 ± 0	0 ± 0	3.5 ± 0.3	26.9 ±6.8	30.9 ± 2.8	0.6 ± 0.1	4.6 ± 0.5	1.2 ± 0.3	0.2 ± 0	0.8 ± 0	0.7 ± 0.2	1.1 ± 0.2	0.4 ± 0.1	2.4 ± 0.4	1.4 ± 0.3	4.5 ± 0.9	9.1 ± 2.1	0.3 ± 0	0 ± 0	1 ± 0.2	3 ± 0.6	0 ± 0	1.6 ± 0.4	0.5 = 0.1							
LAMIIC (n=9)	5.8 ± 0.4	0.2 ± 0.1	0 ± 0	3.1 ± 0.5	28.5 ±6.6	33.1 ± 3.9	0.6 ± 0.2	5.3 ± 1.2	1.3 ± 0.6	0.2 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.5 ± 0.3	0.1 ± 0.1	2.6 ± 0.4	1.3 ± 0.3	3.9 ± 1.3	6.2 ± 1.8	0.3 ± 0.1	0 ± 0	0.9 ± 0.3	2.6 ± 1	0 ± 0	1.4 ± 0.4	0.4 ± 0.1							
LAMPJB (n=13)	4.8 ± 0.1	0.1 ± 0	0 ± 0	3.1 ± 0.3	24.1 ±1.7	33.6 ± 0.9	0.9 ± 0.2	3.7 ± 0.5	2.2 ± 0.5	0.3 ± 0.1	0.8 ± 0.1	0.7 ± 0	0.4 ± 0.3	2.8 ± 0.4	3.7 ± 0.4	1.2 ± 0.2	5.5 ± 2.2	8.7 ± 1	0 ± 0.1	0 ± 0	1.5 ± 0.3	3.2 ± 0.4	0 ± 0	2 ± 0.3	0.1 ± 0.3							
LAMQDL (n=14)	5 ± 0.5	0.2 ± 0	0 ± 0	2.7 ± 0.4	26.4 ±2.2	31.2 ± 1	0.2 ± 0	5.2 ± 0.6	0.4 ± 0.1	0.1 ± 0	0.7 ± 0.1	1 ± 0.1	2.8 ± 0.5	1 ± 0.2	3.7 ± 0.4	2.1 ± 0.4	4.7 ± 2	6.7 ± 1.2	0.3 ± 0	0 ± 0	0.4 ± 0.1	2.7 ± 0.7	0 ± 0	0.8 ± 0.2	0.4 ± 0.2	1.1 ± 0.1							
LAMQNE (n=14)	6.9 ± 1.3	0.3 ± 0.1	0 ± 0	2.7 ± 0.3	27.8 ±2.6	31.8 ± 0.7	0.8 ± 0.1	4.9 ± 0.6	1.3 ± 0.1	0.2 ± 0	0.7 ± 0.1	0.7 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	4 ± 1.7	1.9 ± 0.8	2.7 ± 1	5.9 ± 1.1	0.3 ± 0	0 ± 0	1.2 ± 0.2	2.8 ± 0.6	0 ± 0	1.2 ± 0.3	0.8 ± 0.6	0.4 ± 0.1							
LAMQSF (n=14)	5.2 ± 0.1	0.3 ± 0	0 ± 0	3.4 ± 0.3	24.3 ±2.7	31.5 ± 1.2	0.8 ± 0.4	4.5 ± 0.7	2 ± 0.8	0.3 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	0.2 ± 0.2	1.9 ± 0.6	1.3 ± 0.5	4.1 ± 1.5	10.6 ± 1.7	0.3 ± 0	0 ± 0	1.1 ± 0.2	3.6 ± 0.5	0 ± 0	1.8 ± 0.5	0.3 ± 0.3							
LAMRCO (n=14)	5.5 = 0.6	0.3 ± 0.1	0 ± 0	4.4 ± 0.6	23.9 ±6.8	28.4 ± 3.1	1.3 ± 0.4	2.7 ± 0.4	3.4 ± 0.8	0.3 ± 0.1	1 ± 0.2	0.6 ± 0.1	0.4 ± 0.2	0.1 ± 0.1	3.7 ± 0.8	1.3 ± 0.3	5.7 ± 1.3	8.7 ± 1.7	0.4 ± 0.1	0 ± 0	1.8 ± 0.4	3.3 ± 0.8	0 ± 0	2.1 ± 0.6	0.4 ± 0.2	0.4 ± 0.1							
LAMRDS (n=15)	4.8 = 0.1	0 ± 0	0 ± 0	3.6 ± 0.4	23.4 ±1.7	32.7 ± 1.3	1.1 ± 0.2	3.4 ± 0.5	2.3 ± 0.3	0.2 ± 0	0.9 ± 0.1	0.7 ± 0	0.2 ± 0.1	5.4 ± 0.9	0 ± 0.1	1.2 ± 0.1	3.4 ± 0.8	8.2 ± 0.7	0.4 ± 0.1	0 ± 0	2 ± 0.4	3.5 ± 0.3	0 ± 0	1.8 ± 0.3	0.5 ± 0.3							
LAMRHL (n=14)	6 ± 1	0.3 ± 0.1	0 ± 0	3.5 ± 0.5	26.5 ±6	26.2 ± 3.9	0.6 ± 0.1	3.7 ± 0.8	1 ± 0.3	0.2 ± 0.1	0.9 ± 0.1	0.8 ± 0.2	1.6 ± 0.6	0.4 ± 0.1	3 ± 0.6	1.9 ± 0.4	4.6 ± 0.8	10.5 ± 1.9	0.4 ± 0	0 ± 0	1 ± 0.3	3.9 ± 1	0 ± 0	1.9 ± 0.5	0.3 ± 0.2	1.4 ± 0.4							
LAMRJK (n=15)	4.9 ± 0.2	0.1 ± 0	0 ± 0	3.6 ± 0.4	26.4 ±2.9	33 ± 1	1 ± 0.2	4.1 ± 0.6	2 ± 0.4	0.2 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0 ± 0.1	0.5 ± 0.1	2.6 ± 1.4	1.3 ± 0.3	4.4 ± 1.3	7.7 ± 1.2	0.1 ± 0.2	0 ± 0	1.3 ± 0.4	3.2 ± 0.6	0 ± 0	1.5 ± 0.2	0.2 ± 0.4							
LAMRNQ (n=9)	4.8 ± 0.1	0.2 ± 0	0 ± 0	2.7 ± 0.4	29.6 ±3.8	32.2 ± 0.9	0.5 ± 0.1	5.1 ± 0.8	0.9 ± 0.2	0.1 ± 0	0.8 ± 0.1	0.9 ± 0	1.2 ± 0.2	0.3 ± 0.1	2.8 ± 0.7	1.3 ± 0.3	4.6 ± 1.3	6 ± 1.2	0.3 ± 0	0 ± 0	0.9 ± 0.1	2.4 ± 0.3	0 ± 0	1.2 ± 0.3	0.4 ± 0.2	0.6 ± 0.2							
LAMYUB (n=15)	4.9 ± 0.3	0.2 ± 0	0 ± 0	3.6 ± 0.4	24.6 ±1.9	33.4 ± 0.5	0.2 ± 0.1	4.8 ± 0.4	0.4 ± 0.1	0 ± 0	0.9 ± 0.1	1 ± 0.1	2.8 ± 0.5	1 ± 0.2	4 ± 0.4	2.4 ± 0.3	4 ± 1.4	6.7 ± 1	0.4 ± 0	0 ± 0	0.5 ± 0.1	2.3 ± 0.4	0 ± 0	1.3 ± 0.3	0.6 ± 0.2							
LAMYDP (n=14)	5.1 ± 0.5	0.2 ± 0.1	0 ± 0	4 ± 0.8	23.1 ±2.3	31.7 ± 1.1	1.2 ± 0.1	4.1 ± 0.5	2.4 ± 0.3	0.3 ± 0	0.9 ± 0.1	0.6 ± 0	0.3 ± 0.1	2.5 ± 0.4	1.3 ± 0.3	4.1 ± 0.8	10.3 ± 1.6	0.3 ± 0.1	0 ± 0	1.3 ± 0.3	3.6 ± 0.7	0 ± 0	2.1 ± 0.5	0.4 ± 0.2							
LANBCH (n=14)	4.7 ± 0.8	0.2 ± 0.1	0 ± 0	3.4 ± 0.4	25 ± 3.9	32.7 ± 1.5	0.8 ± 0.2	4.7 ± 0.5	1.5 ± 0.3	0.2 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.5 ± 0.2	0.2 ± 0.1	2.3 ± 0.4	1.5 ± 0.2	3.5 ± 0.5	9.2 ± 1.8	0.3 ± 0	0 ± 0	1.3 ± 0.4	3.7 ± 0.9	0 ± 0	2 ± 0.6	0.5 ± 0.1	0.3 ± 0							

LANCEG (n=4)	4.9 ± 0.1	0.1 ± 0.1	0 ± 0	3.3 ± 0.2	23.8 ± 1.2	33.2 ± 0.5	0.9 ± 0.1	3.7 ± 0.4	2 ± 0.3	0.3 ± 0	0.8 ± 0	0.7 ± 0	0.2 ± 0.1	5.5 ± 0.2	0.2 ± 0.1	0.7 ± 0.1	1.5 ± 0.4	10.8 ± 0.4	0.3 ± 0.1	0 ± 0	1.2 ± 0.1	3.8 ± 0.2	0 ± 0	1.7 ± 0.2	0 ± 0					
LANCOX (n=10)	5.1 ± 0.7	0.2 ± 0.1	0 ± 0	3.1 ± 0.2	25 ± 2.5	35.5 ± 1.3	0.7 ± 0.1	4.1 ± 0.3	2.1 ± 0.3	0.3 ± 0	0.8 ± 0.1	0.7 ± 0		7.2 ± 2.4	0.2 ± 0.1	0.6 ± 0.1	1.6 ± 0.3	7.3 ± 1.5	0.3 ± 0.1	0 ± 0	0.9 ± 0.4	2.7 ± 0.7	0 ± 0.1	1.4 ± 0.5	0 ± 0					
LANFEF (n=15)	5.1 ± 0.3	0.1 ± 0.1	0 ± 0	3.5 ± 0.4	27.2 ± 1	34.3 ± 1.1	0.2 ± 0.1	4.5 ± 0.9	0.3 ± 0	0 ± 0	0.8 ± 0.1	1 ± 0	2.5 ± 0.3	0.8 ± 0.2	3.5 ± 0.5	1.6 ± 0.4	3.3 ± 1.4	5.5 ± 0.5	0.2 ± 0.1	0 ± 0	1 ± 0.1	2.2 ± 0.3	0 ± 0	1.1 ± 0.1	0.8 ± 0.2	0.9 ± 0				
LANMGC (n=15)	5.6 ± 0.9	0.2 ± 0.1	0 ± 0	4 ± 0.5	25.1 ± 1.9	33.1 ± 1.8	1 ± 0.2	4.3 ± 0.3	2.1 ± 0.2	0.2 ± 0	0.9 ± 0.1	0.7 ± 0	0.2 ± 0	0 ± 0	7.9 ± 1.3	3.6 ± 0.7	1.4 ± 0.5	3.7 ± 1.3	0.3 ± 0	0 ± 0	0.7 ± 0.2	1.5 ± 0.6	0 ± 0	0.8 ± 0.4	2.4 ± 0.6					
LANMOM (n=10)	5.4 ± 0.4	0.2 ± 0	0 ± 0	2.8 ± 0.2	26.9 ± 3.1	31.8 ± 1.3	0.8 ± 0.2	4.9 ± 0.5	1.4 ± 0.4	0.2 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.6 ± 0.3	0.2 ± 0.1	2 ± 0.3	1.2 ± 0.2	3.9 ± 0.5	8.6 ± 1.5	0.3 ± 0	0 ± 0	1.2 ± 0.2	3.7 ± 0.7	0 ± 0	1.5 ± 0.4	0.4 ± 0.1	0.3 ± 0.1				
LANPMZ (n=13)	4.8 ± 0.6	0.2 ± 0.1	0 ± 0	3.7 ± 0.3	26.2 ± 1.4	34.4 ± 1	0.3 ± 0.1	5 ± 0.4	0.5 ± 0.1	0.1 ± 0	0.9 ± 0	0.9 ± 0.1	1.5 ± 0.3	0.5 ± 0.2	3 ± 0.2	2 ± 0.3	2.7 ± 0.3	6.9 ± 0.8	0.4 ± 0	0 ± 0	0.8 ± 0.2	2.8 ± 0.6	0 ± 0	1.5 ± 0.3	0.7 ± 0.1	1 ± 0.1				
LANTLE (n=15)	5 ± 0.4	0.2 ± 0.1	0 ± 0	2.4 ± 0.4	26.7 ± 4	32.7 ± 1.2	0.4 ± 0.3	6.1 ± 0.8	1 ± 0.5	0.1 ± 0.1	0.6 ± 0.1	0.8 ± 0.2	1.4 ± 1	0.5 ± 0.5	2.5 ± 0.5	1.7 ± 0.4	3.9 ± 1.4	7.9 ± 1.9	0 ± 0.1	0 ± 0	0.7 ± 0.4	3.3 ± 0.8	0 ± 0	1.5 ± 0.4	0.1 ± 0.2	0.4 ± 0.2				
LANTSP (n=12)	4.9 ± 0.2	0.2 ± 0	0.1 ± 0	3.3 ± 0.3	35.9 ± 6.9	26.8 ± 4	0.8 ± 0.2	4.6 ± 0.9	1.1 ± 0.4	0.2 ± 0.1	0.9 ± 0.1	0.9 ± 0.2	0.8 ± 0.3	0.2 ± 0.1	2.1 ± 0.4	1.2 ± 0.2	3.4 ± 0.9	6.6 ± 1.8	0.4 ± 0	0 ± 0	1 ± 0.4	2.5 ± 0.7	0 ± 0	1.3 ± 0.4	0.4 ± 0.2	0.4 ± 0.3				
LANUCB (n=14)	5 ± 0.5	0.3 ± 0	0 ± 0	2.9 ± 0.4	28.2 ± 4.5	28.8 ± 2.2	1.5 ± 0.6	3.8 ± 0.8	2.7 ± 1.5	0.4 ± 0.3	0.7 ± 0.1	0.7 ± 0.1	0.5 ± 0.5	0.1 ± 0.1	2.2 ± 0.7	1.1 ± 0.3	5.7 ± 1.9	9.2 ± 1.4	0.2 ± 0.1	0 ± 0	0.9 ± 0.3	3.3 ± 0.5	0 ± 0	1.3 ± 0.4	0 ± 0.1	0.6 ± 0.5				
LAOBGQ (n=15)	5.2 ± 0.4	0.2 ± 0.1	0 ± 0	3.5 ± 0.3	31.8 ± 7.9	29.2 ± 4.2	1.2 ± 0.4	3.9 ± 0.6	2.5 ± 0.7	0.4 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	0.2 ± 0.2	0 ± 0	2.1 ± 0.9	1.1 ± 0.5	3.9 ± 0.6	7.3 ± 1.5	0.2 ± 0.2	0 ± 0	1.1 ± 0.3	2.9 ± 0.6	0 ± 0	1.4 ± 0.4	0.1 ± 0.3	0.1 ± 0.1				
LAOHLR (n=14)	5.1 ± 0.2	0.2 ± 0	0 ± 0	3.5 ± 0.3	26.5 ± 6.1	33 ± 2.7	1.1 ± 0.2	3.7 ± 0.7	2.1 ± 0.4	0.2 ± 0	0.9 ± 0.1	0.7 ± 0.1	0.1 ± 0.1	4.1 ± 1.6	1.4 ± 0.5	5.3 ± 0.8	6.8 ± 1.2	0.3 ± 0.1	0 ± 0	0.8 ± 0.2	2.3 ± 0.4	0 ± 0	1.2 ± 0.3	0.3 ± 0.3						
LAOJAT (n=15)	5.1 ± 0.5	0.1 ± 0.1	0 ± 0	2.8 ± 0.7	28.7 ± 4	34 ± 1.8	5 ± 0.8	6.1 ± 0.8	1 ± 0.4	0.1 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.4	0.1 ± 0.2	1.5 ± 0.5	0.9 ± 0.2	4.3 ± 0.7	7.9 ± 1.3	0 ± 0	0 ± 0	0.8 ± 0.3	2.9 ± 0.4	0 ± 0	1.4 ± 0.3	0 ± 0	0.3 ± 0.1				
LAOKLP (n=15)	4.7 ± 0.4	0.2 ± 0	0 ± 0	3.1 ± 0.2	26 ± 2.2	32.5 ± 1.3	0.2 ± 0	5.2 ± 0.5	0.3 ± 0	0 ± 0	0.8 ± 0	1 ± 0	2.5 ± 0.7	1 ± 0.2	3.5 ± 0.4	2.1 ± 0.3	4.4 ± 1.6	6.5 ± 0.9	0.3 ± 0	0 ± 0	0.5 ± 0.1	2.5 ± 0.6	0 ± 0	1.2 ± 0.3	0.5 ± 0.2	1.1 ± 0.6				
LAOKTE (n=15)	4.7 ± 0.3	0.2 ± 0.1	0 ± 0	2.8 ± 0.4	27.1 ± 7.8	31.4 ± 2.3	0.5 ± 0.3	5.5 ± 1.7	0.9 ± 0.6	0.1 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	1.2 ± 0.7	0.4 ± 0.2	2.5 ± 0.8	1.6 ± 0.4	4.1 ± 2.2	8.2 ± 2.9	0.3 ± 0.1	0 ± 0	0.9 ± 0.4	3.3 ± 1	0 ± 0	1.6 ± 0.6	0.4 ± 0.2	0.5 ± 0.2				
LAPKLS (n=12)	5.2 ± 0.3	0.2 ± 0.1	0 ± 0	3.7 ± 0.5	23.8 ± 1.9	33.6 ± 0.7	0.9 ± 0.1	4.1 ± 0.5	1.9 ± 0.4	0.2 ± 0.1	0.8 ± 0	0.7 ± 0.3	0.4 ± 0.1	0.1 ± 0.5	3.1 ± 0.5	1.5 ± 0.4	6 ± 2.3	7.9 ± 1.5	0.2 ± 0.1	0 ± 0	0.8 ± 0.3	2.6 ± 0.7	0 ± 0	1.4 ± 0.5	0.3 ± 0.3	0.4 ± 0.2				
LAPKXM (n=15)	4.6 ± 0.3	0.2 ± 0	0 ± 0	3.2 ± 0.3	26.1 ± 2	32 ± 0.9	0.7 ± 0.2	5.2 ± 0.5	1.2 ± 0.4	0.2 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.7	0.3 ± 0.1	2.3 ± 0.4	1.7 ± 0.2	3.1 ± 0.7	9.8 ± 2	0.3 ± 0	0 ± 0	0.9 ± 0.3	3.4 ± 0.7	0 ± 0	1.4 ± 0.4	0.4 ± 0.3	0.5 ± 0.2				
LAPKZJ (n=15)	5.1 ± 0.5	0.1 ± 0.1	0 ± 0	3.6 ± 0.4	24.6 ± 1.9	31.8 ± 1.6	0.4 ± 0.2	4.7 ± 0.7	0.6 ± 0.3	0 ± 0	0.8 ± 0.1	0.9 ± 0.2	2.1 ± 0.8	0.8 ± 0.4	3.4 ± 0.5	2.1 ± 0.5	4.9 ± 2.1	7.7 ± 1.1	0.2 ± 0.2	0 ± 0	0.6 ± 0.3	2.8 ± 0.5	0 ± 0	1.4 ± 0.3	0.4 ± 0.5	1 ± 0.2				
LAPWJD (n=14)	5.1 ± 0.4	0.1 ± 0.1	0 ± 0	3.6 ± 0.1	27 ± 0.9	34 ± 0.7	0.3 ± 0.1	4.9 ± 0.2	0.5 ± 0.1	0 ± 0	0.8 ± 0.1	0.9 ± 0.1	1.6 ± 0.4	0.6 ± 0.2	3.4 ± 0.2	2.2 ± 0.2	2.5 ± 0.3	6.2 ± 0.3	0.2 ± 0.2	0 ± 0	0.7 ± 0.1	2.5 ± 0.2	0 ± 0	1.3 ± 0.1	0.7 ± 0.1	0.8 ± 0.1				

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LAMJIC (n=1)	6.3	0.3	0.0	2.9	24.5	28.3	0.7	6.4	1.3	0.3	0.7	0.7	0.7	0.2	2.7	1.7	4.9	9.4	0.3	0.0	1.1	4.3	0.0	1.8	0.5	0.0			
LAMPJB (n=1)	4.9	0.2	0.0	3.2	24.2	32.0	1.1	4.0	2.7	0.4	0.8	0.7	0.0	0.0	2.2	1.1	4.0	10.3	0.0	0.0	1.8	4.0	0.0	2.4	0.0	0.0			
LAMQDL (n=1)	4.3	0.2	0.0	2.4	24.7	28.5	0.2	6.1	0.5	0.1	0.8	1.1	2.6	1.4	3.6	2.9	2.5	9.4	0.4	0.0	0.7	4.7	0.0	1.3	0.8	1.1			
LAMQNE (n=1)	7.6	0.4	0.0	2.7	25.4	30.7	0.7	4.7	1.4	0.2	0.7	0.6	0.5	0.2	3.0	1.5	3.6	7.8	0.3	0.0	1.4	3.7	0.0	1.7	0.8	0.3			
LAMQSF (n=1)	5.4	0.3	0.0	3.8	20.3	30.2	0.5	3.9	3.6	0.5	0.8	0.6	0.4	0.1	1.3	0.9	6.1	13.3	0.3	0.0	1.0	4.0	0.0	2.4	0.0	0.0			
LAMRCO (n=1)	6.7	0.4	0.0	4.2	14.8	26.6	1.0	2.4	4.7	0.5	1.0	0.4	0.3	0.0	3.9	1.5	6.7	12.3	0.4	0.0	2.6	5.3	0.0	3.5	0.5	0.0			
LAMRDS (n=1)	4.9	0.0	0.0	3.4	20.9	31.3	1.4	2.7	2.7	0.2	0.9	0.6	0.3	6.6	0.0	1.2	4.0	9.1	0.3	0.0	2.7	3.8	0.0	2.1	0.7	0.0			
LAMRHL (n=1)	6.4	0.3	0.0	4.0	19.7	23.4	0.8	2.6	1.1	0.2	1.0	0.7	1.9	0.5	4.4	2.7	4.7	13.7	0.3	0.0	1.3	5.0	0.0	2.8	0.6	1.8			
LAMRJK (n=1)	5.0	0.2	0.0	3.6	25.5	31.1	1.5	4.3	2.6	0.4	0.9	0.7	0.0	0.0	2.8	1.4	4.0	9.5	0.0	0.0	1.3	3.8	0.0	1.7	0.0				
LAMRNQ (n=1)	5.0	0.2	0.1	3.1	23.5	31.7	0.6	4.2	1.2	0.2	0.8	0.8	1.4	0.4	3.4	1.8	5.5	8.5	0.3	0.0	1.1	3.0	0.0	1.8	0.5	0.9			
LAMVUB (n=1)	4.3	0.2	0.0	3.2	21.9	33.5	0.2	4.8	0.4	0.1	0.8	1.0	3.0	1.2	3.7	2.4	4.7	8.4	0.3	0.0	0.6	3.0	0.0	1.7	0.6				
LAMYDP (n=1)	3.4	0.1	0.0	2.9	19.2	29.9	1.1	4.6	2.8	0.4	0.7	0.7	0.4	0.1	2.0	1.5	4.2	14.1	0.3	0.0	1.9	5.7	0.0	3.3	0.5	0.2			
LANBCH (n=1)	3.7	0.1	0.0	3.2	19.5	31.2	1.0	4.7	2.2	0.4	0.8	0.7	0.3	0.0	1.9	1.6	4.5	12.5	0.3	0.0	2.1	5.7	0.0	3.2	0.0	0.3			
LANCEG (n=1)	5.0	0.0	0.0	3.7	22.4	33.6	0.8	4.1	2.0	0.3	0.9	0.6	0.1	5.5	0.4	0.7	1.3	11.2	0.2	0.0	1.2	4.0	0.0	2.0	0.0	0.0			
LANCOX (n=1)	4.9	0.2	0.0	3.2	22.1	33.9	0.6	4.4	2.3	0.4	0.8	0.6	0.0	4.9	0.3	0.8	1.6	10.5	0.3	0.0	1.4	4.1	0.0	2.6	0.0	0.0			
LANFEF (n=1)	5.3	0.0	0.0	2.5	28.8	32.0	0.0	6.0	0.3	0.0	0.6	1.0	2.7	1.1	2.7	1.6	2.4	6.3	0.0	0.0	1.0	2.9	0.0	1.1	0.9	0.8			
LANMGC (n=1)	5.2	0.2	0.0	3.4	25.2	33.1	1.0	4.1	2.2	0.2	0.8	0.6	0.2	0.0	5.1	2.3	2.7	6.5	0.3	0.0	1.1	2.6	0.0	1.4	1.3	0.0			
LANPMZ (n=1)	3.6	0.1	0.0	3.1	22.5	32.7	0.3	5.8	0.4	0.1	0.8	1.0	2.0	0.8	3.0	2.6	2.7	8.8	0.4	0.0	1.2	4.1	0.0	2.2	0.9	1.0			
LANMOM (n=1)	5.3	0.2	0.0	2.8	19.6	32.5	0.8	4.5	2.2	0.4	0.7	0.6	0.4	0.2	2.3	1.6	4.3	11.9	0.3	0.0	1.5	4.6	0.0	2.5	0.4	0.3			
LANTLE (n=1)	4.4	0.3	0.0	2.3	19.0	31.5	0.5	5.3	1.6	0.3	0.6	0.7	1.4	0.7	3.2	2.5	5.1	11.6	0.2	0.0	1.0	4.7	0.0	2.6	0.2	0.4			
LANTSP (n=1)	5.3	0.2	0.0	2.9	23.5	32.1	0.8	4.6	1.9	0.3	0.7	0.7	0.4	0.1	2.3	1.5	3.6	9.9	0.3	0.0	1.8	3.9	0.0	2.3	0.6	0.1			
LANUCB (n=1)	4.8	0.3	0.0	3.4	25.7	27.9	2.1	3.6	4.2	0.6	0.8	0.7	0.1	0.0	1.9	1.1	4.2	10.7	0.3	0.0	1.3	4.0	0.0	1.9	0.0	0.3			
LAOBGQ (n=1)	4.8	0.2	0.0	3.4	23.0	34.0	0.9	3.9	2.0	0.2	0.9	0.7	0.4	0.0	2.4	1.2	4.8	9.2	0.4	0.0	1.6	3.8	0.0	1.9	0.0	0.2			
LAOHLR (n=1)	5.5	0.2	0.0	3.2	21.7	34.5	1.1	3.2	2.3	0.2	0.8	0.6	0.0	0.0	4.6	1.6	6.0	8.4	0.3	0.0	1.1	2.6	0.0	1.6	0.3	0.0			
LAOJAT (n=1)	5.3	0.2	0.0	2.9	22.5	34.1	0.8	5.0	2.3	0.4	0.6	0.6	0.4	0.0	2.1	1.3	4.3	10.3	0.0	0.0	1.0	3.6	0.0	2.0	0.0	0.2			
LAOKLP (n=1)	5.0	0.2	0.0	3.1	22.9	32.5	0.2	5.5	0.4	0.1	0.8	1.0	3.0	1.3	3.2	2.5	3.4	8.4	0.3	0.0	0.7	3.3	0.0	1.8	0.6	0.0			
LAOKTE (n=1)	4.9	0.2	0.0	2.6	21.3	29.8	0.9	4.6	1.9	0.3	0.7	0.6	0.3	0.2	2.3	1.6	5.4	12.6	0.3	0.0	1.4	4.8	0.0	2.4	0.4	0.3			
LAPKLS (n=1)	5.7	0.3	0.0	3.8	20.0	32.5	1.1	3.9	3.0	0.4	0.8	0.6	0.0	0.0	3.1	2.0	4.0	10.6	0.2	0.0	1.4	3.5	0.0	2.3	0.6	0.1			
LAPKXM (n=1)	4.4	0.2	0.0	3.6	23.0	33.1	0.8	4.3	1.7	0.2	0.9	0.7	0.5	0.0	2.3	1.6	4.3	13.3	0.4	0.0	0.6	2.5	0.0	1.2	0.0	0.3			
LAPKZJ (n=1)	4.8	0.1	0.0	3.7	23.2	30.4	0.4	4.7	0.6	0.1	0.9	1.0	2.2	0.9	3.4	2.7	2.9	9.2	0.4	0.0	0.9	3.7	0.0	1.9	0.8	1.1			
LAPWJD (n=1)	5.5	0.1	0.0	3.5	25.6	35.2	0.4	4.6	0.7	0.0	0.8	0.8	1.5	0.6	3.4	2.1	2.7	6.6	0.2	0.0	0.8	2.4	0.0	1.4	0.6	0.7			
LAPWLP (n=1)	4.5	0.1	0.0	2.9	28.2	18.2	0.5	4.7	0.5	0.2	0.8	1.2	2.9	1.1	1.9	2.1	3.6	15.9	0.4	0.0	0.7	4.8	0.0	3.2	0.0	1.7			
LAQYTA (n=1)	5.3	0.0	0.0	3.0	24.8	30.1	1.2	4.3	2.1	0.1	0.7	0.6	0.4	0.0	2.1	1.1	7.5	10.2	0.0	0.0	0.9	3.7	0.0	1.5	0.0	0.5			
LAQYUT (n=1)	5.6	0.2	0.0	3.5	21.5	32.5	1.4	3.0	3.1	0.3	0.7	0.6	0.0	0.0	4.6	1.6	6.8	8.9	0.0	0.0	1.0	2.6	0.0	1.6	0.0	0.3			
LAQYWQ (n=1)	5.8	0.3	0.0	4.3	22.3	30.0	2.1	3.6	4.1	0.6	0.8	0.5	0.3	0.0	1.7	1.1	3.8	11.5	0.0	0.0	1.5	3.5	0.0	1.9	0.0	0.1			
LAQZME (n=1)	6.3	0.3	0.0	2.5	21.7	30.7	0.7	3.3	2.7	0.4	0.6	0.6	0.7	0.2	2.7	1.6	6.4	10.3	0.3	0.0	1.2	3.8	0.0	2.6	0.1	0.2			

Table 44: Fatty acid profiles of T2 seeds harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column; as defined in Table 41. The number of T1 plants fullfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=296)	4.9 ± 0.5	0.2 ± 0.1	0 ± 0	3.2 ± 0.6	28.5 ± 5.4	31 ± 4	0.5 ± 0.3	4.7 ± 1	0.8 ± 0.5	0.1 ± 0.1	0.8 ± 0.1	0.9 ± 0.2	1.6 ± 0.9	0.7 ± 1.2	2.7 ± 0.9	1.6 ± 0.6	4.6 ± 2.3	7.2 ± 1.9	0.3 ± 0.1	0 ± 0	0.7 ± 0.3	2.7 ± 0.7	0 ± 0	1.3 ± 0.4	0.3 ± 0.3	0.9 ± 0.5
dc (n=198)	5.1 ± 0.6	0.2 ± 0.1	0 ± 0	3.5 ± 0.6	23.7 ± 3.1	32.2 ± 2.5	1.1 ± 0.3	4.1 ± 0.7	2.3 ± 0.6	0.3 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.3 ± 0.2	0.4 ± 1.4	3 ± 2	1.6 ± 0.8	4 ± 1.8	9.1 ± 2.8	0.3 ± 0.1	0 ± 0	1.4 ± 0.5	3.4 ± 1.2	0 ± 0	1.8 ± 0.6	0.6 ± 0.7	0.3 ± 0.2
tc (n=2)	5.6 ± 0.4	0.3 ± 0	0 ± 0	4.4 ± 0.7	22 ± 1.5	30.2 ± 0.1	1.6 ± 0.7	3.6 ± 1.2	3.1 ± 0.7	0.4 ± 0.1	0.9 ± 0	0.6 ± 0	0.3 ± 0.1	0.1 ± 0.1	3.5 ± 1.5	1.7 ± 0.4	3.4 ± 1.1	9.7 ± 3	0.3 ± 0	0 ± 0	1.6 ± 0.2	3.6 ± 1.4	0 ± 0	2.2 ± 0.7	0.7 ± 0	

Table 45: Fatty acid profiles of T2 seeds harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column; as defined in Table 41. For each category, the fatty acid profile of the plant having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1n -7	16:3n -3	18:0	18:1n -9	18:2n -6	18:2n -9	18:3n -3	18:3n -6	18:4n -3	20:0	20:1n -9	20:2n -6	20:3n -3	20:3n -6	20:4n -3	20:4n -6	20:5n -3	22:0	22:1n -9	22:4n -6	22:5n -3	22:5n -6	22:6n -3	unknown peak a	unknown peak b
sc (n=1)	5.3	0.3	0.0	3.2	26.0	32.3	0.6	4.5	1.0	0.2	0.8	0.8	1.1	0.4	1.8	1.1	5.7	9.3	0.4	0.0	0.7	3.2	0.0	1.4	0.0	0.0
dc (n=1)	4.5	0.1	0.0	2.9	28.2	18.2	0.5	4.7	0.5	0.2	0.8	1.2	2.9	1.1	1.9	2.1	3.6	15.9	0.4	0.0	0.7	4.8	0.0	3.2	0.0	1.7
tc (n=1)	3.5	0.1	0.0	2.7	18.2	26.4	0.4	4.6	0.9	0.2	0.7	0.9	2.0	0.7	2.2	1.9	5.5	16.7	0.3	0.0	1.2	6.6	0.0	3.3	0.2	1.0

Table 46: Phenotypic rating of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where rated per event. DFF: days to first flower (days), DF:

deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoL: number of lobes(#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad)

Event	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC
LALHCY (n=15)	59.3 ± 2.6	1 ± 0	1 ± 0	1 ± 0	1.3 ± 0.5	3 ± 0	3 ± 0	5 ± 0	1.7 ± 1.1	4 ± 0	136 ± 3.9	4.7 ± 0.2	3.3 ± 1.1
LALIAO (n=15)	65.9 ± 3.2	1 ± 0	1.2 ± 0.8	1.3 ± 0.5	1.8 ± 0.7	3 ± 0	3 ± 0	5 ± 0	1.3 ± 1.3	3.5 ± 0.9	124.3 ± 3.7	4 ± 0.5	3 ± 0.7
LALIKA (n=15)	62.1 ± 5.5	1 ± 0	1 ± 0	1.1 ± 0.3	2.5 ± 2.2	3 ± 0	3 ± 0	5 ± 0	1.6 ± 1.8	3.6 ± 0.7	126 ± 5.1	4.6 ± 0.2	3.4 ± 2
LALLTL (n=11)	55.3 ± 2.2	1 ± 0	1 ± 0	1 ± 0	1.5 ± 0.7	3 ± 0	3 ± 0	5 ± 0	1.2 ± 0.6	4 ± 0	128.6 ± 5.5	4.5 ± 0.2	2.2 ± 1.2
LALQAM (n=15)	58.3 ± 2.8	1 ± 0	1 ± 0	1 ± 0	2.7 ± 2.1	3 ± 0	3 ± 0	5 ± 0	2.1 ± 2.3	4 ± 0	133.7 ± 4	4.8 ± 0.2	4.7 ± 1.8
LALQDS (n=14)	54.5 ± 3.1	1 ± 0	1 ± 0	1.6 ± 0.9	2.6 ± 1.8	3 ± 0	3 ± 0	5 ± 0	1.9 ± 1.6	4 ± 0	127.1 ± 5.4	4.6 ± 0.2	3.4 ± 1.4
LALRCQ (n=14)	61.7 ± 1.7	1 ± 0	1 ± 0	1 ± 0	2.1 ± 1.1	3 ± 0	3 ± 0	5 ± 0	1.9 ± 1.5	3.7 ± 0.7	127.1 ± 3.8	4.8 ± 0.3	3.3 ± 1.2
LALWKF (n=14)	62.1 ± 3.2	1 ± 0	1 ± 0	1.4 ± 1.1	1.4 ± 0.8	3 ± 0	3 ± 0	5 ± 0	1.2 ± 0.6	4 ± 0	132.9 ± 5.8	4.5 ± 0.2	3.1 ± 0.8
LAMABL (n=8)	56.1 ± 1.6	1 ± 0	2.9 ± 1.6	2.1 ± 0.6	6.5 ± 2	3 ± 0	3 ± 0	5 ± 0	5 ± 2.3	4 ± 0	123.1 ± 4.6	4.1 ± 0.4	5.3 ± 1.6
LAMCKI (n=10)	61.5 ± 2.4	1 ± 0	1 ± 0	1 ± 0	1.6 ± 0.7	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	131 ± 3.9	4.1 ± 0.2	2.8 ± 0.6
LAMCLE (n=14)	65.4 ± 1.9	1 ± 0	1.4 ± 1.1	1.5 ± 0.8	2 ± 0.7	3 ± 0	3 ± 0	5 ± 0	1.3 ± 0.6	3.7 ± 0.7	124.3 ± 4.3	4.3 ± 0.3	3 ± 1
LAMCLF (n=9)	66.4 ± 4.3	1 ± 0	1.3 ± 1	1 ± 0	1.1 ± 0.3	3 ± 0	3 ± 0	5 ± 0	1 ± 0	3.1 ± 1.1	121.1 ± 3.3	4 ± 0.5	2.1 ± 0.3
LAMEUB (n=15)	57.7 ± 2.4	1 ± 0	1 ± 0	1.5 ± 0.7	4.5 ± 2.3	3 ± 0	3 ± 0	5 ± 0	3.9 ± 2.3	4 ± 0	128.3 ± 3.6	4.2 ± 0.4	3.1 ± 1
LAMEUU (n=13)	65.8 ± 3.6	1 ± 0	1.5 ± 1.1	1.5 ± 0.9	2.2 ± 1.9	3 ± 0	3 ± 0	5 ± 0	2.1 ± 1.7	3.7 ± 0.8	126.2 ± 4.2	4.2 ± 0.4	3.2 ± 0.7
LAMFJO (n=15)	64.7 ± 2.9	1 ± 0	1.6 ± 1.2	1.1 ± 0.3	3.7 ± 2.4	3 ± 0	3 ± 0	5 ± 0	2.3 ± 1.7	4 ± 0	123.7 ± 3.5	4.1 ± 0.5	4.1 ± 2.4
LAMIRY (n=10)	54.7 ± 2.3	1 ± 0	1 ± 0	1 ± 0	2.2 ± 2.1	3 ± 0	3 ± 0	5 ± 0	1.6 ± 1.9	4 ± 0	123 ± 4.2	4.9 ± 0.2	3.3 ± 1.7
LAMJIC (n=9)	60.1 ± 2.9	1 ± 0	2 ± 1.5	2.7 ± 2.5	5.3 ± 3.1	3 ± 0	3 ± 0	5 ± 0	3.6 ± 2	3.3 ± 1	111.1 ± 26.8	3.9 ± 0.7	4.4 ± 1.1
LAMPJB (n=13)	64.8 ± 2.5	1 ± 0	1.1 ± 0.3	1 ± 0	2.1 ± 0.8	3 ± 0	3 ± 0	5 ± 0	1.5 ± 0.9	4 ± 0	123.5 ± 2.4	4.6 ± 0.3	3.8 ± 1.5
LAMQDL (n=14)	67.9 ± 4.5	1 ± 0	1.2 ± 0.8	1.6 ± 0.8	2.6 ± 2.3	3 ± 0	3 ± 0	5 ± 0	1.6 ± 0.9	4 ± 0	117.1 ± 6.4	3.5 ± 0.4	3.8 ± 1.6
LAMQNE (n=14)	71.9 ± 6.2	1 ± 0	1.2 ± 0.8	1 ± 0	1.5 ± 1.3	3 ± 0	3 ± 0	5 ± 0	1.1 ± 0.5	4 ± 0	119.3 ± 3.3	4.2 ± 0.3	3.6 ± 1.3
LAMQSF (n=14)	61.1 ± 1.8	1 ± 0	1 ± 0	1 ± 0	1.6 ± 0.7	3 ± 0	3 ± 0	5 ± 0	1.1 ± 0.5	4 ± 0	123.9 ± 4	3.9 ± 0.5	2.6 ± 0.7
LAMRCO (n=14)	62.1 ± 2.9	1 ± 0	1 ± 0	1.6 ± 1	2.9 ± 1.7	3 ± 0	3 ± 0	5 ± 0	2.1 ± 1.3	4 ± 0	119.3 ± 4.3	3.9 ± 0.4	3.9 ± 2.1
LAMRDS (n=15)	63.7 ± 2.3	1 ± 0	1.4 ± 1.1	1.1 ± 0.3	1.9 ± 1.4	3 ± 0	3 ± 0	5 ± 0	1.4 ± 1.1	4 ± 0	125.7 ± 3.7	4.4 ± 0.4	2.7 ± 1.3
LAMRHL (n=14)	67.7 ± 4	1 ± 0	1 ± 0	1 ± 0	2.9 ± 2	3 ± 0	3 ± 0	5 ± 0	2.7 ± 2.4	4 ± 0	122.1 ± 2.6	4.1 ± 0.5	4.1 ± 1.1
LAMRJK (n=15)	63.4 ± 2.6	1 ± 0	1.3 ± 1	1 ± 0	3.5 ± 2	3 ± 0	3 ± 0	5 ± 0	2.5 ± 1.6	4 ± 0	124.7 ± 4.4	4.5 ± 0.4	3.8 ± 1.1
LAMRNQ (n=9)	63.1 ± 3	1 ± 0	1 ± 0	1 ± 0	1.1 ± 0.4	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	121.9 ± 2.6	3.8 ± 0.5	2.6 ± 1.3
LAMVUB (n=15)	65.5 ± 3.3	1 ± 0	1 ± 0	1 ± 0	3.3 ± 2.6	3 ± 0	3 ± 0	5 ± 0	2.3 ± 1.9	4 ± 0	124.3 ± 4.2	4.6 ± 0.3	4.2 ± 2.2
LAMYDP (n=14)	64.1 ± 4.6	1 ± 0	1 ± 0	1 ± 0	1.9 ± 0.7	3 ± 0	3 ± 0	5 ± 0	1.4 ± 0.9	4 ± 0	123.2 ± 3.7	4.5 ± 0.3	3.9 ± 1.4
LANBCH (n=14)	64.4 ± 2.8	1 ± 0	1 ± 0	1 ± 0	1.1 ± 0.4	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	123.9 ± 3.5	4.2 ± 0.2	3.1 ± 0.7

LANCEG (n=4)	67.3 ± 3.3	1 ± 0	1 ± 0	1.3 ± 0.5	1.3 ± 0.5	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	123.8 ± 2.5	4.1 ± 0.4	2.5 ± 0.6
LANCOX (n=10)	66.3 ± 3.2	1 ± 0	1 ± 0	1.6 ± 0.8	4.2 ± 2.3	3 ± 0	3 ± 0	5 ± 0	2.9 ± 1.4	3.6 ± 0.8	127.5 ± 2.6	4.6 ± 0.3	4 ± 1.5
LANFEF (n=15)	67.4 ± 2.4	1 ± 0	1.2 ± 0.8	1.1 ± 0.4	1.5 ± 0.6	3 ± 0	3 ± 0	5 ± 0	1.1 ± 0.5	4 ± 0	127 ± 3.2	4.7 ± 0.3	2.9 ± 0.5
LANMGC (n=15)	64.2 ± 2	1 ± 0	1 ± 0	2.3 ± 2.2	3 ± 0	3 ± 0	5 ± 0	1.7 ± 1.6	4 ± 0	123.7 ± 4	4.4 ± 0.3	3 ± 0.8
LANMOM (n=10)	65.9 ± 3.1	1 ± 0	1 ± 0	1.4 ± 1	3 ± 0	3 ± 0	5 ± 0	1.1 ± 0.3	4 ± 0	119.5 ± 3.7	3.4 ± 0.7	3.3 ± 1.2
LANPMZ (n=13)	65.3 ± 3.1	1 ± 0	1 ± 0	1.2 ± 0.4	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	124.2 ± 3.4	4.4 ± 0.2	2.4 ± 0.5
LANTLE (n=15)	55.6 ± 2	1 ± 0	2.5 ± 1.7	3.1 ± 0.9	1.9 ± 1.8	3 ± 0	3 ± 0	5 ± 0	1.3 ± 0.6	3.9 ± 0.5	106 ± 9.3	3.2 ± 0.3	2.6 ± 0.8
LANTSP (n=12)	65.3 ± 3.4	1 ± 0	1 ± 0	1.2 ± 0.4	1.3 ± 0.5	3 ± 0	3 ± 0	5 ± 0	1.2 ± 0.6	4 ± 0	124.6 ± 4	4.1 ± 0.4	3.8 ± 1.3
LANUCB (n=14)	58.3 ± 2.2	1 ± 0	1.2 ± 0.8	2.6 ± 0.6	1.9 ± 0.8	3 ± 0	3 ± 0	5 ± 0	1.4 ± 0.7	4 ± 0	117.9 ± 5.4	3.6 ± 0.3	3.1 ± 1
LAOBGQ (n=15)	67.8 ± 3.4	1 ± 0	1 ± 0	1.1 ± 0.4	1.2 ± 0.4	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	127 ± 3.2	4.2 ± 0.3	3.1 ± 0.6
LAOHLR (n=14)	61.7 ± 2.7	1 ± 0	1 ± 0	1 ± 0	1.1 ± 0.4	3 ± 0	3 ± 0	5 ± 0	1 ± 0	3.9 ± 0.5	123.2 ± 4.2	4.2 ± 0.5	2.8 ± 0.7
LAOJAT (n=15)	60.3 ± 2.8	1 ± 0	1 ± 0	1.4 ± 0.6	1.4 ± 0.5	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	124.3 ± 5	4 ± 0.4	4.8 ± 1.6
LAOKLP (n=15)	62 ± 2.9	1 ± 0	1 ± 0	1.1 ± 0.4	1.9 ± 1.5	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	125.7 ± 3.2	4.1 ± 0.3	3.8 ± 1.9
LAOKTE (n=15)	62.9 ± 3.7	1 ± 0	1.4 ± 1.1	1.7 ± 0.6	2.1 ± 1.1	3 ± 0	3 ± 0	5 ± 0	1.3 ± 1	4 ± 0	122.3 ± 4.2	3.7 ± 0.5	5.6 ± 2.7
LAPKLS (n=12)	66.3 ± 2.7	1 ± 0	1 ± 0	1.7 ± 0.9	1.9 ± 0.7	3 ± 0	3 ± 0	5 ± 0	1.2 ± 0.6	2.8 ± 1	118.3 ± 6.2	4.2 ± 0.3	4.5 ± 1.4
LAPKXM (n=15)	68.2 ± 3.2	1 ± 0	1.2 ± 0.8	1.9 ± 0.7	1.3 ± 0.5	3 ± 0	3 ± 0	5 ± 0	1 ± 0	3.2 ± 1	127.3 ± 2.6	4 ± 0.4	3.5 ± 0.5
LAPKZJ (n=15)	67.9 ± 2.7	1 ± 0	1.2 ± 0.8	1.1 ± 0.3	1.5 ± 0.5	3 ± 0	3 ± 0	5 ± 0	1 ± 0	3.9 ± 0.5	127 ± 3.2	4.2 ± 0.3	3.3 ± 0.8
LAPWJD (n=14)	68.3 ± 2.9	1 ± 0	1 ± 0	1.1 ± 0.4	1.7 ± 0.9	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	126.1 ± 4	4.6 ± 0.3	3.1 ± 0.3
LAPWLP (n=14)	64.9 ± 4	1 ± 0	1.5 ± 1.3	2 ± 1.4	3.6 ± 3	3 ± 0	3 ± 0	5 ± 0	2.6 ± 2.8	3.6 ± 0.9	119.3 ± 8.7	3.5 ± 0.4	4.1 ± 0.9
LAQYTA (n=15)	59.2 ± 3.9	1 ± 0	1.2 ± 0.8	2.6 ± 0.8	2.1 ± 1.5	3 ± 0	3 ± 0	5 ± 0	1.7 ± 1	3.7 ± 0.7	111.3 ± 6.1	3.6 ± 0.3	4.2 ± 1.3
LAQYUT (n=15)	66.3 ± 3	1 ± 0	1 ± 0	1.1 ± 0.3	1.6 ± 0.5	3 ± 0	3 ± 0	5 ± 0	1 ± 0	3.7 ± 0.7	126.3 ± 3	4.3 ± 0.2	3.7 ± 0.6
LAQYWQ (n=15)	66.7 ± 4.6	1 ± 0	1 ± 0	1.6 ± 0.7	2.5 ± 1.8	3 ± 0	3 ± 0	5 ± 0	1.2 ± 0.6	4 ± 0	124 ± 3.9	4.1 ± 0.4	3.8 ± 0.7
LAQZME (n=12)	64.3 ± 4.7	3 ± 3.6	1.8 ± 1.5	1.5 ± 0.5	4.1 ± 3.4	3 ± 0	3 ± 0	5 ± 0	2.8 ± 3.3	4 ± 0	124.2 ± 5.1	4.2 ± 0.4	4.8 ± 2.8

Table 47: Phenotypic rating of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column; as defined in Table 41. The number of T1 plants fullfilling these criteria are displayed in parentheses. DFF: days to first flower (days), DF: deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoI: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad)

Category of T1 plants	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC
sc (n=296)	62.9 ± 4.8	1 ± 0	1.2 ± 0.7	1.4 ± 0.9	2.5 ± 2	3 ± 0	3 ± 0	5 ± 0	1.7 ± 1.6	3.9 ± 0.4	123.9 ± 8	4.2 ± 0.6	3.6 ± 1.6

dc (n=198)	63.4 ± 4.5	1 ± 0.6	1.2 ± 0.7	1.2 ± 0.5	2 ± 1.6	3 ± 0	3 ± 0	5 ± 0	1.6 ± 1.4	3.8 ± 0.5	123.9 ± 10.4	4.2 ± 0.4	3.6 ± 1.6
tc (n=2)	59.5 ± 0.7	1 ± 0	1 ± 0	1 ± 0	1 ± 0	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	125 ± 0	3.7 ± 0.1	3.5 ± 0.7

Fatty acid profiles, copy number measurements, and phenotypic observations of T2 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc cultivated in greenhouses during summer

Table 48 shows the copy number analysis of select events. The events comprised one to two homozygous insertions and some had additional insertions still segregating. For example LANBCH segregated as homozygous for one T-DNA insertions for each construct, while LANPMZ segregated as homozygous for two T-DNA insertions for each construct. LALXOL seems to segregate for one insertion of VC-LLM337-1qcz rc, not homozygous, and for one homozygous insertion of LJB2197-1qcz_F with another copy which was not homozygous with the exception of the region around j-t-StCAT_p2_p-LuPXR, which seems to be a double copy event homozygous for each copy. For the T2 events selected, combined DHA and EPA levels were from nine to thirteen percent of the total fatty acids present in the seed. Whereas the selected T3 events had combined DHA and EPA levels varying from eleven to twenty three percent, with LALWPA having a DHA level of five percent and an EPA level of eighteen percent with respect to total fatty acid content in the seed, see Table 50. The selected events exhibited no morphological or anatomical defects relative to one another or to wild type.

Table 48: Copy number measurement of T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where measured per event. As the T2 plants underwent two cycles of selecting homozygous plants, all plants of all events are homozygous for all T-DNA insertions. A copy number of ~2 therefore was indicative of one homozygous locus, a copy number of ~4 indicative for two homozygous loci or indicative for one homozygous locus containing two copies of the target gene measured by the assay, and so forth. Odd results of 3 and 5 indicate that at least some of the selected T1 plants carry a heterozygous locus.

Event	Copy number assays targeting the T-DNA of VC-LJB2197-1qcz. Assays are listed according to the position of the assay target along the T-DNA, with left T-DNA border and target j-i-Atss18_c-d6Elo(Pp_GA2) near the right T-DNA border.								Copy number assays targeting the T-DNA of VC-LLM337-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target j-t-PvARC-p3 located near the left T-DNA border and target c-d4Des(Eg_GA) near the right T-DNA border.					
	c-AHAS	j-t-E9-p3-2	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-t-CaMV_p-LucNl-2	c-d6Elo(Pp_GA)	j-i-Atss18_c-d6Elo(Pp_GA2)	j-t-PvARC-p3	c-d4Des(Tc_GA)	c-o3Des(Pl_GA2_SNP)	j-i-Atss15_c-o3Des(Pl_GA2)	c-o3Des(Plr_GA)	j-p-VfSBPperm3_c-o3Des(Plr_GA)	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Eg_GA)
LANBCH (n=30)	4.0	3.7	4.1	4.0	4.4	4.0	3.8	2.9	3.9				3.8	3.8	3.8
LANPMZ (n=74)	2.0	2.0	2.0	2.0	1.6	2.0	2.0	1.8	2.1				2.0	2.0	2.1
LAOIKC (n=29)	2.1	4.0	3.9	4.2	4.3	4.1	4.3	2.3	2.0				2.1	2.3	2.2
LALHBO (n=29)	4.1	3.9	4.1	4.0	3.9	3.9	3.8	3.9	3.5				3.8	2.1	2.0
LALRCH (n=30)	4.1	3.8	4.0	3.9	3.4	4.1	4.0	3.9	4.1				3.6	3.8	4.0
LALWPA (n=29)	4.0	3.9	4.1	6.0	5.7	4.1	3.9	2.3	3.6				3.5	3.4	3.6
LALXOL (n=15)	2.1	2.1	2.1	4.1	2.7	2.0	2.1	0.9	1.2				1.2	1.1	1.2
LALXVM (n=30)	3.8	3.6	3.9	3.5	1.8	2.0	1.9	2.5	2.5				2.4	2.2	2.3
LALZGS (n=24)	1.9	4.0	3.8	4.0	1.8	2.0	2.0	2.1	2.0				2.0	2.1	2.2
LAMADR (n=44)	4.0	3.9	3.8	4.0	4.6	4.0	3.9	2.5	3.4				3.1	3.0	3.1
LAMQJH (n=30)	4.0	4.0	4.0	3.9	2.9	4.1	4.1	4.5	4.1				4.0	3.9	4.2
LAMQUI (n=36)	2.2	2.1	4.0	3.7	3.5	3.6	3.8	1.5	1.3				2.9	5.2	5.5
LAMRUR (n=30)	3.6	3.7	3.4	3.7	4.1	3.6	3.8	3.7	3.6				3.8	4.3	3.9

LANPSF (n=30)	4.0	4.0	3.8	3.9	4.1	3.9	4.1	1.5	3.5			3.4	4.3	3.9

Table 49: Fatty acid profiles of T3 seeds harvested from T2 cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc The events are indicated in the first column, along with the number of T3 seed batches that were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unkn own peak a	unkn own peak b
LANBCH (n=30)	4.5 ± 0.6	0.2 ± 0	0.1 ± 0	4 ± 0.2	22.2 ± 1.1	31.1 ± 0.7	1 ± 0.1	4 ± 0.2	1.8 ± 0.2	0.2 ± 0	0.8 ± 0	0.6 ± 0	0.5 ± 0.1	0.1 ± 0	2.6 ± 0.3	1.8 ± 0.1	3.3 ± 0.1	11.4 ± 0.5	0.3 ± 0	0 ± 0	1.7 ± 0.1	4.4 ± 0.2	0 ± 0	2.3 ± 0.2	0.6 ± 0.1	0.4 ± 0.1
LANPMZ (n=74)	5 ± 0.4	0.2 ± 0	0 ± 0	3.6 ± 0.4	26.3 ± 2.1	35.2 ± 1.6	0.4 ± 0.1	4.6 ± 0.6	0.7 ± 0.2	0.1 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	1.1 ± 0.4	0.3 ± 0.2	2.4 ± 0.3	1.5 ± 0.2	3.1 ± 0.4	7.2 ± 0.8	0.3 ± 0.1	0 ± 0	1 ± 0.1	2.9 ± 0.5	0 ± 0	1.5 ± 0.3	0.4 ± 0.1	0.5 ± 0.1
LAOIKC (n=29)	5.1 ± 0.6	0.2 ± 0	0.1 ± 0	3.6 ± 0.3	22.1 ± 1.7	30.8 ± 1	0.9 ± 0.1	4 ± 0.3	1.8 ± 0.2	0.2 ± 0	0.8 ± 0.1	0.6 ± 0	0.5 ± 0.1	0.1 ± 0	3.1 ± 0.8	1.6 ± 0.3	5.9 ± 0.5	10.3 ± 0.8	0.3 ± 0	0 ± 0	1.3 ± 0.1	4 ± 0.3	0 ± 0	1.8 ± 0.2	0.4 ± 0.1	0.3 ± 0
LALHBO (n=29)	4.4 ± 0.3	0.2 ± 0.1	0 ± 0	3.9 ± 0.4	19.6 ± 2.3	28.8 ± 1.5	0.6 ± 0.1	4.9 ± 0.3	1.3 ± 0.3	0.2 ± 0.1	0.9 ± 0.1	0.7 ± 0	0.9 ± 0.3	0.3 ± 0.1	2.4 ± 0.3	2 ± 0.2	4.3 ± 0.3	13.4 ± 1.9	0.3 ± 0	0 ± 0	1.3 ± 0.2	5.7 ± 0.8	0 ± 0	2.7 ± 0.4	0.4 ± 0.1	0.6 ± 0.1
LALRCH (n=30)	4.6 ± 0.2	0.2 ± 0	0.1 ± 0	3.7 ± 0.3	22.3 ± 1.2	30.1 ± 1	0.9 ± 0.1	4.2 ± 0.2	1.6 ± 0.2	0.2 ± 0	0.8 ± 0	0.7 ± 0	0.7 ± 0.2	0.2 ± 0.1	2.7 ± 0.3	1.8 ± 0.2	4 ± 0.4	11.2 ± 0.8	0.3 ± 0	0 ± 0	1.8 ± 0.1	4.5 ± 0.4	0 ± 0	2.3 ± 0.4	0.6 ± 0.1	0.5 ± 0.2
LALWPA (n=29)	4.4 ± 0.2	0.2 ± 0.1	0 ± 0	3.3 ± 0.2	17.3 ± 1.6	25.6 ± 1.4	1 ± 0.1	4.4 ± 0.4	2.3 ± 0.3	0.4 ± 0.1	0.8 ± 0	0.7 ± 0	0.5 ± 0.2	0.2 ± 0	2 ± 0.2	1.6 ± 0.1	5.7 ± 0.7	14.8 ± 1.5	0.4 ± 0	0 ± 0	2.1 ± 0.2	7.3 ± 0.8	0 ± 0	4 ± 0.7	0.5 ± 0.1	0.3 ± 0.1
LALXOL (n=15)	4.4 ± 0.3	0.2 ± 0	0 ± 0	4.4 ± 0.6	21.4 ± 1.1	28.4 ± 1.2	0.4 ± 0.1	3.5 ± 0.3	0.8 ± 0.2	0.1 ± 0	0.9 ± 0.1	0.9 ± 0.1	2.5 ± 0.5	0.5 ± 0.2	2.2 ± 0.3	1.3 ± 0.3	8.7 ± 2.1	11.6 ± 1.3	0.3 ± 0	0 ± 0	0.8 ± 0.2	3.5 ± 0.8	0 ± 0	1.8 ± 0.4	0.1 ± 0.1	1 ± 0.2
LALXVM (n=30)	4.5 ± 1	0.2 ± 0	0 ± 0	3.8 ± 0.4	21.3 ± 1.6	28.8 ± 1.7	0.7 ± 0.2	4.5 ± 0.6	1.2 ± 0.6	0.2 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	1.5 ± 0.8	0.5 ± 0.3	2.7 ± 0.3	1.9 ± 0.3	4.7 ± 0.9	11.7 ± 1.3	0.3 ± 0	0 ± 0	1.4 ± 0.4	5.1 ± 0.8	0 ± 0	2.3 ± 0.3	0.4 ± 0.2	0.8 ± 0.4
LALZGS (n=24)	4.5 ± 0.7	0.3 ± 0	0 ± 0	3.4 ± 0.5	21.8 ± 1.8	29.4 ± 1.4	0.6 ± 0.1	4.2 ± 0.5	1.2 ± 0.3	0.2 ± 0	0.8 ± 0.1	0.7 ± 0.1	1.3 ± 0.3	0.3 ± 0.1	2.6 ± 0.3	1.8 ± 0.3	5.6 ± 0.6	12.1 ± 1.2	0.3 ± 0	0 ± 0	1.2 ± 0.1	4.7 ± 0.6	0 ± 0	2.1 ± 0.4	0.2 ± 0.2	0.8 ± 0.2
LAMADR (n=44)	3.7 ± 1.6	0.2 ± 0	0.1 ± 0	4 ± 0.5	22.3 ± 2.8	32.5 ± 1.5	0.8 ± 0.2	4.3 ± 0.6	1.9 ± 0.4	0.3 ± 0.1	0.9 ± 0.1	0.7 ± 0	0.5 ± 0.2	0.1 ± 0	2.2 ± 0.4	1.4 ± 0.2	4.4 ± 0.2	10.8 ± 2	0.3 ± 0	0 ± 0	1.6 ± 0.2	4.1 ± 0.5	0 ± 0	2.1 ± 0.3	0.5 ± 0.2	0.3 ± 0.1
LAMQJH (n=30)	4.9 ± 0.3	0.3 ± 0	0.1 ± 0.1	3.8 ± 0.3	21.9 ± 1.7	30.9 ± 1.1	1 ± 0.2	4.3 ± 0.3	2.2 ± 0.2	0.3 ± 0.1	0.8 ± 0	0.6 ± 0.1	0.2 ± 0.1	3 ± 0.5	1.7 ± 0.2	3.4 ± 0.4	10.7 ± 1	0.3 ± 0.1	0 ± 0	1.8 ± 0.2	4.4 ± 0.6	0 ± 0	2.3 ± 0.5	0.7 ± 0.1	0.2 ± 0
LAMQUI (n=36)	3.2 ± 2	0.2 ± 0.1	0.1 ± 0	3.1 ± 0.8	25.1 ± 2.7	26 ± 1.4	1.1 ± 0.3	4.5 ± 0.9	1.5 ± 0.4	0.2 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	1.1 ± 0.7	0.3 ± 0.2	2.2 ± 0.4	1.3 ± 0.3	4.7 ± 0.7	11.6 ± 2.2	0.2 ± 0	0 ± 0	2.4 ± 0.4	6.1 ± 0.9	0 ± 0	2.3 ± 0.6	0.6 ± 0.1	0.7 ± 0.3

LAMRUR (n=30)	4.9 ± 0.3	0.2 ± 0	0.2 ± 0.1	3.9 ± 0.3	23.4 ± 1.7	32 ± 1.1	1.1 ± 0.2	4 ± 0.5	2.3 ± 0.3	0.3 ± 0	0.8 ± 0.1	0.7 ± 0	0.2 ± 0.1	0 ± 0	2.9 ± 0.6	1.5 ± 0.2	4 ± 0.4	9.4 ± 0.9	0.2 ± 0.1	0 ± 0	1.6 ± 0.2	3.7 ± 0.3	0 ± 0	1.9 ± 0.3	0.6 ± 0.1	0.2 ± 0.1
LANPSF (n=30)	4.7 ± 0.2	0.3 ± 0	0.1 ± 0	3.9 ± 0.2	23.2 ± 1.3	30.9 ± 0.9	0.8 ± 0.1	4.5 ± 0.5	1.7 ± 0.3	0.3 ± 0	0.8 ± 0.1	0.7 ± 0	0.5 ± 0.1	0.1 ± 0	2.2 ± 0.2	1.5 ± 0.1	3.7 ± 0.7	9.9 ± 0.8	0.3 ± 0	0 ± 0	2.1 ± 0.2	4.4 ± 0.4	0 ± 0	2.3 ± 0.2	0.7 ± 0.2	0.3 ± 0.1
WT Kumily (n=46)	5 ± 0.1	0.4 ± 0	0.2 ± 0	2.6 ± 0.1	66.3 ± 1.7	16.8 ± 1.3	0 ± 0	6.1 ± 0.4	0 ± 0	0 ± 0	0.9 ± 0	1.2 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.5 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Table 50: Fatty acid profiles of one T3 seed batch per event harvested from T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T3 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LANBCH (n=1)	4.6	0.2	0.1	3.9	21.3	30.3	1.1	4.0	2.0	0.3	0.8	0.6	0.4	0.1	2.5	1.8	3.3	12.4	0.3	0.0	1.8	4.8	0.0	2.5	0.7	0.3
LANPMZ (n=1)	4.6	0.3	0.1	4.0	22.6	32.2	0.3	5.2	0.7	0.1	0.9	0.8	1.4	0.5	2.6	2.1	3.1	9.7	0.3	0.0	1.1	4.2	0.0	2.3	0.5	0.5
LAOIKC (n=1)	7.9	0.5	0.1	3.2	19.0	28.4	0.8	3.4	1.9	0.2	0.7	0.5	0.5	0.1	3.0	1.6	6.5	12.2	0.3	0.0	1.5	4.6	0.0	2.2	0.3	0.4
LALHBO (n=1)	4.1	0.1	0.0	3.6	16.4	26.5	0.6	4.8	1.5	0.3	0.9	0.7	1.1	0.4	2.7	2.3	4.3	16.6	0.4	0.0	1.4	7.0	0.0	3.5	0.5	0.6
LALRCH (n=1)	4.1	0.2	0.1	3.6	20.5	28.5	0.8	4.3	1.3	0.2	0.7	0.7	1.2	0.3	2.7	2.0	4.6	12.3	0.3	0.0	1.8	5.0	0.0	3.3	0.6	1.0
LALWPA (n=1)	4.7	0.2	0.0	3.0	14.6	20.2	1.1	4.7	2.8	0.7	0.8	0.7	0.7	0.2	1.6	1.6	5.4	18.1	0.4	0.0	2.0	10.0	0.0	5.7	0.5	0.4
LALXOL (n=1)	4.6	0.2	0.0	4.9	21.0	26.0	0.5	4.2	0.7	0.2	1.0	0.9	2.3	0.9	2.1	1.9	3.4	14.0	0.4	0.0	1.1	5.4	0.0	2.7	0.4	1.1
LALXVM (n=1)	4.7	0.3	0.0	3.7	18.3	26.9	0.5	4.5	0.8	0.2	0.8	0.9	2.4	0.8	2.7	2.2	4.7	14.3	0.3	0.0	1.1	5.6	0.0	2.8	0.4	1.1
LALZGS (n=1)	4.6	0.3	0.0	2.5	19.0	28.4	0.6	3.7	1.5	0.2	0.6	0.7	1.2	0.3	2.7	1.9	7.0	14.1	0.2	0.0	1.4	5.5	0.0	2.9	0.0	0.7
LAMADR (n=1)	4.2	0.2	0.1	3.6	17.2	29.3	0.9	3.4	3.1	0.4	0.7	0.7	0.5	0.1	2.6	1.5	5.8	14.7	0.3	0.0	2.1	5.1	0.0	2.7	0.5	0.3
LAMQJH (n=1)	4.8	0.3	0.1	3.0	17.7	28.1	0.7	4.6	2.3	0.4	0.7	0.6	0.4	0.1	2.2	1.5	4.7	14.4	0.4	0.0	2.2	6.8	0.0	3.5	0.5	0.2
LAMQUI (n=1)	0.2	0.1	0.1	3.8	24.4	25.8	1.2	2.4	1.9	0.4	0.7	0.8	1.1	0.3	2.2	1.6	4.0	14.9	0.2	0.0	2.2	7.3	0.0	3.1	0.6	0.8
LAMRUR (n=1)	4.9	0.2	0.3	4.2	20.3	32.0	1.1	3.2	2.7	0.3	0.8	0.6	0.0	0.0	3.0	1.7	4.0	11.3	0.3	0.0	1.8	3.8	0.0	2.7	0.6	0.3
LANPSF (n=1)	4.7	0.3	0.0	3.9	20.8	30.7	0.8	4.1	2.0	0.3	0.8	0.6	0.5	0.1	2.4	1.4	4.5	11.4	0.3	0.0	2.1	4.7	0.0	2.6	0.6	0.4
LANBCH (n=1)	4.6	0.2	0.1	3.9	21.3	30.3	1.1	4.0	2.0	0.3	0.8	0.6	0.4	0.1	2.5	1.8	3.3	12.4	0.3	0.0	1.8	4.8	0.0	2.5	0.7	0.3

Table 51: Phenotypic rating of T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where rated per event. DFF: days to first flower (days), DF:

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deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoI: number of lobes(#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad), Oil: oil content (% of seed weight), Protein: Protein content (% of seed cake without oil)

Event	DFF	DF	DL	DP	D5	FC	LD	LGC	LF	NoL	PH	TKW	SC	Oil	Protein
LALHCY (n=1)	59.1 ± 3.3	9 ± 0	8.9 ± 0.4	9 ± 0	7 ± 0.9	3 ± 0	3 ± 0	5 ± 0	7.5 ± 1.4	4 ± 0.2	129.2 ± 3.5				
LANBCH (n=30)	51.9 ± 3.1	9 ± 0.2	8.5 ± 0.7	9 ± 0.2	8.1 ± 0.7	3 ± 0	3 ± 0	5 ± 0	8 ± 0.7	5 ± 0.8	127.7 ± 6.6			35.2 ± 2.8	30 ± 2
LANPMZ (n=74)	57.3 ± 1.6	9 ± 0	8.9 ± 0.4	9 ± 0	7.3 ± 0.7	3 ± 0	3 ± 0	5 ± 0	7.9 ± 1.1	4 ± 0	125.3 ± 5				
LAOIKC (n=29)	65.2 ± 2.1	9 ± 0	7.1 ± 1.7	7.4 ± 1.1	6.2 ± 2.9	3 ± 0	3 ± 0	5 ± 0	7.7 ± 2	4.1 ± 0.4	118.8 ± 5.8	4.9 ± 0.5	5.3 ± 2.3		
LALHBO (n=29)	61.3 ± 1.9	9 ± 0	7.9 ± 1.3	8.8 ± 0.4	7.3 ± 0.5	3 ± 0	3 ± 0	5.1 ± 0.4	8 ± 0.9	3.9 ± 0.4	128.3 ± 3.3				
LALRCH (n=30)	61.3 ± 1.8	9 ± 0	8.2 ± 1.4	7.8 ± 1	7.7 ± 1.9	3 ± 0	3 ± 0	5 ± 0	8.2 ± 1.3	3.8 ± 0.6	125 ± 3	5.2 ± 0.4	3.7 ± 1		
LALWPA (n=29)	60.4 ± 3.9	9 ± 0	9 ± 0	9 ± 0	7.6 ± 1.1	3 ± 0	3 ± 0	5 ± 0	7.6 ± 1.1	4.9 ± 0.8	127.3 ± 3.7				
LALXOL (n=15)	60.7 ± 4.6	9 ± 0	8.9 ± 0.7	8.2 ± 0.8	7.6 ± 0.9	3 ± 0	3 ± 0	5 ± 0	8.4 ± 0.9	4 ± 0.6	126 ± 4.6	5.4 ± 0.4	3.3 ± 1.3		
LALXVM (n=30)	56.7 ± 4	8.7 ± 1.3	9 ± 0	8.9 ± 0.4	7.3 ± 1.3	3 ± 0	3 ± 0	5 ± 0	7.3 ± 1.4	5.6 ± 1.3	121 ± 24.4				
LALZGS (n=24)	56.9 ± 6	9 ± 0	8.4 ± 1.3	9 ± 0	7.8 ± 1	3 ± 0	3 ± 0	5 ± 0	7.9 ± 0.9	5.1 ± 0.7	128.4 ± 5.5				
LAMADR (n=44)	59.9 ± 2.6	9 ± 0	8.7 ± 0.9	8.8 ± 0.5	7.5 ± 1.1	3 ± 0	3 ± 0	5 ± 0	8.2 ± 1.2	4 ± 0.2	126.5 ± 5.3				
LAMQJH (n=30)	60.3 ± 3.1	9 ± 0	8.5 ± 1.1	8.3 ± 0.6	8.1 ± 0.9	3 ± 0	3 ± 0	5 ± 0	8.6 ± 0.9	3.9 ± 0.3	124 ± 4.6	5.3 ± 0.4	4.7 ± 1.3		
LAMQUI (n=36)	59.6 ± 1.9	9 ± 0	9 ± 0	9 ± 0	8.3 ± 0.7	3 ± 0	3 ± 0	5 ± 0	8.3 ± 0.7	4.9 ± 0.5	129.3 ± 4.5				
LAMRUR (n=30)	55.1 ± 2.9	9 ± 0	9 ± 0	9 ± 0	7.3 ± 0.8	3 ± 0	3 ± 0	5 ± 0	7.4 ± 0.9	5.7 ± 0.8	126.8 ± 4.4				
LANPSF (n=30)	59.1 ± 3.3	9 ± 0	8.9 ± 0.4	9 ± 0	7 ± 0.9	3 ± 0	3 ± 0	5 ± 0	7.5 ± 1.4	4 ± 0.2	129.2 ± 3.5				
WT Kumily (n=46)														34.9 ± 1.1	32.2 ± 1

Fatty acid profiles of T2 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc cultivated in field trials in USDA growth zones 3a-4b and 5a during summer.

Field data from the T3 seed indicate that field values are lower for EPA and DHA than what was observed in the greenhouse, with values ranging from six to thirteen percent of the total fatty acid content of the seed for EPA and DHA combined. These data show a difference in seed oil content observed in field studies compared to the greenhouse (e.g. comparing Table 54 with Table 51), see also Example 10. Results of this analysis are described in Example 20.

Table 52: Fatty acid profiles of T3 seeds harvested from T2 cultivated in the field in field trials, corresponding to USDA zones 3a-4b and zone 5a , of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc The events are indicated in the first column, along with the number of T3 seed aliquots representing a plot were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unkn own peak a	unkn own peak b
LANBCH (n=30)	5.1 ± 0.2	0.4 ± 0	0 ± 0	2.6 ± 0.1	26 ± 1.9	31.3 ± 1	0.7 ± 0.1	6.2 ± 0.4	1.2 ± 0.2	0.2 ± 0	0.7 ± 0	0.7 ± 0	0.7 ± 0.1	0.2 ± 0	1.9 ± 0.1	1.1 ± 0.1	3.4 ± 0.3	8.9 ± 0.8	0.3 ± 0	0 ± 0	1.4 ± 0.2	4.7 ± 0.4	0 ± 0	1.5 ± 0.2	0.4 ± 0.1	0.3 ± 0.1
LANPMZ (n=30)	4.9 ± 0.1	0.3 ± 0	0 ± 0	2.4 ± 0.1	30.7 ±1.5	32.1 ±0.8	0.3 ± 0	6.9 ± 0.3	0.5 ± 0.1	0.1 ± 0	0.7 ± 0	0.9 ± 0	1.4 ± 0.3	0.5 ± 0.1	2 ± 0.1	1.2 ± 0.1	2.9 ± 0.2	5.8 ± 0.5	0.3 ± 0	0 ± 0	0.8 ± 0.1	3.3 ± 0.2	0 ± 0	1 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
LAOIKC (n=31)	5.2 ± 0.2	0.3 ± 0	0 ± 0	2.5 ± 0.1	26 ± 2.2	31.4 ±1.1	0.8 ± 0.1	5.5 ± 0.3	1.4 ± 0.2	0.2 ± 0	0.7 ± 0	0.7 ± 0	0.6 ± 0.1	0.1 ± 0	2.4 ± 0.2	1.1 ± 0.1	5.7 ± 0.5	8 ± 0.6	0.2 ± 0	0 ± 0	1.1 ± 0.1	4.1 ± 0.3	0 ± 0	1.2 ± 0.2	0.1 ± 0.1	0.4 ± 0.1
LALHBO (n=29)	5.1 ± 0.2	0.2 ± 0.1	0 ± 0	2.5 ± 0.2	28 ± 6.4	30.3 ± 2	0.6 ± 0.2	6.7 ± 1.1	1.1 ± 0.4	0.2 ± 0.1	0.7 ± 0	0.8 ± 0.1	0.9 ± 0.2	0.3 ± 0.1	1.9 ± 0.4	1.1 ± 0.2	3.8 ± 1	8.5 ± 2.1	0.3 ± 0	0 ± 0	0.9 ± 0.3	4.2 ± 1	0 ± 0	1.2 ± 0.3	0.1 ± 0.1	0.4 ± 0.1
LALWPA (n=30)	5 ± 0.4	0.3 ± 0	0 ± 0	2.2 ± 0.2	21.3 ±2.7	28.8 ±1.8	0.8 ± 0.1	5.5 ± 0.5	1.6 ± 0.3	0.3 ± 0.1	0.7 ± 0	0.8 ± 0	1 ± 0.2	0.3 ± 0	2.3 ± 0.3	1.3 ± 0.1	6 ± 1.4	11.1 ± 1.3	0.3 ± 0	0 ± 0	1.5 ± 0.1	5.9 ± 0.7	0 ± 0	2 ± 0.3	0.3 ± 0.2	0.5 ± 0.1
LALXOL (n=29)	5.1 ± 0.2	0.3 ± 0.1	0 ± 0	2.6 ± 0.2	26.8 ±1.9	29.7 ±1.8	0.4 ± 0.1	6 ± 0.8	0.8 ± 0.1	0.2 ± 0	0.7 ± 0	1 ± 0.1	2.1 ± 0.7	0.5 ± 0.2	1.9 ± 0.2	1 ± 0.1	5.5 ± 1.6	8.5 ± 1	0.3 ± 0	0 ± 0	0.8 ± 0.1	3.8 ± 0.6	0 ± 0	1.1 ± 0.2	0 ± 0	0.8 ± 0.2
LALXVM (n=30)	5 ± 0.3	0.3 ± 0	0 ± 0	2.4 ± 0.1	29 ± 4	29.9 ±1.3	0.5 ± 0.1	6.3 ± 0.9	0.8 ± 0.2	0.2 ± 0	0.7 ± 0	0.9 ± 0.1	1.3 ± 0.4	0.4 ± 0.1	2 ± 0.3	1.1 ± 0.1	4.4 ± 1.3	7.7 ± 1.8	0.3 ± 0	0 ± 0	0.8 ± 0.2	3.9 ± 0.9	0 ± 0	1.1 ± 0.3	0.2 ± 0.1	0.6 ± 0.2
LALZGS (n=29)	5.1 ± 0.2	0.2 ± 0.1	0 ± 0	2.6 ± 0.2	25.1 ±1.4	30.6 ±1.2	0.6 ± 0.1	5.9 ± 0.4	1 ± 0.1	0.2 ± 0	0.7 ± 0	0.8 ± 0	1 ± 0	0.3 ± 0.1	2.3 ± 0.1	1.2 ± 0.1	5.7 ± 0.5	8.8 ± 0.5	0.2 ± 0.1	0 ± 0	0.9 ± 0.1	4.5 ± 0.2	0 ± 0	1.2 ± 0.1	0 ± 0.1	0.7 ± 0.1
LAMADR (n=30)	5.1 ± 0.1	0.2 ± 0.1	0 ± 0	2.7 ± 0.2	25.9 ± 1	32 ± 1.2	0.7 ± 0.1	5.9 ± 0.4	1.3 ± 0.2	0.2 ± 0	0.7 ± 0	0.8 ± 0	0.7 ± 0	0.2 ± 0	2.1 ± 0.1	1 ± 0.1	4.7 ± 0.6	8 ± 0.7	0.3 ± 0	0 ± 0	1.3 ± 0.1	4.1 ± 0.3	0 ± 0	1.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.1
LAMQUI (n=30)	4.7 ± 0.2	0.3 ± 0.1	0 ± 0	2.5 ± 0.2	28.8 ±1.9	29 ± 1.6	0.8 ± 0.2	5.7 ± 0.5	1.2 ± 0.2	0.2 ± 0	0.6 ± 0	0.9 ± 0.1	1 ± 0.3	0.2 ± 0.1	1.7 ± 0.3	0.8 ± 0.1	4.6 ± 0.9	7.6 ± 1	0.2 ± 0	0 ± 0	2.1 ± 0.3	4.6 ± 0.7	0 ± 0	1.4 ± 0.2	0.5 ± 0.1	0.5 ± 0.1
LAMRJR (n=29)	5.1 ± 0.3	0.3 ± 0	0 ± 0	2.6 ± 0.1	28.5 ±2.3	32 ± 1.9	0.8 ± 0.2	5.8 ± 0.8	1.3 ± 0.2	0.2 ± 0	0.7 ± 0.1	0.7 ± 0.1	0.4 ± 0.1	0 ± 0.1	2.1 ± 0.2	1 ± 0.1	3.8 ± 0.6	7.2 ± 0.7	0.2 ± 0.1	0 ± 0	1.3 ± 0.2	3.9 ± 0.4	0 ± 0	1.2 ± 0.2	0.3 ± 0.1	0.3 ± 0.1
LANPSF (n=28)	5.1 ± 0.2	0.3 ± 0.1	0 ± 0	2.6 ± 0.1	25.9 ±1.8	31.4 ±1.4	0.7 ± 0.2	6.1 ± 0.7	1.2 ± 0.3	0.2 ± 0	0.7 ± 0	0.8 ± 0.1	0.7 ± 0.2	0.2 ± 0.1	1.9 ± 0.2	1 ± 0.1	3.9 ± 0.9	7.6 ± 1.1	0.3 ± 0	0 ± 0	1.7 ± 0.2	4.2 ± 0.4	0 ± 0	1.4 ± 0.2	0.6 ± 0.2	0.4 ± 0.1
WT Kumily (n=60)	5 ± 0.3	0.4 ± 0	0.1 ± 0.1	2 ± 0.1	56.2 ±4.2	23.3 ±1.7	0 ± 0.1	9.5 ± 0.7	0.1 ± 0.2	0 ± 0	0.7 ± 0	1.1 ± 0.1	0.1 ± 0.1	0 ± 0	0.2 ± 0.3	0.1 ± 0.3	0.2 ± 0.5	0.4 ± 1.1	0.3 ± 0.1	0 ± 0	0 ± 0.2	0.2 ± 0.6	0 ± 0	0 ± 0.2		

Table 53: Fatty acid profiles of one T3 seed batch per event harvested from T2 plants cultivated in field trials, corresponding to USDA zones 3a-4b and zone 5a, of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T3 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unkown peak a	unkown peak b
LANBCH (n=30)	5.3	0.4	0.1	2.5	25.5	29.5	0.7	6.1	1.3	0.3	0.6	0.7	0.8	0.2	1.9	1.1	3.7	10.1	0.2	0.0	1.4	5.2	0.0	1.6	0.2	0.4
LANPMZ (n=30)	5.1	0.3	0.0	2.3	27.1	31.5	0.2	6.9	0.4	0.1	0.7	0.9	2.1	0.8	2.4	1.5	3.0	6.8	0.3	0.0	0.9	4.1	0.0	1.4	0.5	0.6
LAOIKC (n=31)	5.3	0.3	0.0	2.6	23.3	32.0	0.8	5.3	1.5	0.2	0.7	0.7	0.5	0.1	2.5	1.2	6.1	8.9	0.2	0.0	1.2	4.5	0.0	1.4	0.1	0.4
LALHBO (n=29)	5.2	0.4	0.1	2.5	25.8	29.0	0.8	5.5	1.5	0.3	0.7	0.7	0.7	0.2	2.1	1.2	4.5	10.6	0.3	0.0	1.1	4.9	0.0	1.6	0.0	0.4
LALWPA (n=30)	5.9	0.4	0.0	2.0	15.4	27.9	0.8	5.2	2.5	0.5	0.7	0.7	0.9	0.2	2.7	1.4	7.1	13.2	0.4	0.0	1.8	6.9	0.0	2.7	0.0	0.4
LALXOL (n=29)	5.2	0.2	0.0	2.7	24.3	28.8	0.5	5.9	0.9	0.2	0.7	1.0	2.3	0.6	1.9	1.1	5.3	10.1	0.3	0.0	0.9	4.8	0.0	1.4	0.0	1.0
LALXVM (n=30)	4.9	0.3	0.1	2.4	26.7	28.2	0.5	5.3	0.8	0.2	0.7	0.9	1.6	0.5	2.1	1.2	5.3	10.2	0.3	0.0	0.8	4.5	0.0	1.4	0.1	0.9
LALZGS (n=29)	5.4	0.3	0.0	2.4	24.5	29.1	0.6	5.6	1.1	0.2	0.6	0.8	1.1	0.3	2.5	1.3	6.3	9.6	0.2	0.0	1.0	4.9	0.0	1.5	0.0	0.7
LAMADR (n=30)	5.2	0.3	0.0	2.6	25.6	30.1	0.7	5.5	1.4	0.3	0.7	0.8	0.8	0.1	2.1	1.0	5.4	9.1	0.3	0.0	1.3	4.5	0.0	1.5	0.0	0.4
LAMQUI (n=30)	4.4	0.4	0.0	2.3	26.7	26.2	0.9	5.3	1.2	0.2	0.6	0.9	1.3	0.3	2.0	1.0	5.0	9.8	0.2	0.0	2.4	6.0	0.0	1.7	0.6	0.7
LAMRUR (n=29)	6.3	0.3	0.0	2.8	34.5	23.3	0.5	4.5	1.1	0.2	0.5	0.5	0.4	0.1	2.3	1.2	3.5	9.6	0.2	0.0	1.1	5.1	0.0	1.7	0.0	0.3
LANPSF (n=28)	5.0	0.3	0.1	2.5	26.6	29.7	0.8	4.9	1.6	0.3	0.7	0.7	0.5	0.1	1.9	1.0	5.4	9.2	0.3	0.0	1.7	4.6	0.0	1.5	0.4	0.3

Table 54: Phenotypic rating of T2 plants cultivated in the field of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column, along with the number of field plots that where rated per event. Oil: oil content (% of seed weight), protein: Protein content (% of seed cake without oil)

Event	Oil	protein
LANBCH (n=30)	37.9 ± 1.2	27.9 ± 0.9
LANPMZ (n=30)	38.7 ± 1	27.8 ± 0.9
LAOIKC (n=31)	38.8 ± 1.1	28.1 ± 2.7
LALHBO (n=29)	37.9 ± 1.4	28.2 ± 0.7
LALWPA (n=30)	36.5 ± 1.4	28 ± 0.7
LALXOL (n=29)	38.4 ± 1.1	27.7 ± 0.6
LALXVM (n=30)	38.3 ± 1.1	27.7 ± 1
LALZGS (n=29)	39.5 ± 0.7	27.2 ± 0.6
LAMADR (n=30)	38.7 ± 0.9	27.6 ± 0.5
LAMQUI (n=30)	38.3 ± 0.9	28.7 ± 0.8
LAMRUR (n=29)	38.3 ± 1.1	27.8 ± 0.8
LANPSF (n=28)	38.4 ± 1.1	27.6 ± 0.8
WT Kumily (n=60)	38.7 ± 1.1	

Fatty acid profiles copy number measurements, and phenotypic observations of T3 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc cultivated in greenhouses during winter

The data indicate that EPA and DHA are still being synthesized by the plant in the T4 seed/generation.

Table 55: Copy number measurement of T3 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column, along with the number of T3 plants that were measured per event. As the T3 plants underwent two cycles of selecting homozygous plants, all plants of all events are homozygous for all T-DNA insertions. A copy number of ~2 therefore was indicative for one homozygous locus, a copy number of ~4 indicative for two homozygous loci or indicative for one homozygous locus containing two copies of the target gene measured by the assay, and so forth.

Event	Copy number assays targeting the T-DNA of VC-LJB2197-1qcz. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target j-i-Atss18_c-d6Elo(Pp_GA2) near the right T-DNA border.						Copy number assays targeting the T-DNA of VC-LLM337-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target j-t-PvARC-p3 located near the left T-DNA border and target c-d4Des(Eg_GA) near the right T-DNA border.					
	c-AHAS	j-t-E9-p3-2	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-t-CaMV_p-LuCnl 2	c-d6Elo(Pp_GA)	j-i-Atss18_c-d6Elo(Pp_GA2)	j-t-PvARC-p3	c-d4Des(Tc_GA)	c-o3Des(Pi_GA2_SNP)	j-i-Atss15_c-o3Des(Pi_GA2)	c-o3Des(Plr_GA)	j-p-VfSBPperm3_c-o3Des(Plr_GA)	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Eg_GA)
LANPMZ (n=74)	1.9					2	2.02		1.94				2.04		1.88

Table 56: Fatty acid profiles of T4 seeds harvested from T3 plants cultivated in greenhouses of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc The events are indicated in the first column, along with the number of T4 seed aliquots representing a plot were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LANPMZ (n=30)	5.3 ± 0.3	0.2 ± 0	0 ± 0	3.6 ± 0.8	25.2 ± 5.3	38.1 ± 2.8	0.5 ± 0.1	4.1 ± 0.6	1.1 ± 0.3	0.1 ± 0.1	0.8 ± 0.2	0.7 ± 0	0.5 ± 0.1	0.1 ± 0.1	1.9 ± 0.2	0.9 ± 0.1	3.8 ± 0.6	6.9 ± 1.3	0.3 ± 0.1	0 ± 0	1.2 ± 0.4	2.6 ± 0.5	0 ± 0	1.5 ± 0.6	0.2 ± 0.1	0.3 ± 0.1

Table 57: Fatty acid profiles of one T4 seed batch per event harvested from T3 plants cultivated in greenhouses of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T4 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LANPMZ (n=1)	4.9	0.2	0.0	4.0	19.5	38.5	0.5	3.6	1.6	0.2	1.0	0.7	0.4	0.0	1.6	0.8	4.8	9.2	0.4	0.0	1.7	3.8	0.0	2.5	0.0	0.2

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Table 58: Phenotypic rating of T3 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where rated per event. DFF: days to first flower (days), DF: deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoL: number of lobes(#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad), Oil: oil content (% of seed weight), Protein: Protein content (% of seed cake without oil)

Event	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC	Oil	Protein
LANPMZ (n=30)	42.4 ± 2	8.2 ± 0.8	8.7 ± 0.7	9 ± 0	8 ± 0.7	3 ± 0	5 ± 0	5 ± 0		5.2 ± 0.8	128.5 ± 8.5				

Fatty acid profiles and phenotypes of T3 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc cultivated in field trials in USDA growth zones 8a-9a during winter

The data indicate that in the field the T4 seed are making EPA and DHA, but at lower levels than seen in the summer field trial (Part D). The greenhouse data show higher oil content compared to the summer field trials (Comparison of Table 61 with Table 54). This data was analyzed in detail in Example 20.

Table 59: Fatty acid profiles of T4 seeds harvested from T3 plants cultivated in the field in USDA growth zones 8a-9a of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc The events are indicated in the first column, along with the number of T4 seed aliquots representing a plot were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LANPMZ (n=56)	5.2 ± 0.5	0.3 ± 0.1	0.1 ± 0.2	2.6 ± 0.5	35.6 ± 2.7	30.4 ± 1.8	0.3 ± 0.1	7.1 ± 0.5	0.5 ± 0.3	0.1 ± 0.1	0.6 ± 0	0.9 ± 0.1	1.3 ± 0.3	0.4 ± 0.1	1.6 ± 0.2	1 ± 0.1	2.5 ± 0.3	4.5 ± 0.6	0.2 ± 0.1	0 ± 0	0.5 ± 0.3	2.6 ± 0.3	0 ± 0	0.7 ± 0.2	0.3 ± 0.1	0.5 ± 0.1
LAOIKC (n=16)	5.3 ± 0.2	0.3 ± 0	0 ± 0	2.5 ± 0.2	32.6 ± 2.2	29.9 ± 0.9	0.7 ± 0.1	5.9 ± 0.4	1 ± 0.2	0.2 ± 0	0.6 ± 0	0.7 ± 0	0.6 ± 0.1	0 ± 0	2.1 ± 0.1	1 ± 0.1	4.6 ± 0.4	6.2 ± 0.5	0.2 ± 0	0 ± 0	0.8 ± 0.1	3.3 ± 0.2	0 ± 0	0.9 ± 0.2	0 ± 0.1	0.4 ± 0.1
WT Kumily (n=83)	5.1 ± 0.5	0.4 ± 0.1	0.1 ± 0.1	2.1 ± 0.5	59.1 ± 1.8	21.3 ± 1.5	0 ± 0.1	9.5 ± 0.7	0 ± 0.1	0 ± 0	0.6 ± 0.1	1 ± 0.1	0.1 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0.1	0.1 ± 0.1	0.3 ± 0	0 ± 0	0 ± 0.1	0 ± 0.1	0 ± 0	0 ± 0	5.1 ± 0.5	0.4 ± 0.1

Table 60: Fatty acid profiles of one T4 seed batch per event harvested from T3 plants cultivated in the field in USDA growth zones 8a-9a of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T4 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LANPMZ (n=1)	4.9	0.3	0.0	2.3	31.2	32.0	0.2	7.6	0.4	0.1	0.6	0.9	1.6	0.6	1.6	1.1	2.8	5.7	0.2	0.0	0.8	3.3	0.0	1.1	0.4	0.5
LAOIKC (n=1)	5.4	0.3	0.0	2.4	29.6	30.3	0.7	5.5	1.4	0.2	0.5	0.7	0.5	0.0	2.2	1.1	5.4	7.3	0.2	0.0	1.0	3.6	0.0	1.3	0.0	0.3

Table 61: Phenotypic rating of T3 plants cultivated in the field in USDA growth zones 8a-9b of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column, along with the number of field plots that where rated per event. Oil: oil content in T4 seeds harvested from T3 plants (% of seed weight), Protein: Protein content in T4 seeds harvested from T3 plants (% of seed cake without oil)

Event	Oil	Protein
LANPMZ (n=56)	43.9 ± 7.8	23.2 ± 3.2
LAOIKC (n=16)	42.6 ± 4	23.1 ± 3

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WT Kumily (n=83)	45.3 ± 3.9	

Fatty acid profiles and phenotypes of T4 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc cultivated in field trials in USDA growth zones 3a-4b and 5a during summer.

The data indicate that through the T5 generation the transformants are still producing EPA and DHA at a level consistent with the field trial in summer 2012 (described in part D). An additional observation is that the oil levels are comparable between these two field trials.

Table 62: Fatty acid profiles of T5 seeds harvested from T4 plants cultivated in the filed in USDA growth zones 3a-4b and 5a of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc The events are indicated in the first column, along with the number of T4 seed aliquots representing a plot were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LANPMZ (n=124)	4.5 ± 0.4	0.2 ± 0.1	0 ± 0	2.8 ± 0.4	34.7 ± 3.9	33 ± 4.8	0.1 ± 0.2	6.5 ± 0.8	0.6 ± 0.2	0.1 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	0.7 ± 0.3	0.2 ± 0.1	1.7 ± 0.3	1.2 ± 1.3	2.8 ± 0.6	4.3 ± 1.9	0.3 ± 0.1	0 ± 0	0.7 ± 0.4	2.8 ± 0.6	0 ± 0.2	0.8 ± 0.3	0.1 ± 0.2	0.4 ± 0.2
WT Kumily (n=56)	4.5 ± 0.5	0.1 ± 0.1	0 ± 0	1.8 ± 1	60 ± 3.8	22.5 ± 3.6	0.6 ± 1.5	7.9 ± 2.2	0.1 ± 0.1	0 ± 0	0.8 ± 0.2	1 ± 0.4	0.1 ± 0	0 ± 0	0.2 ± 0.8	0 ± 0	0 ± 0	0 ± 0.1	0.2 ± 0.2	0 ± 0	0 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Table 63: Fatty acid profiles of one T5 seed batch per event harvested from T3 plants cultivated in the field in USDA growth zones 3a-4b and 5a of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T5 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LANPMZ (n=1)	5.6	0.3	0.0	2.7	21.8	32.3	0.0	6.3	0.9	0.0	0.8	0.8	1.5	0.5	2.2	1.5	4.7	8.0	0.4	0.0	1.6	5.1	0.0	2.7	0.0	0.4

Table 64: Phenotypic rating of T4 plants cultivated in the field in USDA growth zones 3a-4b and 5a of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column, along with the number of field plots that were rated per event. Oil: oil content in T4 seeds harvested from T3 plants (% of seed weight), Protein: Protein content in T4 seeds harvested from T3 plants (% of seed cake without oil)

Event	Oil	Protein
LANPMZ (n=124)	39.7 ± 2.7	25.8 ± 1.4
WT Kumily (n=56)	40.6 ± 2.3	26.4 ± 1.3

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Example 12: Plants containing the T-DNAs of plasmid VC-LJB2197-1qcz and VC-LLM338-3qcz rc (combination C in example 5) for production of EPA and DHA in seeds

In this example, the genetic elements required for EPA and DHA synthesis were transferred into the plant genome on two different T-DNA. To this end, the two different plasmids VC-LJB2197-1qcz and VC-LLM338-3qcz rc containing two different T-DNAs where cloned into Agrobacteria, and plant tissue was incubated according to example 5 at the same time with these two agrobacterial cultures that are identical apart from containing either VC-LJB2197-1qcz or VC-LLM338-3qcz rc. Due to the selectable herbicide resistance marker, regenerated plants contained the T-DNA of VC-LJB2197-1qcz. Only those plants where kept, that also contained the T-DNA of plasmid VC-LLM338-3qcz rc as confirmed by PCR, conducted as described in example 5. Only plants containing the T-DNA of plasmid VC-LJB2197-1qcz as well as the T-DNA of plasmid VC-LLM338-3qcz rc combine all the genetic elements required for EPA and DHA synthesis in seeds. The genetic elements of VC-LJB2197-1qcz and the function of each element are listed in Table 1. The genetic elements of VC-LLM338-3qcz rc and the function of each element was listed in Table 5. For convenience, all enzymes expressed in seeds of plants carrying both T-DNA of VC-LJB2197-1qcz and VC-LLM338-3qcz rc that are required for EPA and DHA synthesis are additionally listed in Table 65.

Table 65: Combined list of genes essential of EPA and DHA synthesis carried by the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM338-3qcz rc.

Genes encoding enzmyes for EPA and DHA synthesis	Plasmid containing T-DNA with the gene	Length (bp)	Enzymatic function and source of encoded protein
c-d12Des(Ps_GA)	VC-LJB2197-1qcz	1196	Delta-12 desaturase from *Phythophthora sojae*
c-d15Des(Ch_ERTp_GA)	VC-LLM338-3qcz rc	1238	Delta-15 desaturase from *Cochliobolus heterostrophus* C5
c-d6Des(Ot_febit)	VC-LJB2197-1qcz	1370	Delta-6 desaturase from *Ostreococcus tauri*
c-d6Elo(Pp_GA2)	VC-LJB2197-1qcz	872	Delta-6 elongase from *Physcomitrella patens*
c-d6Elo(Tp_GA2)	VC-LJB2197-1qcz	818	Delta-6 elongase from *Thalassiosira pseudonana*
c-d5Des(Tc_GA2)	VC-LJB2197-1qcz	1319	Delta-5 desaturase from

Genes encoding enzmyes for EPA and DHA synthesis	Plasmid containing T-DNA with the gene	Length (bp)	Enzymatic function and source of encoded protein
			Thraustochytrium sp. ATCC21685
c-o3Des(Pi_GA2)	VC-LLM338-3qcz rc	1085	Omega-3-desaturase from *Phythophthora infestans*
c-d5Elo(Ot_GA3)	VC-LLM338-3qcz rc	902	Delta-5 elongase from *Ostreococcus tauri*
c-d4Des(Eg_GA)	VC-LLM338-3qcz rc	1625	Delta-4 desaturase from *Euglena gracilis*
c-d4Des(Tc_GA)	VC-LLM338-3qcz rc	1559	Delta-4 desaturase from *Thraustochytrium* sp.

Fatty acid profiles, copy number measurements, and phenotypic observations of T0 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM338-3qcz rc cultivated in greenhouses during summer

The data in Table 67 and Table 68 indicate that for this construct the increase in EPA and DHA, when comparing single copy to double copy events, was more subtle, but double copy events still had an increase in EPA and DHA over single copy events. As observed in the other examples, there was no significant observed alteration of the phenotype of the plants bearing the T-DNA from both constructs..

Table 66: Copy number measurement of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM338-3qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column; sc: all T1 plants where the average of all copy number assays listed in this table was 0.51-1.49, dc: all T1 plants where the average of all copy number assays listed in this table was 1.51-2.49, tc: all T1 plants where the average of all copy number assays listed in this table was 2.51-3.49.

Category of T0 plants	Copy number assays targeting the T-DNA of VC-LJB2197-1qcz. Assays are listed according to the position of the assay target along the T-DNA, with assay of target c-AHAS was located near the left T-DNA border and assay of target c-d6Elo(Pp_GA) near the right T-DNA border.		Copy number assays targeting the T-DNA of VC-LLM338-3qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with assay of target c-d4Des(Eg_GA) was located near the left T-DNA border and assay of target c-d15Des(Ch_ERTp_GA) near the right T-DNA border.	
	c-AHAS	c-d6Elo(Pp_GA)	c-d4Des(Eg_GA)	c-d15Des(Ch_ERTp_GA)
sc (n=240)	1.0	1.0	1.2	1.2
dc (n=117)	1.6	1.7	1.8	2.0

Table 67: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM338-3qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 66. The number of T1 plants/events fullfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=240)	5.8 ± 0.9	0.4 ± 0.3	0 ± 0	2.8 ± 0.9	34.2 ± 5.7	30.5 ± 3.3	0.2 ± 1.2	7.9 ± 1.6	0.7 ± 0.5	0.2 ± 0.2	0.9 ± 0.1	0.9 ± 0.1	1 ± 0.6	0.3 ± 0.3	1.6 ± 0.6	1 ± 0.5	3.5 ± 1.7	3 ± 1.1	0.4 ± 0.2	0 ± 0	0.5 ± 0.3	2.1 ± 0.8	0 ± 0	1.4 ± 0.6	0.4 ± 0.4	
dc (n=117)	5.8 ± 0.9	0.4 ± 0.2	0 ± 0	2.9 ± 0.4	31.7 ± 6.5	30.3 ± 3.3	0.2 ± 0.2	7.9 ± 1.9	1.3 ± 1.1	0.4 ± 0.5	0.9 ± 0.1	0.8 ± 0.1	0.7 ± 0.5	0.2 ± 0.2	1.8 ± 1	1.1 ± 0.6	4 ± 2.1	3.2 ± 1.3	0.4 ± 0.2	0 ± 0	0.8 ± 0.4	2.6 ± 0.9	0 ± 0.1	1.8 ± 0.6	0.6 ± 0.6	

Table 68: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM338-3qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 66. For each category, the fatty acid profile of the plant/event having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=1)	6.0	0.3	0.1	3.8	19.5	34.3	0.2	4.4	1.0	0.2	0.8	0.7	1.9	0.6	2.0	1.3	8.0	8.9	0.3	0.0	0.7	3.1	0.0	1.9	0.0	
dc (n=1)	5.8	0.4	0.0	3.2	19.5	29.5	0.1	8.5	1.6	0.7	0.9	0.7	1.7	0.7	1.6	1.2	6.5	7.2	0.4	0.0	0.9	4.9	0.0	3.9	0.3	

Table 69: Phenotypic rating of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM338-3qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 66. The number of T1 plants/events fullfilling these criteria are displayed in parentheses. DFF: days to first flower (days), DF: deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoL: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad)

Category of T1 plants	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC
sc (n=240)	81.4 ± 12.3	1.3 ± 0.6	1.9 ± 1.1	2.2 ± 1.3	2.1 ± 1.3	3 ± 0.1	4.6 ± 0.7	5 ± 0.3	3.1 ± 1.4	3.6 ± 0.9	93.7 ± 15.3	2.3 ± 0.7	7.4 ± 1.7
dc (n=117)	78.6 ± 10.1	1.3 ± 0.5	1.9 ± 1	2.3 ± 1.4	2.4 ± 1.2	3 ± 0.1	4.6 ± 0.7	5 ± 0	3.1 ± 1.4	3.9 ± 4.6	93.1 ± 17.4	2.2 ± 0.6	7.5 ± 1.5

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Example 13: Plants containing the T-DNAs of plasmid VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc (combination D in example 5) for production of EPA and DHA in seeds

In this example, the genetic elements required for EPA and DHA synthesis were transferred into the plant genome on two different T-DNAs. To this end, the two different plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc containing two different T-DNAs where cloned into agrobacteria, and plant tissue was incubated according to example 5 at the same time with these two agrobacterial cultures that are identical apart from containing either VC-LJB2755-2qcz rc or VC-LLM391-2qcz rc. Due to the selectable herbicide resistance marker, regenerated plants contained the T-DNA of VC-LJB2755-2qcz rc. Only those plants where kept, that also contained the T-DNA of plasmid VC-LLM391-2qcz rc as confirmed by PCR, conducted as described in example 5. Only plants containing the T-DNA of plasmid VC-LJB2755-2qcz rc as well as the T-DNA of plasmid VC-LLM391-2qcz rc combine all the genetic elements required for EPA and DHA synthesis in seeds. The genetic elements of VC-LJB2755-2qcz rc and the function of each element are listed in Table 2. The genetic elements of VC-LLM391-2qcz rc and the function of each element was listed in Table 6. For convenience, all enzymes expressed in seeds of plants carrying both T-DNA of VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc that are required for EPA and DHA synthesis are additionally listed Table 70.

Table 70: Combined list of genes essential of EPA and DHA synthesis carried by the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc.

Genes encoding enzmyes for EPA and DHA synthesis	Plasmid containing T-DNA with the gene	Length (bp)	Enzymatic function and source of encoded protein
c-d12Des(Ps_GA)	VC-LJB2755-2qcz rc	1196	Delta-12 desaturase from *Phythophthora sojae*
c-d5Des(Tc_GA2)	VC-LJB2755-2qcz rc	1319	Delta-5 desaturase from *Thraustochytrium* sp. ATCC21685
c-d6Des(Ot_febit)	VC-LJB2755-2qcz rc	1370	Delta-6 desaturase from *Ostreococcus tauri*
c-d6Elo(Tp_GA2)	VC-LJB2755-2qcz rc	818	Delta-6 elongase from *Thalassiosira pseudonana*
c-o3Des(Pi_GA2)	VC-LJB2755-2qcz rc	1085	Omega-3-desaturase from *Phythophthora infestans*
c-o3Des(Pir_GA)	VC-LJB2755-2qcz rc	1091	Omega-3 desaturase from

Genes encoding enzmyes for EPA and DHA synthesis	Plasmid containing T-DNA with the gene	Length (bp)	Enzymatic function and source of encoded protein
			Pythium irregulare
c-d5Elo(Ot_GA3)	VC-LLM391-2qcz rc	902	Delta-5 elongase from *Ostreococcus tauri*
c-d4Des(Eg_GA)	VC-LLM391-2qcz rc	1625	Delta-4 desaturase from *Euglena gracilis*
c-d4Des(Tc_GA)_T564G	VC-LLM391-2qcz rc	1559	Delta-4 desaturase from *Thraustochytrium* spp.

Fatty acid profiles, copy number measurements, and phenotypic observations of T0 plants carrying T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc cultivated in greenhouses during summer

The data in Table 72, Table 73 and Table 74 show that this combination of constructs was able to insert the T-DNA into the genome, but EPA and DHA accumulation was again at a more subtle level than observed in previous examples. None the less the constructs successfully recapitulated the pathway to generate EPA and DHA and ARA with no impact on the aerial portion of the plant.

Table 71: Copy number measurement of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. Considering that each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column; sc: all T0 plants where the average of all copy number assays listed in this table was 0.51-1.49, dc: all T0 plants where the average of all copy number assays listed in this table was 1.51-2.49

Event	Copy number assays targeting the T-DNA of VC-LJB2755-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target j-p-LuPXR_i-Atss15 near the right T-DNA border.							Copy number assays targeting the T-DNA of VC-LLM391-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-d4Des(Eg_GA) located near the left T-DNA border and target j-i-Atss1_c-d5Elo(Ot_GA3) near the right T-DNA border.					
	c-AHAS	c-o3Des(Pi_GA)	j-i-Atss18_c-o3Des(Pi_GA2)	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-i-Atss1_c-d6Elo(Tp_GA2)	c-o3Des(Plr_GA)	j-p-LuPXR_i-Atss15	c-d4Des(Eg_GA)	j-t-PvARC-p-LuCnl	j-i-Atss2_c-d4Des(Tc_GA3)	c-d4Des(Tc_GA)	j-p-PvARCS_t-BnFAE	c-d5Elo(Ot_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)
sc (n=184)	1.0							1.1	1.0	1.0		1.0		1.1	
dc (n=6)	1.0							2.1	1.1	1.5				2.2	

Table 72: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. Considering that each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 71. The number of T1 plants/events fullfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=184)	5.4 ± 0.5	0.3 ± 0.1	0.2 ± 0.1	3 ± 0.4	39.5 ± 5.1	31.2 ± 2.9	0.4 ± 0.1	6.1 ± 0.9	1.3 ± 0.5	0.3 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.1 ± 0.1	0 ± 0	1.3 ± 0.4	0.8 ± 0.2	1.3 ± 0.6	4.2 ± 1.4	0.3 ± 0	0 ± 0	0.4 ± 0.2	1.5 ± 0.5	0 ± 0	0.6 ± 0.3	0.2 ± 0.1	
dc (n=6)	4.7 ± 0.8	0.3 ± 0.1	0.1 ± 0.1	2.9 ± 0.4	34.2 ± 5.2	30.6 ± 3.9	0.8 ± 0.6	5.8 ± 0.9	3.8 ± 3.4	0.9 ± 0.8	0.7 ± 0.1	0.8 ± 0	0.1 ± 0.1	0 ± 0	1.9 ± 1.3	1.2 ± 0.8	1.2 ± 1	5.9 ± 5.8	0.3 ± 0	0 ± 0	0.5 ± 0.2	1.9 ± 1.3	0 ± 0	0.6 ± 0.4	0.6 ± 0.6	

Table 73: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 71. For each category, the fatty acid profile of the plant/event having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=1)	3.8	0.1	0.1	2.8	41.9	22.2	1.0	5.5	1.9	0.4	0.8	1.0	0.1	0.0	2.1	1.6	1.4	8.4	0.4	0.0	0.5	2.6	0.0	1.0	0.3	
dc (n=1)	3.6	0.1	0.0	2.6	25.9	28.4	1.3	4.7	3.9	1.0	0.7	0.8	0.1	0.1	2.3	1.6	2.7	14.0	0.4	0.0	0.6	3.6	0.0	1.4	0.2	

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Table 74: Phenotypic rating of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 71. The number of T1 plants/events fullfilling these criteria are displayed in parentheses. DFF: days to first flower (days), DF: deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoI: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad)

Category of T1 plants	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC
sc (n=184)	61.7 ± 7.8	1 ± 0	1.4 ± 1.2	1.6 ± 1.4	2.9 ± 2.5	3 ± 0	3 ± 0.1	5 ± 0	3.2 ± 2.5	3.7 ± 0.7	121.7 ± 9.4	4.9 ± 0.6	5.5 ± 2.1
dc (n=6)	63.7 ± 8	1 ± 0	1 ± 0	1.8 ± 1.3	2.8 ± 0.4	3 ± 0	3 ± 0	5 ± 0	2.8 ± 1	3.3 ± 1	123.3 ± 5.2	4.9 ± 1.2	5.5 ± 1.8

Fatty acid profiles, copy number measurements, and phenotypic observations of T1 plants carrying T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc cultivated in greenhouses during winter.

The data on Table 75, Table 76, Table 77, Table 78, Table 79, Table 80, Table 81 and Table 82 demonstrate that this pair of constructs was successful in recapitulating the pathway to generate VLC-PUFA (C20 and C22, including EPA, DHA and ARA). The copy number for each gene varied from homozygous single insertion of the T-DNA to insertions of parts of the T-DNA's and/or deletions of the T-DNA after insertion into the genome. The fatty acid profile indicated that some events (see Table 78, event LAPCSC) were able to accumulate up to 18 percent EPA and DHA combined). Table 75 indicates that LAPCSC was largely homozygous for a single insertion of each T-DNA with the exception of region of j-p-LuPXR_i-Atss15 on construct VC-LJB2755-2qcz, which contained at least four copies of the regions around that marker. The data presented on Table 81 indicate there was no obvious alteration of the phenotype of the plants bearing T-DNA corresponding to the constructs VC-LJB2755-2qcz and VC-LLM391-2qcz rc.

Table 75: Copy number measurement of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column, along with the number of T1 plants that were measured per event. The T1 plants underwent a selection from 250 segregating T1 seedlings using half-kernel analysis, where the correlation of VCL-PUFA levels with copy number was employed to select for homozygous plants, or on case of multilocus events to selecect for plants where one or more loci are homozygous. A copy number of ~2 therefore was indicative for one homozygous locus, a copy number of ~4 indicative for two homozygous loci or indicative for one homozygous locus containing two copies of the target gene measured by the assay, and so forth. Odd results of 3 and 5 indicate that at least some of the selected T1 plants carry a heterozygous locus.

	Copy number assays targeting the T-DNA of VC-LJB2755-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target j-p-LuPXR_i-Atss15 near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity of all plants per event was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.						Copy number assays targeting the T-DNA of VC-LLM391-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-d4Des(Eg_GA) located near the left T-DNA border and target j-i-Atss1_c-d5Elo(Ot_GA3) near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.				
Event	c-AHAS	c-o3Des(Pi_GA)	j-i-Atss18_c-o3Des(Pi_GA2)	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-i-Atss1_c-d6Elo(Tp_GA2)	c-o3Des(Pir_GA)	j-p-LuPXR_i-Atss15	c-d4Des(Eg_GA)	j-t-PvARC-p-LuCnl	j-i-Atss2_c-d4Des(Tc_GA3)	c-d4Des(Tc_GA)	j-p-PvARCS_t-BnFAE	c-d5Elo(Ot_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)
LAPCTC (n=11)	1.3 (T0: 1.1)		2.3		2.1		4.6 (T0: 3.6)	1.7 (T0: 1)	N/A (T0: 1.2)		1.7		1.7 (T0: 1.1)		
LAPCSC (n=11)	1.4 (T0: 1)		2.7		2.6		5.1 (T0: 4)	1.6 (T0: 1)	N/A (T0: 1)		1.7		1.6 (T0: 1.1)		
LAPYTJ (n=15)	1.7 (T0: 0.9)		3.2		3.3		3.2 (T0: 2.1)	1.7 (T0: 1.1)	N/A (T0: 1.1)		1.8		1.7 (T0: 1.1)		
LAQKQ5 (n=15)	1.5 (T0: 1.1)		1.7		1.4		2 (T0: 1.3)	1.4 (T0: 1)	N/A (T0: 1.1)		1.5		1.4 (T0: 1)		
LAPARV (n=15)	2 (T0: 1.1)		1.9		2.0		2 (T0: 1.1)	1.2 (T0: 1.1)	N/A (T0: 0.9)		1.2		1.2 (T0: 1.1)		
LAPCMY (n=15)	2 (T0: 1)		2.0		2.1		2.2 (T0: 1.1)	1.7 (T0: 1)	N/A (T0: 1.1)		1.7		1.9 (T0: 1.2)		
LAPBOW (n=15)	2 (T0: 1)		1.9		2.1		2 (T0: 1)	2 (T0: 1)	N/A (T0: 1.2)		1.9		2.1 (T0: 1.1)		
LAPAWA (n=15)	1.9 (T0: 1.1)		2.0		2.0		2.1 (T0: 1.1)	1.5 (T0: 1)	N/A (T0: 1)		1.5		1.5 (T0: 1.2)		
LAPBYW (n=13)	2 (T0: 1)		2.0		2.2		2.3 (T0: 1)	1.3 (T0: 1)	N/A (T0: 0.8)		1.3		1.5 (T0: 1.1)		
LAPQEP (n=15)	1.4 (T0: 0.9)		2.8		2.6		4 (T0: 3.1)	1.3 (T0: 0.9)	N/A (T0: 1.2)		1.4		1.4 (T0: 1.1)		
LAODDN (n=15)	2.1 (T0: 1.1)		2.0		2.1		2.1 (T0: 1.1)	2 (T0: 1)	N/A (T0: 1.1)		2.0		2.1 (T0: 1.1)		
LAPAUX (n=10)	1.5 (T0: 1)		1.6		1.7		1.7 (T0: 1)	1 (T0: 0.9)	N/A (T0: 0.8)		1.0		1.1 (T0: 0.6)		
LAPZOJ (n=10)	1.8 (T0: 1)		2.1		1.9		2 (T0: 1.1)	1.8 (T0: 1)	N/A (T0: 1.2)		1.9		1.9 (T0: 1)		

Table 76: Copy number measurement of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column; sc: all T1 plants where the average of all copy number assays listed in this table was 1.51-2.49, dc: all T1 plants where the average of all copy number assays listed in this table was 3.51-4.49, tc: all T1 plants where the average of all copy number assays listed in this table was 5.51-6.49. The number of T1 plants fullfilling these criteria are displayed in parentheses.

	Copy number assays targeting the T-DNA of VC-LJB2755-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target j-p-LuPXR_i-Atss15 near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity of all plants per event was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.							Copy number assays targeting the T-DNA of VC-LLM391-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-d4Des(Eg_GA) located near the left T-DNA border and target j-i-Atss1_c-d5Elo(Ot_GA3) near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.					
Category of T1 plants	c-AHAS	c-o3Des(Pi_GA)	j-i-Atss18_c-o3Des(Pi_GA2)	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-i-Atss1_c-d6Elo(Tp_GA2)	c-o3Des(Pir_GA)	j-p-LuPXR_i-Atss15	c-d4Des(Eg_GA)	j-t-PvARC-p-LuCnl	j-i-Atss2_c-d4Des(Tc_GA3)	c-d4Des(Tc_GA)	j-p-PvARC5_t-BnFAE	c-d5Elo(Ot_GA3)
sc (n=140)	1.8		2.1		2.1		2.6	1.6				1.6		1.6

Table 77: Fatty acid profiles of T2 seeds harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc The events are indicated in the first column, along with the number of T2 seed batches that were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unkn own peak a	unkn own peak b
LAPCTC (n=11)	5.1 ± 0.2	0.3 ± 0	0 ± 0	3 ± 0.3	28.2 ±5.5	28.9 ±1.6	1.3 ± 0.2	5 ± 0.2	3 ± 0.5	0.8 ± 0.2	0.9 ± 0.1	0.7 ± 0.1	0.2 ± 0	0.1 ± 0	2.2 ± 0.4	1.8 ± 0.5	1.2 ± 0.4	10.9 ± 2.1	0.4 ± 0	0 ± 0	0.6 ± 0.2	3.5 ± 1.1	0 ± 0	1.3 ± 0.4	0.4 ± 0.2	0.2 ± 0.1
LAPCSC (n=11)	5.1 ± 0.1	0.3 ± 0	0 ± 0	3.2 ± 0.4	27.9 ±4.2	28.8 ±2.3	1.3 ± 0.2	4.9 ± 0.4	3.2 ± 0.6	0.8 ± 0.2	0.9 ± 0.1	0.7 ± 0.1	0.2 ± 0.1	0.1 ± 0	2.1 ± 0.4	1.7 ± 0.5	1.3 ± 0.3	11.4 ± 2.4	0.4 ± 0.1	0 ± 0	0.6 ± 0.1	3.4 ± 1	0 ± 0	1.2 ± 0.3	0.3 ± 0.1	0.2 ± 0.1

LAPYTJ (n=15)	5.9 ± 0.3	0.3 ± 0.1	0 ± 0	3.3 ± 0.6	26.5 ± 1.9	34 ± 1.6	0.9 ± 0.2	4.9 ± 0.7	2.1 ± 0.3	0.4 ± 0.1	0.8 ± 0.1	0.6 ± 0	0 ± 0		2.2 ± 0.3	1.3 ± 0.3	2.9 ± 0.5	8.4 ± 1.7	0.1 ± 0.1	0 ± 0		0.9 ± 0.3	2.8 ± 0.5	0 ± 0		1.2 ± 0.3	0.2 ± 0.2	0.1 ± 0.1
LAQKQ5 (n=15)	5.3 ± 0.3	0.3 ± 0.1	0 ± 0	3.2 ± 0.6	32.7 ± 8	29.7 ± 2.7	1.1 ± 0.3	5.3 ± 0.5	2.6 ± 0.6	0.6 ± 0.1	0.9 ± 0.2	0.7 ± 0.1	0.1 ± 0.1	0 ± 0	1.7 ± 0.6	1.3 ± 0.5	1.1 ± 0.2	8.8 ± 3.2	0.2 ± 0.2	0 ± 0	0.5 ± 0.3	2.4 ± 0.6	0 ± 0	1 ± 0.4	0.2 ± 0.2	0.1 ± 0.1
LAPARV (n=15)	5.3 ± 0.2	0.3 ± 0	0 ± 0	3.5 ± 0.3	26.5 ± 1.9	36 ± 1.2	0.7 ± 0.1	5.4 ± 0.3	1.9 ± 0.2	0.4 ± 0.1	0.9 ± 0.1	0.7 ± 0	0 ± 0	0 ± 0	1.9 ± 0.3	1.4 ± 0.2	1.7 ± 0.3	8.4 ± 1.6	0.4 ± 0	0 ± 0	0.6 ± 0.2	2.4 ± 0.5	0 ± 0	1.2 ± 0.3	0.3 ± 0.2	
LAPCMY (n=15)	6.2 ± 1.3	0.3 ± 0.1	0 ± 0	2.4 ± 1.4	34 ± 8.6	26.7 ± 14.5	0.5 ± 0.3	6.5 ± 1.4	1.9 ± 0.4	0.4 ± 0.1	1 ± 0.2	0.7 ± 0.1	0.1 ± 0.1	0 ± 0	2.5 ± 0.5	1.8 ± 0.4	1.6 ± 0.4	7.9 ± 2	0.4 ± 0.1	0 ± 0	0.6 ± 0.3	2.6 ± 0.8	0 ± 0	1.2 ± 0.4	0.3 ± 0.2	
LAPBOW (n=15)	5.1 ± 0.6	0.2 ± 0.1	0 ± 0	2.9 ± 0.2	27.3 ± 2.6	34.9 ± 0.9	0.7 ± 0	5.6 ± 0.6	1.9 ± 0.2	0.4 ± 0	0.8 ± 0.1	0.7 ± 0	0.1 ± 0	0 ± 0	2 ± 0.3	1.4 ± 0.2	1.6 ± 0.1	7.8 ± 1.1	0.3 ± 0	0 ± 0	0.9 ± 0.2	3.1 ± 0.7	0 ± 0	1.3 ± 0.4	0.6 ± 0.1	0.1 ± 0
LAPAWA (n=15)	5.2 ± 0.1	0.3 ± 0	0 ± 0	3.1 ± 0.2	28.3 ± 1.3	35.9 ± 1.1	0.7 ± 0.1	5.3 ± 0.3	1.7 ± 0.2	0.3 ± 0	0.8 ± 0.1	0.7 ± 0	0.1 ± 0	0 ± 0	2.1 ± 0.2	1.4 ± 0.2	1.6 ± 0.1	7.4 ± 0.7	0.3 ± 0	0 ± 0	0.7 ± 0.2	2.2 ± 0.5	0 ± 0	1 ± 0.3	0.5 ± 0.2	
LAPBYW (n=13)	5.6 ± 0.3	0.3 ± 0.1	0 ± 0	2.6 ± 1.2	28.4 ± 1.2	36 ± 1.2	0.7 ± 0.1	5.4 ± 0.3	1.7 ± 0.2	0.4 ± 0	0.8 ± 0.1	0.7 ± 0	0.1 ± 0.1	0 ± 0	1.9 ± 0.2	1.4 ± 0.2	1.6 ± 0.1	7.9 ± 0.8	0.4 ± 0.1	0 ± 0	0.5 ± 0.2	2 ± 0.4	0 ± 0	1 ± 0.2	0.3 ± 0.2	
LAPQEP (n=15)	5 ± 0.3	0.3 ± 0	0 ± 0	2.7 ± 0.3	38.1 ± 5.8	26.7 ± 4	1.1 ± 0.3	6.1 ± 0.9	1.2 ± 0.2	0.3 ± 0	0.8 ± 0.1	0.8 ± 0.1	0.2 ± 0.1	0.1 ± 0	1.6 ± 0.4	1.5 ± 0.4	0.7 ± 0.2	7.7 ± 1.6	0.4 ± 0	0 ± 0	0.4 ± 0.1	2.8 ± 0.7	0 ± 0	0.7 ± 0.2	0.3 ± 0.1	0.2 ± 0.1
LAODDN (n=15)	5.2 ± 0.6	0.2 ± 0.1	0 ± 0	3.1 ± 0.3	28 ± 2	35.8 ± 1.1	0.7 ± 0.1	5.9 ± 0.4	1.6 ± 0.2	0.4 ± 0	0.8 ± 0.1	0.7 ±	0 ± 0	0 ± 0	2 ± 0.2	1.4 ± 0.2	1.2 ± 0.1	7.1 ± 0.9	0.4 ± 0	0 ± 0	0.8 ± 0.1	2.8 ± 0.4	0 ± 0	1.1 ± 0.3	0.6 ± 0.1	0.1 ± 0
LAPAUX (n=10)	5.5 ± 0.5	0.3 ± 0	0 ± 0	2.8 ± 0.3	31.3 ± 7.3	34.9 ± 2.5	0.5 ± 0.1	6.1 ± 0.6	1.5 ± 0.5	0.4 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.1 ± 0	0 ± 0	1.7 ± 0.5	1.2 ± 0.4	1.7 ± 0.5	8.3 ± 3.4	0.4 ± 0	0 ± 0	0.2 ± 0.1	1.1 ± 0.4	0 ± 0	0.4 ± 0.2	0 ± 0.1	
LAPZOJ (n=10)	5.6 ± 0.6	0.3 ± 0	0 ± 0	3 ± 0.3	34.1 ± 6.8	33.5 ± 2.8	0.6 ± 0.2	6.5 ± 0.6	1.3 ± 0.3	0.3 ± 0.1	0.8 ± 0.1	0.7 ±	0 ± 0	0 ± 0	1.6 ± 0.4	1.1 ± 0.3	1 ± 0.2	5.5 ± 1.5	0.2 ± 0.1	0 ± 0	0.4 ± 0.1	2.2 ± 0.6	0 ± 0	0.8 ± 0.3	0.3 ± 0.2	0 ± 0

Table 78: Fatty acid profiles of one T2 seed batch per event harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column. Fatty acid profiles of T2 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unkn own peak a	unkn own peak b
LAPCTC (n=1)	5.1	0.3	0.0	2.7	25.2	29.7	1.3	4.8	2.6	0.5	0.8	0.6	0.3	0.1	2.4	1.9	1.9	14.1	0.3	0.0	0.4	3.3	0.0	1.0	0.2	0.3
LAPCSC (n=1)	5.0	0.3	0.0	3.4	20.8	25.8	1.7	5.2	3.5	1.0	0.8	0.6	0.3	0.1	2.5	2.7	1.0	16.1	0.3	0.0	0.6	5.3	0.0	1.9	0.4	0.3
LAPYTJ (n=1)	5.5	0.3	0.0	3.0	24.0	31.1	1.1	4.9	2.4	0.5	0.8	0.6	0.0	0.0	2.4	1.7	2.8	10.8	0.3	0.0	1.1	4.1	0.0	1.7	0.5	0.3
LAQKQS (n=1)	5.8	0.3	0.0	3.6	24.4	31.3	1.4	4.7	3.9	0.9	0.8	0.6	0.0	0.0	2.2	1.8	1.5	13.8	0.0	0.0	0.0	1.8	0.0	1.0	0.0	0.0

LAPARV (n=1)	5.3	0.2	0.0	3.2	24.3	34.2	0.6	5.6	2.0	0.5	0.8	0.6	0.1	0.0	2.0	1.5	2.3	11.9	0.3	0.0	0.6	2.4	0.0	1.1	0.3	
LAPCMY (n=1)	8.1	0.5	0.0	0.0	51.4	1.0	0.0	9.5	2.3	0.6	1.2	1.0	0.1	0.0	2.9	2.1	2.4	12.5	0.5	0.0	0.3	2.6	0.0	0.8	0.0	
LAPBOW (n=1)	3.8	0.1	0.0	2.3	21.5	33.7	0.7	6.7	2.3	0.6	0.7	0.7	0.1	0.1	2.2	1.8	2.0	10.8	0.4	0.0	1.5	5.2	0.0	2.1	0.7	0.1
LAPAWA (n=1)	5.3	0.3	0.0	3.4	26.2	36.5	0.7	4.8	1.8	0.4	0.9	0.7	0.0	0.0	2.7	1.9	1.7	8.7	0.4	0.0	0.5	1.7	0.0	0.9	0.3	
LAPBYW (n=1)	5.5	0.3	0.0	0.0	29.4	36.1	0.6	5.8	1.8	0.4	0.8	0.8	0.1	0.0	1.9	1.5	1.7	9.5	0.4	0.0	0.4	1.7	0.0	0.8	0.3	
LAPQEP (n=1)	4.9	0.4	0.0	2.3	36.6	23.9	1.5	5.7	1.3	0.4	0.7	0.8	0.3	0.1	1.9	1.6	1.0	10.9	0.4	0.0	0.3	3.7	0.0	0.7	0.3	0.3
LAODDN (n=1)	3.8	0.1	0.0	2.6	24.1	36.0	0.8	5.7	2.0	0.4	0.8	0.7	0.0	0.0	2.3	1.8	1.6	9.4	0.4	0.0	1.1	3.8	0.0	1.6	0.6	0.1
LAPAUX (n=1)	5.5	0.3	0.0	3.1	25.6	33.6	0.7	5.4	1.9	0.4	0.8	0.6	0.1	0.0	2.4	1.7	2.3	12.8	0.3	0.0	0.2	1.5	0.0	0.5	0.0	
LAPZOJ (n=1)	5.7	0.3	0.0	2.1	25.8	31.3	0.4	7.9	2.2	0.7	0.6	0.6	0.0	0.0	1.3	1.0	2.1	9.3	0.0	0.0	1.0	5.6	0.0	2.2	0.0	0.0

Table 79: Fatty acid profiles of T2 seeds harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column; as defined in Table 76. The number of T1 plants fullfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=143)	5.4 ± 0.7	0.3 ± 0.1	0 ± 0	2.9 ± 0.7	29.6 ± 5.5	32.8 ± 6.2	0.8 ± 0.3	5.6 ± 0.8	1.9 ± 0.6	0.4 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	0.1 ± 0.1	0 ± 0	2 ± 0.4	1.5 ± 0.3	1.5 ± 0.5	8.3 ± 2	0.3 ± 0.1	0 ± 0	0.6 ± 0.3	2.6 ± 0.7	0 ± 0	1.1 ± 0.4	0.4 ± 0.2	0.1 ± 0.1

Table 80: Fatty acid profiles of T2 seeds harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column; as defined in Table 76. For each category, the fatty acid profile of the plant having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=1)	5.1	0.3	0.0	2.7	25.2	29.7	1.3	4.8	2.6	0.5	0.8	0.6	0.3	0.1	2.4	1.9	1.9	14.1	0.3	0.0	0.4	3.3	0.0	1.0	0.2	0.3

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Table 81: Phenotypic rating of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column, along with the number of T1 plants that where rated per event. DFF: days to first flower (days), DF: deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoI: number of lobes(#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad), Oil: oil content (% of seed weight), Protein: Protein content (% of seed cake without oil)

Event	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC	Oil	Protein
LAPCTC (n=11)	67.5 ± 1.5	1 ± 0	1 ± 0	1 ± 0	1.8 ± 0.6	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	129.5 ± 3.5	3.6 ± 0.6	3.6 ± 0.9		
LAPCSC (n=11)	68.6 ± 3.3	1 ± 0	1 ± 0	1 ± 0	1.7 ± 0.6	3 ± 0	3 ± 0	5 ± 0	1 ± 0	3.6 ± 0.8	126.4 ± 3.2	3.2 ± 0.3	3.9 ± 0.5		
LAPYTJ (n=15)	72.3 ± 7.1	1 ± 0	1.4 ± 1.1	1.1 ± 0.5	1.6 ± 0.8	3 ± 0	3 ± 0	5 ± 0	1.3 ± 0.7	4 ± 0	123 ± 4.9	3.6 ± 0.3	3.5 ± 0.8		
LAQKQS (n=15)	67.4 ± 4.4	1 ± 0	1.4 ± 0.8	1.1 ± 0.3	3.1 ± 1.6	3 ± 0	3 ± 0	5 ± 0	1.8 ± 1.5	4 ± 0	130.3 ± 4.8	3.8 ± 0.5	3.7 ± 1		
LAPARV (n=15)	68.1 ± 2.6	1 ± 0	1.2 ± 0.8	1 ± 0	2.3 ± 2.1	3 ± 0	3 ± 0	5 ± 0	1.3 ± 0.9	4 ± 0	128 ± 3.2	4 ± 0.6	3.9 ± 1.3		
LAPCMY (n=15)	68.7 ± 2.4	1 ± 0	1 ± 0	1 ± 0	1.7 ± 0.6	3 ± 0	3 ± 0	5 ± 0	1 ± 0	3.9 ± 0.5	130.7 ± 1.8	3.6 ± 0.4	3.3 ± 0.7		
LAPBOW (n=15)	68.1 ± 0.9	1 ± 0	1 ± 0	1 ± 0	1.2 ± 0.6	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	127.3 ± 3.2	4 ± 0.4	3.7 ± 1	35.3 ± 1.3	29.2 ± 0.3
LAPAWA (n=15)	66.5 ± 1.4	1 ± 0	1 ± 0	1 ± 0	1.2 ± 0.4	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	128.7 ± 3.5	3.7 ± 0.4	2.6 ± 0.5		
LAPBYW (n=13)	65.7 ± 2.6	1 ± 0	1.2 ± 0.8	1.2 ± 0.6	1.7 ± 0.6	3 ± 0	3 ± 0	5 ± 0	1.2 ± 0.6	3.8 ± 0.6	128.1 ± 5.2	4 ± 0.5	3.9 ± 1.7		
LAPQEP (n=15)	66.9 ± 2.6	1 ± 0	1.2 ± 0.8	1 ± 0	1.7 ± 0.7	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	128 ± 4.6	3.2 ± 0.3	3.3 ± 0.6		
LAODDN (n=15)	69.3 ± 2	1 ± 0	1 ± 0	1 ± 0	1.2 ± 0.4	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	125.7 ± 3.2	3.9 ± 0.5	3.8 ± 1.2	36.6 ± 1.2	27.9 ± 0.4
LAPAUX (n=10)	70 ± 4.5	1 ± 0	1.3 ± 0.9	1 ± 0	3.1 ± 2.3	3 ± 0	3 ± 0	5 ± 0	2.4 ± 2.1	3.8 ± 0.6	130 ± 2.4	4.3 ± 0.5	4.5 ± 1.4		
LAPZOJ (n=10)	68 ± 6.7	1 ± 0	1.2 ± 0.6	1 ± 0	1.1 ± 0.3	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	126.5 ± 3.4	3.8 ± 0.6	3.3 ± 0.9		

Table 82: Phenotypic rating of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column; as defined in Table 76. The number of T1 plants fullfilling these criteria are displayed in parentheses. DFF: days to first flower (days), DF: deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoL: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad), Oil: oil content (% of seed weight), Protein: Protein content (% of seed cake without oil)

Category of T1 plants	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC	Oil	Protein
sc (n=143; n=30 for oil and protein)	67.9 ± 3	1 ± 0	1.1 ± 0.5	1 ± 0.2	1.7 ± 1.1	3 ± 0	3 ± 0	5 ± 0	1.1 ± 0.7	3.9 ± 0.3	127.9 ± 4	3.6 ± 0.4	3.5 ± 1.1	36 ± 1.4	28.5 ± 0.8

Fatty acid profiles, copy number measurements, and phenotypic observations of T2 plants carrying T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc cultivated in greenhouses during summer.

The data in Table 83 indicate the copy number of the selected events was a single insertion which was homozygous in the T3 seed. Fatty acid profile measurements, see Table 84 and Table 85, indicated the combination of T-DNAs from VC-LJB2755-2qcz and VC-LLM391-2qcz rc are capable of bringing in the VLC-PUFA pathway to successfully accumulate ARA, EPA and DHA. The data on Table 86 show that there was no significant impact on the aerial portion of the plant caused by VC-LJB2755-2qcz and VC-LLM391-2qcz rc.

Table 83: Copy number measurement of T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column, along with the number of T1 plants that were measured per event. As the T2 plants underwent two cylces of selecting homozygous plants, all plants of all events are homozygous for all T-DNA insertions. A copy number of ~2 therefore was indicated for one homozygous locus, a copy number of ~4 indicative for two homozygous loci or indicative for one homozygous locus containing two copies of the target gene measured by the assay, and so forth. Odd results of 3 and 5 indicate that at least some of the selected T1 plants carry a heterozygous locus.

Event	Copy number assays targeting the T-DNA of VC-LJB2755-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target j-p-LuPXR_i-Atss15 near the right T-DNA border.					Copy number assays targeting the T-DNA of VC-LLM391-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-d4Des(Eg_GA) located near the left T-DNA border and target j-i-Atss1_c-d5Elo(Ot_GA3) near the right T-DNA border.				
	c-AHAS	c-o3Des(Pi_GA)	j-i-Atss18_c-	j-i-Atss14_c-	c-d6Elo(Tp_GA)	j-i-Atss1_c-d6Elo(Tp_GA2)	c-o3Des(Plr_GA)	j-p-LuPXR_i-Atss15	c-d4Des(Eg_GA)	j-t-PvARC-p-LuCnl	j-i-Atss2_c-d4Des(Tc_GA3)	c-d4Des(Tc_GA)	j-p-PvARC5_t-BnFAE	c-d5Elo(Ot_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)
LAPBOW (n=54)	1.9	2.0					2.0	2.4	2.0				2.0		2.0
LAODDN (n=63)	1.9	2.0					2.0	2.1	2.0				2.0		1.9

Table 84: Fatty acid profiles of T3 seeds harvested from T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc The events are indicated in the first column, along with the number of T3 seed batches that where measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LAPBOW (n=54)	5.3 ± 0.3	0.3 ± 0.1	0.2 ± 0.1	3.4 ± 0.2	26.8 ± 1.9	35.6 ± 2.1	0.6 ± 0.1	6.1 ± 0.8	1.6 ± 0.2	0.4 ± 0.1	0.8 ± 0.1	0.7 ± 0	0.1 ± 0.1	0 ± 0	1.8 ± 0.3	1.4 ± 0.3	1.4 ± 0.2	7.1 ± 0.8	0.3 ± 0.1	0 ± 0	1 ± 0.1	3.2 ± 0.5	0 ± 0	1.3 ± 0.3	0.6 ± 0.2	0.1 ± 0.1
LAODDN (n=63)	5.4 ± 0.4	0.3 ± 0.1	0.2 ± 0.1	3.5 ± 0.4	27.7 ± 2.4	35.4 ± 1.8	0.6 ± 0.1	6.5 ± 0.8	1.4 ± 0.2	0.4 ± 0	0.8 ± 0.1	0.7 ± 0	0.1 ± 0.1	0 ± 0	1.8 ± 0.2	1.4 ± 0.2	1 ± 0.2	6.8 ± 0.6	0.3 ± 0.1	0 ± 0	0.8 ± 0.1	3 ± 0.3	0 ± 0	1.1 ± 0.3	0.6 ± 0.1	0.1 ± 0.1
WT Kumily (n=46)	5 ± 0.1	0.4 ± 0	0.2 ± 0	2.6 ± 0.1	66.3 ± 1.7	16.8 ± 1.3	0 ± 0	6.1 ± 0.4	0 ± 0	0 ± 0	0.9 ± 0	1.2 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.5 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Table 85: Fatty acid profiles of one T3 seed batch per event harvested from T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column. Fatty acid profiles of T3 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LAPBOW (n=1)	5.7	0.3	0.2	3.3	24.1	34.3	0.6	6.1	2.0	0.5	0.7	0.6	0.0	0.0	2.1	1.7	1.6	8.7	0.1	0.0	1.1	3.8	0.0	1.7	0.7	0.0
LAODDN (n=1)	5.3	0.3	0.2	3.1	25.1	34.7	0.7	6.0	1.8	0.5	0.7	0.7	0.0	0.0	2.2	2.0	1.1	8.7	0.3	0.0	0.8	3.6	0.0	1.5	0.7	0.1

Table 86: Phenotypic rating of T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column, along with the number of T1 plants that where rated per event. Oil: oil content (% of seed weight), protein: Protein content (% of seed cake without oil)

Event	Oil	protein
LAPBOW (n=54)	34.8 ± 2.9	30.8 ± 2.3
LAODDN (n=63)	36.6 ± 3	28.6 ± 2.4

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WT Kumily (n=46)	34.9 ± 1.1	32.2 ± 1

Fatty acid profiles, copy number measurements, and phenotypic observations of T2 plants carrying T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc cultivated in field trials in USDA growth zones 3a-4b and 5a during the summer

Field data for the T3 seed from the events carrying the T-DNA from VC-LJB2755-2qcz and VC-LLM391-2qcz rc, shown in Table 87 and Table 88, indicate that the plants are capable of making VLC-PUFAs in the field (ARA, EPA and DHA), though not at the level observed in the greenhouse. However, there was also a difference in seed oil content observed compared to the greenhouse (e.g. comparing Table 89 with Table 86). These observations are in agreement with previous examples where it was observed that increased oil contents in the field grown plants concomitant with a decrease in VLC-PUFAs, in particular EPA, DHA and ARA. A more detailed description of the observations regarding oil content and VLC-PUFAs is given in Example 20.

Table 87: Fatty acid profiles of T3 seeds harvested from T2 cultivated in the field, corresponding to USDA growth zones 3a-4b and 5a, for field trials of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc The events are indicated in the first column, along with the number of T3 seed aliquots representing a plot where measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LAPBOW (n=31)	5.3 ± 0.2	0.4 ± 0.1	0 ± 0	2.4 ± 0.1	31.9 ± 1.6	33.3 ± 1	0.6 ± 0.1	7.7 ± 0.5	1.4 ± 0.2	0.4 ± 0.1	0.6 ± 0	0.7 ± 0	0.1 ± 0.1	0 ± 0	1.5 ± 0.1	0.9 ± 0.1	1.6 ± 0.2	5.5 ± 0.5	0.2 ± 0.1	0 ± 0	0.9 ± 0.1	3.1 ± 0.3	0 ± 0	0.8 ± 0.1	0.4 ± 0.1	0.1 ± 0.1
LAODDN (n=31)	5.1 ± 0.2	0.4 ± 0	0.1 ± 0	2.5 ± 0.2	32.1 ± 2.3	33.4 ± 1.4	0.6 ± 0.1	7.9 ± 0.5	1.3 ± 0.2	0.4 ± 0.1	0.6 ± 0	0.7 ± 0	0.2 ± 0.1	0.1 ± 0.1	1.3 ± 0.1	0.8 ± 0.1	1.4 ± 0.2	5.7 ± 0.7	0.3 ± 0	0 ± 0	0.8 ± 0.1	3.1 ± 0.4	0 ± 0	0.8 ± 0.1	0.4 ± 0.1	0.1 ± 0
WT Kumily (n=60)	5 ± 0.3	0.4 ± 0	0.1 ± 0.1	2 ± 0.1	56.2 ± 4.2	23.3 ± 1.7	0 ± 0.1	9.5 ± 0.7	0.1 ± 0.2	0 ± 0	0.7 ± 0	1.1 ± 0.1	0.1 ± 0.1	0 ± 0	0.2 ± 0.3	0.1 ± 0.3	0.2 ± 0.5	0.4 ± 1.1	0.3 ± 0.1	0 ± 0	0 ± 0.2	0.2 ± 0.6	0 ± 0	0 ± 0.2		

Table 88: Fatty acid profiles of one T3 seed batch per event harvested from T2 plants cultivated in USDA growth zones 3a-4b and 5a for field trials of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column. Fatty acid profiles of T3 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LAPBOW (n=1)	5.5	0.4	0.1	2.3	30.3	32.9	0.6	7.6	1.8	0.5	0.6	0.7	0.1	0.0	1.6	1.0	1.7	6.5	0.1	0.0	1.0	3.5	0.0	1.1	0.2	0.1
LAODDN (n=1)	5.4	0.4	0.0	2.6	30.1	33.3	0.7	6.9	1.5	0.4	0.6	0.6	0.1	0.0	1.5	0.9	1.8	6.8	0.2	0.0	1.0	3.5	0.0	0.9	0.4	0.1

Table 89: Phenotypic rating of T2 plants cultivated in USDA growth zones 3a-4b and 5a for field trials of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column, along with the number of field plots that where rated per event. Oil: oil content (% of seed weight), protein: Protein content (% of seed cake without oil)

Event	Oil	protein
LAPBOW (n=31)	38.7 ± 1.2	28 ± 1.1
LAODDN (n=31)	38.3 ± 1.4	27.9 ± 1.2

WT Kumily (n=50)	38.7 ± 1.1

Fatty acid profiles, copy number measurements, and phenotypic observations of T3 plants carrying T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc cultivated in greenhouses during winter.

T4 seed from T3 plants from the event LAODDN, which was homozygous for T-DNA from both VC-LJB2755-2qcz and VC-LLM391-2qcz rc (see Table 90) accumulated VLC-PUFAs (in particular ARA, EPA and DHA, see Table 91 and Table 92). The combination of EPA and DHA was up to approximately ten percent of the total fatty acid content in the seed for this event.

Table 90: Copy number measurement of T3 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column, along with the number of T1 plants that where measured per event. As the T3 plants underwent two cylces of selecting homozygous plants, all plants of all events are homozygous for all T-DNA insertions. A copy number of ~2 therefore was indicative for one homozygous locus, a copy number of ~4 indicative for two homozygous loci or indicative for one homozygous locus containing two copies of the target gene measured by the assay, and so forth. Odd results of 3 and 5 indicate that at least some of the selected T1 plants carry a heterozygous locus.

Event	Copy number assays targeting the T-DNA of VC-LJB2755-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target j-p-LuPXR_i-Atss15 near the right T-DNA border.								Copy number assays targeting the T-DNA of VC-LLM391-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-d4Des(Eg_GA) located near the left T-DNA border and target j-i-Atss1_c-d5Elo(Ot_GA3) near the right T-DNA border.						
	c-AHAS	c-o3Des(Pi_GA)	j-i-Atss18_c-o3Des(Pi_GA2)	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-i-Atss1_c-d6Elo(Tp_GA2)	c-o3Des(Pir_GA)	j-p-LuPXR_i-Atss15	c-d4Des(Eg_GA)	j-t-PvARC-p-LuCnl	j-i-Atss2_c-d4Des(Tc_GA3)	c-d4Des(Tc_GA)	j-p-PvARC5_t-BnFAE	c-d5Elo(Ot_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)
LAODDN (n=30)	1.92		2.0					1.9	1.8			1.9	2.1		1.9

Table 91: Fatty acid profiles of T4 seeds harvested from T3 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc The events are indicated in the first column, along with the number of T4 seed batches that were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LAODDN (n=30)	5.3 ± 0.5	0.3 ± 0	0 ± 0	3.3 ± 0.7	27.2 ± 2.5	37.7 ± 4.1	2.3 ± 5.1	5.1 ± 0.7	1.7 ± 0.3	0.4 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.1 ± 0.1	0 ± 0	1.2 ± 0.2	0.3 ± 0.5	1.3 ± 0.2	6.5 ± 0.8	0.2 ± 0.2	0 ± 0	1 ± 0.3	2.7 ± 0.4	0 ± 0	1.3 ± 0.4	0.4 ± 0.1	0 ± 0

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Table 92: Fatty acid profiles of one T4 seed batch per event harvested from T3 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column. Fatty acid profiles of T4 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1n-7	16:3n-3	18:0	18:1n-9	18:2n-6	18:2n-9	18:3n-3	18:3n-6	18:4n-3	20:0	20:1n-9	20:2n-6	20:3n-3	20:3n-6	20:4n-3	20:4n-6	20:5n-3	22:0	22:1n-9	22:4n-6	22:5n-3	22:5n-6	22:6n-3	unknown peak a	unknown peak b
LAODDN (n=1)	5.2	0.2	0.0	3.7	24.2	39.5	0.4	4.8	2.0	0.5	0.9	0.6	0.1	0.1	1.0	0.0	1.5	7.7	0.4	0.0	1.3	3.3	0.0	1.9	0.3	0.1

Table 93: Phenotypic rating of T3 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column, along with the number of T1 plants that where rated per event. DFF: days to first flower (days), DF: deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoI: number of lobes (#), PH: plant height (cm)

Event	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH
LAODDN (n=30)	43.5 ± 4.1	8.5 ± 0.5	7.8 ± 0.8	9 ± 0	7.9 ± 1	3 ± 0	4.6 ± 0.7	4.5 ± 0.7	8.8 ± 0.4	5 ± 0.8	115 ± 12.5

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Fatty acid profiles, copy number measurements, and phenotypic observations of T3 plants carrying T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc cultivated in field trials in USDA growth zones 8a-9a in the winter.

Field data for T4 seed of two events carrying homozygous T-DNA insertions from VC-LJB2755-2qcz and VC-LLM391-2qcz rc (see Table 83 and Table 90 and Table 84, Table 87, Table 91) indicate these events do accumulate EPA, DHA and ARA when grown in the greenhouse and field, though as consistently observed, the field grown material did not accumulate the VLC-PUFAs (ARA, EPA, DHA) to the extent observed in the greenhouse (see Table 94 and Table 95 in comparison with Table 91, Table 92, Table 87 and Table 88). As observed in in Example 11 part F, higher oil content was observed compared to the summer field trials (Comparison Table 96 with Table 89). This phenomenon is analyzed in detail in Example 20.

Table 94: Fatty acid profiles of T4 seeds harvested from T3 cultivated in the field corresponding to USDA growth zones 8a-9a for field trials of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc The events are indicated in the first column, along with the number of T4 seed aliquots representing a plot were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LAPBOW (n=16)	5.3 ± 0.2	0.4 ± 0	0 ± 0	2.4 ± 0.2	36.1 ± 3.1	31.3 ± 2	0.5 ± 0.1	7.9 ± 0.5	1.3 ± 0.3	0.4 ± 0.1	0.6 ± 0	0.7 ± 0	0.1 ± 0.1	0 ± 0	1.2 ± 0.1	0.8 ± 0.1	1.4 ± 0.2	4.5 ± 0.5	0.2 ± 0	0 ± 0	0.7 ± 0.2	2.7 ± 0.2	0 ± 0	0.7 ± 0.2	0.3 ± 0.2	0.1 ± 0.1
LAODDN (n=47)	5.5 ± 0.6	0.5 ± 0.2	0.2 ± 0.3	2.7 ± 0.5	36.8 ± 3.2	30.3 ± 2.2	0.6 ± 0.2	7.9 ± 0.5	1.3 ± 0.3	0.4 ± 0.1	0.6 ± 0.1	0.7 ± 0	0.1 ± 0.1	0 ± 0	1 ± 0.1	0.8 ± 0.1	1.2 ± 0.2	4.7 ± 0.7	0.2 ± 0	0 ± 0	0.4 ± 0.3	2.6 ± 0.3	0 ± 0	0.6 ± 0.2	0.2 ± 0.1	0.1 ± 0.1
WT Kumily (n=83)	5.1 ± 0.5	0.4 ± 0.1	0.1 ± 0.1	2.1 ± 0.5	59.1 ± 1.8	21.3 ± 1.5	0 ± 0.1	9.5 ± 0.7	0 ± 0.1	0 ± 0	0.6 ± 0.1	1 ± 0.1	0.1 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0.1	0.1 ± 0.1	0.3 ± 0	0 ± 0	0 ± 0.1	0 ± 0.1	0 ± 0	0 ± 0	5.1 ± 0.5	0.4 ± 0.1

Table 95: Fatty acid profiles of one T4 seed batch per event harvested from T3 plants cultivated in the field corresponding to USDA growth zones 8a-9a for field trials of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column. Fatty acid profiles of T4 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LAPBOW (n=1)	5.3	0.4	0.0	2.3	31.5	34.0	0.5	7.3	1.8	0.6	0.6	0.7	0.1	0.0	1.2	0.8	1.8	5.5	0.2	0.0	1.0	3.1	0.0	1.1	0.2	0.0
LAODDN (n=1)	5.6	0.4	0.0	2.1	33.8	31.1	0.7	8.0	1.5	0.5	0.5	0.7	0.1	0.0	1.2	0.8	1.6	6.2	0.1	0.0	0.7	3.3	0.0	1.0	0.2	0.1

Table 96: Phenotypic rating of T3 plants cultivated in the field corresponding to USDA growth zones 8a-9a for trials of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column, along with the number of field plots that were rated per event. Oil: oil content (% of seed weight), protein: Protein content (% of seed cake without oil)

Event	Oil	protein
LAPBOW (n=16)	42.9 ± 4.2	23.3 ± 3.1
LAODDN (n=47)	43.5 ± 3.8	22.7 ± 2.7

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WT Kumily (n=83)	45.3 ± 3.9	

Fatty acid profiles, copy number measurements, and phenotypic observations of T4 plants carrying T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc cultivated in field trials in USDA zones 3a-4b and 5a during the summer.

The data indicate that through the T5 generation the event LAODDN was still producing EPA and DHA at a level consistent with the field trial (described in part D). Also oil content was comparable between these two field trials.

Table 97: Fatty acid profiles of T5 seeds harvested from T4 cultivated in the field corresponding to USDA growth zones 3a-3b and 5a for field trials of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc The events are indicated in the first column, along with the number of T4 seed aliquots representing a plot where measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LAODDN (n=142)	4.7 ± 0.4	0.2 ± 0.1	0 ± 0	2.8 ± 0.4	34.8 ± 3.5	33.8 ± 4.8	0.3 ± 0.3	7.7 ± 1	1.1 ± 0.4	0.2 ± 0.2	0.7 ± 0.1	0.8 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	1.2 ± 0.3	1.1 ± 1.2	1.4 ± 0.4	4.5 ± 1.9	0.3 ± 0.1	0 ± 0	0.5 ± 0.4	2.9 ± 0.5	0.1 ± 0.2	0.7 ± 0.2	0.1 ± 0.2	0.1 ± 0.1
WT Kumily (n=56)	4.5 ± 0.5	0.1 ± 0.1	0 ± 0	1.8 ± 1	60 ± 3.8	22.5 ± 3.6	0.6 ± 1.5	7.9 ± 2.2	0.1 ± 0.1	0 ± 0	0.8 ± 0.2	1 ± 0.4	0.1 ± 0	0 ± 0	0.2 ± 0.8	0 ± 0	0 ± 0	0 ± 0.1	0.2 ± 0.2	0 ± 0	0 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Table 98: Fatty acid profiles of one T5 seed batch per event harvested from T4 plants cultivated in the field corresponding to USDA growth zones 3a-3b and 5a for field trials of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column. Fatty acid profiles of T5 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LAODDN (n=1)	4.5	0.2	0.0	2.6	30.3	30.8	0.4	5.8	0.6	0.1	0.6	0.9	0.3	0.4	2.4	1.3	3.7	8.1	0.3	0.0	0.0	4.2	1.2	1.2	0.0	0.0

Table 99: Phenotypic rating of T4 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column, along with the number of field plots that were rated per event. Oil: oil content (% of seed weight), protein: Protein content (% of seed cake without oil)

Event	Oil	protein
LAODDN (n=47)	39.9 ± 4.4	25.4 ± 1.8
WT Kumily (n=56)	40.6 ± 2.3	26.4 ± 1.3

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Example 14: Plants containing the T-DNAs of plasmid VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc (combination E in example 5) for production of EPA and DHA in seeds

In this example, the genetic elements required for EPA and DHA synthesis were transferred into the plant genome on two different T-DNAs. To this end, the two different plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc containing two different T-DNAs were cloned into Agrobacteria, and plant tissue was incubated according to example 5 at the same time with these two agrobacterial cultures that are identical apart from containing either VC-LJB2755-2qcz rc or VC-LTM217-1qcz rc rc. Due to the selectable herbicide resistance marker, regenerated plants contained the T-DNA of VC-LJB2755-2qcz rc. Only those plants were kept, that also contained the T-DNA of plasmid VC-LTM217-1qcz rc rc as confirmed by PCR, conducted as described in example 5. Only plants containing the T-DNA of plasmid VC-LJB2755-2qcz rc as well as the T-DNA of plasmid VC-LTM217-1qcz rc combined all the genetic elements required for EPA and DHA synthesis in seeds. The genetic elements of VC-LJB2755-2qcz rc and the function of each element are listed in Table 2. The genetic elements of VC-LTM217-1qcz rc and the function of each element were listed in Table 7. For convenience, all enzymes expressed in seeds of plants carrying both T-DNA of VC-LJB2755-2qcz rc and VC- LTM217-1qcz rc that were required for EPA and DHA synthesis are additionally listed Table 100.

Table 100: Combined list of genes essential of EPA and DHA synthesis carried by the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc.

Genes encoding enzmyes for EPA and DHA synthesis	Plasmid containing T-DNA with the gene	Length (bp)	Enzymatic function and source of encoded protein
c-d12Des(Ps_GA)	VC-LJB2755-2qcz rc	1196	Delta-12 desaturase from *Phythophthora sojae*
c-d6Des(Ot_febit)	VC-LJB2755-2qcz rc	1370	Delta-6 desaturase from *Ostreococcus tauri*
c-d6Elo(Tp_GA2)	VC-LJB2755-2qcz rc	818	Delta-6 elongase from *Thalassiosira pseudonana*
c-d5Des(Tc_GA2)	VC-LJB2755-2qcz rc	1319	Delta-5 desaturase from *Thraustochytrium* sp. ATCC21685
c-o3Des(Pi_GA2)	VC-LJB2755-2qcz rc	1085	Omega-3-desaturase from *Phythophthora infestans*
c-o3Des(Pir_GA)	VC-LJB2755-2qcz rc	1091	Omega-3 desaturase from *Pythium irregulare*

Genes encoding enzmyes for EPA and DHA synthesis	Plasmid containing T-DNA with the gene	Length (bp)	Enzymatic function and source of encoded protein
c-d5Elo(Ot_GA3)	VC-LTM217-1qcz rc	902	Delta-5 elongase from *Ostreococcus tauri*
c-d4Des(Pl_GA)2	VC-LTM217-1qcz rc	1338	Delta-4 desaturase from *Pavlova lutheri*
c-d4Des(Tc_GA)	VC-LTM217-1qcz rc	1560	Delta-4 desaturase from *Thraustochytrium* spp.

Fatty acid profiles, copy number measurements, and phenotypic observations of T0 plants carrying T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc cultivated in greenhouses during winter.

Table 102 and Table 103 indicate the single copy events for insertions of VC-LJB2755-2qcz and VC-LTM217-1qcz rc did not accumulate as much EPA and DHA as the double copy events. Table 103 indicates that the combined EPA and DHA content, for the highest producers in T1 seed, was in the range of 15 percent of the total fatty acid content of the seed (5% of the total seed fatty acid content being DHA and 10% being EPA).

Table 101: Copy number measurement of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column; sc: all T1 plants where the average of all copy number assays listed in this table was 0.51-1.49, dc: all T1 plants where the average of all copy number assays listed in this table was 1.51-2.49, tc: all T1 plants where the average of all copy number assays listed in this table was 2.51-3.49.

	Copy number assays targeting the T-DNA of VC-LJB2755-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target j-p-LuPXR_i-Atss15 near the right T-DNA border.						Copy number assays targeting the T-DNA of VC-LTM217-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-d4Des(Eg_GA) located near the left T-DNA border and target j-i-Atss1_c-d5Elo(Ot_GA3) near the right T-DNA border.					
Event	c-AHAS	c-o3Des(Pi_GA)	j-i-Atss18_c-o3Des(Pi_GA2)	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-i-Atss1_c-d6Elo(Tp_GA2)	c-o3Des(Plr_GA)	j-p-LuPXR_i-Atss15	c-d4Des(Pl_GA)2	j-t-PvARC-p-LuCnl	j-i-Atss2_c-d4Des(Tc_GA3)	c-d4Des(Tc_GA)	j-p-PvARC5_t-BnFAE	c-d5Elo(Ot_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)
sc (n=139)	1.2	1.2					1.2	1.2	1.2		1.1	1.2			1.1
dc (n=77)	1.6	1.8			2.0		1.8	1.8	1.9		1.9	1.7			1.9

Table 102: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 101. The number of T1 plants/events fullfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=139)	5.1 ± 0.5	0.3 ± 0.1	0 ± 0.1	3.7 ± 0.8	37.8 ± 7.2	32 ± 4.4	0.4 ± 0.3	4.6 ± 0.6	1.7 ± 1.2	0.3 ± 0.3	0.9 ± 0.1	0.8 ± 0.1	0 ± 0.1	0 ± 0	1.4 ± 0.6	0.8 ± 0.4	1.8 ± 0.7	4.8 ± 1.8	0.4 ± 0.1	0 ± 0.3	0.2 ± 0.2	0.8 ± 0.5	0.3 ± 0.4	1.4 ± 0.6	0.1 ± 0.2	0 ± 0.1
dc (n=77)	5 ± 0.4	0.3 ± 0.1	0 ± 0.1	3.7 ± 0.6	35.5 ± 7.4	32.8 ± 4.4	0.5 ± 0.2	4.7 ± 0.8	1.8 ± 0.7	0.3 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0 ± 0.1	0 ± 0	1.5 ± 0.8	0.9 ± 0.5	1.7 ± 0.6	5.5 ± 2.6	0.3 ± 0.1	0 ± 0	0.3 ± 0.2	1 ± 0.5	0.4 ± 0.4	1.7 ± 0.6	0.2 ± 0.3	0 ± 0.1

Table 103: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 101. For each category, the fatty acid profile of the plant/event having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=1)	5.0	0.4	0.0	2.9	32.0	26.7	1.3	3.3	3.4	0.6	0.9	0.8	0.1	0.0	1.6	1.0	2.7	10.3	0.4	0.0	1.2	0.0	0.0	5.2	0.2	0.1
dc (n=1)	5.1	0.2	0.1	5.1	23.3	31.7	0.7	3.8	2.9	0.6	1.0	0.8	0.2	0.1	1.7	1.3	2.2	12.5	0.4	0.0	0.5	1.9	0.7	3.0	0.2	0.1

Table 104: Phenotypic rating of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column; as defined inTable 101. The number of T1 plants fullfilling these criteria are displayed in parentheses. TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad), Oil: oil content (% of seed weight), Protein: Protein content (% of seed cake without oil)

Category of T1 plants	TKW	SC	Oil	Protein
sc (n=139)	4.3 ± 0.5	5 ± 1.8	33.3 ± 1.6	30.5 ± 0.6

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dc (n=77)	4.1 ± 0.5	5.3 ± 2	33.5 ± 3	29.7 ± 1.1

Fatty acid profiles, copy number measurements, and phenotypic observations of T1 plants carrying T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc cultivated in greenhouses during summer.

Measurements carried out on plants from selected T1 events indicated single copy homozygous insertions of the T-DNA corresponding to VC-LJB2755-2qcz and VC-LTM217-1qcz rc (see table 104, in particular the table legend) and the T2 accumulates EPA and DHA in similar levels as the T1. Measurements on T2 seed (see Table 106 and Table 107) showed that EPA and DHA accumulated up to 13% of the total fatty acid content of the seed (with ca. 3% of the seed total fatty acid content being DHA).

Table 105: Copy number measurement of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc. Homozygous plants of the T1 generation of these two events have been selected using Half Kernel Analysis (Example 9). Based on the proven ability to discriminate homozygous and heterozygous seeds using half kernel analysis, it can be assumed all T1 plants in Table 106, Table 107, and Table 108 are homozygous, but no copy number analysis has been performed to confirm this.

Event	Copy number assays targeting the T-DNA of VC-LJB2755-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target j-p-LuPXR_i-Atss15 near the right T-DNA border.								Copy number assays targeting the T-DNA of VC-LTM217-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-d4Des(Eg_GA) located near the left T-DNA border and target j-i-Atss1_c-d5Elo(Ot_GA3) near the right T-DNA border.					
	c-AHAS	c-o3Des(Pi_GA)	j-i-Atss18_c-o3Des(Pi_GA2)	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-i-Atss1_c-d6Elo(Tp_GA2)	c-o3Des(Pir_GA)	j-p-LuPXR_i-Atss15	c-d4Des(Pl_GA)2	j-t-PvARC-p-LuCnI	j-i-Atss2_c-d4Des(Tc_GA3)	c-d4Des(Tc_GA)	j-p-PvARC5_t-BnFAE	c-d5Elo(Ot_GA3)
LBAIID (n=1)	1.1	1.0					1.0	1.0	1.2		1.0			1.1
LBAPPG (n=1)	1.0	1.0					1.0	1.0	1.2		1.1			0.9

Table 106: Fatty acid profiles of T2 seeds harvested from homozygous T1 cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc The events are indicated in the first column, along with the number of T2 seed batches that where measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LBAIID (n=20)	5.1 ± 0.2	0.2 ± 0	0 ± 0	3.6 ± 0.3	23.6 ± 1.8	38.4 ± 0.9	0.5 ± 0.1	4.9 ± 0.3	2.1 ± 0.2	0.5 ± 0.1	0.9 ± 0.1	0.6 ± 0	0.1 ± 0	0.1 ± 0	1.2 ± 0.1	0.9 ± 0.1	1.8 ± 0.2	8.1 ± 0.8	0.4 ± 0	0 ± 0	0.7 ± 0.1	1.8 ± 0.2	0.8 ± 0.1	3.4 ± 0.4	0.3 ± 0.1	0 ± 0
LBAPPG (n=18)	5.6 ± 0.5	0.3 ± 0.1	0 ± 0	4.3 ± 0.3	24.2 ± 2.7	39.9 ± 1.2	0.3 ± 0.1	4.7 ± 0.3	2 ± 0.3	0.4 ± 0.1	1 ± 0.1	0.6 ± 0	0.1 ± 0	0 ± 0	1 ± 0.1	0.7 ± 0.2	1.8 ± 0.2	6.9 ± 1.2	0.4 ± 0	0 ± 0	0.6 ± 0.1	1.6 ± 0.2	0.6 ± 0.2	2.9 ± 0.5	0 ± 0.1	0 ± 0

Table 107: Fatty acid profiles of one T2 seed batch per event harvested from homozygous T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T2 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LBAIID (n=1)	5.2	0.3	0.0	3.7	20.9	37.3	0.4	5.3	2.4	0.6	0.9	0.6	0.1	0.1	1.0	0.8	2.0	9.2	0.4	0.0	0.8	2.2	1.0	4.5	0.3	0.0
LBAPPG (n=1)	4.9	0.2	0.0	4.6	22.3	38.8	0.3	4.7	2.1	0.5	1.2	0.7	0.1	0.0	1.2	0.9	2.0	8.2	0.5	0.0	0.6	1.8	0.7	3.7	0.0	0.0

Table 108: Fatty acid profiles of T2 seeds harvested from homozygous T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc. Plants of all events combined have been grouped into the category "sc". The number of T1 plants fullfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1n -7	16:3n -3	18:0	18:1n -9	18:2n -6	18:2n -9	18:3n -3	18:3n -6	18:4n -3	20:0	20:1n -9	20:2n -6	20:3n -3	20:3n -6	20:4n -3	20:4n -6	20:5n -3	22:0	22:1n -9	22:4n -6	22:5n -3	22:5n -6	22:6n -3	unknown peak a	unknown peak b
sc (n=38)	2.6 ± 0.2	0.1 ± 0	0 ± 0	2 ± 0.2	12 ± 1.1	19.5 ± 0.6	0.2 ± 0	2.4 ± 0.1	1 ± 0.1	0.2 ± 0	0.5 ± 0	0.3 ± 0	0 ± 0	0 ± 0	0.5 ± 0.1	0.4 ± 0.1	0.9 ± 0.1	3.7 ± 0.6	0.2 ± 0	0 ± 0	0.3 ± 0	0.8 ± 0.1	0.3 ± 0.1	1.6 ± 0.3	0.1 ± 0.1	0 ± 0

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Example 15: Plants containing the T-DNA of plasmid RTP10690-1qcz_F for production of EPA and DHA in seeds

All genetic elements required for EPA and DHA synthesis described in this example, were transferred on a single T-DNA using a BiBAC plasmid into the plant genome. To this end, the plasmid RTP10690-1qcz_F was cloned into agrobacteria, and plant tissue was incubated according to example 6 with this agrobacterial culture. Due to the selectable herbicide resistance marker, regenerated plants contained the T-DNA of RTP10690-1qcz_F. The genetic elements of RTP10690-1qcz_F and the function of each element are listed in Table 8. For convenience, all enzymes expressed in seeds of plants carrying both T-DNA of RTP10690-1qcz_F that required for EPA and DHA synthesis are additionally listed Table 109.

Table 109: List of genes essential of EPA and DHA synthesis carried by the T-DNA of plasmid RTP10690-1qcz_F.

Genes encoding enzmyes for EPA and DHA synthesis	Length (bp)	Enzymatic function and source of encoded protein
c-d12Des(Ps_GA2)	1197	Delta-12 desaturase from *Phythophthora sojae*
c-d6Des(Ot_febit)	1371	Delta-6 desaturase from *Ostreococcus tauri*
c-d6Elo(Pp_GA2)	873	Delta-6 elongase from *Physcomitrella patens*
c-d6Elo(Tp_GA2)	819	Delta-6 elongase from *Thalassiosira pseudonana*
c-d5Des(Tc_GA2)	1320	Delta-5 desaturase from *Thraustochytrium* sp. ATCC21685
2 copies of c-d5Des(Tc_GA2)	1320	Delta-5 desaturase from *Thraustochytrium* sp. ATCC21685
c-o3Des(Pi_GA2)	1086	Omega-3-desaturase from *Phythophthora infestans*
2 copies of c-o3Des(Pir_GA)	1092	Omega-3 desaturase from *Pythium irregulare*
c-d5Elo(Ot_GA3)	903	Delta-5 elongase from *Ostreococcus tauri*

Genes encoding enzmyes for EPA and DHA synthesis	Length (bp)	Enzymatic function and source of encoded protein
c-d4Des(Pl_GA)2	1338	Delta-4 desaturase from *Pavlova lutheri*
c-d4Des(Tc_GA3)	1560	Delta-4 desaturase from *Thraustochytrium* sp.

Fatty acid profiles, copy number measurements, and phenotypic observations of T0 plants carrying T-DNA of plasmids RTP10690-1qcz_F cultivated in greenhouses during winter

As Table 110 indicates there were fewer insertion events of this construct observed than for other constructs, with more single copy events than double copy, by approximately four fold. Fatty acid profile data on Table 111 and Table 112 indicated that DHA and EPA can accumulate up to 4% of the total seed fatty acid content in the T1 with similar performance between single and double copy events, within error. Table 113 demonstrates that there was no significant aerial phenotype associated with this construct in the T0.

Table 110: Copy number measurement of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid RTP10690-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column; sc: all T0 plants where the average of all copy number assays listed in this table was 0.51-1.49, dc: all T0 plants where the average of all copy number assays listed in this table was 1.51-2.49, tc: all T0 plants where the average of all copy number assays listed in this table was 2.51-3.49.

	Copy number assays targeting the T-DNA of RTP10690-1qcz_F. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHA5 located near the left T-DNA border and target c-d6Elo(Pp_GA)near the right T-DNA border.													
Category of T0 plants	c-AHAS	c-d5Des(Tc_GA)	j-i-Atss18_c-o3Des(Pi_GA2)	j-i-Atss2_c-d4Des(Tc_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Pl_GA)2	j-i-Atss2_c-d4Des(Pl_GA)-195R	j-p-LuPXR_i-Atss15	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-i-Atss2_c-d6Des(Otfebit_GA)	c-d5Des(Tc_GA)	c-d6Elo(Pp_GA)
sc (n=52)	1.1	2.0	1.2	1.0	1.1	1.0		1.0	1.2	1.0	1.1	1.0	2.0	1.1
dc (n=12)	1.6	3.5	2.0	1.8	1.9	2.0		1.9	2.2	1.8	2.1	1.8	3.5	1.8

Table 111: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid RTP10690-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 110. The number of T0 plants/events fullfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=52)	5.1 ± 0.7	0.3 ± 0.1	0 ± 0	3.1 ± 0.4	43.8 ± 7.3	28.3 ± 4.5	0.3 ± 0.2	5.1 ± 0.8	0.8 ± 0.4	0.1 ± 0.1	0.9 ± 0.1	1 ± 0.1	0.5 ± 0.6	0.2 ± 0.3	2.2 ± 0.7	1.1 ± 0.4	1.6 ± 0.5	3.2 ± 1	0.4 ± 0.1	0 ± 0	0.1 ± 0.1	1.1 ± 0.4	0 ± 0	0.5 ± 0.3	0.1 ± 0.1	0.2 ± 0.2
dc (n=12)	5.2 ± 0.4	0.3 ± 0.1	0 ± 0.1	3.5 ± 0.5	39 ± 6.7	30.1 ± 3	0.5 ± 0.2	4.5 ± 0.6	1.6 ± 0.6	0.2 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.3 ± 0.4	0.1 ± 0.1	3.1 ± 1	1.2 ± 0.4	2 ± 1.4	3.7 ± 1.3	0.4 ± 0	0 ± 0	0.3 ± 0.2	1.2 ± 0.4	0 ± 0	0.7 ± 0.3	0.2 ± 0.2	0.1 ± 0.2

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Table 112: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid RTP10690-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 110. For each category, the fatty acid profile of the plant/event having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=1)	5.5	0.3	0.0	4.1	27.2	33.6	0.7	4.1	1.9	0.3	0.9	0.7	0.3	0.2	4.5	2.1	2.7	5.8	0.4	0.0	0.3	2.2	0.1	1.4	0.4	0.2
dc (n=1)	5.3	0.3	0.0	3.8	31.9	32.9	0.6	3.8	1.6	0.2	0.9	0.8	0.4	0.2	3.6	1.5	2.7	5.7	0.4	0.0	0.3	1.7	0.0	1.1	0.2	0.3

Table 113: Phenotypic rating of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid RTP10690-1qcz rc. Considering each event was in this generation represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 110. The number of T0 plants/events fullfilling these criteria are displayed in parentheses. DFF: days to first flower (days), DF: deformed flower (1=deformed, 9=normal), DL: deformed leaf (1=deformed, 9=normal), DP: deformed plant (1=deformed, 9=normal), DS: deformed silique (1=deformed, 9=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoI: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad), Oil: oil content (% of seed weight), Protein: Protein content (% of seed cake without oil)

Category of T1 plants	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC	Oil	Protein
sc (n=52)	61.4 ± 15.5	8.7 ± 1.2	8.7 ± 1.2	8.8 ± 0.9	7.6 ± 2.5	3 ± 0	5.4 ± 7.9	5.9 ± 6.9	7.3 ± 2.6	4.9 ± 1.3	123.8 ± 7.5			35.4 ± 2.4	30.1 ± 1.1
dc (n=12)	56.3 ± 9.6	9 ± 0	9 ± 0	9 ± 0	8.1 ± 2.1	3 ± 0	4.3 ± 1	5 ± 0	8.1 ± 2.1	5 ± 0.9	125.4 ± 5.4			34.8 ± 1.1	29.9 ± 1

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Fatty acid profiles, copy number measurements, and phenotypic observations of T1 plants carrying T-DNA of plasmids RTP10690-1qcz_F cultivated in greenhouses during summer

Data in Table 115 and Table 116 indicate a similar performance of the T2 seed as that of the T1 seed with respect to EPA and DHA, see also Table 111 and Table 112 for comparison. The selected events all performed at a similar level and segregated for one to two copies of each gene.

Table 114: Copy number measurement of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid RTP10690-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that were measured per event.

	Copy number assays targeting the T-DNA of RTP10690-1qcz_F. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target c-d6Elo(Pp_GA)near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity of all plants per event was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.																			
Event	c-AHAS	c-dSDes(Tc_GA)		j-i-Atss18_c-o3Des(Pl_GA2)		j-i-Atss2_c-d4Des(Tc_GA3)		j-i-Atss1_c-d5Elo(Ot_GA3)		c-d4Des(Pl_GA)2		j-i-Atss2_c-d4Des(Pl_GA)-19SR		j-p-LuPXR_i-Atss15		j-i-Atss14_c-d12Des(Ps_GA)		c-d6Elo(Tp_GA)	
LBDGCA (n=94)	1.5 (T0: 1)	3.2 (T0: 2)		1.6 (T0: 1.4)		(T0: 1)		(T0: 1.2)		1.7 (T0: 0.9)		1.6		1.6 (T0: 1)		(T0: 1.2)		1.7 (T0: 1)	
LBDGBP (n=20)	(T0: 1.1)	(T0: 2.1)		(T0: 1.4)		(T0: 1.2)		(T0: 1.3)		(T0: 1.1)				(T0: 1.1)		(T0: 1.3)		(T0: 1)	
LBDIHN (n=114)	1.6 (T0: 1.2)	3.3 (T0: 2.2)		1.6 (T0: 1.7)		(T0: 1.1)		(T0: 1.2)		1.7 (T0: 1)		1.6		1.6 (T0: 1.1)				1.7 (T0: 1)	

	c-AHAS		c-dSDes(Tc_GA)		j-i-Atss18_c-o3Des(Pl_GA2)		j-i-Atss2_c-d4Des(Tc_GA3)		j-i-Atss1_c-d5Elo(Ot_GA3)		c-d4Des(Pl_GA)2		j-i-Atss2_c-d4Des(Pl_GA)-19SR		j-p-LuPXR_i-Atss15		j-i-Atss14_c-d12Des(Ps_GA)		c-d6Elo(Tp_GA)		j-t-StCAT_p2_p-LuPXR		j-i-Atss2_c-d6Elo(Ot febit_GA)		c-d5Des(Tc_GA)		c-d6Elo(Pp_GA)	
Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unkn own peak a	unkn own peak b
LBDGCA (n=90)	5.7 ± 0.5	0.2 ± 0.1	0 ± 0.1	4.1 ± 0.7	36.4 ± 7.3	32.5 ± 3.9	0.5 ± 0.2	4.5 ± 0.9	1.7 ± 0.7	0.2 ± 0.1	1.1 ± 0.2	0.8 ± 0.1	0.2 ± 0.1	0 ± 0	2.6 ± 0.6	1 ± 0.2	1.9 ± 0.7	3.3 ± 1.2	0.4 ± 0.1	0 ± 0	0.4 ± 0.2	1.4 ± 0.5	0 ± 0	0.6 ± 0.3	0.3 ± 0.1	0.1 ± 0.1
LBDGBP (n=20)	5.4 ± 0.5	0.3 ± 0	0 ± 0	3.9 ± 0.4	25.4 ± 2.3	37.1 ± 1.6	0.5 ± 0	3.4 ± 0.2	2.4 ± 0.3	0.2 ± 1 ± 0	0.1	0.6 ± 0	0.1 ± 0	0 ± 0	2.3 ± 0.2	0.9 ± 0.2	3.8 ± 0.5	7.5 ± 1.6	0.4 ± 0	0 ± 0	0.6 ± 0.1	2.4 ± 0.4	0 ± 0.2	1.7 ± 0.7	0 ± 0	0 ± 0
LBDIHN (n=110)	4.9 ± 0.4	0.2 ± 0.1	0 ± 0	3.6 ± 0.5	32.5 ± 7.6	34.5 ± 4.1	0.6 ± 0.2	4.4 ± 1	1.7 ± 0.7	0.2 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	2.3 ± 0.4	1.1 ± 0.4	2.7 ± 0.9	5.2 ± 1.6	0.4 ± 0.1	0 ± 0	0.4 ± 0.2	1.9 ± 0.6	0 ± 0	0.9 ± 0.4	0.2 ± 0.1	0.1 ± 0.1

Table 115: Fatty acid profiles of T2 seeds harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid RTP10690-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that were measured per event.

Table 116: Fatty acid profiles of T2 seeds harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid RTP10690-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T2 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Events	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LBDGCA (n=1)	5.5	0.2	0.0	3.3	29.2	31.8	0.9	5.3	2.8	0.4	0.7	0.7	0.1	0.1	2.9	1.2	3.6	5.9	0.3	0.0	0.6	2.6	0.0	1.3	0.4	0.1
LBDGBP (n=1)	5.2	0.3	0.0	3.9	20.3	35.1	0.5	3.6	2.7	0.3	1.0	0.6	0.1	0.0	2.6	1.3	4.4	10.8	0.4	0.0	0.7	3.3	0.0	3.0	0.0	0.0
LBDIHN (n=1)	5.0	0.3	0.0	3.5	23.8	36.3	0.6	2.6	2.6	0.2	0.9	0.6	0.1	0.0	2.8	1.1	4.1	9.1	0.4	0.0	1.0	2.8	0.0	2.1	0.0	0.1

Table 117: Phenotypic rating of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid RTP10690-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that were measured per event. Oil: oil content (% of seed weight), Protein: Protein content (% of seed cake without oil)

Events	Oil	Protein
LBDGCA (n=90)	34.1 ± 3.1	30.1 ± 1.9
LBDIHN (n=110)	35.3 ± 2.7	29.9 ± 1.8

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Example 16: Plants containing the T-DNA of plasmid RTP10691-2qcz for production of EPA and DHA in seeds

All genetic elements required for EPA and DHA synthesis described in this example, were transferred on a single T-DNA using a BiBAC plasmid into the plant genome. To this end, the plasmid RTP10691-2qcz was cloned into agrobacteria, and plant tissue was incubated according to example 6 with this agrobacterial culture. Due to the selectable herbicide resistance marker, regenerated plants contained the T-DNA of RTP10691-2qcz. The genetic elements of RTP10691-2qcz and the function of each element are listed in Table 9. For convenience, all enzymes expressed in seeds of plants carrying both T-DNA of RTP10691-2qcz that required for EPA and DHA synthesis are additionally listed in Table 118.

Table 118: List of genes essential of EPA and DHA synthesis carried by the T-DNA of plasmid RTP10691-2qcz.

Genes encoding enzmyes for EPA and DHA synthesis	Length (bp)	Enzymatic function and source of encoded protein
c-d12Des(Ps_GA2)	1196	Delta-12 desaturase from *Phythophthora sojae*
c-d6Des(Ot_febit)	1371	Delta-6 desaturase from *Ostreococcus tauri*
c-d6Elo(Pp_GA2)	873	Delta-6 elongase from *Physcomitrella patens*
c-d6Elo(Tp_GA2)	819	Delta-6 elongase from *Thalassiosira pseudonana*
2 copies of c-d5Des(Tc_GA2)	1320	Delta-5 desaturase from *Thraustochytrium* sp. ATCC21685
c-o3Des(Pi_GA2)	1086	Omega-3-desaturase from *Phythophthora infestans*
2 copies of c-o3Des(Pir_GA)	1092	Omega-3 desaturase from *Pythium irregulare*
c-d5Elo(Ot_GA3)	903	Delta-5 elongase from *Ostreococcus tauri*
c-d4Des(Pl_GA)2	1337	Delta-4 desaturase from *Pavlova lutheri*
c-d4Des(Tc_GA3)	1560	Delta-4 desaturase from

Genes encoding enzmyes for EPA and DHA synthesis	Length (bp)	Enzymatic function and source of encoded protein
		Thraustochytrium sp.

Example 17: Plants containing the T-DNA of plasmid VC-LTM595-1qcz rc for production of EPA and DHA in seeds

All genetic elements required for EPA and DHA synthesis described in this example, were transferred on a single T-DNA using a BiBAC plasmid into the plant genome. To this end, the plasmid VC-LTM595-1qcz rc was cloned into agrobacteria, and plant tissue was incubated according to example 6 with this agrobacterial culture. Due to the selectable herbicide resistance marker, regenerated plants contained the T-DNA of VC-LTM595-1qcz rc. The genetic elements of VC-LTM595-1qcz rc and the function of each element are listed in Table 10. For convenience, all enzymes expressed in seeds of plants carrying both T-DNA of VC-LTM595-1qcz rc that required for EPA and DHA synthesis are additionally listed Table 119.

Table 119: List of genes essential of EPA and DHA synthesis carried by the T-DNA of plasmid VC-LTM595-1qcz rc.

Genes encoding enzmyes for EPA and DHA synthesis	Length (bp)	Enzymatic function and source of encoded protein
c-d12Des(Ps_GA2)	1197	Delta-12 desaturase from *Phythophthora sojae*
c-d6Des(Ot_febit)	1371	Delta-6 desaturase from *Ostreococcus tauri*
c-d6Elo(Pp_GA2)	873	Delta-6 elongase from *Physcomitrella patens*
c-d6Elo(Tp_GA2)	819	Delta-6 elongase from *Thalassiosira pseudonana*
c-d5Des(Tc_GA2)	1320	Delta-5 desaturase from *Thraustochytrium* sp. ATCC21685
c-d5Des(Tc_GA2)	1320	Delta-5 desaturase from *Thraustochytrium* sp. ATCC21685
c-o3Des(Pi_GA2)	1086	Omega-3-desaturase from

Genes encoding enzmyes for EPA and DHA synthesis	Length (bp)	Enzymatic function and source of encoded protein
		Phythophthora infestans
2 copies of c-o3Des(Pir_GA)	1092	Omega-3 desaturase from *Pythium irregulare*
c-d5Elo(Ot_GA3)	903	Delta-5 elongase from *Ostreococcus tauri*
c-d4Des(Pl_GA)2	1338	Delta-4 desaturase from *Pavlova lutheri*
c-d4Des(Tc_GA3)	1560	Delta-4 desaturase from *Thraustochytrium* sp.

Fatty acid profiles, copy number measurements, and phenotypic observations of T0 plants carrying T-DNA of plasmids VC-LTM595-1qcz rc cultivated in greenhouses during summer

Similar to VC-RTP10690-1qcz_F, the number of insertions of the entire T-DNA was not as high as obtained for the multi-construct transformations, see Table 120. Table 121 and Table 122 show the fatty acid profile measurements for single, double and triple copy T-DNA, and indicate that the double copy constructs perform marginally better than the single copy constructs and perhaps marginally better than the triple copy constructs in terms of EPA and DHA accumulation. The fatty acid profile data further indicate that the accumulation of EPA and DHA in the T1 seed was up to 10% of the total fatty acids with up to 2% of the total fatty acids in the seed being DHA. Phenotypic measurements, shown on Table 123, indicated some variability in flowering time (as represented by DFF).

Table 120: Copy number measurement of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM595-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column; sc: all T0 plants where the average of all copy number assays listed in this table except the assay c-d5Des(Tc_GA) was 0.51-1.49, dc: all T0 plants where the average of all copy number assays listed in this table was 1.51-2.49, tc: all T0 plants where the average of all copy number assays listed in this table was 2.51-3.49.

	Copy number assays targeting the T-DNA of VC-LTM595-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target c-d6Elo(Pp_GA) near the right T-DNA border.									
Category of T0 plants	c-AHAS	j-p-BnSETL-v1_c-o3Des(Pir_GA)	c-d4Des(Pl_GA)2	j-i-Atss18_c-o3Des(Pi_GA2)	j-p-PvARCS_t-BnFAE	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d5Des(Tc_GA)	j-p-LuPXR_i-Atss15	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-i-Atss2_c-d6Des(Ot_febit_GA)	c-d5Des(Tc_GA)	c-d6Elo(Pp_GA)
sc (n=24)	1.1		1.0	1.0	0.9	0.9	1.9	1.1		1.0	0.9	1.1	1.9	1.0
dc (n=7)	2.2		2.3	2.1	2.0	1.9	3.8	2.3		2.2	2.3	2.1	3.8	1.9
tc (n=3)	2.4		2.4	1.9	2.5	2.0	4.6	3.3		2.4	2.9	2.4	4.6	2.4

Table 121: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM595-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 120. The number of T0 plants/events fulfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1n-7	16:3n-3	18:0	18:1n-9	18:2n-6	18:2n-9	18:3n-3	18:3n-6	18:4n-3	20:0	20:1n-9	20:2n-6	20:3n-3	20:3n-6	20:4n-3	20:4n-6	20:5n-3	22:0	22:1n-9	22:4n-6	22:5n-3	22:5n-6	22:6n-3	unknown peak a	unknown peak b
sc (n=24)	5.2 ± 0.6	0.3 ± 0.1	0 ± 0	3 ± 0.4	41.2 ± 4.8	28.6 ± 2.3	0.3 ± 0.1	5.9 ± 0.6	0.6 ± 0.3	0.1 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.6 ± 0.3	0.3 ± 0.1	1.9 ± 0.7	1.2 ± 0.5	1.5 ± 0.3	3.5 ± 0.8	0.4 ± 0.1	0 ± 0	0.3 ± 0.1	1.2 ± 0.3	0 ± 0.1	1.1 ± 0.3	0.4 ± 0.2	0.3 ± 0.2
dc (n=7)	5.6 ± 1.4	0.3 ± 0.2	0 ± 0	3.7 ± 0.7	34.4 ± 7.3	31.2 ± 3.4	0.4 ± 0.2	5.7 ± 1.4	1.5 ± 0.5	0.2 ± 0.2	0.9 ± 0.2	0.8 ± 0.2	0.3 ± 0.2	0.1 ± 0.1	2.7 ± 0.8	1.5 ± 1	1.6 ± 0.8	3.8 ± 2.2	0.4 ± 0	0 ± 0	0.6 ± 0.4	1.5 ± 0.8	0.2 ± 0.1	1.6 ± 0.6	0.7 ± 0.1	0.2 ± 0.1
tc (n=3)	4.1 ± 1.3	0.2 ± 0.1	0 ± 0	3 ± 0.1	45.4 ± 8.4	26.2 ± 2.2	0.4 ± 0.3	6 ± 0.9	1.7 ± 2	0.2 ± 0.3	0.8 ± 0	1 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	1.5 ± 0.4	0.7 ± 0.1	1.5 ± 0.7	3.3 ± 1.6	0.4 ± 0	0 ± 0	0.5 ± 0.3	1 ± 0.4	0.2 ± 0.1	1.1 ± 0.5	0.3 ± 0.1	0.2 ± 0.1

Table 122: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM595-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 120. For each category, the fatty acid profile of the plant/event having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1n-7	16:3n-3	18:0	18:1n-9	18:2n-6	18:2n-9	18:3n-3	18:3n-6	18:4n-3	20:0	20:1n-9	20:2n-6	20:3n-3	20:3n-6	20:4n-3	20:4n-6	20:5n-3	22:0	22:1n-9	22:4n-6	22:5n-3	22:5n-6	22:6n-3	unknown peak a	unknown peak b
sc (n=1)	6.3	0.4	0.0	3.4	34.6	29.8	0.4	5.3	1.0	0.1	0.8	0.8	0.6	0.3	2.9	1.6	1.8	4.6	0.3	0.0	0.5	1.6	0.2	1.6	0.7	0.3
dc (n=1)	4.9	0.2	0.0	2.8	25.8	33.9	0.3	4.8	1.7	0.3	0.7	0.7	0.5	0.2	3.2	1.7	2.8	7.4	0.4	0.0	1.2	2.9	0.2	2.3	0.8	0.3
tc (n=1)	4.5	0.2	0.0	3.1	37.8	28.0	0.7	5.7	3.9	0.5	0.8	0.9	0.1	0.0	1.6	0.6	1.8	4.8	0.4	0.0	0.8	1.3	0.3	1.5	0.4	0.1

Table 123: Phenotypic rating of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM595-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 120. The number of T0 plants/events fullfilling these criteria are displayed in parentheses. DFF: days to first flower (days), DF: deformed flower (1=deformed, 9=normal), DL: deformed leaf (1=deformed, 9=normal), DP: deformed plant (1=deformed, 9=normal), DS: deformed silique (1=deformed, 9=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoI: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad)

Category of T1 plants	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC
sc (n=24)	44.8 ± 17.3	9 ± 0	8.7 ± 1.1	9 ± 0	6.7 ± 2.4	3 ± 0	4.7 ± 0.8	5 ± 0	6.7 ± 2.4	4.7 ± 1	123.5 ± 7.7	4.4 ± 0.5	1.6 ± 0.9
dc (n=7)	47.1 ± 10.3	9 ± 0	8.7 ± 0.8	9 ± 0	4.7 ± 2.6	3 ± 0	5 ± 0	5 ± 0	4.7 ± 2.6	4.7 ± 1.1	122.9 ± 5.7	4.3 ± 0.9	2.1 ± 1.5
tc (n=3)	42.3 ± 2.5	9 ± 0	9 ± 0	9 ± 0	6 ± 4.4	3 ± 0	5 ± 0	5 ± 0	6 ± 4.4	5 ± 1.7	125 ± 10	4.2 ± 0.2	4.3 ± 3.2

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Fatty acid profiles, copy number measurements, and phenotypic observations of T1 plants carrying T-DNA of plasmids VC-LTM595-1qcz rc cultivated in greenhouses during winter

The data Table 124, Table 125 and Table 126 indicate that a variety of copy numbers for the genes contained on the T-DNA were obtained, with copy number values ranging from 1 to 3. The performance of the events selected for measurements of fatty acid profiles (see Table 127 and Table 128) was similar to that observed in the previous example with the EPA and DHA combined value being approximately ten percent of the total fatty acid content of the seed, for the upper end of values. The data in Table 129 indicate, as observed in prior generations for this construct (see example 17 part A), that there was some variation in flowering time among the various events.

Table 124: Copy number measurement of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LTM595-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where measured per event. The T1 plants underwent a selection from 250 segregating T1 seedlings, using zygosity analysis as illustrated in Table 125, keeping only plants that are homozygous for the desired number of loci (which are indicated in the last column of Table 125). A copy number of ~2 therefore was indicative for one homozygous copy, a copy number of ~4 indicative for two homozygous copies (located either at on or at two different loci) and so forth. Odd results of 3, 5, 7, 9 etc indicate that at least some of the selected T1 plants carry at least one heterozygous locus. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the the result oberved in the T0 generation (indicated in parentheses). For some events this was not the case because during selection of T1 plants, undesired loci have been segregated out while retaining only desired loci in a homozygous state.

Event	Copy number assays targeting the T-DNA of VC-LTM595-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target c-d6Elo(Pp_GA) near the right T-DNA border.														
	c-AHAS	c-d5Des(Tc_GA)	j-i-Atss18_c-o3Des(Pl_GA2)	j-i-Atss2_c-d4Des(Tc_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Pl_GA)2	j-i-Atss2_c-d4Des(Pl_GA)-195R	j-p-LuPXR_i-Atss15	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-i-Atss2_c-d6Des(Otfebit_GA)	c-d5Des(Tc_GA)	c-d6Elo(Pp_GA)	Conclusion from individual assays: number of T-DNA copies inserted into the genome
LBEDTZ	4.1 (T0: 2.2)	4	4 (T0: 2)	4 (T0: 1.8)	(T0: 1.8)	4.3 (T0: 1.8)	8.1 (T0: 3.8)	3.8 (T0: 2.5)	5.7	5 (T0: 2.8)	4.7 (T0: 2.8)	5.5 (T0: 3.1)	8.1 (T0: 3.8)	4.1 (T0: 2)	partial double copy
LBEDUA	4.3 (T0: 2.7)	4.2	4 (T0: 2.3)	3 (T0: 2.2)	(T0: 2)	4.5 (T0: 2.3)	8 (T0: 4)	4 (T0: 2.4)	4.3	3.7 (T0: 2.2)	3.6 (T0: 2.4)	4 (T0: 2.1)	8 (T0: 4)	3.8 (T0: 2.4)	partial double copy
LBEDWU	4.2 (T0: 1.8)	3.4	3.3 (T0: 1.9)	2.7 (T0: 1.6)	(T0: 1.7)	3.6 (T0: 1.7)	6.7 (T0: 3.5)	3.2 (T0: 1.9)	3.2	3.3 (T0: 1.7)	3 (T0: 1.7)	3.3 (T0: 1.8)	6.7 (T0: 3.5)	3.9 (T0: 2.1)	partial double copy
LBEFME	(T0: 2.6)		(T0: 2.5)	(T0: 2.6)	(T0: 2.4)		(T0: 3.7)	(T0: 3)		(T0: 2.4)	(T0: 2.7)	(T0: 2)	(T0: 3.7)	(T0: 1.1)	partial double copy
LBEEIL	(T0: 2.4)		(T0: 2.7)	(T0: 2.5)	(T0: 2.5)		(T0: 3.7)	(T0: 2)		(T0: 1.9)	(T0: 2)	(T0: 1.8)	(T0: 3.7)	(T0: 2.1)	partial double copy
LBEEBO	(T0: 2.1)		(T0: 2.7)	(T0: 1.8)	(T0: 1.6)	(T0: 1.9)	(T0: 3.6)	(T0: 1.9)		(T0: 2)	(T0: 2.1)	(T0: 1.8)	(T0: 3.6)	(T0: 2.1)	partial double copy
LBERLW	(T0: 2.1)		(T0: 1.4)	(T0: 1.7)	(T0: 1.6)		(T0: 2.6)	(T0: 2)		(T0: 1.2)	(T0: 0.9)	(T0: 1.6)	(T0: 2.6)	(T0: 1.6)	partial double copy

Table 125: Observed Medelian segregation of the genotype of T1 seeds of events from construct VC-LTM595-1qcz rc. The segregation has been analysed at three positions of the T-DNA. For each position, the number of seedlings have been counted that have a copy number (arithmetically rounded) of 0, 1, 2, 3, 4, 5,

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6, 7, 8, 9, 10, 11 and 12. The of seedlings counted for each copy number category are separated by colon, displaying the categories in the following order: 0 : 1 : 2 : 3 : 4 : 5 : 6 : 7 : 8 : 9 : 10 : 11 : 12. Listed are the observed copy number segregation ratios for T1 seeds segregating for one or more unlinked genomic loci, which contain one or more linked copies of T-DNA insertions. The observed frequencies for each assay have been compared against expected frequencies for various locus configurations listed in Table 136 using Chi-Square analysis. The last column displays the total number of loci that are segregating in the genome of a given event. Many events contain truncated insertions, as was evident when some assays indicate single copy insertion at e.g. the left border (e.g. event LBEDTZ), while other position on the T-DNA clearly indicate a double copy insertion that are either inserted in one locus (e.g. event LBEDTZ), or in two loci (e.g. event LBEDWU)

Event	Copy number ratios measured near the left border of the T-DNA using an assay targetting c-AHAS	Copy number ratios measured using T-DNA internal assays targeting reagions that had copy number results indicating truncated T-DNA insertions using assay A04 (target: j-i-Atss18_c-o3Des(Pi_GA2)), or A10 (target: c-d6Elo(Tp_GA)), or A11 (target j-t-StCAT_p2_p-LuPXR)	Copy number ratios measured near the right border of the T-DNA using an assay targetting c-d6Elo(Pp_GA)	Most likely number of loci containing one or more T-DNA copies, tested using Chi-Square test
LBEDTZ	084:001:111:002:050:000:000:000:000:000:000:000	A10: 085:000:001:111:002:048:002:000:000:000:000:000	084:000:111:000:052:001:000:000:000:000:000:000	single locus
LBEDUA				double locus
LBEDWU	023:071:098:043:014:000:000:000:000:000:000:000	A10: 023:075:098:048:004:000:000:000:000:000:000:000	023:071:099:045:011:000:000:000:000:000:000:000	double locus
LBEFME	011:025:039:062:040:041:023:005:001:000:000:001:000	A11: 010:024:043:063:073:027:004:001:001:000:000:000:000	059:137:052:000:000:000:000:000:000:000:000:000	triple locus
LBEEIL	075:020:044:051:039:018:001:000:000:000:000:000:000	A04: 073:020:046:050:046:010:001:000:000:000:000:000:000	075:020:043:052:039:017:002:000:000:000:000:000:000	triple locus
LBEEBO	019:072:090:055:012:000:000:000:000:000:000:000:000	A10: 019:073:090:052:015:000:000:000:000:000:000:000:000	063:118:059:007:000:000:000:000:000:000:000:000:000	double locus
LBERLW	012:039:057:058:050:023:010:000:000:000:000:000:000	A10: 010:046:065:034:009:000:000:000:000:000:000:000:000	011:072:096:054:014:000:000:000:000:000:000:000:000	double locus

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Table 126: Copy number measurement of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM595-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that were measured per event.

	Copy number assays targeting the T-DNA of VC-LTM595-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target c-d6Elo(Pp_GA)near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity of all plants per event was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.													
Event	c-AHAS	c-d5Des(Tc_GA)	j-i-Atss18_c-o3Des(Pi_GA2)	j-i-Atss2_c-d4Des(Tc_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Pl_GA)2	j-i-Atss2_c-d4Des(Pl_GA)-195R	j-p-LuPXR_i-Atss15	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-i-Atss2_c-d6Des(Ot febit_GA)	c-d5Des(Tc_GA)	c-d6Elo(Pp_GA)
LBDGCA (n=94)	1.5 (T0: 1)	3.2 (T0: 2)	1.6 (T0: 1.4)	(T0: 1)	(T0: 1.2)	1.7 (T0: 0.9)	1.6	1.6 (T0: 1)	(T0: 1.2)	1.7 (T0: 1)	(T0: 1.1)	(T0: 1.1)	3.2 (T0: 2)	1.6 (T0: 1)
LBDGBP (n=20)	(T0: 1.1)	(T0: 2.1)	(T0: 1.4)	(T0: 1.2)	(T0: 1.3)	(T0: 1.1)		(T0: 1.1)	(T0: 1.3)	(T0: 1)	(T0: 1)	(T0: 1.2)	(T0: 2.1)	(T0: 1)
LBDIHN (n=114)	1.6 (T0: 1.2)	3.3 (T0: 2.2)	1.6 (T0: 1.7)	(T0: 1.1)	(T0: 1.2)	1.7 (T0: 1)	1.6	1.6 (T0: 1.1)		1.7 (T0: 1)	(T0: 0.9)	(T0: 1.2)	3.3 (T0: 2.2)	1.6 (T0: 1.1)

Table 127: Fatty acid profiles of T2 seeds harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM595-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that were measured per event.

event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LBEDTZ (n=34)	4.9 ± 0.2	0.2 ± 0	0 ± 0	2.4 ± 0.2	28 ± 1.7	31.1 ± 2.2	1.3 ± 0.2	4.9 ± 0.5	4.9 ± 2	0.8 ± 0.3	0.8 ± 0.1	0.7 ± 0.1	0.1 ± 0.1	0.1 ± 0	3.9 ± 0.8	1.5 ± 0.5	2.4 ± 0.4	4.7 ± 1.6	0.4 ± 0	0 ± 0	0.9 ± 0.1	1.8 ± 0.6	0.8 ± 0.1	1.7 ± 0.6	1.2 ± 0.3	0.2 ± 0.1
LBEDUA (n=4)	7.7 ± 0.2	0.4 ± 0.1	0 ± 0	3.6 ± 0.5	24.3 ± 0.7	32.6 ± 1	0.9 ± 0.1	4.1 ± 0.4	2.4 ± 0.2	0.3 ± 0	1.1 ± 0.1	0.7 ± 0	0.1 ± 0	0.1 ± 0	3.9 ± 0.6	1.4 ± 0.1	2.2 ± 0.2	6.5 ± 0.3	0.5 ± 0	0 ± 0	1.1 ± 0	1.8 ± 0.2	0.7 ± 0.1	2.5 ± 0.1	0.8 ± 0.1	0.1 ± 0
LBEDWU (n=6)	6.4 ± 0.4	0.3 ± 0.1	0 ± 0	5.1 ± 0.9	26.4 ± 3.4	31.5 ± 1.2	0.8 ± 0.2	4.4 ± 0.2	2.1 ± 0.5	0.3 ± 0.1	1.4 ± 0.2	0.7 ± 0.1	0.1 ± 0	0.2 ± 0.1	3.3 ± 0.3	1.4 ± 0.2	1.7 ± 0.2	6.1 ± 1.4	0.6 ± 0.1	0 ± 0	1 ± 0.2	1.8 ± 0.4	0.6 ± 0.2	2.6 ± 0.5	0.9 ± 0.2	0.1 ± 0

Table 128: Fatty acid profiles of T2 seeds harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM595-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where measured per event. For each event, the fatty acid profile of the plant having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Events	16:0	16:1n-7	16:3n-3	18:0	18:1n-9	18:2n-6	18:2n-9	18:3n-3	18:3n-6	18:4n-3	20:0	20:1n-9	20:2n-6	20:3n-3	20:3n-6	20:4n-3	20:4n-6	20:5n-3	22:0	22:1n-9	22:4n-6	22:5n-3	22:5n-6	22:6n-3	unknown peak a	unknown peak b
LBEDTZ (n=1)	4.9	0.2	0.0	2.5	26.4	27.0	1.3	5.6	2.2	0.3	0.8	0.7	0.1	0.1	4.3	2.4	2.0	8.5	0.3	0.0	0.9	3.2	0.9	3.3	1.7	0.3
LBEDUA (n=1)	8.0	0.4	0.0	3.9	23.6	32.3	0.9	4.2	2.1	0.2	1.2	0.7	0.1	0.1	3.9	1.5	2.2	6.7	0.5	0.0	1.1	2.0	0.7	2.7	0.9	0.1
LBEDWU (n=1)	6.1	0.3	0.0	4.9	22.2	32.8	0.8	4.8	1.9	0.3	1.2	0.6	0.1	0.1	3.0	1.4	1.7	7.7	0.5	0.0	1.3	2.5	0.9	3.4	1.2	0.1

Table 129: Phenotypic rating of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM595-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where measured per event. DFF: days to first flower (days), DF: deformed flower (1=deformed, 9=normal), DL: deformed leaf (1=deformed, 9=normal), DP: deformed plant (1=deformed, 9=normal), DS: deformed silique (1=deformed, 9=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoL: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad), Oil: oil content (% of seed weight), Protein: Protein content (% of seed cake without oil)

Events	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC	Oil	Protein
LBEDTZ (n=34)	46.7 ± 3.7	8.9 ± 0.5	7.8 ± 0.6	7.9 ± 0.4	8.8 ± 0.7	3 ± 0	4 ± 0	5 ± 0	9 ± 0	5.2 ± 0.6	102.6 ± 7.1	3.4 ± 1.2	3.7 ± 1.1	36.3	30.5
LBEDUA (n=4)	60.8 ± 2.6	5.3 ± 1.5	8 ± 0	3.8 ± 0.5	5.5 ± 0.6	3 ± 0	5 ± 0	5 ± 0	6.3 ± 0.5	4.5 ± 0.6	83.8 ± 2.5	3.7 ± 0	8 ± 0.8	32.4	30.9
LBEDWU (n=6)	47.2 ± 2.7	6 ± 0	8 ± 0	3.5 ± 0.8	5.8 ± 0.4	3 ± 0	4 ± 0	4.8 ± 0.4	6.7 ± 1.8	4.3 ± 0.5	71.7 ± 12.1	2.5 ± 0.3	6.7 ± 2.1	33.8	30.0

Example 18: Plants containing the T-DNA of plasmid VC-LTM593-1qcz rc for production of EPA and DHA in seeds

All genetic elements required for EPA and DHA synthesis described in this example, were transferred on a single T-DNA using a BiBAC plasmid into the plant genome. To this end, the plasmid VC-LTM593-1qcz rc where cloned into agrobacteria, and plant tissue was incubated according to example 6 with this agrobacterial culture. Due to the selectable herbicide resistance marker, regenerated plants contained the T-DNA of VC-LTM593-1qcz rc . The genetic elements of VC-LTM593-1qcz rc and the function of each element are listed in Table 11. For convenience, all enzymes expressed in seeds of plants carrying both T-DNA of VC-LTM593-1qcz rc that are required for EPA and DHA synthesis are additionally listed Table 130.

Table 130: List of genes essential of EPA and DHA synthesis carried by the T-DNA of plasmid VC-LTM593-1qcz rc .

Genes encoding enzmyes for EPA and DHA synthesis	Length (bp)	Enzymatic function and source of encoded protein
c-d12Des(Ps_GA2)	1197	Delta-12 desaturase from *Phythophthora sojae*
c-d6Des(Ot_febit)	1371	Delta-6 desaturase from *Ostreococcus tauri*
c-d6Elo(Pp_GA2)	873	Delta-6 elongase from *Physcomitrella patens*
c-d6Elo(Tp_GA2)	819	Delta-6 elongase from *Thalassiosira pseudonana*
2 copies of c-d5Des(Tc_GA2)	1320	Delta-5 desaturase from *Thraustochytrium* sp. ATCC21685
c-o3Des(Pi_GA2)	1086	Omega-3-desaturase from *Phythophthora infestans*
2 copies of c-o3Des(Pir_GA)	1092	Omega-3 desaturase from *Pythium irregulare*
c-d5Elo(Ot_GA3)	903	Delta-5 elongase from *Ostreococcus tauri*
c-d4Des(Pl_GA)2	1338	Delta-4 desaturase from *Pavlova lutheri*
c-d4Des(Tc_GA3)	1560	Delta-4 desaturase from

Genes encoding enzmyes for EPA and DHA synthesis	Length (bp)	Enzymatic function and source of encoded protein
		Thraustochytrium sp.

Fatty acid Profile in selected T1 seed batches

Fatty acid profiles, copy number measurements, and phenotypic observations of T0 plants carrying T-DNAs of plasmid VC-LTM593-1qcz rc cultivated in greenhouses during summer

One observation from the data on Table 131 was that there was a higher number of insertion events obtained from VC-LTP593-1qcz than obtained from the BiBAC constructs in examples 16 or 17. The data on Table 132 and Table 133 indicate that with respect to VLC-PUFA accumulation, in particular EPA and DHA, double copy events accumulated more than single copy events and that triple copy events accumulated more than double copy events, with accumulation for the triple copy events being approximately eight percent of total fatty acids (EPA and DHA combined, with 1.6% accumulation of total fatty acids being DHA). The highest amount accumulated was approximately fifteen percent of the total fatty acid content in the seed being EPA and DHA combined, with 3 percent of the total seed fatty acid content being DHA, see Table 133. The aerial phenotype of this construct in the T0 plant and T1 seed showed less variation than that seen in examples 16 or 17.

Table 131: Copy number measurement of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. Considering each event was in this generation represented by only 1 plant, all events have been grouped into the categories indicated in the first column; sc: all T1 plants where the average of all copy number assays listed in this table except the assay c-d5Des(Tc_GA) was 0.51-1.49, dc: all T1 plants where the average of all copy number assays listed in this table was 1.51-2.49, tc: all T1 plants where the average of all copy number assays listed in this table was 2.51-3.49.

Event	Copy number assays targeting the T-DNA of VC-LTM593-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target c-d6Elo(Pp_GA) near the right T-DNA border.													
	c-AHAS	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Pi_GA)2	j-p-LuPXR_i-Atss15	j-p-PvARC5_t-BnSETL	c-d5Des(Tc_GA)	j-i-Atss18_c-o3Des(Pi_GA2)	j-p-BnSET1-v1_c-o3Des(Pir_GA)	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-i-Atss2_c-d6Des(Otfebit_GA)	c-d5Des(Tc_GA)	c-d6Elo(Pp_GA)
sc (n=275)	1.1	1.2	1.1	1.1	1.0	2.1	1.1			1.1	1.1	1.0	2.1	1.1
dc (n=49)	1.7	1.6	1.8	1.7	1.6	3.5	1.9			1.9	2.3	1.8	3.5	1.7
tc (n=11)	2.6		3.0	3.2	2.5	5.3	3.0			2.9	3.8	2.6	5.3	2.8

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Table 132: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM593-1qcz rc. Considering each event was in this generation represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 131. The number of T0 plants/events fullfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=275)	5.1 ± 0.5	0.3 ± 0.1	0.1 ± 0.1	3.2 ± 0.5	41.3 ± 5.3	29.6 ± 2.9	0.4 ± 0.2	6 ± 0.9	0.8 ± 0.4	0.1 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.4 ± 0.2	0.1 ± 0.1	2 ± 0.5	1.2 ± 0.3	1.4 ± 0.5	3.8 ± 1.2	0.3 ± 0.1	0 ± 0	0 ± 0.1	0.9 ± 0.3	0 ± 0	0.9 ± 0.3	0.1 ± 0.1	0.2 ± 0.1
dc (n=49)	5.3 ± 0.6	0.3 ± 0.1	0 ± 0	3.5 ± 0.5	34.4 ± 5.9	31 ± 2.6	0.5 ± 0.2	5.5 ± 1.1	1.5 ± 0.5	0.2 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.3 ± 0.3	0.1 ± 0.1	2.7 ± 0.8	1.5 ± 0.5	2.2 ± 1.4	5.9 ± 2.4	0.3 ± 0	0 ± 0	0.1 ± 0.1	1.3 ± 0.4	0 ± 0	1.2 ± 0.5	0.2 ± 0.2	0.2 ± 0.1
tc (n=11)	5.6 ± 0.6	0.3 ± 0.1	0 ± 0.1	3.7 ± 0.6	28 ± 5.6	31.6 ± 2.1	0.9 ± 0.4	5.1 ± 0.5	2.2 ± 0.8	0.3 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	4.7 ± 1.7	2.8 ± 1.3	1.7 ± 1.2	6.6 ± 3.7	0.3 ± 0.1	0 ± 0	0.2 ± 0.2	1.5 ± 0.7	0 ± 0	1.6 ± 0.9	0.6 ± 0.9	0.3 ± 0.1

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Table 133: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. Considering each event was in this generation represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 131. For each category, the fatty acid profile of the plant/event having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unkn own peak a	unkn own peak b
sc (n=1)	4.7	0.2	0.2	3.2	25.1	36.0	0.6	5.0	2.2	0.3	0.7	0.6	0.4	0.1	2.6	1.3	4.1	8.6	0.2	0.0	0.2	1.8	0.0	1.6	0.0	0.2
dc (n=1)	5.4	0.2	0.0	3.1	23.8	29.4	0.5	4.9	1.8	0.2	0.7	0.7	0.8	0.1	3.5	2.2	4.1	12.3	0.3	0.0	0.2	2.7	0.0	2.4	0.1	0.5
tc (n=1)	5.7	0.2	0.1	3.7	21.5	32.7	0.6	4.8	1.9	0.3	0.9	0.6	0.9	0.4	1.8	1.3	3.0	12.4	0.4	0.0	0.5	2.6	0.1	3.2	0.0	0.4

Table 134: Phenotypic rating of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM593-1qcz rc. Considering each event was in this generation represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 131. The number of T0 plants/events fulfilling these criteria are displayed in parentheses. DFF: days to first flower (days), DF: deformed flower (1=deformed, 9=normal), DL: deformed leaf (1=deformed, 9=normal), DP: deformed plant (1=deformed, 9=normal), DS: deformed silique (1=deformed, 9=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoL: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad), Oil: oil content (% of seed weight), Protein: Protein content (% of seed cake without oil)

Category of T1 plants	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC	Oil	Protein
sc (n=275)	56.5 ± 6.1	9 ± 0	8.6 ± 1.1	9 ± 0	5 ± 4.1	3 ± 0	4.9 ± 0.5	5 ± 0	4.8 ± 2.9	5 ± 1.1	123.9 ± 8.9	4.6 ± 0.6	1.8 ± 1.7	36.5 ± 1.1	29.1 ± 0.8
dc (n=49)	57.4 ± 6	8.9 ± 1	8.4 ± 1.4	8.9 ± 0.5	4.3 ± 2.7	3 ± 0	4.8 ± 0.8	5 ± 0	4.3 ± 2.7	5 ± 1.2	119.1 ± 15.7	4.5 ± 0.6	1.6 ± 1.2	36.2 ± 1.5	28.7 ± 1.2
tc (n=11)	60.3 ± 5.5	9 ± 0	8.5 ± 1	9 ± 0	3.5 ± 2.2	3 ± 0	5 ± 0	5 ± 0	3.5 ± 2.2	4.8 ± 1.5	125.9 ± 7	4.4 ± 0.6	3.5 ± 2.9		

Fatty acid profiles, copy number measurements, and phenotypic observations of T1 plants carrying T-DNAs of plasmids VC-LTM593-1qcz rc cultivated in greenhouses during winter

Specific events were examined further for copy number and displayed a variation in insertion number for the T-DNA from single insertion to partial double insertions along with double insertions. Additionally there were some variations in gene copy number (corresponding to the partial insertions and possible deletions), see Table 135, Table 136 and Table 137. The fatty acid profile data shown on Table 138 and Table 139 indicate an upper range of accumulation of combined EPA and DHA of eighteen percent of the total seed fatty acid content (event LBFDAU). In the event LBFDAU the percent of total seed fatty acid content being EPA is 15% and total seed fatty acid content being DHA is 3% in the T1. LBFDAU was analysed with a copy number indicative of a partial double copy. Another example of specific events having higher levels of EPA and DHA was LBFGKN with approximately 12 percent of the total seed fatty acid content being EPA and DHA, with 10 percent of the total seed fatty acid content being EPA and 2% being DHA. The T1 generation LBFGKN had only a single copy insertion event for VC-LTM593-1qcz rc, though data on Table 140, Table 141 and Table 142 indicate that double copy double locus events tended to accumulate more EPA and DHA combined than other copy and locus numbers with respect to the T2 seed fatty acid profile. This observation likely reflects the nature of insertion site effects and the various factors that affect the generation of elite events. Table 142 indicates that with respect to the aerial phenotype of the plants there was a range of flowering times, as indicated by DFF (days to the first flower) from 36-48. Event LBFDAU did not vary significantly from the majority of other events with a DFF value of 43, thus showing no significant effect on the aerial phenotype or significant impact on total oil or protein accumulation in the seed in the T1 plant and T2 seed.

Table 135: Copy number measurement of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where measured per event. The T1 plants underwent a selection from 250 segregating T1 seedlings, using zygocity analysis as illustrated in Table 137, keeping only plants that are homozygous for the desired number of loci (which are indicated in the last column of Table 137). A copy number of ~2 therefore was indicative for one homozygous copy, a copy number of ~4 indicative for two homozygous copies (located either at on or at two different loci) and so forth. Odd results of 3, 5, 7, 9 etc indicate that at least some of the selected T1 plants carry at least one heterozygous locus. Homozygocity was indicated if the average result of the selected T1 plants was about two fold higher than the the result observed in the T0 generation (indicated in parentheses). For some events this was not the case because during selection of T1 plants, undesired loci have been segregated out while retaining only desired loci in a homozygous state.

Event	Copy number assays targeting the T-DNA of VC-LTM593-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target c-d6Elo(Pp_GA) near the right T-DNA border.													Conclusion from individual assays: number of T-DNA copies inserted into the genome
	c-AHAS	i-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Pl_GA)2	i-p-LuPXR_i-Atss15	i-p-PvARCS_t-BnSETL	c-d5Des(Tc_GA)	i-i-Atss18_c-o3Des(Pl_GA2)	i-p-BnSETL-v1_c-o3Des(Plr_GA)	i-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	i-t-StCAT_p2_p-LuPXR	i-i-Atss2_c-d6Des(Ot_Tebit_GA)	c-d5Des(Tc_GA)	c-d6Elo(Pp_GA)	
LBFDGG (n=50)	2.1 (T0: 1.1)	2	2 (T0: 1.3)	1.8 (T0: 1.1)	(T0: 1)	4.2 (T0: 2.2)	2 (T0: 1.1)	1.9	2	2.1 (T0: 1.1)	2.2 (T0: 1)	2.1 (T0: 1)	4.2 (T0: 2.2)	1.9 (T0: 1)	single copy
LBFGKN (n=50)	2.1 (T0: 1)	2.2	2.1 (T0: 1.3)	2.2 (T0: 1.1)	(T0: 0.8)	4.2 (T0: 2)	2.2 (T0: 1.2)	2.1	2.1	2.1 (T0: 1)	2.2 (T0: 1)	2.1 (T0: 1.1)	4.2 (T0: 2)	1.9 (T0: 1.1)	single copy
LBFIHE (n=34)	2 (T0: 1)	2.2	2.1 (T0: 1.1)	2.1 (T0: 1.2)	(T0: 1)	4.1 (T0: 1.9)	2.2 (T0: 1.1)	2.3	2.3	2.1 (T0: 1)	2.1 (T0: 1.2)	2.3 (T0: 1)	4.1 (T0: 1.9)	2.1 (T0: 1.1)	single copy
LBFLDI (n=60)	2.5 (T0: 1)	2.3	2.4 (T0: 1.1)	2.4 (T0: 1)	(T0: 1)	4.4 (T0: 1.9)	2.2 (T0: 1.2)	2.3	2.4	2.3 (T0: 0.8)	2.3 (T0: 1)	2.3 (T0: 0.9)	4.4 (T0: 1.9)	2.5 (T0: 1)	single copy
LBFPNF (n=52)	1.9 (T0: 1.1)	2.1	2.1 (T0: 1.1)	2.1 (T0: 1.3)	(T0: 1)	5.6 (T0: 2.8)	1.9 (T0: 1)	2.1	2	2 (T0: 1.2)	4 (T0: 1.9)	4.1 (T0: 1.7)	5.6 (T0: 2.8)	4.2 (T0: 1.9)	partial double copy
LBFNSQ (n=51)	2 (T0: 1.1)	2	1.9 (T0: 1.2)	1.9 (T0: 1.1)	(T0: 1.6)	7.1 (T0: 3.1)	3.8 (T0: 1.9)	4	3.9	3.7 (T0: 1.9)	4.1 (T0: 1.9)	3.8 (T0: 1.9)	7.1 (T0: 3.1)	2 (T0: 0.7)	partial double copy
LBFDGL (n=57)	2.1 (T0: 1)	2.2	3.9 (T0: 2)	3.9 (T0: 1.7)	(T0: 1.9)	6.7 (T0: 2.9)	3.8 (T0: 2)	3.8	4.1	3.8 (T0: 1.7)	4 (T0: 1.9)	3.8 (T0: 1.4)	6.7 (T0: 2.9)	1.8 (T0: 0.7)	partial double copy

LBFIEF (n=6)	4.2 (T0: 2.5)	3.7	3.6 (T0: 2.7)	3.7 (T0: 2.7)	(T0: 2)	6.4 (T0: 3.5)	3.8 (T0: 2.3)	4.2	4.1	3.6 (T0: 2.3)	3.8 (T0: 2.6)	3.7 (T0: 1.6)	6.4 (T0: 3.5)	2.1 (T0: 0.8)	partial double copy
LBFBAV (n=50)	2 (T0: 1.1)	2.2 (T0: 1.3)	3.7 (T0: 1.5)	4 (T0: 1.7)	(T0: 1.4)	8.2 (T0: 3.7)	3.8 (T0: 1.8)	3.9	3.9	4 (T0: 1.9)	3.7 (T0: 2)	4.1 (T0: 1.7)	8.2 (T0: 3.7)	6.3 (T0: 2.9)	partial double copy
LBFPNC (n=32)	2.1 (T0: 1.1)	2	2.3 (T0: 1.2)	2 (T0: 1)	(T0: 1.4)	7.5 (T0: 3.4)	4.2 (T0: 1.7)	4	3.8	3.8 (T0: 1.8)	4.1 (T0: 1.8)	4.2 (T0: 1.4)	7.5 (T0: 3.4)	4 (T0: 1.9)	partial double copy
LBFGHQ (n=46)	3.6 (T0: 1.8)	3.8	4.1 (T0: 1.8)	4 (T0: 2.2)	(T0: 1.7)	6.1 (T0: 3.1)	3.8 (T0: 1.7)	3.8	3.7	2.1 (T0: 1.1)	2 (T0: 1.1)	2 (T0: 1.1)	6.1 (T0: 3.1)	2 (T0: 1)	partial double copy
LBFAZB (n=49)	3.9 (T0: 1.8)	4 (T0: 1.7)	3.9 (T0: 1.8)	3.9 (T0: 1.7)	(T0: 1.3)	6.6 (T0: 3.1)	3.7 (T0: 1.8)	4.1	4	3.8 (T0: 1.7)	4 (T0: 1.8)	3.7 (T0: 1.3)	6.6 (T0: 3.1)	1.9 (T0: 0.6)	partial double copy
LBFGKW (n=72)	3.8 (T0: 1.8)	3.8	4.2 (T0: 2.1)	4.1 (T0: 1.9)	(T0: 1.6)	7 (T0: 3.2)	3.9 (T0: 2)	4	4	4 (T0: 1.7)	3.6 (T0: 1.8)	3.7 (T0: 1.5)	7 (T0: 3.2)	1.7 (T0: 0.6)	partial double copy
LBFNRU (n=58)	3.8 (T0: 1.9)	4	3.8 (T0: 1.9)	3.7 (T0: 2)	(T0: 1.7)	7 (T0: 3.2)	4 (T0: 1.7)	3.7	3.9	3.8 (T0: 1.7)	4.2 (T0: 1.9)	3.8 (T0: 1.6)	7 (T0: 3.2)	1.9 (T0: 0.8)	partial double copy
LBFGIZ (n=43)	3.8 (T0: 1.9)	4.1	3.9 (T0: 1.9)	3.8 (T0: 2.1)	(T0: 1.6)	7 (T0: 2.8)	4.2 (T0: 1.8)	3.8	3.9	3.8 (T0: 1.7)	4.3 (T0: 1.5)	3.8 (T0: 1.5)	7 (T0: 2.8)	1.9 (T0: 0.8)	partial double copy
LBFIGM (n=56)	4.1 (T0: 2)	3.9	3.8 (T0: 2)	3.8 (T0: 1.7)	4 (T0: 1.5)	7.6 (T0: 2.8)	3.9 (T0: 1.7)	3.9	3.8	3.8 (T0: 1.8)	4.2 (T0: 2.2)	4.1 (T0: 1.6)	7.6 (T0: 2.8)	1.9 (T0: 0.6)	partial double copy
LBFNRR (n=61)	3.7 (T0: 1.9)	4.2	4 (T0: 2.2)	3.8 (T0: 2)	(T0: 1.7)	7.1 (T0: 2.8)	4.3 (T0: 2)	3.8	4.1	3.9 (T0: 1.7)	4.3 (T0: 1.6)	3.9 (T0: 1.6)	7.1 (T0: 2.8)	1.9 (T0: 0.7)	partial double copy
LBFNTK (n=69)	4.1 (T0: 1.9)	4.1	3.9 (T0: 2.1)	3.9 (T0: 2)	4 (T0: 1.7)	7.6 (T0: 2.9)	4 (T0: 1.8)	3.9	4.1	3.8 (T0: 1.7)	4.3 (T0: 1.9)	4.1 (T0: 1.5)	7.6 (T0: 2.9)	1.9 (T0: 0.7)	partial double copy
LBFGJA (n=42)	3.7 (T0: 1.6)	3.7	3.9 (T0: 2.1)	3.6 (T0: 2.1)	(T0: 1.7)	7.6 (T0: 3.6)	3.4 (T0: 1.7)	3.8	3.7	4.1 (T0: 1.9)	3.9 (T0: 1.9)	3.6 (T0: 1.9)	7.6 (T0: 3.6)	3.9 (T0: 2.1)	double copy
LBFIFV (n=58)	3.9 (T0: 1.7)	4	3.9 (T0: 2.4)	1.9 (T0: 1)	(T0: 1.8)	7.7 (T0: 3.9)	3.9 (T0: 1.8)	3.9	4	3.9 (T0: 2)	4 (T0: 2.6)	4.2 (T0: 1.8)	7.7 (T0: 3.9)	3.9 (T0: 2)	double copy
LBFLER (n=52)	3.8 (T0: 1.9)	3.9	3.9 (T0: 2.3)	3.8 (T0: 1.9)	(T0: 1.7)	7.7 (T0: 3.3)	3.9 (T0: 2.2)	4	4	4.1 (T0: 1.8)	4.2 (T0: 2.3)	4 (T0: 1.6)	7.7 (T0: 3.3)	3.8 (T0: 1.9)	double copy
LBFLDL (n=44)	3.8 (T0: 1.7)	3.9	3.8 (T0: 2)	3.8 (T0: 1.8)	(T0: 1.8)	7.8 (T0: 3.5)	3.9 (T0: 2.1)	3.8	4.1	4.1 (T0: 1.8)	4.1 (T0: 2.1)	3.9 (T0: 1.6)	7.8 (T0: 3.5)	3.8 (T0: 1.9)	double copy
LBFNQW (n=51)	3.8 (T0: 1.8)	3.9	3.9 (T0: 1.9)	3.9 (T0: 1.9)	(T0: 1.6)	7.9 (T0: 3.4)	3.9 (T0: 1.8)	3.9	4	4.2 (T0: 1.6)	4.2 (T0: 2)	3.9 (T0: 1.9)	7.9 (T0: 3.4)	3.9 (T0: 1.8)	double copy
LBFBAP (n=19)	2 (T0: 1.1)	2 (T0: 1.2)	2 (T0: 1)	1.8 (T0: 1.3)	(T0: 1.1)	7.5 (T0: 2.8)	3.8 (T0: 1.8)	3.9	3.8	5.7 (T0: 2.3)	5.5 (T0: 2.3)	5.7 (T0: 1.8)	7.5 (T0: 2.8)	4 (T0: 2.4)	partial triple copy
LBFDAU (n=10)	2 (T0: 1.1)	2	3.8 (T0: 1.9)	3.8 (T0: 1.8)	(T0: 1.5)	7.3 (T0: 3.7)	3.8 (T0: 2)	3.9	3.9	3.7 (T0: 1.8)	3.9 (T0: 2.4)	4.1 (T0: 1.7)	7.3 (T0: 3.7)	3.7 (T0: 1.9)	partial double copy

LBFPRA (n=16)	2.1 (T0: 2)	2	6 (T0: 3)	1.8 (T0: 1.8)	(T0: 2)	7.4 (T0: 4.7)	3.8 (T0: 2.8)	4.1	3.7	3.7 (T0: 2.4)	3.9 (T0: 3.1)	3.7 (T0: 2.3)	7.4 (T0: 4.7)	3.8 (T0: 2.8)	partial double copy
LBFIFU (n=11)	2.2 (T0: 2)	2.5	2.4 (T0: 2.2)	2.4 (T0: 2.1)	(T0: 1.7)	6.5 (T0: 4.2)	2.6 (T0: 1.9)	3.2	4.9	4.3 (T0: 2.3)	4.2	4 (T0: 2.6)	6.5 (T0: 4.2)	3.8 (T0: 3)	partial double copy
LBFDKD (n=2)	3.6 (T0: 1.6)	3.7	3.6 (T0: 2.1)	3.9 (T0: 2.2)	(T0: 1.8)	7.6 (T0: 3.4)	2.9 (T0: 2.1)	3.5	3.6	3.8 (T0: 1.9)	4.3 (T0: 2)	4.3 (T0: 1.8)	7.6 (T0: 3.4)	4 (T0: 1.6)	double copy
LBFDJG (n=12)	3.6 (T0: 1.6)	3.7	4 (T0: 1.8)	3.7 (T0: 1.6)	(T0: 1.9)	8 (T0: 3.6)	3.6 (T0: 2)	4	3.9	4.1 (T0: 1.9)	4.1 (T0: 2.2)	3.7 (T0: 1.9)	8 (T0: 3.6)	4.5 (T0: 1.9)	double copy
LBFLFK (n=15)	3.7 (T0: 2)	3.9	3.7 (T0: 2.4)	3.6 (T0: 2.3)	(T0: 2)	7.5 (T0: 5.2)	4.1 (T0: 2.2)	3.7	3.8	3.8 (T0: 1.3)	4.1 (T0: 2.4)	3.9 (T0: 1.8)	7.5 (T0: 5.2)	4 (T0: 2)	double copy
LBFLCG (n=15)	3.8 (T0: 1.9)	3.9	3.7 (T0: 2.3)	3.7 (T0: 1.9)	(T0: 1.7)	9 (T0: 5.1)	3.8 (T0: 1.9)	3.9	3.9	3.7 (T0: 2)	3.9 (T0: 2.5)	5.6 (T0: 2.2)	9 (T0: 5.1)	3.9 (T0: 2)	double copy
LBFPQM (n=12)	4 (T0: 2)	3.8	4.1 (T0: 2)	4.1 (T0: 2)	(T0: 1.5)	7.6 (T0: 3.7)	3.9 (T0: 2)	4	3.8	3.8 (T0: 1.8)	4 (T0: 2)	4.2 (T0: 1.5)	7.6 (T0: 3.7)	4.1 (T0: 1.8)	double copy
LBFDHG (n=5)	4.2 (T0: 1.9)	4	3.8 (T0: 2.3)	3.8 (T0: 1.8)	(T0: 1.6)	7.7 (T0: 3.3)	3.9 (T0: 1.9)	3.7	3.6	3.8 (T0: 2)	3.9 (T0: 2.3)	3.9 (T0: 1.7)	7.7 (T0: 3.3)	3.9 (T0: 1.9)	double copy
LBFCYO (n=0)	(T0: 3)		(T0: 2.2)	(T0: 2)	(T0: 2)	(T0: 3)	(T0: 3.4)			(T0: 2)	(T0: 2.1)	(T0: 1.8)	(T0: 3)	(T0: 0.6)	partial double copy
LBFBAJ (n=0)	(T0: 1)	(T0: 1.1)	(T0: 1.1)	(T0: 1)	(T0: 1.7)	(T0: 4.8)	(T0: 2)			(T0: 2)	(T0: 2.5)	(T0: 1.8)	(T0: 4.8)	(T0: 2)	partial double copy
LBFDJI (n=0)	(T0: 1.1)		(T0: 1.2)	(T0: 1)	(T0: 1)	(T0: 3)	(T0: 2)			(T0: 1.5)	(T0: 2.5)	(T0: 2.1)	(T0: 3)	(T0: 1.7)	partial double copy
LBFGGO (n=0)	(T0: 1)		(T0: 1)	(T0: 1.1)	(T0: 1)	(T0: 2.6)	(T0: 1)			(T0: 2.1)	(T0: 2.1)	(T0: 2)	(T0: 2.6)	(T0: 1.9)	partial double copy
LBFLFP (n=0)	(T0: 0.9)		(T0: 1.2)	(T0: 1.2)	(T0: 1.8)	(T0: 3.5)	(T0: 2)			(T0: 1.8)	(T0: 2.1)	(T0: 1.7)	(T0: 3.5)	(T0: 1.7)	partial double copy
LBFNQV (n=0)	(T0: 1.1)		(T0: 1.2)	(T0: 1)	(T0: 1)	(T0: 2.7)	(T0: 1.8)			(T0: 1.7)	(T0: 2.3)	(T0: 1.7)	(T0: 2.7)	(T0: 2)	partial double copy
LBFNLT (n=0)	(T0: 1.1)		(T0: 1.2)	(T0: 1)	(T0: 1.8)	(T0: 3.3)	(T0: 1.8)			(T0: 1.8)	(T0: 2.2)	(T0: 1.8)	(T0: 3.3)	(T0: 1.9)	partial double copy
LBFLGC (n=0)	(T0: 1)		(T0: 1.1)	(T0: 1.1)	(T0: 1)	(T0: 2.5)	(T0: 1.1)			(T0: 3)	(T0: 2.6)	(T0: 2.2)	(T0: 2.5)	(T0: 1.8)	partial double copy
LBFLCW (n=0)	(T0: 0.9)		(T0: 1.2)	(T0: 1)	(T0: 0.8)	(T0: 3.6)	(T0: 1.7)			(T0: 1.5)	(T0: 2)	(T0: 1.6)	(T0: 3.6)	(T0: 1.5)	partial double copy
LBFZPJ (n=0)	(T0: 2)		(T0: 1.8)	(T0: 1.9)	(T0: 1)	(T0: 3.5)	(T0: 1)			(T0: 2.3)	(T0: 3)	(T0: 2.4)	(T0: 3.5)	(T0: 2.4)	partial double copy

LBFN5S (n=0)	(TO: 1.1)		(TO: 1)	(TO: 1.2)	(TO: 1.1)	(TO: 2.5)	(TO: 1.8)			(TO: 2.3)	(TO: 2.5)	(TO: 2)	(TO: 2.5)	(TO: 2)	partial double copy
LBGAOR (n=0)	(TO: 1.1)		(TO: 1.8)	(TO: 2)	(TO: 1.7)	(TO: 2.9)	(TO: 1.8)			(TO: 1.8)	(TO: 1.1)	(TO: 1)	(TO: 2.9)	(TO: 1.1)	partial double copy
LBFZOE (n=0)	(TO: 1)		(TO: 1)	(TO: 1.1)	(TO: 1.1)	(TO: 2.8)	(TO: 1)			(TO: 1.1)	(TO: 2.1)	(TO: 1.7)	(TO: 2.8)	(TO: 1.8)	partial double copy
LBFDJS (n=0)	(TO: 1)		(TO: 1.2)	(TO: 1)	(TO: 1)	(TO: 2.9)	(TO: 1.2)			(TO: 0.9)	(TO: 2.2)	(TO: 1.9)	(TO: 2.9)	(TO: 1.9)	partial double copy
LBFDKC (n=0)	(TO: 1)		(TO: 1)	(TO: 1.2)	(TO: 1.8)	(TO: 2.5)	(TO: 2.2)			(TO: 1.7)	(TO: 1.6)	(TO: 1.4)	(TO: 2.5)	(TO: 0.6)	partial double copy
LBFDKA (n=5)	4.2 (TO: 1.6)	3.8	5.4 (TO: 2.6)	5.2 (TO: 3.1)	(TO: 2.4)	10.1 (TO: 5.6)	4.8 (TO: 3.1)	6	5.7	5.7 (TO: 3.3)	5.7 (TO: 3.9)	5.9 (TO: 2.9)	10.1 (TO: 5.6)	5.7 (TO: 3.5)	partial triple copy
LBFLGH (n=0)	(TO: 0.9)		(TO: 1)	(TO: 1.1)	(TO: 1.1)	(TO: 2.9)	(TO: 2.3)			(TO: 2.5)	(TO: 3)	(TO: 2.1)	(TO: 2.9)	(TO: 2.5)	partial triple copy
LBFNUS (n=0)	(TO: 1.1)		(TO: 1)	(TO: 0.9)	(TO: 1.4)	(TO: 3.9)	(TO: 2.2)			(TO: 2.3)	(TO: 3.1)	(TO: 2.3)	(TO: 3.9)	(TO: 3.3)	partial triple copy
LBFLCH (n=0)	(TO: 1.8)		(TO: 2.1)	(TO: 2.1)	(TO: 2.3)	(TO: 5)	(TO: 3.1)			(TO: 2.3)	(TO: 5.3)	(TO: 3.3)	(TO: 5)	(TO: 3.4)	partial triple copy
LBFZMI (n=0)	(TO: 3.3)		(TO: 2.1)	(TO: 2)	(TO: 1.5)	(TO: 4.6)	(TO: 2.2)			(TO: 1.7)	(TO: 1.9)	(TO: 2.9)	(TO: 4.6)	(TO: 2.6)	partial triple copy
LBFIDT (n=7)	3.8 (TO: 1.5)	4	4.3 (TO: 2.2)	4.2 (TO: 1.9)	(TO: 1.5)	8.5 (TO: 4.5)	4 (TO: 2.5)	4.3	4.3	4.3 (TO: 2.5)	3.8 (TO: 3.8)	4.2 (TO: 2.4)	8.5 (TO: 4.5)	3.9 (TO: 2.4)	partial triple copy
LBFAZW (n=0)	(TO: 1.8)	(TO: 2)	(TO: 2.1)	(TO: 1.8)	(TO: 1.4)	(TO: 4.5)	(TO: 1.7)			(TO: 2.5)	(TO: 3.3)	(TO: 2.4)	(TO: 4.5)	(TO: 2.5)	partial triple copy
LBFBBI (n=0)	(TO: 2)	(TO: 2)	(TO: 1.9)	(TO: 1.5)	(TO: 1.6)	(TO: 4.2)	(TO: 1.9)			(TO: 1.9)	(TO: 2.7)	(TO: 1.9)	(TO: 4.2)	(TO: 1.7)	partial triple copy
LBFAZW (n=0)	(TO: 1.8)	(TO: 2)	(TO: 2.1)	(TO: 1.8)	(TO: 1.4)	(TO: 4.5)	(TO: 1.7)			(TO: 2.5)	(TO: 3.3)	(TO: 2.4)	(TO: 4.5)	(TO: 2.5)	partial triple copy

Table 136: Expected Mendelian segregation of the genotype in T1 seeds for some possible T-DNA insertion scenarios. Listed are the expected copy number segregation ratios for T1 seeds segregating for one or more unlinked genomic loci, which contain one or more linked copies of T-DNA insertions. sc: single copy, dc: double copy

	Ratio of copy numbers (cn) expected T1 seed segregating for given locus configuration												
Locus configuration	cn=0	cn=1	cn=2	cn=3	cn=4	cn=5	cn=6	cn=7	cn=8	cn=9	cn=10	cn=11	cn=12
1 sc locus	1	2	1										
1 dc locus	1		2		1								
2 sc loci	1	4	6	4	1								
2 sc loci, 1 dc locus	1	2	3	4	3	2	1						
3 sc loci, 1 dc locus	1	4	8	12	14	12	8	4	1				

Table 137: Observed Medelian segregation of the genotype of T1 seeds of events from construct VC-LTM593-1qcz rc. The segregation has been analysed at three positions of the T-DNA. For each position, the number of seedlings have been counted that have a copy number (aritmetically rounded) of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12. The of seedlings counted for each copy number category are separated by colon, displaying the categories in the following order: 0 : 1 : 2 : 3 : 4 : 5 : 6 : 7 : 8 : 9 : 10 : 11 : 12. Listed are the observed copy number segregation ratios for T1 seeds segregating for one or more unlinked genomic loci, which contain one or more linked copies of T-DNA insertions. The observed frequencies for each assay have been compared against expected frequencies for various locus configurations listed in Table 136 using Chi-Square analysis. The last column displays the total number of loci that are segregating in the genome of a given event. Many events contain truncated insertions, as was evident when some assays indicate single copy insertion at e.g. the left border (e.g. event LBFDAU, LPFPNC), while other positions on the T-DNA clearly indicate a double copy insertion that was either inserted in one locus (e.g. event LBFPNC), or in two loci (e.g. event LBFDAU)

Event	Copy number ratios measured near the left border of the T-DNA using assay A1	Copy number ratios measured using T-DNA internal assays targeting reagions that had copy number results indicating truncated T-DNA insertions using assay A06, or A08, or A09, or A10	Copy number ratios measured near the right border of the T-DNA using assay A12	Most likely number of loci containing one or more T-DNA

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				copies, tested using Chi-Square test
LBFDGG	073 : 122 : 053 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A07 : 076 : 119 : 053 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	073 : 123 : 052 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFGKN	057 : 131 : 059 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A07 : 057 : 130 : 059 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	057 : 130 : 060 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFIHE	076 : 125 : 042 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 075 : 123 : 035 : 009 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	076 : 122 : 036 : 010 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFLDI	068 : 101 : 059 : 018 : 003 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 075 : 094 : 062 : 015 : 002 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	068 : 106 : 053 : 018 : 002 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFPNF	065 : 128 : 055 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 066 : 000 : 127 : 001 : 053 : 002 : 000 : 000 : 000 : 000 : 000 : 000 : 000	064 : 001 : 127 : 001 : 051 : 002 : 002 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFNSQ	063 : 109 : 066 : 005 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 063 : 000 : 110 : 011 : 049 : 010 : 000 : 000 : 000 : 000 : 000 : 000 : 000	063 : 114 : 061 : 005 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFDGL	072 : 113 : 062 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 072 : 000 : 108 : 007 : 061 : 000 : 001 : 000 : 000 : 000 : 000 : 000 : 000	073 : 113 : 061 : 002 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFIEF	042 : 000 : 018 : 020 : 013 : 002 : 004 : 000 : 001 : 000 : 000 : 000 : 000	A12 : 042 : 000 : 025 : 015 : 012 : 005 : 000 : 001 : 000 : 000 : 000 : 000 : 000	041 : 032 : 020 : 005 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFBAV	068 : 128 : 053 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A04 : 068 : 000 : 128 : 000 : 053 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	068 : 000 : 000 : 122 : 006 : 000 : 044 : 009 : 000 : 000 : 000 : 000 : 000	one locus
LBFPNC	043 : 074 : 035 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 043 : 002 : 072 : 001 : 034 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	043 : 000 : 074 : 001 : 034 : 000 : 001 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFGHQ	067 : 000 : 113 : 007 : 051 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 065 : 110 : 057 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	066 : 104 : 059 : 003 : 003 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFAZB	078 : 001 : 158 : 002 : 055 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 078 : 001 : 151 : 008 : 055 : 002 : 001 : 000 : 000 : 000 : 000 : 000 : 000	078 : 161 : 054 : 003 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFGKW	047 : 000 : 124 : 007 : 070 : 000 : 001 : 000 : 000 : 000 : 000 : 000 : 000	A13 : 047 : 000 : 124 : 012 : 065 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	047 : 129 : 073 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus

LBFNRU	070 : 000 : 120 : 003 : 056 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A11 : 069 : 000 : 121 : 001 : 056 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	070 : 121 : 058 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFGIZ	073 : 000 : 108 : 001 : 045 : 004 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A11 : 073 : 000 : 108 : 002 : 044 : 004 : 000 : 000 : 000 : 000 : 000 : 000 : 000	072 : 109 : 048 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFIGM	055 : 000 : 133 : 004 : 056 : 000 : 001 : 000 : 000 : 000 : 000 : 000 : 000	A04 : 054 : 000 : 133 : 004 : 056 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	055 : 137 : 057 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFNRR	070 : 000 : 116 : 004 : 057 : 000 : 000 : 000 : 001 : 000 : 000 : 000 : 000	A11 : 070 : 000 : 115 : 001 : 062 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	070 : 127 : 061 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFNTK	054 : 003 : 119 : 005 : 063 : 004 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A04 : 053 : 003 : 115 : 003 : 070 : 002 : 000 : 000 : 000 : 000 : 000 : 000 : 000	053 : 122 : 072 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFGJA	088 : 000 : 111 : 006 : 042 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 088 : 002 : 111 : 003 : 041 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	089 : 001 : 113 : 003 : 039 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFIFV	070 : 000 : 119 : 000 : 056 : 002 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A04 : 070 : 119 : 057 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	070 : 000 : 119 : 000 : 056 : 001 : 001 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFLER	073 : 000 : 123 : 002 : 051 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A13 : 074 : 000 : 121 : 002 : 050 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	073 : 000 : 123 : 002 : 051 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFLDL	069 : 000 : 129 : 008 : 043 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A13 : 069 : 000 : 130 : 002 : 045 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	068 : 000 : 130 : 006 : 041 : 001 : 001 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFNQW	070 : 001 : 123 : 007 : 045 : 002 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A13 : 070 : 000 : 123 : 001 : 053 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	070 : 001 : 122 : 005 : 047 : 003 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFBAP	059 : 123 : 064 : 001 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A07 : 059 : 001 : 123 : 015 : 049 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	009 : 037 : 068 : 057 : 051 : 022 : 002 : 000 : 000 : 000 : 000 : 000 : 000	double locus, isolation of one locus
LBFDAU	059 : 123 : 062 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 013 : 069 : 090 : 062 : 010 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	013 : 072 : 087 : 062 : 010 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFPRA	024 : 075 : 087 : 057 : 005 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 024 : 041 : 060 : 058 : 052 : 012 : 001 : 000 : 000 : 000 : 000 : 000 : 000	024 : 041 : 060 : 051 : 049 : 022 : 001 : 000 : 000 : 000 : 000 : 000 : 000	double locus, isolation of one locus
LBFIFU	019 : 075 : 105 : 034 : 013 : 000 : 000 : 000 : 000 : 000 : 000 : 000 :	A13 : 019 : 040 : 060 : 067 : 044 : 013 : 003 : 000 : 000 : 000 : 000 :	019 : 040 : 060 : 072 : 039 : 013 : 003 : 000 : 000 : 000 : 000 : 000 :	double locus,

	000		000 : 000	000	isolation of one locus
LBFDKD	007 : 031 : 045 : 018 : 002 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000		A07 : 007 : 031 : 046 : 017 : 002 : 000 : 000 : 000 : 000 : 000 : 000 : 000	007 : 031 : 044 : 017 : 003 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFDJG	026 : 078 : 088 : 036 : C14 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000		A12 : 029 : 072 : 085 : 035 : 017 : 001 : 000 : 000 : 000 : 000 : 000 : 000	026 : 076 : 077 : 037 : 022 : 004 : 001 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFLFK	018 : 062 : 096 : 058 : C15 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000		A11 : 018 : 061 : 096 : 059 : 015 : 000 : 000 : 000 : 000 : 000 : 000 : 000	018 : 061 : 098 : 057 : 015 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFLCG	015 : 059 : 087 : 068 : C15 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000		A12 : 016 : 058 : 087 : 067 : 016 : 000 : 000 : 000 : 000 : 000 : 000 : 000	016 : 056 : 090 : 065 : 015 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFPQM	008 : 058 : 094 : 060 : C14 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000		A12 : 008 : 058 : 094 : 062 : 012 : 000 : 000 : 000 : 000 : 000 : 000 : 000	008 : 058 : 095 : 06[illegible] : 012 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFDHG	022 : 064 : 084 : 037 : C04 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000		A07 : 023 : 062 : 090 : 033 : 005 : 000 : 000 : 000 : 000 : 000 : 000 : 000	023 : 063 : 089 : 034 : 005 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFCYO	091 : 001 : 002 : 013 : 090 : 001 : 006 : 031 : 001 : 000 : 000 : 000 : 000		A14 : 059 : 126 : 051 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	059 : 126 : 051 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFBAJ	056 : 140 : 048 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000		A12 : 017 : 056 : 093 : 064 : 014 : 000 : 000 : 000 : 000 : 000 : 000 : 000	016 : 056 : 091 : 032 : 043 : 005 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFDJI	151 : 285 : 171 : 003 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000		A12 : 35[illegible] : 073 : 118 : 157 : 149 : 036 : 023 : 002 : 00[illegible] : 000 : 000 : 000	049 : 152 : 228 : 129 : 041 : 003 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFGGO	017 : 034 : 030 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000		A12 : 009 : 01[illegible] : 030 : 023 : 008 : 000 : 000 : 000 : 000 : 000 : 000 : 000	009 : 011 : 031 : 023 : 007 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFLFP	047 : 094 : 053 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000		A04 : 046 : 092 : 056 : 002 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	010 : 038 : 069 : 049 : 027 : 001 : 001 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFNQV	174 : 289 : 138 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000		A04 : 174 : 286 : 138 : 002 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	049 : 163 : 210 : 126 : 048 : 003 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFNLT	149 : 279 : 145 : 010 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000		A07 : 015 : 067 : 130 : 063 : 033 : 001 : 000 : 000 : 000 : 000 : 000 : 000	029 : 146 : 214 : 136 : 055 : 007 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFLGC	064 : 12[illegible] : 057 : 005 : 002 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000		A12 : 013 : 034 : 059 : 060 : 049 : 026 : 006 : 000 : 002 : 000 : 000 : 000	313 : 066 : 112 : 048 : 008 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFLCW	207 : 283 : 098 : 003 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000		A12 : 065 : 152 : 224 : 131 : 018 : 000 : 000 : 000 : 000 : 000 : 000 : 000	364 : 156 : 232 : 110 : 022 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus

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LBFGKW (n=72)	5.1 ± 0.2	0.2 ± 0	0 ± 0	2.5 ± 0.2	30.1 ± 1.3	32.1 ± 1.6	1.1 ± 0.1	6.1 ± 0.4	1.7 ± 0.3	0.2 ± 0.1	0.8 ± 0.1	0.7 ± 0	0.1 ± 0	0.1 ± 0	6 ± 0.7	3.3 ± 0.4	0.5 ± 0.1	4.1 ± 0.8	0.4 ± 0	0 ± 0	0.4 ± 0.1	1.5 ± 0.3	0.1 ± 0	0.8 ± 0.2	1.7 ± 0.3	0.3 ± 0.1
LBFNRU (n=58)	5 ± 0.2	0.2 ± 0	0 ± 0	2.6 ± 0.2	31.8 ± 2.5	29.2 ± 1.9	1 ± 0.1	7.1 ± 0.5	1.2 ± 0.2	0.2 ± 0	0.8 ± 0.1	0.8 ± 0	0.1 ± 0	0.1 ± 0	5.9 ± 0.7	3.6 ± 0.5	0.4 ± 0.1	4.2 ± 0.9	0.4 ± 0	0 ± 0	0.4 ± 0	1.7 ± 0.4	0.1 ± 0	0.9 ± 0.3	1.9 ± 0.4	0.3 ± 0.1
LBFGIZ (n=43)	4.9 ± 0.6	0.2 ± 0.1	0 ± 0	2.6 ± 0.4	30.5 ± 3	30 ± 2.6	1 ± 0.2	6.2 ± 0.8	1.3 ± 0.2	0.2 ± 0	0.8 ± 0.1	0.7 ± 0.1	0.2 ± 0	0.2 ± 0	5.7 ± 0.8	3.5 ± 0.5	0.6 ± 0.1	5.3 ± 1.1	0.4 ± 0	0 ± 0	0.4 ± 0.1	2.1 ± 0.4	0.1 ± 0	1 ± 0.2	1.7 ± 0.4	0.4 ± 0.1
LBFIGM (n=56)	4.8 ± 0.4	0.2 ± 0	0 ± 0	2.3 ± 0.2	31.9 ± 2.7	30.6 ± 2.7	1 ± 0.1	6.3 ± 0.7	1.3 ± 0.2	0.2 ± 0	0.7 ± 0.1	0.8 ± 0.1	0.1 ± 0	0.1 ± 0	5 ± 0.7	2.5 ± 0.3	1 ± 0.2	5.4 ± 1	0.4 ± 0	0 ± 0	0.4 ± 0.1	2 ± 0.5	0.4 ± 2.2	1 ± 0.2	1.2 ± 0.2	0.3 ± 0.1
LBFNRR (n=61)	5 ± 0.2	0.2 ± 0	0 ± 0	2.8 ± 0.4	31.4 ± 1.5	30 ± 2.1	1.1 ± 0.1	7 ± 0.6	1.3 ± 0.2	0.3 ± 0	0.9 ± 0.1	0.8 ± 0.1	0.1 ± 0	0.1 ± 0	5.3 ± 1.2	3.5 ± 0.3	0.4 ± 0.1	4.4 ± 1.1	0.4 ± 0	0 ± 0	0.3 ± 0	1.7 ± 0.4	0 ± 0	0.8 ± 0.2	1.7 ± 0.3	0.3 ± 0.1
LBFNTK (n=69)	5.1 ± 0.4	0.2 ± 0	0 ± 0	2.5 ± 0.2	29.6 ± 1.4	29 ± 1.8	1.3 ± 0.2	6.4 ± 0.6	1.5 ± 0.3	0.1 ± 0.1	0.8 ± 0.1	0.8 ± 0	0.1 ± 0	0.1 ± 0	6.7 ± 0.8	3.8 ± 0.5	0.6 ± 0.2	5 ± 0.9	0.4 ± 0	0 ± 0	0.5 ± 0.1	2 ± 0.4	0.1 ± 0	0.9 ± 0.2	1.8 ± 0.3	0.5 ± 0.1
LBFGJA (n=42)	5.2 ± 0.2	0.2 ± 0	0 ± 0	3.1 ± 0.2	27.7 ± 0.8	34.5 ± 0.8	1.9 ± 0.2	3.3 ± 0.2	5.8 ± 0.9	0.5 ± 0.2	0.8 ± 0	0.7 ± 0.1	0.1 ± 0	0.1 ± 0	4.7 ± 0.6	1.5 ± 0.2	1.3 ± 0.2	3.8 ± 0.6	0.3 ± 0	0 ± 0	0.7 ± 0.1	1.4 ± 0.2	0.1 ± 0	0.8 ± 0.2	1.2 ± 0.2	0.1 ± 0
LBFIFV (n=58)	4.8 ± 0.4	0.1 ± 0	0 ± 0	3.2 ± 0.3	27.6 ± 1.5	37.3 ± 0.9	0.8 ± 0.1	3.7 ± 0.2	1.8 ± 0.3	0.2 ± 0	0.9 ± 0.1	0.7 ± 0.2	0.1 ± 0	0.1 ± 0	6.3 ± 0.6	2.5 ± 0.2	1.3 ± 0.2	4.2 ± 0.7	0.4 ± 0	0 ± 0	0.4 ± 0.1	1.1 ± 0.2	0.1 ± 0	1.1 ± 0.3	1 ± 0.2	0.2 ± 0
LBFLER (n=52)	4.7 ± 0.1	0.1 ± 0	0 ± 0	3.1 ± 0.2	28.7 ± 2	33.9 ± 1.1	1 ± 0.1	4.4 ± 0.3	1.9 ± 0.5	0.3 ± 0	0.9 ± 0	0.7 ± 0	0.1 ± 0	0.1 ± 0	5.9 ± 0.6	2.9 ± 0.3	1.1 ± 0.2	5.3 ± 0.7	0.4 ± 0	0 ± 0	0.5 ± 0.1	1.7 ± 0.2	0 ± 0	1 ± 0.2	1.1 ± 0.2	0.2 ± 0.1
LBFLDL (n=44)	4.8 ± 0.1	0.1 ± 0	0 ± 0	3.4 ± 0.3	28 ± 1	34.6 ± 1	1 ± 0.1	4.2 ± 0.2	2.2 ± 0.5	0.3 ± 0	0.9 ± 0.1	0.7 ± 0	0.1 ± 0	0.1 ± 0	5.2 ± 0.6	2.5 ± 0.4	1.2 ± 0.3	5.5 ± 0.7	0.4 ± 0	0 ± 0	0.6 ± 0.1	1.9 ± 0.2	0.1 ± 0	0.8 ± 0.2	1 ± 0.2	0.2 ± 0
LBFNQW (n=51)	4.8 ± 0.2	0.1 ± 0	0 ± 0	3.2 ± 0.3	27.6 ± 1.5	33.5 ± 1.1	1 ± 0.1	4.2 ± 0.3	2 ± 0.3	0.3 ± 0	1.3 ± 0.1	0.9 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	5.3 ± 0.5	2.7 ± 0.4	1.1 ± 0.2	6.2 ± 1	0.4 ± 0	0 ± 0	0.5 ± 0.1	2 ± 0.3	0.1 ± 0	0.9 ± 0.2	0.8 ± 0.4	0.5 ± 0.1
LBFBAP (n=19)	4.8 ± 0.1	0.1 ± 0	0 ± 0	3 ± 0.2	26 ± 0.9	33.8 ± 1	1.2 ± 0.1	3.4 ± 0.2	2.4 ± 0.3	0.3 ± 0	0.8 ± 0	0.7 ± 0	0.2 ± 0	0.1 ± 0	7.4 ± 0.7	2.9 ± 0.3	2.1 ± 0.3	6.2 ± 0.8	0.3 ± 0	0 ± 0	0.3 ± 0	1.7 ± 0.2	0.1 ± 0	1.1 ± 0.1	0.6 ± 0.1	0.4 ± 0.1
LBFDAU (n=10)	5.2 ± 1	0.2 ± 0	0 ± 0	3.3 ± 0.3	20.5 ± 0.9	31.8 ± 0.5	0.9 ± 0.1	4.1 ± 0.2	2.5 ± 0.2	0.3 ± 0	0.9 ± 0.1	0.6 ± 0.1	0.2 ± 0	0.1 ± 0	4.2 ± 0.2	3.1 ± 0.3	1.6 ± 0.3	13.9 ± 1	0.4 ± 0	0 ± 0	0.2 ± 0	2.3 ± 0.2	0.1 ± 0	2.6 ± 0.4	0.4 ± 0.1	0.3 ± 0
LBFPRA (n=16)	4.9 ± 0.2	0.2 ± 0	0 ± 0	2.6 ± 0.6	25.4 ± 2.2	31 ± 1.3	1.3 ± 0.2	5.4 ± 0.8	2 ± 0.4	0.2 ± 0.1	0.8 ± 0	0.8 ± 0	0.1 ± 0	0.1 ± 0	2.9 ± 0.6	1.6 ± 0.3	3 ± 0.3	12.3 ± 1.4	0.4 ± 0	0 ± 0	0.2 ± 0	2.7 ± 0.2	0 ± 0	1.4 ± 0.2	0.1 ± 0	0.4 ± 0.1
LBFIFU (n=11)	4.7 ± 0.2	0.1 ± 0	0 ± 0	3.8 ± 0.9	25.9 ± 2.6	34.7 ± 1.3	1.1 ± 0.3	3.6 ± 0.6	2.7 ± 0.9	0.3 ± 0.1	0.9 ± 0.1	0.7 ± 0	0.1 ± 0	0.1 ± 0	4.2 ± 0.6	1.7 ± 0.3	2.7 ± 0.5	8 ± 1.8	0.3 ± 0	0 ± 0	0.4 ± 0.1	2 ± 0.3	0.1 ± 0.1	1.3 ± 0.4	0.3 ± 0.2	0.2 ± 0
LBFDKD (n=2)	5.1 ± 0.2	0.2 ± 0	0 ± 0	3 ± 0.9	26.1 ± 3.9	35.6 ± 0.6	0.6 ± 0	4.8 ± 0.7	1.3 ± 0.4	0.2 ± 0.1	0.9 ± 0.2	0.8 ± 0	0.3 ± 0	0.2 ± 0	5.1 ± 1.6	3 ± 1.3	0.9 ± 0.4	5.7 ± 0.7	0.4 ± 0	0 ± 0	0.5 ± 0.1	2.2 ± 0.5	0.1 ± 0	1.1 ± 0.2	1.4 ± 0.7	0.3 ± 0
LBFDJG (n=12)	4.4 ± 0.1	0.1 ± 0	0 ± 0	3.4 ± 0.3	27.3 ± 1.4	33.1 ± 1.9	1.1 ± 0.2	3.6 ± 0.1	2.1 ± 0.4	0.4 ± 0.1	0.9 ± 0.1	0.6 ± 0.2	0.1 ± 0	0 ± 0	6.1 ± 1.1	2.6 ± 0.4	1.6 ± 0.4	6.8 ± 1.4	0.4 ± 0	0 ± 0	0.4 ± 0.1	1.7 ± 0.2	0.1 ± 0	1.3 ± 0.4	1 ± 0.4	0.4 ± 0.1
LBFLFK (n=15)	5.1 ± 0.3	0.2 ± 0	0 ± 0	2.5 ± 0.1	28.7 ± 1.6	29 ± 1.7	1.2 ± 0.2	5.6 ± 0.4	1.5 ± 0.2	0.2 ± 0	0.8 ± 0	0.7 ± 0	0.1 ± 0	0.1 ± 0	3.8 ± 0.3	2.4 ± 0.1	1.3 ± 0.2	9.9 ± 0.8	0.4 ± 0.1	0 ± 0	0.5 ± 0	3 ± 0.3	0.1 ± 0	1.8 ± 0.2	0.6 ± 0.1	0.3 ± 0.1

LBFLCG (n=15)	4.9 ± 0.3	0.1 ± 0	0 ± 0	3.6 ± 0.3	25.8 ± 1.4	34.1 ± 0.8	0.9 ± 0.1	4.4 ± 0.2	1.9 ± 0.2	0.3 ± 0	0.9 ± 0	0.6 ± 0	0.1 ± 0	0.1 ± 0	3.7 ± 0.5	2.2 ± 0.3	1.4 ± 0.2	9 ± 0.7	0.3 ± 0	0 ± 0	0.4 ± 0.1	2.5 ± 0.2	0.1 ± 0	1.6 ± 0.3	0.6 ± 0.2	0.2 ± 0
LBFPQM (n=12)	5.2 ± 0.3	0.1 ± 0	0 ± 0	3.8 ± 0.4	26.3 ± 1.2	31.6 ± 1	1.2 = 0.1	4.3 ± 0.4	1.8 ± 0.2	0.2 ± 0	1.1 ± 0.1	0.9 ± 0.1	0.2 ± 0.1	0.2 ± 0	4.8 ± 0.6	3 ± 0.4	1.1 ± 0.2	8.2 ± 1.1	0.4 ± 0	0 ± 0	0.4 ± 0.1	2.2 ± 0.2	0.1 ± 0	1.5 ± 0.2	0.8 ± 0.1	0.5 ± 0.2
LBFDHG (n=5)	5.4 ± 0.1	0.2 ± 0	0 ± 0	2.3 ± 0.2	24 ± 0.6	34 ± 0.7	1 ± 0.1	4.1 ± 0.2	2.7 ± 0.1	0.3 ± 0	0.7 ± 0	0.7 ± 0	0.1 ± 0	0.1 ± 0	3 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	10.4 ± 0.8	0.4 ± 0	0 ± 0	0.6 ± 0.1	2.4 ± 0.1	0.3 ± 0	2.7 ± 0.2	0.5 ± 0	0.2 ± 0
LBFDKA (n=5)	5.1 ± 0.3	0.1 ± 0	0 ± 0	3.7 ± 0.4	24.2 ± 2.8	29.7 ± 1.1	1.5 ± 0.3	4.4 ± 0.3	1.8 ± 0.1	0.2 ± 0	1.1 ± 0.1	0.9 ± 0.1	0.2 ± 0.1	0.2 ± 0	7.6 ± 0.6	5.1 ± 0.8	0.6 ± 0.1	6.6 ± 1	0.4 ± 0	0 ± 0	0.3 ± 0.1	2 ± 0.3	0.1 ± 0	1.3 ± 0.5	1.8 ± 0.2	0.9 ± 0
LBFIDT (n=7)	5 ± 0.1	0.2 ± 0	0 ± 0	2.6 ± 0.1	27 ± 1.7	30.8 ± 1.2	1.1 ± 0.2	6.2 ± 0.4	1.8 ± 0.2	0.4 = 0.1	0.8 ± 0	0.7 ± 0	0.1 ± 0	0.1 ± 0	4.5 ± 1.3	3 ± 0.9	1 ± 0.2	8.2 ± 0.6	0.4 ± 0	0 ± 0	0.5 ± 0.1	2.6 ± 0.2	0.1 ± 0	1.7 ± 0.2	1 ± 0.3	0.3 ± 0.1

Table 139: Fatty acid profiles of one T2 seed batch per event harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T2 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1n -7	16:3n -3	18:0	18:1n -9	18:2n -6	18:2n -9	18:3n -3	18:3n -6	18:4n -3	20:0	20:1n -9	20:2n -6	20:3n -3	20:3n -6	20:4n -3	20:4n -6	20:5n -3	22:0	22:1n -9	22:4n -6	22:5n -3	22:5n -6	22:6n -3	unkn own peak a	unkn own peak b
LBFDGG (n=1)	4.6	0.2	0.0	2.6	26.6	34.2	0.8	4.6	2.0	0.3	0.8	0.7	0.1	0.1	2.7	1.6	2.8	9.7	0.4	0.0	0.3	2.2	0.2	1.9	0.2	0.3
LBFGKN (n=1)	4.7	0.2	0.0	3.0	25.0	34.6	0.8	4.3	2.2	0.3	0.8	0.7	0.2	0.1	3.1	1.7	2.5	10.4	0.3	0.0	0.4	2.4	0.1	2.0	0.2	0.2
LBFIHE (n=1)	4.8	0.1	0.0	3.0	23.7	34.1	0.9	3.6	4.0	0.4	1.0	0.9	0.2	0.1	3.1	1.3	3.3	10.3	0.4	0.0	0.3	1.9	0.1	1.8	0.2	0.3
LBFLDI (n=1)	6.4	0.1	0.0	2.1	28.5	28.4	0.9	5.9	1.2	0.2	0.7	0.7	0.3	0.2	3.0	1.9	1.7	10.8	0.3	0.0	0.4	3.3	0.2	1.7	0.4	0.4
LBFPNF (n=1)	5.4	0.2	0.0	2.3	26.9	28.9	1.3	5.1	3.0	0.4	0.8	0.7	0.1	0.0	3.3	1.6	2.8	11.0	0.4	0.0	0.2	3.2	0.1	1.7	0.3	0.2
LBFNSQ (n=1)	4.7	0.1	0.0	2.4	23.6	34.2	1.0	4.7	2.2	0.4	0.7	0.0	0.3	0.1	4.3	2.3	2.3	10.2	0.4	0.0	0.5	2.2	0.1	2.0	0.4	0.7
LBFDGL (n=1)	4.9	0.1	0.0	2.5	23.9	32.2	1.0	6.0	1.7	0.3	0.7	0.7	0.1	0.1	4.6	2.9	1.4	10.1	0.4	0.0	0.3	2.0	0.2	2.2	1.0	0.3
LBFIEF (n=1)	4.7	0.1	0.0	3.0	27.0	37.2	0.6	5.1	1.4	0.2	1.0	0.7	0.2	0.3	4.3	2.6	0.9	5.9	0.4	0.0	0.5	1.4	0.1	1.2	0.7	0.2
LBFBAV (n=1)	5.9	0.2	0.0	2.5	23.9	27.6	1.0	5.7	1.8	0.3	0.9	0.7	0.1	0.3	10.2	7.6	0.3	4.2	0.5	0.0	0.0	2.0	0.0	1.1	2.6	0.4
LBFPNC (n=1)	4.7	0.1	0.0	2.3	19.8	35.1	0.6	3.9	2.7	0.3	0.6	0.7	0.2	0.1	4.3	1.8	4.6	11.4	0.3	0.0	0.6	3.9	0.1	1.1	0.4	0.3
LBFGHQ (n=1)	5.4	0.2	0.0	2.3	28.7	31.5	0.5	9.0	1.4	0.6	0.9	0.8	0.1	0.1	2.6	2.1	0.6	6.5	0.4	0.0	0.3	2.8	0.2	1.6	0.9	0.4
LBFAZB (n=1)	5.1	0.1	0.0	3.1	26.6	30.1	1.6	4.7	2.5	0.4	0.8	0.6	0.0	0.1	5.4	2.9	1.6	8.7	0.4	0.0	0.4	2.2	0.1	1.5	0.9	0.3
LBFGKW (n=1)	4.4	0.2	0.0	2.0	26.5	31.5	1.2	6.8	1.9	0.3	0.6	0.7	0.1	0.1	6.7	4.1	0.5	5.9	0.3	0.0	0.4	2.0	0.2	1.2	2.0	0.3
LBFNRU (n=1)	5.0	0.2	0.0	2.5	27.2	26.6	0.9	8.0	1.4	0.3	0.8	0.7	0.1	0.2	6.0	4.6	0.4	7.0	0.4	0.0	0.4	3.0	0.1	1.8	1.9	0.3
LBFGIZ (n=1)	5.2	0.2	0.0	2.3	27.3	31.2	0.7	6.1	1.5	0.2	0.7	0.4	0.2	0.2	5.3	3.1	1.0	7.4	0.4	0.0	0.5	2.8	0.1	1.4	1.4	0.5

LBFIGM (n=1)	4.9	0.1	0.0	2.5	28.9	29.6	1.6	5.8	1.8	0.3	0.8	0.8	0.1	0.1	5.0	2.5	1.5	7.9	0.4	0.0	0.4	2.2	0.1	1.5	0.9	0.4
LBFNRR (n=1)	4.8	0.1	0.0	2.8	27.3	26.9	1.9	6.5	1.9	0.3	0.9	0.8	0.1	0.1	5.4	3.7	0.8	8.9	0.4	0.0	0.3	2.5	0.1	1.6	1.1	0.5
LBFNTK (n=1)	4.9	0.2	0.0	2.4	29.0	27.5	1.1	6.8	1.4	0.2	0.7	0.7	0.2	0.1	5.9	3.6	1.0	7.3	0.4	0.0	0.6	3.0	0.1	1.1	1.5	0.4
LBFGJA (n=1)	5.2	0.2	0.0	3.0	27.1	33.5	2.1	3.4	5.2	0.6	0.8	0.7	0.1	0.1	4.2	1.6	1.5	5.4	0.3	0.0	0.7	1.6	0.2	1.2	1.0	0.2
LBFIFV (n=1)	4.8	0.1	0.0	3.4	21.6	34.4	0.8	3.5	2.8	0.3	0.9	0.7	0.2	0.1	6.8	2.9	2.2	7.6	0.4	0.0	0.6	1.8	0.3	2.6	1.0	0.2
LBFLER (n=1)	5.1	0.2	0.0	2.8	22.8	32.5	1.1	3.7	4.1	0.5	0.8	0.7	0.1	0.1	6.4	3.2	1.7	7.9	0.3	0.0	0.7	2.1	0.1	1.9	1.1	0.2
LBFLDL (n=1)	4.7	0.1	0.0	3.4	26.9	33.7	1.0	4.4	1.8	0.2	0.9	0.7	0.1	0.1	4.6	2.6	1.2	7.8	0.4	0.0	0.6	2.4	0.1	1.3	0.8	0.3
LBFNQW (n=1)	4.8	0.1	0.0	2.7	24.8	33.0	1.1	4.3	2.0	0.3	1.6	0.9	0.2	0.1	5.1	3.0	1.3	8.6	0.3	0.0	0.5	2.5	0.1	1.4	0.9	0.4
LBFBAP (n=1)	4.9	0.1	0.0	3.2	24.5	31.9	1.4	3.2	2.7	0.3	0.8	0.7	0.2	0.1	7.6	3.1	2.5	7.6	0.3	0.0	0.3	1.9	0.0	1.3	0.7	0.4
LBFDAU (n=1)	4.6	0.2	0.0	3.4	18.7	31.8	0.9	4.1	2.9	0.4	0.8	0.7	0.2	0.1	4.2	3.4	1.5	15.1	0.3	0.0	0.2	2.5	0.1	3.0	0.4	0.3
LBFPRA (n=1)	4.8	0.1	0.0	3.2	21.9	28.7	1.8	4.4	2.7	0.3	0.9	0.8	0.1	0.1	3.6	2.0	3.4	15.6	0.4	0.0	0.2	2.5	0.0	1.8	0.1	0.5
LBFIFU (n=1)	4.8	0.1	0.0	3.2	22.4	34.7	0.9	4.5	2.5	0.3	0.7	0.6	0.2	0.1	4.2	2.3	2.1	9.6	0.2	0.0	0.5	2.5	0.2	2.3	0.6	0.2
LBFDKD (n=1)	5.0	0.2	0.0	2.4	28.9	35.2	0.6	5.3	1.0	0.1	0.7	0.8	0.3	0.2	4.0	2.1	1.2	6.2	0.4	0.0	0.6	2.5	0.1	1.0	0.9	0.3
LBFDJG (n=1)	4.4	0.1	0.0	3.3	25.4	32.3	1.2	3.5	2.3	0.5	0.9	0.7	0.1	0.1	5.5	2.6	2.1	9.0	0.4	0.0	0.4	1.8	0.1	1.9	0.6	0.4
LBFLFK (n=1)	5.2	0.2	0.0	2.5	26.2	29.0	1.2	5.9	1.7	0.3	0.8	0.7	0.1	0.1	3.4	2.4	1.3	11.4	0.4	0.0	0.5	3.4	0.1	2.3	0.6	0.3
LBFLCG (n=1)	5.0	0.2	0.0	4.4	22.6	34.7	0.7	4.1	2.1	0.3	1.1	0.6	0.1	0.1	3.4	2.1	1.6	10.1	0.4	0.0	0.5	2.6	0.1	2.3	0.6	0.2
LBFPQM (n=1)	5.1	0.2	0.0	3.3	25.7	30.4	1.4	4.4	1.9	0.3	1.0	0.9	0.2	0.2	4.3	2.5	1.8	10.2	0.4	0.0	0.4	2.6	0.1	1.8	0.6	0.5
LBFDHG (n=1)	5.4	0.2	0.0	2.1	23.9	33.5	1.1	4.0	2.6	0.3	0.7	0.7	0.1	0.1	3.0	1.8	1.7	11.4	0.4	0.0	0.6	2.4	0.3	2.8	0.5	0.2
LBFDKA (n=1)	5.6	0.2	0.0	3.2	20.9	30.0	1.3	3.9	1.9	0.2	1.0	1.0	0.3	0.2	8.3	5.8	0.7	7.6	0.3	0.0	0.4	2.2	0.1	1.8	2.0	0.9
LBFIDT (n=1)	5.1	0.2	0.0	2.6	26.4	32.0	1.0	5.7	2.0	0.4	0.8	0.7	0.1	0.1	3.5	2.2	1.3	9.1	0.4	0.0	0.6	2.8	0.1	2.0	0.7	0.2

Table 140: Fatty acid profiles of T2 seeds harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column; as defined in Table 131. In addition to those categories, the catergory "dc" was sub-divided into the category dc sl: all T1 plants where the average of all copy number assays listed in Table 135 was 3.51-4.49, and the zygocity analysis listed in Table 137 revelead a single locus insertion of both copies, and into the category dc dl: all T1 plants where the average of all copy number assays listed in Table 135 was 3.51-4.49, and the zygocity analysis listed in Table 137 revelead a double locus insertion of both copies. The number of T1 plants fullfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b

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sc (n=172)	5.1 ± 0.9	0.2 ± 0.1	0 ± 0	2.8 ± 0.5	29.1 ± 3	33.8 ± 2.8	0.8 ± 0.2	4.8 ± 0.9	1.9 ± 0.7	0.3 ± 0.2	0.8 ± 0.1	0.8 ± 0.1	0.2 ± 0.2	0.1 ± 0.1	2.8 ± 0.3	1.5 ± 0.2	2.1 ± 0.4	7.9 ± 1.3	0.4 ± 0	0 ± 0	0.3 ± 0.1	1.9 ± 0.4	0.1 ± 0.1	1.4 ± 0.3	0.3 ± 0.1	0.3 ± 0.1
dc (n=813)	4.9 ± 0.4	0.2 ± 0	0 ± 0	2.9 ± 0.5	29.1 ± 2.6	32 ± 3.1	1.1 ± 0.3	5.3 ± 1.3	1.9 ± 1	0.3 ± 0.1	0.9 ± 0.2	0.7 ± 0.1	0.1 ± 0	0.1 ± 0	5.9 ± 1.7	3.1 ± 1.1	0.9 ± 0.5	5.1 ± 2.1	0.4 ± 0	0 ± 0	0.4 ± 0.1	1.7 ± 0.5	0.1 ± 0.6	1 ± 0.4	1.4 ± 0.7	0.3 ± 0.1
dc sl (n=700)	4.9 ± 0.4	0.2 ± 0	0 ± 0	2.8 ± 0.4	29.4 ± 2.5	31.9 ± 3.2	1.1 ± 0.3	5.4 ± 1.4	1.9 ± 1.1	0.3 ± 0.1	0.8 ± 0.2	0.7 ± 0.1	0.1 ± 0	0.1 ± 0	6 ± 1.7	3.2 ± 1.2	0.9 ± 0.6	4.8 ± 1.7	0.4 ± 0	0 ± 0	0.4 ± 0.2	1.7 ± 0.5	0.1 ± 0.6	0.9 ± 0.3	1.4 ± 0.7	0.3 ± 0.2
dc dl (n=64)	5 ± 0.5	0.2 ± 0	0 ± 0	3.2 ± 0.6	26.3 ± 2.5	32.2 ± 2.4	1.1 ± 0.2	4.5 ± 0.7	1.9 ± 0.4	0.3 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.1 ± 0	0.1 ± 0	4.3 ± 1.1	2.5 ± 0.5	1.4 ± 0.3	9 ± 2	0.4 ± 0	0 ± 0	0.4 ± 0.1	2.4 ± 0.5	0.1 ± 0.1	1.7 ± 0.5	0.7 ± 0.3	0.3 ± 0.1

Table 141: Fatty acid profiles of T2 seeds harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column as defined in the description of Table 140. For each category, the fatty acid profile of the plant having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=1)	6.4	0.1	0.0	3.0	23.7	34.1	0.9	3.6	4.0	0.4	1.0	0.9	0.2	0.1	3.1	1.3	3.3	10.3	0.4	0.0	0.3	1.9	0.1	1.8	0.2	0.3
dc (n=1)	4.6	0.1	0.0	2.3	19.8	35.1	0.6	3.9	2.7	0.3	0.6	0.7	0.2	0.1	4.3	1.8	4.6	11.4	0.3	0.0	0.6	3.9	0.1	1.1	0.4	0.3
dc sl (n=1)	4.8	0.1	0.0	2.3	19.8	35.1	0.6	3.9	2.7	0.3	0.6	0.7	0.2	0.1	4.3	1.8	4.6	11.4	0.3	0.0	0.6	3.9	0.1	1.1	0.4	0.3
dc dl (n=1)	4.6	0.3	0.0	2.7	20.4	31.3	0.8	3.9	2.3	0.3	0.7	0.5	0.2	0.1	3.9	2.5	2.4	13.7	0.3	0.0	0.3	2.6	0.2	2.9	0.4	0.2

Table 142: Phenotypic rating of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where rated per event. DFF: days to first flower (days), DF: deformed flower (1=deformed, 9=normal), DL: deformed leaf (1=deformed, 9=normal), DP: deformed plant (1=deformed, 9=normal), DS: deformed silique (1=deformed, 9=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoL: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad), Oil: oil content (% of seed weight), protein: Protein content (% of seed cake without oil)

Event	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC	Oil	Protein
LBFDGG (n=50)	43.6 ± 2.7	9 ± 0	9 ± 0	9 ± 0	8.1 ± 1.6	3 ± 0	5 ± 0	5 ± 0	8.1 ± 1.6	4.5 ± 0.8	118.8 ± 6.1	4.4 ± 0.3	3.5 ± 0.9	36.1	30.2

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LBFGKN (n=50)	43.3 ± 1.7	9 ± 0	9 ± 0	8.1 ± 0.7	3 ± 0	5 ± 0	5 ± 0	8.1 ± 0.7	5.1 ± 0.8	112.4 ± 4.1	4.4 ± 0.3	3 ± 0.8	36.9	30.1
LBFIHE (n=34)	47.4 ± 5.1	9 ± 0	9 ± 0	6.6 ± 1.6	3 ± 0	5 ± 0	5 ± 0	6.6 ± 1.6	5.6 ± 0.9	105.7 ± 6.3	4.2 ± 0.5	3.8 ± 0.6	36.0	29.4
LBFLDI (n=60)	35.8 ± 2.9	9 ± 0	7.5 ± 0.6	8.2 ± 1.1	3 ± 0	4 ± 0.5	4 ± 0	8.4 ± 1.2	5.4 ± 0.9	94 ± 5.1	3.6 ± 0.8	4.8 ± 1.8	36.9	30.1
LBFPNF (n=52)	35.3 ± 2.2	9 ± 0	7.6 ± 0.6	8.4 ± 0.6	3 ± 0	3.9 ± 0.3	4 ± 0	8.3 ± 1.2	5.3 ± 0.9	88.4 ± 7.9	3.6 ± 0.9	3.5 ± 0.7	39.1	28.3
LBFNSQ (n=51)	45.2 ± 7	9 ± 0	7.7 ± 0.9	6.8 ± 1.2	3 ± 0	5 ± 0.2	4.1 ± 0.5	8.7 ± 0.6	5 ± 1	109.6 ± 10.9	3.4 ± 0.3	4.4 ± 1.8	37.5	28.7
LBFDGL (n=57)	43.9 ± 2.4	9 ± 0	9 ± 0	7.7 ± 0.8	3 ± 0	5 ± 0	5 ± 0	7.7 ± 0.8	5.1 ± 0.8	109.4 ± 6.6	4.1 ± 0.4	2.8 ± 0.7	36.9	29.9
LBFIEF (n=6)	47.2 ± 5	9 ± 0	9 ± 0	4 ± 2	3 ± 0	5 ± 0	5 ± 0	4 ± 2	6.2 ± 0.8	98.3 ± 4.1	3.4 ± 0.3	3.8 ± 1	38.9	29.9
LBFBAV (n=50)	46.6 ± 1.6	9 ± 0	7.4 ± 0.8	8.9 ± 0.3	3 ± 0	5 ± 0	4 ± 0	9 ± 0	3.5 ± 0.5	125.1 ± 5.6	2.7 ± 0.3	3.9 ± 1	39.0	28.0
LBFPNC (n=32)	44.6 ± 4.2	9 ± 0	9 ± 0	8.3 ± 0.7	3 ± 0	5 ± 0	5 ± 0	8.3 ± 0.7	5.5 ± 0.9	105.6 ± 5.6	4.1 ± 0.3	2.8 ± 0.5	39.4	28.3
LBFGHQ (n=46)	44.4 ± 4	9 ± 0	7.3 ± 1.9	8.9 ± 0.3	3 ± 0	5 ± 0	4 ± 0	9 ± 0.1	3.9 ± 0.7	124.5 ± 6.2	2.6 ± 0.3	3.7 ± 0.9	38.3	27.8
LBFAZB (n=49)	47.6 ± 3.2	9 ± 0	9 ± 0	7.9 ± 0.8	3 ± 0	5 ± 0	5 ± 0	7.9 ± 0.8	5.1 ± 0.8	112.3 ± 4.9	3.9 ± 0.4	2.6 ± 0.6	38.2	29.4
LBFGKW (n=72)	46.7 ± 1.9	9 ± 0	7.3 ± 0.5	8.9 ± 0.3	3 ± 0	5 ± 0	4 ± 0	8.9 ± 0.3	4.1 ± 0.6	125.2 ± 6.2	2.6 ± 0.3	3.7 ± 0.8	38.8	27.9
LBFNRU (n=58)	41.4 ± 1.7	9 ± 0	7.6 ± 0.6	8.2 ± 0.7	3 ± 0	5 ± 0	4 ± 0	8.9 ± 0.8	5.6 ± 0.8	117 ± 5	2.6 ± 0.3	3.6 ± 1.3	39.5	27.6
LBFGIZ (n=43)	41.5 ± 2.8	9 ± 0	7.6 ± 0.6	8.3 ± 0.5	3 ± 0	5 ± 0	4 ± 0	8.9 ± 0.3	5.3 ± 0.9	113.5 ± 5.9	2.4 ± 0.2	3.9 ± 0.7	38.4	28.6
LBFIGM (n=56)	36.1 ± 1.3	9 ± 0	6.7 ± 0.8	8.8 ± 0.4	3 ± 0	2.7 ± 0.7	4 ± 0	8.8 ± 0.5	3.9 ± 0.7	107.6 ± 9	2.7 ± 0.3	3.4 ± 0.8	39.1	28.6
LBFNRR (n=61)	41.3 ± 2.2	9 ± 0	7.8 ± 0.6	8 ± 0.6	3 ± 0	5 ± 0	4 ± 0	9 ± 0	5.3 ± 0.6	116.5 ± 6.3	2.9 ± 0.3	3.2 ± 0.9	40.6	27.1
LBFNTK (n=69)	34.5 ± 2.8	8 ± 0	6.6 ± 1.1	8.7 ± 0.5	3 ± 0	2.4 ± 0.6	4 ± 0	8.8 ± 0.7	3.7 ± 0.6	93.4 ± 8.2	2.8 ± 0.3	3.8 ± 0.8	39.8	28.3
LBFGJA (n=42)	45.1 ± 1	9 ± 0	9 ± 0	7.6 ± 0.5	3 ± 0	5 ± 0	5 ± 0	7.6 ± 0.5	4.3 ± 1.1	113.3 ± 2.4	4.9 ± 0.2	2.4 ± 0.8	32.9	25.6
LBFIFV (n=58)	42.4 ± 0.6	9 ± 0	9 ± 0	8.8 ± 0.6	3 ± 0	5 ± 0	5 ± 0	8.8 ± 0.6	4.5 ± 0.8	113.1 ± 2.4	4.2 ± 0.2	2.6 ± 0.6	37.3	29.4
LBFLER (n=52)	45.1 ± 3.5	9 ± 0	9 ± 0	8.5 ± 0.7	3 ± 0	5 ± 0	5 ± 0	8.5 ± 0.7	5.6 ± 1	108.3 ± 6.8	3.9 ± 0.3	2.6 ± 0.7	40.8	27.7
LBFLDL (n=44)	44.7 ± 3.7	9 ± 0	9 ± 0	8.5 ± 0.6	3 ± 0	5 ± 0	5 ± 0	8.5 ± 0.6	5.1 ± 0.8	109.3 ± 5.3	4 ± 0.2	2.6 ± 0.8	38.6	29.2
LBFNQW (n=51)	42.8 ± 1.9	9 ± 0	9 ± 0	8.7 ± 0.5	3 ± 0	5 ± 0	5 ± 0	8.7 ± 0.5	5.7 ± 0.9	105.5 ± 6.3	4.1 ± 0.3	3.1 ± 0.5	38.4	28.9
LBFBAP (n=19)	43.3 ± 1.8	9 ± 0	9 ± 0	8.9 ± 0.2	3 ± 0	5 ± 0	5 ± 0	8.9 ± 0.2	4.6 ± 0.8	117.4 ± 6.1	4 ± 0.5	2.4 ± 0.5	38.7	28.8
LBFDAU (n=10)	43.6 ± 1.7	9 ± 0	9 ± 0	9 ± 0	3 ± 0	5 ± 0	5 ± 0	8.5 ± 1.6	4.4 ± 0.8	110 ± 5.8	4.5 ± 0.5	2.6 ± 0.5	34.5	30.2
LBFPRA (n=16)	47.8 ± 2.5	9 ± 0	7.9 ± 0.8	7.8 ± 0.4	3 ± 0	5.2 ± 0.4	4 ± 0	9 ± 0	5.6 ± 0.8	123.4 ± 4	4.2 ± 0.6	2.3 ± 0.8	38.1	28.2
LBFIFU (n=11)	46.8 ± 6.4	9 ± 0	8.7 ± 0.9	7.3 ± 1.1	3 ± 0	5 ± 0	5 ± 0	7.3 ± 1.1	5.6 ± 1.1	103.6 ± 8.1	3.8 ± 0.5	3.5 ± 0.9	37.4	28.7
LBFDKD (n=2)	43 ± 4.2	9 ± 0	9 ± 0	7.5 ± 0.7	3 ± 0	5 ± 0	5 ± 0	7.5 ± 0.7	5 ± 1.4	107.5 ± 10.6	4.3 ± 0.8	4 ± 1.4	36.6	31.2
LBFDJG (n=12)	45.1 ± 1	9 ± 0	9 ± 0	8.8 ± 0.6	3 ± 0	5 ± 0	5 ± 0	8.8 ± 0.6	4.3 ± 1	104.2 ± 29.8	3.8 ± 0.2	2.8 ± 0.5	37.7	29.8
LBFLFK (n=15)	42.5 ± 5.5	9 ± 0	7.9 ± 1.2	8.1 ± 1.3	3 ± 0	4.9 ± 0.5	4 ± 0	8.9 ± 0.4	5.1 ± 1.2	113.7 ± 9	4.1 ± 1	3.7 ± 0.6	39.3	27.2
LBFLCG (n=15)	42.5 ± 0.6	9 ± 0	9 ± 0	9 ± 0	3 ± 0	5 ± 0	5 ± 0	8.7 ± 1	4.5 ± 0.8	113.7 ± 2.3	4.3 ± 0.5	2.7 ± 0.8	37.7	29.1
LBFPQM (n=12)	42.7 ± 2.5	9 ± 0	9 ± 0	8.7 ± 0.5	3 ± 0	5 ± 0	5 ± 0	8.7 ± 0.5	5.5 ± 1	110 ± 6	4.2 ± 0.5	2.8 ± 0.4	39.2	27.7
LBFDHG (n=5)	44.8 ± 3	9 ± 0	9 ± 0	6.4 ± 0.9	3 ± 0	5 ± 0	5 ± 0	6.4 ± 0.9	4.2 ± 0.8	115 ± 0	4.2 ± 0.2	5 ± 0	33.5	28.0
LBFDKA (n=5)	48.6 ± 1.7	9 ± 0	9 ± 0	9 ± 0	3 ± 0	5 ± 0	5 ± 0	9 ± 0	5.6 ± 0.9	108 ± 4.5	3.8 ± 0.2	2.4 ± 0.5	40.0	26.6

LBFIDT (n=7)	46.3 ± 1.3	9 ± 0	7.7 ± 0.5	9 ± 0	8 ± 0	3 ± 0	5 ± 0	4 ± 0	9 ± 0	4.1 ± 0.7	125.7 ± 6.7	2.5 ± 0.2	3.7 ± 0.5	38.1	28.0

Table 143: Phenotypic rating of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column as defined in the description of Table 140. The number of T1 plants fullfilling these criteria are displayed in parentheses. DFF: days to first flower (days), DF: deformed flower (1=deformed, 9=normal), DL: deformed leaf (1=deformed, 9=normal), DP: deformed plant (1=deformed, 9=normal), DS: deformed silique (1=deformed, 9=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoI: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad), Oil: oil content (% of seed weight), protein: Protein content (% of seed cake without oil)

Category of T1 plants	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC	oil	protein
sc (n=177)	41.5 ± 5.2	9 ± 0	8.6 ± 0.7	9 ± 0.4	7.9 ± 1.5	3 ± 0	4.8 ± 0.5	4.8 ± 0.4	8 ± 1.5	5.1 ± 1	109.1 ± 10.6	3.9 ± 0.6	3.8 ± 1.4	37 ± 1.4	29.5 ± 0.9
dc (n=781)	43.4 ± 4.4	8.9 ± 0.3	8.1 ± 1.1	9 ± 0.3	8.4 ± 0.8	3 ± 0	4.6 ± 0.9	4.5 ± 0.5	8.6 ± 0.8	4.7 ± 1.1	112.2 ± 11	3.3 ± 0.8	3.2 ± 1	38 ± 2.1	28.5 ± 1.2
dc sl (n=677)	43.1 ± 4.4	8.9 ± 0.3	8 ± 1.1	9 ± 0.2	8.5 ± 0.8	3 ± 0	4.6 ± 1	4.4 ± 0.5	8.7 ± 0.8	4.7 ± 1.1	112.3 ± 11	3.3 ± 0.8	3.2 ± 1	38.4 ± 1.8	28.3 ± 1.1
dc dl (n=55)	43.3 ± 3.3	9 ± 0	8.7 ± 0.8	8.9 ± 0.6	8.4 ± 1	3 ± 0	5 ± 0.3	4.8 ± 0.4	8.5 ± 1.1	4.8 ± 1.1	110.8 ± 14.3	3.5 ± 0.7	3.2 ± 0.9	36.6 ± 2.7	28.9 ± 1.7

Fatty acid profiles, copy number measurements, and phenotypic observations of T1 plants carrying T-DNAs of plasmids VC-LTM593-1qcz rc cultivated in field trials in USDA growth zone 11 during winter.

Certain events that had higher levels of EPA and DHA were tested in the field and examined for fatty acid profile, aerial phenotype (if any) and copy number in the T1 generation. A variety of constructs were examined including those with partial double copy insertions, single copy insertions and double copy insertions being represented (see Table 144). Table 145 indicates that LBFDAU had an EPA content of ca. 13% and a DHA content of ca. 3% of the total seed fatty acid content, and a maximum content for DHA of 3.6% and EPA of 17% of total seed fatty acids (Table 146). Measurements of single seeds from LBFDAU had as much as 26% EPA and 4.6% DHA, see Table 147. Overall the field performance of LBFDAU matched or exceeded that of the greenhouse.

Table 144: Copy number measurement of T1 plants cultivated in field, corresponding to USDA growth zone 11, during the winter for field trials of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where measured per event. The T1 plants underwent a selection from ~80 segregating T1 seedlings, using zygocity analysis similar to the selection performed in the greenhouse (which was illustrated in Table 137), keeping only plants that are homozygous for the desired number of loci. A copy number of ~2 therefore was indicative for one homozygous copy, a copy number of ~4 indicative for two homozygous copies (located either at on or at two different loci) and so forth. Odd results of 3, 5, 7, 9 etc indicate that at least some of the selected T1 plants carry at least one heterozygous locus. Homozygocity was indicated if the average result of the selected T1 plants was about two fold higher than the the result oberved in the T0 generation (indicated in parentheses). For some events this was not the case because during selection of T1 plants, undesired loci have been segregated out while retaining only desired loci in a homozygous state.

Event	Copy number assays targeting the T-DNA of VC-LTM593-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target c-d6Elo(Pp_GA) near the right T-DNA border.											Conclusion from individual assays: number of T-DNA copies inserted into the genome
	c-AHAS	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Pl_GA)2	j-p-LuPXR_i-Atss15	j-p-PvARC5 t-BnSETL	c-d5Des(Tc_GA)	j-i-Atss18_c-o3Des(Pi_GA2)	j-p-BnSETL-v1_c-o3Des(Pir_GA)	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-i-Atss2_c-d6Des(Ot febit_GA)	c-d5Des(Tc_GA)	c-d6Elo(Pp_GA)	
LBFDGG (n=5)	2 (T0: 1.1)										1.9 (T0: 1)			1.9 (T0: 1)	single copy
LBFGKN (n=12)	2.1 (T0: 1)										2 (T0: 1)			1.9 (T0: 1.1)	single copy
LBFHJE (n=8)	2.1 (T0: 1)										1.9 (T0: 1.2)			1.9 (T0: 1.1)	single copy
LBFLDJ (n=13)	2 (T0: 1)										1.9 (T0: 1)			1.8 (T0: 1)	single copy
LBFPNF (n=9)	2 (T0: 1.1)										3.7 (T0: 1.9)			3.3 (T0: 1.9)	partial double copy
LBFDAU (n=4)	2 (T0: 1.1)										3.8 (T0: 2.4)			3.6 (T0: 1.9)	partial double copy
LBFPRA (n=5)	3.2 (T0: 2)										4.9 (T0: 3.1)			4 (T0: 2.8)	partial double copy
LBFLFK (n=4)	3.9 (T0: 2)										3.4 (T0: 2.4)			3.4 (T0: 2)	partial double copy
LBFLCG (n=3)	4.3 (T0: 1.9)										2.9 (T0: 2.5)			3.4 (T0: 2)	partial double copy
LBFPQM (n=1)	3.7 (T0: 2)										(T0: 2)			3.9 (T0: 1.8)	partial double copy

Table 145: Fatty acid profiles of T2 seeds harvested from T1 cultivated in the field, corresponding to USDA growth zone 11, during winter of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. The events are indicated in the first column, along with the number of T2 seed batches that were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unkn own peak a	unkn own peak b
LBFDGG (n=5)	4.4 ± 0.2	0.2 ± 0	0.1 ± 0	2.4 ± 0.1	32.3 ± 1.9	34.4 ± 1.6	0.7 ± 0	5.3 ± 0.6	1.5 ± 0.1	0.4 ± 0.1	0.6 ± 0	0.7 ± 0	0.1 ± 0	2 ± 0 ± 0	1.1 ± 0.2	2.7 ± 0.3	6.9 ± 0.7	0.3 ± 0	0 ± 0	0.3 ± 0	1.9 ± 0.2	0.1 ± 0	1.4 ± 0.3	0 ± 0	0.1 ± 0
LBFGKN (n=12)	4.8 ± 0.3	0.2 ± 0	0.1 ± 0	2.6 ± 0.3	27.8 ± 1.9	36 ± 1.3	0.5 ± 0.1	4.8 ± 0.7	1.5 ± 0.2	0.4 ± 0.1	0.7 ± 0.1	0.7 ± 0	0.2 ± 0.1	0.1 ± 0	2.3 ± 0.2	1.1 ± 0.2	3.3 ± 0.4	8 ± 0.8	0.3 ± 0.1	0 ± 0	0.4 ± 0.1	1.9 ± 0.3	0.1 ± 0	1.8 ± 0.4	0.1 ± 0	0.2 ± 0
LBFIHE (n=8)	4.9 ± 0.8	0.3 ± 0.1	0.1 ± 0	2.4 ± 0.4	30.8 ± 3.1	36.1 ± 1.2	0.5 ± 0.1	5.4 ± 1.3	1.5 ± 0.5	0.3 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.2 ± 0	0.1 ± 0	1.9 ± 0.5	0.9 ± 0.1	2.8 ± 0.7	6.4 ± 1.1	0.3 ± 0	0 ± 0	0.5 ± 0.1	1.7 ± 0.3	0.1 ± 0	1.3 ± 0.2	0 ± 0.1	0.1 ± 0
LBFLDI (n=13)	4.5 ± 1.1	0.2 ± 0.1	0.1 ± 0	2.3 ± 0.6	30 ± 5.9	35.4 ± 6.1	0.5 ± 0.1	4.1 ± 0.8	1.3 ± 0.3	0.3 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.2 ± 0.1	0.1 ± 0	1.9 ± 0.5	0.8 ± 0.2	2.9 ± 0.8	6.4 ± 1.6	0.3 ± 0	0 ± 0	0.4 ± 0.1	1.6 ± 0.3	0.1 ± 0.1	1.3 ± 0.4	0.1 ± 0	0.1 ± 0
LBFPNF (n=9)	4.7 ± 0.2	0.2 ± 0	0.1 ± 0	2.4 ± 0.3	29.2 ± 1.8	31.7 ± 1	1.1 ± 0.1	4 ± 0.6	2.6 ± 1.4	0.6 ± 0.1	0.7 ± 0	0.7 ± 0	0.1 ± 0	0 ± 0	3.3 ± 0.4	1.2 ± 0.1	4.2 ± 0.5	8.4 ± 1.1	0.3 ± 0	0 ± 0	0.5 ± 0.1	2.3 ± 0.4	0.1 ± 0	1.5 ± 0.3	0.1 ± 0.1	0.1 ± 0
LBFDAU (n=4)	4.6 ± 0.2	0.2 ± 0	0.1 ± 0	2.3 ± 0.3	24.3 ± 3.7	31.2 ± 2.5	0.8 ± 0.1	5.2 ± 0.6	2.2 ± 0.5	0.4 ± 0.1	0.6 ± 0	0.7 ± 0.1	0.1 ± 0	0.1 ± 0	2.5 ± 0.4	1.7 ± 0.5	2.9 ± 0.4	13.4 ± 3.4	0.3 ± 0	0 ± 0	0.3 ± 0	2.7 ± 0.7	0.1 ± 0	2.7 ± 0.7	0.1 ± 0.1	0.2 ± 0
LBFPRA (n=5)	4.8 ± 0.2	0.2 ± 0	0.1 ± 0	2.5 ± 0.4	25.1 ± 0.9	34.5 ± 2.4	0.9 ± 0.2	4.4 ± 0.7	2.2 ± 0.2	0.4 ± 0.1	0.6 ± 0.1	0.7 ± 0	0.1 ± 0	0.1 ± 0	2.6 ± 0.6	1.3 ± 0.3	3.6 ± 0.6	10.7 ± 1.9	0.3 ± 0	0 ± 0	0.5 ± 0.1	2.3 ± 0.3	0.1 ± 0	1.7 ± 0.2	0.1 ± 0.1	0.2 ± 0.1
LBFLFK (n=4)	5.1 ± 0.8	0.2 ± 0.1	0.1 ± 0	2.1 ± 0.4	27 ± 4.3	32.3 ± 0.5	0.8 ± 0.1	5.4 ± 0.8	1.9 ± 0.5	0.4 ± 0	0.6 ± 0.1	0.7 ± 0	0.2 ± 0	0.1 ± 0	3.1 ± 0.6	1.7 ± 0.3	2.7 ± 0.5	9.5 ± 1.8	0.3 ± 0	0 ± 0	0.5 ± 0.2	2.9 ± 0.4	0.2 ± 0.1	2.1 ± 0.5	0.3 ± 0.1	0.2 ± 0
LBFLCG (n=3)	4.8 ± 0.2	0.2 ± 0	0.1 ± 0	3 ± 0.3	26.4 ± 1.3	33.3 ± 1	0.8 ± 0	4.7 ± 0.7	2 ± 0.2	0.5 ± 0.1	0.7 ± 0	0.7 ± 0	0.1 ± 0	0.1 ± 0	2.3 ± 0.1	1.2 ± 0.1	2.9 ± 0.2	9.9 ± 0	0.3 ± 0	0 ± 0	0.7 ± 0.1	2.6 ± 0.3	0.2 ± 0	2.3 ± 0.5	0.2 ± 0	0.2 ± 0
LBFPQM (n=1)	5.0	0.2	0.1	2.7	26.1	30.8	1.0	4.3	2.3	0.5	0.7	0.6	0.1	0.1	3.4	1.8	2.8	10.8	0.3	0.0	0.5	2.5	0.3	2.5	0.4	0.2

Table 146: Fatty acid profiles of one T2 seed batch per event harvested from T1 plants cultivated in the field, corresponding to USDA growth zone 11, during winter of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T2 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1n-9	18:2n-6	18:2n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4n-3	20:4 n-6	20:5n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LBFDGG (n=1)	4.5	0.21	0.1	2.4	29.86	33.45	0.71	5.74	1.69	0.45	0.62	0.74	0.12	0.05	2.25	1.38	2.83	8.02	0.29	0	0.35	2.21	0.1	1.79	0	0.13
LBFGKN (n=1)	4.5	0.21	0.09	2.28	26.68	34.29	0.65	4.99	1.73	0.42	0.61	0.69	0.23	0.1	2.43	1.22	3.34	9.74	0.31	0	0.48	2.27	0.17	2.31	0	0.23
LBFIHE (n=1)	4.8	0.22	0.09	2.95	26.2	38.09	0.52	3.19	2.12	0.22	0.78	0.69	0.15	0	2.73	1.03	3.67	8.16	0.35	0	0.59	1.72	0.12	1.55	0	0.09
LBFLDI (n=1)	4.8	0.24	0.09	2.51	27.6	35.76	0.54	4.15	1.66	0.26	0.7	0.69	0.29	0.1	2.63	1.16	3.5	8.32	0.31	0	0.47	1.89	0.19	1.85	0.15	0.21
LBFPNF (n=1)	4.9	0.27	0.13	2.11	26.29	31.14	0.91	5.04	1.72	0.68	0.67	0.7	0.11	0.04	2.8	1.34	4.26	10.24	0.36	0	0.54	3.24	0.14	2.09	0.15	0.12
LBFDAU (n=1)	4.8	0.23	0.14	1.94	20.37	28.27	0.98	5.23	2.78	0.5	0.63	0.62	0.13	0.13	3.08	2.3	2.51	17.57	0.36	0	0.29	3.16	0.09	3.62	0	0.25
LBFPRA (n=1)	4.7	0.16	0.14	1.92	25.56	32.27	1.21	4.87	2.09	0.44	0.51	0.66	0.13	0.07	2.34	1.29	3.67	12.46	0.28	0	0.44	2.58	0.08	1.8	0.08	0.26
LBFLFK (n=1)	4.9	0.21	0.12	2.34	22.43	32.9	0.9	4.55	2.5	0.42	0.66	0.62	0.13	0.07	3.86	2.18	2.37	11.06	0.3	0	0.76	3.2	0.24	2.66	0.46	0.21
LBFLCG (n=1)	5.0	0.27	0.12	2.65	24.88	32.2	0.74	5.16	2.3	0.46	0.69	0.65	0.14	0.09	2.24	1.36	2.85	10.87	0.31	0	0.69	2.92	0.22	2.82	0.21	0.15
LBFPQM (n=1)	5.0	0.22	0.11	2.67	26.07	30.83	1.02	4.34	2.31	0.45	0.69	0.63	0.12	0.07	3.4	1.75	2.78	10.78	0.3	0	0.54	2.53	0.27	2.5	0.4	0.23

Table 147: Fatty acid profiles of 95 single seeds of the one seedbatch of event LBFDAU shown in Table 146 having highest EPA+DHA levels .

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1n-9	18:2n-6	18:2n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4n-3	20:4 n-6	20:5n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LBFDAU seed 1	4.2	0.2	0.2	2.1	11.3	26.5	1.2	3.8	4.3	0.6	0.7	0.5	0.1	0.1	3.3	3.0	1.9	26.2	0.4	0.0	0.6	3.7	0.2	4.9	0.0	0.2
LBFDAU seed 2	5.5	0.3	0.1	2.5	14.3	25.0	0.8	4.5	2.5	0.5	0.7	0.5	0.2	0.1	3.0	2.5	2.5	23.6	0.5	0.0	0.5	3.8	0.3	5.8	0.0	0.2
LBFDAU seed 3	5.1	0.3	0.1	2.0	16.0	25.5	1.2	4.4	3.4	0.6	0.7	0.5	0.1	0.1	3.4	2.5	2.5	23.1	0.3	0.0	0.6	3.3	0.1	4.2	0.0	0.2
LBFDAU seed 4	6.7	0.4	0.2	3.8	15.8	23.5	1.5	4.9	3.3	0.9	1.2	0.5	0.0	0.0	1.7	1.3	1.9	21.2	0.7	0.0	0.8	3.5	0.4	5.8	0.0	0.0
LBFDAU seed 5	5.4	0.4	0.2	1.5	16.1	24.4	1.0	5.4	3.4	0.8	0.6	0.5	0.1	0.1	2.9	2.1	3.5	22.0	0.3	0.0	0.5	3.7	0.2	4.8	0.0	0.2
LBFDAU seed 6	5.5	0.3	0.1	1.9	16.6	25.6	0.9	5.3	3.3	0.7	0.7	0.6	0.1	0.1	2.9	2.3	2.4	21.8	0.3	0.0	0.4	3.4	0.2	4.4	0.0	0.2
LBFDAU seed 7	5.0	0.4	0.2	2.4	16.3	25.3	1.1	4.6	3.5	0.6	0.7	0.5	0.1	0.0	3.6	3.1	2.0	21.9	0.5	0.0	0.5	3.5	0.0	4.3	0.0	0.1
LBFDAU seed 8	5.3	0.3	0.1	2.3	17.4	26.0	1.2	4.1	3.7	0.7	0.7	0.5	0.1	0.1	2.6	2.0	2.7	21.7	0.4	0.0	0.4	3.1	0.2	4.0	0.0	0.2
LBFDAU seed 9	5.3	0.2	0.2	2.4	14.3	27.8	1.0	4.0	4.2	0.6	0.7	0.5	0.1	0.1	3.6	2.9	2.3	21.1	0.4	0.0	0.5	2.8	0.3	4.4	0.0	0.1

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LBFDAU seed 10	6.7	0.5	0.2	2.8	15.8	23.3	0.8	5.7	2.9	0.8	0.9	0.5	0.0	0.0	3.1	2.4	2.2	19.8	0.8	0.0	0.6	4.7	0.1	5.4	0.0	0.0
LBFDAU seed 11	5.9	0.6	0.2	3.4	19.3	22.2	1.5	4.0	3.1	0.7	0.9	0.6	0.0	0.0	3.0	2.1	2.0	20.8	0.8	0.0	0.2	4.6	0.0	4.2	0.0	0.0
LBFDAU seed 12	5.2	0.4	0.2	2.2	16.1	25.2	0.8	5.5	3.3	0.7	0.8	0.5	0.1	0.1	3.7	2.9	2.4	20.1	0.4	0.0	0.4	4.2	0.2	4.7	0.0	0.2
LBFDAU seed 13	4.3	0.2	0.1	2.0	17.5	27.7	1.2	4.9	2.9	0.5	0.6	0.6	0.1	0.1	2.9	2.3	2.6	20.7	0.3	0.0	0.4	3.6	0.2	4.1	0.0	0.2
LBFDAU seed 14	4.2	0.3	0.1	2.0	16.4	27.9	0.9	5.1	2.9	0.5	0.6	0.5	0.1	0.1	3.5	3.2	2.0	20.5	0.3	0.0	0.4	3.7	0.1	4.3	0.0	0.2
LBFDAU seed 15	4.5	0.4	0.1	1.5	17.0	25.7	1.1	5.3	2.9	0.6	0.5	0.6	0.1	0.1	3.4	2.4	2.9	19.7	0.3	0.0	0.5	4.8	0.3	5.1	0.0	0.3
LBFDAU seed 16	4.7	0.6	0.2	2.0	17.2	25.9	1.1	5.9	3.0	0.7	0.7	0.5	0.1	0.0	3.2	2.6	2.3	20.1	0.6	0.0	0.7	3.4	0.1	4.3	0.0	0.2
LBFDAU seed 17	4.5	0.3	0.1	2.3	18.4	27.0	1.5	4.6	3.4	0.7	0.7	0.6	0.1	0.0	2.7	1.9	2.8	20.8	0.5	0.0	0.6	2.8	0.1	3.5	0.0	0.2
LBFDAU seed 18	5.1	0.2	0.2	2.9	16.1	27.1	1.5	4.7	4.2	0.7	0.9	0.7	0.0	0.0	2.3	2.3	2.1	19.5	0.7	0.0	0.4	3.4	0.0	4.8	0.0	0.2
LBFDAU seed 19	4.5	0.3	0.1	1.7	18.7	27.0	1.4	4.5	2.9	0.4	0.5	0.6	0.1	0.1	3.1	2.1	2.9	19.8	0.2	0.0	0.5	3.7	0.2	4.3	0.0	0.4
LBFDAU seed 20	5.3	0.2	0.1	2.6	17.9	26.4	1.1	4.4	2.9	0.5	0.7	0.6	0.1	0.1	3.4	2.7	2.8	20.8	0.3	0.0	0.4	3.0	0.1	3.3	0.0	0.2
LBFDAU seed 21	5.2	0.3	0.2	2.1	16.3	27.8	0.7	5.1	3.5	0.7	0.7	0.6	0.2	0.1	3.1	2.4	2.7	19.7	0.4	0.0	0.5	3.6	0.2	4.0	0.0	0.1
LBFDAU seed 22	4.8	0.2	0.1	2.4	17.7	28.9	0.9	4.9	3.1	0.5	0.7	0.6	0.1	0.1	2.4	2.1	2.7	19.9	0.3	0.0	0.4	3.0	0.1	3.8	0.0	0.2
LBFDAU seed 23	4.5	0.2	0.1	1.6	19.4	26.9	1.3	5.6	2.8	0.5	0.5	0.6	0.1	0.1	3.0	2.3	3.1	19.1	0.2	0.0	0.4	3.7	0.1	3.7	0.0	0.2
LBFDAU seed 24	6.6	0.6	0.2	3.4	18.4	23.0	1.0	3.9	4.6	1.1	1.1	0.7	0.0	0.0	3.6	2.0	1.6	17.9	0.7	0.0	0.9	3.7	0.0	4.8	0.0	0.0
LBFDAU seed 25	5.2	0.3	0.2	2.1	16.0	26.6	0.9	4.9	3.7	0.8	0.7	0.6	0.0	0.0	3.9	2.6	2.8	18.0	0.6	0.0	0.7	4.6	0.3	4.4	0.0	0.1
LBFDAU seed 26	5.5	0.6	0.2	2.6	19.9	23.4	2.1	3.8	3.0	0.6	0.8	0.6	0.0	0.0	4.0	2.0	2.8	18.5	0.6	0.0	1.0	3.8	0.2	3.9	0.0	0.2
LBFDAU seed 27	4.5	0.3	0.1	1.7	19.5	26.6	1.5	5.2	2.3	0.5	0.5	0.6	0.1	0.1	3.6	2.5	2.6	18.6	0.3	0.0	0.5	4.1	0.2	3.8	0.0	0.4

LBFDAU seed 28	5.1	0.3	0.2	2.5	17.2	27.3	0.8	4.6	2.9	0.5	0.9	0.5	0.1	0.0	3.7	2.3	3.6	18.6	0.5	0.0	0.5	3.7	0.2	3.8	0.0	0.2
LBFDAU seed 29	5.0	0.3	0.2	3.2	20.0	28.0	1.3	4.7	2.9	0.5	0.9	0.6	0.1	0.0	2.2	1.5	2.3	18.5	0.4	0.0	0.5	2.8	0.2	3.6	0.0	0.2
LBFDAU seed 30	4.7	0.2	0.1	2.4	18.6	29.3	1.6	4.4	3.2	0.5	0.7	0.6	0.1	0.1	2.9	2.1	2.5	18.6	0.3	0.0	0.7	2.7	0.2	3.2	0.0	0.2
LBFDAU seed 31	4.9	0.3	0.1	2.5	18.8	28.0	1.5	4.1	3.0	0.4	0.8	0.6	0.1	0.1	3.9	2.8	1.8	18.2	0.4	0.0	0.6	3.1	0.2	3.6	0.0	0.3
LBFDAU seed 32	4.8	0.3	0.1	2.2	19.6	28.3	1.0	5.5	2.6	0.5	0.6	0.6	0.1	0.1	3.1	2.5	2.4	18.3	0.3	0.0	0.4	3.1	0.1	3.4	0.0	0.2
LBFDAU seed 33	4.6	0.3	0.1	2.3	19.6	28.4	1.3	5.2	3.1	0.6	0.8	0.6	0.1	0.0	2.7	2.0	2.4	18.2	0.4	0.0	0.5	3.1	0.1	3.5	0.0	0.2
LBFDAU seed 34	4.9	0.3	0.1	3.4	19.2	25.9	1.8	4.0	3.1	0.5	1.0	0.7	0.1	0.0	3.9	2.7	2.1	17.9	0.5	0.0	0.6	3.0	0.2	3.7	0.0	0.3
LBFDAU seed 35	4.6	0.3	0.2	2.0	19.1	28.5	1.1	5.7	3.0	0.6	0.6	0.6	0.1	0.1	3.0	2.3	2.6	17.9	0.3	0.0	0.4	3.3	0.1	3.6	0.0	0.2
LBFDAU seed 36	4.2	0.2	0.1	2.1	18.1	30.5	1.0	4.9	2.9	0.4	0.6	0.6	0.1	0.1	3.5	3.0	2.1	17.6	0.3	0.0	0.4	3.2	0.1	3.8	0.0	0.2
LBFDAU seed 37	4.9	0.2	0.1	2.0	19.0	29.0	1.1	5.0	3.0	0.5	0.6	0.6	0.1	0.1	3.1	2.2	2.9	17.8	0.3	0.0	0.4	3.0	0.2	3.5	0.0	0.2
LBFDAU seed 38	4.4	0.3	0.1	1.9	19.7	29.9	1.2	4.5	2.6	0.4	0.6	0.5	0.1	0.1	3.0	2.0	2.6	17.8	0.3	0.0	0.5	3.4	0.2	3.5	0.0	0.2
LBFDAU seed 39	4.8	0.2	0.1	2.2	19.2	28.6	1.0	5.0	2.4	0.4	0.6	0.6	0.1	0.1	4.0	3.2	1.9	17.8	0.3	0.0	0.5	3.1	0.1	3.4	0.0	0.3
LBFDAU seed 40	4.3	0.3	0.1	1.8	20.2	28.8	1.4	4.8	2.6	0.4	0.6	0.6	0.1	0.1	3.2	2.2	2.5	17.7	0.3	0.0	0.5	3.6	0.2	3.5	0.0	0.3
LBFDAU seed 41	5.8	0.4	0.2	3.0	19.3	26.9	0.9	4.6	3.6	0.7	1.0	0.6	0.0	0.0	3.1	1.8	2.1	17.7	0.7	0.0	1.0	3.1	0.1	3.5	0.0	0.0
LBFDAU seed 42	4.6	0.3	0.1	2.3	21.3	27.8	1.2	5.2	2.8	0.6	0.6	0.6	0.1	0.1	2.7	2.1	2.3	18.0	0.3	0.0	0.7	2.9	0.1	3.2	0.0	0.2
LBFDAU seed 43	5.3	0.3	0.2	2.0	18.1	29.2	1.0	4.2	3.1	0.5	0.7	0.6	0.1	0.1	3.4	2.1	3.7	17.6	0.3	0.0	0.5	3.5	0.2	3.4	0.0	0.2
LBFDAU seed 44	4.7	0.3	0.2	1.9	19.3	29.3	1.0	5.7	2.7	0.5	0.7	0.7	0.1	0.1	2.7	2.1	2.6	17.3	0.3	0.0	0.5	3.6	0.2	3.6	0.0	0.2
LBFDAU seed 45	4.3	0.2	0.1	2.2	19.4	29.8	1.1	5.0	2.5	0.4	0.7	0.6	0.1	0.1	3.3	2.6	2.4	17.7	0.3	0.0	0.5	3.2	0.2	3.2	0.0	0.2

LBFDAU seed 46	4.5	0.2	0.2	2.4	18.8	29.0	1.1	5.0	3.7	0.6	0.7	0.6	0.1	0.1	3.1	2.2	2.8	17.1	0.3	0.0	0.5	3.1	0.2	3.5	0.0	0.2
LBFDAU seed 47	4.0	0.2	0.1	1.8	19.2	30.1	1.3	4.9	2.9	0.4	0.6	0.6	0.1	0.1	3.5	2.5	2.6	17.0	0.3	0.0	0.5	3.5	0.2	3.5	0.0	0.3
LBFDAU seed 48	4.3	0.2	0.1	2.0	20.0	30.1	1.3	4.5	2.7	0.4	0.6	0.5	0.1	0.1	3.5	2.3	2.7	16.9	0.3	0.0	0.4	3.1	0.2	3.5	0.0	0.3
LBFDAU seed 49	4.4	0.2	0.1	2.5	18.3	30.1	1.6	3.7	3.2	0.5	0.8	0.6	0.1	0.1	4.3	3.3	2.0	17.2	0.4	0.0	0.4	2.5	0.2	3.2	0.0	0.3
LBFDAU seed 50	4.3	0.3	0.1	1.9	21.5	29.2	1.3	5.2	2.5	0.5	0.6	0.6	0.1	0.1	2.8	2.1	2.3	17.0	0.3	0.0	0.4	3.2	0.2	3.3	0.0	0.2
LBFDAU seed 70	5.3	0.3	0.2	3.1	20.0	28.7	1.4	4.1	3.0	0.4	1.0	0.6	0.1	0.1	3.5	2.3	2.3	16.8	0.5	0.0	0.5	2.4	0.2	3.2	0.0	0.3
LBFDAU seed 71	4.2	0.2	0.1	2.0	22.1	29.1	1.7	4.9	2.3	0.3	0.6	0.6	0.1	0.1	2.9	2.3	2.2	16.7	0.3	0.0	0.7	2.7	0.2	3.3	0.0	0.3
LBFDAU seed 75	4.7	0.2	0.1	2.4	21.4	28.0	2.2	4.8	2.5	0.4	0.7	0.6	0.1	0.1	3.1	2.2	2.4	17.2	0.4	0.0	0.5	2.7	0.2	2.8	0.0	0.3
LBFDAU seed 76	4.7	0.3	0.1	2.1	20.9	28.1	1.0	5.4	3.1	0.6	0.6	0.6	0.1	0.1	2.9	2.1	2.6	16.3	0.3	0.0	0.6	3.4	0.2	3.7	0.0	0.2
LBFDAU seed 77	5.0	0.2	0.1	2.1	18.5	29.7	1.6	4.8	2.9	0.5	0.7	0.6	0.1	0.1	3.9	2.9	2.4	16.7	0.3	0.0	0.5	2.8	0.2	3.3	0.0	0.2
LBFDAU seed 78	4.8	0.2	0.1	1.9	20.1	29.2	1.1	5.1	2.6	0.4	0.6	0.7	0.1	0.1	3.4	2.4	2.7	16.7	0.3	0.0	0.7	3.2	0.2	3.2	0.0	0.2
LBFDAU seed 79	4.3	0.2	0.1	1.9	21.6	28.7	1.5	5.1	2.6	0.4	0.5	0.6	0.1	0.1	3.3	2.3	2.6	16.6	0.3	0.0	0.6	2.9	0.2	3.3	0.0	0.4
LBFDAU seed 80	4.7	0.3	0.2	1.9	19.0	31.0	0.7	4.6	2.7	0.5	0.6	0.6	0.1	0.0	3.3	2.2	2.5	16.1	0.4	0.0	0.6	3.8	0.3	3.7	0.0	0.1
LBFDAU seed 81	4.3	0.2	0.1	2.1	21.0	29.5	1.4	5.1	2.6	0.4	0.6	0.6	0.1	0.1	3.4	2.5	2.3	16.7	0.3	0.0	0.4	3.1	0.1	2.9	0.0	0.2
LBFDAU seed 82	4.5	0.3	0.1	2.0	21.0	29.6	1.4	5.5	2.5	0.5	0.6	0.6	0.1	0.1	2.7	1.9	2.7	16.2	0.3	0.0	0.5	3.2	0.2	3.5	0.0	0.3
LBFDAU seed 83	5.0	0.3	0.1	2.3	20.6	28.3	0.9	5.8	2.8	0.6	0.6	0.6	0.1	0.1	3.0	2.4	2.5	16.2	0.4	0.0	0.5	3.3	0.1	3.3	0.0	0.2
LBFDAU seed 84	6.2	0.5	0.2	2.9	20.3	27.1	0.8	5.6	3.0	0.9	0.9	0.6	0.0	0.0	3.0	2.1	2.1	15.4	0.7	0.0	0.5	3.5	0.0	3.9	0.0	0.0
LBFDAU seed 85	4.1	0.2	0.1	2.2	21.5	30.4	1.4	4.8	2.6	0.4	0.6	0.6	0.1	0.1	3.1	2.4	2.3	16.1	0.4	0.0	0.5	2.6	0.2	3.1	0.0	0.4

LBFDAU seed 86	4.7	0.3	0.2	2.2	20.6	30.6	1.0	5.5	2.8	0.5	0.7	0.6	0.1	0.1	2.7	2.0	2.7	15.5	0.4	0.0	0.5	2.7	0.2	3.2	0.0	0.2
LBFDAU seed 87	4.3	0.3	0.1	2.2	20.9	31.0	1.6	4.5	2.7	0.3	0.6	0.6	0.1	0.1	3.3	2.2	2.1	15.6	0.3	0.0	0.9	2.8	0.2	3.0	0.0	0.3
LBFDAU seed 88	4.1	0.2	0.1	1.9	22.2	30.6	1.3	4.6	2.5	0.5	0.6	0.6	0.1	0.1	2.9	1.7	3.1	15.6	0.3	0.0	0.7	3.0	0.2	2.9	0.0	0.3
LBFDAU seed 89	4.4	0.3	0.1	2.0	21.6	30.5	1.2	5.4	2.3	0.4	0.6	0.6	0.1	0.1	2.9	2.1	2.2	15.3	0.4	0.0	0.7	3.3	0.1	3.1	0.0	0.2
LBFDAU seed 90	4.8	0.2	0.1	2.4	22.3	29.6	1.1	5.2	2.3	0.4	0.7	0.6	0.1	0.1	3.2	2.6	2.2	15.6	0.3	0.0	0.5	2.7	0.1	2.7	0.0	0.3
LBFDAU seed 91	4.8	0.2	0.1	2.2	22.0	30.2	1.3	4.9	2.7	0.6	0.7	0.7	0.1	0.0	3.2	2.2	2.5	15.1	0.3	0.0	0.4	2.5	0.2	3.0	0.0	0.3
LBFDAU seed 92	5.2	0.3	0.2	2.0	20.3	29.7	1.1	5.1	3.2	0.6	0.6	0.6	0.1	0.0	3.1	1.9	3.4	14.8	0.4	0.0	0.6	3.3	0.2	3.2	0.0	0.2
LBFDAU seed 93	4.7	0.2	0.1	2.4	23.0	30.3	1.1	5.3	2.1	0.5	0.7	0.7	0.1	0.1	3.0	2.2	2.2	15.1	0.3	0.0	0.4	2.7	0.1	2.5	0.0	0.3
LBFDAU seed 94	5.0	0.3	0.2	2.3	21.9	30.6	1.3	5.2	2.4	0.7	0.8	0.6	0.1	0.1	2.9	2.1	2.4	14.7	0.4	0.0	0.4	2.7	0.2	2.8	0.0	0.3
LBFDAU seed 95	4.8	0.2	0.1	2.5	24.6	29.6	1.3	5.2	2.1	0.5	0.7	0.7	0.1	0.1	3.2	2.2	2.4	13.5	0.3	0.0	0.5	2.2	0.1	2.5	0.0	0.3

Fatty acid profiles, copy number measurements, and phenotypic observations of T2 plants carrying T-DNAs of plasmids VC-LTM593-1qcz rc cultivated in greenhouses during the summer

The data in Table 148 indicate the copy number of the selected events was a single insertion which was homozygous in the T3 seed. Fatty acid profile measurements, see Table 149 and Table 150, indicated the combination of T-DNA from VC-LTM593-1qcz rc are capable of bringing in the VLC-PUFA pathway to successfully accumulate ARA, EPA and DHA. The data on Table 151 show that there was no significant impact on the aerial portion of the plant caused by VC-LTM593-1qcz rc.

Table 148: Copy number measurement of T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. The events are indicated in the first column, along with the number of T2 plants that where measured per event. For each event, T2 seedbatches of two homozygous T1 plants where selected for seeding. Comparison of the results with Table 135 confirmes homozygozity of all T2 plants.

Event	Copy number assays targeting the T-DNA of VC-LTM593-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target c-d6Elo(Pp_GA) near the right T-DNA border.														
	c-AHAS	f-i-Atss1_c-d6Elo(Ot_GA3)	c-d4Des(Pl_GA)2	f-p-LuPXR_i-Atss1S	f-p-PvARCS_t-BnSETL	c-d5Des(Tc_GA)	f-i-Atss18_c-o3Des(Pl_GA2)	c-o3Des(Pl_GA)	f-p-BnSETL-v1_c-o3DES(Plr_GA)	f-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	f-t-StCAT_p2_p-LuPXR	f-i-Atss2_c-d6Des(Ot_febit_GA)	c-d5Des(Tc_GA)	c-d6Elo(Pp_GA)	f-i-Atss18_c-d6Elo(Pp_GA2)
LBFDAU (n=218)	2.0	2.1	4.1		3.8	8.1	4.0	4.0			4.1	4.2	4.0	8.1	4.1	4.0
LBFDGG (n=200)	2.0	2.0	2.0		2.0	4.0	2.0	2.0			2.1	2.1	2.0	4.0	2.2	2.1
LBFGKN (n=182)	2.0	2.0	2.0		2.0	4.2	2.1	1.9			2.0	2.2	2.0	4.2	2.1	2.1
LBFIHE (n=157)	2.1	2.0	2.0		2.1	4.3	2.0	2.0			2.2	2.1	2.0	4.3	2.1	2.0
LBFLDI (n=229)	2.3	2.2	2.3		2.3	4.7	2.3	2.3			2.4	2.4	2.3	4.7	2.4	2.2
LBFPRA (n=196)	2.0	1.9	5.8		3.9	7.9	4.0	4.1			3.9	4.0	3.9	7.9	4.0	1.9
LBFDHG (n=177)	4.1	4.2	4.2		4.0	8.3	4.1	4.1			4.2	4.1	4.0	8.3	4.1	4.0
LBFLFK (n=195)	4.0	4.1	4.0		4.0	8.2	4.0	4.0			4.0	4.0	4.0	8.2	4.0	4.0

Table 149: Fatty acid profiles of T3 seeds harvested from T2 cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM593-1qcz rc. The events are indicated in the first column, along with the number of T3 seed batches representing a plant measured per event. Per seed batch a random selection of ~15 seed was measured in five technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unkn own peak a	unkn own peak b

LBFDAU (n=218)	4.8 ± 0.2	0.2 ± 0	0 ± 0	4.2 ± 0.3	23 ± 1.6	31.5 ± 1.2	1.1 ± 0.2	4.6 ± 0.4	2.2 ± 0.4	0.4 ± 0.1	1 ± 0.1	0.7 ± 0	0.1 ± 0	0.1 ± 0	4.5 ± 0.8	3.2 ± 0.6	1.4 ± 0.3	10.5 ± 1.4	0.4 ± 0	0 ± 0	0.4 ± 0.1	2.3 ± 0.3	0.2 ± 0.1	2.1 ± 0.4	0.6 ± 0.2	0.4 ± 0.1
LBFDGG (n=200)	5 ± 0.2	0.2 ± 0	0 ± 0	3.2 ± 0.3	30.4 ± 2.2	34.1 ± 1.6	0.8 ± 0.1	5 ± 0.5	1.6 ± 0.2	0.3 ± 0	0.9 ± 0.1	0.7 ± 0	0.1 ± 0	0.1 ± 0	2.4 ± 0.2	1.4 ± 0.2	2 ± 0.3	7 ± 0.8	0.4 ± 0	0 ± 0	0.4 ± 0.1	2 ± 0.2	0.1 ± 0	1.3 ± 0.3	0.3 ± 0	0.1 ± 0
LBFGKN (n=182)	4.8 ± 0.2	0.2 ± 0	0 ± 0	3.6 ± 0.4	28.3 ± 1.9	34.6 ± 1.6	0.7 ± 0.2	4.7 ± 0.5	1.8 ± 0.4	0.3 ± 0.2	0.9 ± 0.1	0.7 ± 0	0.2 ± 0.1	0.1 ± 0	2.4 ± 0.3	1.3 ± 0.2	2.4 ± 0.3	7.9 ± 0.9	0.4 ± 0	0 ± 0	0.4 ± 0.1	2 ± 0.2	0.1 ± 0.1	1.6 ± 0.3	0.3 ± 0.1	0.2 ± 0.1
LBFIHE (n=157)	4.9 ± 0.3	0.2 ± 0	0 ± 0	3.7 ± 0.6	25.9 ± 1.9	32.9 ± 1.8	0.8 ± 0.1	4.7 ± 0.6	2.4 ± 0.7	0.3 ± 0.1	0.8 ± 0.1	0.7 ± 0	0.2 ± 0.1	0.1 ± 0.1	2.7 ± 0.3	1.7 ± 0.3	2.5 ± 0.4	9.6 ± 1.3	0.4 ± 0	0 ± 0	0.4 ± 0.1	2.2 ± 0.3	0.2 ± 0.1	2 ± 0.5	0.3 ± 0.1	0.3 ± 0.1
LBFLDI (n=229)	5.9 ± 1	0.3 ± 0.1	0 ± 0.1	2.9 ± 0.3	27.8 ± 2.5	32.5 ± 1.8	0.7 ± 0.1	4.7 ± 0.5	1.7 ± 0.3	0.4 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.1 ± 0	0.1 ± 0	2.4 ± 0.3	1.4 ± 0.2	2.4 ± 0.4	9.2 ± 1.5	0.3 ± 0	0 ± 0	0.5 ± 0.1	2.6 ± 0.4	0.1 ± 0	1.7 ± 0.4	0.3 ± 0.1	0.2 ± 0
LBFPRA (n=196)	5 ± 0.2	0.2 ± 0	0 ± 0	3.9 ± 0.7	23.2 ± 2	30.9 ± 1.9	1.1 ± 0.2	3.8 ± 0.6	2.6 ± 0.5	0.3 ± 0.1	0.9 ± 0.1	0.6 ± 0	0.1 ± 0	0.1 ± 0	3 ± 0.4	1.7 ± 0.2	3.5 ± 0.4	13.5 ± 2	0.4 ± 0	0 ± 0	0.3 ± 0.1	2.6 ± 0.3	0.1 ± 0.1	1.7 ± 0.3	0.1 ± 0	0.3 ± 0.1
LBFDHG (n=177)	5.7 ± 0.2	0.3 ± 0	0 ± 0	3.2 ± 0.5	24.1 ± 0.8	34.5 ± 1.4	0.8 ± 0.1	4.4 ± 0.3	2.5 ± 0.5	0.3 ± 0.1	0.9 ± 0.1	0.7 ± 0	0.1 ± 0	0.1 ± 0	3.1 ± 0.6	1.9 ± 0.4	1.5 ± 0.3	8.5 ± 1.2	0.4 ± 0	0 ± 0	0.8 ± 0.1	2.5 ± 0.3	0.2 ± 0.1	2.4 ± 0.3	0.8 ± 0.2	0.2 ± 0.1
LBFLFK (n=195)	5 ± 0.1	0.2 ± 0	0.1 ± 0.1	4 ± 0.4	25.9 ± 1.8	31.9 ± 1.3	1 ± 0.2	4.7 ± 0.4	1.8 ± 0.3	0.4 ± 0.1	1 ± 0.1	0.7 ± 0	0.1 ± 0	0.1 ± 0	4 ± 0.9	2.4 ± 0.5	1.4 ± 0.3	8.3 ± 1	0.4 ± 0	0 ± 0	0.6 ± 0.1	2.7 ± 0.5	0.2 ± 0	1.5 ± 0.4	0.8 ± 0.3	0.3 ± 0.1

Table 150: Fatty acid profiles of one T3 seed batch per event harvested from T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM593-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T3 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LBFDAU (n=1)	5.1	0.3	0.0	4.7	20.6	27.6	1.1	4.3	2.5	0.5	1.3	0.6	0.2	0.1	4.0	2.8	1.6	14.1	0.5	0.0	0.4	2.8	0.3	3.5	0.5	0.3
LBFDGG (n=1)	5.2	0.3	0.0	2.9	25.6	33.8	0.7	5.2	2.0	0.4	0.8	0.7	0.2	0.1	2.5	1.6	2.9	9.1	0.4	0.0	0.5	2.4	0.2	2.2	0.3	0.1
LBFGKN (n=1)	4.8	0.2	0.0	3.7	26.5	30.2	1.2	4.9	2.3	0.3	0.8	0.6	0.2	0.1	2.5	1.4	3.2	10.9	0.3	0.0	0.4	2.4	0.1	2.5	0.2	0.3
LBFIHE (n=1)	4.4	0.1	0.0	3.0	20.2	29.0	0.8	4.7	2.8	0.5	0.7	0.7	0.4	0.3	2.9	2.1	3.3	15.3	0.3	0.0	0.5	2.9	0.4	3.9	0.2	0.5
LBFLDI (n=1)	6.7	0.3	0.0	3.0	22.2	28.3	1.1	4.0	2.7	0.7	0.8	0.5	0.1	0.1	3.2	2.1	2.5	13.8	0.4	0.0	0.5	3.4	0.3	2.7	0.5	0.2
LBFPRA (n=1)	5.0	0.2	0.0	3.5	20.0	27.6	1.4	3.1	3.4	0.4	0.8	0.6	0.1	0.1	2.9	1.9	3.8	19.0	0.3	0.0	0.3	2.7	0.1	2.4	0.1	0.4
LBFDHG (n=1)	6.0	0.3	0.0	2.6	23.3	29.4	1.1	5.0	2.3	0.4	0.8	0.7	0.1	0.1	2.8	1.9	1.6	12.8	0.4	0.0	0.7	3.3	0.4	3.3	0.6	0.3
LBFLFK (n=1)	4.9	0.2	0.2	3.8	23.0	31.9	1.0	4.4	2.3	0.5	0.9	0.7	0.1	0.1	3.2	2.1	2.0	11.6	0.3	0.0	0.7	3.0	0.2	2.3	0.5	0.3

Table 151: Phenotypic rating of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where rated per event. DFF: days to first flower (days), DF: deformed flower (1=deformed, 9=normal), DL: deformed leaf (1=deformed, 9=normal), DP: deformed plant (1=deformed, 9=normal), DS: deformed silique (1=deformed, 9=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoI: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad), Oil: oil content (% of seed weight), protein: Protein content (% of seed cake without oil)

Event	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC	Oil	Protein
LBFDAU (n=218)	43.2 ± 2.9	9 ± 0	9 ± 0	8 ± 0	9 ± 0	3 ± 0	4 ± 0.1	5 ± 0	8.6 ± 0.8	5.1 ± 0.6	107.5 ± 7.6	3 ± 0.3	3.7 ± 1.2	35.6	
LBFDGG (n=200)	43 ± 3.7	8.6 ± 1.5	8.5 ± 0.5	8 ± 0	8.6 ± 1.3	3 ± 0	4 ± 0	5 ± 0	8.7 ± 0.7	4.5 ± 0.5	112.1 ± 5.5	3 ± 0.3	3.7 ± 0.7	N/A	
LBFGKN (n=182)	42.5 ± 4.3	8 ± 2.7	8.5 ± 1	7.6 ± 1.6	8.1 ± 1.9	2.9 ± 0.3	4.1 ± 0.8	5 ± 0.3	8.5 ± 1	5 ± 0	111.6 ± 15.2	3.3 ± 0.4	3.3 ± 0.6	36.1	
LBFIHE (n=157)	51.1 ± 5.2	8.9 ± 0.4	8.9 ± 0.5	8.9 ± 0.5	5.2 ± 2.6	3 ± 0	5.1 ± 0.4	4.9 ± 0.4	5.2 ± 2.6	6.6 ± 1.1	139.4 ± 14.7	4 ± 0.5	3.8 ± 1.9	34.1	
LBFLDI (n=229)	48.6 ± 4.5	9 ± 0	9 ± 0	9 ± 0	7.6 ± 2.5	3 ± 0	5 ± 0.1	5 ± 0.1	7.6 ± 2.5	6.2 ± 1.1	145.5 ± 15.9	4.2 ± 0.4	3.1 ± 1.3	N/A	
LBFPRA (n=196)	51.2 ± 2.4	9 ± 0	9 ± 0	9 ± 0	5.8 ± 3.2	3 ± 0	5 ± 0	5 ± 0	5.8 ± 3.2	7.3 ± 1	138.7 ± 16.6	4.2 ± 0.4	2.4 ± 1.5	34.7	
LBFDHG (n=177)	46 ± 3.5	9 ± 0	9 ± 0	8 ± 0	4.7 ± 1.6	3 ± 0	4 ± 0	5 ± 0	8.5 ± 0.8	5.2 ± 0.8	112.5 ± 5.4	3.4 ± 0.4	5.3 ± 1	32.3	
LBFLFK (n=195)	42.3 ± 2.8	9 ± 0	9 ± 0	9 ± 0.3	8.9 ± 0.7	3 ± 0	4 ± 0.1	5 ± 0	8.7 ± 0.9	5.3 ± 0.5	113.4 ± 8	3.3 ± 0.3	3.9 ± 1.5	37.1	

Fatty acid profiles, copy number measurements, and phenotypic observations of T2 plants carrying T-DNAs of plasmids VC-LTM593-1qcz rc cultivated in field trials in USDA growth zones 3a-4b and 5a during the summer

Field data for the T3 seed from the events carrying the T-DNA from VC-LTM593-1qcz rc, shown in Table 152 and Table 153, indicate that the plants are capable of making VLC-PUFAs in the field (ARA, EPA and DHA), though not at the level observed in the greenhouse. ANOVA was conducted with using the software JMP 11.0. Analysis was conducted at the 95% confidence level using Tukey test. To compensate for unbalance in the data obtained from the field trial (e.g. due to e.g. weather), Least Square menas instead of means where used in the statistical analysis. Common letters in the Table 154, Table 155and Table 156 indidcate no significant difference of the least square means. Table 154 shows the statistical analysis of agronomical parameters.

There was a difference in seed oil content observed compared to the greenhouse (e.g. comparing Table 154 with Table 151), indicating oil content and the fatty acid profile could be linked. These observations are in agreement with previous examples where it was observed that increased oil contents in the field grown plants concomitant with a decrease in VLC-PUFAs, in particular EPA, DHA and ARA. A more detailed description of the observations regarding oil content and VLC-PUFAs is given in Example 20.

For seed yield (kg per ha, data not shown), no statisticaly relevant difference was found comparing the events against wildtype Kumily (tested using Tukey, 0.05% level).

Table 152: Fatty acid profiles of T3 seeds harvested from T2 cultivated in the field, corresponding to USDA growth zones 3a-4b and 5a, for field trials of canola events containing the T-DNAs of plasmid VC-LTM593-1qcz rc. The events are indicated in the first column, along with the number of T3 seed aliquots representing a plot where measured per event. For event LBFGKN, 36 plots and 60 single plants from those plots where measured. Per seed batch a random selection of ~15 seed was measured in five technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unkn own peak a	unkn own peak b
LBFDAU (n=16)	4.7 ± 0.1	0.2 ± 0	0 ± 0	2.7 ± 0.1	28.6 ± 1.5	29.2 ± 0.7	1 ± 0.1	6.1 ± 0.3	1.6 ± 0.1	0.3 ± 0	0.7 ± 0	0.7 ± 0	0.1 ± 0	0.1 ± 0	3.3 ± 0.3	2.2 ± 0.2	2 ± 0.2	10.7 ± 0.7	0.3 ± 0	0 ± 0	0.3 ± 0	2.9 ± 0.2	0.1 ± 0	1.6 ± 0.2	0.3 ± 0.1	0.3 ± 0
LBFDGG (n=36)	4.7 ± 0.1	0.2 ± 0	0 ± 0	2.5 ± 0.2	34.2 ± 1.9	32.3 ± 1.2	0.6 ± 0.1	7 ± 0.5	1.2 ± 0.1	0.2 ± 0	0.6 ± 0	0.8 ± 0	0.1 ± 0	0.1 ± 0	2 ± 0.3	1.3 ± 0.2	1.9 ± 0.2	6.1 ± 0.7	0.3 ± 0	0 ± 0	0.3 ± 0	2.1 ± 0.2	0.1 ± 0	1.1 ± 0.2	0.2 ± 0.1	0.1 ± 0
LBFGKN (n=36+60)	4.6 ± 0.2	0.2 ± 0	0 ± 0	2.6 ± 0.2	33.7 ± 1.7	32.8 ± 1.4	0.6 ± 0.1	7.5 ± 0.6	0.9 ± 0.1	0.2 ± 0	0.7 ± 0	0.8 ± 0.1	0.2 ± 0	0.1 ± 0	2.1 ± 0.3	1.2 ± 0.1	1.8 ± 0.2	6 ± 0.6	0.3 ± 0	0 ± 0	0.3 ± 0.1	2.1 ± 0.2	0.1 ± 0	1 ± 0.1	0.2 ± 0	0.2 ± 0
LBFIHE (n=36)	4.8 ± 0.2	0.2 ± 0	0 ± 0	2.6 ± 0.2	31.2 ± 1.7	33.9 ± 1.2	0.6 ± 0.1	6.7 ± 0.7	1.3 ± 0.2	0.3 ± 0	0.7 ± 0.1	0.8 ± 0	0.2 ± 0	0.1 ± 0	2.1 ± 0.2	1.2 ± 0.1	2.4 ± 0.3	6.7 ± 0.6	0.3 ± 0	0 ± 0	0.3 ± 0.1	1.9 ± 0.2	0.1 ± 0	1.2 ± 0.2	0.2 ± 0.1	0.2 ± 0
LBFLDI (n=36)	4.9 ± 0.2	0.3 ± 0	0 ± 0	2.5 ± 0.2	33.4 ± 1.7	32.7 ± 1.2	0.6 ± 0.1	6.8 ± 0.6	1 ± 0.1	0.2 ± 0	0.7 ± 0.1	0.8 ± 0	0.2 ± 0	0.1 ± 0	2 ± 0.3	1.2 ± 0.2	2 ± 0.2	6.2 ± 0.7	0.3 ± 0	0 ± 0	0.3 ± 0.1	2.1 ± 0.2	0.1 ± 0	1.1 ± 0.2	0.2 ± 0.1	0.2 ± 0
LBFIDT (n=32)	4.6 ± 0.1	0.2 ± 0	0 ± 0	2.7 ± 0.2	30 ± 1.4	29.9 ± 1	0.9 ± 0.1	6.5 ± 0.5	1.6 ± 0.1	0.3 ± 0	0.7 ± 0.1	0.7 ± 0	0.1 ± 0	0.1 ± 0	4.4 ± 0.4	2.6 ± 0.2	1.5 ± 0.2	6.8 ± 0.7	0.3 ± 0	0 ± 0	0.6 ± 0.1	2.9 ± 0.3	0.1 ± 0	1.2 ± 0.2	1 ± 0.1	0.3 ± 0
LBFPRA (n=36)	4.8 ± 0.2	0.2 ± 0	0 ± 0	2.6 ± 0.2	28.4 ± 2.1	32.7 ± 1.4	0.8 ± 0.1	5.7 ± 0.4	1.6 ± 0.2	0.3 ± 0.1	0.7 ± 0	0.8 ± 0	0.2 ± 0	0.1 ± 0	2.3 ± 0.3	1.2 ± 0.2	3.8 ± 0.5	9.6 ± 1	0.3 ± 0	0 ± 0	0.3 ± 0	2.4 ± 0.3	0.1 ± 0	1.1 ± 0.2	0.1 ± 0	0.2 ± 0.1
LBFDHG (n=4)	5.2 ± 0.1	0.2 ± 0	0 ± 0	2.4 ± 0.1	28.2 ± 1.4	29.5 ± 1	0.9 ± 0.2	6.5 ± 0	1.6 ± 0.1	0.3 ± 0	0.6 ± 0	0.7 ± 0	0.2 ± 0	0.1 ± 0	2.9 ± 0	1.7 ± 0.1	2 ± 0.1	9.6 ± 0.4	0.3 ± 0	0 ± 0	0.6 ± 0	3.5 ± 0.1	0.2 ± 0	1.9 ± 0.2	0.5 ± 0.1	0.3 ± 0
LBFLFK (n=36)	4.7 ± 0.2	0.2 ± 0	0 ± 0	2.6 ± 0.2	30.1 ± 1.9	30.2 ± 1.1	0.9 ± 0.1	6.2 ± 0.4	1.5 ± 0.2	0.3 ± 0.1	0.6 ± 0	0.8 ± 0	0.1 ± 0	0.1 ± 0	3.3 ± 0.3	1.9 ± 0.2	1.9 ± 0.2	8.2 ± 1	0.3 ± 0	0 ± 0	0.5 ± 0	3.2 ± 0.4	0.1 ± 0	1.4 ± 0.3	0.5 ± 0.1	0.3 ± 0.1

Table 153: Fatty acid profiles of one T3 seed batch per event harvested from T2 plants cultivated in USDA growth zones 3a-4b and 5a for field trials of canola events containing the T-DNAs of plasmid VC-LTM593-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T3 seed batches representing a field plot having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unkn own peak a	unkn own peak b
LBFDAU (n=1)	4.9	0.2	0.0	2.6	26.0	28.9	0.8	6.2	1.7	0.3	0.7	0.8	0.2	0.1	3.6	2.4	2.0	12.0	0.3	0.0	0.3	3.2	0.1	2.1	0.4	0.3
LBFDGG (n=1)	5.0	0.3	0.0	2.7	30.1	33.1	0.5	6.5	1.5	0.3	0.7	0.8	0.2	0.1	2.1	1.4	2.4	7.5	0.3	0.0	0.4	2.4	0.1	1.6	0.2	0.1
LBFGKN (n=1)	4.4	0.2	0.0	2.6	32.6	31.1	0.7	7.3	1.1	0.3	0.6	0.8	0.2	0.1	2.1	1.3	2.1	7.7	0.3	0.0	0.3	2.5	0.1	1.3	0.2	0.2
LBFIHE (n=1)	4.8	0.2	0.0	2.5	28.0	33.9	0.6	6.3	1.6	0.3	0.8	0.8	0.2	0.1	2.4	1.5	2.7	8.2	0.3	0.0	0.4	2.3	0.1	1.6	0.2	0.2
LBFIDI (n=1)	5.2	0.3	0.0	2.5	29.8	33.3	0.4	6.8	1.3	0.4	1.1	0.8	0.2	0.1	1.8	1.2	2.4	7.2	0.4	0.0	0.4	2.4	0.1	1.6	0.2	0.2
LBFIDT (n=1)	4.7	0.2	0.0	2.6	28.6	28.8	1.0	7.1	1.6	0.3	0.7	0.7	0.1	0.1	4.3	2.6	1.5	8.1	0.3	0.0	0.6	3.4	0.1	1.4	1.0	0.4
LBFPRA (n=1)	5.1	0.2	0.0	2.6	26.0	31.5	0.7	5.3	1.8	0.3	0.7	0.7	0.2	0.1	2.4	1.3	4.3	11.7	0.3	0.0	0.3	2.8	0.0	1.3	0.1	0.2
LBFDHG (n=1)	5.1	0.2	0.0	2.4	29.0	28.4	1.0	6.6	1.6	0.3	0.6	0.8	0.1	0.1	2.9	1.7	1.9	10.2	0.3	0.0	0.5	3.5	0.2	1.9	0.5	0.3
LBFLFK (n=1)	5.1	0.2	0.0	2.6	26.9	29.3	0.8	5.9	1.9	0.4	0.7	0.8	0.1	0.1	3.1	2.0	2.3	10.3	0.3	0.0	0.6	3.9	0.1	2.0	0.5	0.2

Table 154: Phenotypic rating of T2 plants cultivated in USDA growth zones 3a-4b and 5a for field trials of canola events containing the T-DNAs of plasmid VC-LTM593-1qcz rc. The events are indicated in the first column, along with the number of field plots that where rated per event. Stand: the number of plants that emerged within a 1 meter section of row at GS 14 (4 leaves unfolded), rating of a seedlings ability to grow and develop at GS 14 (4 leaves unfolded, 1= All plants at different growth stages and all plants appear unhealthy, 9= All plants at same growth stage and all plants appear healthy), First flower: date at which plants are at GS 60 (days after emergence), Last flower: date at which plants are at GS 69 (days after emergence), Maturity: Days to Maturity (when average seed moisture is 30 to 35%, and 30 to 40% if the seeds in the pods have developed their mature seed color, either black or yellow),Plant Heigth: the average height (cm) of five plants in a plot measured from soil level to the top of the plant at GS 69 (cm), Lodging: rating of the average standability of plants in a plot at GS 83 (30% of pods ripe, 1=0 – 10% average lean of plants in plot from horizontal (standing erect), 9=81%+ average lean of plants in plot from horizontal (nearly prostrate)), Shatter: rating of the ability of plants to retain seed in the pod at GS 89 (all pods fully ripe, 1=0 – 10% perished pods, 9=81%+ perished pods), Agron Score: scale of 1-5 with 1 being the best, 5 the worst ranking of plant phenotype (measurements occurred post flower during pods formation and seed development),Moisture (% of seed weight), TKW: thousand kernel weight (g), Oil: oil content (% of seed weight), protein: Protein content (% of seed cake without oil).

Event	Stand		Vigor		First Flower		Last Flower		Maturity		Plant Height		Lodging		Shatter		Agron Score		Moisture		TWK		Oil		Protein	
LBFDAU (n=16)	23.7	a	5.7	bcd	43.8	bc	77.3	abcde	94.5	a	115.5	b	2.3	ab	1.5	a	4.7	ab	9.0	a	3.5	abcd	38.5	bc	26.6	c
LBFDGG (n=36)	23.0	a	5.9	bcd	44.0	bc	77.1	bcd	94.3	a	116.2	b	2.3	b	1.6	a	4.2	ab	8.9	a	3.8	a	38.8	abc	26.3	c
LBFDHG (n=4)	23.4		5.8		43.9		77.2				115.9		2.3		1.5		4.4		9.0		3.7		39.3	abc	26.5	c
LBFGKN (n=36)	22.8	a	6.3	bcd	43.3	c	76.5	cde	94.2	a	118.8	b	2.8	ab	1.8	a	4.1	ab	8.5	a	3.7	ab	36.7	c	26.5	c
LBFIHE (n=36)	21.7	a	5.6	cd	45.7	b	79.7	ab	94.7	a	115.2	b	2.3	b	1.6	a	4.1	ab	8.7	a	3.4	bcd	39.8	ab	26.5	c
LBFLFK (n=36)	22.5	a	5.1	d	45.6	b	78.0	abc	94.3	a	118.4	b	2.6	ab	1.5	a	4.1	ab	8.6	a	3.7	a	38.3	bc	27.1	c
LBFPRA (n=36)	22.5	a	6.3	bcd	44.2	bc	78.1	abc	94.3	a	121.2	b	2.9	ab	1.9	a	4.2	ab	8.5	a	3.5	abc	39.2	abc	26.5	c
Topas	24.8	a	5.2	d	48.1	a	80.9	a	95.3	a	139.7	a	3.2	ab	2.6	a	4.4	a	7.3	b	3.3	cd	37.8	c	26.9	c
Kumily	28.2	a	6.9	ab	43.4	c	76.6	cde	94.2	a	119.9	b	2.8	ab	1.8	a	4.5	a	7.4	b	3.7	ab	39.8	ab	28.5	a
Control 1	28.1	a	7.7	a	42.9	c	73.8	e	89.2	b	121.1	b	3.9	a	2.7	a	3.4	b	7.0	b	3.2	d	39.9	ab	27.1	c
Control 2	25.3	a	6.6	abc	40.7	d	74.3	de	90.3	b	119.1	b	3.8	a	2.7	a	3.4	b	7.0	b	3.1	d	40.7	a	27.4	bc

Table 155: Compositional analysis of T3 seeds of T2 plants cultivated in USDA growth zones 3a-4b and 5a for field trials of canola events containing the T-DNAs of plasmid VC-LTM593-1qcz rc. The events are indicated in the first column. The analysis has been done on 4 BULK, whereby each BULK is a representative sample of all seeds harvedted from 4 different geographic reagions. Alpha-Tocopherol (mg/100g seed), Beta-Tocopherol (mg/100g seed), Delta-

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Tocopherol (mg/100g seed), Gamma-Tocopherol (mg/100g seed), Tocopherol (mg/100g seed), Sinapine (µg/g (ppm)), Phytate (% of seed weight (w/w)), Ash (% of seed weight (w/w)), Crude Fiber (% of seed weight (w/w)), ADF: acid detergent fiber (% of seed weight (w/w)), NDF: neutral detergent fiber (% of seed weight (w/w)). All results have been normalized to the seed weight of seeds having 0% moisture.

Event	Alpha-Tocopherol		Beta-Tocopherol		Delta-Tocopherol		Gamma-Tocopherol		Tocopherols (VitE)		Sinapine		Glucosinolate		Phytate		Ash		Crude Fiber		ADF		NDF	
LBFDAU	13.3	ab	0.25	a	0.58	a	29.5	a	43.7	a	0.64	cd	11.0	bcd	2.3	B	4.5	ab	9.4	a	11.8	ab	14.5	a
LBFDGG	14.1	ab	0.23	a	0.45	bcd	25.6	b	40.4	abc	0.69	bcd	13.1	ab	2.3	B	4.3	ab	9.2	a	10.9	cdef	13.6	ab
LBFGKN	12.9	b	0.23	a	0.52	abc	26.9	ab	40.6	abc	0.70	bc	11.3	bc	2.3	b	4.4	ab	9.3	a	11.0	bcdef	13.5	ab
LBFIHE	13.2	ab	0.23	a	0.45	bcd	22.0	cd	35.9	cde	0.65	cd	15.7	a	2.2	b	4.7	ab	9.1	a	10.8	cdef	13.4	ab
LBFLFK	12.5	b	0.23	a	0.52	abc	25.7	b	38.9	abc	0.68	bcd	12.6	abc	2.3	b	4.3	b	9.6	a	11.3	abcde	13.7	ab
LBFPRA	13.6	ab	0.22	a	0.47	bcd	24.9	bc	39.2	abc	0.62	d	10.9	bcd	2.2	b	4.3	ab	9.6	a	12.0	a	14.6	a
Topas	14.7	ab	0.25	a	0.36	d	16.6	e	31.9	e	0.69	bcd	7.6	d	2.7	a	4.8	a	9.0	a	10.5	ef	12.9	b
Kumily	12.3	b	0.23	a	0.54	ab	24.4	bc	37.5	bcd	0.78	a	11.0	bcd	2.2	b	4.3	b	9.1	a	11.5	abc	14.3	a
Control 1	16.6	a	0.25	a	0.43	cd	24.1	bc	41.4	ab	0.73	ab	9.5	cd	2.3	b	4.6	ab	8.6	a	10.2	f	12.8	b
Control 2	12.0	b	0.20	a	0.45	bcd	20.8	d	33.5	de	0.72	ab	11.9	bc	2.3	b	4.4	ab	9.2	a	10.6	def	13.8	ab

Table 156: Herbicde tolerance of T2 plants cultivated in USDA growth zones 3a-4b and 5a for field trials of canola events containing the T-DNAs of plasmid VC-VC-LTM593-1qcz rc. The events are indicated in the first column. IMI Injury: injury according to the scale detailed in Table 157 (DAT= days after treatment). Herbicide imazamox was aplied at a 2x rate of 70 g imazamox /ha. Brassica napus cv Kumily, which is the non-transgeneic comparator line that is otherwise isogenic to the events, was rated at 6 to 7, and was removed from the statistical analysis to make the Tukey test more sensitive to detect significant differences between events that are very similar in their tolerance.

Event	IMI Injury 7 DAT		IMI Injury 14 DAT		IMI Injury 21 DAT	
LBFDAU	2	a	1	ab	1	a
LBFDGG	2	a	1	ab	1	a
LBFDHG	2		1		1	
LBFGKN	2	a	1	ab	1	a
LBFIHE	2	a	2	a	1	a
LBFLFK	2	a	1	b	1	a

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Event	IMI Injury 7 DAT		IMI Injury 14 DAT		IMI Injury 21 DAT	
LBFPRA	2	a	1	ab	1	a
Topas	1	a	1	b	1	a
Kumily	6		6		7	

Table 157: Canola rating scale for herbicide

% Injury	1-7 Scale	Category	Injury Symptoms	Growth Rates and Recovery Effects
0	1	Excellent	None	None
1-6	2	Very Good	Leaf and petiole epinasty, chlorosis.	Minor or temporary growth effects. Injury and effects should be minor enough to not cause commercialization concerns.
7-14	3	Good	Leaf, petiole and stem epinasty, chlorosis, stem swelling. Leaf cupping may be observed.	This would be the maximum allowable injury for commercial evaluations. Fairly temporary in nature without any effect on final yield and minimal delay in maturity,
15-20	4	Fair	Above symptoms plus stunting in height, smaller leaf size or impact on LAI, in this class: Basal swelling may be observed. Expect recovery and seed production with this set of symptoms but delayed, reduced growth and reduced seed set. Plant stand may be non-uniform upon recovery.	Appearance of unaffected new growth impeded for <7 days. Slight delay in bolting and flower production. Yield impact minimal or small at harvest.
21-40	5	Poor	Injury in this class would be as above and more than evaluator's estimate of the level of commercial acceptance.	Significant delay in plant development, significant malformation s in growth and development vs. control. Malformations persist Serious reduction in maturity, height and harvest yield.
41-79	6	Non Tolerant		Equivalent to suppression as a volunteer crop in a weed control assessment. Minimal regrowth following application. Plants survive but fail to flower and mature as normal.
80-100	7	Susceptible	Severe injury or death.	Severe injury or death.

Example 19: Gene copy number effects on observed conversion efficiencies at each pathway step.

When analysing the VLC-PUFA levels of the various constructs listed in this invention, distinct differences were observed in conversion efficiency at the pathway steps when the T-DNA copy number increased. This was observed when comparing the VCL-PUFA levels of (1) events having a single copy T-DNA insertion vs a double copy T-DNA insertion, (2) heterozygous plants vs homozygous plants, and (3) when analysing segregating single seeds as described in Example 9 and shown in Figure 22 and Figure 23. This demonstrates a relationship between 'gene dosage', that is the number of T-DNA copies present in the genome, and VLC-PUFA levels. Upon further investigation it is clear that certain genes/steps of the VLC-PUFA pathway benefit more in terms of impact on the pathway when the gene number and/or expression level of the gene or activity at that step is increased. While the contribution from each gene present, when multiple genes which encode enzymes with identical activities are present, are difficult to assess (e.g. two different omega-3-desaturases are technically difficult to assess), it was possible to calculate conversion efficiencies for each pathway step, by using the equations shown in Figure 2. These conversion efficiencies for each pathway step were calculated for various populations of plants that represent a certain gene dosage, in order to investigate if the observed increase in conversion efficiency can be assigned to one or more individual pathway steps (e.g. increasing conversion efficiency due to increased gene dosage at one early step in the pathway may increase conversion efficiency at a later step simply be providing more of a limiting substrate).

The conversion efficiencies are sometimes referred to as "apparent" conversion efficiencies because for some of the calculations it is recognized that the calculations do not take into account all factors that could be influencing the reaction. As an example of factors that may be influencing a reaction but are not taken into account during the conversion efficiency calculation; the substrates of the delta-6-desaturase are produced while bound at the sn-2 positions of phospholipids, whereas the product of the delta-6-desaturase is formed while the fatty acid is bound to CoA. It is therefore not immediately clear if the low delta-6-conversion conversion efficiencies shown in Figure 33 are due to inefficient provision of substrate to the delta-6-desaturase via trans-esterification from PC (phosphatidylcholine) bound LA/ALA to CoA bound LA/ALA, or whether the delta-6-desature had low activity/low conversion efficiency.

For the interpretation of the conversion efficiencies it was also important to note that any catalyzed conversion of substrate to product was dependent on the substrate concentration, catalyst (enzyme) concentration, and product concentration.

Analysing the data pathway step by pathway step, the following observations can be made in Figure 32 to Figure 39:

Conversion efficiency of delta-12-desaturation

The delta-12-desaturase conversion efficiency encompasses all steps that influence substrate concentration, product concentration, and enzyme concentrations. From the data in example 21 it is evident that the delta-12-desaturase, c-d12Des(Ps_GA), enzymatically produces phosphatidylcholine bound 18:2n-6 (Figure 26, Panel A), which implies the product was phosphatidylcholine bound 18:1n-9. The primary flux of newly formed 18:1n-9 was derived from plastid produced 18:1n-9, which was exported into the cytosol, where it was bound to CoA. Therefore, a prerequisite of delta-12-desaturation was the incorporation of CoA-bound 18:1n-9 into the sn2 position of phosphatidylcholine via LPCAT (Lysophosphatidylcholine acetyltransferase EC 2.3.1.23), or via LPAAT (Lysophosphatidic acid acyltransferase E.C. 2.3.1.51), with subsequent conversion of the formed (sn2)18:1n-9-phosphatidic acid into (sn2)18:1n-9-DAG (DAG is an abbreviation for diacylglycerol), whereby (sn2)18:1n-9-DAG was either directly converted into (sn2)18:1n-9-PC (PC is an abbreviation for phosphatidylcholine) substrate for the delta-12-desaturases via PDCT (phosphatidylcholine diacylglycerol cholinephosphotransferase EC 2.7.8.2), or obtains a phosphocholine headgroup via CPT (CPT is an abbreviation for sn-1,2-diacylglycerol:cholinephosphotransferase EC 2.7.8.2) to be converted into (sn2)18:1n-9-PC. The efficiency of this incorporation of plastid synthesized 18:1n-9 directly affects delta-12-desaturase substrate concentration. As mentioned already, PC is converted by PDCT into DAG (demonstrated by Bates et al), which was subsequently converted into TAG. The amount of PC bound delta-12-desaturase substrate and product that was part of the delta-12-desaturase reaction was therefore also directly linked to the substrate specificity of PDCT.

There was a strong correlation of the delta-12-desaturation conversion efficiency with the copy number of the delta-12-desaturase (Figure 32). This was evident when comparing the average conversion efficiency of T0 single copy plants (across all events per construct), with the average conversion efficiency of T0 double copy plants (across all events per

construct). The same correlation can be seen in the T1 generation. The strong correlation of the delta-12-desaturation conversion efficiency with the copy number of the delta-12-desaturase was also seen when comparing copy number differences due to heterozygous vs homozygous T-DNA integrations: The T0 generation always consist of heterozygous plants, whereas the T1 generation largely consists of homozygous plants due to the selection applied in Example 10 through Example 18. The delta-12-desaturation conversion efficiency increases when comparing the average conversion efficiency of T0 single copy plants (across all events per construct), with the average conversion efficiency of T1 single copy plants (across all events per construct). Similarly, the delta-12-desaturation conversion efficiency increases when comparing the average conversion efficiency of T0 double copy plants (across all events per construct), with the average conversion efficiency of T1 double copy plants (across all events per construct). The only exceptions are:

T0 single copy events vs T0 double copy events containing the T-DNAs of VC-LJB2197-1qcz and VC-LLM337-1qcz rc: while not as pronounced as for VC-LJB2197-1qcz and VC-LLM306-1qcz rc, there are also subtle differences here in delta-12-desaturase conversion efficiencies. The slight differences are due to insufficient data in the T0 generation to accurately assign the events to the two copy number categories for events containing the T-DNAs of construct VC-LJB2197-1qcz and VC-LLM337-1qcz rc

T0 single copy events vs T0 double copy events containing the T-DNAs of VC-LJB2197-1qcz and VC-LLM338-3qcz rc: while not as pronounced as for VC-LJB2197-1qcz and VC-LLM306-1qcz rc, also here the subtle differences in delta-12-desaturase conversion efficiencies are due to insufficient data in the T0 generation to accurately assign the events to the two copy number categories for events containing the T-DNAs of construct VC-LJB2197-1qcz and VC-LLM338-3qcz rc

T1 single copy events vs T1 single copy (single locus) events containing the construct VC-LTM593-1qcz rc: , there was no effect on the conversion efficiency due to the 2-fold copy number difference between these groups. The group of single copy (single locus) events is affected by an additional phenomenon whereby the average VLC-PUFA level in T2 seeds of T1 plants of this homozygous group decreases instead of increasing compared to segregating T1 seeds of heterozygous T0 plants. A further indication that this group was an outlier group can be seen in Figure 35, where it was evident that the delta-5-desaturase

conversion efficiency was significantly lower in homozygous plants compared to heterozygous plants.

Considering the high conversion efficiency of the delta-12-desaturase, it is likely hat the provision of substrate via LPCAT is not a bottleneck. Also, the data suggest that efficient removal of delta-12-desaturase products contributes to the high conversion efficiency. However; taking into account the high levels of 18:2n-6 accumulated in the oil and the low delta-6-conversion efficiency, it was evident that not all delta-12-desaturated 18:2n-6 was removed from PC via trans-esterification to CoA (the site of action for the next pathway step). With respect to the fate of 18:2n-6; our observations indicate that the delta-12-desaturated 18:2n-6 was likely removed by the direct conversion of unsaturated PC into DAG via PDCT as discussed by Bates et al. (2014). This activity of PDCT strongly supports a high delta-12-desaturase conversion efficiency by providing fresh 18:1n-9-PC substrate to the delta-12-desaturase, while simultaneously removing 18:2n-6-PC product (by conversion into DAG). However, the latter activity poses a strong bottleneck for the continuation of the pathway, which is evident by the low delta-6-desaturase conversion efficiencies.

This effect of increased conversion efficiency of the delta-12-desaturase has been observed when analyzing the PUFA profile of more than 6.000 plants, that have been obtained by transforming more than 300 multi-gene constructs into Canloa and Arabidopsis, whereby all of these constructs caried genes having the essentail activities requiered for Arachidonic acid synthesis. Across these more then 300 constructs, more than 10 different delta-12-desaturase enzymes have been investigated.

Conversion efficiency of delta-6-desaturation

For the delta-6-desaturation, it was of particular importance to highlight that conversion efficiencies observed for this step are dependent on the substrate concentration (which was CoA bound linoleic acid), catalyst concentration (which was the delta-6-desaturase), and product concentration. It was, that PC bound 18:2n-6 produced by the delta-12-desaturase needs to be trans-esterified to CoA before delta-6-desaturation can take place. The absence of accumulation of 18:3n-6 and 18:4n-3 in the examples 10 -18 strongly indicates that CoA-bound 18:3n-6 and CoA-bound 18:4n-3 was efficiently converted by the delta-6-elongase, effectively preventing any significant accumulation of 18:3n-6 and 18:4n-3. In general, delta-6-desaturase conversion efficiencies are low for all constructs. Considering efficient removal of product was not a bottleneck, the low conversion efficiencies can be

either due to low activity of the delta-6-desaturase enzyme, or because of inefficient conversion of PC-bound substrate into CoA-bound substrate. Regardless of this, there was a clear correlation of the delta-6-desaturation conversion efficiency with the copy number of the delta-6-desaturase. In a manner similar to that of the delta-12-desaturase, this was seen regardless of the reason for the copy number increases (heterozygous vs homozygous, single copy genomic integrations vs double copy genomic integrations). In fact, plants of the construct combination VC-LJB2197-1qcz and VC-LLM306-1qcz rc contain an additional copy of the delta-6-desaturase on the T-DNA. It was therefore consistent that the group of 22 double copy T1 plants would have in the T2 seeds the highest delta-6-desaturase conversion efficiencies of all similar double copy groups of different constructs. For the delta-6-desaturase, the same exceptions to this copy number effect as for the delta-12-desaturase are seen. The same for the interpretation mentioned for the delta-12-desaturase of why these exceptions are observed are assumed to be the reasons for the delta-6-desaturase also applies in this case.

T0 single copy events vs T0 double copy events containing the T-DNAs of VC-LJB2197-1qcz and VC-LLM306-1qcz rc

T0 single copy events vs T0 double copy events containing the T-DNAs of VC-LJB2197-1qcz and VC-LLM337-1qcz rc

T0 single copy events vs T0 double copy events containing the T-DNAs of VC-LJB2197-1qcz and VC-LLM338-3qcz rc

T1 single copy events vs T1 single copy(single locus) events containing the construct VC-LTM593-1qcz rc

As demonstrated for the delta-12-desaturase, the amount of delta-12-desaturase product increases with increasing copy number of the delta-12-desaturases, which not only indicates an increased amount of delta-12-desaturase protein, but also implies an increased amount of substrate for the delta-6-desaturase. As both the delta-12-desaturases and the delta-6-desaturase are contained on the same T-DNA in all examples 10 to 18, the copy number of the delta-6-desatuases will also increase when copy number of the delta-12-desaturase increases, with the exceptions of events where truncated insertion of the T-DNA contain either the delta-12-desaturase and not the delta-6-desaturases, or vice versa.

In summary, not only was the effect of the gene copy duplication of the delta-12-desaturase surprising, but it was also unexpected that the increased copy number of delta-6-

desaturase led to an increased amount of delta-6-desaturase protein, and this increased amount of protein can convert the additional amount of delta-12-desaturated fatty acids with an increased conversion rate. To illustrate the effect, here as an example the comparison of two groups shown in examples 10 to 18 that have a gene copy number difference of "four":

All 275 heterozygous T0 plants containing a single copy of the T-DNA of construct VC-LTM593-1qcz rc

All 64 homozygous T1 plants containing two copies the T-DNA of of construct VC-LTM593-1qcz rc at two different chromosomal loci

In group A, the total sum of fatty acid that contain a double bond due to the activity of the delta-12-desaturase was 47.3% and total sum of fatty acids that contain a double bond due to the activity of the delta-6-desaturase was 11.2%. Consequently, 23.7% of all fatty acids that underwent delta-12-desaturation also underwent delta-6-desaturation.

In group B, the total sum of fatty acid that contain a double bond due to the activity of the delta-12-desaturase was 61 % and total sum of fatty acid that contain a double bond due to the activity of the delta-6-desaturase was 23.7%. Consequently, 38.8% of all fatty acids that underwent delta-12-desaturation also underwent delta-6-desaturation.

As a result of that, the sum of all fatty acids downstream of the delta-6-desaturase in the pathway more than doubled in group B versus group A.

This effect of increased conversion efficiency of the delta-6-desaturase has been observed when analyzing the PUFA profile of more than 6.000 plants, that have been obtained by transforming more than 300 multi-gene constructs into Canloa and Arabidopsis, whereby all of these constructs caried genes having the essentail activities requiered for Arachidonic acid synthesis. Across these more then 300 constructs, more than 5 different delta-6-desaturase enzymes have been investigated.

Conversion efficiency of delta-6-elongation

As was evident in Figure 34, the conversion efficiency of the delta-6-elongation was very high regardless of the copy number. Because of that, copy number mediated effects on the conversion efficiency are sometimes insignificant, but are sometimes clearly observed. E.g. the events obtained for the construct combinations VC-LJB2755-2qcz rc + VC-LLM391-2qcz rc and for the combination LJB2755-2qcz rc and VC-LTM217-1qcz rc contain the

lowest delta-6-elongation conversion efficiencies of all constructs, which can be attributed to the fact that these construct combinations encode just one delta-6-elongation enzyme [c-d6Elo(Tp_GA2)], instead of two [(c-d6Elo(Tp_GA2), c-d6Elo(Pp_GA2)].

Conversion efficiency of delta-5-desaturation

The data in Figure 35 do not indicate a consistent construct independent copy number dependence of the delta-5-desaturase.

Conversion efficiency of omega-3-desaturation

Figure 36 shows the omega-3-desaturase conversion efficiencies that have been calculated by excluding the C18 substrates and products shown in Figure 2, thereby excluding the delta-15-desaturase specific activity of omega-3-desaturases. Figure 37 shows the omega-3-desaturase conversion efficiencies that have been calculated by including the C18 substrates and products, thereby including the delta-15-desaturase specific activity of omega-3-desaturases. As both omega-3-desaturases used in the invention have virtually no delta-15-desaturase activity, conclusions on the effect of copy number on the omega-3-desaturase are best drawn from Figure 36. While not as pronounced as observed for the delta-12-desaturase or delta-6-desaturase, there is, in many cases, a clear link between omega-3-desaturases copy number and the omega-3-desaturase conversion efficiency. This was particularly evident as plants of the construct combination as VC-LJB2197-1qcz + VC-LLM338-3qcz rc display the lowest omega-3-desaturase conversion efficiency of all other constructs, which can be attributed to the fact that this construct combination encodes just one omega-3-desaturase enzyme [c-o3Des(Pi_GA2)], instead of two [(c-o3Des(Pi_GA2), c-o3Des(Pir_GA)]. Exceptions to the copy number dependence of the omega-3-desaturase conversion efficiency are:

Plants containing the T-DNAs of construct RTP10690-1qcz_f

Plants containing the T-DNAs of construct VC-LTM595-1qcz rc

Plants containing the T-DNAs of VC-LJB2197-1qcz and VC-LLM338-3qcz rc

Conversion efficiency of delta-5-elongation

The complex interdependency of all the pathway step conversion efficiencies between each other can be seen by comparing Figure 38 with Figure 35: the high delta-5-elongation conversion efficiency for two groups of plants containing the T-DNAs of construct VC-LTM593-1qcz rc (813 T1 plants of the double copy group, 700 plants of the double

copy/single locus group) was in fact not due to a high delta-5-elongation conversion efficiency, but rather due to an inefficient supply of substrate to the delta-5-elogase at the delta-5-desaturase pathway step. Similar to the omega-3-desaturase, while not as pronounced as for the delta-12-desaturase or delta-6-desaturase, there was a link between delta-5-elongase copy number and the delta-5-elongase conversion efficiency.

Conversion efficiency of delta-4-desaturation

As can be seen in Figure 39, there was no direct evidence for an increase in delta-4-desaturase conversion efficiency to delta-4-desaturase copy number. The highest delta-4-desaturase conversion efficiency has been observed for plants containing the T-DNAs of construct LJB2755-2qcz rc and VC-LTM217-1qcz rc. This construct combination differs only from the combination VC-LJB2755-2qcz rc + VC-LLM391-2qcz rc in replacing the d4Des(Eg) by the c-d4Des(PI_GA)2. As a conclusion of the plant data shown in Example 10 to 18 and the yeast data shown in Example 22, it can be clearly seen that the delta-4-desaturation conversion efficiency reaches an upper limit using the genes c-d4Des(Tc_GA) and d4Des(Eg), that cannot be overcome by increased gene copy numbers. All constructs that encode the c-d4Des(PI_GA)2 instead of d4Des(Eg) have a higher delta-4-desaturation conversion efficiency, and the only exception to that are plants containing the construct RTP10690-1qcz_F. As shown in Example 25, this was due to very low expression of both delta-4-desaturases. This in turn highlights that the conversion efficiency does depend on the gene expression, but cannot overcome an upper limit.

Example 20: Environmental effects on VLC-PUFA levels. Correlation between oil content, VLC-PUFA levels observed conversion efficiencies at each pathway step

Analysing the VLC-PUFA levels presented in the Examples 10 to 18, we have oberserved that there is, in most cases, a level of variability, within a genetically identical population of plants (e.g. a genetically stable event where all plants are genetically identical), even when these plants are grown in a controlled greenhouse environment. To illustrate the effect more clearly, VLC-PUFA profiles of seed batches having highest EPA+DHA levels among several type of plant populations are shown in the examples 10 to 18. Such plant populations are (1) either genetically identical due to the T-DNA integrations (i.e. all plants of the same event, where either all or at least most are homozygous for all T-DNA integrations in those events), or (2) identical related to the T-DNA copy number, and these populations have been grown in the same environment. For example, out of 249 segregating T1 seedlings,

10 T1 plants for event LBFDAU (see Example 18) have been identified in the greenhouse to be identical in having one complete T-DNA integration, and one truncated T-DNA integration, whereby both T-DNA insertions where found to be homozygous (Table 135). In a field trial conducted in parallel, out of about segregating T1 seedlings, 4 plants have been identified as being homozygous for both T-DNA insertions (Table 144). The average EPA+DHA content measured in randomly selected seed from seed batches of these 10 greenhouse grown plants was found to be 13.9% EPA + 2.6% DHA. Similarly, the EPA+DHA content of the 4 field grown plants was found to be 13.4% EPA + 2.7% DHA. Among the 10 greenhouse grown plants was a plant that produced up to 15.1% EPA + 3.0% DHA (Table 139). Similarly, among the 4 field grown plants was a plant that produced up to 17.6% EPA + 3.6% DHA (Table 146). Analysing single seed of this field grown plant, single seeds having up to 26.2% EPA + 4.9% DHA, or 23.6%EPA + 5.8% DHA where found (Table 147). The lowest EPA+DHA content found among these 95 seed contained 13.5% EPA and 2.5% DHA, and the average content among these seed was 18.2% EPA and 3.7% DHA, which matched the content measured in the random selection of twice 15 seed as shown in Table 146 (17.6% EPA + 3.6% DHA). This event LBFDAU is just a representative example to illustrate for that every event and all constructs exhibit plant-to-plant variability, and seed-to-seed variability. The extent of the plant to plant variability is shown in the examples 10 to 18, where it is shown that the average PUFA profile of a certain population (e.g. all plants belong to the same event and are genetically identical, or all plants are single copy, heterozygous) is always different than the profile observed in individual plants. To ilustrate the magnitude of this variability, examples 10 to 18 show the average PUFA profile of such populations and also the profile of the single plant having the highest EPA+DHA levels.

In conclusion, while the event LBFDAU was capable of producing in a genetically identical plant population ~13.5% EPA + 2.6% DHA in a given environment, there are individual plants within this population that produce up to ~18% EPA + 3.7% DHA as an average of all seeds produced by this plant, and single seed of this plant can reach up to ~26% EPA and ~5% DHA. These differences in EPA+DHA levels are due to the interaction of the genetic constituents of the event with the environment. However, it was consistently observed that comparable populations yield higher VLC-PUFA levels in the greenhouse compared to the field. This trend usually correlates with lower oil content in the greenhouse, compared to the field. To investigate this observation in more detail, oil content measured in all homozygous seed batches of the single copy event LANPMZ (event described in example 11) was

plotted in Figure 40 against the sum of all VLC-PUFA that are downstream of the delta-12-desaturase (see Figure 2) measured in the same seed batches. The same has been done for event LAODDN (event described in example 13), and is plotted Figure 41. This analysis was alos done for two events described in example 18, namely for event LBFGKN (Figure 42), and for event LBFLFK (Figure 43). What was observed is a strong correlation between these two parameters. The same analysis for wildtypes (Figure 72) reveals no such correlation, instead, Figure 72 shows a difference between greehouse grown and field grown wildtype plants, in that field grown wildtype plants have higher levels of 18:2n-6 and 18:3n-3 and lower 18:1n-9 compared to greenhouse grown wildype plants.

To analyse in detail if this correlation depicted for event LANPMZ in Figure 40 can be attributed to certain pathway steps, conversion efficiencies for each pathway step and for each seedbatch analysed in Figure 40 have been calculated, and plotted in Figure 44, Figure 48, Figure 52, Figure 56, Figure 60, Figure 64, and Figure 68. The same analysis has been done for event LAODDN, and is plotted in Figure 45, Figure 49, Figure 53, Figure 57, Figure 61, Figure 65 and Figure 69. The same analysis was also been done for event LBFGKN, (plotted in Figure 46, Figure 50, Figure 54, Figure 58, Figure 62, Figure 66 and Figure 70), and for event LBFLFK (Figure 47, Figure 51, Figure 55, Figure 59, Figure 63, Figure 67 and Figure 71). Comparing all these figures, one can see that the correlation observed in Figure 40 and Figure 41 is largely attributed to the delta-12-desaturase pathway step (Figure 44 to Figure 47), the delta-6-desaturase pathway step (Figure 48 to Figure 51), and the delta-4-desaturase pathway step (Figure 68 to Figure 71): in these figures, there is a construct and event independent negative correlation between the pathway steps and the seed oil content visible, which cannot be observed for the other pathway steps.

The negative correlation of the delta-12-desaturase pathway with seed oil content in event LANPMZ was observed in wild type plants, as showin in Figure 72. Moreover, Figure 72 shows that field grown wildtype plants have higher levels of 18:2n-6 and 18:3n-3 and lower 18:1n-9 compared to greenhouse grown wildype plants. This can most likely be explained with previous observations (Xiao et al. 2014), in that the native Canola endogenous delta-12-desaturase was regulated by temperature and that temperature regulation of delta-12-desaturase at the transcript level and protein level is a recurring them in plants (Kargiotidou et al. 2008, Tang et al. 2005, Sanchez-Garcia et al. 2004) for the regulation of this enzyme, whereby under field conditions the delta-12-desaturase be would up-regulated compared to

greenhouse conditions. However, it can be concluded that there was no regulation of the delta-12-desaturase in dependance of the oil content observed in wildtype canola plant. Consequently comparing Figure 72 with Figure 40 to Figure 43), it can furthermore be concluded that only the introduced transgeneic delta-12-desaturase was subject to a regulation that was seed oil content dependant.

A further obersvation is that field grown transgenic plants do not have a higher delta-12-desaturase conversion efficiency compared to greenhouse grown plants, even though this was clearly oberved for wildtype plants in Figure 72. It can be concluded that the endogenous native delta-12-desaturase does not significantly contribute to the overall observed delta-12-desaturase conversion efficiency in the transgenic plants of the invention.

In addition to plotting pathway step conversion efficiencies vs seed oil content, levels of some key fatty acids have been plotted agains seed oil content (Figure 81, Figure 82, Figure 83, Figure 84). The data have been fitted in those figures by using all datapoints of all depicted events to determine the slope of the regression, and by subsequently using all datapoints for single events to determine the intercept of the regression line with the x-axis (0% oil content) of the regressions shown in (Figure 81, Figure 82, Figure 83, Figure 84). The results of this analysis are shown in Table 158.

Table 158: Regressions equations of fitting the data in Figure 81, Figure 82, Figure 83, Figure 84 as described in example 20. Listed are the parameters for the equation (Fatty acid) = (seed oil content) * Slope + Intercept.

Event		EPA+DHA vs oil		ARA vs oil		EPA vs oil		DHA vs oil	
	N	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope
LBFDAU	23	28.054	-0.398	5.774	-0.098	22.053	-0.288	6.003	-0.110
LBFPRA	44	26.075	-0.398	7.399	-0.098	20.762	-0.288	5.315	-0.110
LBFLFK	42	25.280	-0.398	5.788	-0.098	19.578	-0.288	5.703	-0.110
LBFDHG	9	25.058	-0.398	5.235	-0.098	19.167	-0.288	5.892	-0.110
LANBCH	60	24.910	-0.398	7.142	-0.098	19.305	-0.288	5.606	-0.110
LBFGKN	291	23.481	-0.398	5.856	-0.098	17.994	-0.288	5.488	-0.110
LBFIHE	47	22.841	-0.398	6.086	-0.098	17.489	-0.288	5.353	-0.110
LBFDGG	44	22.800	-0.398	5.789	-0.098	17.403	-0.288	5.398	-0.110

Example 21: In vitro demonstration of enzyme activity

Desaturase Enzyme Activity in Microsomes Isolated from Transgenic Yeast

Expression of desaturases and elongases was accomplished in *Saccharomyces cerevisiae*. Briefly, yeast strains containing the appropriate plasmid were grown overnight at 30 °C (in SD-medium-uracil + raffinose) and then used to inoculate a larger culture at a starting OD_{600}

= 0.2 (in SD-medium-uracil+raffinose+galactose). After 24 hours at 30 °C the culture (typically OD_{600} = 0.6-0.8) was harvested by centrifugation and washed once in 25 mM Tris Buffer (pH 7.6). Preparation of crude extracts and microsomes from yeast expressing genes encoding desaturases and elongases was accomplished using standard procedures. Briefly, cells expressing desaturases were resuspended in 2 ml Desaturase Disruption Buffer (0.1M potassium phosphate pH 7.2, 0.33 M sucrose, 4 mM NADH, 1 mg/ml BSA (fatty acid free), 4000 U/ml catalase and protease inhibitors (Complete EDTA-free (Roche)) and disrupted using silica/zirconium beads in a BeadBeater. The crude extract was clarified by centrifugation twice at 8,000xg, 4 °C). After an additional centrifugation at 100,000xg (30 minutes at 4 °C) the microsomes were pelleted and ultimately resuspended in Desaturase Disruption Buffer (300 microliters). Protein concentrations in both the crude extract and microsomes were measured using the bicinchoninic acid (BCA) procedure (Smith, P. K., et al (1985) *Anal. Biochem.* (150): 76–85).

General Desaturase Activity Assays:
In the desaturase assay a [^{14}C]-labeled acyl-CoA was provided as a substrate and after the reaction the acyl-CoAs (and phospholipids) were hydrolyzed and methylated to fatty acid methyl esters (FAMEs), which were analyzed using argentation-TLC. The general assay conditions were modified from Banas et al. (Banas et al. (1997) *Physiology, Biochemistry and Molecular Biology of Plant Lipids* (Williams, J.P., Khan, M.U. and Lem, N.W. eds.) pp. 57-59).

The assay contained: 1 mg enzyme (crude extract) or 150 µg (microsomal fraction), 10 nmol [^{14}C]-acyl-CoA (3000 dpm/nmol), 7.2 mM NADH (total), 0.36 mg BSA (total) in a buffer comprised of 0.1 M K-phosphate pH 7.2, 0.33 M sucrose, 4 mM NADH, 1 mg/ml BSA and protease inhibitors in a total volume of 200 µl. After incubation at 30 °C for the desired time, 200 µl of 2 M KOH in MeOH:H_2O (1:4) was added and incubated for 20 minutes at 90 °C. Fatty acids were extracted by addition of 3 M HCl (200 µl), 1.5 ml of MeOH:$CHCl_3$ (2:1) and $CHCl_3$ (500 µl). The chloroform phase was recovered, dried under N_2(g) and fatty acids were methylated by addition of 2 ml MeOH containing 2% H_2SO_4 and incubation of 30 minutes at 90 °C. FAMEs were extracted by addition of 2 ml H_2O and 2 ml hexane and separated by $AgNO_3$-TLC and Heptane:Diethyl ether:Acetic Acid (70:30:1) as a solvent. The radioactive lipids were visualized and quantified by electronic autoradiography using Instant Imager.

Delta-12 desaturase (*Phytophthora sojae*), c-d12Des(Ps_GA) Enzyme Activity: Enzyme assays were performed using re-suspended microsomes isolated from a yeast strain expressing the c-d12Des(Ps_GA) protein and compared to microsomes isolated from a control yeast strain containing an empty vector (LJB2126). In the presence of [^{14}C]18:1n-9-CoA, 16:0-lysphosphatidylcholine (LPC), and NADH membranes containing c-d12Des(Ps_GA) form an [^{14}C]18:2-fatty acid that can be isolated as a methyl ester and resolves on $AgNO_3$-TLC [heptane:diethyl ether (90:10)] similar to known synthetic standards. This enzyme activity requires NADH and was not observed in membranes isolated from the empty vector control strain. Control assays without 16:0-LPC contain a small-amount of activity, presumably due to endogenous 16:0-LPC found in yeast microsomes. Furthermore, separation of the phospholipids from the free-fatty acids after the enzymatic reaction and characterization of the isolabled fatty acid methyl esters demonstrated that all of the c-d12Des(Ps_GA) enzymatically produced 18:2n-6-fatty acid methyl ester (FAME) was found in the phosphatidylcholine fraction. c-d12Des(Ps_GA) enzyme activity may also be demonstrated using other [^{14}C]acyl-CoA's which may include, but are not limited to: [^{14}C]18:2n-6-CoA, [^{14}C]20:3n-6-CoA, [^{14}C]20:4n-6-CoA, [^{14}C]22:5n-3-CoA.

Delta-6 desaturase (*Ostreococcus tauri*), c-d6Des(Ot_febit) Enzyme Activity: c-d6Des(Ot_febit) enzyme activity and substrate specificity can be demonstrated in microsomes isolated from a yeast strain expressing the c-d6Des(Ot_febit) protein using an [^{14}C]acyl-CoA in the general assay described above. [^{14}C]Acyl-CoA's may include, but are not limited to: [^{14}C]18:1n-9-CoA, [^{14}C]18:2n-6-CoA, [^{14}C]20:3n-6-CoA, [^{14}C]20:4n-6-CoA, [^{14}C]22:5n-3-CoA. Isolated fatty acid methyl esters derived from enzymatic substrates and products can be resolved using $AgNO_3$-TLC and Heptane:Diethyl ether:Acetic Acid (70:30:1) as a solvent. Furthermore, the c-d6Des(Ot_febit) enzyme can be shown to directly desaturate an acyl-CoA substrate, as described in section C, as suggested in previous reports (Domergue et al. (2005) Biochem. J. 389: 483-490).

Delta-5 desaturase (*Thraustochytrium* ssp.), c-d5Des(Tc_GA2) Enzyme Activity: c-d5Des(Tc_GA2) enzyme activity and substrate specificity can be demonstrated in microsomes isolated from a yeast strain expressing the c-d5Des(Tc_GA2) protein using an [^{14}C]acyl-CoA general assay as described above. [^{14}C]Acyl-CoA's may include, but are not limited to: [^{14}C]18:1n-9-CoA, [^{14}C]18:2n-6-CoA, [^{14}C]20:3n-6-CoA, [^{14}C]20:4n-6-CoA,

[^{14}C]22:5n-3-CoA. Isolated fatty acid methyl esters derived from enzymatic substrates and products can be resolved using Reverse Phase-TLC (Silica gel 60 RP-18) and acetonitrile (100%) as a solvent.

Omega-3 desaturase (*Phytophthora infestans*), c-o3Des(Pi_GA2) Enzyme Activity: c-o3Des(Pi_GA2) enzyme activity and substrate specificity can be demonstrated in microsomes isolated from a yeast strain expressing the c-o3Des(Pi_GA2) protein using [^{14}C]acyl-CoA in the general assay described above. [^{14}C]Acyl-CoA's may include, but are not limited to: [^{14}C]18:1n-9-CoA, [^{14}C]18:2n-6-CoA, [^{14}C]20:3n-6-CoA, [^{14}C]20:4n-6-CoA, [^{14}C]22:5n-3-CoA. Isolated fatty acid methyl esters derived from enzymatic substrates and products can be resolved using Reverse Phase-TLC (Silica gel 60 RP-18) and acetonitrile (100%) as a solvent.

Omega-3 desaturase (*Pythium irregulare*), c-o3Des(Pir_GA) Enzyme Activity: c-o3Des(Pir_GA) enzyme activity and substrate specificity can be demonstrated in microsomes isolated from a yeast strain expressing the c-o3Des(Pir_GA) protein using an [^{14}C]acyl-CoA in the general assay described above. [^{14}C]Acyl-CoA's may include, but are not limited to: [^{14}C]18:1n-9-CoA, [^{14}C]18:2n-6-CoA, [^{14}C]20:3n-6-CoA, [^{14}C]20:4n-6-CoA, [^{14}C]22:5n-3-CoA. Isolated fatty acid methyl esters derived from enzymatic substrates and products can be resolved using Reverse Phase-TLC (Silica gel 60 RP-18) and acetonitrile (100%) as a solvent.

Delta-4 desaturase (*Thraustochytrium* ssp.), c-d4Des(Tc_GA) Enzyme Activity: c-d4Des(Tc_GA) enzyme activity and substrate specificity can be demonstrated in microsomes isolated from a yeast strain expressing the c-d4Des(Tc_GA) protein using an [^{14}C]acyl-CoA in the general assay described above. [^{14}C]Acyl-CoA's may include, but are not limited to: [^{14}C]18:1n-9-CoA, [^{14}C]18:2n-6-CoA, [^{14}C]20:3n-6-CoA, [^{14}C]20:4n-6-CoA, [^{14}C]22:5n-3-CoA. Isolated fatty acid methyl esters derived from enzymatic substrates and products can be resolved using Reverse Phase-TLC (Silica gel 60 RP-18) and acetonitrile (100%) as a solvent.

Delta-4 desaturase (*Pavlova lutheri*), c-d4Des(Pl_GA)2 Enzyme Activity: c-d4Des(Pl_GA)2 enzyme activity and substrate specificity can be demonstrated in microsomes isolated from a yeast strain expressing the c-d4Des(Pl GA)2 protein using an [^{14}C]acyl-CoA in the general assay described above. [^{14}C]Acyl-CoA's may include, but are not limited to:

[^{14}C]18:1n-9-CoA, [^{14}C]18:2n-6-CoA, [^{14}C]20:3n-6-CoA, [^{14}C]20:4n-6-CoA, [^{14}C]22:5n-3-CoA. Isolated fatty acid methyl esters derived from enzymatic substrates and products can be resolved using Reverse Phase-TLC (Silica gel 60 RP-.18) and acetonitrile (100%) as a solvent.

Delta-4 desaturase (*Euglena gracilis*), c-d4Des(Eg) Enzyme Activity: c-d4Des(Eg) enzyme activity and substrate specificity can be demonstrated in microsomes isolated from a yeast strain expressing the c-d4Des(Eg) protein using an [^{14}C]acyl-CoA in the general assay described above. [^{14}C]Acyl-CoA's may include, but are not limited to: [^{14}C]18:1n-9-CoA, [^{14}C]18:2n-6-CoA, [^{14}C]20:3n-6-CoA, [^{14}C]20:4n-6-CoA, [^{14}C]22:5n-3-CoA. Isolated fatty acid methyl esters derived from enzymatic substrates and products can be resolved using Reverse Phase-TLC (Silica gel 60 RP-18) and acetonitrile (100%) as a solvent.

Desaturase Activity in Microsomes isolated from Transgenic *Brassica napus*.

Microsomes containing recombinant desaturases and elongases capable of synthesizing docosahexaenoic acid (22:6n-3) were isolated from immature seeds from transgenic *B. napus* using a procedure adopted from Bafor, M. et al. Biochem J. (1991) 280, 507-514. Briefly, immature seeds were first seperated from canola pods and then the developing embryos were isolated from the seed coat and transferred to ice-cold 0.1M Phosphate buffer (pH 7.2). The developing embryos were then washed with fresh Phosphate buffer, transferred to an ice-cold mortar, and ground to a homogenous solution in Extraction Buffer (0.1 M Phosphate, pH 7.2, 0.33 M sucrose, 1 mg/ml BSA (essentially fatty acid free), 4000U/ml catalase, 4 mM NADH and protease inhibitor-Complete EDTA-free (Roche)). The lysed developing embryo's were diluted 20-fold with additional Extraction Buffer and passed through 2 layers of Miracloth into a centrifuge tube. Following centrifugation at 18,000 x g for 10 minutes at 4 °C, the clarified supernatant was passed through Miracloth into an ultracentrifuge tube. Following centrifugation at 105, 000 xg for 60 minutes at 4 °C, the supernatant was removed from the microsomal pellet, which was then washed once with Extraction Buffer, and then using a Dounce homogenizer resuspended as a homogenous solution in Extraction Buffer (about 1 ml per 500 embryo's).

Enzyme activity can be demonstrated for the desaturases using the assays described above in Section A. for microsomes isolated from yeast expression strains.

In summary in Section A we have provided a method that allows unambiguous demonstration of fatty acyl desaturase enzyme activity. We provide data demonstrating that: (1) gene c-d12Des(Ps_GA) encodes a delta-12 desaturase protein from *Phytophthora sojae* (c-d12Des(Ps_GA) that desaturates oleic acid (18:1n-9) to form linoleic acid (18:2n-6) in both microsomes isolated from a transgenic yeast (Figure 24, panel A) and from a transgenic *B. napus* event (Figure 25, panel A) expressing this protein, (2) gene c-o3Des(Pi_GA2) encodes a protein from *Phytophthora infestans* (c-o3Des(Pi_GA2)) that desaturates arachidonic acid (20:4n-6) to form eicosapentaenoic acid (20:5n-3) in microsomes isolated from a transgenic yeast (Figure 24, panel B) expressing this protein, (3) gene c-o3Des(Pir_GA) encodes an omega-3 desaturase protein from *Pythium irregulare* (c-o3Des(Pir_GA)) that desaturates arachidonic acid (20:4n-6) to form eicosapentaenoic acid (20:5n-3) in microsomes isolated from a transgenic yeast (Figure 24, panel B) expressing this protein, (4) gene c-d4Des(Tc_GA) encodes a delta-4 desaturase protein from *Thraustochytrium* sp. (c-d4Des(Tc_GA)) that desaturates docosapentaenoic acid (22:5n-3) to form docosahexaenoic acid (22:6n-3) in microsomes isolated from a transgenic yeast (Figure 24, panel C) expressing this protein, (5) a transgenic *B. napus* event containing both the gene encoding the delta-4 desaturase protein from *Thraustochytrium* sp, gene c-d4Des(Tc_GA), and the gene c-d4-Des(Pl_GA) from *Pavlova Lutheri* contains at least one enzyme, localized to the microsomes, capable of desaturating docosapentaenoic acid (22:5n-3) to form docosahexaenoic acid (22:6n-3) (Figure 25, panel C). Except for the c-d12Des(Ps_GA), which has a known endogenous enzyme in Brassica, all other examples presented contain no detectable endogenous desaturase activity in microsomes isolated from either control yeast strains (Figure 24) or control *Brassica* lines (Figure 25).

Using the methods described in Section A for desaturase proteins the level of expression or detected enzyme activity may be influenced by the presence or absence of fusion tags to the native protein. Fusion tags or proteins to the desaturases may be attached the amino-terminus (N-terminal fusions) or the carboxy-terminus (C-terminal fusions) of the protein and may include but are not limited to: FLAG, hexa-Histidine, Maltose Binding Protein, and Chitin Binding Protein.

We have provided methods to establish enzyme catalyzed desaturation reactions required in an engineered pathway to biosynthesize docosohexaenoic acid (DHA, 22:6n-3) from oleic acid (18:1n-9) in canola. The methods presented in Example 21, Section A were developed to demonstrate desaturase activity in yeast strains expressing individual

desaturases and can be further used to confirm the respective desaturase enzyme activities in transgenic canola, as described and demonstrated in Example 21, Section B. Furthermore these methods can be incorporated, by one skilled in the art, to measure desaturase enzyme activities in other organisms including, but not limited to: *Saccharomyces cerevisiae*, *Arabidopsis thaliana*, *Brassica* spp., *Camelina sativa*, *Carthamus tinctorius*, and *Salvia hispanica*.

Desaturase Headgroup (CoA vs PC) Preference

Fatty acid desaturases catalyze the abstraction of two hydrogen atoms from the hydrocarbon chain of a fatty acid to form a double bond in an unsaturated fatty acid and can be classified according to the backbone that their substrate was connected to: an acyl-CoA, an acyl-ACP (ACP, acyl carrier protein) or an acyl-lipid. To date a few examples exist where the acyl-CoA substrate has been confirmed. These involve purified enzymes and examples include a Linoleoyl-CoA Desaturase (Okayasu et al. (1981) Arch. Biochem. Biophys. 206: 21-28), a stearoyl-CoA desaturase from rat liver (Strittmatter et al (1974) Proc. Nat. Acad. Sci. USA 71: 4565-4569), and a Stearoyl-ACP desaturase from avocado (Shanklin J and Somerville C (1991) Proc Natl Acad Sci USA 88:2510-2514).

Alternatively, Heinz and coworkers have reported a strategy employing *in vivo* feeding of substrates to yeast strains expressing desaturases to examine substrate specificity of desaturases (Domergue et al. (2003) J. Biol. Chem. 278: 35115-35126 , Domergue et al. (2005) Biochem. J. 389: 483-490). In these studies predictions of a desaturases's preference for acyl-lipid substrates were based on data obtained from a thorough analysis of the desaturated products in the acyl-CoA, phospholipid and neutral lipid pools over a growth time course. However, highly active acyltransferases which transfer acyl-groups between various pools (e.g. CoA, ACP, and lipid) may influence or convolute these data (Domergue et al. (2005) Biochem. J. 389: 483-490, Meesapyodsuk, D., Qui, X. (2012) Lipids 47: 227-237). Therefore this approach was still limited by the absence of direct evidence, such as obtained from *in vitro* assays, needed for conclusive determination of the substrate form of the desatrurase.

Herein, we provide a previously unreported method to distinguish between enzymes that desaturate acyl-CoA fatty acids from enzymes that desaturate phospholipid linked fatty acids using microsomal preparations of proteins. We have improved upon initial reports of similar strategies to generate [^{14}C]-phosphatidylcholine analogs *in situ* (Stymne, S., and

Stobart, A. K. (1986) Biochem. J. 240: 385-393, Griffiths, G., Stobart, A. K., and Stymne, S. (1988) Biochem. J. 252: 641-647) by: (1) monitoring the initial acyl-transfer reaction catalyzed by LPCAT to establish that all of the [^{14}C]-acyl-CoA has been consumed, and (2) including exogenous lysophosphatidyl choline (LPC). Our improvements therefore establish that only [^{14}C]-phosphatidylcholine analogs are present upon initiation of the desaturase assay and allow for testing of other phospholipids by adding their corresponding lysolipid.

Demonstration of Phosphatidylcholine Specificity:

To test if a desaturase accepts an acyl-lipid (e.g. a phospholipid) substrate the enzyme reaction was performed as described above (Section A, General Desaturase Activity Assays), but after a pre-incubation and in the presence and absence of exogenous lysophosphatidyl choline (LPC). The microsomal fraction of the yeast strain expressing the enzyme of interest was pre-incubated with a [^{14}C]-labelled acyl-CoA substrate in the presence of 16:0-lysophosphatidyl choline, which was typically 50 μM but may vary from 0 – 500 μM. During the pre-incubation endogenous lysophosphatidyl choline acyl transferase (LPCAT), present in the microsomes, transfers the [^{14}C]fatty acid from CoA to 16:0-LPC generating, *in situ*, a [^{14}C]fatty acid-phosphatidylcholine (PC) (Jain et al. (2007) *J. Biol. Chem.* 282:30562-30569, Riekhof et al. (2007) *J. Biol. Chem.* 282:36853-36861, Tamaki et al. (2007) *J. Biol. Chem.* 282:34288-34298)). After a pre-incubation (typically 15 minutes, but may vary from 1 – 300 minutes) essentially all of the [^{14}C]-labelled acyl-CoA substrate was consumed, as measured by scintillation counting and TLC of the aqueous phase. Control reactions are performed in the absence of exogenous 16:0-LPC.

The reaction was stopped and lipids were extracted using the method of Bligh and Dyer (Bligh, E. G., and Dyer, J. J. (1959) *Can J. Biochem. Physiol.* 37: 911-918), by addition of 200 μl 0.15 M acetic acid and 1 ml MeOH:$CHCl_3$ (1:1). Part (about 10%) of the $CHCl_3$ phase (containing phosphatidyl choline (PC) and free fatty acids (FFA's)) was analyzed by scintillation counting and the rest was applied to a silica thin layer chromatography (TLC) plate. The plate was first developed in a polar solvent [$CHCl_3$:MeOH:acetic acid (90:15:10:3) and then in Heptane:diethylether:acetic acid (70:30:1) to measure incorporation into PC and the amount of FFA's (likely generated by thioesterases). PC and FFA's were scraped off the the plate and methylated by addition of MeOH containing 2% H_2SO_4 at 90 °C for 30 minutes. The methyl esters were extracted in

hexane and analyzed as as described above for the respective enzymes (Example 21, Section A). The upper (aqueous) phase of the reaction mixture extraction contains acyl-CoA's and was hydrolyzed by addition of an equal volume of 2 M KOH in MeOH:H_2O (1:4) and incubated for 20 minutes at 90 °C. Part of the aqueous phase was then analyzed by scintillation counting before fatty acids were extracted by addition of 3 M HCl (1 ml), 3.75 ml of MeOH:$CHCl_3$ (2:1) and $CHCl_3$ (1 ml). The chloroform phase was recovered, dried under N_2(g) and fatty acids were methylated by addition of 2 ml MeOH containing 2% H_2SO_4 and incubation of 30 minutes at 90 °C. FAMEs were extracted by addition of 2 ml H_2O and 2 ml hexane and separated by $AgNO_3$-TLC and Heptane:Diethyl ether:acetic acid (70:30:1) as a solvent or Reverse Phase-TLC (Silica gel 60 RP-18 using acetonitrile (100%)). The radioactive lipids were visualized and quantified by electronic autoradiography using Instant Imager.

Delta-12 desaturase (*Phytophthora sojae*), c-d12Des(Ps_GA) Substrate Preference:
The c-d12Des(Ps_GA) enzyme activity demonstrated in Section A can be further characterized to establish the backbone of the oleic acid substrate. In the desaturase assay described in Section C containing 16:0-lysphosphatidylcholine (LPC) substantial desaturation was observed. A significantly reduced, but detectable, desaturase activity was observed in control reactions lacking 16:0-LPC which likely results from acylation of endogenous LPC present in the yeast microsomes containing the d12Des(Ps_GA) protein. Additionally, separation of the phospholipids from the free-fatty acids after the enzymatic reaction and characterization of the isolable fatty acid methyl esters demonstrated that all of the d12Des(Ps_GA) enzymatically produced 18:2n-6-fatty acid methyl ester (FAME) was found in the phosphatidylcholine fraction (Figure 26, Panel A).

Delta-4 desaturase (*Thraustochytrium* ssp.), c-d4Des(Tc_GA) Substrate Preference:
The c-d4Des(Tc_GA) enzyme activity demonstrated in Section A can be further characterized to establish the backbone of the docosopentaenoic acid substrate. In the desaturase assay described in Section C without additional 16:0-lysphosphatidylcholine (LPC), desaturation was observed (Figure 26, Panel B), and likely results from the presence of endogenous 16:0-LPC present in the membranes containing the c-d4Des(Tc_GA) protein. The c-d4Des(Tc_GA) desaturase activity was dramatically stimulated by including additional 16:0-LPC in the assay (Figure 26, Panel B),

consistent with the observation endogenous lysophosphatidyl choline acyl transferase (LPCAT), present in the microsomes, transfers the [^{14}C]22:5n-3 from CoA to 16:0-LPC generating a [^{14}C]22:5n-3-phosphatidylcholine (PC) that was desaturated. Additionally, separation of the phospholipids from the free-fatty acids after the enzymatic reaction and characterization of the isolable fatty acid methyl esters demonstrated that essentially all of the c-d4Des(Tc_GA) enzymatically produced 22:6n-3-fatty acid methyl ester (FAME) was found in the phosphatidylcholine fraction (Figure 26, Panel B and Panel C).

Demonstration of acyl-CoA specificity:

The assay conditions were as described above in Section A (General Desaturase Activity Assays). Microsomal protein from yeast expressing d9Des(Sc) was used and to the assay, up to 5 nmol of 20:1-CoA was added. This assay may also include alternative acyl-CoA's such as: 18:1n-9-CoA, 18:2n-6-CoA, 20:3n-6-CoA, 20:4n-6-CoA, 22:5n-3-CoA. The reaction was stopped and lipids were extracted using the method of Bligh and Dyer (Bligh, E. G., and Dyer, J. J. (1959) *Can J. Biochem. Physiol.* **37**, 911-918), by addition of 200 µl 0.15 M acetic acid and 1 ml MeOH:$CHCl_3$ (1:1). Part (about 10%) of the $CHCl_3$ phase (containing phosphatidyl choline (PC) and free fatty acids (FFA's)) was analyzed by scintillation counting and the rest was applied to a silica thin layer chromatography (TLC) plate. The plate was first developed in a polar solvent [$CHCl_3$:MeOH:acetic acid (90:15:10:3) and then in Heptane:diethylether:acetic acid (70:30:1) to measure incorporation into PC and the amount of FFA's (likely generated by thioesterases). PC and FFA's were scraped off the plate and methylated by addition of MeOH containing 2% H_2SO_4 at 90 °C for 30 minutes. The methyl esters were extracted in hexane and analyzed as described above for the respective enzymes (SectionA). The upper (aqueous) phase of the reaction mixture extraction contains acyl-CoA's and was hydrolyzed by addition of an equal volume of 2 M KOH in MeOH:H_2O (1:4) and incubated for 20 minutes at 90 °C. Fatty acids were extracted by addition of 3 M HCl (1 ml), 3.75 ml of MeOH:$CHCl_3$ (2:1) and $CHCl_3$ (1 ml). The chloroform phase was recovered, dried under N_2(g) and fatty acids were methylated by addition of 2 ml MeOH containing 2% H_2SO_4 and incubation of 30 minutes at 90 °C. FAMEs were extracted by addition of 2 ml H_2O and 2 ml hexane and separated by $AgNO_3$-TLC and Heptane:Diethyl ether:acetic acid (70:30:1) as a solvent or Reverse Phase-TLC (Silica

gel 60 RP-18 using acetonitrile (100%)). The radioactive lipids were visualized and quantified by electronic autoradiography using Instant Imager.

To demonstrate acyl-CoA dependency both methods are tested. If desaturation does not occur in the method for determining PC-specificity (LPC addition and preincubation before adding NADH) and the method for determining acyl-CoA specificity (20:1-CoA addition) leads to the desaturated product in the H_2O-phase, it can be concluded that the enzyme was acyl-CoA dependent (see Figure 27).

In summary, we presented a previously unreported method to distinguish between enzymes that desaturate acyl-CoA fatty acids from enzymes that desaturate phospholipid linked fatty acids. This embodment of the invention t uses microsomal preparations of enzymes and does not, as in previous examples, require purification of the enzyme of interest. Furthermore, this embodiment allows isolation of the intact desaturated enzymatic product, allowing characterization of the backbone to which it was linked (e.g. lipid-, CoA-, or free fatty acid). An important consideration was that the endogenous lysophosphatidyl choline acyl transferase (LPCAT) present in yeast-derived microsomes can utilize a broad range of acyl-CoA's (Jain et al. (2007) *J. Biol. Chem.* 282:30562-30569, Riekhof et al. (2007) *J. Biol. Chem.* 282:36853-36861, Tamaki et al. (2007) *J. Biol. Chem.* 282:34288-34298)) making it suitable for generating an extensive variety of different phosphatidylcholine derivatives for assaying desaturase enzymes. LPCAT is able to accept 18:1n-9-CoA and 20:4n-6-CoA and this enzyme can acylate LPC with 22:5n-3-CoA.. Microsomes isolated from any cells or tissue can be used in this embodiment of the invention, including but not limited to bacterial cells (e.g. *Escherichia coli, Psuedomonas aeruginose*, *Bacillus thuringiensis*), mammalian tissue (e.g. liver) and plant tissue (e.g. leafs, roots, seeds, and pods) and could use exogenously supplied lysophosphatidyl choline acyl transferase from *Saccharomyces cerevisiae*, if necessary. Slight modifications to the general method presented here may include a pre-incubation with alternate acyl-CoA's, not the potential desaturase substrate, which could reduce the observed background due to endogenous LPC present in the membranes and also minimize thioesterase degradation of enzyme substrate or product acyl-coA's.

Elongase Activity.

Expression of elongase enzymes in yeast was performed as described above for the desaturase enzymes in Section A. Isolation of microsomes containing expressed elongases was generally as described above in Section 1 and by Denic (Denic, V. and Weissman (2007) Cell 130, 663-677). Briefly, cells from a yeast expression culture (50 ml) were resuspended in 1 ml of Elongase Disruption Buffer (20 mM Tris-HCl, pH 7.9, 10 mM $MgCl_2$, 1 mM EDTA, 5% glycerol, 0.3 M ammonium sulfate, protease inhibitor), mixed with 1 ml silica/zirconium beads (0.5mm) and disrupted in a BeadBeater. After centrifugation (two times for 5 minutes at 8000xg, 4 °C) the crude extract was recovered and after a second centrifugation (100,000xg, 2 hours at 4 °C), the microsomal fraction was resuspended in 500 µl of assay buffer (50 mM HEPES-KOH pH 6.8, 150 mM KOAc, 2 mM MgOAc, 1 mM $CaCl_2$, protease inhibitor). The protein concentrations in the microsomes were measured according to the BCA method. Resuspended microsomes were aliquoted and frozen in N_2(l) and stored at -80 °C.

In the elongase assay [^{14}C]-labeled malonyl-CoA and non-labeled acyl-CoA were provided as substrates. After the reaction has proceeded an appropriate time, which may vary between 0 - 300 minutes depending on the purpose of the experiment, the reaction mixture was subjected to hydrolysis and methylation and the FAMEs were analyzed by RP-TLC combined with by electronic autoradiography using Instant Imager.

The assay contains about 170 µg microsomal protein, 7.5 nmol [^{14}C]malonyl-CoA (3000 dpm/nmol), 5 nmol acyl-CoA in a total volume of 100 µl. After incubation for the desired time at 30 °C, the reaction was stopped with the addition of 100 µl of 2 M KOH in MeOH (1:4) followed by a 20 minute incubation at 90 °C. Fatty acids were extracted by addition of 3 M HCl (100 µl), 0.75 ml of MeOH:$CHCl_3$ (2:1) and $CHCl_3$ (250 µl). The chloroform phase was recovered, dried under N_2(g), and fatty acids were methylated by addition of 2 ml MeOH containing 2% H_2SO_4 and incubation of 30 minutes at 90 °C. FAMEs were extracted by addition of 2 ml H_2O and 2 ml hexane and separated by Reverse Phase-TLC (Silica gel 60 RP-18) using a solvent of acetonitrile:tetrahydrofuran (85:15). The radioactive lipids were visualized and quantified by electronic autoradiography using Instant Imager.

Furthermore, assays may include additional components (e.g. 1 mM NADPH, 2 mM $MgCl_2$, and 100 μM cerulenin) to complete the fatty acid reduction cycle by endogenous yeast enzymes, but limit further elongation of the acyl- CoA.

Delta-6 Elongase (*Thalassiosira pseudonana*), c-d6Elo(Tp_GA2) Enzyme Activity: c-d6Elo(Tp_GA2) enzyme activity and substrate specificity can be demonstrated in microsomes isolated from a yeast strain expressing the c-d6Elo(Tp_GA2) protein using [^{14}C]malonyl-CoA and an acyl-CoA in the general elongase assay described above. Acyl-CoA's may include, but are not limited to: 18:1n-9-CoA, 18:2n-6-CoA, 18:3n-6-CoA, 20:3n-6-CoA, 20:4n-6-CoA, 20:5n-3-CoA, 22:5n-3-CoA.

Delta-6 Elongase (*Physcomitrella patens*), c-d6Elo(Pp_GA2) Enzyme Activity: c-d6Elo(Pp_GA2) enzyme activity and substrate specificity can be demonstrated in microsomes isolated from a yeast strain expressing the c-d6Elo(Tp_GA2) protein using [^{14}C]malonyl-CoA and an acyl-CoA in the general elongase assay described above. Acyl-CoA's may include, but are not limited to: 18:1n-9-CoA, 18:2n-6-CoA, 18:3n-6-CoA, 20:3n-6-CoA, 20:4n-6-CoA, 20:5n-3-CoA, 22:5n-3-CoA.

Delta-5 Elongase (*Ostreococcus tauri*), c-d5Elo(Ot_GA3) Enzyme Activity: c-d5Elo(Ot_GA3) enzyme activity and substrate specificity can be demonstrated in microsomes isolated from a yeast strain expressing the c-d5Elo(Ot_GA3) protein using [^{14}C]malonyl-CoA and an acyl-CoA in the general elongase assay described above. Acyl-CoA's may include, but are not limited to: 18:1n-9-CoA, 18:2n-6-CoA, 18:3n-6-CoA, 20:3n-6-CoA, 20:4n-6-CoA, 20:5n-3-CoA, 22:5n-3-CoA.

In the presence of NADPH and [^{14}C]malonyl-CoA, 18:3n-6-CoA was elongated to 20:3 n-6-CoA by the delta-6 Elongases isolated from *Thalassiosira pseudonana* (Tp) and *Physcomitrella patens* (Pp) as shown if Figure 28, panels A and B. In both delta-6 elongase reactions the observed FAME-product co-migrates with 20:3n-6-methyl ester standards and was radioactive, consistent with transfer of two-carbons from [^{14}C]-malonyl-CoA to 18:3n-6-CoA. In the presence of NADPH the fatty acid reduction cycle was completed resulting in a saturated enzymatic product. However in the absence of NADPH a derivative of the direct enzymatic product, 3-keto-20:3n-6-CoA, was isolated

as a FAME. The isolated enzymatic product was decarboxylated and converted to the 2-keto-19:3n-6-FAME as described previously (Bernert, J.T and Sprecher, H. (1977) J. Biol. Chem. 252:6736-6744 and Paul et al (2006) J. Biol. Chem. 281: 9018-9029). Appropriate controls demonstrate that this elongation reaction was dependent upon either the Delta-6 Elo (Tp) or the Delta-6 Elo (Pp) and not catalyzed by endogenous yeast enzymes.

In the presence of NADPH and [^{14}C]malonyl-CoA , 20:5n-3-CoA was elongated to 22:5 n-3-CoA by the c-d5Elo(Ot_GA3), and containing either an N-terminal FLAG tag or a C-terminal FLAG tag, as shown in Figure 28, panel C. In the Delta-5 elongase reaction the observed FAME-product co-migrates with a 22:5n-3-methyl ester standard and was radioactive, consistent with transfer of two-carbons from [^{14}C]-malonyl CoA to 20:5n-3-CoA. In the presence of NADPH the fatty acid reduction cycle was completed resulting in a saturated enzymatic product. However in the absence of NADPH a derivative of the direct 3-keto-22:5n-3-CoA product was isolated as a FAME. The isolated enzymatic product was decarboxylated and a 2-keto-21:5n-3-FAME as described previously (Bernert, J.T and Sprecher, H. (1977) J. Biol. Chem. 252:6736-6744 and Paul et al (2006) J. Biol. Chem. 281: 9018-9029). Appropriate controls demonstrate that this elongation reaction was dependent upon the Delta-5 Elo (Ot) and not catalyzed by endogenous yeast enzymes.

Herein, using a highly sensitive elongase assay, we have demonstrated the enzyme activities of the Delta-6 Elongases used (Figure 28, panel A and B) and a Delta-5 Elongase (Figure 28, panel C), enzymes that are central to engineering canola to biosynthesize docosahexaenoic acid. For each of these elongases we have shown that in the presence of [^{14}C]malonyl-CoA and the appropriate fatty-acyl CoA ester substrate these enzymes can transfer two-carbons (containing [^{14}C]) from malonyl-CoA to the appropriate fatty-acyl-CoA ester to synthesize a new fatty acid which has been elongated by two carbons. In some cases a derivative (decarboxylated 2-keto compound) of the direct enzymatic product (3-Keto-acylCoA ester) of the elongase was observed, however in the absence of NADPH only this decarboxylated 2-keto compound was observed, consistent with previous observations by Napier (Bernert, J.T and Sprecher, H. (1977) J. Biol. Chem. 252:6736-6744 and Paul et al (2006) J. Biol.

Chem. 281: 9018-9029).

In summary we have provided a method that allows unequivocal demonstration of fatty acyl elongation enzyme activity. We provide data demonstrating that: (1) gene c-d6Elo(Tp_GA2) encodes a delta-6 elongase protein from *Thalassiosira pseudonana* (c-d6Elo(Tp_GA2)) that converts 18:3n-6-CoA to 20:3n-6-CoA in microsomes isolated from a transgenic yeast (Figure 28, panel A), (2) gene c-d6Elo(Pp_GA2) encodes a delta-6 elongase protein from *Physcomitrella patens* (c-d6Elo(Pp_GA2)) that converts 18:3n-6-CoA to 20:3n-6-CoA in microsomes isolated from a transgenic yeast (Figure 28, panel B), (3) a transgenic *B. napus* event containing both the gene encoding for the delta-6 elongase protein from *Thalassiosira pseudonana*, gene c-d6Elo(Tp_GA2), and the gene encoding for the gene the delta-6 elongase protein from *Physcomitrella patens*, gene c-d6Elo(Pp_GA2), contains at least one enzyme, localized to the microsomes, capable of elongating 18:3n-6-CoA to 20:3n-6-CoA (Figure 28, panel A) (4) gene c-d5-Elo(Ot_GA3) encodes a delta-5 elongase protein from *Ostreococcus tauri* (c-d5Elo(Ot_GA3)) that converts 20:5n-3-CoA to 22:5n-3-CoA in microsomes isolated from both a transgenic yeast (Figure 28, Panel C) and transgenic *B. napus* event (Figure 29, Panel B). In all examples presented no endogenous elongase activity was detected in microsomes isolated from either control yeast strains (Figure 28) or control Brassica lines (Figure 29).

Using the methods described in Section D for elongase proteins the level of expression or detected enzyme activity may be influenced by the presence or absence of fusion tags to the native protein. Fusion tags or proteins to the desaturases may be attached the amino-terminus (N-terminal fusions) or the carboxy-terminus (C-terminal fusions) of the protein and may include but are not limited to: FLAG, hexa-Histidine, Maltose Binding Protein, and Chitin Binding Protein.

We have provided methods to establish enzyme catalyzed elongase reactions required in an engineered pathway to biosynthesize docosohexaenoic acid (DHA, 22:6n-3) from oleic acid (18:1n-9) in canola. The methods presented in Example 21 were developed to demonstrate elongase activity in yeast strains expressing individual elongases and can be further used to confirm the respective elongase enzyme activities in transgenic

canola. Furthermore these methods can be incorporated, by one skilled in the art, to establish elongase enzyme activities in other organisms including, but not limited to: *Saccharomyces cerevisiae*, *Arabidopsis thaliana*, *Brassica* spp., *Camelina sativa*, *Carthamus tinctorius*, and *Salvia hispanica*.

Example 22: In vivo demonstration of mode of action: substrate specificity, substrate selectivity

Cloning of Genes into Yeast Expression Vectors:

For single gene expression, the yeast expression vector pYES2.1/V5-His-TOPO (Invitrogen) was used. Flanking primers were designed according to the manufacturer's instructions, and genes were amplified from plant expression vectors using the proof-reading polymerase Phusion high-fidelity polymerase (New England Biolabs). After agarose gel electrophoresis PCR fragments were cut out and purified using an EZ-10 spin column gel extraction kit (Bio Basic Inc.), cloned into the yeast expression vector pYES2.1/V5-His-TOPO (Invitrogen), transformed into *E. coli*, and gene orientation was checked by PCR. For co-expression of multiple genes, genes were cloned into pESC yeast expression vectors (Stratagene) under the control of the GAL1 promotor. To do this, appropriate restriction sites were introduced upstream and downstream of coding regions via PCR, followed by fragment isolation, TA-cloning into the pGEM-T vector, release of the gene fragment by enzyme digestion, and ligation into the pESC vector. For all constructs plasmids were isolated using an EZ-10 spin column plasmid DNA miniprep kit (Bio Basic Inc.). All constructs were sequenced prior to yeast transformation. After sequencing, plasmids were transformed into Saccharomyces cerevisiae (yeast) strain INVSc1 (Invitrogen), using the Sc EasyComp Transformation Kit (Invitrogen) according to the manufacturer's protocol, and selected on plates lacking the appropriate amino acids.

Expression of Heterologous Genes in Yeast:

Yeast cultures were grown overnight at 30°C in drop out base (DOB-URA: 1.7 g/L yeast nitrogen base, 5 g ammonium sulfate, and complete supplement mixture minus appropriate amino acids for selection) containing 2 % glucose. The OD600 of the overnight yeast cultures were obtained and culture concentrations were standardized between samples. The samples were washed with DOB-URA containing 2 % galactose

and expression was carried out for 3 days at 20 °C in the same media supplemented with exogenous fatty acids and 0.01 % tergitol. For exogenous fatty acid feeding, cells were fed with 0.25 mM of the appropriate fatty acids, except where indicated otherwise. Fatty acid substrates and FAME standards were purchased from Nu-Chek Prep Inc (Elysian, MN).

Fatty Acid Analysis by Gas Chromatography:

5 mL cultures were precipitated by centrifugation and washed once with induction buffer and once with water. The supernatant was removed, and 2 mL of 3N methanolic-HCl (Supelco) was added to the cell pellet. After gentle mixing, the mixture was incubated at 80°C for 40 min, cooled to room temperature, and 1 mL 0.9% NaCl plus 2 mL hexane was added. The sample was vortexed and centrifuged, and the hexane phase was removed and dried under nitrogen gas. Fatty acid methyl esters (FAMEs) were resuspended in 100 µl hexane and analyzed by gas chromatography using an Agilent 6890N gas chromatograph equipped with a DB-23 column. The thermal program used was 160 °C for 1 min, then temperature was increased to 240 °C at a rate of 4 °C/min. FAMEs were identified based on known standards and the conversion percent was calculated as: [(Product)x100%]/(Substrate + Product).

Time Course Studies:

Samples were grown overnight at 30°C, sample concentrations were standardized prior to feeding, and cultures were induced at 20°C. For some time course studies, samples were pre-induced overnight prior to feeding as indicated in results. Samples (1 ml of culture) were collected at the indicated time intervals, washed once with 0.5% tergitol and once with double distilled water, and pellets were stored at -80°C until the completion of the time course. Fatty acid extraction and GC analysis basically followed section 3, however 1 mL methanolic HCl and 0.5 mL 0.9% NaCl and 1mL hexane were used for fatty acid extraction, and the final resuspension was performed in 100 µL hexane.

Feeding Studies with Individual Fatty Acids:

Samples were grown overnight at 30°C, sample concentrations were standardized prior to feeding, and cultures were induced at 20°C for 3 days. Measurements from at least 3 clones were used to calculate averages.

Feeding Studies with Multiple Fatty Acids:

Fatty acids for co-feeding were selected based on results from experiments described above where cells expressing each construct were fed with all possible substrates. The positive substrates for a given enzyme were fed together, however, if one substrate formed a product that also was a known substrate (for example, with certain elongases), separate mixtures were used. For omega-3-desaturases, two mixtures, each containing SDA as a standard, were used for feeding. Initially, cultures were fed mixtures containing 0.25 mM total fatty acid substrate. However, due to differences in uptake, it was necessary to optimize fatty acid mixtures so that the levels of Substrate A + Product A was equal to Substrate B + Product B within ± 5%. The final fatty acid mixtures used were also subjected to GC to obtain an estimation of relative uptake. After appropriate fatty acid mixtures were obtained, induction and feeding was performed as described above (Expression of Heterologous Genes in Yeast). After induction, yeast cells were induced for 3 days at 20 °C before harvesting of cells and GC analysis as described above (Expression of Heterologous Genes in Yeast and Fatty Acid Analysis by Gas Chromatography). All experiments were replicated three times.

Co-expression Heterologous Genes in Yeast:

Protocols for co-expression of genes basically followed those provided for single gene expression experiments. Cultures were grow overnight at 30°C, sample concentrations were standardized prior to feeding, and cultures were induced at 20°C for 3 days. The following sets of genes were co-expressed (pY = pYES2.1/V5-His-TOPO ; pT= pESC-Typ, pL=pESC-Leu):

1) pY-c-d12Des(Ps_GA) /pT-c-d6Des(Pir_GA)
2) pY-c-d12Des(Ps_GA)/pT-c-d6Des(Ot_febit)
3) pY-c-d6Elo(Tp_GA2)/pT-c-d6Des(Ot_febit)
4) pY-c-d6Elo(Tp_GA2)/pT-c-d6Des(Pir_GA)
5) pL- c-d6Elo(Tp_GA2)/pY-c-d5Des(Tc_GA2)
6) pL-c-d8Des(Eg) /pY-c-d5Des(Tc_GA2)
7) pL- c-d6Elo(Tp_GA2)/pY-c-d5Des(Sa)

8) pL-c-d8Des(Eg)/pY-c-d5Des(Sa)

Isolation and Analysis of Lipids:

For expression of desaturases or elongases, yeast cultures were grown overnight at 30°C in drop out base (DOB-ura) containing 2% glucose. The samples were washed with induction media (DOB-ura) containing 2% galactose and expression was induced for 3 days at 20 °C in the same media supplemented with the appropriate fatty acids and 0.01 % tergitol (NP-40). After 3 days of incubation, yeast cells were collected by centrifugation and washed twice with distilled water. Fifteen mL of chloroform:methanol (1:1) was added to the collected yeast pellet and the sample was incubated at room temperature with shaking for 3 hr. After the 3 hr incubation, the sample was centrifuged and the aqueous phase was collected and stored at -20°C. Chloroform:methanol (2:1) was added to the pellet, and it was incubated overnight at 4°C, then subjected to centrifugation. The aqueous phase was collected and pooled with the aqueous phase collected previously. Nine mL of 0.45% NaCl was added to the pooled aqueous phase, the sample was vortexed and the organic phase was collected after separated by centrifugation at 1500 x g for 3 min. The organic phase was dried under nitrogen gas and resuspended in 100 uL of chloroform to give the total fatty acid fraction. 5 uL of the total fatty acid fraction was analyzed by GC, and the remainder was separated by TLC as described below.

TLC plates were heat activated for at least 3 hrs at 120°C before use. The mobile phase used to separate the fatty acids consisted of chloroform : methanol : acetic acid (65:35:8) and primuline (5mg in 100mL acetone : water, 80:20) was used to visualize the separated lipid fractions under UV light. The individual lipid fractions (PC, PE, PI+PS, and neutral lipids) were identified using standards and removed separately from the TLC plate. To extract the lipids from the silica, 400 uL water, 2 mL chloroform and 2 mL methanol were added, the sample was vortexed vigorously, and 2 mL of 0.2 M H_3PO_4/ 1M KCl was added. The lower organic phase was collected and the remaining aqueous phase was re-extracted by adding 2 mL of chloroform. The pooled organic phase was dry under nitrogen gas, and the fatty acid profile of individual lipid fractions was analyzed by gas chromatography. GC was performed by adding 2 mL of 3N methanolic HCl, followed by incubation at 80 °C for 40 min, then adding 1 ml of 0.9% NaCl and 2 mL of hexane and vortexing. The hexane phase was collected, dried under nitrogen gas, and resuspended in 100 µL of hexane for GC analysis. Gas

chromatography was performed using an Agilent 6890N gas chromatograph equipped with a DB-23 column and a thermal program consisting of: 160 °C for 1 min, then temperature was increased to 240 °C at a rate of 4 °C/min. After the initial GC analysis, samples with low concentrations were re-suspended in 40µL of hexane and re-analyzed.

Time Course studies:

Initially, a range of conditions were tested with the c-d5Des(Tc_GA2) (Figure 30). When cultures were fed with 0.25 mM exogenous substrate at the beginning of induction the amounts of product and substrate and the desaturation percentage remained steady from approximately 48-92 hours (Figure 30, Panel A). Therefore, these conditions were used for further time course experiments.

To determine if these conditions were acceptable for all gene constructs, time courses were conducted with yeast cultures expressing all 10 genes individually. The preferred substrate for each enzyme was supplied exogenously (Figure 31).

Although the rate of uptake of fatty acids and the activity of enzymes varied, the levels of product, substrate and percent elongation or desaturation was relatively stable at the 72 hour time point (Figure 31). Therefore data collection at 72 hours was used in all further experiments, except where noted otherwise.

Analysis of all enzymes with all fatty acids:

The major activity of each desaturase and elongase used in the plant construct was determined upon gene isolation. However, many desaturases and elongases have secondary activities. When all enzymes are present together, such secondary activities affect the overall production rates of desired fatty acids, or lead to the production of side-products. In this experiment, we tested all enzymes with all fatty acids that might be expected to be present in a plant expressing all 10 genes. The enzymes and fatty acids used in this experiment are listed below:

Enzyme	Fatty acid
c-d12Des(Ps_GA)	18:1n-9
c-d6Des(Ot_febit)	LA (18:2n-6)
c-d6Elo(Pp_GA2)	ALA (18:3n-3)
c-d6Elo(Tp_GA2)	GLA (18:3n-6)
c-d5Des(Tc_GA2)	SDA (18:4n-3)
c-o3Des(Pi_GA2)	DHGLA (20:3n-6)
c-o3Des(Pir_GA)	ARA (20:4n-6)
c-d5Elo(Ot_GA3)	DTA (22:4n-6)
c-d4Des(Pl_GA)2	DPAn-3 (22:5n-3)
c-d4Des(Tc_GA)	DPAn-6 (22:5n-6)
	DHAn-3 (22:6n-3)

Although each individual substrate fatty acid was tested with each individual enzyme, results are only given for fatty acid/enzyme combinations where a detectable level of activity was obtained (see Table 159, Table 160, and Table 161).

Although we saw some unexpected activities with the desaturases, such as the conversion of GLA to SDA by the c-d5Des(Tc_GA2) and conversion of DHGLA to ARA by the c-d4Des(Tc_GA), these activity levels were low and produce fatty acids that would be expected in the DHA synthesis pathway (Table 159).

Table 159. Conversion percentages of exogenous fatty acids by desaturases.

Enzyme	Substrate	Product	% desaturation
c-o3Des(Pir_GA)	GLA	SDA	2.7 ± 0.05
	DPAn-6	DHAn-6	6.93 ± 0.85
	ARA	EPA	45.44 ± 2.40
	LA	ALA	6.29 ± 0.28
	DTA	DPAn-3	25.57 ± 1.16
	DHGLA	ETA	31.64 ± 3.38
c-o3Des(Pi_GA2)	GLA	SDA	7.84 ± 0.53
	DPAn-6	DHAn-3	4.77 ± 0.78
	ARA	EPA	40.0 ± 3.38

Enzyme	Substrate	Product	% desaturation
	LA	ALA	5.47 ± 0.76
	DTA	DPAn-3	17.86 ± 0.43
	DHGLA	ETA	39.71 ± 2.37
c-d5Des(Tc_GA2)	DHGLA	ARA	58.03 ± 2.01
	GLA	SDA	0.23 ± 006
c-d4Des(Tc_GA)	DPAn-3	DHAn-3	20.74 ± 1.43
	DTA	DPAn-6	17.33 ± 0.24
	DHGLA	ARA	1.15 ± 0.12
c-d4Des(Pl_GA)2	DPAn-3	DHAn-3	43.78 ± 3.85
	DTA	DPAn-6	44.26 ± 4.83
c-d12Des(Ps_GA)	18:1	LA	57.04 ± 0.49
	16:1*	16:2	32.42 ± 0.64
c-d6Des(Ot_febit)	LA	GLA	68.82 ± 1.89
	ALA	SDA	71.0 ± 0.68

The elongases generally showed a higher level of side activities (Table 160, Table 161). While the two delta-6 elongases showed similar conversion percentages for the main substrates GLA and SDA, the P. patens enzyme showed higher levels of activity on LA and ALA, and only the P. patens delta-6 elongases showed detectable activity of ARA and EPA. Nonetheless, activity levels were substantially higher with GLA and SDA, suggesting that if these fatty acids, which were expected to be available in the pathway, would be the preferred substrate of the enzyme.

Table 160. Conversion percentages of exogenous fatty acids by Delta-6 Elongases

Enzyme	Substrate	Product	% Elongation
c-d6Elo(Pp_GA2)	GLA	DHGLA	80.23 ± 0.81
	SDA	20:4n-3	85.67 ± 1.18

Enzyme	Substrate	Product	% Elongation
	LA	20:2n-6	20.86 ± 1.23
	ALA	20:3n-3	31.19 ± 0.23
	ARA	DTA	2.42 ± 0.08
	EPA	DPAn-3	5.16 ± 0.29
c-d6Elo(Tp_GA2)	GLA	DHGLA	70.11 ± 1.37
	SDA	20:4n-3	85.55 ± 0.32
	LA	20:2n-6	2.22 ± 0.15
	ALA	20:3n-3	4.63 ± 0.35

The c-d5Elo(Ot_GA3) was capable of consecutive elongations of most fatty acids (Table 161). However, the highest conversion by a large margin was for the desired EPA to DPAn-3 conversion. In the engineered plant system, the two delta-4 desaturases are available to desaturate DPAn-3, preventing further elongation. The levels of fatty acids containing 24 carbons produced by this elongase are small given that high levels of substrate are present in the yeast system and no competing enzymes are present.

Table 161. Conversion percentages of exogenous fatty acids by c-d5Elo(Ot_GA3) elongase. Substrate and Product values represent % of total fatty acids.

Substrate	Product 1	Product 2	Product 3	% Elongation
LA	**20:2n-6**			
24.34 ± 1.28	0.41 ± 0.07			1.64 ± 0.18
DHGLA	**22:3n-6**			
12.92 ± 0.93	1.24 ± 0.22			8.73 ± 1.10
DHAn-3	**24:6n-3**			
9.41 ± 0.86	1.37 ± 0.12			12.84 ± 1.84
DPAn-3	**24:5n-3**			
10.85 ± 0.46	2.15 ± 0.28			16.59 ± 2.37
GLA	**DHGLA**	**22:3n-6**		
23.95 ± 3.22	1.0 ± 0.13	0.24 ± 0.03		4.91 ± 0.24
ARA	**22:4n-6**	**24:4n-6**		
7.12 ± 1.33	11.23 ± 0.28	0.26 ± 0.05		61.92 ± 5.22
EPA	**DPAn-3**	**24:5n-3**		

Substrate	Product 1	Product 2	Product 3	% Elongation
0.85 ± 0.01	6.76 ± 0.85	1.74 ± 0.21		90.8 ± 0.98
SDA	**20:4n-3**	**22:4n-3**	**24:4n-3**	
14.42 ± 1.18	0.47 ± 0.04	4.17 ± 0.12	0.26 ± 0.03	25.48 ± 1.72
ALA	**20:3n-3**	**22:3n-3**	**24:3n-3**	
20.09 ± 3.23	1.79 ± 0.28	1.70 ± 0.35	0.15 ± 0.03	15.38 ± 2.06

Since 20:4n-3 was not commercially available was generated in vivo by co-expression of c-d6Elo(Tp_GA2) with each of the other desaturases or elongases. Therefore upon supplying exogenous SDA, 20:4n-3 was produced by the c-d6Elo(Tp_GA2) and the efficiency of conversion of this fatty acid by the second enzyme was measured.

Table 162. Enzyme activity with 20:4n-3.

Helper Enzyme (delta-6 elongase) pL vector	2nd enzyme in pY vector	delta-6 elongase conversion efficiency (%)	Conversion efficiency of 2nd enzyme (%)	Gene dosage effect
c-d6Elo(Tp_GA2)	c-d4Des(Pl_GA)2	70.0 ± 1.2	N/A	N/A
c-d6Elo(Tp_GA2)	c-d4Des(Tc_GA)	69.1 ± 0.7	3.3 ± 0.2	N/A
c-d6Elo(Tp_GA2)	c-d5Des(Tc_GA2)	64.3 ± 1.4	86.2 ± 1.0	N/A
c-d6Elo(Tp_GA2)	c-d6Des(Ot_febit)	72.9 ± 2.1	2.9 ± 0.2	N/A
c-d6Elo(Tp_GA2)	c-d12Des(Ps_GA)	70.3 ± 1.3	N/A	N/A
c-d6Elo(Tp_GA2)	c-o3Des(Pi_GA2)	67.4 ± 3.6	N/A	N/A
c-d6Elo(Tp_GA2)	c-o3Des(Pir_GA)	70.0 ± 1.1	N/A	N/A
c-d6Elo(Tp_GA2)	c-d5Elo(Ot_GA3)	68.8 ± 1.4	80.2 ± 2.5	N/A
c-d6Elo(Tp_GA2)	c-d6Elo(Pp_GA2)	89.8 ± 0.7	N/A	YES (Δ6 ELO)
c-d6Elo(Tp_GA2)	c-d6Elo(Tp_GA2)	90.3 ± 0.8	N/A	YES (Δ6 ELO)

* Cultures were fed with SDA and 20:4n-3 (delta-6 elongation) was produced as a substrate for either desaturases or elongases. Three repeats were measured. pY: pYES2.1/V5-His-TOPO expression vector; pL: pESC-Leu expression vector. All genes were placed under the control of the Gal1 promoter. N/A: not applicable; DES: desaturase; ELO: elongase

The c-d4Des(Tc_GA), the c-d5Des(Tc_GA2), the c-d6Des(Ot_febit), and the c-d5Elo(Ot_GA3) were able to use 20:4n-3 as a substrate (Table 162). The c-d5Des(Tc_GA2) showed the highest conversion percentage, which would be expected as 20:4n-3 was a known substrate for delta-5 desaturases. The c-d5Des(Tc_GA2) was also able to produce 22:4n-3. When using two delta-6 elongases in one construct, a gene dosage effect was observed; the delta-6 elongase activity increased in the presence of two c-d6Elo(Tp_GA2), or a gene pair consisting of a c-d6Elo(Tp_GA2) and a c-d6Elo(Pp_GA2).

Fatty acid specificity of desaturases and elongases of this invention

Enzymes were provided with mixtures of fatty acids to allow comparisons of relative activity when two substrates were present concurrently; an enzyme may be more active on substrate A than substrate B when they are supplied individually, but when they are supplied at the same time the relative activity of the enzyme on each substrate could change. Fatty acids of the same length were preferably provided at the same time, since they would be most likely to be present in the plant concurrently. Mixtures of fatty acids were adjusted so that the level of (substrate 1 + product 1) = (substrate 2 + product 2) within ± 5%.

Table 163. Fatty acid preferences of elongases and desaturases

Gene (Fatty Acid Mix)	Substrate	% conversion
c-d5Elo(Ot_GA3)	EPA	90.36 ± 2.17
(EPA/ARA/DHGLA)	ARA	40.96 ± 4.50
	DHGLA	3.14 ± 0.47
c-d5Elo(Ot_GA3)	DPAn-3	4.75 ± 0.57
(DPAn-3/DHA)	DHA	5.69 ± 0.56
c-d5Elo(Ot_GA3)	LA	0.57 ± 0.06
(LA/ALA)	ALA	13.43 ± 1.65
c-d5Elo(Ot_GA3)	GLA	2.89 ± 0.06
(GLA/SDA)	SDA	29.24 ± 0.73
c-d4-Des(Tc_GA)	DTA	21.01 ± 2.28
(DTA/DPAn-3)	DPAn-3	25.18 ± 2.08
c-d4-Des(Pl_GA)2	DTA	34.41 ± 1.63
(DTA/DPAn-3)		
	DPAn-3	41.27 ± 2.07
c-d6-Des(Ot_febit)	LA	56.62 ± 2.06

Gene (Fatty Acid Mix)	Substrate	% conversion
(LA/ALA)	ALA	77.66 ± 0.80
c-d6-Elo(Pp_GA2)	LA	11.38 ± 0.94
(LA/ALA)	ALA	24.41 ± 1.84
c-d6-Elo(Pp_GA2)	GLA	81.59 ± 1.66
(GLA/SDA)	SDA	85.23 ± 1.28
c-d6-Elo(Tp_GA2)	LA	1.93 ± 0.15
(LA/ALA)	ALA	6.41 ± 0.68
c-d6-Elo(Tp_GA2)	GLA	81.61 ± 1.03
(GLA/SDA)	SDA	87.70 ± 0.87
c-d5-Des(Tc_GA2)	DHGLA	85.0 ± 2.38
(DHGLA/ETA)	ETA (20:4n-3)	90.75 ± 1.15
c-o3-Des(Pi_GA2)	DHGLA	28.82 ± 1.26
(DHGLA/ARA/DTA)	ARA	39.15 ± 1.19
	DTA	7.32 ± 0.63
c-o3Des(Pir_GA)	DHGLA	25.22 ± 1.70
(DHGLA/ARA/DTA)	ARA	53.19 ± 2.52
	DTA	12.66 ± 1.17

As described in Table 163 the relative activities of the omega-3 desaturases follow the same order as described earlier (Table 159), but that the activity of both omega-3 desaturases was lower on DTA when DHGLA and ARA were available. Both delta-4 desaturases showed a preference for the omega-3 substrate when the omega-3 and omega-6 substrates were present in equal amounts. The preference of the c-d6Des(Ot_febit) for the omega-3 substrate was also increased when both substrates are available (Table 163). For the delta-5 elongase, the relative elongation of secondary substrates generally dropped when the primary, or preferred substrate was available (Table 163).

Additive enzyme activity supporting MoA (CoA vs PC)

The delta-5 desaturase genes from *S. arctica* and *Thraustochytrium* sp. were cloned into the pYES2.1/V5-His-TOPO expression vector, and both the c-d6Elo(Tp_GA2) and the c-d8Des(Eg) were cloned into the pESC-Leu expression vector. Yeast was transformed with the appropriate vector pairs and selection on DOB-uracil-leucine.

Positive cultures were grown and induced and GC analysis was conducted as described above in Example 23. All cultures were grown concurrently.

Table 164. Deduced mode of action of the Delta-5 desaturases.

Helper Enyzme known to work in PC (delta-8 DES)	delta-5 DES	delta-% DES Conversion efficiency	Deduced MoA of delta-5 DES
c-d8Des(Eg)	c-d5Des(Sa)	38 %	
c-d8Des(Eg)	c-d5Des(Tc_GA2)	36 %	
Helper Enyzme known to work in CoA (delta-6 ELO)	**delta-5 DES**	**delta-% DES Conversion efficiency**	
c-d6Elo(Tp_GA2)	c-d5Des(Sa)	32 %	PC
c-d6Elo(Tp_GA2)	c-d5Des(Tc_GA2)	80 %	CoA

As described in Table 164, the delta-5 desaturase activity of the c-d5Des(Sa) on HGLA was similar regardless if the DHGLA was derived from elongation in the coenzyme A (CoA) pool or desaturation in the phosphatidylcholine (PC) pool. Conversely, Table 164 also shows that the delta-5 desaturase activity of the c-d5Des(Tc_GA2) was much higher if the DHGLA substrate was derived from elongation in the CoA pool. In combination with data from lipid analysis by TLC, this suggests that the c-d5Des(Tc_GA2) was capable of desaturating substrates in the CoA pool, although it does not indicate that desaturation was limited to this pool.

The level of delta-6-elongation following desaturation by a desaturase active in the PC pool (c-d6Des(Pir_GA)) and a desaturase thought to be active in the CoA pool (c-d6Des(Ot_febit)) was determined. Since elongation takes place in the acyl-CoA pool, more efficient elongation can occur following desaturation by an acyl-CoA dependant desaturase. The two desaturases were also expressed in the presence of a delta -12 desaturase that was active on PC substrates, to allow the efficiency of subsequent delta -6 desaturation to be compared. Finally, the effects of individual desaturase genes and pairs of desaturase genes on elongase and desaturase activity were compared to determine dosage effects.

Table 165. Effects of various delta-6 desaturases on subsequent delta-6 elongation*.

Construct	delta- 6 Des %	delta- 6 ELO %
pT-c-d6Des(Ot_febit) & pY-c-d6Elo(Tp_GA2)	44.9 ± 1.0	89.3 ± 1.4
pT-c-d6Des(Pir_GA) & pY-c-d6Elo(Tp_GA2)	19.7 ± 0.5	63.7 ± 4.8

* Cultures were fed with LA and production of GLA (delta-6 desaturation) and HGLA (delta-6 elongation) were measured. pY: pYES2.1/V5-His-TOPO expression vector pT: pESC-trp expression vector. All genes were under the control of the Gal1 promoter.

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Table 166. Effects of delta-12 desaturation on subsequent delta-6 elongation*.

Construct	delta-12 DES %	delta-6 DES %
pT-c-d6Des(Ot_febit) & pY-c-d12Des(Ps_GA)	60.2 ± 2.9	23.2 ± 0.1
pT-c-d6Des(Pir_GA) & pY-c-d12Des(Ps_GA)	62.8 ± 1.4	17.1 ± 1.7

* Cultures were supplied with exogenous 18:1n-9. The production of LA (delta-12 desaturation) and GLA (delta-6 desaturation) were measured. pY: pYES2.1/V5-His-TOPO expression vector pT: pESC-trp expression vector. All genes were under the control of the Gal1 promoter.

GLA conversion by the c-d6Elo(Tp_GA2) was higher with GLA derived from desaturation by the c-d6Des(Ot_febit) compared to GLA derived by desaturation with the c-d6Des(Pir_GA) (Table 165). This shows that c-d6Des(Ot_febit) acts in the acyl-CoA pool, making the resulting substrate more readily available for the delta-6 elongase, and therefore resulting in a higher elongase activity. Correspondingly, the delta-6 desaturase activity of the c-d6Des(Ot_febit) was reduced by almost half if the substrate was derived from delta-12 desaturation in the PC pool (Table 165 and Table 166), whereas the the delta-6 desaturase activity of the c-d6Des(Pir_GA) was similar whether the substrate (LA) was derived from delta-12-desaturation of OA in the PC pool, or was supplied exogenously (Table 165 and Table 166). Exogenously supplied substrate was believed to enter the yeast cell in the form of acyl-CoA. In combination with data from lipid analysis by TLC, this data suggests that the c-d6Des(Ot_febit) was acyl-CoA-dependent enzyme, consistent with a previous prediction (Domergue et al. (2005) Biochem. J. 389: 483-490).

Table 167. Effect of substrate pool and gene dosage on delta-6-desaturation and subsequent delta-6-elongation of ALA.

Helper Enzyme (delta-6 elongase) pL vector	First delta-6 desaturase pY vector	Second delta-6 desaturase pT vector	delta-6 desaturase conversion efficiency	delta-6 elongase conversion efficiency	Gene dosage effect	Deduced MoA of 1st delta-6 desaturase	Deduced MoA of 2nd delta-6 desaturase
c d6Elo(Tp_GA2)	c-d6Des(Ot_febit)	pT vector	64.3 ± 1.5	73.3 ± 0.2	N/A	CoA	N/A
c-d6Elo(Tp_GA2)	c-d6Des(Pir_GA)	pT vector	26.8 ± 11.0	53.0 ± 8.8	N/A	PC	N/A
c-d6Elo(Tp_GA2)	pY vector	c-d6Des(Ot_febit)	60.8 ± 2.3	80.8 ± 0.6	N/A	N/A	CoA

Helper Enzyme (delta-6 elongase) pL vector	First delta-6 desaturase pY vector	Second delta-6 desaturase pT vector	delta-6 desaturase conversion efficiency	delta-6 elongase conversion efficiency	Gene dosage effect	Deduced MoA of 1st delta-6 desaturase	Deduced MoA of 2nd delta-6 desaturase
c-d6Elo(Tp_GA2)	pY vector	c-d6Des(Pir_GA)	27.9 ± 0.9	52.0 ± 0.9	N/A	N/A	PC
c-d6Elo(Tp_GA2)	c-d6Des(Ot_febit)	c-d6Des(Pir_GA)	72.5 ± 2.2	71.1 ± 2.1	Yes (DES)	CoA	PC
c-d6Elo(Tp_GA2)	c-d6Des(Pir_GA)	c-d6Des(Pir_GA)	40.5 ± 1.3	47.5 ± 2.0	Yes (DES)	PC	PC
c-d6Elo(Tp_GA2)	c-d6Des(Ot_febit)	c-d6Des(Ot_febit)	72.4 ± 0.9	78.1 ± 0.7	Yes (DES)	CoA	CoA

* Cultures were fed with ALA and production of SDA (delta-6 desaturation) and 20:4n-3 (delta-6 elongation) was measured. pY: pYES2.1/V5-His-TOPO expression vector; pL: pESC-Leu expression vector; pT: PESC-trp. All regulated by Gal1 promoter. N/A: not applicable; DES: desaturation conversion percent. Data from at least 3 clones were used for each measurement.

Table 168. Effect of substrate pool and gene dosage on delta-6-desaturation and subsequent delta-6-elongation of LA.

Helper Enzyme (delta-6 elongase) pL vector	First delta-6 desaturase pY vector	Second delta-6 desaturase pT vector	delta-6 desaturase conversion efficiency	delta-6 elongase conversion efficiency	Gene dosage effect	Deduced MoA of 1st delta-6 desaturase	Deduced MoA of 2nd delta-6 desaturase
c-d6Elo(Tp_GA2)	c-d6Des(Ot_febit)	pT vector	60.3 ± 7.0	65.6 ± 1.8	N/A	CoA	N/A
c-d6Elo(Tp_GA2)	c-d6Des(Pir_GA)	pT vector	23.4 ± 15.5	43.2 ± 8.6	N/A	PC	N/A
c-d6Elo(Tp_GA2)	pY vector	c-d6Des(Ot_febit)	48.5 ± 0.2	75.6 ± 1.5	N/A	N/A	CoA
c-d6Elo(Tp_GA2)	pY vector	c-d6Des(Pir_GA)	23.0 ± 0.8	36.9 ± 2.2	N/A	N/A	PC
c-d6Elo(Tp_GA2)	c-d6Des(Ot_febit)	c-d6Des(Pir_GA)	3.0 ± 1.4	60.1 ± 1.9	Yes (DES)	CoA	PC
c-d6Elo(Tp_GA2)	c-d6Des(Pir_GA)	c-d6Des(Pir_GA)	35.1 ± 2.2	35.4 ± 4.8	Yes (DES)	PC	PC
c-d6Elo(Tp_GA2)	c-d6Des(Ot_febit)	c-d6Des(Ot_febit)	62.5 ± 1.4	66.8 ± 2.5	Yes (DES)	CoA	CoA

* Cultures were fed with LA and production of GLA (delta-6 desaturation) and DHGLA (delta-6 elongation), three repeats were measured. pY: pYES2.1/V5-His-TOPO expression vector; pL: pESC-Leu expression vector; pT: PESC-trp. All genes were placed under the control of the Gal1 promoter. N/A: not applicable; DES: desaturation conversion percent.

When co-expressing the c-d6Elo(Tp_GA2) and the c-d6Des(Ot_febit) (CoA), a higher elongase conversion efficiency was achieved compared to co-expressing the c-d6Elo(Tp_GA2) and the c-d6Des(Pir_GA) (PC), regardless of whether n-3 or omega-6 substrate was supplied exogenously (Table 167, Table 168). This was observed whether the relevant desaturase gene was cloned into the pYes vector or the pESC-trp vector. A slight increase in desaturation efficiency was observed in the presence of two

desaturase genes, particularly in cultures supplied with the omega-3 substrate ALA. Compared to cultures carrying a single c-d6Des(Ot_febit), the proportion of substrate elongated was only slightly enhanced in the presence of two acyl-CoA dependant c-d6Des(Ot_febit), or in the presence of both desaturases. Co-expression of the c-d6Elo(Tp_GA2) with two copies of c-d6Des(Pir_GA) (PC) resulted in the lowest elongation conversion level among the cultures carrying two desaturases. The presence of the with acyl-CoA dependent delta-6 desaturase contributed to a higher delta-6 elongation activity whether it was expressed alone or along with a second desaturase, further indicating the usefulness of this gene for VLC-PUFA synthesis.

The effect of substrate pool for various delta-4 desaturases was determined in the presence of a helper enzyme, the c-d5Elo(Ot_GA3), which was known to work in the CoA pool. The gene dosage effect of delta-4 desaturase genes was also determined.

Table 169. Effect of substrate pool and gene dosage effect for delta-4 desaturases (exogenous EPA supplied).

Helper Enzyme (delta-5 elongase) pL vector	First delta-4 desaturase pY vector	Second delta-4 desaturase pT vector	delta-4 desaturase conversion efficiency	Gene dosage effect	Deduced MoA of 1st delta-4 desaturase	Deduced MoA of 2nd delta-4 desaturase
c-d5Elo(Ot_GA3)	c-d4Des(Pl_GA)2	pT vector	29.9 ± 4.0	N/A	CoA	NA
c-d5Elo(Ot_GA3)	c-d4Des(Tc_GA)	pT vector	31.2 ± 2.8	N/A	PC	NA
c-d5Elo(Ot_GA3)	c-d4Des(Pl_GA)2	c-d4Des(Tc_GA)	53.6 ± 1.0	++ (0.88)	CoA	PC
c-d5Elo(Ot_GA3)	c-d4Des(Eg)	c-d4Des(Tc_GA)	36.9 ± 3.5	Yes	PC	PC
c-d5Elo(Ot_GA3)	c-d4Des(Tc_GA)	c-d4Des(Tc_GA)	40.2 ± 0.2	+ (0.64)	PC	PC

* Cultures were fed with EPA and production of DPA (delta-5 elongation) and DHA (delta-4 desaturation) was measured for at least three samples. pY: pYES2.1/V5-His-TOPO expression vector; pL: pESC-Leu expression vector; pT: PESC-trp. All genes were placed under the control of the Gal1 promoter. N/A: not applicable. Dosage effect: number in brackets = conversion efficiency of two-desaturase construct/ (conversion efficiency of single gene A construct + conversion efficiency of single gene B construct).

Table 170. Effect of substrate pool and gene dosage effect for delta-4 desaturases (exogenous ARA supplied).

Helper Enzyme (delta-5 elongase) pL vector	First delta-4 desaturase pY vector	Second delta-4 desaturase pT vector	delta-4 desaturase conversion efficiency	Gene dosage effect	Deduced MoA of 1st delta-4 desaturase	Deduced MoA of 2nd delta-4 desaturase

Helper Enzyme (delta-5 elongase) pL vector	First delta-4 desaturase pY vector	Second delta-4 desaturase pT vector	delta-4 desaturase conversion efficiency	Gene dosage effect	Deduced MoA of 1st delta-4 desaturase	Deduced MoA of 2nd delta-4 desaturase
c-d5Elo(Ot_GA3)	c-d4Des(Pl_GA)2	pT vector	28.8 ± 1.0	N/A	CoA	N/A
c-d5Elo(Ot_GA3)	c-d4Des(Tc_GA)	pT vector	27.0 ± 2.5	N/A	PC	N/A
c-d5Elo(Ot_GA3)	c-d4Des(Pl_GA)2	c-d4Des(Tc_GA)	44.0 ± 0.9	++ (0.79)	CoA	PC
c-d5Elo(Ot_GA3)	c-d4Des(Eg)	c-d4Des(Tc_GA)	29.4 ± 0.6	-	PC	PC
c-d5Elo(Ot_GA3)	c-d4Des(Tc_GA)	c-d4Des(Tc_GA)	32.2 ± 0.8	-	PC	PC

*Cultures were fed with ARA and production of DTA (delta-5 elongation) and DPAn-6 (delta-4 desaturation) were measured. pY: pYES2.1/V5-His-TOPO expression vector; pL: pESC-Leu expression vector; pT: PESC-Trp expression vector. All genes were placed under control of the Gal1 promoter. N/A: not applicable. Dosage effect: number in brackets = conversion efficiency of two-desaturase construct/ (conversion efficiency of single gene A construct + conversion efficiency of single gene B construct).

The desaturation levels achieved by the *P. lutheri* and c-d4Des(Tc_GA)s were similar. When two delta-4 desaturases were used, a significant gene dosage effects only observed with the *P. lutheri/Thraustochyrium* sp. pair, and the lowest with two copies of the *Thraustocytrium* sp desaturase. These trends were observed in the presence of elongated ARA (Table 170) and EPA (Table 169). However, with the n-6 elongation substrate (ARA), the desaturation conversion percentage was generally slightly lower compared to the omega-3 substrate (EPA).

Example 23: Spacer Regions Containing Transcription Factor Binding Motifs

As indicated in examples 3 and 4, between each expression cassette in the constructs used in the invention there is a spacer region of 100-200 base pairs. For the BiBAC T-DNAs, the short stretches of sequence in between the gene cassettes, which had been multi-cloning sites and Gateway site sequences in the co-transformation vectors, were subjected to randomization to maintain the GC content and to remove multiple repeats of identical sequence. The skilled worker would recognize that the presence of multiple repeats within a construct, in particular large multi-gene constructs, could result in internal deletions and rearrangements as the plasmid was cloned into *Escherichia coli* and passaged through the *Agrobacterium* strain/species used. After removal of repeats and adjustment of GC content, the sequences of the spacer regions were then examined for any possible binding sites for transcription factors and other DNA

interacting proteins. The spacer region sequences used are RTP10690_1 through RTP10690_12, including the AtAHAS promoter and terminator, RTP10690_5', LJB2197_5' and LJB2197_1 through LJB2197_4. The underscore numbers also represent the order in which that spacer region appears in the cassette reading from the right border towards the left border, in the sense orientation of the GOI in the proceeding cassette. For example, RTP10690_1 was the sequence after the terminator of the first cassette and before the promoter of the second cassette in the construct VC-RTP10690-1qcz_F. The terminator of the AHAS selection cassette and the 5' region of the first cassette were also examined.

>RTP10690_1
TTAATTCAGCTAGCTAGCCTCAGCTGACGTTACGTAACGCTAGGTAGCGTCACGTGAC
GTTAGCTAACGCTAGGTAGCGTCAGCTGAGCTTACGTAAGCGCTTAGCAGATATTT

>RTP10690_2
TTACTGATTGTCTACGTAGGCTCAGCTGAGCTTACCTAAGGCTACGTAGGCTCACGTG
ACGTTACGTAAGGCTACGTAGCGTCACGTGAGCTTACCTAACTCTAGCTAGCCTCACG
TGACCTTAGCTAACACTAGGTAGCGTCAGCTCGACGGCCCG

>RTP10690_3
GGCGGAGTGGGGCTACGTAGCGTCACGTGACGTTACCTAAGCCTAGGTAGCCTCAGC
TGACGTTACGTAACGCTAGGTAGGCTCAGCTGACACGGGCAGGACATAG

>RTP10690_4
GAATGAAACCGATCTACGTAGGCTCAGCTGAGCTTAGCTAAGCCTACCTAGCCTCACG
TGAGATTATGTAAGGCTAGGTAGCGTCACGTGACGTTACCTAACACTAGCTAGCGTCA
GCTGAGCTTAGCTAACCCTACGTAGCCTCACGTGAGCTTACCTAACGCTACGTAGCCT
CACGTGACTAAGGATGACCTACCCATTCTT

>RTP10690_5
GCGGCCGCTAGCTAGCCTCAGCTGACGTTACGTAACGCTAGGTAGCGTCACGTGACG
TTAGCTAACGCTAGGTAGCGTCAGCTGAGCTTACGTAAGCGCCACGGGCAGGACATA
GGGACTACTACAA

>RTP10690_6

CGAATGAAACCGATCTACGTAGGCTCAGCTGAGCTTACCTAAGGCTACGTAGGCTCAC
GTGACGTTACGTAAGGCTACGTAGCGTCACGTGAGCTTACCTAACTCTAGCTAGCCTC
ACGTGACCTTAGCTAACACTAGGTAGCGTCAGCTTAGCAGATAT

>RTP10690_7
CCGTTCAATTTACTGATTGTCTACGTAGCGTCACCTGACGTTACGTAAGGCTACCTAGG
CTCACGTGACGTTACGTAACGCTACGTAGCGTCAGGTGAGGTTAGCTAACGCTAGCTA
GCCTCACCTGACGTTAGGTAAGGCTACGTAGCGTCACCTGAGATTAGCTAAGCCTACC
TAGACTCACGTGACCTTAGGTAACGCTACGTAGCGTCAAAGCTTTACAACGCTACACA
AA

>RTP10690_8
TTACCTAACTTAGAACTAAAATCAACTCTTTGTGACGCGTCTACCTAGAGTCAGCTGAG
CTTAGCTAACGCTAGCTAGTGTCAGCTGACGTTACGTAAGGCTAACTAGCGTCACGTG
ACCTTACGTAACGCTACGTAGGCTCAGCTGAGCTTAGCTAACCCTAGCTAGTGTCACG
TGAGCTTACGCTACTATAGAAAATGTGTTATAT

>RTP10690_9
TTCAGTCTAAAACAACTACGTAGCGTCACGTGACGTTACCTAAGCCTAGGTAGCCTCA
GCTGACGTTACGTAACGCTAGGTAGGCTCAGCTGACTGCAGCAAATTTACACATTGCC
A

>RTP10690_10
TAATTCGGCGTTAATTCAGCTACGTAGGCTCAGCTGAGCTTACCTAAGGCTACGTAGG
CTCACGTGACGTTACGTAAGGCTACGTAGCGTCACGTGAGCTTACCTAACTCTAGCTA
GCCTCACGTGACCTTAGCTAACACTAGGTAGCGTCAGCACAGATGAATACTAGCTGTT
GTTCA

>RTP10690_11
ATCATGATGCTTCTCTGAGCCGTGTTTGCTAGCTAGCCTCAGCTGACGTTACGTAACG
CTAGGTAGCGTCACGTGACGTTAGCTAACGCTAGGTAGCGTCAGCTGAGCTTACGTAA
GCGCACAGATGAATACTAGCTGTTGTTCACA

>RTP10690_12

CTTCTCTGAGCCGTGTTTGCTAGCTAGCCTCAGCTGACGTTACGTAACGCTAGGTAGC
GTCACGTGACGTTAGCTAACGCTAGGTAGCGTCAGCTGAGCTTACGTAAGCGCTTAAT
TAAAGTACTGATATCGGTACCAAATCGAATCCAAAAATTACGGATATGAATAT

>RTP10690_5'
ATTACAACGGTATATATCCTGCCAGTCAGCATCATCACACCAAAAGTTAGGCCCGAATA
GTTTGAAATTAGAAAGCTCGCAATTGAGGTCTACAGGCCAAATTCGCTCTTAGCCGTAC
AATATTACTCACCGGTGCGATGCCCCCCATCGTAGGTGAAGGTGGAAATTAATGGCGC
GCCTGATCACTGATTAGTAACTATTACGTAAGCCTACGTAGCGTCACGTGACGTTAGCT
AACGCTACGTAGCCTCAGCTGACGTTACGTAAGCCTACGTAGCGTCACGTGAGCTTAG
CTAACGCTACCTAGGCTCAGCTGACGTTACGTAACGCTAGCTAGCGTCACTCCTGCAG
CAAATTTACACA

>t-AtAHASL
GAGATGAAACCGGTGATTATCAGAACCTTTTATGGTCTTTGTATGCATATGGTAAAAAA
ACTTAGTTTGCAATTTCCTGTTTGTTTTGGTAATTTGAGTTTCTTTTAGTTGTTGATCTGC
CTGCTTTTTGGTTTACGTCAGACTACTACTGCTGTTGTTGTTTGGTTTCCTTTCTTTCAT
TTTATAAATAAATAATCCGGTTCGGTTTACTCCTTGTGACTGGCTCAGTTTGGTTATTGC
GAAATGCGAATGGTAAATTGAGTAATTGAAATTCGTTATTAGGGTTCTAAGCTGTTTTAA
CAGTCACTGGGTTAATATCTCTCGAATCTTGCATGGAAAATGCTCTTACCATTGGTTTTT
AATTGAAATGTGCTCATATGGGCCGTGGTTTCCAAATTAAATAAAACTACGATGTCATC
GAGAAGTAAAATCAACTGTGTCCACATTATCAGTTTTGTGTATACGATGAAATAGGGTA
ATTCAAAATCTAGCTTGATATGCCTTTTGGTTCATTTTAACCTTCTGTAAACATTTTTTCA
GATTTTGAACAAGTAAATCCAAAAAAAAAAAAAAAAATCTCAACTCAACACTAAATTATTT
TAATGTATAAAAGATGCTTAAAACATTTGGCTTAAAAGAAAGAAGCTAAAAACATAGAGA
ACTCTTGTAAATTGAAGTATGAAAATATACTGAATTGGGTATTATATGAATTTTTCTGATT
TAGGATTCACATGATCCAAAAAGGAAATCCAGAAGCACTAATCAGACATTGGAAGTAG
G

>LJB2197_5'
ACATACAAATGGACGAACGGATAAACCTTTTCACGCCCTTTTAAATATCCGATTATTCTA
ATAAACGCTCTTTTCTCTTAGGTTTACCCGCCAATATATCCTGTCAAACACTGATAGTTT
AAACTGAAGGCGGGAAACGACAATCTGATCACTGATTAGTAACTAAGGCCTTTAATTAA
TCTAGAGGCGCGCCGGGCCCCCTGCAGGGAGCTCGGCCGGCCAATTTAAATTGATAT
CGGTACATCGATTACGCCAAGCTATCAACTTTGTATAGAAAAGTTGCCATGATTACGCC
AAGCTTGGCCACTAAGGCCAATTTCGCGCCCTGCAGCAAATTTACACA

>LJB2197_1
CAATTTACTGATTGTGTCGACGCGATCGCGTGCAAACACTGTACGGACCGTGGCCTAA
TAGGCCGGTACCCAAGTTTGTACAAAAAAGCAGGCTCCATGATTACGCCAAGCTTGGC
CACTAAGGCCAATTTAAATCTACTAGGCCGGCCATCGACGGCCCGGACTGTA
>LJB2197_2
GGCGGAGTGGGGGGCGCCTACTACCGGTAATTCCCGGGATTAGCGGCCGCTAGTCT
GTGCGCACTTGTATCCTGCAGGTTAGGCCGGCCACACGGGCAGGACATAGGG
>LJB2197_3
AAACCGAATGAAACCGATGGCGCCTACCGGTATCGGTCCGATTGCGGCCGCTTAAAG
GGCGAATTCGTTTAAACACTGTACGGACCGTGGCCTAATAGGCCGGTACCACCCAGCT
TTCTTGTACAAAGTGGCCATGATTACGCCAAGCTTGGCCACTAAGGCCAATTTAAATCT
ACTAGGCCGGCCATAAGGATGACCTACCCATTCTT
>LJB2197_4
TTAATTCAGGGCCGGCCAAAGTAGGCGCCTACTACCGGTAATTCCCGGGATTAGCGG
CCGCTAGTCTGTGCGCACTTGTATCCTGCAGGTTAGGCCGGCCATTAGCAGATATTT

Table 171: Cassettes contained in the constructs VC-RTP10960-1qcz_F and VC-LJB2197-1qcz and the spacer regions in front of them, which was 5' of the promoter region. The bold lettering indicates the spacer, which corresponds to the Seq ID's listed above above.

VC-RTP10960-1qcz_F
p-VfUSP_684bp::i-Atss18_252bp::c-d6-Elo[Pp_GA2]::t-CaMV35S
RTP10690_1
p-LuCnl[1064bp]::i-Atss14_377bp::c-d5Des[Tc_GA2]::t-AgrOCS_192bp
RTP10690_2
p-SBP::i-Atss2_455bp::c-d6-Des[Ot_febit]::t-StCATHD-pA
RTP10690_3
p-LuPXR_1727bp::i-Atss1_847bp::c-d6-Elo[Tp_GA2]::t-AtPXR_400bp
RTP10690_4
p-Napin_A/B::i-Atss14_377bp::c-d12Des[Ps_GA2]::t-E9
RTP10690_5
p-LuPXR_1727bp::i-Atss15_758bp::c-

w3Des[pPir_GA]::AtPXR_400bp
RTP10690_6
p-LuCnl[1064bp]::i-Atss2_455bp::c-d4Des[Pl_GA]2::t-AgrOCS_192bp
RTP10690_7
p-BnFAE1::i-Atss1_847bp::c-d5Elo[Ot_GA3]::t-bnFAE1
RTP10690_8
p-ARC5_perm1::c-d4Des[Tc_GA3]::t-pvarc
RTP10690_9
p-VfUSP_684bp::i-Atss18_252::c-w3-Des[Pi_GA2]::t-CaMV35S
RTP10690_10
p-BnSETL-v1[1234bp]::c-d5-Des[Tc_GA2]::t-BnSETL
RTP10690_11
p-BnSETL-v1[1234bp]::c-w3Des[Pir_GA]::t-BnSETL
RTP10690_12
RTP10690_5'
t-AtAHASL
VC-LJB2197-1qcz
LJB2197_5'
p-VfUSP_684bp::i-Atss18_252bp::c-d6-Elo[PpGA2]::t-CaMV35S
LJB2197_1
p-LuCnl[1064bp]::i-Atss14_377bp::c-d5Des[Tc_GA2]::t-AgrOCS_192bp
LJB2197_2
p-SBP::i-Atss2_455bp::c-d6-Des[Ot_febit]::StCATHD-pA
LJB2197_3
p-LuPXR_1727bp::i-Atss1_847bp::c-d6-Elo[Tp_GA2]::t-AtPXR_400bp
LJB2197_4
p-Napin_A/B::i-Atss14_377bp::c-d12-Des[Ps_GA]::t-E9

Transcription factors are known to bind certain sequences of DNA and from this point of interaction with the chromosome they regulate transcription of a certain gene or subset of genes. The specific sequences which are bound by the transcription factor are referred to as DNA binding motifs and can be in the range of tens of base pairs to as few as four base pairs. The majority of DNA binding motifs consist of less than ten base pairs but those motifs have been documented to be necessary and sufficient for up (or down) regulation of transcription by the transcription factor binding to them. For examples of transcription factor motifs and the enabling data that are used to support the motifs see; Hattori et al., 2002, Keller et al., 1995, Kim et al., 2014, Lopez et al., 2013, Machens et al., 2014, Muino, 2013, Sarkar, 2013, Dubos et al., 2014). Identified motifs have been curated and archived in various data bases, such as PLACE (Higo et al., 1999), which can be accessed and queried by the public. While certain characterized promoters are used as the primary means to regulate expression of a given transgene in the invention, it was possible that alternative and unexpected regulation of a transgene was occurring in the plants described in the invention due to the presence of one or more active transcription factor binding motifs in the spacer region. Examples of artificial/synthetic promoters in the public literature support this line of reasoning; see Kong et al. (2014), Nishikata et al. (2013), and Brown et al. (2014) for examples of promoters made by random assembly of nucleotides and iterative testing as well as specific fragments of known transcriptional activity assembled to make an artificial promoter. Thus the regulatory region of a given transgene in the invention also includes, in addition to the intron, terminator and the promoter used, the spacer region in front of the cassette containing the transgene.

Table 172: lists the spacer region and the transcription factor binding motif present in the region and the nucleotide sequenceUpper case letters denote the motif while surrounding bases indicate examples of sequences that might neighbor the predicted motif but are not necessary for the motif to function in the regulation of transcription

Spacer ID	Detailed Family Information	Sequence
RTP10690_1	Plant G-box/C-box bZIP proteins	gttacgtaACGTcagctgagg
RTP10690_1	Plant G-box/C-box bZIP proteins	ctcagcTGACgttacgtaacg
RTP10690_1	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcctcagctgacgtTACGtaacgcta
RTP10690_1	Opaque-2 like transcriptional activators	cagctgACGTtacgtaa
RTP10690_1	GT-box elements	gctgacGTTAcgtaacg
RTP10690_1	Cell-death specification 2	gttacGTAAcg
RTP10690_1	Plant G-box/C-box bZIP proteins	ctagcgttACGTaacgtcagc

RTP10690_1	Opaque-2 like transcriptional activators	agcgttACGTaacgtca
RTP10690_1	Cell-death specification 2	gttacGTAAcg
RTP10690_1	Opaque-2 like transcriptional activators	gacgttACGTaacgcta
RTP10690_1	GT-box elements	cctagcGTTAcgtaacg
RTP10690_1	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gacgctacctagcgtTACGtaacgtca
RTP10690_1	Plant G-box/C-box bZIP proteins	gtcacgTGACgctacctagcg
RTP10690_1	Plant G-box/C-box bZIP proteins	gctaggtaGCGTcacgtgacg
RTP10690_1	ABA response elements	tagcgtcaCGTGacgtt
RTP10690_1	Plant G-box/C-box bZIP proteins	ctaacgtcACGTgacgctacc
RTP10690_1	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgacgctac
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_1	Opaque-2 like transcriptional activators	aacgtcACGTgacgcta
RTP10690_1	ABA response elements	taacgtcaCGTGacgct
RTP10690_1	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgacgttagc
RTP10690_1	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgacgttag
RTP10690_1	Opaque-2 like transcriptional activators	agcgtcACGTgacgtta
RTP10690_1	Plant G-box/C-box bZIP proteins	gttagctaACGTcacgtgacg
RTP10690_1	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgttagctaacgtCACGtgacgcta
RTP10690_1	Opaque-2 like transcriptional activators	tagctaACGTcacgtga
RTP10690_1	Plant G-box/C-box bZIP proteins	gtcacgTGACgttagctaacg
RTP10690_1	MYB-like proteins	cacgtgacGTTAgctaa
RTP10690_1	Plant G-box/C-box bZIP proteins	ctcagcTGACgctacctagcg
RTP10690_1	Plant G-box/C-box bZIP proteins	gctaggtaGCGTcagctgagc
RTP10690_1	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgtcagctgagctTACGtaagcgct
RTP10690_1	Vertebrate TATA binding protein factor	gcttacgTAAGctcagc
RTP10690_1	Cell-death specification 2	cttacGTAAgc
RTP10690_1	Plant G-box/C-box bZIP proteins	aagcgcttACGTaagctcagc
RTP10690_1	Opaque-2 like transcriptional activators	gcgcttACGTaagctca
RTP10690_1	Cell-death specification 2	cttacGTAAgc
RTP10690_1	Plant G-box/C-box bZIP proteins	ctgagcttACGTaagcgctta
RTP10690_1	Opaque-2 like transcriptional activators	gagcttACGTaagcgct
RTP10690_1	Vertebrate TATA binding protein factor	gcttacgTAAGcgctta
RTP10690_1	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	tatctgctaagcgctTACGtaagctca
RTP10690_1	Enhancer element first identified in the promoter of the octopine synthase gene (OCS) of the Agrobacterium	tctgctaagcgcttACGTaag
RTP10690_1	Arabidopsis CDC5 homolog	tgctaAGCGct
RTP10690_2	Cell-death specification 2	cttagGTAAgc
RTP10690_2	Opaque-2 like transcriptional activators	gagcctACGTagcctta
RTP10690_2	ABA response elements	taggctcaCGTGacgtt
RTP10690_2	Plant G-box/C-box bZIP proteins	gtaacgtcACGTgagcctacg
RTP10690_2	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgagcctac
RTP10690_2	Opaque-2 like transcriptional activators	aacgtcACGTgagccta
RTP10690_2	Plant G-box/C-box bZIP proteins	gtaggctcACGTgacgttacg
RTP10690_2	Myc-like basic helix-loop-helix binding factors	taggctCACGtgacgttac
RTP10690_2	Opaque-2 like transcriptional activators	aggctcACGTgacgtta
RTP10690_2	Cell-death specification 2	gtaacGTCAcg
RTP10690_2	Plant G-box/C-box bZIP proteins	cttacgtaACGTcacgtgagc
RTP10690_2	Opaque-2 like transcriptional activators	tacgtaACGTcacgtga
RTP10690_2	Plant G-box/C-box bZIP proteins	ctcacgTGACgttacgtaagg

RTP10690_2	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	aggctcacgtgacgtTACGtaaggcta
RTP10690_2	Opaque-2 like transcriptional activators	cacgtgACGTtacgtaa
RTP10690_2	GT-box elements	cgtgacGTTAcgtaagg
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_2	Cell-death specification 2	cttacGTAAcg
RTP10690_2	Plant G-box/C-box bZIP proteins	gtagccttACGTaacgtcacg
RTP10690_2	Opaque-2 like transcriptional activators	agccttACGTaacgtca
RTP10690_2	Cell-death specification 2	gttacGTAAgg
RTP10690_2	Opaque-2 like transcriptional activators	gacgttACGTaaggcta
RTP10690_2	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gacgctacgtagcctTACGtaacgtca
RTP10690_2	Opaque-2 like transcriptional activators	gacgctACGTagcctta
RTP10690_2	Plant G-box/C-box bZIP proteins	gtaaggctACGTagcgtcacg
RTP10690_2	Opaque-2 like transcriptional activators	aaggctACGTagcgtca
RTP10690_2	Plant G-box/C-box bZIP proteins	ctcacgTGACgctacgtagcc
RTP10690_2	Plant G-box/C-box bZIP proteins	gctacgtaGCGTcacgtgagc
RTP10690_2	Plant G-box/C-box bZIP proteins	gtaagctcACGTgacgctacg
RTP10690_2	Myc-like basic helix-loop-helix binding factors	taagctCACGtgacgctac
RTP10690_2	Opaque-2 like transcriptional activators	aagctcACGTgacgcta
RTP10690_2	ABA response elements	taagctcaCGTGacgct
RTP10690_2	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgagcttacc
RTP10690_2	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgagcttac
RTP10690_2	Opaque-2 like transcriptional activators	agcgtcACGTgagctta
RTP10690_2	GT-box elements	cgtcacGTGAgcttacc
RTP10690_2	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agagttaggtaagctCACGtgacgcta
RTP10690_2	Cell-death specification 2	gttagGTAAgc
RTP10690_2	MYB IIG-type binding sites	tagagttaGGTAagc
RTP10690_2	MYB-like proteins	tagctagAGTTaggtaa
RTP10690_2	ABA response elements	tagcctcaCGTGacctt
RTP10690_2	Plant G-box/C-box bZIP proteins	ctaaggtcACGTgaggctagc
RTP10690_2	Myc-like basic helix-loop-helix binding factors	taaggtCACGtgaggctag
RTP10690_2	Opaque-2 like transcriptional activators	aaggtcACGTgaggcta
RTP10690_2	Dc3 promoter binding factors	cTCACgtgacc
RTP10690_2	Plant G-box/C-box bZIP proteins	ctagcctcACGTgaccttagc
RTP10690_2	Myc-like basic helix-loop-helix binding factors	tagcctCACGtgaccttag
RTP10690_2	Opaque-2 like transcriptional activators	agcctcACGTgacctta
RTP10690_2	GT-box elements	cctcacGTGAccttagc
RTP10690_2	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agtgttagctaaggtCACGtgaggcta
RTP10690_2	Plant G-box/C-box bZIP proteins	tcgagcTGACgctacctagtg
RTP10690_2	Plant G-box/C-box bZIP proteins	actaggtaGCGTcagctcgac
RTP10690_3	Opaque-2 like transcriptional activators	gacgctACGTagcccca
RTP10690_3	Plant G-box/C-box bZIP proteins	gtggggctACGTagcgtcacg
RTP10690_3	Opaque-2 like transcriptional activators	ggggctACGTagcgtca
RTP10690_3	Plant G-box/C-box bZIP proteins	gtcacgTGACgctacgtagcc
RTP10690_3	Plant G-box/C-box bZIP proteins	gctacgtaGCGTcacgtgacg
Spacer ID	**Detailed Family Information**	**Sequence**

RTP10690_3	ABA response elements	tagcgtcaCGTGacgtt
RTP10690_3	Plant G-box/C-box bZIP proteins	gtaacgtcACGTgacgctacg
RTP10690_3	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgacgctac
RTP10690_3	Opaque-2 like transcriptional activators	aacgtcACGTgacgcta
RTP10690_3	ABA response elements	taacgtcaCGTGacgct
RTP10690_3	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgacgttacc
RTP10690_3	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgacgttac
RTP10690_3	Opaque-2 like transcriptional activators	agcgtcACGTgacgtta
RTP10690_3	Cell-death specification 2	gtaacGTCAcg
RTP10690_3	Plant G-box/C-box bZIP proteins	cttaggtaACGTcacgtgacg
RTP10690_3	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	aggcttaggtaacgtCACGtgacgcta
RTP10690_3	Opaque-2 like transcriptional activators	taggtaACGTcacgtga
RTP10690_3	Plant G-box/C-box bZIP proteins	gtcacgTGACgttacctaagc
RTP10690_3	Opaque-2 like transcriptional activators	cacgtgACGTtacctaa
RTP10690_3	GT-box elements	cgtgacGTTAcctaagc
RTP10690_3	Cell-death specification 2	cttagGTAAcg
RTP10690_3	Vertebrate TATA binding protein factor	cgttaccTAAGcctagg
RTP10690_3	Plant G-box/C-box bZIP proteins	gttacgtaACGTcagctgagg
RTP10690_3	Plant G-box/C-box bZIP proteins	ctcagcTGACgttacgtaacg
RTP10690_3	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcctcagctgacgtTACGtaacgcta
RTP10690_3	Opaque-2 like transcriptional activators	cagctgACGTtacgtaa
RTP10690_3	GT-box elements	gctgacGTTAcgtaacg
RTP10690_3	Cell-death specification 2	gttacGTAAcg
RTP10690_3	Plant G-box/C-box bZIP proteins	ctagcgttACGTaacgtcagc
RTP10690_3	Opaque-2 like transcriptional activators	agcgttACGTaacgtca
RTP10690_3	Cell-death specification 2	gttacGTAAcg
RTP10690_3	Opaque-2 like transcriptional activators	gacgttACGTaacgcta
RTP10690_3	GT-box elements	cctagcGTTAcgtaacg
RTP10690_3	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gagcctacctagcgtTACGtaacgtca
RTP10690_3	Calmodulin binding / CGCG box binding proteins	gccCGTGtcagctgagc
RTP10690_3	Bel-1 similar region	tgtcctgcccgtgTCAGctgagc
RTP10690_4	Dehydration responsive element binding factors	gaatgaaaCCGAtctacgtag
RTP10690_4	Light responsive element motif, not modulated by different light qualities	cgATCTacgta
RTP10690_4	Plant G-box/C-box bZIP proteins	ataatctcACGTgaggctagg
RTP10690_4	Myc-like basic helix-loop-helix binding factors	taatctCACGtgaggctag
RTP10690_4	Opaque-2 like transcriptional activators	aatctcACGTgaggcta
RTP10690_4	ABA response elements	taatctcACGTgaggct
RTP10690_4	Plant G-box/C-box bZIP proteins	ctagcctcACGTgagattatg
RTP10690_4	Myc-like basic helix-loop-helix binding factors	tagcctCACGtgagattat
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_4	Opaque-2 like transcriptional activators	agcctcACGTgagatta
RTP10690_4	Brassinosteroid (BR) response element	cctcaCGTGagattatg
RTP10690_4	Cell-death specification 2	attatGTAAgg
RTP10690_4	Vertebrate TATA binding protein factor	gattatgTAAGgctagg
RTP10690_4	Plant G-box/C-box bZIP proteins	gtcacgTGACgctacctagcc
RTP10690_4	Plant G-box/C-box bZIP proteins	gctaggtaGCGTcacgtgacg

RTP10690_4	ABA response elements	tagcgtcaCGTGacgtt
RTP10690_4	Plant G-box/C-box bZIP proteins	gtaacgtcACGTgacgctacc
RTP10690_4	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgacgctac
RTP10690_4	Opaque-2 like transcriptional activators	aacgtcACGTgacgcta
RTP10690_4	ABA response elements	taacgtcaCGTGacgct
RTP10690_4	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgacgttacc
RTP10690_4	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgacgttac
RTP10690_4	Opaque-2 like transcriptional activators	agcgtcACGTgacgtta
RTP10690_4	Cell-death specification 2	gtaacGTCAcg
RTP10690_4	Plant G-box/C-box bZIP proteins	gttaggtaACGTcacgtgacg
RTP10690_4	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agtgttaggtaacgtCACGtgacgcta
RTP10690_4	Opaque-2 like transcriptional activators	taggtaACGTcacgtga
RTP10690_4	Plant G-box/C-box bZIP proteins	gtcacgTGACgttacctaaca
RTP10690_4	Opaque-2 like transcriptional activators	cacgtgACGTtacctaa
RTP10690_4	GT-box elements	cgtgacGTTAcctaaca
RTP10690_4	Cell-death specification 2	gttagGTAAcg
RTP10690_4	Plant G-box/C-box bZIP proteins	ctcagcTGACgctagctagtg
RTP10690_4	Plant G-box/C-box bZIP proteins	actagctaGCGTcagctgagc
RTP10690_4	Telo box (plant interstitial telomere motifs)	gctaaCCCTacgtag
RTP10690_4	Opaque-2 like transcriptional activators	aaccctACGTagcctca
RTP10690_4	Brassinosteroid (BR) response element	gctcaCGTGaggctacg
RTP10690_4	Plant G-box/C-box bZIP proteins	gtaagctcACGTgaggctacg
RTP10690_4	Myc-like basic helix-loop-helix binding factors	taagctCACGtgaggctac
RTP10690_4	Opaque-2 like transcriptional activators	aagctcACGTgaggcta
RTP10690_4	Plant G-box/C-box bZIP proteins	gtagcctcACGTgagcttacc
RTP10690_4	Myc-like basic helix-loop-helix binding factors	tagcctCACGtgagcttac
RTP10690_4	Opaque-2 like transcriptional activators	agcctcACGTgagctta
RTP10690_4	Brassinosteroid (BR) response element	cctcaCGTGagcttacc
RTP10690_4	GT-box elements	cctcacGTGAgcttacc
RTP10690_4	Cell-death specification 2	gttagGTAAgc
RTP10690_4	MYB IIG-type binding sites	tagcgttaGGTAagc
RTP10690_4	Core promoter motif ten elements	cttacctAACGctacgtagcc
RTP10690_4	Plant G-box/C-box bZIP proteins	gtgaggctACGTagcgttagg
RTP10690_4	Opaque-2 like transcriptional activators	gaggctACGTagcgtta
RTP10690_4	Opaque-2 like transcriptional activators	aacgctACGTagcctca
RTP10690_4	Root hair-specific cis-elements in angiosperms	aacgctacgtagcctCACGtgacta
RTP10690_4	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	aacgctacgtagcctCACGtgactaag
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_4	ABA response elements	tagcctcaCGTGactaa
RTP10690_4	Plant G-box/C-box bZIP proteins	ccttagtcACGTgaggctacg
RTP10690_4	Myc-like basic helix-loop-helix binding factors	cttagtCACGtgaggctac
RTP10690_4	Opaque-2 like transcriptional activators	ttagtcACGTgaggcta
RTP10690_4	Plant G-box/C-box bZIP proteins	gtagcctcACGTgactaagga
RTP10690_4	Myc-like basic helix-loop-helix binding factors	tagcctCACGtgactaagg
RTP10690_4	Opaque-2 like transcriptional activators	agcctcACGTgactaag
RTP10690_4	GT-box elements	cctcacGTGActaagga
RTP10690_4	MYB IIG-type binding sites	aatgGGTAggtcatc
RTP10690_5	Plant G-box/C-box bZIP proteins	gttacgtaACGTcagctgagg
RTP10690_5	Plant G-box/C-box bZIP proteins	ctcagcTGACgttacgtaacg

RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcctcagctgacgtTACGtaacgcta
RTP10690_5	Opaque-2 like transcriptional activators	cagctgACGTtacgtaa
RTP10690_5	GT-box elements	gctgacGTTAcgtaacg
RTP10690_5	Cell-death specification 2	gttacGTAAcg
RTP10690_5	Plant G-box/C-box bZIP proteins	ctagcgttACGTaacgtcagc
RTP10690_5	Opaque-2 like transcriptional activators	agcgttACGTaacgtca
RTP10690_5	Cell-death specification 2	gttacGTAAcg
RTP10690_5	Opaque-2 like transcriptional activators	gacgttACGTaacgcta
RTP10690_5	GT-box elements	cctagcGTTAcgtaacg
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gacgctacctagcgtTACGtaacgtca
RTP10690_5	Plant G-box/C-box bZIP proteins	gtcacgTGACgctacctagcg
RTP10690_5	Plant G-box/C-box bZIP proteins	gctaggtaGCGTcacgtgacg
RTP10690_5	ABA response elements	tagcgtcaCGTGacgtt
RTP10690_5	Plant G-box/C-box bZIP proteins	ctaacgtcACGTgacgctacc
RTP10690_5	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgacgctac
RTP10690_5	Opaque-2 like transcriptional activators	aacgtcACGTgacgcta
RTP10690_5	ABA response elements	taacgtcaCGTGacgct
RTP10690_5	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgacgttagc
RTP10690_5	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgacgttag
RTP10690_5	Opaque-2 like transcriptional activators	agcgtcACGTgacgtta
RTP10690_5	Plant G-box/C-box bZIP proteins	gttagctaACGTcacgtgacg
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgttagctaacgtCACGtgacgcta
RTP10690_5	Opaque-2 like transcriptional activators	tagctaACGTcacgtga
RTP10690_5	Plant G-box/C-box bZIP proteins	gtcacgTGACgttagctaacg
RTP10690_5	MYB-like proteins	cacgtgacGTTAgctaa
RTP10690_5	Plant G-box/C-box bZIP proteins	ctcagcTGACgctacctagcg
RTP10690_5	Plant G-box/C-box bZIP proteins	gctaggtaGCGTcagctgagc
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgtcagctgagctTACGtaagcgcc
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_5	Vertebrate TATA binding protein factor	gcttacgTAAGctcagc
RTP10690_5	Cell-death specification 2	cttacGTAAgc
RTP10690_5	Plant G-box/C-box bZIP proteins	tggcgcttACGTaagctcagc
RTP10690_5	Opaque-2 like transcriptional activators	gcgcttACGTaagctca
RTP10690_5	Cell-death specification 2	cttacGTAAgc
RTP10690_5	Plant G-box/C-box bZIP proteins	ctgagcttACGTaagcgccac
RTP10690_5	Opaque-2 like transcriptional activators	gagcttACGTaagcgcc
RTP10690_5	Vertebrate TATA binding protein factor	gcttacgTAAGcgccac
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	cctgcccgtggcgctTACGtaagctca
RTP10690_5	Enhancer element first identified in the promoter of the octopine synthase gene (OCS) of the Agrobacterium tumefaciens T-DNA	tgcccgtggcgctTACGtaag
RTP10690_5	Calmodulin binding / CGCG box binding proteins	gccCGTGgcgcttacgt
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcttacgtaagcgccACGGgcaggac

RTP10690_5	Plant G-box/C-box bZIP proteins	tgtcctgcCCGTggcgcttac
RTP10690_6	Dehydration responsive element binding factors	gaatgaaaCCGAtctacgtag
RTP10690_6	Light responsive element motif, not modulated by different light qualities	cgATCTacgta
RTP10690_6	Cell-death specification 2	cttagGTAAgc
RTP10690_6	Opaque-2 like transcriptional activators	gagcctACGTagcctta
RTP10690_6	ABA response elements	taggctcaCGTGacgtt
RTP10690_6	Plant G-box/C-box bZIP proteins	gtaacgtcACGTgagcctacg
RTP10690_6	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgagcctac
RTP10690_6	Opaque-2 like transcriptional activators	aacgtcACGTgagccta
RTP10690_6	Plant G-box/C-box bZIP proteins	gtaggctcACGTgacgttacg
RTP10690_6	Myc-like basic helix-loop-helix binding factors	taggctCACGtgacgttac
RTP10690_6	Opaque-2 like transcriptional activators	aggctcACGTgacgtta
RTP10690_6	Cell-death specification 2	gtaacGTCAcg
RTP10690_6	Plant G-box/C-box bZIP proteins	cttacgtaACGTcacgtgagc
RTP10690_6	Opaque-2 like transcriptional activators	tacgtaACGTcacgtga
RTP10690_6	Plant G-box/C-box bZIP proteins	ctcacgTGACgttacgtaagg
RTP10690_6	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	aggctcacgtgacgtTACGtaaggcta
RTP10690_6	Opaque-2 like transcriptional activators	cacgtgACGTtacgtaa
RTP10690_6	GT-box elements	cgtgacGTTAcgtaagg
RTP10690_6	Cell-death specification 2	cttacGTAAcg
RTP10690_6	Plant G-box/C-box bZIP proteins	gtagccttACGTaacgtcacg
RTP10690_6	Opaque-2 like transcriptional activators	agccttACGTaacgtca
RTP10690_6	Cell-death specification 2	gttacGTAAgg
RTP10690_6	Opaque-2 like transcriptional activators	gacgttACGTaaggcta
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_6	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gacgctacgtagcctTACGtaacgtca
RTP10690_6	Opaque-2 like transcriptional activators	gacgctACGTagcctta
RTP10690_6	Plant G-box/C-box bZIP proteins	gtaaggctACGTagcgtcacg
RTP10690_6	Opaque-2 like transcriptional activators	aaggctACGTagcgtca
RTP10690_6	Plant G-box/C-box bZIP proteins	ctcacgTGACgctacgtagcc
RTP10690_6	Plant G-box/C-box bZIP proteins	gctacgtaGCGTcacgtgagc
RTP10690_6	Plant G-box/C-box bZIP proteins	gtaagctcACGTgacgctacg
RTP10690_6	Myc-like basic helix-loop-helix binding factors	taagctCACGtgacgctac
RTP10690_6	Opaque-2 like transcriptional activators	aagctcACGTgacgcta
RTP10690_6	ABA response elements	taagctcaCGTGacgct
RTP10690_6	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgagcttacc
RTP10690_6	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgagcttac
RTP10690_6	Opaque-2 like transcriptional activators	agcgtcACGTgagctta
RTP10690_6	GT-box elements	cgtcacGTGAgcttacc
RTP10690_6	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agagttaggtaagctCACGtgacgcta
RTP10690_6	Cell-death specification 2	gttagGTAAgc
RTP10690_6	MYB IIG-type binding sites	tagagttaGGTAagc
RTP10690_6	MYB-like proteins	tagctagAGTTaggtaa
RTP10690_6	ABA response elements	tagcctcaCGTGacctt

RTP10690_6	Plant G-box/C-box bZIP proteins	ctaaggtcACGTgaggctagc
RTP10690_6	Myc-like basic helix-loop-helix binding factors	taaggtCACGtgaggctag
RTP10690_6	Opaque-2 like transcriptional activators	aaggtcACGTgaggcta
RTP10690_6	Dc3 promoter binding factors	cTCACgtgacc
RTP10690_6	Plant G-box/C-box bZIP proteins	ctagcctcACGTgaccttagc
RTP10690_6	Myc-like basic helix-loop-helix binding factors	tagcctCACGtgaccttag
RTP10690_6	Opaque-2 like transcriptional activators	agcctcACGTgacctta
RTP10690_6	GT-box elements	cctcacGTGAccttagc
RTP10690_6	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agtgttagctaaggtCACGtgaggcta
RTP10690_6	Plant G-box/C-box bZIP proteins	ctaagcTGACgctacctagtg
RTP10690_7	L1 box, motif for L1 layer-specific expression	aatCAGTaaattgaacg
RTP10690_7	Arabidopsis homeobox protein	caaTTTActga
RTP10690_7	Sucrose box	gaCAATcagtaaattgaac
RTP10690_7	Enhancer element first identified in the promoter of the octopine synthase gene (OCS) of the Agrobacterium tumefaciens T-DNA	tttactgattgtctACGTagc
RTP10690_7	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	aatttactgattgtcTACGtagcgtca
RTP10690_7	Plant G-box/C-box bZIP proteins	gattgtctACGTagcgtcacc
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_7	Plant G-box/C-box bZIP proteins	gtcaggTGACgctacgtagac
RTP10690_7	Plant G-box/C-box bZIP proteins	tctacgtaGCGTcacctgacg
RTP10690_7	Plant G-box/C-box bZIP proteins	cgtagcgtcaccTGACgttac
RTP10690_7	Plant G-box/C-box bZIP proteins	cttacgtaACGTcaggtgacg
RTP10690_7	Plant G-box/C-box bZIP proteins	gtcaccTGACgttacgtaagg
RTP10690_7	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgtcacctgacgtTACGtaaggcta
RTP10690_7	Opaque-2 like transcriptional activators	cacctgACGTtacgtaa
RTP10690_7	GT-box elements	cctgacGTTAcgtaagg
RTP10690_7	Cell-death specification 2	cttacGTAAcg
RTP10690_7	Plant G-box/C-box bZIP proteins	gtagccttACGTaacgtcagg
RTP10690_7	Opaque-2 like transcriptional activators	agccttACGTaacgtca
RTP10690_7	Cell-death specification 2	gttacGTAAgg
RTP10690_7	Opaque-2 like transcriptional activators	gacgttACGTaaggcta
RTP10690_7	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gagcctaggtagcctTACGtaacgtca
RTP10690_7	ABA response elements	taggctcaCGTGacgtt
RTP10690_7	Plant G-box/C-box bZIP proteins	gtaacgtcACGTgagcctagg
RTP10690_7	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgagcctag
RTP10690_7	Opaque-2 like transcriptional activators	aacgtcACGTgagccta
RTP10690_7	Plant G-box/C-box bZIP proteins	ctaggctcACGTgacgttacg
RTP10690_7	Myc-like basic helix-loop-helix binding factors	taggctCACGtgacgttac
RTP10690_7	Opaque-2 like transcriptional activators	aggctcACGTgacgtta
RTP10690_7	Cell-death specification 2	gtaacGTCAcg
RTP10690_7	Plant G-box/C-box bZIP proteins	gttacgtaACGTcacgtgagc

RTP10690_7	Opaque-2 like transcriptional activators	tacgtaACGTcacgtga
RTP10690_7	Plant G-box/C-box bZIP proteins	ctcacgTGACgttacgtaacg
RTP10690_7	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	aggctcacgtgacgtTACGtaacgcta
RTP10690_7	Opaque-2 like transcriptional activators	cacgtgACGTtacgtaa
RTP10690_7	GT-box elements	cgtgacGTTAcgtaacg
RTP10690_7	Cell-death specification 2	gttacGTAAcg
RTP10690_7	Plant G-box/C-box bZIP proteins	gtagcgttACGTaacgtcacg
RTP10690_7	Opaque-2 like transcriptional activators	agcgttACGTaacgtca
RTP10690_7	Cell-death specification 2	gttacGTAAcg
RTP10690_7	Opaque-2 like transcriptional activators	gacgttACGTaacgcta
RTP10690_7	GT-box elements	cgtagcGTTAcgtaacg
RTP10690_7	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gacgctacgtagcgtTACGtaacgtca
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_7	Plant G-box/C-box bZIP proteins	ctgacgctACGTagcgttacg
RTP10690_7	Opaque-2 like transcriptional activators	gacgctACGTagcgtta
RTP10690_7	Plant G-box/C-box bZIP proteins	gtaacgctACGTagcgtcagg
RTP10690_7	Opaque-2 like transcriptional activators	aacgctACGTagcgtca
RTP10690_7	Plant G-box/C-box bZIP proteins	ctcaccTGACgctacgtagcg
RTP10690_7	Plant G-box/C-box bZIP proteins	gctacgtaGCGTcaggtgagg
RTP10690_7	MYB-like proteins	caggtgagGTTAgctaa
RTP10690_7	Plant G-box/C-box bZIP proteins	cttacctaACGTcaggtgagg
RTP10690_7	Plant G-box/C-box bZIP proteins	ctcaccTGACgttaggtaagg
RTP10690_7	MYB IIG-type binding sites	tgacgttaGGTAagg
RTP10690_7	Cell-death specification 2	gttagGTAAgg
RTP10690_7	GT-box elements	cgttagGTAAggctacg
RTP10690_7	Opaque-2 like transcriptional activators	gacgctACGTagcctta
RTP10690_7	Plant G-box/C-box bZIP proteins	gtaaggctACGTagcgtcacc
RTP10690_7	Opaque-2 like transcriptional activators	aaggctACGTagcgtca
RTP10690_7	Plant G-box/C-box bZIP proteins	ctcaggTGACgctacgtagcc
RTP10690_7	Plant G-box/C-box bZIP proteins	gctacgtaGCGTcacctgaga
RTP10690_7	Myc-like basic helix-loop-helix binding factors	tagcgtCACCtgagattag
RTP10690_7	ABA response elements	tagactcaCGTGacctt
RTP10690_7	Plant G-box/C-box bZIP proteins	ctaaggtcACGTgagtctagg
RTP10690_7	Myc-like basic helix-loop-helix binding factors	taaggtCACGtgagtctag
RTP10690_7	Opaque-2 like transcriptional activators	aaggtcACGTgagtcta
RTP10690_7	TFF-box	ctAAGGtcacgtgagtctagg
RTP10690_7	Dc3 promoter binding factors	cTCACgtgacc
RTP10690_7	Plant G-box/C-box bZIP proteins	ctagactcACGTgaccttagg
RTP10690_7	Myc-like basic helix-loop-helix binding factors	tagactCACGtgaccttag
RTP10690_7	Opaque-2 like transcriptional activators	agactcACGTgaccta
RTP10690_7	GT-box elements	actcacGTGAccttagg
RTP10690_7	Cell-death specification 2	cttagGTAAcg
RTP10690_7	GT-box elements	ccttagGTAAcgctacg
RTP10690_7	GT-box elements	cgtagcGTTAcctaagg
RTP10690_7	Plant G-box/C-box bZIP proteins	ttgacgctACGTagcgttacc
RTP10690_7	Opaque-2 like transcriptional activators	gacgctACGTagcgtta
RTP10690_7	Plant G-box/C-box bZIP proteins	gtaacgctACGTagcgtcaaa
RTP10690_7	Opaque-2 like transcriptional activators	aacgctACGTagcgtca
RTP10690_7	Plant specific NAC [NAM (no apical meristem), ATAF172,	gtaaagctttgacgctACGTagcgtta

	CUC2 (cup-shaped cotyledons 2)] transcription factors	
RTP10690_7	Plant G-box/C-box bZIP proteins	gctacgtaGCGTcaaagcttt
RTP10690_7	Calcium regulated NAC-factors	taaaGCTTtgacgctacgtag
RTP10690_7	W Box family	aagctTTGAcgctacgt
RTP10690_7	Tracheary-element-regulating cis-elements, conferring TE-specific expression	cgtcAAAGctt
RTP10690_7	Vertebrate TATA binding protein factor	cgttgTAAAgctttgac
RTP10690_7	DNA binding with one finger (DOF)	agcgttgtAAAGctttg
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_7	Mitochondral HMG-box transcription factor	tgtagcgTTGTaa
RTP10690_8	Secondary wall NACS	gattttagttcTAAGttag
RTP10690_8	AS1/AS2 repressor complex	gagTTGAtt
RTP10690_8	Calmodulin binding / CGCG box binding proteins	agaCGCGtcacaaagag
RTP10690_8	Coupling element 3 sequence	ggtagaCGCGtcacaaaga
RTP10690_8	Plant G-box/C-box bZIP proteins	aggtagacGCGTcacaaagag
RTP10690_8	Plant G-box/C-box bZIP proteins	tctttgTGACgcgtctaccta
RTP10690_8	Calmodulin binding / CGCG box binding proteins	tgaCGCGtctacctaga
RTP10690_8	Plant G-box/C-box bZIP proteins	gctagtgtcagcTGACgttac
RTP10690_8	Plant G-box/C-box bZIP proteins	cttacgtaACGTcagctgaca
RTP10690_8	Plant G-box/C-box bZIP proteins	gtcagcTGACgttacgtaagg
RTP10690_8	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agtgtcagctgacgtTACGtaaggcta
RTP10690_8	Opaque-2 like transcriptional activators	cagctgACGTtacgtaa
RTP10690_8	GT-box elements	gctgacGTTAcgtaagg
RTP10690_8	Cell-death specification 2	cttacGTAAcg
RTP10690_8	Plant G-box/C-box bZIP proteins	ttagccttACGTaacgtcagc
RTP10690_8	Opaque-2 like transcriptional activators	agccttACGTaacgtca
RTP10690_8	Cell-death specification 2	gttacGTAAgg
RTP10690_8	Opaque-2 like transcriptional activators	gacgttACGTaaggcta
RTP10690_8	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gacgctagttagcctTACGtaacgtca
RTP10690_8	MYB-like proteins	tgacgctAGTTagcctt
RTP10690_8	Plant G-box/C-box bZIP proteins	gtcacgTGACgctagttagcc
RTP10690_8	ABA response elements	tagcgtcaCGTGacctt
RTP10690_8	Plant G-box/C-box bZIP proteins	gtaaggtcACGTgacgctagt
RTP10690_8	Myc-like basic helix-loop-helix binding factors	taaggtCACGtgacgctag
RTP10690_8	Opaque-2 like transcriptional activators	aaggtcACGTgacgcta
RTP10690_8	ABA response elements	taaggtcaCGTGacgct
RTP10690_8	Dc3 promoter binding factors	gTCACgtgacc
RTP10690_8	Plant G-box/C-box bZIP proteins	ctagcgtcACGTgaccttacg
RTP10690_8	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgaccttac
RTP10690_8	Opaque-2 like transcriptional activators	agcgtcACGTgacctta
RTP10690_8	GT-box elements	cgtcacGTGAccttacg
RTP10690_8	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgttacgtaaggtCACGtgacgcta
RTP10690_8	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgtcacgtgacctTACGtaacgcta
RTP10690_8	Cell-death specification 2	gttacGTAAgg

RTP10690_8	Plant G-box/C-box bZIP proteins	gtagcgttACGTaaggtcacg
RTP10690_8	Opaque-2 like transcriptional activators	agcgttACGTaaggtca
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_8	Cell-death specification 2	cttacGTAAcg
RTP10690_8	Opaque-2 like transcriptional activators	gaccttACGTaacgcta
RTP10690_8	GT-box elements	cgtagcGTTAcgtaagg
RTP10690_8	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gagcctacgtagcgtTACGtaaggtca
RTP10690_8	Plant G-box/C-box bZIP proteins	ctgagcctACGTagcgttacg
RTP10690_8	Opaque-2 like transcriptional activators	gagcctACGTagcgtta
RTP10690_8	Telo box (plant interstitial telomere motifs)	gctaACCCtagctag
RTP10690_8	ABA response elements	tagtgtcACGTgagctt
RTP10690_8	Plant G-box/C-box bZIP proteins	gtaagctcACGTgacactagc
RTP10690_8	Myc-like basic helix-loop-helix binding factors	taagctCACGtgacactag
RTP10690_8	Opaque-2 like transcriptional activators	aagctcACGTgacacta
RTP10690_8	ABA response elements	taagctcaCGTGacact
RTP10690_8	Plant G-box/C-box bZIP proteins	ctagtgtcACGTgagcttacg
RTP10690_8	Myc-like basic helix-loop-helix binding factors	tagtgtCACGtgagcttac
RTP10690_8	Opaque-2 like transcriptional activators	agtgtcACGTgagctta
RTP10690_8	GT-box elements	tgtcacGTGAgcttacg
RTP10690_8	Enhancer element first identified in the promoter of the octopine synthase gene (OCS) of the Agrobacterium tumefaciens T-DNA	tgtcacgtgagctTACGctac
RTP10690_8	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agtgtcacgtgagctTACGctactata
RTP10690_8	Cell-death specification 2	gtagcGTAAgc
RTP10690_8	Pollen-specific regulatory elements	ctataGAAAatgtgtta
RTP10690_9	CA-rich motif	agtctaaAACAactacgta
RTP10690_9	General transcription factor IID, GTF2D	tcagtctaaaacaactacgtagcgtcacg tGACGttacc
RTP10690_9	Plant G-box/C-box bZIP proteins	gtcacgTGACgctacgtagtt
RTP10690_9	Plant G-box/C-box bZIP proteins	actacgtaGCGTcacgtgacg
RTP10690_9	ABA response elements	tagcgtcaCGTGacgtt
RTP10690_9	Plant G-box/C-box bZIP proteins	gtaacgtcACGTgacgctacg
RTP10690_9	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgacgctac
RTP10690_9	Opaque-2 like transcriptional activators	aacgtcACGTgacgcta
RTP10690_9	ABA response elements	taacgtcaCGTGacgct
RTP10690_9	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgacgttacc
RTP10690_9	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgacgttac
RTP10690_9	Opaque-2 like transcriptional activators	agcgtcACGTgacgtta
RTP10690_9	Cell-death specification 2	gtaacGTCAcg
RTP10690_9	Plant G-box/C-box bZIP proteins	cttaggtaACGTcacgtgacg
RTP10690_9	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	aggcttaggtaacgtCACGtgacgcta
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_9	Opaque-2 like transcriptional activators	taggtaACGTcacgtga

RTP10690_9	Plant G-box/C-box bZIP proteins	gtcacgTGACgttacctaagc
RTP10690_9	Opaque-2 like transcriptional activators	cacgtgACGTtacctaa
RTP10690_9	GT-box elements	cgtgacGTTAcctaagc
RTP10690_9	Cell-death specification 2	cttagGTAAcg
RTP10690_9	Vertebrate TATA binding protein factor	cgttaccTAAGcctagg
RTP10690_9	Plant G-box/C-box bZIP proteins	gttacgtaACGTcagctgagg
RTP10690_9	Plant G-box/C-box bZIP proteins	ctcagcTGACgttacgtaacg
RTP10690_9	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcctcagctgacgtTACGtaacgcta
RTP10690_9	Opaque-2 like transcriptional activators	cagctgACGTtacgtaa
RTP10690_9	GT-box elements	gctgacGTTAcgtaacg
RTP10690_9	Cell-death specification 2	gttacGTAAcg
RTP10690_9	Plant G-box/C-box bZIP proteins	ctagcgttACGTaacgtcagc
RTP10690_9	Opaque-2 like transcriptional activators	agcgttACGTaacgtca
RTP10690_9	Cell-death specification 2	gttacGTAAcg
RTP10690_9	Opaque-2 like transcriptional activators	gacgttACGTaacgcta
RTP10690_9	GT-box elements	cctagcGTTAcgtaacg
RTP10690_9	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gagcctacctagcgtTACGtaacgtca
RTP10690_9	Upstream sequence element of U-snRNA genes	aaatttACACattgcca
RTP10690_10	Opaque-2 like transcriptional activators	gagcctACGTagctgaa
RTP10690_10	Cell-death specification 2	cttagGTAAgc
RTP10690_10	Opaque-2 like transcriptional activators	gagcctACGTagcctta
RTP10690_10	ABA response elements	taggctcaCGTGacgtt
RTP10690_10	Plant G-box/C-box bZIP proteins	gtaacgtcACGTgagcctacg
RTP10690_10	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgagcctac
RTP10690_10	Opaque-2 like transcriptional activators	aacgtcACGTgagccta
RTP10690_10	Plant G-box/C-box bZIP proteins	gtaggctcACGTgacgttacg
RTP10690_10	Myc-like basic helix-loop-helix binding factors	taggctCACGtgacgttac
RTP10690_10	Opaque-2 like transcriptional activators	aggctcACGTgacgtta
RTP10690_10	Cell-death specification 2	gtaacGTCAcg
RTP10690_10	Plant G-box/C-box bZIP proteins	cttacgtaACGTcacgtgagc
RTP10690_10	Opaque-2 like transcriptional activators	tacgtaACGTcacgtga
RTP10690_10	Plant G-box/C-box bZIP proteins	ctcacgTGACgttacgtaagg
RTP10690_10	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	aggctcacgtgacgtTACGtaaggcta
RTP10690_10	Opaque-2 like transcriptional activators	cacgtgACGTtacgtaa
RTP10690_10	GT-box elements	cgtgacGTTAcgtaagg
RTP10690_10	Cell-death specification 2	cttacGTAAcg
RTP10690_10	Plant G-box/C-box bZIP proteins	gtagccttACGTaacgtcacg
RTP10690_10	Opaque-2 like transcriptional activators	agccttACGTaacgtca
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_10	Cell-death specification 2	gttacGTAAgg
RTP10690_10	Opaque-2 like transcriptional activators	gacgttACGTaaggcta
RTP10690_10	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gacgctacgtagcctTACGtaacgtca
RTP10690_10	Opaque-2 like transcriptional activators	gacgctACGTagcctta
RTP10690_10	Plant G-box/C-box bZIP proteins	gtaaggctACGTagcgtcacg

RTP10690_10	Opaque-2 like transcriptional activators	aaggctACGTagcgtca
RTP10690_10	Plant G-box/C-box bZIP proteins	ctcacgTGACgctacgtagcc
RTP10690_10	Plant G-box/C-box bZIP proteins	gctacgtaGCGTcacgtgagc
RTP10690_10	Plant G-box/C-box bZIP proteins	gtaagctcACGTgacgctacg
RTP10690_10	Myc-like basic helix-loop-helix binding factors	taagctCACGtgacgctac
RTP10690_10	Opaque-2 like transcriptional activators	aagctcACGTgacgcta
RTP10690_10	ABA response elements	taagctcaCGTGacgct
RTP10690_10	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgagcttacc
RTP10690_10	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgagcttac
RTP10690_10	Opaque-2 like transcriptional activators	agcgtcACGTgagctta
RTP10690_10	GT-box elements	cgtcacGTGAgcttacc
RTP10690_10	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agagttaggtaagctCACGtgacgcta
RTP10690_10	Cell-death specification 2	gttagGTAAgc
RTP10690_10	MYB IIG-type binding sites	tagagttaGGTAagc
RTP10690_10	MYB-like proteins	tagctagAGTTaggtaa
RTP10690_10	ABA response elements	tagcctcaCGTGaccttt
RTP10690_10	Plant G-box/C-box bZIP proteins	ctaaggtcACGTgaggctagc
RTP10690_10	Myc-like basic helix-loop-helix binding factors	taaggtCACGtgaggctag
RTP10690_10	Opaque-2 like transcriptional activators	aaggtcACGTgaggcta
RTP10690_10	Dc3 promoter binding factors	cTCACgtgacc
RTP10690_10	Plant G-box/C-box bZIP proteins	ctagcctcACGTgaccttagc
RTP10690_10	Myc-like basic helix-loop-helix binding factors	tagcctCACGtgaccttag
RTP10690_10	Opaque-2 like transcriptional activators	agcctcACGTgacctta
RTP10690_10	GT-box elements	cctcacGTGAccttagc
RTP10690_10	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agtgttagctaaggtCACGtgaggcta
RTP10690_10	Plant G-box/C-box bZIP proteins	ctgtgcTGACgctacctagtg
RTP10690_10	Plant G-box/C-box bZIP proteins	actaggtaGCGTcagcacaga
RTP10690_10	Myc-like basic helix-loop-helix binding factors	agtattCATCtgtgctgac
RTP10690_10	GAP-Box (light response elements)	acagATGAatactag
RTP10690_11	VIP1 responsive elements	acacgGCTCag
RTP10690_11	Plant G-box/C-box bZIP proteins	gttacgtaACGTcagctgagg
RTP10690_11	Plant G-box/C-box bZIP proteins	ctcagcTGACgttacgtaacg
RTP10690_11	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcctcagctgacgtTACGtaacgcta
RTP10690_11	Opaque-2 like transcriptional activators	cagctgACGTtacgtaa
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_11	GT-box elements	gctgacGTTAcgtaacg
RTP10690_11	Cell-death specification 2	gttacGTAAcg
RTP10690_11	Plant G-box/C-box bZIP proteins	ctagcgttACGTaacgtcagc
RTP10690_11	Opaque-2 like transcriptional activators	agcgttACGTaacgtca
RTP10690_11	Cell-death specification 2	gttacGTAAcg
RTP10690_11	Opaque-2 like transcriptional activators	gacgttACGTaacgcta
RTP10690_11	GT-box elements	cctagcGTTAcgtaacg
RTP10690_11	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gacgctacctagcgtTACGtaacgtca
RTP10690_11	Plant G-box/C-box bZIP proteins	gtcacgTGACgctacctagcg
RTP10690_11	Plant G-box/C-box bZIP proteins	gctaggtaGCGTcacgtgacg

RTP10690_11	ABA response elements	tagcgtcaCGTGacgtt
RTP10690_11	Plant G-box/C-box bZIP proteins	ctaacgtcACGTgacgctacc
RTP10690_11	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgacgctac
RTP10690_11	Opaque-2 like transcriptional activators	aacgtcACGTgacgcta
RTP10690_11	ABA response elements	taacgtcaCGTGacgct
RTP10690_11	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgacgttagc
RTP10690_11	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgacgttag
RTP10690_11	Opaque-2 like transcriptional activators	agcgtcACGTgacgtta
RTP10690_11	Plant G-box/C-box bZIP proteins	gttagctaACGTcacgtgacg
RTP10690_11	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgttagctaacgtCACGtgacgcta
RTP10690_11	Opaque-2 like transcriptional activators	tagctaACGTcacgtga
RTP10690_11	Plant G-box/C-box bZIP proteins	gtcacgTGACgttagctaacg
RTP10690_11	MYB-like proteins	cacgtgacGTTAgctaa
RTP10690_11	Plant G-box/C-box bZIP proteins	ctcagcTGACgctacctagcg
RTP10690_11	Plant G-box/C-box bZIP proteins	gctaggtaGCGTcagctgagc
RTP10690_11	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgtcagctgagctTACGtaagcgca
RTP10690_11	Vertebrate TATA binding protein factor	gcttacgTAAGctcagc
RTP10690_11	Cell-death specification 2	cttacGTAAgc
RTP10690_11	Plant G-box/C-box bZIP proteins	gtgcgcttACGTaagctcagc
RTP10690_11	Opaque-2 like transcriptional activators	gcgcttACGTaagctca
RTP10690_11	Cell-death specification 2	cttacGTAAgc
RTP10690_11	Plant G-box/C-box bZIP proteins	ctgagcttACGTaagcgcaca
RTP10690_11	Opaque-2 like transcriptional activators	gagcttACGTaagcgca
RTP10690_11	Vertebrate TATA binding protein factor	gcttacgTAAGcgcaca
RTP10690_11	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	attcatctgtgcgctTACGtaagctca
RTP10690_11	Plant G-box/C-box bZIP proteins	tcatctgtgcgcTTACgtaag
RTP10690_11	Enhancer element first identified in the promoter of the octopine synthase gene (OCS) of the Agrobacterium tumefaciens T-DNA	tcatctgtgcgctTACGtaag
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_11	Myc-like basic helix-loop-helix binding factors	agtattCATCtgtgcgctt
RTP10690_11	GAP-Box (light response elements)	acagATGAatactag
RTP10690_11	MYB-like proteins	tactagctGTTGttcac
RTP10690_12	VIP1 responsive elements	acacgGCTCag
RTP10690_12	Plant G-box/C-box bZIP proteins	gttacgtaACGTcagctgagg
RTP10690_12	Plant G-box/C-box bZIP proteins	ctcagcTGACgttacgtaacg
RTP10690_12	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcctcagctgacgtTACGtaacgcta
RTP10690_12	Opaque-2 like transcriptional activators	cagctgACGTtacgtaa
RTP10690_12	GT-box elements	gctgacGTTAcgtaacg
RTP10690_12	Cell-death specification 2	gttacGTAAcg
RTP10690_12	Plant G-box/C-box bZIP proteins	ctagcgttACGTaacgtcagc
RTP10690_12	Opaque-2 like transcriptional activators	agcgttACGTaacgtca

RTP10690_12	Cell-death specification 2	gttacGTAAcg
RTP10690_12	Opaque-2 like transcriptional activators	gacgttACGTaacgcta
RTP10690_12	GT-box elements	cctagcGTTAcgtaacg
RTP10690_12	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gacgctacctagcgtTACGtaacgtca
RTP10690_12	Plant G-box/C-box bZIP proteins	gtcacgTGACgctacctagcg
RTP10690_12	Plant G-box/C-box bZIP proteins	gctaggtaGCGTcacgtgacg
RTP10690_12	ABA response elements	tagcgtcaCGTGacgtt
RTP10690_12	Plant G-box/C-box bZIP proteins	ctaacgtcACGTgacgctacc
RTP10690_12	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgacgctac
RTP10690_12	Opaque-2 like transcriptional activators	aacgtcACGTgacgcta
RTP10690_12	ABA response elements	taacgtcaCGTGacgct
RTP10690_12	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgacgttagc
RTP10690_12	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgacgttag
RTP10690_12	Opaque-2 like transcriptional activators	agcgtcACGTgacgtta
RTP10690_12	Plant G-box/C-box bZIP proteins	gttagctaACGTcacgtgacg
RTP10690_12	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgttagctaacgtCACGtgacgcta
RTP10690_12	Opaque-2 like transcriptional activators	tagctaACGTcacgtga
RTP10690_12	Plant G-box/C-box bZIP proteins	gtcacgTGACgttagctaacg
RTP10690_12	MYB-like proteins	cacgtgacGTTAgctaa
RTP10690_12	Plant G-box/C-box bZIP proteins	ctcagcTGACgctacctagcg
RTP10690_12	Plant G-box/C-box bZIP proteins	gctaggtaGCGTcagctgagc
RTP10690_12	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgtcagctgagctTACGtaagcgct
RTP10690_12	Vertebrate TATA binding protein factor	gcttacgTAAGctcagc
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_12	Cell-death specification 2	cttacGTAAgc
RTP10690_12	Plant G-box/C-box bZIP proteins	aagcgcttACGTaagctcagc
RTP10690_12	Opaque-2 like transcriptional activators	gcgcttACGTaagctca
RTP10690_12	Cell-death specification 2	cttacGTAAgc
RTP10690_12	Plant G-box/C-box bZIP proteins	ctgagcttACGTaagcgctta
RTP10690_12	Opaque-2 like transcriptional activators	gagcttACGTaagcgct
RTP10690_12	Vertebrate TATA binding protein factor	gcttacgTAAGcgctta
RTP10690_12	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	ctttaattaagcgctTACGtaagctca
RTP10690_12	Enhancer element first identified in the promoter of the octopine synthase gene (OCS) of the Agrobacterium tumefaciens T-DNA	ttaattaagcgcttACGTaag
RTP10690_12	Vertebrate TATA binding protein factor	ctttaatTAAGcgctta
RTP10690_12	DNA binding with one finger (DOF)	gcttaattAAAGtactg
RTP10690_12	Arabidopsis homeobox protein	gtaCTGAtatc
RTP10690_12	Dehydration responsive element binding factors	atttggtaCCGAtatcagtac
RTP10690_12	SBP-domain proteins	tatcgGTACcaaatcga
RTP10690_12	Sucrose box	aaAAATtacggatatgaat
RTP10690_12	MYB proteins with single DNA binding repeat	attcatATCCgtaattt
RTP10690_5	M-phase-specific activator elements	attacAACGgtatat

RTP10690_5	Yeast TATA binding protein factor	aacggtaTATAtcctgc
RTP10690_5	Yeast TATA binding protein factor	ggcaggaTATAtaccgt
RTP10690_5	Vertebrate TATA binding protein factor	cggtaTATAtcctgcca
RTP10690_5	MYB proteins with single DNA binding repeat	gtatatATCCtgccagt
RTP10690_5	Caenorhabditis maternal gene product SKN-1	cagcATCAtcaca
RTP10690_5	Fungal and oomycete pathogen response cluster - promoter motif	ctattcGGGCctaactt
RTP10690_5	Retroviral upstream element	ggcccgaatagTTTGaaatta
RTP10690_5	SBP-domain proteins	tagcCGTAcaatattac
RTP10690_5	Enhancer element first identified in the promoter of the octopine synthase gene (OCS) of the Agrobacterium tumefaciens T-DNA	gtgagtaatattgtACGGcta
RTP10690_5	Arabidopsis homeobox protein	aatATTActca
RTP10690_5	Opaque-2 like transcriptional activators	accggTGAGtaatattg
RTP10690_5	Epstein-Barr virus transcription factor R	atgccccccatcgtaGGTGaa
RTP10690_5	Retroviral PolyA signal	gCCATtaatttccaccttcac
RTP10690_5	Retroviral upstream element	cgcgccattaaTTTCcacctt
RTP10690_5	L1 box, motif for L1 layer-specific expression	cgcCATTaatttccacc
RTP10690_5	Arabidopsis homeobox protein	gaaATTAatgg
RTP10690_5	GT-box elements	gtaataGTTActaatca
RTP10690_5	MYB-like proteins	acgtaatAGTTactaat
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	cactgattagtaactatTACGtaagcc
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	ctgattagtaactatTACGtaagccta
RTP10690_5	Cell-death specification 2	cttacGTAAta
RTP10690_5	Plant G-box/C-box bZIP proteins	gtaggcttACGTaatagttac
RTP10690_5	Cell-death specification 2	attacGTAAgc
RTP10690_5	Vertebrate TATA binding protein factor	tattacgTAAGcctacg
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gacgctacgtaggctTACGtaatagtt
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	actattacgtaagccTACGtagcgtca
RTP10690_5	Plant G-box/C-box bZIP proteins	gtaagcctACGTagcgtcacg
RTP10690_5	Opaque-2 like transcriptional activators	aagcctACGTagcgtca
RTP10690_5	Plant G-box/C-box bZIP proteins	gtcacgTGACgctacgtaggc
RTP10690_5	Plant G-box/C-box bZIP proteins	cctacgtaGCGTcacgtgacg
RTP10690_5	ABA response elements	tagcgtcaCGTGacgtt
RTP10690_5	Plant G-box/C-box bZIP proteins	ctaacgtcACGTgacgctacg
RTP10690_5	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgacgctac
RTP10690_5	Opaque-2 like transcriptional activators	aacgtcACGTgacgcta
RTP10690_5	ABA response elements	taacgtcaCGTGacgct
RTP10690_5	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgacgttagc
RTP10690_5	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgacgttag
RTP10690_5	Opaque-2 like transcriptional activators	agcgtcACGTgacgtta
RTP10690_5	Plant G-box/C-box bZIP proteins	gttagctaACGTcacgtgacg
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgttagctaacgtCACGtgacgcta

RTP10690_5	Opaque-2 like transcriptional activators	tagctaACGTcacgtga
RTP10690_5	Plant G-box/C-box bZIP proteins	gtcacgTGACgttagctaacg
RTP10690_5	MYB-like proteins	cacgtgacGTTAgctaa
RTP10690_5	Plant G-box/C-box bZIP proteins	ctgaggctACGTagcgttagc
RTP10690_5	Opaque-2 like transcriptional activators	gaggctACGTagcgtta
RTP10690_5	Opaque-2 like transcriptional activators	aacgctACGTagcctca
RTP10690_5	Plant G-box/C-box bZIP proteins	cttacgtaACGTcagctgagg
RTP10690_5	Plant G-box/C-box bZIP proteins	ctcagcTGACgttacgtaagc
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcctcagctgacgtTACGtaagccta
RTP10690_5	Opaque-2 like transcriptional activators	cagctgACGTtacgtaa
RTP10690_5	GT-box elements	gctgacGTTAcgtaagc
RTP10690_5	Cell-death specification 2	cttacGTAAcg
RTP10690_5	Plant G-box/C-box bZIP proteins	gtaggcttACGTaacgtcagc
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_5	Opaque-2 like transcriptional activators	aggcttACGTaacgtca
RTP10690_5	Cell-death specification 2	gttacGTAAgc
RTP10690_5	Plant G-box/C-box bZIP proteins	ctgacgttACGTaagcctacg
RTP10690_5	Opaque-2 like transcriptional activators	gacgttACGTaagccta
RTP10690_5	Vertebrate TATA binding protein factor	cgttacgTAAGcctacg
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gacgctacgtaggctTACGtaacgtca
RTP10690_5	Plant G-box/C-box bZIP proteins	gtaagcctACGTagcgtcacg
RTP10690_5	Opaque-2 like transcriptional activators	aagcctACGTagcgtca
RTP10690_5	Plant G-box/C-box bZIP proteins	ctcacgTGACgctacgtaggc
RTP10690_5	Plant G-box/C-box bZIP proteins	cctacgtaGCGTcacgtgagc
RTP10690_5	Plant G-box/C-box bZIP proteins	ctaagctcACGTgacgctacg
RTP10690_5	Myc-like basic helix-loop-helix binding factors	taagctCACGtgacgctac
RTP10690_5	Opaque-2 like transcriptional activators	aagctcACGTgacgcta
RTP10690_5	ABA response elements	taagctcaCGTGacgct
RTP10690_5	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgagcttagc
RTP10690_5	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgagcttag
RTP10690_5	Opaque-2 like transcriptional activators	agcgtcACGTgagctta
RTP10690_5	GT-box elements	cgtcacGTGAgcttagc
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgttagctaagctCACGtgacgcta
RTP10690_5	Plant G-box/C-box bZIP proteins	gttacgtaACGTcagctgagc
RTP10690_5	Plant G-box/C-box bZIP proteins	ctcagcTGACgttacgtaacg
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	aggctcagctgacgtTACGtaacgcta
RTP10690_5	Opaque-2 like transcriptional activators	cagctgACGTtacgtaa
RTP10690_5	GT-box elements	gctgacGTTAcgtaacg
RTP10690_5	Cell-death specification 2	gttacGTAAcg
RTP10690_5	Plant G-box/C-box bZIP proteins	ctagcgttACGTaacgtcagc
RTP10690_5	Opaque-2 like transcriptional activators	agcgttACGTaacgtca
RTP10690_5	Cell-death specification 2	gttacGTAAcg
RTP10690_5	Opaque-2 like transcriptional activators	gacgttACGTaacgcta
RTP10690_5	GT-box elements	gctagcGTTAcgtaacg
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172,	gacgctagctagcgtTACGtaacgtca

	CUC2 (cup-shaped cotyledons 2)] transcription factors	
RTP10690_5	Plant G-box/C-box bZIP proteins	caggagTGACgctagctagcg
RTP10690_5	Plant G-box/C-box bZIP proteins	gctagctaGCGTcactcctgc
RTP10690_5	Enhancer element first identified in the promoter of the octopine synthase gene (OCS) of the Agrobacterium tumefaciens T-DNA	ttgctgcaggagtGACGctag
t-AtAHASL	Retroviral PolyA signal	aCCATaaaaggttctgataat
t-AtAHASL	Vertebrate TATA binding protein factor	gaccaTAAAaggttctg
t-AtAHASL	Papillioma virus E2 transcriptional activator	agaccataaaAGGTtct
Spacer ID	**Detailed Family Information**	**Sequence**
t-AtAHASL	MADS box proteins	aaagaccatAAAAggttctga
t-AtAHASL	Papillioma virus E2 transcriptional activator	gaacctttaTGGTctt
t-AtAHASL	GT-box elements	ttttATGGtctttgtat
t-AtAHASL	MYB proteins with single DNA binding repeat	taccaTATGcatacaaa
t-AtAHASL	Arabidopsis homeobox protein	aaaCTTAgttt
t-AtAHASL	MYB-like proteins	aaaactTAGTttgcaat
t-AtAHASL	GT-box elements	gttttgGTAAtttgagt
t-AtAHASL	MYB-like proteins	ttcttttAGTTgttgat
t-AtAHASL	Retroviral CCAAT binding factors	aCCAAaaagcaggcagatcaacaac
t-AtAHASL	Sucrose box	aaAAAGcaggcagatcaac
t-AtAHASL	Legumin Box family	ttgatctGCCTgctttttggtttacgt
t-AtAHASL	DNA binding with one finger (DOF)	taaaccaaAAAGcaggc
t-AtAHASL	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gcctgctttttggttTACGtcagacta
t-AtAHASL	Cell-death specification 2	ctgacGTAAac
t-AtAHASL	Plant G-box/C-box bZIP proteins	gtagtcTGACgtaaaccaaaa
t-AtAHASL	Cell-death specification 2	tttacGTCAga
t-AtAHASL	Plant G-box/C-box bZIP proteins	tttggtttACGTcagactact
t-AtAHASL	Plant G-box/C-box bZIP proteins	gcagtagtagtcTGACgtaaa
t-AtAHASL	Secondary wall NACS	tttataaaatgAAAGaaag
t-AtAHASL	Core promoter initiator elements	ttTCATtttat
t-AtAHASL	Vertebrate TATA binding protein factor	atttaTAAAatgaaaga
t-AtAHASL	Plant TATA binding protein factor	atttTATAaataaat
t-AtAHASL	Vertebrate TATA binding protein factor	ttttaTAAAtaaataat
t-AtAHASL	L1 box, motif for L1 layer-specific expression	tataaaTAAAtaatccg
t-AtAHASL	Retroviral PolyA signal	ataaaTAAAtaatccggttcg
t-AtAHASL	Dehydration responsive element binding factors	ggagtaaaCCGAaccggatta
t-AtAHASL	MYB-like proteins	tcagtttgGTTAttgcg
t-AtAHASL	GT-box elements	agtttgGTTAttgcgaa
t-AtAHASL	Cell-death specification 2	atttcGCAAta
t-AtAHASL	Cell-death specification 2	attgcGAAAtg
t-AtAHASL	Plant nitrate-responsive cis-elements	tcaatttaccattcgcatttcgcAATAac caaa
t-AtAHASL	TEF-box	gaATGGtaaattgagtaattg
t-AtAHASL	Cell-death specification 2	attgaGTAAtt
t-AtAHASL	MYB-like proteins	tgaaatTCGTtattagg
t-AtAHASL	Telo box (plant interstitial telomere motifs)	tagaaCCCTaataac
t-AtAHASL	Mitochondral HMG-box transcription factor	attagggTTCTaa
t-AtAHASL	VIP1 responsive elements	aaacaGCTTag
t-AtAHASL	CA-rich motif	ctgttaaAACAgcttagaa

t-AtAHASL	MYB-like proteins	cagtgaCTGTtaaaaca
t-AtAHASL	GT-box elements	cactggGTTAatatctc
t-AtAHASL	Circadian control factors	tgggttAATAtctct
t-AtAHASL	Myb-related DNA binding proteins (Golden2, ARR, Psr)	AGATtcgag
Spacer ID	**Detailed Family Information**	**Sequence**
t-AtAHASL	Legumin Box family	gcattttcCATGcaagattcgagagat
t-AtAHASL	GT-box elements	ttgcATGGaaaatgctc
t-AtAHASL	Retroviral upstream element	ggtaagagcatTTTCcatgca
t-AtAHASL	Mitochondral HMG-box transcription factor	aagagcaTTTTcc
t-AtAHASL	MYB proteins with single DNA binding repeat	tggaAAATgctcttacc
t-AtAHASL	LFY binding site	aACCAatggtaag
t-AtAHASL	CCAAT binding factors	aaCCAAtgg
t-AtAHASL	LFY binding site	tACCAttggtttt
t-AtAHASL	Retroviral PolyA signal	ttcaaTTAAaaaccaatggta
t-AtAHASL	Soybean embryo factor 4	ggTTTTtaatt
t-AtAHASL	DNA-binding proteins with the plant specific TCP-domain	cacgGCCCatatg
t-AtAHASL	Root hair-specific cis-elements in angiosperms	atttaatttggaaacCACGgcccat
t-AtAHASL	MADS box proteins	ggtTTCCaaattaaataaaac
t-AtAHASL	Vertebrate TATA binding protein factor	ttttattTAATttggaa
t-AtAHASL	Sulphur limitation, elements found in genes inducible during sulphur deprivation	agtttTATTtaatttggaaac
t-AtAHASL	High mobility group factors	gtttTATTtaatttg
t-AtAHASL	L1 box, motif for L1 layer-specific expression	ccaaatTAAAtaaaact
t-AtAHASL	Retroviral PolyA signal	ttaaaTAAAactacgatgtca
t-AtAHASL	Opaque-2 like transcriptional activators	actacgatgTCATcgag
t-AtAHASL	Caenorhabditis maternal gene product SKN-1	cgatGTCAtcgag
t-AtAHASL	Sucrose box	taAAATcaactgtgtccac
t-AtAHASL	Lentiviral Poly A downstream element	aaCTGTgtccacatt
t-AtAHASL	MYB-like proteins	acaaaactGATAatgtg
t-AtAHASL	Retroviral upstream element	cacattatcagTTTTgtgtat
t-AtAHASL	Core promoter initiator elements	taTCAGttttg
t-AtAHASL	GT-box elements	aataggGTAAttcaaaa
t-AtAHASL	Circadian control factors	taattcaaAATCtag
t-AtAHASL	Lentiviral Tata upstream element	aaggttaaaatgAACCaaaaggc
t-AtAHASL	GT-box elements	cagaaggTTAAaatgaa
t-AtAHASL	Heat shock factors	tttaacCTTCtgtaaac
t-AtAHASL	Nodulin consensus sequence 1	aAAATgtttac
t-AtAHASL	Circadian control factors	ttgttcaaAATCtga
t-AtAHASL	L1 box, motif for L1 layer-specific expression	aacaagTAAAtccaaaa
t-AtAHASL	Circadian control factors	aaaaaaaaAATCtca
t-AtAHASL	Mitochondral HMG-box transcription factor	ttgagatTTTTtt
t-AtAHASL	Arabidopsis homeobox protein	taaTTTAgtgt
t-AtAHASL	L1 box, motif for L1 layer-specific expression	caaCACTaaattatttt
t-AtAHASL	Storekeeper motif	cacTAAAttatttta
t-AtAHASL	Arabidopsis homeobox protein	aaaATAAttta
t-AtAHASL	Arabidopsis homeobox protein	taaATTAtttt
t-AtAHASL	Arabidopsis homeobox protein	tatttTAATgt
t-AtAHASL	Vertebrate TATA binding protein factor	atgtaTAAAagatgctt
t-AtAHASL	Nodulin consensus sequence 1	tAAAAgatgct
t-AtAHASL	Storekeeper motif	gctTAAAacatttgg
t-AtAHASL	Myc-like basic helix-loop-helix binding factors	ttaaaACATttggcttaaa
Spacer ID	**Detailed Family Information**	**Sequence**
t-AtAHASL	Sucrose box	ttAAAAcatttggcttaaa

t-AtAHASL	Secondary wall NACS	catttggcttaAAAGaaag
t-AtAHASL	Tracheary-element-regulating cis-elements, conferring TE-specific expression	cttaAAAGaaa
t-AtAHASL	Heat shock factors	taaaaacatagAGAAct
t-AtAHASL	DNA binding with one finger (DOF)	actcttgtAAATtgaag
t-AtAHASL	MYB proteins with single DNA binding repeat	cagtATATtttcatact
t-AtAHASL	MADS box proteins	taataCCCAattcagtatatt
t-AtAHASL	MADS box proteins	ataTACTgaattgggtattat
t-AtAHASL	CCAAT binding factors	acCCAAttc
t-AtAHASL	Sucrose box	ctAAATcagaaaaattcat
t-AtAHASL	MADS box proteins	tgaatcctaaatcaGAAAaat
UB2197_5	M-phase-specific activator elements	ggacgAACGgataaa
UB2197_5	MYB proteins with single DNA binding repeat	aggtttATCCgttcgtc
UB2197_5	Nodulin consensus sequence 1	gAAAAggttta
UB2197_5	DNA binding with one finger (DOF)	gggcgtgaAAAGgttta
UB2197_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	acggataaacctttttCACGccctttta
UB2197_5	E2F-homolog cell cycle regulators	ccttTTCAcgccctt
UB2197_5	Transposase-derived transcription factors	ttttCACGccctttttaa
UB2197_5	Vertebrate TATA binding protein factor	atattTAAAagggcgtg
UB2197_5	DNA binding with one finger (DOF)	gatatttaAAAGggcgt
UB2197_5	Vertebrate TATA binding protein factor	cctttTAAAtatccgat
UB2197_5	Arabidopsis homeobox protein	ccgATTAttct
UB2197_5	Retroviral PolyA signal	tctaaTAAAcgctcttttctc
UB2197_5	Retroviral CCAAT binding factors	tCTAAtaaacgctcttttctcttag
UB2197_5	DNA binding with one finger (DOF)	ctaagagaAAAGagcgt
UB2197_5	Nodulin consensus sequence 2	tcttttCTCTtaggt
UB2197_5	Ethylen respone element factors	gGCGGgtaaacctaagaga
UB2197_5	DNA binding with one finger (DOF)	tggcgggtAAACctaag
UB2197_5	MYB proteins with single DNA binding repeat	caggATATattggcggg
UB2197_5	MYB proteins with single DNA binding repeat	caatatATCCtgtcaaa
UB2197_5	TALE (3-aa acid loop extension) class homeodomain proteins	tttGACAggatat
UB2197_5	W Box family	agtgtTTGAcaggatat
UB2197_5	E2F-homolog cell cycle regulators	tcgtTTCCgccttc
UB2197_5	Circadian control factors	gaaacgacAATCtga
UB2197_5	MYB-like proteins	aggcctTAGTtactaat
UB2197_5	Vertebrate TATA binding protein factor	tagtaacTAAGgccttt
UB2197_5	Retroviral PolyA signal	attaaTTAAaggccttagtta
UB2197_5	DNA binding with one finger (DOF)	gattaattAAAGgcctt
UB2197_5	Arabidopsis homeobox protein	ttaatTAATct
UB2197_5	Arabidopsis homeobox protein	tagATTAatta
UB2197_5	Light responsive element motif, not modulated by different light qualities	taATCTagagg
Spacer ID	**Detailed Family Information**	**Sequence**
UB2197_5	Vertebrate TATA binding protein factor	caattTAAAttggccgg
UB2197_5	L1 box, motif for L1 layer-specific expression	tcaattTAAAttggccg
UB2197_5	Retroviral upstream element	accgatatcaaTTTAaattgg
UB2197_5	MYB proteins with single DNA binding repeat	accgaTATCaatttaaa
UB2197_5	Dehydration responsive element binding factors	tcgatgtaCCGAtatcaattt
UB2197_5	Plant specific NAC [NAM (no apical meristem), ATAF172,	tatcggtacatcgatTACGccaagcta

	CUC2 (cup-shaped cotyledons 2)] transcription factors	
UB2197_5	Cell-death specification 2	ttggcGTAAtc
UB2197_5	MADS box proteins	cttTTCTatacaaagttgata
UB2197_5	Plant TATA binding protein factor	tttcTATAcaaagtt
UB2197_5	Pollen-specific regulatory elements	gtataGAAAagttgcca
UB2197_5	GT-box elements	aatcATGGcaacttttc
UB2197_5	Arabidopsis homeobox protein	gtaATCAtggc
UB2197_5	Arabidopsis homeobox protein	gccatgATTAc
UB2197_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	aaaagttgccatgatTACGccaagctt
UB2197_5	Cell-death specification 2	ttggcGTAAtc
UB2197_5	Calcium regulated NAC-factors	ccaaGCTTggccactaaggcc
UB2197_5	E2F-homolog cell cycle regulators	caatTTCGcgccctg
UB2197_1	Arabidopsis homeobox protein	caaTTTActga
UB2197_1	Motifs of plastid response elements	cacgCGATcgcgtcgacacaatcagtaaatt
UB2197_1	Calmodulin binding / CGCG box binding proteins	gatCGCGtcgacacaat
UB2197_1	Core promoter motif ten elements	gcacgcgATCGcgtcgacaca
UB2197_1	Calmodulin binding / CGCG box binding proteins	gatCGCGtgcaaacact
UB2197_1	Root hair-specific cis-elements in angiosperms	tccgtacagtgtttgCACGcgatcg
UB2197_1	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	ggtccgtacagtgtttgCACGcgatcg
UB2197_1	SBP-domain proteins	ggtcCGTAcagtgtttg
UB2197_1	SBP-domain proteins	ggccgGTACccaagttt
UB2197_1	SBP-domain proteins	agtttGTACaaaaaagc
UB2197_1	DNA binding with one finger (DOF)	tgtacaaaAAAGcaggc
UB2197_1	Legumin Box family	caaaaaaGCAGgctccatgattacgcc
UB2197_1	Arabidopsis homeobox protein	tccatgATTAc
UB2197_1	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcaggctccatgatTACGccaagctt
UB2197_1	Cell-death specification 2	ttggcGTAAtc
UB2197_1	Calcium regulated NAC-factors	ccaaGCTTggccactaaggcc
Spacer ID	**Detailed Family Information**	**Sequence**
UB2197_1	L1 box, motif for L1 layer-specific expression	tagattTAAAttggcct
UB2197_1	Circadian control factors	ccaatttaAATCtac
UB2197_1	Vertebrate TATA binding protein factor	caattTAAAtctactag
UB2197_1	Light responsive element motif, not modulated by different light qualities	aaATCTactag
UB2197_2	E2F-homolog cell cycle regulators	gtaaTTCCcgggatt
UB2197_2	Brassinosteroid (BR) response element	ctgccCGTGtggccggc
UB2197_3	Core promoter initiator elements	ttTCATtcggt
UB2197_3	Retroviral upstream element	ggcgccatcggTTTCattcgg
UB2197_3	Papillioma virus E2 transcriptional activator	ggaccgatacCGGTagg
UB2197_3	Papillioma virus E2 transcriptional activator	ctaccggtatCGGTccg
UB2197_3	Dehydration responsive element binding factors	caatcggaCCGAtaccggtag

UB2197_3	Core promoter motif ten elements	ggccgcaATCGgaccgatacc
UB2197_3	TEF-box	taAAGGgcgaattcgtttaaa
UB2197_3	SBP-domain proteins	ggtcCGTAcagtgttta
UB2197_3	Activator-, mediator- and TBP-dependent core promoter element for RNA polymerase II transcription from TATA-less promoters	ggGTGGtaccg
UB2197_3	SBP-domain proteins	ggccgGTACcacccagc
UB2197_3	Soybean embryo factor 3	gtaccACCCagcttt
UB2197_3	Arabidopsis homeobox protein	gtaATCAtggc
UB2197_3	Arabidopsis homeobox protein	gccatgATTAc
UB2197_3	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	caaagtggccatgatTACGccaagctt
UB2197_3	Cell-death specification 2	ttggcGTAAtc
UB2197_3	Calcium regulated NAC-factors	ccaaGCTTggccactaaggcc
UB2197_3	L1 box, motif for L1 layer-specific expression	tagattTAAAttggcct
UB2197_3	Circadian control factors	ccaatttaAATCtac
UB2197_3	Vertebrate TATA binding protein factor	caattTAAAtctactag
UB2197_3	Light responsive element motif, not modulated by different light qualities	aaATCTactag
UB2197_3	MYB IIG-type binding sites	aatgGGTAggtcatc
UB2197_4	E2F-homolog cell cycle regulators	gtaaTTCCcgggatt

Of particular note in Table 172 was the surprisingly high number of sequences that contain abscisic acid related transcription factor binding motifs for the construct VC-RTP10690-1qcz_F. Abscisic acid was a plant hormone involved in seed maturation, desiccation, and stress response, see Cutler et al. (2010) for a comprehensive review. Based upon what was known about abscisic acid and its effect on seed maturation, the observed high number of motifs implicated in the regulation of gene expression in response to abscisic acid may have played a role in the observed transcript levels and timing of gene expression for the genes in VC-RTP10690-1qcz_F. In addition to the likely affects on transcription, it is also known in the literature that topography is affected by GC content (see Wachter et al., 2014) and it is prudent to have a GC content in the T-DNA matching that of the host as much as possible. In addition to reducing the possibility of deletions and rearrangements, reducing the amount of repeats would also impact DNA topography and gene regulation (Ramamoorthy et al. 2014). The spacer regions would contribute to the overall impact of the T-DNA upon the local chromatin structure, which in turn will impact how the genes in that region are regulated and transcribed (Parker et al. 2009, Meggendorfer et al. 2010)..

Example 24 Gene Expression, Copy number determination and event detection

Plant material representing wild type, the BiBAC VC-RTP10690-1qcz_F, and the co-transformation constructs: VC-LJB2197-1qcz with VC-LLM337-1qcz rc, VC-LJB2755-2qcz rc with VC-LLM391-2qcz rc and VC-LJB2755-2qcz rc with VC-LTM217-1qcz rc were harvested according to the date after pollination. Construct details (genes and promoters) are found inTable 1, Table 2, Table 4, Table 6, Table 7 and Table 8. Based on the day after pollination, which correlated with developmental stage under the growth conditions employed, the seeds were combined into pools. The pooled seeds were immediately frozen in liquid nitrogen and subsequently stored at -80° C. A third of the frozen immature seeds were homogenized using the Precelly®24-Dual technology in 7ml tubes and two ceramic beads (6500Hz, 2-3 times, 20sec). From each pool three aliquots of 50-70mg of tissue were used to isolate the RNA. Frozen tissue was ground to a fine powder and extracted following basic procedures familiar to one who was skilled in the art (see Ruuska et al. 2000 and Focks and Benning, 1998 as well as Sambrook et al.1989 for an overview on RNA extraction from immature seeds and sample and RNA handling). RNA was extracted according to the protocol "SG-MA_0007-2009 RNA isolation" using Spectrum Plant Total RNA-KIT part number STRN50 (SIGMA-ALDRICH GmbH, Munich, Germany). In average the concentration of total RNA was about 450ng/µl. The 260/280 ratio was at 2.2 and the 260/230 ratio at 2.3.

For cDNA synthesis for qPCR 1µg of total RNA was treated with DNAseI (DEOXYRIBUNUCLEASE I (AMP-D1, Amplification Grade from SIGMA-Aldrich, GmbH) according to the supplier's protocol. After DNAseI treatment, the reverse transcription reaction was performed with the SuperScript™ III First-Strand Synthesis SuperMix for qRT-PCR (Invitrogen, Cat. No. 11752-250) and with a combination of oligo dT and random hexamers to ensure thorough and even representation of all transcripts, regardless of length.

Quantitative real time pcr protocol

Transcript measurement by quantitative real time PCR was carried out using procedures considered standard to those skilled in the art; see Livak and Schmittgen (2001). The qPCR reactions were done as simplex TaqMan reactions. The endogenous

reference gene was isolated in house and used due to predicted stability of the transcript based on the observed stability of the transcript corresponding to the orthologue in *Arabidopsis thaliana* during development. The canola ortholog was isolated and the gene, SEQ ID, was part of the glycosyl-phosphatidyl inositol aminotransferase pathway (GPI). The cDNA reactions, described above, were diluted 1:4. 2µl cDNA, which corresponded to 25ng of total RNA, was used per 10µl qPCR reaction with JumpStart TAQ ReadyMix (P2893-400RXN Sigma-Aldrich, GmbH). Primer/probe concentrations were 900 nmol for forward and reverse primer and 100nmol TaqMan probe. The TaqMan probes for targets of interest were labeled with FAM/BHQ1, and the reference gene was labeled with Yakima Yellow/BHQ1.

Each qPCR assay included a 1:1 dilution curve (5 dilution steps) with cDNA from the pool VC-RTP10690-1qcz_F, a no template control, three -RT controls (VC-RTP10690-1qcz_F, VC-LTM593-1qcz rc (~4w) and co-transformation VC-LJB2197-1qcz + VC-LLM337-1qcz rc). From each pool three independent aliquots of cDNA were measured as technical repeats. The ABI PRISM® 7900 sequence detection system (Applied Biosystem) was used with the following PCR Conditions:

Initial denaturation	95°C for 300 seconds	1 cycle
Amplification	95°C for 15 seconds/60°C for 60 seconds repate	for 40 cycles

The raw data were the Ct values for the target and the endogenous reference gene, respectively. The dCt values were calculated by subtraction: Ct(GOI)-Ct(Ref). The Reference dCt value was set to equal zero, which was interpreted as meaning that if there was no difference between GPI and the gene of interest (dCt = 0) the expression was = 1. The fold expression was equal to 2^{-dCt} (where the dCt = (Ct(GOI)-Ct(Ref)-0)). Three samples from each pool were taken and the geomean as well as the geometric positive and negative deviation were calculated. The slopes of dilution curves were calculated for each gene of interest and the endogenous reference gene (GPI) as a measure for the amplification efficiency. Table 173,

Table 174 and Table 175 indicate the probes and primers used to amplify the genes for qPCR assays.

Table 173: Probes used in the qPCR reactions

Target of Interest	Probe	Probe Oligo
c-o3DES(Pi_GA2)	o3DES-594FAM	CGCTCACTTCTTCGTTGCTGGACTCTC
c-o3Des(Pir_GA)	o3DESPIR-198FAM	ATCATCTCTCTCGGAGTTC
c-o3Des(Pir_GA) 3'	o3DESPIR-962FAM	CGCTGCTCCTATCATCCCAACTTTCTTCA
j-o3DES(PIR_GA)/t-AtPXR	AtPXR-Fam	TCGACCTAGAGGATCCCCGGCC
j-BnSETLprom/o3DES(PIR_GA)	BnSETL-1186FAM	CTCTGCCAGCGACCAAATCGAAGC
j-o3DES(PIR_GA)/t-BnSETL	BnSETL-Fam	CCTTGTGTTAGTTTAATG
c-d5Elo(Ot_GA3)	E011	TGACAAACAAGCCACCAAGCCCAA
c-d4DES(TC_GA)	D4DES-Tc-FAM	TGCTTCCCCAATGTACGTTGCTAGGTTCT
c-d4Des(Eg_GA)	D4DES-Eg-FAM	AAGGCACATCCTCC
c-d4Des(PI_GA2)	D4DES-PI-770FAM	AGCTTCTTTTCTTGGACGCCCTTGAGC
GPI	Exp3-78-YAK	GGATTCGACATTCCATCGGCTTTGA

Table 174: Forward primers used in qPCR

Target of Interest	Forward Primer	Forward Primer Oligo
c-o3DES(Pi_GA2)	o3DES-572F	CCGCTGTGGTTATCTCTTTGC
c-o3Des(Pir_GA)	o3DESPIR-160F	CTTGGGAGGCTATGTATGTTAGAAGA
c-o3Des(Pir_GA) 3'	o3DESPIR-924F	GCTTTCCCTGAGCTTGTTAGGA
j-o3DES(PIR_GA)/t-AtPXR	o3DesPir-F	GGAGGCTAAGGCTGCTGCTA
j-	BnSETL-	GACCCCCTCTCTCTCGTTGTC

Target of Interest	Forward Primer	Forward Primer Oligo
BnSETLprom/o3DES(PIR_GA)	1164-F	
j-o3DES(PIR_GA)/t-BnSETL	BnSETL-1164-F	GACCCCCTCTCTCTCGTTGTC
c-d5Elo(Ot_GA3)	MA54	GCAATCGTTGGTAGCCATGA
c-d4DES(TC_GA)	D4DES-Tc-F	CAAATCGATGCTGAGTGCAGAT
c-d4Des(Eg_GA)	D4DES-EG-F	TGACAAGTAAGCCATCCGTCAGT
c-d4Des(Pl_GA2)	D4DES-Pl-746-F	CTGGTGAGGCTATGTACGCTTTT
GPI	Exp 3-52F	GATGAATATCCTCCTGATGCTAACC

Table 175: Reverse primers used for qPCR

Target of Interest	Reverse Primer	Reverse Primer Oligo
c-o3DES(Pi_GA2)	o3DES-652R	TCTTAAGTCCCAACTGGAGAGACA
c-o3Des(Pir_GA)	o3DESPIR-262R	AAACCAAGGAGCGTCAAGTCTAGA
c-o3Des(Pir_GA) 3'	o3DESPIR-1026-R	TCAACAACTCCGTACTTAGCGTACAT
j-o3DES(PIR_GA)/t-AtPXR	AtPXR-R	TGCATGTACGTTATATAGTAGGCTTTTG
j-BnSETLprom/o3DES(PIR_GA)	o3DESPIR-R	GCAGATGTAGAAGCCATGGTTTG
j-o3DES(PIR_GA)/t-BnSETL	o3DESPIR-R	GCAGATGTAGAAGCCATGGTTTG
c-d5Elo(Ot_GA3)	MA55	CGTGTACCACCACGCTTTGT
c-d4DES(TC_GA)	D4DES-Tc-988R	AACACGGTCAAAGCCTTCATAATC
c-d4Des(Eg_GA)	D4DES-Eg-R	ACTTTTCACCACCGACGAAGTT
c-d4Des(Pl_GA2)	D4DES-Pl-817R	CCTCCCACCTCCAAGCAA
GPI	Exp 3-	CTTGCATGATGATCAGGAAAGC

Target of Interest	Reverse Primer	Reverse Primer Oligo
	128R	

Transcript analysis of a time course for gene expression of the BiBAC and co-transformations.

Transformants from four constructs were assayed as described above and transcript abundance relative to a single standard was measured as described, following standard protocols known to those skilled in the art as well as instructions provided with the kits employed. The graphs in Figure 16, Figure 17, Figure 18, Figure 19, Figure 20 and Figure 21 represent abundance of the gene listed over time with the error represented by the geometric positive and negative deviation. The time was listed on the abscissa and represents days after pollination and the ordinate contains the expression of the gene of interest relative to an internal standard (canola GPI). Lines with two constructs listed represent co-transformation events.

The gene o3Des(Pi_GA2) driven by the VfUSP promoter with the CaMV35S terminator was present in VC-LJB2755-2qcz, VC-LLM337-1qcz rc and VC-RTP10960-1qcz_F. One observes in Figure 16 that VC-RTP10960-1qcz_F has relatively low transcript accumulation while the co-transformation combination of VC-LJB2755-2qcz and VC-LLM391-2qcz rc contains the highest accumulation. The overall trend for this transcript with the promoter, terminator, intron and spacer region in front of the promoter was to increase over seed development.

The gene d4Des(Pl_GA)2 driven by the LuCnl promoter with the AgrOCS 192bp[LED12] terminator was present in both VC-LTM217-1qcz rc and in VC-RTP10960-1qcz_F. One observes in Figure 19 for both constructs that the accumulation relative to the GPI reference was in the range of 4-12 fold with gradual accumulation over seed development, with VC-RTP10960-1qcz_F accumulating less than VC-LTM217-1qcz rc.

The o3Des(Pir_GA) gene was present in VC-LJB2755-2qcz and VC-RTP10960-1qcz_F driven by the LuCnl promoter with the AtPXR 400bp[LLL823] terminator while it was present in VC-LLM337-1qcz rc driven by the VfSBP_perm3 promoter with the StCATHD-pA terminator. Within error it was difficult to ascribe a pattern to any of the constructs, but as shown in Figure 17, single copy events of the construct combination VC-LJB2755-2qcz and VC-LLM391-2qcz had a peak towards seed maturation that was not observed with any of

the other constructs. An additional copy of the gene driven by the BnSETL promoter and terminated by the BnSETL terminator was present in RTP10960-1qcz_F. However, similar to the d5Des(Tc_GA2) gene controlled by the same set of control elements, the o3Des(Pir_GA) copy under control of the p-BnSETL promoter was expressed at lower levels than the other genes with different control elements (Figure 18).

The gene d4Des(Tc_GA) was contained in VC-LLM337-1qcz rc, RTP10960-1qcz_F and VC-LTM217-1qcz rc and was driven by the ARC5_perm1 promoter with the pvarc terminator. A single point mutant of this gene; d4Des(Tc_GA)_T564G was contained in VC-LLM391-2qcz rc and regulated by the ARC5_perm1 promoter with the pvarc terminator. The overall trend of expression with this spacer region, promoter and terminator combination was an increase over seed development and then a decrease towards seed maturation, as depicted in Figure 20. Interestingly, the construct pair VC-LJB2755-2qcz with VC-LLM391-2qcz rc did not adhere to this trend but rather continued to increase over seed development and had a more robust expression than the other constructs and construct combinations. As previously observed VC-RTP10960-1qcz_F had the lowest level of expression.

VC-LLM337-1qcz rc and VC-LLM391-2qcz rc contain d4Des(Eg_GA) driven by the LuCnI(1064) promoter with the AgrOCS 192bp[LED12] terminator. It can be seen in Figure 21, that similar expression levels that increased during seed development where observed of single copy events containing either one of these two constructs.

Copy Number

To assess whether the entire pathway for PUFA biosynthesis was being brought into the plant and to what extent duplication and/or deletion was occurring in selected events; copy analysis of certain genes along the T-DNA was carried out.

DNA extraction

Brown spotted immature seeds from three events were analyzed, in addition to cotyledons of germinating seedlings of two further events. The gDNA was isolated according to standard protocols consistent with Sambrook et al., 1982. To isolate plant tissue DNA the Wizard Magnetic 96 DNA Plant System was used (Promega, Madison, WI USA part number FF3760). The supplied protocols were followed with the listed changes being implemented in order to increase the amount of extracted DNA: the volume of the extraction buffer was

increased to two hundred microliters and the volume of beads used was increased to ninety microliters with the volume of elution buffer being decreased to twenty microliters.

Duplex qPCR reactions were performed using JumpStart TaqReadyMix for Quantitative PCR (Sigma, D7440). qPCR assays were validated according to standard protocols for qPCR, see Demeke and Jenkins (2010) and Livak and Schmittgen (2001). The genomic copy numbers were calculated with the ddCt method based on the mean of the reference dCt value of a pool of known single copy events. Using the Excel function "frequency", groups of 0, 1, 2, 3, >4 were counted both target and event wise. A Chi-square test was performed for testing the hypothesis the family was segregating for a single T-DNA. The cutoff of P value was set at > 0.0499 for a single T-DNA segregation.

PCR for copy number analysis was carried out using the primers and probes listed and according to standard protocols used by those skilled in the art, see Livak and Schmittgen (2001) and Demekes T. and Jenkins RG (2010).
The primer pairs and probes are listed by name below and sequences can be found in the sequence listing:

Target	Forward Primer	Reverse Primer	Probe
c-AHAS	c-AHAS_F	c-AHAS_R	c-AHAS_P
c-d4Des(Eg_GA)	c-d4Des(Eg_GA)_F	c-d4Des(Eg_GA)_R	c-d4Des(Eg_GA)_P
c-d4Des(Pl_GA)2	c-d4Des(Pl_GA)2_F	c-d4Des(Pl_GA)2_R	c-d4Des(Pl_GA)2_P
c-d4Des(Tc_GA)	c-d4Des(Tc_GA)_F	c-d4Des(Tc_GA)_R	c-d4Des(Tc_GA)_P
c-d5Des(Tc_GA)	c-d5Des(Tc_GA)_F	c-d5Des(Tc_GA)_R	c-d5Des(Tc_GA)_P
c-d5Elo(Ot_GA3)	c-d5Elo(Ot_GA3)_F	c-d5Elo(Ot_GA3)_R	c-d5Elo(Ot_GA3)_P
c-d6Des(Ot_febit)	c-d6Des(Ot_febit)_F	c-d6Des(Ot_febit)_R	c-d6Des(Ot_febit)_P
c-d6Elo(Pp_GA)	c-d6Elo(Pp_GA)_F	c-d6Elo(Pp_GA)_R	c-d6Elo(Pp_GA)_P
c-d6Elo(Tp_GA)	c-d6Elo(Tp_GA)_F	c-d6Elo(Tp_GA)_R	c-d6Elo(Tp_GA)_P
c-o3Des(Pi_GA)	c-o3Des(Pi_GA)_F	c-o3Des(Pi_GA)_R	c-o3Des(Pi_GA)_P
c-o3Des(Pir_GA)	c-o3Des(Pir_GA)_F	c-o3Des(Pir_GA)_R	c-o3Des(Pir_GA)_P
j-i-Atss1_c-d5Elo(Ot_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)_F	j-i-Atss1_c-d5Elo(Ot_GA3)_R	j-i-Atss1_c-d5Elo(Ot_GA3)_P
j-i-Atss1_c-d6Elo(Tp_GA2)	j-i-Atss1_c-d6Elo(Tp_GA2)_F	j-i-Atss1_c-d6Elo(Tp_GA2)_R	j-i-Atss1_c-d6Elo(Tp_GA2)_P
j-i-Atss14_c-d12Des(Ps_GA)	j-i-Atss14_c-d12Des(Ps_GA)_F	j-i-Atss14_c-d12Des(Ps_GA)_R	j-i-Atss14_c-d12Des(Ps_GA)_P
j-i-Atss18_c-d6Elo(Pp_GA2)	j-i-Atss18_c-d6Elo(Pp_GA2)_F	j-i-Atss18_c-d6Elo(Pp_GA2)_R	j-i-Atss18_c-d6Elo(Pp_GA2)_P
j-i-Atss18_c-o3Des(Pi_GA2)	j-i-Atss18_c-o3Des(Pi_GA2)_F	j-i-Atss18_c-o3Des(Pi_GA2)_R	j-i-Atss18_c-o3Des(Pi_GA2)_P
j-i-Atss2_c-d4Des(Tc_GA3)	j-i-Atss2_c-d4Des(Tc_GA3)_F	j-i-Atss2_c-d4Des(Tc_GA3)_R	j-i-Atss2_c-d4Des(Tc_GA3)_P
j-i-Atss2_c-d6Des(Ot febit_GA)	j-i-Atss2_c-d6Des(Ot febit_GA)_F	j-i-Atss2_c-d6Des(Ot febit_GA)_R	j-i-Atss2_c-d6Des(Ot febit_GA)_P
j-p-BnFAE_t-PvARC	j-p-BnFAE_t-PvARC_F	j-p-BnFAE_t-PvARC_R	j-p-BnFAE_t-PvARC_P

Target	Forward Primer	Reverse Primer	Probe
j-p-BnSETL-v1_c-o3Des(Pir_GA)	j-p-BnSETL-v1_c-o3Des(Pir_GA)_F	j-p-BnSETL-v1_c-o3Des(Pir_GA)_R	j-p-BnSETL-v1_c-o3Des(Pir_GA)_P
j-p-LuPXR_i-Atss15	j-p-LuPXR_i-Atss15_F	j-p-LuPXR_i-Atss15_R	j-p-LuPXR_i-Atss15_P
j-p-PvARC5_t-BnFAE	j-p-PvARC5_t-BnFAE_F	j-p-PvARC5_t-BnFAE_R	j-p-PvARC5_t-BnFAE_P
j-p-PvARC5_t-BnSETL	j-p-PvARC5_t-BnSETL_F	j-p-PvARC5_t-BnSETL_R	j-p-PvARC5_t-BnSETL_P
j-t-CaMV_p-LuCnl-2	j-t-CaMV_p-LuCnl-2_F	j-t-CaMV_p-LuCnl-2_R	j-t-CaMV_p-LuCnl-2_P
j-t-E9-p3-2	j-t-E9-p3-2_F	j-t-E9-p3-2_R	j-t-E9-p3-2_P
j-t-PvARC-p-LuCnl	j-t-PvARC-p-LuCnl_F	j-t-PvARC-p-LuCnl_R	j-t-PvARC-p-LuCnl_P
j-t-StCAT_p2_p-LuPXR	j-t-StCAT_p2_p-LuPXR_F	j-t-StCAT_p2_p-LuPXR_R	j-t-StCAT_p2_p-LuPXR_P
reference (Adh1)	reference (Adh1)_F	reference (Adh1)_R	reference (Adh1)_P
c-o3Des(Pi_GA2_SNP)	c-o3Des(Pi_GA2_SNP)_F	c-o3Des(Pi_GA2_SNP)_R	c-o3Des(Pi_GA2_SNP)_P
j-t-PvARC-p3	j-t-PvARC-p3_F	j-t-PvARC-p3_R	j-t-PvARC-p3_P
j-i-Atss15_c-o3Des(Pi_GA2)	j-i-Atss15_c-o3Des(Pi_GA2)_F	j-i-Atss15_c-o3Des(Pi_GA2)_R	j-i-Atss15_c-o3Des(Pi_GA2)_P
j-p-VfSBPperm3_c-o3Des(Pir_GA)	j-p-VfSBPperm3_c-o3Des(Pir_GA)_F	j-p-VfSBPperm3_c-o3Des(Pir_GA)_R	j-p-VfSBPperm3_c-o3Des(Pir_GA)_P
c-d15Des(Ch_ERTp_GA)	c-d15Des(Ch_ERTp_GA)_F	c-d15Des(Ch_ERTp_GA)_R	c-d15Des(Ch_ERTp_GA)_P
j-i-Atss2_c-d4Des(Pl_GA)-195R)	j-i-Atss2_c-d4Des(Pl_GA)-195R)_F	j-i-Atss2_c-d4Des(Pl_GA)-195R)_R	j-i-Atss2_c-d4Des(Pl_GA)-195R)_P

For Event specific detection the following protocol was used:

Preparation of the Jumpstart™ Taq ReadyMix (Sigma, P2893) for use with the Quantstudio™ 12K Flex Real-Time PCR system from Life Technologies

Prior to use, add to the Jumpstart™Taq ReadyMix :

$MgCl_2$ to a final concentration of 11mM and

ROX (=Sulforhodamine 101) to a final concentration of 120 nM

= prepared jumpstart mix (1)

Preparation of the final reaction mixture for duplex qPCR reactions.

The qPCR reactions are done in a 384-well plate (Life technologies, catalogue number 4309849) in a total volume of 10 microliters per well. Per reaction, 2µl of template DNA is mixed with 8 microliters of qPCR reaction mixture according to the table below. The plates are sealed with MicroAmp® Optical Adhesive Film (Life Technologies, catalogue number 4311971).

reaction components	Final concentration
Prepared jumpstart mix (1)	1x
Transgene target forward primer	900 nM
Transgene target reverse primer	900 nM
Transgene target probe	100 nM
Endogenous reference gene forward primer	900 nM

Endogenous reference gene reverse primer	900 nM
Endogenous reference gene probe	100 nM
Template DNA	2 µl
Nuclease free water	Add up to 10µl

The primers and probes for event specific detection are shows in Table 176.

Table 176: Primers and Probes used for event specific detection.

Event / Locus	Forward Primer	Reverse Primer	Probe
LANBCH locus 1 (N5)	LANBCH locus 1 (N5)_Forward primer	LANBCH locus 1 (N5)_Reverse primer	LANBCH locus 1 (N5)_Probe
LANBCH locus 1 (N9)	LANBCH locus 1 (N9)_Forward primer	LANBCH locus 1 (N9)_Reverse primer	LANBCH locus 1 (N9)_Probe
LANBCH locus 2 (N5)	LANBCH locus 2 (N5)_Forward primer	LANBCH locus 2 (N5)_Reverse primer	LANBCH locus 2 (N5)_Probe
LANBCH locus 2 (N9)	LANBCH locus 2 (N9)_Forward primer	LANBCH locus 2 (N9)_Reverse primer	LANBCH locus 2 (N9)_Probe
LBFDAU locus 1	LBFDAU locus 1_Forward primer	LBFDAU locus 1_Reverse primer	LBFDAU locus 1_Probe
LBFDAU locus 2	LBFDAU locus 2_Forward primer	LBFDAU locus 2_Reverse primer	LBFDAU locus 2_Probe
LBFDGG	LBFDGG_Forward primer	LBFDGG_Reverse primer	LBFDGG_Probe
LBFDHG locus 1	LBFDHG locus 1_Forward primer	LBFDHG locus 1_Reverse primer	LBFDHG locus 1_Probe
LBFDHG locus 2	LBFDHG locus 2_Forward primer	LBFDHG locus 2_Reverse primer	LBFDHG locus 2_Probe
LBFGKN	LBFGKN_Forward primer	LBFGKN_Reverse primer	LBFGKN_Probe
LBFIHE	LBFIHE_Forward primer	LBFIHE_Reverse primer	LBFIHE_Probe
LBFLFK locus 1	LBFLFK locus 1_Forward primer	LBFLFK locus 1_Reverse primer	LBFLFK locus 1_Probe
LBFLFK locus 2	LBFLFK locus 2_Forward primer	LBFLFK locus 2_Reverse primer	LBFLFK locus 2_Probe
LBFPRA Locus 1	LBFPRA Locus 1_Forward primer	LBFPRA Locus 1_Reverse primer	LBFPRA Locus 1_Probe
LBFPRA locus 2	LBFPRA locus 2_Forward primer	LBFPRA locus 2_Reverse primer	LBFPRA locus 2_Probe
LBFPRA Locus 3e	LBFPRA Locus 3e_Forward primer	LBFPRA Locus 3e_Reverse primer	LBFPRA Locus 3e_Probe

PCR conditions for event specific detection were as follows:

The qPCR is performed on the QuantStudio™ 12K Flex Real-Time PCR System (Life technologies) with following qPCR conditions:

step1: 95°C 5min

step2: 45 cycles

95°C, 18 sec

60°C, 1 min

Expected amplicon sizes are given below in base pairs (plus or minus 10 bp):

Amplicon	Amplicon Size plus or minus 10 bp

Amplicon LANBCH locus 1 (N5)	100 bp
Amplicon LANBCH locus 1 (N9)	150 bp
Amplicon LANBCH locus 2 (N5)	150 bp
Amplicon LANBCH locus 2 (N9)	120 bp
Amplicon LBFDAU locus 1	110 bp
Amplicon LBFDAU locus 2	95 bp
Amplicon LBFDGG	100 bp
Amplicon LBFDHG locus 1	200 bp
Amplicon LBFDHG locus 2	140 bp
Amplicon LBFGKN	140 bp
Amplicon LBFIHE	110 bp
Amplicon LBFLFK locus 1	160 bp
Amplicon LBFLFK locus 2	170 bp
Amplicon LBFPRA Locus 1	570 bp
Amplicon LBFPRA locus 2	190 bp
Amplicon LBFPRA Locus 3e	90 bp

Example 25 Protein levels

Pooled seed material from four week old siliques was used for protein extraction. To enrich the membrane proteins and reduce the background signal, microsomes isolated from ground seed tissue were used to analyze protein levels for the following enzymes: c-o3Des(Pi_GA2), c-o3Des(Pir_GA), d4Des(Eg), c-d4Des(Pl_GA)2, and c-d6Elo(Tp_GA2). For c-o3Des(Pir_GA) and c-d6Elo(Tp_GA2), the microsomes were further extracted with detergent and used for ELISA. The various versions of a given gene (denoted as GA, GA2 etc.) all encoded an identical protein corresponding to the gene listed.

Initially, in a mortar cooled with liquid nitrogen, seeds were ground to a fine powder in Extraction buffer (25 mM Tris, pH 7.4; 140 mM NaCl; 3 mM KCl; 3 mM DTT; 200 mM Sorbitol; and EDTA-free Protease inhibitors (Roche)) at a ratio of 1g seed/4 ml buffer. The plant cell-debris was removed by centrifugation (5000 x g, 10 minutes, 4 °C), re-extracted by grinding a second time as described above with 1.5 ml of Extraction Buffer, and then again separated by centrifugation (5000 x g, 10 minutes, 4 °C). The supernatant from these two extractions was combined and represents the cell-free extract (typically 3.5 ml/g seed). Microsomal pellets were obtained from the cell-free extracts by ultra-centrifugation (100,000 x g, 60 minutes, 4 °C). For the c-o3Des(Pi_GA2), d4Des(Eg), c-d4Des(Pl_GA)2, enzymes the microsomal pellets were suspended in Suspension Buffer (1xTBST+1%TritonX-100). 1X

TBST consists of 50 mM Tris, 150 mM NaCl, 0.05% tween 20 detergent with a final pH of 7.6 at 4 °C.

For c-o3Des(Pir_GA) and c-d6Elo(Tp_GA2) the microsomal fractions were isolated as described above, and proteins were solubilized with 1X TBST buffer and detergent of choice (For details, please see the table below). For c-d4Des(Tc_GA), c-d5Elo(Ot_GA3), c-d5Des(Tc_GA2), c-d6Des(Ot_febit), c-d6Elo(Pp_GA2), c-d12Des(Ps_GA), the seed powder was further ground in liquid nitrogen and extracted with 1xTBST+1%Triton X100+Protease Inhibitor at 1:3 W/V ratio. The supernatant was cleared at 13,000g for 20 minutes at 4°C using a micro-centrifuge and followed by a 10 minute spin at 13,000g. The clarified supernatant was used for ELISA and total protein assay.

Antibody/antigen hybridization conditions: Plates were blocked with 300 µl/well of freshly made blocking solution. The plates were sealed and incubated for 1 hour with continuous shaking. After 1 hour of incubation, the plates were washed twice with 1X TBST. The extract from above was used in the ELISA assay. The plate was incubated with 100 µl of standards or sample extract at 4 °C for overnight, without shaking. Standards were serial diluted 1:3. The plates were sealed and incubated overnight at 4 °C. After the plates were incubated overnight, the plates were washed five times with 1X TBST. After the wash the plates were incubated with 100 µl of detecting antibody in blocking solution for 1 hour, sealed with continuous shaking. The plates were washed five times with 1X TBST at the end of the one hour incubation step. The plates were then incubated with 100 ml of HRP-conjugated donkey anti-animal of the detecting antibody secondary antibody (1:10,000) in blocking solution for 1 hour, in a sealed plate with continuous shaking. The plates were then washed five times with 1X TBST at the end of the blocking step. 100 µl of 1-step TMB was then added and the plates incubated at room temperature for 20 minutes, sealed with continuous shaking. After the twenty minutes, 100 µl of a 1M HCl solution was added and the plates read at 450 nm.

For c-d6Elo(Tp_GA2) only: all steps were the same as for the other proteins but in place of the HRP conjugated donkey anti-body system used for the others, 100 µl of biotin-conjugated donkey anti-rabbit IgG diluted 1:40,000 in 2% BSA/1X TBST was used. The plate was sealed and shaken continuously during this incubation step, which was done for one hour. The plate was washed five times with 1X TBST at the end of this step. To read this plate, the plate was incubated with 100 µl extravidin-peroxidase diluted 1:40,000 in 2%

BSA/1X TBST, sealed and incubated for 1 hour with continuous shaking. The plate was then washed five times with 1X TBST. The plate was read at 450 nm.

Table 177: Overview of hybridization for protein detection and quantification TMB (3,3',5,5'-tetramethylbenzidine) was the substrate for the horse radish peroxidase employed to visualize protein bands, supplied by Sigma-Aldrich T-0565 and used according to the instructions of the manufacturer.

	Coating AB	Blocking Buffer	Sample extraction buffer	Standard ID and dilution	Detecting AB	Secondary AB	TMB
o3Des(Pir)	AB212, 1:5,500	1% BSA in TBST	1% Triton X-100, + 1 xTBS, + Protease Inhibitor	1:3, from 90 ng/ml, full length o3Des(Pir)	AB210 1:1,300, in blocking buffer	1:10,000 HRP-donkey anti chicken	1 step TMB, 20 min
d4Des(Tc)	AB216, 1:15,000	1% BSA in TBST	1% Triton X-100, + 1 xTBS, + Protease Inhibitor	1:3, from 200 ng/ml, RN121	AB217, 1:15,000, in blocking buffer	1:10,000 HRP-donkey anti Rabbit	1 step TMB, 20 min
d5Elo(ot)	AB214, 1:1500,	1% BSA in TBST	1% Triton X-100, + 1 xTBS, + Protease Inhibitor	1:3, from 200 ng/ml, RN134	AB215, 1:1500, in blocking buffer	1:10,000 HRP-donkey anti chicken	1 step TMB, 20 min
d5Des (Tc)	AB90, 1:8000	1% BSA in TBST	1% Triton X-100, + 1 xTBS, + Protease Inhibitor	1:3, from 100 ng/ml, full lenth d5Des	AB 100, 1:40000, in blocking buffer	1:10,000 HRP-donkey anti chicken	1 step TMB, 20 min
d6Des(Ot)	AB113, 1:500	1% BSA in TBST	1% Triton X-100, + 1 xTBS, + Protease Inhibitor	1:3, from 100 ng/ml, full length d6Des(Ot)	AB 112 rabbit, 1:10000, in blocking buffer	1:10,000 HRP-donkey anti rabbit	1 step TMB, 20 min
d6Elo(Pp)	AB 107, 1:1000	1% BSA in TBST	1% Triton X-100, + 1 xTBS, + Protease Inhibitor	1:3, from 110 ng/ml, PA534	AB 121 chicken, 1:5000 in extraction buffer	1:10,000 HRP-donkey anti chicken	1 step TMB, 20 min
d6Elo(Tp)	AB125, 1:2000,	2% BSA in TBST	1 XTBST+1% DDM+Protease inihibitor	1:3, from 200 ng/ml, PA678	AB140, 1:1000, in blocking buffer	1:40,000 biotin-donkey anti rabbit for 1hr, then 1:40,000 extravidin-peroxidase	1 step TMB, 20 min
d12Des (Ps)	AB128, 1:3000	1% BSA in TBST	1% Triton X-100, + 1 xTBS, + Protease Inhibitor	1:3, from 200 ng/ml, PA557	AB 129, 1:3000, in blocking buffer	1:10,000 HRP-donkey anti Rabbit	1 step TMB, 20 min

Table 178: Levels of the proteins of interest were expressed as nanograms (ng) per milligram of total protein. The values were the average and standard deviation from three technical measurements of approximately four week old seed material. As one observes the construct VC-RTP10690-1qcz_F had overall less protein for the expressed genes than the combination of VC-LJB2197-1qcz and VC-LLM337-1qcz rc.

Protein	Wt	LJB2197+LLM337	VC-RTP10690-1qcz_F
c-o3Des(Pir_GA), ng/mg total microsomal protein	9.04 ± 0.5	387.5 ± 10.5	69.7 ± 1.2
c-d4Des(Tc_GA), ng/mg of total protein	0.04 ± 0.01	0.74 ± 0.03	0.1 ± 0.01
c-d5Elo(Ot_GA3), ng/mg of total protein	0.12 ± 0.01	2.85 ± 0.02	1.23 ± 0.01
c-d5Des(Tc_GA2), ng/mg of total protein	0.05 ± 0.01	2.03 ± 0.08	1.54 ± 0.03
c-d6Des(Ot_febit),ng/mg total protein	0 ± 0	4.1 ± 0.1	1.83 ± 0.01
c-d6Elo(Pp_GA2), ng/mg total protein	0 ± 0	3.77 ± 0.3	1.1 ± 0.01
c-d6Elo(Tp_GA2), ng/mg of total microsomal protein	0 ± 0	0.33 ± 0.06	0.38 ± 0.04
c-d12Des(Ps_GA), ng/mg of total protein	0.05 ± 01	1.5 ± 0.07	0.34 ± 0.03

Example 26: Desaturase and Elongase substrate specificity and selectivity observed in yeast compared with fatty acid profiles observed in canola events expressing these enzymes

The engineered biosynthetic pathway described in Examples 10-18 can convert oleic acid (18:1n-9), ALA (18:3n-3), and SDA (18:4n-3) to 20:5n-3 and 22:6n-3. The conversion of ALA and SDA to 20:5n-3 and 22:6n-3 is accomplished by the enzymes encoded in the engineered pathway and results from their ability to accept several substrates. The substrate tolerance of these enzymes cannot be predicted, but instead must be determined empirically as described in Examples 21 and 22. The ability of the desaturases and elongases, described in Examples 10-18, to use alternate substrates also provides a proof-reading mechanism within the engineered pathway that allows re-routing of undesired side-products back into the biosynthesis of 20:5n-3 and 22:6n-3.

Delta-6-desaturase:

Yeast feeding studies (Table 159 and Table 163) show that the delta-6-desaturase (*Ostreococcus tauri*) can accept two substrates, LA (18:2n-6) and ALA (18:3n-3), but prefers ALA slightly. The range of acceptable substrates (Tables 148-152) utilized by the specific combination of elongases and desaturases that were engineered into canola (Examples 10-18) allow the ALA desaturated by the delta-6-desaturase (*Ostreococcus tauri*) to ultimately be converted to DHA. The fatty acid analysis data presented for the engineered canola lines in Examples (10-18) show significantly lower levels of ALA relative to non-transgenic Kumily controls, consistent with the conversion of ALA to 20:5n-3 and 22:6n-3.

Delta-6-elongases:

Yeast feeding studies (Table 160 and Table 163) show that both of the delta-6-elongase's, *Physcomitrella patens* and *Thalassiosira pseudonana*, accept 18-carbon chain substrates but prefer molecules desaturated at carbon-6 (see GLA (18:3n-6) and SDA (18:4n-3) vs. LA (18:2n-6) and ALA (18:3n-3). The delta-6-elongase from *Thalassiosira pseudonana* is much more stringent than the one from *Physcomitrella patens* in recognizing the C-6 desaturation. In the fatty acid analysis of the engineered canola lines presented in Examples (10-18) 20:2n-6 and 20:3n-3, resulting from desaturation of LA and ALA respectively, are detected. In the LTM593 (Example 18) line the levels of 20:2n-6 and 20:3n-3 are about the same as found in wild-type Kumily.

Delta-5-desaturase:

Yeast feeding studies (Table 159 and Table 162 and Table 163) show that the delta-5-desaturase (*Thraustochytrium* sp. ATCC21685) accepts DHGLA (20:3n-6) and 20:4n-3. The ability of the delta-5-desaturase (*Thraustochytrium* sp. ATCC21685) to desaturate 20:4n-3 is important for the conversion of ALA and SDA into 20:5n-3 and 22:6n-3.

Omega-3-desaturases:

Yeast feeding show that both omega-3-desaturase's, (*Phythophthora infestans* and *Pythium irregulare*) can accept ARA (20:4n-6), DTA (22:4n-6), and, DHGLA (20:3n-6). The omega-3-desaturases prefer molecules with 4- double bonds. In the transgenic canola lines described in Examples (10-18), the ability of these omega-3-desaturases to utilize 22:4n-6 as a substrate allows re-direction of ARA (20:4n-6) that has been elongated by the delta-5-elongase (*Ostreococcus tauri*) back into the pathway for synthesis of 22:6n-3.

Delta-5-elongase:

Yeast feeding show (Table 161 and Table 163) that the delta-5-elongase (*Ostreococcus tauri*) prefers 20-carbon chain substrates, EPA (20:5n-3) and ARA (20:4n-6), but can also extend lipids containing 18-carbon chains, like SDA (18:4n-3) and ALA (18:3n-3). In the engineered canola (Examples 10-18) the presence of the omega-3-desaturase's, (*Phythophthora infestans* and *Pythium irregulare*), allows the 22:4n-6 product, resulting from the elongation of ARA, to be converted to 22:5n-3 and which is then desaturated by the delta-4-desaturases (*Thraustochytrium* sp.and *Pavlova lutheri*) to produce the final product, 22:6n-3. Fatty acid analysis of the transgenic canola lines described in Examples 10-18 shows minor levels of 22:4n-6, likely due to the ability of the omega-3 desaturases to redirect this lipid back into the biosynthesis of 22:6n-6. The 20:4n-3 produced from the extension of SDA is substrate recognized by the delta-5-desaturase (*Thraustochytrium* sp. ATCC21685) and can be converted to 20:5n-3, the preferred substrate of the delta-5-elongase (*Ostreococcus tauri*).

Delta-4-desaturases:

Yeast feeding studies (Table 159 and Table 163) show that both delta-4-desaturase's, (*Thraustochytrium* sp.and *Pavlova lutheri*) can accept 22:5n-3 and DTA (22:4n-6) with a slight preference for 22:5n-3.

Example 27: Using differentially labeled heavy peptides to monitor digestion efficiency in quantitative LC-MS.

Introduction:

The described method provides an avenue for quantitating digestion efficiency in targeted proteomics experiments. In most MRM LC-MS applications, measuring digestion efficiency relies on an indirect measurement which examines a peptide profile of the sample by comparing the number of missed cleavage peptides detected to the number of completely digested peptides detected in a separate LC-MS experiment. While providing an overall perspective of the trypsin efficiency within the experiment, the data does little to address specific digestion of the target sequence being monitored in the MRM experiment. Measuring global digestion efficiency does not address possible sequence specific biases in trypsin activity and may result in misrepresentation of target quantity. Indeed, there was a growing appreciation that trypsin digestion kinetics was strongly influenced by sequence context (1, 2). In order to measure sequence specific digestion efficiency we use two differentially-labeled heavy peptides to obtain a ratio for digestion efficiency (scheme 1). Peptide three includes the correct sequence context for the target by including four amino acids both amino and carboxyl to the two trypsin cleavage sites that generate the native peptide. In general, three amino acids were considered sufficient for trypsin to identify its cleavage site (3) and hydrolysis rates were only affected by the closest two amino acids (2). This scheme allows us to simultaneously assess specific digestion efficiency and also obtain quantitation data for the target molecule. In this design, the two peptides were spiked at equimolar concentrations into the test sample prior to digestion. During sample analysis, transitions were monitored for the target peptide and the two heavy peptide products. Peak intensities obtained from the two heavy peptides were compared to get a ratio for digestion efficiency and the target peptide intensities were used for quantitation.

Scheme 1

Example protein: O3D (Pir)

Sequence:

MASTSAAQDAAPYEFPSLTEIKRALPSECFEASVPLSLYYTARSLALAGSLAVALSYARALPLVQ

ANALLDATLCTGYVLLQGIVFWGFFTVGHDCGHGAFSRSHVLNFSVGTLMHSIILTPFESWKLS
HRHHHKNTGNIDKDEIFYPQREADSHPVSRHLVMSLGSAWFAYLFAGFPPRTMNHFNPWEAM
YVRRVAAVIISLGVLFAFAGLYSYLTFVLGFTTMAIYYFGPLFIFATMLVVTTFLHHNDEETPWYA
DSEWTYVKGNLSSVDRSYGALIDNLSHNIGTHQIHHLFPIIPHYKLNDATAAFAKAFPELVRKNA
APIIPTFFRMAAMYAKYGVVDTDAKTFTLKEAKAAAKTKSS

target peptide (underlined), native sequence contained in synthetic peptide for digestion efficiency (redouble underlined)

Peptides

DEIFYPQR (native peptide measured from sample)

DEIFYPQ(15N13CR) (for standard curve, and normalization)

NIDKDEIFY(15N13CP)Q(15N13CR)EADS (for digestion efficiency)

Standard peptides (2) (3) were spiked into the test sample at the same concentration prior to proteolysis. After proteolysis the transitions for the product peptides, (2) and DEIFY(15N13CP)Q(15N13CR) were compared in product ion intensity in order to determine the digestion efficiency of the targeted sequence within the context of its native sequence according to the following equation:

$$\%\ digestion\ efficiency = \frac{\text{peak intensity DEIFY}(^{15}\text{N}^{13}\text{C}\text{P})\text{Q}(^{15}\text{N}^{13}\text{C}\text{R})}{\text{peak intensity DEIFYPQ}(^{15}\text{N}^{13}\text{C}\text{R})} * 100$$

The % digestion efficiency is therefore measured in the test sample directly and can be used to assess the impact on detected target concentration.

1) Proc J. et al. 2010. Journal of Proteome Res. 9:5422-5437.

2) Lowenthal M. et al. 2014. Anal Chem. 86:551-558.

3) Makriyannis T. and Y. Clonis. 1997. Biotech Bioeng. 53:49-57.

Example 28: Use of different germplasm to optimize production of VLC-PUFA

It appears that expression of the same or very similar gene sets in different host plants, such as *Camelina sativa* (Ruiz-Lopez et al., 2014), results in a larger yield of VLC-PUFA than has been attainable in *Brassica napus*. This notion is illustrated by the data in Table 179. Table 179 contains fatty acid data from seeds of transgenic *B. napus* (LBJ1671) and transgenic *C. sativa* (RRes_EPA_line) transformed with nearly identical T-DNAs and the respective wild type controls. The only difference between the constructs used to make the two transgenic lines is that the TDNA used to make the transgenic *B. napus* (LBJ1671) has an extra GFP expression cassette adjacent to the right border. When comparing transgenic *B. napus* (LJB1671) to the WT Kumily control, there is a decrease in the amount of 18:1 and increases in the amounts of 18:2 and

18:3 fatty acids. The 18:2 and 18:3 fatty acids are intermediates of the pathway introduced by LJB1671, and therefore the elongation of 18:2 and 18:3 fatty acids into 20C fatty acids can be considered a bottleneck reaction in *B. napus.* On the other hand, when comparing transgenic *C. sativa* (RRes_EPA_line) to the WT control, there is a decrease in 18:1 and also in 18:2 and 18:3 fatty acids. Therefore, the elongation of 18:2 and 18:3 fatty acids is not a bottleneck in *C. sativa*. The absence of this bottleneck in *C. sativa* results in 10-fold higher 20:5n-3 (EPA) content compared to the transgenic *B. napus*.

The conversion of 18:1 to 18:2 and of 18:2 to 18:3 occurs largely in the phospholipid membrane, while the elongation of 18C fatty acids to 20C fatty acids occurs strictly in the acyl-CoA pool. This difference in substrate specificity means that 18C fatty acids must be released from membrane phospholipids in order to be elongated. This release is apparently a bottleneck in *B. napus*, but not in *C. sativa*. The difference in flux of 18:2 from PC into the CoA pool between these two species could be due to differences in the activities of numerous enzymes, including: 1) phospholipases, 2) acyl-CoA synthetases, 3) lyso-PC acyltransferases, 4) phospholipid-diacylglycerol choline transferases, 5) phospholipid-diacylglycerol acyl transferases, and others. As a consequence of these observations, transforming any of the constructs from example 11 to example 18 into variety of species and germplasms (such as other Brassica germplasms in the so called Triangle of U), would result in a prefered embodiement of the present invention (e.g. higher amounts of EPA and DHA, and/or lower amounts of 18:2n-6).

Table 179: Introduction of a construct that was isogenic for all genes involved in VLC-PUFA synthesis into *Brassica napus* cv Kumily and into *Camelina sativa*.Average fatty acid data of segregating T1 seed of 26 events containing the T-DNA of LJB1671 and wild type (WT) *Brassica napus* cv Kumily are shown in the top two rows of data. For line RRes_EPA, fatty acid profiles of T3 homozygous seeds of selected insertion events, and of the respective WT control, are shown. RRes_EPA line and WT *Camelina sativa* data were taken from supplemental table S1 of Ruiz-Lopez *et al.*, 2014. n.d, not determined.

Event	Other	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9
LJB1671 introduced into Kumily (n=26)	0	5 ± 0.5	0.2 ± 0.1	0.1 ± 0.1	2.6 ± 0.6	37.8 ± 10.9	29.1 ± 4.3	0.4 ± 0.4	7.8 ± 1.2	4.4 ± 5.4	2 ± 2.7	0.9 ± 0.2	1.3 ± 0.4	1.8 ± 1.8	0.6 ± 0.6	1.5 ± 1	1 ± 0.7	0.8 ± 0.5	2.1 ± 1.5	0.5 ± 0.1	0 ± 0
WT Kumily greenhouse (n=46)	0	5 ± 0.1	0.4 ± 0	0.2 ± 0	2.6 ± 0.1	66.3 ± 1.7	16.8 ± 1.3	0 ± 0	6.1 ± 0.4	0	0	0.9 ± 0	1.2 ± 0	0	0	0	0	0	0	0.5 ± 0	0 ± 0
RRes_EPA line	11.9 ± 1.0	8.3 ± 0.6	n.d	n.d	6.7 ± 0.9	5.0 ± 0.8	20.6 ± 1.2	n.d	15.8 ± 2.9	2.4 ± 1.7	1.5 ± 1.2	n.d	7.0 ± 0.9	n.d	n.d	1.1 ± 0.4	3.2 ± 1.1	1.6 ± 0.3	14.7 ± 2.8	n.d	n.d
WT *Camelina sativa*	11.2 ± 0.1	6.5 ± 0.1	n.d	n.d	3.4 ± 0.1	14.5 ± 0.4	19.0 ± 0.6	n.d	30.8 ± 0.6	0	0	n.d	14.5 ± 0.1	n.d	n.d	0	0	0	0	n.d	n.d

CSIRO Exhibit 1007

Example 29: Fatty acid analysis of canola oils and commercial oil and solid samples (Total FAME analysis)

Canola seeds:
10-15 canola seeds were transferred into tubes on a 96-format rack and closed with cap strips. Seeds were ground in a swing mill using 3 mm beads for 2 x 2 min at 30 Hz. The rack was then centrifuged for 5 min at 4000 rpm to remove powder from the lid. Extraction of oil was carried out by adding 800 µL of methyl *tert*-butyl ether (MTBE) to the samples followed by extraction in a swing mill for 2 x 30 sec at 30 Hz. After centrifugation at 4000 rpm for 10 min, 40 µL of the clear supernatant was transferred into a 96-well micro rack and diluted using 260 µL MTBE. Lipids were derivatized into fatty acid methyl esters (FAMEs) by adding 20 µL trimethylsulfonium hydroxide solution (TMSH, 0.2 M in methanol) into each sample. The rack was closed using silicone/PTFE cap mats and incubated for 20 min at room temperature.

Arabidopsis seeds:
20 mg of seeds were transferred into tubes on a 96-format rack. The canola seed protocol was followed in all further steps.

Canola oils and commercial oil samples:
20 µL of oil was transferred into a glass vial and diluted using 1 mL MTBE. 20-80 µL of the sample-solutions were transferred into into a 96-well micro rack and diluted using 260 µL MTBE. Lipids were derivatized into FAMEs by adding 20 µL trimethylsulfonium hydroxide solution (TMSH, 0.2 M in methanol) into each sample. The rack was closed using silicone/PTFE cap mats and incubated for 20 min at room temperature.

Commercial solid samples:
Solid samples were lyophilized prior to analysis. 20 mg of solid samples were transferred into tubes on a 96-format rack. The canola seed protocol was followed in all further steps.

An overview on the samples is provided in the following Table.

Table 180: Samples description

Sample Name	Description	Source/Origin
	Transgenic plant samples (T-DNA identity)	
LANBCH oil	*B. napus* (VC-LJB2197-1qcz, VC-LLM337-1qcz rc)	2014 Field season
LANPMZ seeds	*B. napus* (VC-LJB2197-1qcz, VC-LLM337-1qcz rc)	2012 Field season
LAODDN seeds	*B. napus* (VC-LJB2755-2qcz rc, VC-LLM391-2qcz rc)	2012 Field season
LBFDAU oil	*B. napus* (VC-LTM593-1qcz rc)	2014 Field season
LBFGKN seeds	*B. napus* (VC-LTM593-1qcz rc)	2014 Field season
LBFGKN oil	*B. napus* (VC-LTM593-1qcz rc)	2014 Field season
LBFLFK seeds	*B. napus* (VC-LTM593-1qcz rc)	2014 Field season
LBFLFK oil	*B. napus* (VC-LTM593-1qcz rc)	2014 Field season
Arabidopsis seeds	*A. thaliana* (VC-LTM593-1qcz rc)	Greenhouse
	Non-transgenic plant samples	
Kumily seeds	wild type *B. napus*	2012 Field season
Kumily oil	wild type *B. napus*	2012 Field season
Canola Commodity oil	canola oil blend	
Clear Valley 65 oil	canola oil blend (65% oleic acid)	
Clear Valley 80 oil	canola oil blend (80% oleic acid)	
Brassica juncea seeds	wild type *B. juncea* (high erucic acid variety)	
Borage oil	oil preparation from plants (*Borago officinalis*)	
Echium oil	oil preparation from plants (*Echium plantagineum*)	
Primrose oil	oil preparation from plants (*Oenothera*)	
	Land animal fat samples	
Beef (corned beef)	prepared beef product	
Butter 1	butter from cow milk	
Butter 2	butter from cow milk	
Egg yolk from chicken	egg yolk powder from chicken	
	Fish/Crustacean oil samples	
Menhaden oil	oil preparation from fish (Menhaden)	
Salmon oil capsule	oil preparation from fish (Salmon)	
Tuna 1 oil	oil preparation from fish (Tuna)	
Anchovy 2 oil	oil preparation from fish (Anchovy)	
Krill oil capsule	oil preparation from crustacean (Krill)	
	Algal oil samples	
DHA DHA oil capsule	oil preparation from algae	
Omega EPA/DHA oil capsule	oil preparation from algae	
Omega-3 EPA & DHA	oil preparation from algae	
Omega 3 algae oil	oil preparation from algae	
	Fungal oil sample	
Mortierella alpina ARA-rich oil	oil preparation from fungi	

GC analysis:

An Agilent 7890A gas chromatograph coupled to Agilent flame ionization detector was used for FAME analysis. Separation of FAMEs was carried out on a DB-225 capillary column (20 m x 180 µm x 0.2 µm, Agilent) using H_2 as carrier gas with a flow rate of 0.8mL/min. The GC was operated in split mode using a split ratio of 1:50 at an injector temperature of 250 °C, injection volume was 1 µL. Oven temperature was held at 190 °C for 3 min and increased to 220 °C with 15 °C min^{-1}. Temperature was held at

220 °C for another 6 min. Peak detection and integration was carried out using Agilent GC ChemStation software (Rev. B.04.02 SP1).

Table 181: Fatty acids determined by FAME analysis

Shorthand Name	Trivial Name	Fatty Acid Name (systematic name)	Fatty Acid Souce
14:0	Myristic acid	Tetradecanoic acid	Supelco 37 Component FAME Mix
16:0	Palmitic acid	Hexadecanoic acid	Supelco 37 Component FAME Mix
16:1n-9		(Z)-7-Hexadecenoic acid	single standard
16:1n-7	Palmitoleic acid	(Z)-9-Hexadecenoic acid	Supelco 37 Component FAME Mix
17:0	Margaric acid	Heptadecanoic Acid	Supelco 37 Component FAME Mix
16:3n-3		(Z,Z,Z)-7,10,13-Hexadecatrienoic acid	single standard
18:0	Stearic acid	Octadecanoic acid	Supelco 37 Component FAME Mix
18:1n-9	Oleic acid	(Z)-9-Octadecenoic acid	Supelco 37 Component FAME Mix
18:1n-7	Vaccenic acid	(Z)-11-Octadecenoic acid	single standard
18:2n-9		(Z,Z)-6,9-Octadecadienoic acid	single standard
18:2n-6	Linoleic acid	(Z,Z)-9,12-Octadecadienoic acid	Supelco 37 Component FAME Mix
18:3n-6	gamma-Linolenic acid	(Z,Z,Z)-6,9,12-Octadecatrienoic acid	Supelco 37 Component FAME Mix
18:3n-3	alpha-Linolenic acid	(Z,Z,Z)-9,12,15-Octadecatrienoic acid	Supelco 37 Component FAME Mix
18:4n-3	Stearidonic acid	(Z,Z,Z,Z)-6,9,12,15-Octadecatetraenoic acid	single standard
20:0	Arachidic acid	Eicosanoic acid	Supelco 37 Component FAME Mix
20:1n-9	Gondoic acid	(Z)-11-Eicosenoic acid	Supelco 37 Component FAME Mix
20:2n-9		(Z,Z)-8,11-Eicosadienoic acid	no standard
20:3n-9	Mead acid	(Z,Z,Z)-5,8,11-Eicosatrienoic acid	single standard
20:2n-6		(Z,Z)-11,14-Eicosadienoic acid	Supelco 37

			Component FAME Mix
20:3n-6	Dihomo-gamma-linolenic acid	(Z,Z,Z)-8,11,14-Eicosatrienoic acid	Supelco 37 Component FAME Mix
20:4n-6	Arachidonic acid	(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic acid	Supelco 37 Component FAME Mix
20:3n-3		(Z,Z,Z)-11,14,17-Eicosatrienoic acid	Supelco 37 Component FAME Mix
20:4n-3		(Z,Z,Z,Z)-8,11,14,17-Eicosatetraenoic acid	single standard
20:5n-3	Timnodonic acid	(Z,Z,Z,Z,Z)-5,8,11,14,17-Eicosapentaenoic acid	Supelco 37 Component FAME Mix
22:0	Behenic acid	Docosanoic acid	Supelco 37 Component FAME Mix
22:1n-9	Erucic acid	(Z)-13-Docosenoic acid	Supelco 37 Component FAME Mix
22:2n-6		(Z,Z)-13,16-Docosadienoic acid	Supelco 37 Component FAME Mix
22:4n-6	Adrenic acid	(Z,Z,Z,Z)-7,10,13,16-Docosatetraenoic acid	single standard
22:5n-6	Osbond acid	(Z,Z,Z,Z,Z)-4,7,10,13,16-Docosapentaenoic acid	single standard
22:4n-3		(Z,Z,Z,Z)-10,13,16,19-Docosatetraenoic acid	no standard
22:5n-3	Clupanodonic acid	(Z,Z,Z,Z,Z)-7,10,13,16,19-Docosapentaenoic acid	single standard
22:6n-3		(Z,Z,Z,Z,Z,Z)-4,7,10,13,16,19-Docosahexaenoic acid	Supelco 37 Component FAME Mix
24:0	Lignoceric acid	Tetracosanoic Acid	Supelco 37 Component FAME Mix
24:1n-9	Nervonic acid	Tetracosenoic Acid	Supelco 37 Component FAME Mix

The commercially available Supelco® 37 Component FAME Mix was used to identify retention times of 22 from 34 target fatty acids, 10 further fatty acids were spiked into the mix or analyzed separately to obtain retention times. For two of the target FAs, no commercial standard was available.

Peak areas of the detected target fatty acids were added up to yield the total peak area. The fatty acid profile and percentage of individual fatty acids was then calculated as peak area percent of total peak area.

The results are shown in the table 182.

Table 182 (Part A): Fatty acid profile of 34 target fatty acids from total lipids after MTBE extraction, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area.

Sample name	14:0	16:0	16:1n-9	16:1n-7	17:0	16:3n-3	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6 (LA)	18:3n-6 (GLA)	18:3n-3 (ALA)	18:4n-3 (SDA)	20:0	20:1n-9
LANBCH oil	0,1	4,6	0,0	0,2	0,1	0,1	2,7	26,3	3,1	0,7	31,7	1,3	5,7	0,3	0,6	0,8
LANPMZ seeds	0,1	4,9	0,0	0,2	0,1	0,1	2,7	29,1	2,9	0,5	33,6	0,8	4,3	0,1	0,8	0,9
LAODDN seeds	0,1	5,2	0,0	0,2	0,1	0,2	2,5	28,2	3,2	0,6	35,1	1,4	5,6	0,4	0,8	0,7
LBFDAU oil	0,1	4,6	0,0	0,2	0,0	0,1	2,7	26,9	3,1	1,0	29,5	1,6	5,8	0,3	0,6	0,7
LBFGKN seeds	0,1	4,5	0,0	0,2	0,1	0,1	2,5	30,9	3,7	0,5	33,2	0,9	6,8	0,2	0,6	0,8
LBFGKN oil	0,1	4,4	0,0	0,2	0,0	0,1	2,6	32,2	2,9	0,5	33,2	0,9	6,8	0,2	0,6	0,8
LBFLFK seeds	0,1	4,8	0,0	0,2	0,1	0,1	2,7	26,4	3,9	0,9	30,3	1,6	5,7	0,3	0,6	0,8
LBFLFK oil	0,1	4,6	0,0	0,2	0,1	0,1	2,7	27,9	3,0	0,9	30,6	1,5	5,8	0,3	0,6	0,8
Arabidopsis seeds	0,1	7,0	0,0	0,2	0,1	0,1	5,3	8,9	1,8	0,2	22,3	0,3	16,8	0,1	3,5	17,5
Kumily seeds	0,1	4,7	0,0	0,3	0,1	0,1	1,9	57,1	3,8	0,0	20,1	0,0	9,3	0,0	0,6	1,1
Bovine fat (corned beef)	3,7	24,6	0,3	3,7	1,2	0,0	16,9	43,9	1,9	0,5	1,6	0,2	0,7	0,1	0,1	0,2
Butter 1	14,2	37,7	0,3	1,9	0,7	0,0	12,7	26,8	1,4	0,8	2,0	0,0	0,6	0,0	0,2	0,1
Butter 2	13,4	37,4	0,3	2,0	0,7	0,0	12,3	27,5	1,6	0,8	2,4	0,0	0,5	0,0	0,2	0,1
Egg yolk from chicken	0,5	25,0	0,6	2,2	0,3	0,0	8,7	35,7	1,5	0,1	19,1	0,3	0,8	0,2	0,1	0,3
Menhaden oil	8,9	17,1	0,2	12,6	2,3	0,1	3,0	7,2	3,4	0,1	1,8	0,4	1,5	3,4	0,3	1,1
Salmon oil capsule	7,8	17,0	0,3	8,6	1,7	0,1	3,5	9,4	3,4	0,2	1,9	0,4	1,1	2,8	0,6	1,9

Tuna 1 0525 oil	3,9	19,7	0,4	5,0	1,3	0,0	5,1	14,9	2,5	0,1	2,0	0,3	0,5	1,0	0,4	1,5
Omevital Anchovy oil	7,3	18,0	0,3	8,6	1,8	0,1	3,9	9,6	3,2	0,2	1,8	0,5	0,9	3,3	0,6	1,6
Krill oil capsule	12,7	21,3	0,1	6,8	0,3	0,1	1,2	10,9	7,0	0,2	2,3	0,3	1,3	4,2	0,1	0,7
DHA DHA oil capsule	5,8	16,0	0,0	0,3	0,1	0,2	1,0	15,4	0,3	0,0	1,3	0,3	0,1	0,3	0,1	0,1
Omega EPA/DHA oil capsule	1,3	16,1	0,0	0,2	0,1	0,0	1,9	26,0	0,2	0,0	2,2	0,1	0,2	0,2	0,4	0,1
Omega-3 EPA & DHA	1,9	19,2	0,0	0,3	0,5	0,0	1,7	10,2	0,2	0,0	1,4	0,1	0,1	0,2	0,4	0,1
Omega 3 algae oil	5,4	15,4	0,0	0,3	0,1	0,2	1,0	15,0	0,2	0,0	1,5	0,3	0,1	0,4	0,1	0,1
Mortierella alpina ARA-rich oil	0,4	6,1	0,0	0,1	0,2	0,0	6,2	5,6	0,3	0,0	7,9	2,3	0,1	0,0	0,9	0,3

Table 182 (Part B): Fatty acid profile of 34 target fatty acids from total lipids after MTBE extraction, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area.

Sample name	20:2n-9	20:3n-9	20:2n-6	20:3n-6 (DGLA)	20:4n-6 (ARA)	20:3n-3	20:4n-3 (ETA)	20:5n-3 (EPA)	22:0	22:1n-9	22:2n-6	22:4n-6	22:5n-6	22:4n-3	22:5n-3 (DPA)	22:6n-3 (DHA)	24:0	24:1
LANBCH oil	0,2	0,1	0,3	1,6	3,4	0,1	1,0	7,5	0,3	0,0	0,1	1,5	0,0	0,4	4,1	1,3	0,1	0,1
LANPMZ seeds	0,6	0,2	0,6	2,1	3,3	0,2	1,0	5,6	0,4	0,0	0,1	1,3	0,0	0,4	2,5	1,0	0,2	0,2
LAODDN seeds	0,1	0,1	0,1	1,5	1,2	0,1	1,0	6,0	0,4	0,0	0,0	1,1	0,0	0,5	2,7	0,9	0,2	0,2
LBFDAU oil	0,3	0,1	0,1	2,9	2,2	0,1	1,9	10,1	0,3	0,0	0,0	0,4	0,1	0,2	2,6	1,4	0,1	0,1
LBFGKN seeds	0,2	0,1	0,2	1,8	2,0	0,1	1,1	5,7	0,3	0,0	0,0	0,4	0,0	0,2	2,0	0,9	0,1	0,2
LBFGKN oil	0,2	0,1	0,2	1,7	1,9	0,1	1,0	5,5	0,3	0,0	0,0	0,4	0,0	0,1	1,9	0,9	0,1	0,1
LBFLFK seeds	0,2	0,1	0,1	2,8	2,2	0,1	1,6	8,4	0,3	0,0	0,0	0,7	0,1	0,4	3,3	1,3	0,1	0,2
LBFLFK oil	0,2	0,1	0,1	3,1	2,1	0,1	1,8	7,9	0,3	0,0	0,0	0,6	0,1	0,4	3,0	1,2	0,1	0,1
Arabidopsis seeds	0,6	0,6	1,4	0,3	0,5	1,7	0,5	5,6	0,5	2,0	0,0	0,7	0,0	0,0	0,1	1,3	0,2	0,4
Kumily seeds	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,3	0,0	0,0	0,0	0,0	0,1	0,2
Bovine fat (corned beef)	0,0	0,0	0,0	0,1	0,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,1	0,0	0,0
Butter 1	0,0	0,0	0,0	0,1	0,2	0,0	0,0	0,1	0,1	0,0	0,0	0,2	0,0	0,0	0,1	0,0	0,0	0,0

Butter 2	0,0	0,0	0,0	0,1	0,2	0,0	0,0	0,1	0,1	0,0	0,0	0,1	0,0	0,0	0,1	0,0	0,0	0,0		
Egg yolk from chicken	0,0	0,1	0,2	0,2	1,9	0,0	0,0	0,4	0,0	0,0	0,0	0,1	0,4	0,0	0,1	1,3	0,0	0,0		
Menhaden oil	0,2	0,0	0,3	0,3	1,3	0,3	1,7	16,4	0,2	0,2	0,0	0,3	0,6	0,1	2,8	12,1	0,2	0,3		
Salmon oil capsule	0,2	0,0	0,2	0,2	1,6	0,1	1,0	19,5	0,2	0,3	0,0	0,2	0,4	0,1	2,5	12,7	0,1	0,6		
Tuna 1 oil	0,0	0,0	0,3	0,2	2,2	0,2	0,5	7,5	0,2	0,2	0,0	0,3	1,8	0,1	1,3	26,6	0,2	0,6		
Anchovy 2 oil	0,2	0,0	0,2	0,2	1,4	0,1	1,0	19,5	0,2	0,3	0,0	0,2	0,4	0,1	2,1	12,7	0,1	0,5		
Krill oil capsule	0,0	0,0	0,1	0,2	0,8	0,1	0,5	18,4	0,1	0,5	0,0	0,0	0,2	0,0	0,4	9,2	0,1	0,1		
DHA DHA oil capsule	0,0	0,0	0,0	0,8	0,7	0,1	0,8	1,1	0,2	0,0	0,0	0,1	15,6	0,1	0,4	38,6	0,5	0,0		
Omega EPA/DHA oil capsule	0,0	0,0	0,0	0,2	1,4	0,1	0,6	17,0	0,3	0,0	0,0	0,2	1,2	0,0	3,1	26,8	0,2	0,0		
Omega-3 EPA & DHA	0,0	0,0	0,0	0,3	1,8	0,1	0,8	19,5	0,2	0,1	0,0	0,3	1,9	0,0	3,3	35,6	0,1	0,0		
Omega 3 algae oil	0,0	0,0	0,0	1,1	1,0	0,1	0,9	1,3	0,3	0,0	0,0	0,1	16,0	0,1	0,6	38,2	0,5	0,0		
Mortierella alpina ARA-rich oil	0,0	0,0	0,4	4,4	48,5	0,5	0,0	0,2	3,4	0,1	0,0	0,5	0,0	0,2	0,0	0,1	11,2	0,4		

Example 30: Lipid Profiling of canola oils and commercial oil samples:

Canola seeds:

12-15 canola seeds were transferred into micro tubes on a 96-format rack and closed with cap strips. Seeds were ground in a swing mill using 3 mm beads for 2 x 2 min at 30 Hz. The rack was then centrifuged for 5 min at 4000 rpm to remove powder from the lid. Extraction of oil was carried out by adding 800 µL of dichloromethane/methanol (DCM/MeOH, 2:1 *v/v*) to the samples followed by extraction in a swing mill for 2 x 30 sec at 30 Hz. After centrifugation at 4000 rpm for 10 min, 100 µL of the clear supernatant was transferred into glass vials and dried to remove the solvent.

Arabidopsis seeds:
20 mg of seeds were transferred into tubes on a 96-format rack. The canola seed protocol was followed in all further steps.

Canola oils and commercial oil samples:
200 µL of oil was transferred into into micro tubes on a 96-format rack and closed with cap strips. Extraction of oil was carried out by adding 800 µL of dichloromethane/methanol (DCM/MeOH, 2:1 *v/v*) to the samples followed by extraction in a swing mill for 2 x 30 sec at 30 Hz. After centrifugation at 4000 rpm for 10 min, 100 µL of the clear supernatant was transferred into glass vials and dried to remove the solvent.

Normal Phase Fractionation:

The dry residues were resuspended in 100 µL 2,2,4-trimethylpentane and isopropyl alcohol (TMP/IPA (9:1, v/v)) and vortexed thoroughly. The krill oil sample was further diluted 1:30 using TMP/IPA. For triacylglycerol (TAG) isolation, the oil samples were resuspended in 500 µL TMP/IPA prior to fractionation Separation of lipid classes was performed using normal phase HPLC that leads to interaction of lipids and stationary phase based on lipid species (e.g. head group in case of phospholipids) rather than length of fatty acid chain. An Agilent 1200 series HPLC was used that comprised

G1322A degasser, G1311A quaternary pump, G1310A isocratic pump, G1316A thermostatted column compartment (TCC), G1367B HiP autosampler, G1330B FC/ALS Thermostat and G1364 Fraction Collector (all Agilent) coupled to and Alltech 3300 evaporative light scattering detector (ELSD, Grace). A quaternary gradient from TMP, MTBE, acetonitrile (MeCN), DCM, formic acid (FA), ammonium formiate (0.5 M) and water was used for separation of lipid species on a PVA-Sil column (150 mm x 3 mm x 5 µm, YMC) with an initial flow rate of 0.4 mL/min. The injection volume was 50 µL. Fractions of triacylglycerol (TAG), diacylglycerol (DAG), monoacylglycerol (MAG), phosphatidylcholine (PC), phosphatidylethanolamine (PE) were collected in glass vials. The solvent was removed and the residue was resuspended in 100 µL toluene. Derivatization of lipid species into FAMEs for subsequent fatty acid profiling was carried out using 20 µL TMSH. FAME analysis was performed as described previously for Example 29 with an injection volume of 2 µL.

The results are shown in the tables 183 to 187.

Table 183 (Part A): Fatty acid profile of 34 target fatty acids from monoacylglycerol (MAG) fraction after DCM/MeOH (2:1) extraction, normal phase fractionation, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area

sample name	Fraction	14:0	16:0	16:1n-9	16:1n-7	17:0	16:3n-3	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6 (LA)	18:3n-6 (GLA)	18:3n-3 (ALA)	18:4n-3 (SDA)	20:0	20:1n-9
LANBCH oil	MAG	0,0	6,6	0,0	0,5	0,0	0,2	3,1	24,5	7,6	0,5	30,3	1,2	4,4	0,3	0,7	0,7
LANPMZ seeds	MAG	0,0	10,6	0,0	1,0	0,0	0,5	4,7	23,2	17,6	0,0	26,1	0,6	2,8	0,0	0,5	0,0
LAODDN seeds	MAG	0,4	12,1	0,0	1,1	0,0	0,0	6,4	22,2	15,5	0,0	25,3	1,3	4,0	0,0	1,1	0,0
LBFDAU oil	MAG	0,3	6,2	0,0	0,6	0,0	0,0	2,8	24,6	7,7	0,8	28,7	1,5	5,0	0,3	0,6	0,7
LBFGKN oil	MAG	0,3	6,8	0,0	0,6	0,0	0,0	3,2	28,1	7,6	0,4	28,6	0,8	4,9	0,0	0,7	0,7
LBFLFK seeds	MAG	0,0	12,7	0,0	0,9	0,0	0,0	6,8	22,8	13,2	0,3	23,7	1,1	4,3	0,0	0,9	0,0
LBFLFK oil	MAG	0,2	6,7	0,0	0,6	0,0	0,2	3,0	25,3	8,2	0,7	28,9	1,4	4,7	0,2	0,6	0,7

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Kumily oil	MAG	0,0	7,4	0,0	0,0	0,0	1,9	3,6	51,0	4,5	0,0	19,7	0,0	9,4	0,0	0,0	2,5
Canola Commodity oil	MAG	0,7	7,5	0,0	0,5	0,0	2,3	3,3	47,2	5,1	0,0	20,8	0,0	8,9	0,0	0,0	3,7
Clear Valley 65 oil	MAG	0,5	13,8	0,0	2,8	0,0	1,4	2,6	28,9	31,7	0,0	14,2	0,0	0,7	0,0	0,7	2,0
Clear Valley 80 oil	MAG	0,0	5,7	0,0	0,6	0,0	3,1	3,4	65,0	5,4	0,0	9,8	0,0	3,1	0,0	0,0	4,0
Menhaden oil	MAG	10,4	24,1	0,0	12,5	2,2	0,1	5,2	6,2	3,8	0,0	1,5	0,6	1,4	3,1	0,4	0,5
Salmon oil capsule	MAG	8,2	22,1	0,0	8,8	1,8	0,5	6,3	8,7	3,9	0,0	1,5	0,0	0,8	2,5	0,8	1,1
Krill oil capsule	MAG	5,4	21,0	0,0	3,4	0,0	1,8	3,3	7,2	6,4	0,0	1,9	0,0	0,0	2,6	0,0	1,9
DHA DHA oil capsule	MAG	7,8	24,4	0,0	1,0	0,0	0,0	3,1	10,1	0,0	0,0	3,5	0,0	0,0	0,0	0,0	0,0
Omega EPA/DHA oil capsule	MAG	2,2	25,8	0,0	0,0	0,0	0,0	3,3	16,0	0,0	0,0	3,2	0,0	0,0	0,0	0,0	0,0
Omega 3 algae oil	MAG	5,4	29,9	0,0	0,0	0,0	0,0	3,0	6,2	0,0	0,0	2,7	0,0	0,0	0,0	0,0	0,8

Table 183 (part B): Fatty acid profile of 34 target fatty acids from monoacylglycerol (MAG) fraction after DCM/MeOH (2:1) extraction, normal phase fractionation, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area

sample name	Fraction	20:2n-9	20:3n-9	20:2n-6	20:3n-6	20:4n-6	20:3n-3	20:4n-3	20:5n-3 (EPA)	22:0	22:1n-9	22:2n-6	22:4n-6	22:5n-6	22:4n-3	22:5n-3	22:6n-3	24:0	24:0
LANBCH oil	MAG	0,3	0,3	0,2	1,8	2,8	0,8	1,0	5,4	0,7	0,0	0,0	1,9	0,0	0,3	2,8	1,3	0,2	0,2
LANPMZ seeds	MAG	0,0	0,0	0,0	1,9	2,8	0,0	1,4	3,9	0,0	0,0	0,0	0,0	0,0	0,0	1,4	1,1	0,0	0,0
LAODDN seeds	MAG	0,0	0,0	0,0	1,0	1,0	0,0	1,3	4,1	0,0	0,0	0,0	0,0	0,0	0,0	1,6	1,5	0,0	0,0
LBFDAU oil	MAG	0,3	0,2	0,2	2,8	1,9	0,0	2,0	8,0	0,0	0,0	0,0	1,3	0,0	0,0	2,2	1,4	0,0	0,0
LBFGKN oil	MAG	0,0	0,3	0,2	1,7	1,7	0,7	1,0	3,9	0,0	0,0	0,0	4,9	0,0	0,4	1,5	1,0	0,3	0,3
LBFLFK seeds	MAG	0,0	0,0	0,0	2,4	1,1	0,0	1,0	4,6	0,0	0,0	0,0	0,7	0,0	0,4	2,0	1,3	0,0	0,0
LBFLFK oil	MAG	0,2	0,3	0,2	2,7	1,4	0,0	1,7	5,9	0,0	0,0	0,0	2,1	0,0	0,4	2,3	1,1	0,3	0,3
Kumily oil	MAG	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Canola Commodity oil	MAG	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Clear Valley 65 oil	MAG	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,7	0,7
Clear Valley 80 oil	MAG	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Menhaden oil	MAG	0,1	0,0	0,3	0,5	1,5	0,0	1,4	12,3	0,0	0,0	0,0	0,0	0,0	0,1	2,2	9,6	0,2	0,2	
Salmon oil capsule	MAG	0,0	0,0	0,0	0,4	1,6	0,0	0,0	17,2	0,0	0,0	0,0	0,0	0,0	0,0	2,1	11,7	0,0	0,0	
Krill oil capsule	MAG	0,0	0,0	0,0	0,0	0,0	0,0	0,0	27,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	17,4	0,0	0,0	
DHA DHA oil capsule	MAG	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	12,1	0,0	0,0	38,1	0,0	0,0	
Omega EPA/DHA oil capsule	MAG	0,0	0,0	0,0	0,0	1,4	0,0	0,0	17,6	0,0	0,0	0,0	0,0	0,0	0,0	3,0	27,6	0,0	0,0	
Royal Green Omega 3 algae oil	MAG	0,0	0,0	0,0	0,0	1,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	14,3	0,0	0,0	36,7	0,0	0,0	

Table 184 (part A): Fatty acid profile of 34 target fatty acids from diacylglycerol (DAG) fraction after DCM/MeOH (2:1) extraction, normal phase fractionation, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area

sample name	Fraction	14:0	16:0	16:1n-9	16:1n-7	17:0	16:3n-3	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6 (LA)	18:3n-6 (GLA)	18:3n-3 (ALA)	18:4n-3 (SDA)	20:0	20:1n-9

LANBCH oil	DAG	0,0	5,4	0,0	0,0	0,0	0,0	2,6	22,0	5,0	0,8	32,7	1,4	5,8	0,0	0,0	0,0	
LBFDAU oil	DAG	0,0	4,7	0,0	0,0	0,0	0,0	2,3	22,4	4,9	0,5	29,9	1,7	6,3	0,0	0,0	0,0	
LBFGKN oil	DAG	0,0	5,9	0,0	0,0	0,0	0,0	2,6	25,8	4,9	0,0	33,2	1,1	7,2	0,0	0,0	0,0	
LBFLFK oil	DAG	0,0	6,1	0,0	0,0	0,0	0,0	2,5	22,9	5,1	0,8	30,7	1,7	6,4	0,0	0,0	0,0	
Canola Commodity oil	DAG	0,0	8,3	0,0	0,0	0,0	2,7	0,0	53,9	0,0	0,0	21,6	0,0	10,1	0,0	0,0	3,4	
Clear Valley 65 oil	DAG	0,0	9,4	0,0	1,7	0,0	3,6	0,0	47,6	17,3	0,0	16,8	0,0	0,0	0,0	0,0	3,7	
Clear Valley 80 oil	DAG	0,0	7,0	0,0	0,0	0,0	3,6	0,0	71,6	4,6	0,0	8,8	0,0	0,0	0,0	0,0	4,3	
Menhaden oil	DAG	10,4	23,6	0,0	12,6	2,4	0,0	4,9	6,4	3,8	0,0	1,5	0,0	1,5	3,6	0,0	0,8	
Salmon oil capsule	DAG	8,4	21,0	0,0	8,6	2,1	0,0	5,3	8,3	3,7	0,0	1,4	0,0	0,0	4,0	0,0	0,9	
DHA DHA oil capsule	DAG	6,8	17,5	0,0	0,0	0,0	0,0	0,0	19,7	0,0	0,0	2,3	0,0	0,0	0,0	0,0	1,3	
Omega EPA/DHA oil capsule	DAG	2,0	16,7	0,0	0,0	0,0	0,0	3,3	32,7	0,0	0,0	2,5	0,0	0,0	0,0	0,0	1,1	
Omega 3 algae oil	DAG	5,0	20,7	0,0	0,0	0,0	0,0	3,0	11,1	0,0	0,0	1,9	0,0	0,0	0,0	0,0	0,0	

Table 184 (part B): Fatty acid profile of 34 target fatty acids from diacylglycerol (DAG) fraction after DCM/MeOH (2:1) extraction, normal phase fractionation, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area

sample name	Fraction	20:2n-9	20:3n-9	20:2n-6	20:3n-6 (DGLA)	20:4n-6 (ARA)	20:3n-3	20:4n-3 (ETA)	20:5n-3 (EPA)	22:0	22:1n-9	22:2n-6	22:4n-6	22:5n-6	22:4n-3	22:5n-3 (DPA)	22:6n-3 (DHA)	24:0	24:1n-9
LANBCH oil	DAG	0,0	0,0	0,0	2,2	4,2	0,0	1,1	8,8	0,0	0,0	0,0	2,1	0,0	0,0	4,1	2,0	0,0	0,0
LBFDAU oil	DAG	0,0	0,0	0,0	3,2	2,8	0,0	2,4	11,7	0,0	0,0	0,0	2,1	0,0	0,0	2,7	2,5	0,0	0,0
LBFGKN oil	DAG	0,0	0,0	0,0	2,7	2,8	0,0	0,8	7,4	0,0	0,0	0,0	0,9	0,0	0,0	2,4	2,5	0,0	0,0
LBFLFK oil	DAG	0,0	0,0	0,0	3,5	2,7	0,0	2,2	9,3	0,0	0,0	0,0	1,4	0,0	0,0	3,2	1,7	0,0	0,0
Canola Commodity oil	DAG	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Clear Valley 65 oil	DAG	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Clear Valley 80 oil	DAG	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Menhaden oil	DAG	0,0	0,0	0,0	0,0	1,0	0,0	1,5	13,4	0,0	0,0	0,0	0,0	0,0	0,0	2,3	10,3	0,0	1,2
Salmon oil	DAG	0,0	0,0	0,0	0,0	2,4	0,0	0,0	19,6	0,0	0,0	0,0	0,0	0,0	0,0	1,9	12,3	0,0	0,0

capsule																				
DHA DHA oil capsule	DAG	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	10,4	0,0	0,0	42,0	0,0	0,0
Omega EPA/DHA oil capsule	DAG	0,0	0,0	0,0	0,0	2,3	0,0	0,0	13,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,8	24,0	0,0	0,0
Omega 3 algae oil	DAG	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	13,9	0,0	0,0	44,5	0,0	0,0

Table 185 (part A): Fatty acid profile of 34 target fatty acids from triacylglycerol (TAG) fraction after DCM/MeOH (2:1) extraction, normal phase fractionation, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area

sample name	Fraction	14:0	16:0	16:1n-9	16:1n-7	17:0	16:3n-3	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6 (LA)	18:3n-6 (GLA)	18:3n-3 (ALA)	18:4n-3 (SDA)	20:0	20:1n-9
LANBCH oil	TAG	0,1	4,5	0,0	0,2	0,1	0,1	2,8	26,2	3,1	0,7	31,5	1,3	5,6	0,2	0,7	0,8
LANPMZ seeds	TAG	0,1	4,9	0,0	0,1	0,1	0,1	2,8	27,9	2,4	0,5	34,9	0,9	4,0	0,1	0,8	0,9
LAODDN seeds	TAG	0,1	5,0	0,0	0,2	0,1	0,2	2,8	29,1	2,7	0,6	35,0	1,4	5,4	0,3	0,8	0,8
LBFDAU oil	TAG	0,1	4,5	0,1	0,2	0,1	0,1	2,8	26,9	3,0	1,0	29,1	1,6	5,8	0,3	0,7	0,8
LBFGKN oil	TAG	0,1	4,3	0,0	0,2	0,1	0,1	2,7	32,4	2,9	0,5	33,0	0,9	6,8	0,2	0,7	0,8
LBFLFK seeds	TAG	0,1	4,5	0,1	0,2	0,1	0,1	2,7	28,6	3,4	0,9	30,1	1,3	5,8	0,2	0,7	0,8

LBFLFK oil	TAG	0,1	4,5	0,0	0,2	0,1	0,1	2,8	28,0	2,9	0,9	30,3	1,5	5,7	0,2	0,7	0,8
Arabidopsis seeds	TAG	0,1	6,7	0,1	0,2	0,1	0,1	5,0	7,8	1,6	0,2	21,7	0,2	17,4	0,1	3,6	18,9
Kumily seeds	TAG	0,1	4,8	0,0	0,3	0,1	0,1	2,0	59,2	0,0	0,0	20,5	0,0	10,3	0,0	0,7	1,2
Canola Commodity oil	TAG	0,1	3,9	0,0	0,2	0,1	0,1	1,8	60,0	2,9	0,0	19,0	0,0	9,3	0,0	0,6	1,3
Clear Valley 65 oil	TAG	0,0	4,1	0,0	0,2	0,1	0,1	1,8	69,5	2,2	0,0	17,6	0,0	0,8	0,0	0,8	1,5
Clear Valley 80 oil	TAG	0,0	2,9	0,0	0,1	0,1	0,1	2,6	80,0	0,0	0,0	8,2	0,0	2,3	0,0	0,9	1,8
Brassica juncea seeds	TAG	0,1	2,8	0,0	0,2	0,0	0,1	1,0	7,0	1,2	0,0	15,2	0,0	12,8	0,0	0,8	4,7
Menhaden oil	TAG	8,7	17,0	0,0	12,8	2,3	0,1	3,1	7,0	3,5	0,1	1,7	0,4	1,5	3,7	0,0	1,1
Salmon oil capsule	TAG	7,5	16,8	0,0	8,7	1,7	0,1	3,7	9,2	3,5	0,1	1,6	0,2	0,9	3,0	0,6	2,0
Krill oil capsule	TAG	21,9	21,4	0,0	11,7	0,4	0,1	1,7	17,4	8,0	0,1	2,5	0,3	1,3	5,5	0,1	1,0
DHA DHA oil capsule	TAG	5,7	15,9	0,0	0,2	0,1	0,2	1,0	15,2	0,2	0,0	1,2	0,2	0,1	0,3	0,1	0,1
Omega EPA/DHA oil capsule	TAG	1,1	15,5	0,0	0,1	0,1	0,0	1,9	25,9	0,2	0,0	2,1	0,0	0,1	0,1	0,4	0,1
Omega 3 algae oil	TAG	5,1	14,7	0,0	0,2	0,1	0,2	1,0	14,8	0,2	0,0	1,2	0,3	0,1	0,3	0,1	0,1

Table 185 (part B): Fatty acid profile of 34 target fatty acids from triacylglycerol (TAG) fraction after DCM/MeOH (2:1) extraction, normal phase fractionation, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area

sample name	Fraction	20:2n-9	20:3n-9	20:2n-6	20:3n-6 (DGLA)	20:4n-6 (ARA)	20:3n-3	20:4n-3 (ETA)	20:5n-3 (EPA)	22:0	22:1n-9	22:2n-6	22:4n-6	22:5n-6	22:4n-3	22:5n-3 (DPA)	22:6n-3 (DHA)	24:0	24:1n-9
LANBCH oil	TAG	0,2	0,1	0,3	1,7	3,4	0,1	1,0	7,6	0,3	0,0	0,1	1,5	0,0	0,4	4,3	1,3	0,1	0,1
LANPMZ seeds	TAG	0,5	0,2	0,5	2,2	3,5	0,2	1,0	5,7	0,4	0,0	0,1	1,3	0,0	0,4	2,4	1,0	0,2	0,2
LAODDN seeds	TAG	0,1	0,1	0,1	1,5	1,3	0,1	0,9	6,0	0,4	0,0	0,0	1,1	0,0	0,4	2,7	0,8	0,2	0,2
LBFDAU oil	TAG	0,3	0,1	0,1	3,0	2,3	0,1	1,9	10,3	0,3	0,0	0,0	0,3	0,0	0,2	2,7	1,4	0,1	0,1
LBFGKN oil	TAG	0,2	0,1	0,2	1,8	1,9	0,1	1,0	5,6	0,3	0,0	0,0	0,3	0,0	0,1	1,9	0,9	0,1	0,1
LBFLFK seeds	TAG	0,3	0,1	0,1	3,1	2,0	0,1	1,7	7,7	0,3	0,0	0,0	0,5	0,1	0,5	3,0	1,2	0,1	0,1
LBFLFK oil	TAG	0,3	0,1	0,1	3,2	2,1	0,1	1,8	8,0	0,3	0,0	0,0	0,5	0,1	0,4	3,1	1,2	0,1	0,1
Arabidopsi	TAG	0,7	0,6	1,5	0,2	0,3	1,8	0,5	6,0	0,5	2,3	0,1	0,2	0,2	0,0	0,0	1,2	0,2	0,4

s seeds																					
Kumily seeds	TAG	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,3	0,0	0,0	0,0	0,0	0,2	0,2			
Canola Commodity oil	TAG	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,4	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,2	0,2			
Clear Valley 65 oil	TAG	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,6	0,0	0,0	0,2	0,0	0,0	0,0	0,0	0,4	0,2			
Clear Valley 80 oil	TAG	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,5	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,4	0,2			
Brassica juncea seeds	TAG	0,0	0,0	0,9	0,0	0,0	0,2	0,0	1,2	49,3	1,6	0,0	0,5	0,0	0,0	0,0	0,7	2,2			
Menhaden oil	TAG	0,2	0,0	0,3	0,3	1,3	0,2	1,8	16,7	0,2	0,2	0,0	0,6	0,3	0,1	2,9	12,1	0,1	0,4		
Salmon oil capsule	TAG	0,2	0,0	0,2	0,2	1,7	0,1	1,1	20,0	0,2	0,4	0,0	0,5	0,2	0,1	2,6	13,0	0,1	0,6		
Krill oil capsule	TAG	0,0	0,0	0,1	0,0	0,2	0,1	0,3	3,8	0,0	0,5	0,0	0,0	0,0	0,0	0,2	1,7	0,0	0,0		
DHA DHA	TAG	0,0	0,0	0,0	0,9	0,5	0,0	0,8	0,8	0,2	0,0	0,0	16,0	0,1	0,2	0,4	39,2	0,5	0,0		

oil capsule																				
Omega EPA/DHA oil capsule	TAG	0,0	0,0	0,0	0,3	1,4	0,1	0,6	17,1	0,3	0,0	0,0	0,2	1,2	0,1	3,3	27,4	0,4	0,0	
Omega 3 algae oil	TAG	0,0	0,0	0,0	1,0	0,6	0,1	0,9	1,2	0,2	0,0	0,0	0,1	16,9	0,1	0,6	39,9	0,3	0,0	

Table 186 (part A): Fatty acid profile of 34 target fatty acids from phosphatidylcholine (PC) fraction after DCM/MeOH (2:1) extraction, normal phase fractionation, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area

sample name	Fraction	14:0	16:0	16:1n-9	16:1n-7	17:0	16:3n-3	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6 (LA)	18:3n-6 (GLA)	18:3n-3 (ALA)	18:4n-3 (SDA)	20:0	20:1n-9
LANPMZ seeds	PC	0,0	12,7	0,6	0,3	0,1	0,1	3,4	6,6	5,8	0,0	39,2	1,8	4,5	0,3	0,0	0,0
LAODDN seeds	PC	0,0	11,5	0,4	0,7	0,0	0,0	3,1	9,7	5,9	0,0	37,0	4,5	10,1	1,5	0,0	0,0
LBFLFK seeds	PC	0,0	8,7	0,4	0,5	0,0	0,3	2,6	10,8	7,6	0,0	39,3	1,3	6,4	0,3	0,2	0,2
Arabidopsis seeds	PC	0,0	13,2	0,9	0,7	0,0	0,0	6,6	4,1	5,2	0,0	27,9	1,2	14,4	0,6	0,9	6,8
Kumily seeds	PC	0,0	6,1	0,2	0,6	0,0	0,2	0,7	57,8	2,7	0,0	27,2	0,0	4,5	0,0	0,0	0,0
Brassica juncea seeds	PC	0,0	12,3	0,0	1,8	0,0	1,1	1,5	29,4	4,6	0,0	34,4	0,0	12,0	0,0	0,0	1,9

Krill oil capsule	PC	3,1	26,5	0,0	1,7	0,1	0,0	1,0	4,6	5,5	0,2	2,1	0,3	1,5	3,0	0,0	0,3

Table 186 (part B): Fatty acid profile of 34 target fatty acids from phosphatidylcholine (PC) fraction after DCM/MeOH (2:1) extraction, normal phase fractionation, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area

sample name	Fraction	20:2n-9	20:3n-9	20:2n-6	20:3n-6 (DGLA)	20:4n-6 (ARA)	20:3n-3	20:4n-3 (ETA)	20:5n-3 (EPA)	22:0	22:1n-9	22:2n-6	22:4n-6	22:5n-6	22:4n-3	22:5n-3 (DPA)	22:6n-3 (DHA)	24:0	24:1n-9
LANPM Z seeds	PC	0,0	0,0	0,0	0,7	0,0	0,3	7,0	7,3	0,0	0,0	0,0	0,0	0,0	0,0	0,4	9,0	0,0	0,0
LAODD N seeds	PC	0,0	0,0	0,0	0,2	0,0	0,0	6,8	3,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	5,3	0,0	0,0
LBFLFK seeds	PC	0,1	0,0	0,0	0,9	0,0	0,1	10,9	4,1	0,0	0,0	0,0	0,0	0,0	0,2	1,8	3,3	0,0	0,0
Arabidopsis seeds	PC	0,0	0,0	0,0	0,0	0,0	2,8	1,0	6,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	7,2	0,0	0,0
Kumily seeds	PC	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Brassica	PC	0,0	0,0	0,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

juncea seeds																									
Krill oil capsule	PC	0,1	0,0	0,1	0,2	0,7	0,0	1,0	33,3	0,0	0,6	0,0	0,0	0,0	0,0	0,0	0,6	13,6	0,0	0,1					

Table 187 (part A): Fatty acid profile of 34 target fatty acids from phosphatidylethanolamine (PE) fraction after DCM/MeOH (2:1) extraction, normal phase fractionation, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area

sample name	Fraction	14:0	16:0	16:1n-9	16:1n-7	17:0	16:3n-3	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6 (LA)	18:3n-6 (GLA)	18:3n-3 (ALA)	18:4n-3 (SDA)	20:0	20:1n-9
LANPMZ seeds	PE	0,0	18,3	0,0	0,0	0,0	0,0	3,1	5,8	6,4	0,0	36,4	1,3	4,4	0,0	0,0	0,0
LAODDN seeds	PE	0,0	17,7	0,0	0,0	0,0	0,0	3,3	7,9	6,0	0,0	33,6	3,0	8,5	0,0	0,0	0,0
LBFLFK seeds	PE	0,0	15,5	0,0	0,0	0,0	0,0	2,9	8,6	7,3	0,0	37,6	1,2	6,1	0,0	0,0	0,0
Arabicopsis seeds	PE	0,0	18,7	0,4	0,0	0,0	0,0	5,6	2,4	4,6	0,0	27,1	0,0	13,2	0,0	0,0	4,1
Brassica juncea seeds	PE	0,0	19,1	0,0	1,1	0,0	2,1	0,0	13,4	4,5	0,0	41,4	0,0	13,3	0,0	0,0	4,1
Krill oil capsule	PE	1,1	16,6	0,0	0,0	0,0	2,9	0,0	4,1	13,6	0,0	0,0	0,0	0,0	0,0	0,0	4,0

Table 187 (part B): Fatty acid profile of 34 target fatty acids from phosphatidylethanolamine (PE) fraction after DCM/MeOH (2:1) extraction, normal phase fractionation, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area

sample name	fraction	20:2n-9	20:3n-9	20:2n-6	20:3n-6	20:4n-6 (ARA)	20:3n-3	20:4n-3 (ETA)	20:5n-3 (EPA)	22:0	22:1n-9	22:2n-6	22:4n-6	22:5n-6	22:4n-3	22:5n-3 (DPA)	22:6n-3 (DHA)	24:0	24:1n-9
LANPMZ seeds	PE	0,0	0,0	0,0	1,0	6,5	0,0	4,1	4,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	8,5	0,0	0,0
LAODDN seeds	PE	0,0	0,0	0,0	0,0	7,5	0,0	4,2	2,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	5,9	0,0	0,0
LBFLFK seeds	PE	0,0	0,0	0,0	1,2	3,6	0,0	8,1	3,3	0,0	0,0	0,0	0,0	0,0	0,0	0,9	3,6	0,0	0,0
Arabidopsis seeds	PE	0,0	0,0	0,0	0,0	11,0	0,5	0,0	4,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	7,7	0,0	0,0
Brassica juncea seeds	PE	0,0	0,0	1,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Krill oil capsule	PE	0,0	0,0	0,0	0,0	2,5	0,0	0,0	24,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	30,4	0,0	0,0

Example 31: Phospholipid species profiling

Diluted extracts from Example 30 were used for phospholipid species analysis

PC species profiling

Phosphatidylcholine (PC) species separation was carried out on a 1290 Agilent HPLC coupled to API5500 triple quadrupole MS (ABSciex). HPLC comprised of G4227A Flex Cube, G4226A autosampler, G1330B thermostat and G4220A binary pump. PC species separation was carried out on a Phenomenex Kinetex C8 column (150 mm x 2.1 mm x 1.7 µm). The mobile phase consisted of H_2O/MeOH/sodium acetate (50 mM) as solvent A and MeOH/sodium acetate (50 mM) as solvent B with a flow rate of 0.5 mL/min. The initial conditions were 40% A followed by a linear gradient to 25% A in a total run time of 7 min. MS was operated in positive ESI mode with a source temperature of 550 °C using scheduled multiple reaction monitoring (sMRM) and an injection volume of 1 µL. Data acquisition was carried out using Analyst 1.5.1 whereas data analysis was done in Multiquant 3.0.1 (AB Sciex). All theoretical $[M+Na]^+$ from phosphatidylcholine species containing C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:3, C20:4, C20:5, C22:5 and C22:6 fatty acids were calculated resulting in a total of 78 PC species. In sMRM-mode, the neutral loss of fatty acids was monitored. MRMs of PC species not present in a representative set of test samples from different canola, fish and algae oil sources were removed from the transition list.

All peak areas of target PC species were added to a total peak area. Species with peak areas lower than 10.000 counts were not included in the calculation as they were considered below limit of quantitation having a poor signal-to-noise ratio. PC species profile was then calculated as PC species percent of total PC species.

LPC species profiling

Lysophosphatidylcholine (LPC) species separation was carried out on a 1290 Agilent HPLC coupled to API5500 triple quadrupole MS (ABSciex). HPLC comprised of G4227A Flex Cube, G4226A autosampler, G1330B thermostat and G4220A binary pump. LPC species separation was carried out on a Phenomenex Kinetex C8 column

(150 mm x 2.1 mm x 1.7 µm). The mobile phase consisted of H_2O/MeOH/formic as solvent A and MeOH as solvent B with a flow rate of 0.5 mL/min and a linear gradient from 80% A to 0% A over a total run time of 4 min. MS was operated in positive ESI mode with a source temperature of 550 °C using multiple reaction monitoring (MRM) and an injection volume of 1 µL. Data acquisition was carried out using Analyst 1.5.1 whereas data analysis was done in Multiquant 3.0.1 (AB Sciex). All theoretical $[M+H]^+$ from LPC species containing C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:3, C20:4, C20:5, C22:5 and C22:6 fatty acids were calculated. In sMRM-mode, the neutral loss of fatty acids was monitored.

All peak areas of target LPC species were added to a total peak area. Species with peak areas lower than 12.500 counts were not included in the calculation as they were considered below limit of quantitation having a poor signal-to-noise ratio. LPC species profile was then calculated as LPC species percent of total LPC species.

PE/LPE species profiling

Phosphatidylethanolamine (PE) and lysophosphatidylethanolamine (LPE) species separation was carried out on a 1290 Agilent HPLC coupled to API5500 triple quadrupole MS (ABSciex). HPLC comprised of G4227A Flex Cube, G4226A autosampler, G1330B thermostat and G4220A binary pump. PE/LPE species separation was carried out on a Phenomenex Kinetex C8 column (150 mm x 2.1 mm x 1.7 µm). The mobile phase consisted of H_2O/MeOH/ammonium formate as solvent A and MeOH/ ammonium formate as solvent B with a flow rate of 0.5 mL/min. The initial conditions were 100% A followed by a linear gradient to 0% A over a total run time of 16 min. MS was operated in negative ESI mode with a source temperature of 550 °C using scheduled multiple reaction monitoring (sMRM) and an injection volume of 1 µL. Data acquisition was carried out using Analyst 1.5.1 whereas data analysis was done in Multiquant 3.0.1 (AB Sciex). All theoretical $[M-H]^-$ from PE and LPE species containing C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:3, C20:4, C20:5, C22:5 and C22:6 fatty acids were calculated resulting in a total of 78 PE species and 12 LPE species.

MRMs of PE/LPE species not present in a representative set of test samples from different canola, fish and algae oil sources were removed from the transition list.

All peak areas of target PE species and LPE species were added to a total peak area separately. The peak area cut-off for LPE not included into the calculation due to poor signal-to-noise was 2000 counts, for PE it was 5000 counts. PE and LPE species profile were then calculated as PE species percent of total PE species and LPE species percent of total LPE species.

The results are shown in Tables 188 to 191.

Table 188: Phosphatidylcholin species profile of occurring PC species comprising 12 target fatty acids from DCM/MeOH (2:1) extracts analyzed by +ESI-HPLC-MS/MS in sMRM mode. Data are mean, n=2-3 and expressed as relative abundance of species. Species that were not detected in any samples were removed from the table

Sample Name	LANBCH oil	LANPMZ seeds	LAODDN seeds	LBFDAU oil	LBFGKN seeds	LBFGKN oil	LBFLFK seeds	LBFLFK oil	Arabidopsis seeds	Kumily seeds	Kumily oil	Canola Commodity oil	Clear Valley 65 oil	Clear Valley 80 oil	Brassica juncea seeds	Salmon oil capsule	Krill oil capsule	DHA DHA oil capsule	Omega EPA/DHA oil capsule	Omega 3 algae oil
PC140 161	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,9	0,0	0,0	0,0
PC140 181	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,4	0,0	0,0	0,0
PC140 182	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,0	0,0	0,0	0,0
PC140 183	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,8	0,0	0,0	0,0
PC140 204	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,0	0,0	0,0
PC140 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	8,5	0,0	0,0	0,0
PC140 225	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,0
PC140 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,7	0,0	0,0	0,0
PC160 160	0,5	0,7	0,4	0,4	0,3	0,0	0,4	0,9	1,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,0	0,0	0,0
PC160 161	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,0	0,0	0,0	0,0
PC160 180	0,0	0,3	0,2	0,0	0,2	0,0	0,3	0,0	0,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
PC160 181	4,9	4,8	5,2	4,6	6,6	7,5	5,8	4,5	3,7	11,5	12,9	12,5	15,9	9,0	11,8	0,0	10,8	3,4	6,1	6,4
PC160 182	12,4	11,7	10,3	12,6	8,8	10,1	7,6	11,5	11,6	7,5	6,7	7,3	7,4	0,0	11,4	0,0	3,9	14,7	12,9	20,5
PC160 183	3,9	2,3	4,7	3,5	2,0	4,8	1,8	3,4	8,2	3,2	1,9	1,7	0,7	0,0	8,1	0,0	1,8	0,0	0,0	0,0
PC160 203	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,4	0,0	0,0	0,0
PC160 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	13,0	0,0	0,0	0,0

PC160 225	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,9	0,0	0,0	0,0	0,0	0,0	0,0
PC160 226	0,0	2,7	1,5	0,0	1,3	0,0	1,2	0,0	2,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	8,4	0,0	0,0	0,0	0,0	0,0	0,0
PC161 161	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,0	0,0	0,0	0,0	0,0	0,0
PC161 180	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
PC161 181	0,0	0,3	0,4	0,0	0,4	0,0	0,5	0,0	0,7	0,0	0,8	0,3	0,8	0,4	0,0	0,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
PC161 182	0,0	0,8	0,7	0,0	0,5	0,0	0,4	0,0	1,7	0,0	0,8	0,6	0,0	1,6	0,0	0,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,3
PC161 183	0,0	0,3	0,7	0,0	0,3	0,0	0,4	0,0	0,6	0,5	0,0	0,0	0,0	1,2	0,0	2,7	0,0	0,1	0,0	0,0	0,0	0,0	0,0	0,0
PC161 203	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,0	4,4	0,0	0,0	0,0	0,0	0,0	0,0
PC161 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	4,4	0,0	0,7	0,0	0,0	0,0	0,0	0,0	0,0
PC161 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,7	0,0	0,2	0,0	0,0	0,0	0,0	0,0	1,2
PC180 181	0,0	0,5	0,6	0,0	1,4	0,0	1,2	0,0	0,8	1,8	0,0	0,8	0,0	1,7	0,0	0,2	0,0	0,3	3,2	3,3	6,1			
PC180 182	3,6	5,2	4,3	3,7	3,8	0,0	4,0	2,9	6,6	0,2	0,0	0,1	0,0	0,8	0,0	0,3	3,2	3,3	6,1					
PC180 183	0,0	1,0	1,8	0,0	0,7	0,0	0,8	0,0	4,1	0,0	0,0	0,0	0,0	0,5	0,0	0,3	0,0	0,0	0,0	0,0	0,0			
PC180 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,2	0,0	0,0	0,0	0,0	0,0			
PC180 226	0,0	0,8	0,6	0,0	0,4	0,0	0,3	0,0	1,3	0,0	0,0	0,0	0,0	0,0	0,0	1,0	0,0	0,0	0,0	0,0	0,0			
PC181 181	5,8	3,4	4,2	7,0	7,8	15,5	8,0	6,6	2,3	26,8	36,1	28,0	32,9	66,0	15,2	29,5	4,4	9,0	18,6	7,5				
PC181 182	15,8	13,5	13,1	17,8	16,7	19,4	16,0	17,6	9,3	24,4	27,4	27,0	28,6	19,1	18,7	0,0	2,2	19,2	16,9	24,5				
PC181 183	3,4	2,7	5,1	4,1	3,5	4,3	3,8	3,9	4,4	7,0	5,0	6,0	1,8	4,9	7,4	0,0	0,7	0,0	0,0	0,0				
PC181 204	3,0	1,9	1,8	4,9	2,6	3,5	4,6	4,2	0,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,0				
PC181 205	0,0	1,2	0,7	0,0	1,2	0,0	1,3	0,0	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	7,5	0,0	0,0	0,0				
PC181 226	0,0	1,4	1,0	0,0	1,1	0,0	1,2	0,0	1,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	4,1	0,0	0,0	0,0				
PC182 182	17,8	12,5	10,7	17,4	13,8	16,8	12,4	17,2	10,8	8,4	6,8	10,2	10,3	0,0	8,7	32,1	0,1	49,7	35,2	33,3				
PC182 183	7,6	7,2	12,4	7,4	8,6	7,6	8,4	9,1	11,6	5,9	3,1	4,3	0,7	0,2	9,3	0,0	0,1	0,0	0,0	0,1				
PC182 203	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,4	0,0	0,0	0,0				
PC182 204	5,7	4,7	4,5	7,2	4,9	4,0	7,3	7,9	0,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0				
PC182 205	9,7	8,6	3,0	5,8	4,5	3,3	4,1	5,3	2,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,0	0,0	0,0	0,0				
PC182 226	5,9	3,9	2,2	2,4	2,2	3,3	1,4	2,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,0	0,0				
PC183 183	0,0	1,6	5,2	0,0	2,3	0,0	2,2	0,6	5,9	1,2	0,0	0,5	0,0	0,0	3,3	0,0	0,0	0,0	0,0	0,0				

PC183 205	0,0	2,4	1,9	0,0	1,7	0,0	1,6	0,7	2,6	0,0	0,0	0,0	0,0	0,0	0,0	0,5	0,0	0,0	0,0	0,0
PC183 226	0,0	1,4	1,8	0,0	1,0	0,0	0,7	0,0	2,0	0,0	0,0	0,0	0,0	0,0	38,4	0,1	0,8	7,0	0,0	
PC204 204	0,0	0,7	0,7	1,3	0,8	0,0	1,9	1,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
PC205 205	0,0	0,7	0,1	0,0	0,3	0,0	0,4	0,0	0,4	0,0	0,0	0,0	0,0	0,0	0,0	4,5	0,0	0,0	0,0	0,0
PC205 225	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,0	0,0
PC205 226	0,0	1,0	0,3	0,0	0,4	0,0	0,3	0,0	0,9	0,0	0,0	0,0	0,0	0,0	0,0	3,9	0,0	0,0	0,0	0,0
PC226 226	0,0	0,4	0,1	0,0	0,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,9	0,0	0,0	0,0	0,0

Table 189: Lysophosphatidylcholin species profile of occuring LPC species comprising 12 target fatty acids from DCM/MeOH (2:1) extracts analyzed by +ESI-HPLC-MS/MS in MRM mode. Data are mean, n=2-3 and expressed as relative abundance of species. Species that were not detected in any samples were removed from the table

Sample Name	LANBCH oil	LANPMZ seeds	LAODDN seeds	LBFDAU oil	LBFGKN seeds	LBFGKN oil	LBFLFK seeds	LBFLFK oil	Arabidopsis seeds	Kumily seeds	Menhaden oil	Salmon oil capsule	Krill oil capsule	DHA DHA oil capsule	Omega EPA/DHA oil capsule	Omega 3 algae oil
LPC 14:0	0,0	0,1	0,1	0,0	0,0	0,0	0,0	0,0	0,2	0,0	0,0	0,0	4,4	0,0	0,0	0,0
LPC 16:0	2,8	14,5	14,2	2,9	11,0	2,3	11,7	1,9	18,1	7,4	28,7	47,3	37,2	10,8	11,1	13,4
LPC 16:1	2,0	2,3	2,0	2,0	1,6	2,2	1,6	2,0	2,8	1,3	0,0	0,0	3,2	0,0	0,0	0,0
LPC 18:0	0,8	2,5	3,1	1,0	2,2	0,6	2,5	0,5	9,1	0,8	48,0	0,0	1,4	5,5	11,4	5,0
LPC 18:1	13,4	16,9	16,8	15,8	22,1	26,5	21,8	14,0	13,0	56,2	13,2	21,1	13,7	23,4	29,1	20,4
LPC 18:2	46,4	33,8	32,9	48,8	38,5	47,4	34,8	45,3	23,7	28,9	0,0	0,0	2,2	60,4	48,4	60,9
LPC 18:3	7,0	6,7	14,7	6,8	8,2	6,7	8,0	8,9	14,0	5,5	0,0	0,0	1,6	0,0	0,0	0,0
LPC 20:3	1,7	0,9	0,4	1,0	0,8	0,5	1,0	0,9	3,0	0,0	0,0	0,0	0,2	0,0	0,0	0,0
LPC 20:4	9,1	6,5	6,3	10,8	6,7	5,9	10,3	13,3	2,4	0,0	0,0	0,0	1,0	0,0	0,0	0,0

LPC 20:5	5,6	6,7	3,5	4,0	3,8	2,6	3,7	4,8	5,6	0,0	10,2	21,1	22,8	0,0	0,0	0,4		
LPC 22:5	1,4	0,8	0,7	1,3	0,7	0,6	1,2	3,1	0,5	0,0	0,0	0,0	1,2	0,0	0,0	0,0		
LPC 22:6	9,8	8,4	5,4	5,6	4,4	4,7	3,4	5,3	7,7	0,0	0,0	10,5	11,1	0,0	0,0	0,0		

Table 190: Phosphatidylethanolamine species profile of occurring PE species comprising 12 target fatty acids from DCM/MeOH (2:1) extracts analyzed by -ESI-HPLC-MS/MS in sMRM mode. Data are mean, n=2-3 expressed as relative abundance of species. Species that were not detected in any samples were removed from the table

Sample Name	LANBCH oil	LANPMZ seeds	LAODDN seeds	LBFDAU oil	LBFGKN seeds	LBFGKN oil	LBFLFK seeds	LBFLFK oil	Arabidopsis seeds	Kumily seeds	Kumily oil	Canola Commodity oil	Clear Valley 65 oil	Clear Valley 80 oil	Brassica juncea seeds	Menhaden oil	Salmon oil capsule	Krill oil capsule	DHA DHA oil capsule	Omega EPA/DHA oil capsule	Omega 3 algae oil
PE140 160	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,4	0,0	0,0	0,0
PE140 161	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,4	0,0	0,0	0,0
PE140 181	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,0	0,0	0,3	0,0	0,0	0,0	0,0	2,1	0,0	0,0	0,0
PE140 182	0,0	1,1	0,6	0,4	0,6	0,0	0,5	0,4	0,5	0,4	0,0	0,4	0,5	0,0	0,5	0,0	0,0	0,0	0,0	0,0	0,0
PE140 183	0,0	0,1	0,2	0,0	0,1	0,0	0,1	0,0	0,2	0,1	0,0	0,0	0,0	0,0	0,2	0,0	0,0	0,1	0,0	0,0	0,0
PE140 203	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,0	0,0	0,0
PE140 204	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
PE140 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	5,7	0,0	0,0	0,0
PE140 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,9	0,0	0,0	0,0
PE160 160	0,3	0,1	0,1	0,2	0,1	0,1	0,1	0,1	0,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,9	0,0	0,0	0,3
PE160 161	0,5	0,5	0,4	0,0	0,4	0,0	0,4	0,5	1,1	0,3	0,0	0,0	0,0	0,0	1,0	0,0	0,0	4,2	0,0	0,0	0,0
PE160 180	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,0

PE160 181	5,0	1,9	2,4	5,4	3,0	6,6	2,6	6,0	1,8	9,7	14,7	19,0	11,4	13,0	7,2	0,0	0,0	9,9	3,1	0,0	2,5
PE160 182	26,6	22,2	17,9	24,7	17,6	23,2	21,2	22,0	26,0	9,8	13,5	12,9	12,3	4,9	19,5	0,0	0,0	6,9	25,7	35,7	30,0
PE160 183	3,0	4,6	10,4	2,9	4,7	3,7	4,8	3,7	14,6	2,4	2,4	2,0	0,3	0,0	8,4	0,0	0,0	6,1	0,0	0,0	0,0
PE160 203	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,4	0,0	0,0	0,0
PE160 204	1,4	2,0	1,1	1,4	1,6	0,0	2,3	1,6	0,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,0
PE161 161	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,1	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,0	0,0
PE161 180	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
PE161 181	0,2	0,2	0,3	0,3	0,5	0,0	0,4	0,5	0,5	1,0	1,0	0,8	0,8	1,8	0,9	0,0	0,0	4,0	0,0	0,0	0,0
PE161 182	0,9	1,1	0,9	0,8	1,2	1,0	0,9	0,9	1,6	1,5	1,0	1,1	1,1	0,0	2,1	0,0	0,0	0,5	0,0	0,0	0,0
PE161 204	0,0	0,0	0,0	0,0	0,1	0,0	0,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,2	0,0	0,0	0,0
PE180 181	0,0	0,1	0,1	0,1	0,1	0,0	0,1	0,0	0,0	0,0	0,0	0,0	0,3	0,0	0,2	0,0	0,0	0,0	0,0	0,0	0,0
PE180 182	2,6	2,5	1,8	2,7	1,7	2,0	2,2	2,5	3,6	0,4	0,7	0,4	0,4	0,0	1,0	0,0	0,0	0,0	9,5	0,0	11,6
PE180 183	0,0	0,3	0,7	0,2	0,4	0,0	0,4	0,0	2,3	0,1	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,0	0,0	0,0	0,0
PE180 226	0,0	0,1	0,1	0,0	0,1	0,0	0,0	0,0	0,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
PE181 181	1,9	1,0	1,2	2,4	2,8	4,6	2,0	3,4	0,6	20,6	14,1	13,2	22,3	38,9	4,9	100,0	100,0	3,4	10,0	23,0	8,7
PE181 182	13,7	12,8	13,5	14,2	16,3	17,0	14,8	14,5	6,9	26,7	27,6	25,6	35,1	28,7	19,8	0,0	0,0	1,4	10,1	2,4	7,2
PE181 183	2,2	1,9	4,8	2,6	3,3	3,2	3,7	2,9	3,8	5,2	5,6	4,0	0,8	3,9	5,2	0,0	0,0	1,2	0,0	0,0	0,0
PE181 204	0,8	0,5	0,5	1,8	1,3	1,0	1,9	1,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,4	0,0	0,0	0,0
PE181 226	0,5	0,6	0,5	0,2	0,7	0,0	0,6	0,4	0,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	20,9	0,0	0,0	0,0
PE182 182	17,8	21,0	11,6	16,0	18,8	19,0	15,7	15,7	13,5	14,6	13,3	15,7	13,7	7,5	16,9	0,0	0,0	0,5	41,6	38,9	39,9
PE182 183	11,0	13,1	18,2	11,5	14,1	12,3	12,0	12,0	13,7	6,3	5,6	4,6	0,9	1,4	9,4	0,0	0,0	0,0	0,0	0,0	0,0
PE182 203	0,7	0,4	0,0	0,6	0,4	0,0	0,5	0,5	0,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
PE182 204	4,7	4,9	4,4	7,1	4,0	4,7	7,0	5,7	0,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
PE182 225	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,0
PE182 226	2,1	3,3	1,4	0,9	1,6	0,0	1,1	0,6	0,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,6	0,0	0,0	0,0
PE183 183	1,8	1,1	4,0	1,6	1,9	1,7	1,4	1,9	4,6	0,8	0,6	0,4	0,0	0,0	2,5	0,0	0,0	0,0	0,0	0,0	0,0
PE183 204	1,0	1,0	1,7	1,4	1,5	0,0	2,2	1,7	0,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,0

PE183 226	0,7	0,9	0,9	0,3	0,6	0,0	0,4	0,3	0,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,8	0,0	0,0	0,0	
PE203 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,2	0,0	0,0	0,0	
PE203 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,6	0,0	0,0	0,0	
PE204 205	0,3	0,2	0,1	0,3	0,3	0,0	0,2	0,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,5	0,0	0,0	0,0	
PE204 226	0,0	0,3	0,2	0,1	0,2	0,0	0,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	6,1	0,0	0,0	0,0	
PE205 205	0,2	0,1	0,1	0,1	0,1	0,0	0,1	0,1	0,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	10,6	0,0	0,0	0,0	
PE226 226	0,0	0,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,6	0,0	0,0	0,0	

Table 191: Lysophosphatidylethanolamine species profile of occurring LPE species comprising 12 target fatty acids from DCM/MeOH (2:1) extracts analyzed by -ESI-HPLC-MS/MS in sMRM mode. Data are mean, n=2-3 expressed as relative abundance of species. Species that were not detected in any samples were removed from the table

Sample Name	LANBCH oil	LANPMZ seeds	LAODDN seeds	LBFDAU oil	LBFGKN seeds	LBFGKN oil	LBFLFK seeds	LBFLFK oil	Arabidopsis seeds	Kumily seeds	Canola Commodity oil	Clear Valley 65 oil	Clear Valley 80 oil	Brassica juncea seeds	Salmon oil capsule	Krill oil capsule	DHA DHA oil capsule	Omega EPA/DHA oil	Omega 3 algae oil
LPE14:0	0,0	0,2	0,2	0,0	0,1	0,0	0,1	0,0	0,2	0,1	0,1	0,1	0,0	0,1	0,0	0,6	0,0	0,0	0,0
LPE16:0	1,9	18,8	20,8	2,5	14,8	1,6	14,7	1,7	22,5	9,7	5,0	8,2	7,7	24,8	19,1	23,4	9,9	17,0	20,5
LPE16:1	0,6	0,7	0,6	0,6	0,5	0,7	0,5	0,7	0,8	1,1	0,5	0,6	0,3	1,1	0,0	1,2	0,0	0,0	0,0
LPE18:0	1,2	3,0	3,6	1,7	2,7	1,2	3,0	1,0	9,5	0,8	1,4	1,7	4,6	8,6	0,0	1,9	2,5	1,2	4,2
LPE18:1	7,1	15,1	16,4	9,8	19,2	13,6	19,5	7,6	12,1	42,1	48,5	61,1	71,7	23,6	25,6	23,7	14,8	17,9	11,9
LPE18:2	54,5	35,7	29,7	57,8	38,1	59,8	33,8	62,3	24,3	39,6	40,5	27,7	14,2	32,4	42,6	1,6	72,8	64,0	63,4
LPE18:3	6,7	6,8	13,8	7,2	9,0	7,2	8,5	8,9	12,7	6,6	4,0	0,6	1,5	9,4	0,0	0,7	0,0	0,0	0,0
LPE20:3	0,3	0,6	0,2	0,2	0,5	0,1	0,7	0,1	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,0
LPE20:4	3,4	5,1	5,9	4,6	6,2	2,7	10,6	3,5	1,5	0,0	0,0	0,0	0,0	0,0	0,0	2,0	0,0	0,0	0,0

LPE20:5	6,3	4,9	3,1	5,3	3,9	3,3	3,7	5,0	5,2	0,0	0,0	0,0	0,0	0,0	12,8	24,8	0,0	0,0	0,0	
LPE22:5	0,2	0,1	0,1	0,2	0,2	0,1	0,5	0,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,4	0,0	0,0	0,0	
LPE22:6	18,0	9,0	5,6	10,1	4,8	9,6	4,3	8,4	10,2	0,0	0,0	0,0	0,0	0,0	0,0	19,3	0,0	0,0	0,0	

Example 32: TAG species analysis

Diluted extracts from Example 29 were used for TAG (triacylglycerol) species analysis. TAG species analysis was carried out on a 1290 Agilent HPLC coupled to API5500 triple quadrupole MS (ABSciex). HPLC comprised of G4227A Flex Cube, G4226A autosampler, G1330B thermostat and G4220A binary pump. TAG species separation was carried out on a Thermo Accucore C30 column (250 mm x 2.1 mm x 2.6 µm). The mobile phase consisted of MeCN/IPA as solvent A and IPA as solvent B with a flow rate of 0.4 mL/min. Starting conditions were 100% followed by a linear gradient to 20% A with a total run time of 30 min. The Injection volume was 1 µL. The mass spectrometer was operated in positive APCI mode with ion source temperature of 300 °C using scheduled multiple reaction monitoring (sMRM). Data acquisition was carried out using Analyst 1.5.1 whereas data analysis was done in Multiquant 3.0.1 (AB Sciex). All theoretical $[M+H]^+$ from TAG species containing C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:3, C20:4, C20:5, C22:5 and C22:6 fatty acids were calculated resulting in a total of 364 TAG species. In MRM-mode, the neutral loss of fatty acids was monitored, therefore all theoretical product ions were calculated as well. The first quadrupole was set to filter only those $[M+H]^+$ of target TAG species that were subsequently fragmented in the second quadrupole which led to the loss of fatty acid. For TAG species containing three different fatty acids, three product ions were monitored in the third quadrupole whereas those species with two different fatty acids led to two product ions. In case of TAG species with only one fatty acid, only one transition could be monitored.

Single and mixed standards of TAGs (16:0/16:0/16:0), (16:1/16:1/16:1), (17:0/17:0/17:0), (18:0/18:0/18:0), (18:1/18:1/18:1), (18:2/18:2/18:2), (18:3/18:3/18:3), (20:5/20:5/20:5), (22:6/22:6/22:6), (16:0/16:0/18:1), (16:0/18:0/18:2), (16:0/18:1/18:1), (16:0/18:2/18:2), (18:0/18:0/18:2), (18:0/18:1/18:1), (18:0/18:1/18:2), (18:1/18:1/18:3) and (18:1/18:2/18:2) were run to identify retention time patterns among TAG species. MRMs of TAG species not present in a representative set of test samples from different canola, fish and algae oil sources were removed from the transition list. For all other species, a retention time was identified to allow for scheduled monitoring of MRM in a

given time window which allows for more data points per transition thus leading to a better quality of data.

Baseline separation of all TAG species could not achieved within a reasonable runtime, therefore peak heights were used instead of peak areas. The sums of all transitions with were used to calculate a total peak height. Species with peak height lower than 1000 counts were not included in the calculation as they were considered below limit of quantitation having a poor signal-to-noise ratio. TAG species profile was then calculated as TAG species percent of total TAG species.

The results are shown in Table 192.

Table 192: Triacylglycerol sspecies profile of occurring TAG species comprising 12 target fatty acids from MTBE extracts analyzed by +APCI-HPLC-MS/MS in sMRM mode. Data are mean, n=2 expressed as relative abundance of species. Species that were not detected in any samples were removed from the table

Sample name	LANBCH oil	LANPMZ seeds	LAODDN seeds	LANBCH oil	LBFGKN oil	LBFLFK seeds	LBFLFK oil	Arabidopsis seeds	Kumily seeds	Kumily oil	Canola Commodity oil	Clear Valley 65 oil	Clear Valley 80 oil	Brassica juncea seeds	Menhaden oil	Salmon oil capsule	Krill oil capsule	DHA DHA oil capsule	Omega EPA/DHA oil	Omega 3 algae oil
TAG 140 140 140	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,1
TAG 140 140 160	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,1
TAG 140 140 161	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,8	0,7	10,8	0,0	0,0	0,0
TAG 140 140 181	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,5	0,4	10,5	0,0	0,0	0,0
TAG 140 140 183	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,6	0,2	2,1	0,0	0,0	0,0
TAG 140 140 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	6,7	7,0	3,3	0,3	0,4	0,4
TAG 140 140 225	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,5	0,0	2,7
TAG 140 140 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,4	2,0	1,2	10,5	1,0	10,4
TAG 140 160 161	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,0	0,6	15,4	0,0	0,0	0,0
TAG 140 160 181	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,6	1,4	15,7	0,0	0,0	0,0
TAG 140 160 182	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,0	2,5	0,0	0,0	0,0
TAG 140 160 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	8,3	10,1	8,8	0,0	2,0	0,0
TAG 140 160 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	5,1	4,4	2,5	23,9	3,7	21,2
TAG 140 161 161	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,5	0,7	2,4	0,0	0,0	0,0
TAG 140 161 183	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,6	0,3	0,0	0,0	0,0	0,0
TAG 140 161 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	8,4	7,0	2,9	0,0	0,0	0,0
TAG 140 161 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,7	1,7	0,0	0,0	0,0	0,4
TAG 140 181 181	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,5	0,4	5,0	0,0	0,0	0,0

TAG 140 205 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,6	3,4	0,0	0,1	1,2	0,2			
TAG 140 205 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,4	2,2	0,0	1,3	3,6	1,1			
TAG 160 160 181	0,1	0,1	0,2	0,1	0,1	0,0	0,1	0,2	0,2	0,1	0,3	0,2	0,5	0,2	0,3	0,9	0,0	0,0	0,0	0,0	0,0			
TAG 160 160 182	0,5	0,3	0,3	0,5	0,4	0,3	0,4	1,1	0,4	0,2	0,4	0,3	0,2	2,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0			
TAG 160 160 183	0,3	0,2	0,2	0,3	0,3	0,3	0,3	2,4	0,8	0,5	0,5	0,0	0,0	7,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0			
TAG 160 160 205	0,2	0,2	0,2	0,2	0,1	0,3	0,2	0,6	0,0	0,0	0,0	0,0	0,0	0,0	2,9	3,6	1,4	0,0	5,9	0,0				
TAG 160 160 225	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,7	0,0				
TAG 160 160 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,1	2,6	0,0	11,3	12,6	8,8				
TAG 160 161 161	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,8	0,8	0,0	0,0	0,0	0,0				
TAG 160 161 181	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,4	0,7	3,3	0,0	0,0	0,0				
TAG 160 161 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	6,1	8,3	1,5	0,0	0,0	0,0				
TAG 160 161 225	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,7	1,0	0,0	0,0	0,0	0,0				
TAG 160 161 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	5,7	0,0	0,0	1,1	0,3	0,7				
TAG 160 180 181	0,1	0,1	0,1	0,1	0,1	0,1	0,3	0,2	0,2	0,2	0,3	0,5	0,4	0,1	0,1	0,1	0,1	0,1	0,1	0,1				
TAG 160 180 182	0,1	0,2	0,3	0,1	0,1	0,1	0,5	0,1	0,1	0,1	0,1	0,0	0,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0				
TAG 160 180 183	0,2	0,2	0,2	0,2	0,2	0,1	2,6	0,3	0,2	0,2	0,0	0,1	1,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0				
TAG 160 180 205	0,0	0,1	0,1	0,0	0,0	0,1	0,0	0,7	0,0	0,0	0,0	0,0	0,5	0,5	0,5	0,0	0,0	0,0	0,8	0,0				
TAG 160 180 225	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,3	0,0				
TAG 160 180 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,0	0,0				
TAG 160 181 181	0,7	2,4	0,8	0,7	0,9	0,8	1,0	0,5	3,4	5,1	2,5	4,7	12,5	1,7	0,2	0,4	1,3	0,8	2,0	1,1				
TAG 160 181 182	2,6	1,9	3,4	2,6	2,7	3,2	2,3	2,0	4,8	2,8	3,7	8,4	3,0	4,0	0,0	0,0	0,0	0,0	0,0	0,0				
TAG 160 181 183	1,3	1,8	0,0	1,3	1,8	1,5	0,0	3,1	6,6	5,1	6,1	1,0	3,4	9,8	0,0	0,0	0,0	0,0	0,0	0,0				
TAG 160 181 205	1,5	1,3	1,2	1,5	1,2	2,0	2,2	1,3	0,0	0,0	0,0	0,0	0,0	0,0	4,9	8,1	3,0	0,0	0,0	0,0				
TAG 160 181 225	0,7	0,4	0,7	0,7	0,4	0,7	0,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0				
TAG 160 182 182	4,1	4,9	4,3	4,1	2,9	3,2	2,1	4,1	1,8	1,6	0,0	1,9	0,0	5,0	0,0	0,0	0,0	0,0	0,0	0,0				
TAG 160 182 183	2,4	2,5	3,1	2,4	2,6	2,3	1,5	9,2	5,8	3,7	3,5	0,5	0,4	15,4	0,0	0,0	0,0	0,0	0,0	0,0				
TAG 160 182 205	4,3	3,3	3,1	4,3	1,7	3,8	2,4	7,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0				
TAG 160 182 225	1,7	1,6	2,1	1,7	0,8	1,4	1,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0				

TAG 160 183 183	0,3	0,3	0,5	0,3	0,6	0,4	0,4	4,6	1,1	1,2	0,0	0,0	0,0	10,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 160 183 205	0,7	0,4	0,5	0,7	0,6	0,6	0,7	2,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 160 184 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,7	2,9	2,0	0,0	0,0	0,0	0,0	0,0
TAG 160 205 205	0,4	0,2	0,2	0,4	0,3	0,4	0,3	1,6	0,0	0,0	0,0	0,0	0,0	0,0	2,8	3,6	0,9	0,0	8,0	8,0	0,0	0,0
TAG 160 205 225	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,1	3,1	0,0	0,0
TAG 160 205 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,5	0,0	0,0	0,0	0,0	0,0	0,0	2,4	3,8	0,0	0,0	13,4	13,4	0,0	0,0
TAG 160 225 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	13,0	1,2	1,2	16,5	16,5
TAG 160 226 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,0	1,1	0,0	21,2	12,0	12,0	20,1	20,1
TAG 161 161 181	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,4	0,3	0,0	0,0	0,0	0,0	0,0	0,0
TAG 161 161 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	4,9	3,4	0,0	0,0	0,0	0,0	0,0	0,0
TAG 161 181 181	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,3	0,2	0,1	0,7	0,6	0,0	0,1	0,2	0,7	0,0	0,0	0,0	0,0	0,0
TAG 161 181 183	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 161 181 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,0	3,1	0,0	0,0	0,0	0,0	0,0	0,0
TAG 161 182 183	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 161 205 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,6	1,7	0,0	0,0	0,0	0,0	0,0	0,0
TAG 161 205 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 161 226 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,3	0,3	0,0	0,6	0,1	0,1	0,5	0,5
TAG 180 180 180	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 180 180 181	0,1	0,1	0,1	0,1	0,2	0,1	0,1	0,4	0,2	0,2	0,1	0,3	0,7	0,2	0,0	0,0	0,0	0,1	0,1	0,1	0,1	0,1
TAG 180 180 182	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,5	0,0	0,0	0,1	0,0	0,1	0,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 180 180 183	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,6	0,0	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 180 181 181	0,2	0,3	0,2	0,2	0,3	0,2	0,2	0,2	0,8	0,6	0,3	1,4	2,8	0,0	0,0	0,0	0,0	0,4	0,6	0,6	0,4	0,4
TAG 180 181 182	0,5	0,8	0,4	0,5	0,6	0,5	0,4	0,8	0,7	0,9	0,9	1,3	1,7	0,0	0,0	0,0	0,0	0,0	0,2	0,2	0,0	0,0
TAG 180 181 183	0,9	0,4	0,4	0,9	0,8	0,4	0,4	1,4	2,0	0,9	0,9	0,0	0,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 180 181 203	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 180 181 205	0,8	0,4	0,4	0,8	0,4	0,9	0,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,5	0,0	0,0	0,0	0,0	0,0	0,0
TAG 180 181 225	0,2	0,1	0,1	0,2	0,1	0,1	0,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,2	0,0	0,0	0,0	0,0	0,0	0,0
TAG 180 182 182	0,9	0,8	1,0	0,9	0,9	0,8	0,6	1,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

TAG 180 182 183	0,8	0,6	0,8	0,8	0,7	0,7	0,6	5,0	0,6	0,5	0,6	0,0	0,0	2,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 180 182 205	0,7	0,4	0,4	0,7	0,6	0,7	0,7	1,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 180 182 225	0,6	0,3	0,5	0,6	0,3	0,9	0,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 180 183 183	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,6	0,3	0,3	0,0	0,0	0,0	1,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 180 183 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 180 205 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,4	0,0	0,0	0,0	0,0	0,0	0,0	0,3	0,7	0,0	0,0	0,0	0,0	0,0	0,0
TAG 180 205 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,3	0,9	0,0	0,0	0,0	0,7	0,0	0,0
TAG 180 226 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,2	0,0	0,5	0,4	0,4		
TAG 181 181 181	1,2	1,4	1,1	1,2	2,9	1,3	2,6	0,5	12,4	8,2	0,0	20,9	29,9	0,3	0,1	0,2	0,3	3,3	5,0	3,3		
TAG 181 181 182	2,1	3,8	4,6	2,1	3,7	3,5	2,6	0,5	10,8	9,1	17,1	33,1	18,8	1,7	0,0	0,0	0,0	0,7	1,1	0,4		
TAG 181 181 183	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	23,1	18,4	6,2	13,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0		
TAG 181 181 203	0,6	0,0	0,5	0,6	0,7	1,0	1,1	0,0	0,0	0,0	4,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0		
TAG 181 181 204	1,5	0,0	0,0	1,5	1,7	0,0	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0		
TAG 181 181 205	5,3	6,6	7,5	5,3	6,9	4,9	7,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,4	1,3	0,0	0,0	0,0	0,0		
TAG 181 181 225	1,9	2,5	1,5	1,9	1,0	1,4	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,1	0,0	0,0	0,0	0,0		
TAG 181 181 226	0,5	0,7	0,3	0,5	1,0	0,8	0,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,6	0,8	0,0	0,0	0,0	0,0		
TAG 181 182 182	5,2	8,0	9,9	5,2	8,1	6,1	4,9	0,0	13,5	5,5	5,0	13,1	4,9	0,0	0,0	0,0	0,0	0,5	0,6	0,3		
TAG 181 182 183	5,7	5,8	4,6	5,7	6,5	5,8	6,0	3,1	16,4	18,6	13,3	3,6	3,8	8,7	0,0	0,0	0,0	0,0	0,0	0,0		
TAG 181 182 203	0,9	1,6	1,6	0,9	1,3	2,0	1,6	0,0	0,0	0,0	1,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0		
TAG 181 182 205	10,1	10,2	10,5	10,1	7,5	12,4	10,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0		
TAG 181 182 225	4,2	3,0	3,8	4,2	2,7	3,5	3,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0		
TAG 181 182 226	1,4	0,9	0,8	1,4	1,2	2,1	1,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0		
TAG 181 183 183	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,9	8,0	4,1	4,5	0,0	1,3	4,4	0,0	0,0	0,0	0,0	0,0	0,0		
TAG 181 183 203	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0		
TAG 181 204 205	1,2	0,0	0,0	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0		
TAG 181 205 205	1,1	0,8	1,0	1,1	1,2	1,8	1,3	0,9	0,0	0,0	0,0	0,0	0,0	0,0	0,8	1,2	0,3	0,0	0,0	0,0		
TAG 181 205 225	0,6	0,4	0,4	0,6	0,5	0,8	0,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,3	0,0	0,0	0,0	0,0		
TAG 181 205 226	0,4	0,3	0,1	0,4	0,1	0,2	0,2	0,6	0,0	0,0	0,0	0,0	0,0	0,0	0,9	1,6	0,0	0,0	0,0	0,0		

TAG 181 226 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,5	0,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 182 182 182	4,2	6,4	5,5	4,2	3,0	3,5	6,0	0,8	2,4	1,7	0,0	1,6	0,3	2,0	0,0	0,0	0,0	0,0	0,0	0,3	0,5	0,5	0,6	
TAG 182 182 183	3,3	3,5	5,7	3,3	5,3	3,4	3,9	1,9	2,9	2,7	3,4	0,3	0,4	8,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
TAG 182 182 203	1,0	0,0	0,0	1,0	0,8	0,0	1,9	0,0	0,0	0,0	0,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
TAG 182 182 205	5,3	8,1	3,6	5,3	7,3	5,1	6,9	2,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
TAG 182 182 225	2,6	2,1	2,5	2,6	2,1	2,6	1,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
TAG 182 182 226	1,2	1,1	0,6	1,2	1,5	1,2	1,4	0,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
TAG 182 183 183	1,5	1,0	1,5	1,5	2,3	1,4	1,1	2,8	2,0	2,1	2,0	0,0	0,2	7,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
TAG 182 183 203	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,6	0,0	0,0	2,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
TAG 182 183 205	2,7	2,0	2,8	2,7	2,7	3,4	3,5	4,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
TAG 182 183 225	1,6	1,0	1,3	1,6	0,7	0,0	0,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
TAG 182 183 226	0,9	0,0	0,0	0,9	1,1	0,0	0,8	2,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
TAG 182 205 205	0,9	0,5	0,6	0,9	0,7	1,1	1,7	2,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
TAG 182 205 225	1,1	0,5	0,4	1,1	0,5	1,1	1,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
TAG 182 205 226	0,0	0,3	0,2	0,0	0,0	0,4	0,0	0,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
TAG 182 225 226	0,2	0,1	0,1	0,2	0,1	0,2	0,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
TAG 183 183 183	0,1	0,0	0,2	0,1	0,3	0,2	0,1	0,7	0,6	0,5	0,0	0,0	0,0	1,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
TAG 183 183 203	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
TAG 183 183 205	0,4	0,2	0,4	0,4	0,3	0,5	0,4	2,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
TAG 183 183 226	0,1	0,1	0,1	0,1	0,1	0,1	0,1	1,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
TAG 183 205 205	0,4	0,1	0,2	0,4	0,3	0,5	0,3	1,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,4	0,0	0,0	0,0	0,0	0,0	0,0	
TAG 183 205 226	0,0	0,1	0,1	0,0	0,0	0,2	0,0	0,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
TAG 205 205 205	0,2	0,1	0,1	0,2	0,1	0,2	0,1	0,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,6	0,9	0,4	0,0	2,5	0,0			
TAG 205 205 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,5	0,8	0,2	0,5	5,1	0,8			
TAG 205 226 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,5	0,0	1,0	5,3	1,5			
TAG 226 226 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,0	4,7	3,8	7,7			

Sequences of the invention

Table 193 shows the sequences of the invention by their name, and the associated SEQ-ID number of the sequence lisiting.

Table 193: Assosiation of sequence names to SEQ-IDs in the sequence listing.

			Continued from column 1 and 2	
SEQ ID NO:	**Sequence Name**		**SEQ ID NO:**	**Sequence Name**
1	RTP10690-1qcz_F		129	j-i-Atss1_c-d5Elo(Ot_GA3)_P
2	RTP10691-2qcz		130	j-i-Atss1_c-d6Elo(Tp_GA2)_P
3	VC-LTM593-1qcz		131	j-i-Atss14_c-d12Des(Ps_GA)_P
4	VC-LTM595-1qcz		132	j-i-Atss18_c-d6Elo(Pp_GA2)_P
5	VC-LJB2197-1qcz		133	j-i-Atss18_c-o3Des(Pi_GA2)_P
6	VC-LJB2755-2qcz		134	j-i-Atss2_c-d4Des(Tc_GA3)_P
7	VC-LLM306-1qcz		135	j-i-Atss2_c-d6Des(Ot febit_GA)_P
8	VC-LLM337-1qcz		136	j-p-BnFAE_ t-PvARC_P
9	VC-LLM338-3qcz		137	j-p-BnSETL-v1_c-o3Des(Pir_GA)_P
10	VC-LLM391-2qcz		138	j-p-LuPXR_i-Atss15_P
11	VC-LLM217-1qcz		139	j-p-PvARC5_t-BnFAE_P
12	o3DES-S94FAM		140	j-p-PvARC5_t-BnSETL_P
13	o3DESPIR-198FAM		141	j-t-CaMV_p-LuCnl-2_P
14	o3DESPIR-962FAM		142	j-t-E9-p3-2_P
15	AtPXR-Fam		143	j-t-PvARC-p-LuCnl_P
16	BnSETL-1186FAM		144	j-t-StCAT_p2_p-LuPXR_P
17	BnSETL-Fam		145	reference (Adh1)_P
18	E011		146	c-o3Des(Pi_GA2_SNP)_F
19	D4DES-Tc-FAM		147	j-t-PvARC-p3_F
20	D4DFS-Eg-FAM		148	j-i-Atss15_c-o3Des(Pi_GA2)_F
21	D4DES-PI-770FAM		149	j-p-VfSBPperm3_c-o3Des(Pir_GA)_F
22	Exp3-78-YAK		150	c-d15Des(Ch_ERTp_GA)_F
23	o3DES-572F		151	j-i-Atss2_c-d4Des(Pl_GA)-195R)_F
24	o3DESPIR-160F		152	c-o3Des(Pi_GA2_SNP)_R
25	o3DESPIR-924F		153	j-t-PvARC-p3_R
26	o3DesPir-F		154	j-i-Atss15_c-o3Des(Pi_GA2)_R
27	BnSETL-1164-F		155	j-p-VfSBPperm3_c-o3Des(Pir_GA)_R
28	MA54		156	c-d15Des(Ch_ERTp_GA)_R
29	D4DES-Tc-F		157	j-i-Atss2_c-d4Des(Pl_GA)-195R)_R
30	D4DES-EG-F		158	c-o3Des(Pi_GA2_SNP)_P
31	D4DES-PI-746-F		159	j-t-PvARC-p3_P
32	Exp 3-52F		160	j-i-Atss15_c-o3Des(Pi_GA2)_P
33	o3DES-652R		161	j-p-VfSBPperm3_c-o3Des(Pir_GA)_P
34	o3DESPIR-262R		162	c-d15Des(Ch_ERTp_GA)_P
35	o3DESPIR-1026-R		163	j-i-Atss2_c-d4Des(Pl_GA)-195R)_P
36	AtPXR-R		164	LANBCH locus 1 (N5) Forward primer
37	o3DESPIR-R		165	LANBCH locus 1 (N9) Forward primer
38	MA55		166	LANBCH locus 2 (N5) Forward primer
39	D4DES-Tc-988R		167	LANBCH locus 2 (N9) Forward primer

			Continued from column 1 and 2	
SEQ ID NO:	Sequence Name		SEQ ID NO:	Sequence Name
40	D4DES-Eg-R		168	LBFDAU locus 1 Forward primer
41	D4DES-PI-817R		169	LBFDAU locus 2 Forward primer
42	Exp 3-128R		170	LBFDGG Forward primer
43	RTP10690_1		171	LBFDHG locus 1 Forward primer
44	RTP10690_2		172	LBFDHG locus 2 Forward primer
45	RTP10690_3		173	LBFGKN Forward primer
46	RTP10690_4		174	LBFIHE Forward primer
47	RTP10690_5		175	LBFLFK locus 1 Forward primer
48	RTP10690_6		176	LBFLFK locus 2 Forward primer
49	RTP10690_7		177	LBFPRA Locus 1 Forward primer
50	RTP10690_8		178	LBFPRA locus 2 Forward primer
51	RTP10690_9		179	LBFPRA Locus 3e Forward primer
52	RTP10690_10		180	LANBCH locus 1 (N5) Reverse primer
53	RTP10690_11		181	LANBCH locus 1 (N9) Reverse primer
54	RTP10690_12		182	LANBCH locus 2 (N5) Reverse primer
55	RTP10690_5'		183	LANBCH locus 2 (N9) Reverse primer
56	t-AtAHASL		184	LBFDAU locus 1 Reverse primer
57	LJB2197_5'		185	LBFDAU locus 2 Reverse primer
58	LJB2197_1		186	LBFDGG Reverse primer
59	LJB2197_2		187	LBFDHG locus 1 Reverse primer
60	LJB2197_3		188	LBFDHG locus 2 Reverse primer
61	LJB2197_4		189	LBFGKN Reverse primer
62	c-AHAS_F		190	LBFIHE Reverse primer
63	c-d4Des(Eg_GA)_F		191	LBFLFK locus 1 Reverse primer
64	c-d4Des(PI_GA)2_F		192	LBFLFK locus 2 Reverse primer
65	c-d4Des(Tc_GA)_F		193	LBFPRA Locus 1 Reverse primer
66	c-d5Des(Tc_GA)_F		194	LBFPRA locus 2 Reverse primer
67	c-d5Elo(Ot_GA3)_F		195	LBFPRA Locus 3e Reverse primer
68	c-d6Des(Ot_febit)_F		196	LANBCH locus 1 (N5) Probe sequence
69	c-d6Elo(Pp_GA)_F		197	LANBCH locus 1 (N9) Probe sequence
70	c-d6Elo(Tp_GA)_F		198	LANBCH locus 2 (N5) Probe sequence
71	c-o3Des(Pi_GA)_F		199	LANBCH locus 2 (N9) Probe sequence
72	c-o3Des(Pir_GA)_F		200	LBFDAU locus 1 Probe sequence
73	j-i-Atss1_c-d5Elo(Ot_GA3)_F		201	LBFDAU locus 2 Probe sequence
74	j-i-Atss1_c-d6Elo(Tp_GA2)_F		202	LBFDGG Probe sequence
75	j-i-Atss14_c-d12Des(Ps_GA)_F		203	LBFDHG locus 1 Probe sequence
76	j-i-Atss18_c-d6Elo(Pp_GA2)_F		204	LBFDHG locus 2 Probe sequence
77	j-i-Atss18_c-o3Des(Pi_GA2)_F		205	LBFGKN Probe sequence
78	j-i-Atss2_c-d4Des(Tc_GA3)_F		206	LBFIHE Probe sequence
79	j-i-Atss2_c-d6Des(Ot febit_GA)_F		207	LBFLFK locus 1 Probe sequence
80	j-p-BnFAE_ t-PvARC_F		208	LBFLFK locus 2 Probe sequence
81	j-p-BnSETL-v1_c-o3Des(Pir_GA)_F		209	LBFPRA Locus 1 Probe sequence
82	j-p-LuPXR_i-Atss15_F		210	LBFPRA locus 2 Probe sequence
83	j-p-PvARC5_t-BnFAE_F		211	LBFPRA Locus 3e Probe sequence
84	j-p-PvARC5_t-BnSETL_F		212	VC-LJB2197-1qcz LANBCH RB6 Locus 1
85	j-t-CaMV_p-LuCnl-2_F		213	VC-LJB2197-1qcz LANBCH LB2 Locus 1

			Continued from column 1 and 2	
SEQ ID NO:	**Sequence Name**		**SEQ ID NO:**	**Sequence Name**
86	j-t-E9-p3-2_F		214	VC-LLM337-1qcz LANBCH RB2 Locus 1
87	j-t-PvARC-p-LuCnl_F		215	VC-LLM337-1qcz LANBCH LB1 Locus 1
88	j-t-StCAT_p2_p-LuPXR_F		216	VC-LIB2197-1qcz LANBCH RB3 Locus 2
89	reference (Adh1)_F		217	VC-LLM337-1qcz LANBCH RB1 Locus 2
90	c-AHAS_R		218	VC-LTM593-1qcz LBFDGG RB
91	c-d4Des(Eg_GA)_R		219	VC-LTM593-1qcz LBFDGG LB
92	c-d4Des(Pl_GA)2_R		220	VC-LTM593-1qcz LBFDHG RB Locus 1
93	c-d4Des(Tc_GA)_R		221	VC-LTM593-1qcz LBFDHG LB Locus 1
94	c-d5Des(Tc_GA)_R		222	VC-LTM593-1qcz LBFDHG RB Locus 2
95	c-d5Elo(Ot_GA3)_R		223	VC-LTM593-1qcz LBFDHG LB Locus 2
96	c-d6Des(Ot_febit)_R		224	VC-LTM593-1qcz LBFGKN RB1
97	c-d6Elo(Pp_GA)_R		225	VC-LTM593-1qcz LBFGKN LB1
98	c-d6Elo(Tp_GA)_R		226	VC-LTM593-1qcz LBFIHE RB1
99	c-o3Des(Pi_GA)_R		227	VC-LTM593-1qcz LBFIHE LB1
100	c-o3Des(Pir_GA)_R		228	VC-LTM593-1qcz LBFLFK RB1 Locus 1
101	j-i-Atss1_c-d5Elo(Ot_GA3)_R		229	VC-LTM593-1qcz LBFLFK LB1 Locus 1
102	j-i-Atss1_c-d6Elo(Tp_GA2)_R		230	VC-LTM593-1qcz LBFLFK RB2 Locus 2
103	j-i-Atss14_c-d12Des(Ps_GA)_R		231	VC-LTM593-1qcz LBFLFK LB2 Locus 2
104	j-i-Atss18_c-d6Elo(Pp_GA2)_R		232	VC-LTM593-1qcz LBFPRA RB1 Locus 1
105	j-i-Atss18_c-o3Des(Pi_GA2)_R		233	VC-LTM593-1qcz LBFPRA LB1 Locus 1
106	j-i-Atss2_c-d4Des(Tc_GA3)_R		234	VC-LTM593-1qcz LBFPRA RB3 Locus 2
107	j-i-Atss2_c-d6Des(Ot febit_GA)_R		235	VC-LTM593-1qcz LBFPRA L1 Locus 2
108	j-p-BnFAE_ t-PvARC_R		236	VC-LTM593-1qcz LBFPRA Locus 3e
109	j-p-BnSETL-v1_c-o3Des(Pir_GA)_R		237	VC-LTM593-1qcz LBFDAU RB1 Locus 1
110	j-p-LuPXR_i-Atss15_R		238	VC-LTM593-1qcz LBFDAU LB1 Locus 1
111	j-p-PvARC5_t-BnFAE_R		239	VC-LTM593-1qcz LBFDAU R Locus 2
112	j-p-PvARC5_t-BnSETL_R		240	VC-LTM593-1qcz LBFDAU L Locus 2
113	j-t-CaMV_p-LuCnl-2_R		241	Amplicon LANBCH locus 1 (N5)
114	j-t-E9-p3-2_R		242	Amplicon LANBCH locus 1 (N9)
115	j-t-PvARC-p-LuCnl_R		243	Amplicon LANBCH locus 2 (N5)
116	j-t-StCAT_p2_p-LuPXR_R		244	Amplicon LANBCH locus 2 (N9)
117	reference (Adh1)_R		245	Amplicon LBFDAU locus 1
118	c-AHAS_P		246	Amplicon LBFDAU locus 2
119	c-d4Des(Eg_GA)_P		247	Amplicon LBFDGG
120	c-d4Des(Pl_GA)2_P		248	Amplicon LBFDHG locus 1
121	c-d4Des(Tc_GA)_P		249	Amplicon LBFDHG locus 2
122	c-d5Des(Tc_GA)_P		250	Amplicon LBFGKN
123	c-d5Elo(Ot_GA3)_P		251	Amplicon LBFIHE
124	c-d6Des(Ot_febit)_P		252	Amplicon LBFLFK locus 1
125	c-d6Elo(Pp_GA)_P		253	Amplicon LBFLFK locus 2
126	c-d6Elo(Tp_GA)_P		254	Amplicon LBFPRA Locus 1
127	c-o3Des(Pi_GA)_P		255	Amplicon LBFPRA locus 2
128	c-o3Des(Pir_GA)_P		256	Amplicon LBFPRA Locus 3e

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Claims

1. T-DNA for expression of a target gene in a plant, wherein the T-DNA comprises a left and a right border element and at lest one expression cassette comprising a promotor, operatively linked thereto a target gene, and downstream thereof a terminator, wherein the length of the T-DNA, measured from left to right border element and comprising the target gene, has a length of at least 30000 bp, and the expression cassette is separated from the closest border of the T-DNA by a separator of at least 500 bp length.

2. T-DNA comprising the coding sequences of any single of the tables given in the examples, preferably comprising the coding sequences and and promotors of any single of the tables given in the examples, more preferably the coding sequences and promotors and terminators of any single of the tables given in the examples, and most preferably the expression cassettes of any single of the tables of the examples.

3. Plant or a seed or part thereof, comprising, integrated in its genome, a heterologous T-DNA of the present invention.

4. Plant comprising one or more T-DNA comprising one or more expression cassetts encoding for one or more d5Des, one or more d6Elo, one or more d5Des, one or more o3Des, one or more d5Elo and one or more D4Des.

5. The plant of claim 3 or 4 comprising one or more T-DNAs encoding for at least two d6Des, at least two d6Elo and/or, at least two o3Des.

6. The plant any one of claims 3 to 5 on or a part thereof comprising a T-DNA encoding for at least one CoA-depenent d4Des and at least one phopho-lipid dependent d4Des.

7 The plant, seed or part thereof any one of claims 3 to 6 the plant, seed or part thereof further encoding for one or more d12Des.

8. Plant or seed thereof of family Brassicaceae, preferably of genus Brassica, with a genotype that confers a heritable phenotype of seed oil VLC-PUFA content, obtainable or obtained from progeny lines prepared by a method comprising the steps of

   i) crossing a plant of family Brassicaceae, preferably of genus Brassica, most preferably of genus Brassica napus, Brassica oleracea, Brassica nigra or Brassica carinata, said plant comprising a T-DNA of any of claims 1 or 2 and/or part of such T-DNA, with a plant of family Brassicaceae, preferably of genus Brassica, most preferably of genus **Brassica** napus, Brassica oleracea, Brassica nigra or Brassica carinata, said plant not comprising said T-DNA and/or part thereof, to yield a F1 hybrid,

   ii) selfing the F1 hybrid for at least one generation, and

   iii) identifying the progeny of step (ii) comprising the T-DNA of the present invention capable of producing seed comprising VLC-PUFA such that the content of all VLC-PUFA downstream of 18:1n-9 is at least 40% (w/w) of the total seed fatty acid content at an oil content of 40% (w/w), or preferably the content of **EPA is at least 6%, preferably at least 7,5%** (w/w) and/or the content of DHA is at least **0,8% (w/w), preferably 1,2%** of the total seed fatty acid content at an oil content of 40% (w/w).

9. Method for creating a plant with a genotype that confers a heritable phenotype of seed oil VLC-PUFA content, obtainable or obtained from progeny lines prepared by a method comprising the steps of

   i) crossing a transgenic plant according to any of claims 3 or 4 with a plant not comprising a T-DNA according to any of claims 1 or 2 or part thereof, said latter plant being of family Brassicaceae, preferably of genus Brassica, most preferably of genus Brassica napus, Brassica oleracea, Brassica nigra or Brassica carinata, to yield a F1 hybrid,

   ii) selfing the F1 hybrid for at least one generation, and

   iii) identifying the progeny of step (ii) comprising the T-DNA of the present invention capable of producing seed comprising VLC-PUFA such that the content of all VLC-PUFA downstream of 18:1n-9 is at least 40% (w/w) of the total seed fatty acid content at an oil content of 40% (w/w), or preferably the content of **EPA is at least 6%, preferably at least 7,5% (w/w) and/or the**

content of DHA is at least 0,8% (w/w), prerferably 1.2% (w/w) of the total seed fatty acid content at an oil content of 40% (w/w).

10. The method of claim 9 o the plant of claim 8, wherein the T-DNA is homozygous

11. Method of plant oil production, comprising the steps of
    i) growing a plant according to any of claims 3 to 8 such as to obtain oil-containing seeds thereof,
    ii) harvesting said seeds, and
    iii) extracting oil from said seeds harvested in step ii), wherein the oil has a DHA content of at least 1% by weight based on the total lipid content and/or a EPA content of at least 8% by weight based on the total lipid content.

12. Plant oil comprising a polyunsaturated fatty acid obtainable or obtained by the method of claim 11.

13. Method for analysing desaturase reaction specificity, comprising the steps of
    i) providing, to a desaturase, a detectably labelled molecule comprising a fatty acid moiety and a headgroup,
    ii) allowing the desaturase to react on the labelled molecule, and
    iii) detecting desaturation products.

14. Method for analysing elongase reaction specificity, comprising the steps of
    i) providing, to an elongase, a detectably labelled elongation substrate and a molecule to be elongated,
    ii) allowing the elongase to elongate the molecule to be elongated using the labelled elongation substrate, and
    iii) detecting elongation products.

15. Method for optimization of a metabolic pathway, comprising the steps of
    i) providing enzymes of a metabolic pathway and one or more substrates to be used by the first enzyme or enzymes of the pathway,
    ii) reacting the enzymes and the substrates to produce products, which in turn are also exposed as potential substrates to the enzymes of the pathway, and

iii) determining the accumulation of products.

16. Method for determining CoA-dependence of a target desaturase, comprising the steps of

i) providing an elongase to produce a substrate for the target desaturase, and determining conversion efficiency of the target desaturase, and

ii) providing a non-CoA dependent desaturase to produce the substrate for the target desaturase, and determining conversion efficiency of the target desaturase, and

iii) comparing the target desaturase conversion efficiencies of step i) and ii).

17. Method for determining CoA-dependence of a target desaturase, comprising the steps of

i) providing an elongase to elongate the products of the target desaturase, and determining conversion efficiency of the elongase,

ii) providing the elongase to elongate the products of a comparison desaturase known to be non-CoA dependent, and determining conversion efficiency of the elongase,

iii) comparing the elongase conversion efficiencies of step i) and ii).

Abstract

MATERIALS AND METHODS FOR PUFA PRODUCTION, AND PUFA-CONTAINING COMPOSITIONS

T-DNA for expression of a target gene in a plant, wherein the T-DNA comprises a left and a right border element and an expression cassette comprising a promotor, operatively linked thereto a target gene or a combination of target genes, and downstream thereof a terminator, wherein the length of the T-DNA, measured from left to right border element and comprising the target gene, has a length of at least 30000 bp, and the expression cassette is separated from the closest border of the T-DNA by a separator of at least 500 bp length.

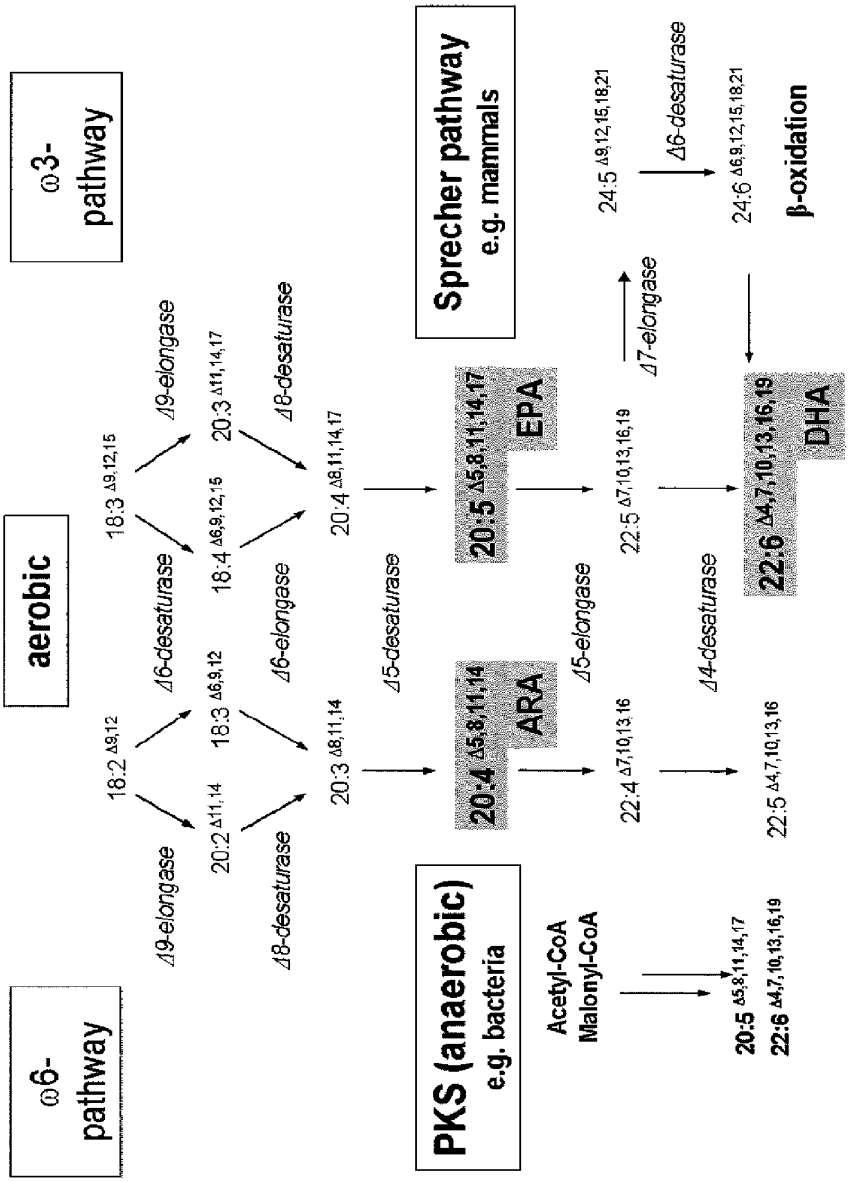


Figure 1: Schematical figure of the different enzymatic activities leading to the production of ARA, EPA and DHA

Formula used for pathway step conversion efficiency Ceff $C_{eff} = \frac{100}{S+P} \times P$

Pathway Step	Product Stream	pre-requisite of product stream										
d12Des	d12Des Product Stream 1	18:1n-9	18:1n-9	18:2n-6	18:3n-6	20:3n-6	20:4n-6	22:4n-6	22:5n-6			
	d12Des Product Stream 2	18:3n-3 from 18:2n-6 via d15Des		18:3n-3	18:4n-3	20:4n-3	20:5n-3	22:5n-3	22:6n-3			
	d12Des Product Stream 3	20:2n-6 from 18:2n-5 via d6Elo		20:2n-6								
	d12Des Product Stream 4	20:3n-3 from 18:3n-3 via d6Elo		20:3n-3								
d6Des	d12Des Product Stream 1	18:2n-6		18:2n-6	18:3n-6	20:3n-6	20:4n-6	22:4n-6	22:5n-6			
	d12Des Product Stream 2	18:3n-3 produced by o3Des		18:3n-3	18:4n-3	20:4n-3	20:5n-3	22:5n-3	22:6n-3			
d6Elo	d6Elo Product Stream 1	18:3n-6 produced by d6Des			18:3n-6	20:3n-6	20:4n-6	22:4n-6	22:5n-6			
	d6Elo Product Stream 2	18:4n-3 produced by d6Des or o3Des			18:4n-3	20:4n-3	20:5n-3	22:5n-3	22:6n-3			
d5Des	d5Des Product Stream 1	20:3n-6 produced by d6Elo				20:3n-6	20:4n-6	22:4n-6	22:5n-6			
	d5Des Product Stream 2	20:4n-3 produced by d6Elo or o3Des				20:4n-3	20:5n-3	22:5n-3	22:6n-3			
d5Elo	d5Elo Product Stream 1	20:4n-6 produced by d5Des					20:4n-6	22:4n-6	22:5n-6			
	d5Elo Product Stream 2	20:4n-3 produced by d5Des or o3Des					20:5n-3	22:5n-3	22:6n-3			
d4Des	d4Des Product Stream 1	20:4n-6 produced by d5Des						22:4n-6	22:5n-6			
	d4Des Product Stream 2	20:4n-3 produced by d5Des or o3Des						22:5n-3	22:6n-3			
o3Des	List of all o6Des Fatty acid substrates		18:2n-6	18:3n-6	20:2n-6	20:3n-6	20:4n-6	22:4n-6	22:5n-6			
	List of all o3Des Fatty acid products		18:3n-3	18:4n-3	20:3n-3	20:4n-3	20:5n-3	22:5n-3	22:6n-3			

Figure 2: Formulas to calculate pathway step conversion efficiencies. S: substrate of pathway step. P: product of pathway step. Product was always the sum of the immediate product of the conversion at this pathway step, and all downstream products that passed this pathway step in order to be formed. E.g. DHA (22:6n-3 does possess a double bond that was a result of the delta-12-desaturation of oleic acid (18:1n-9) to linoleic acid (18:2n-6).

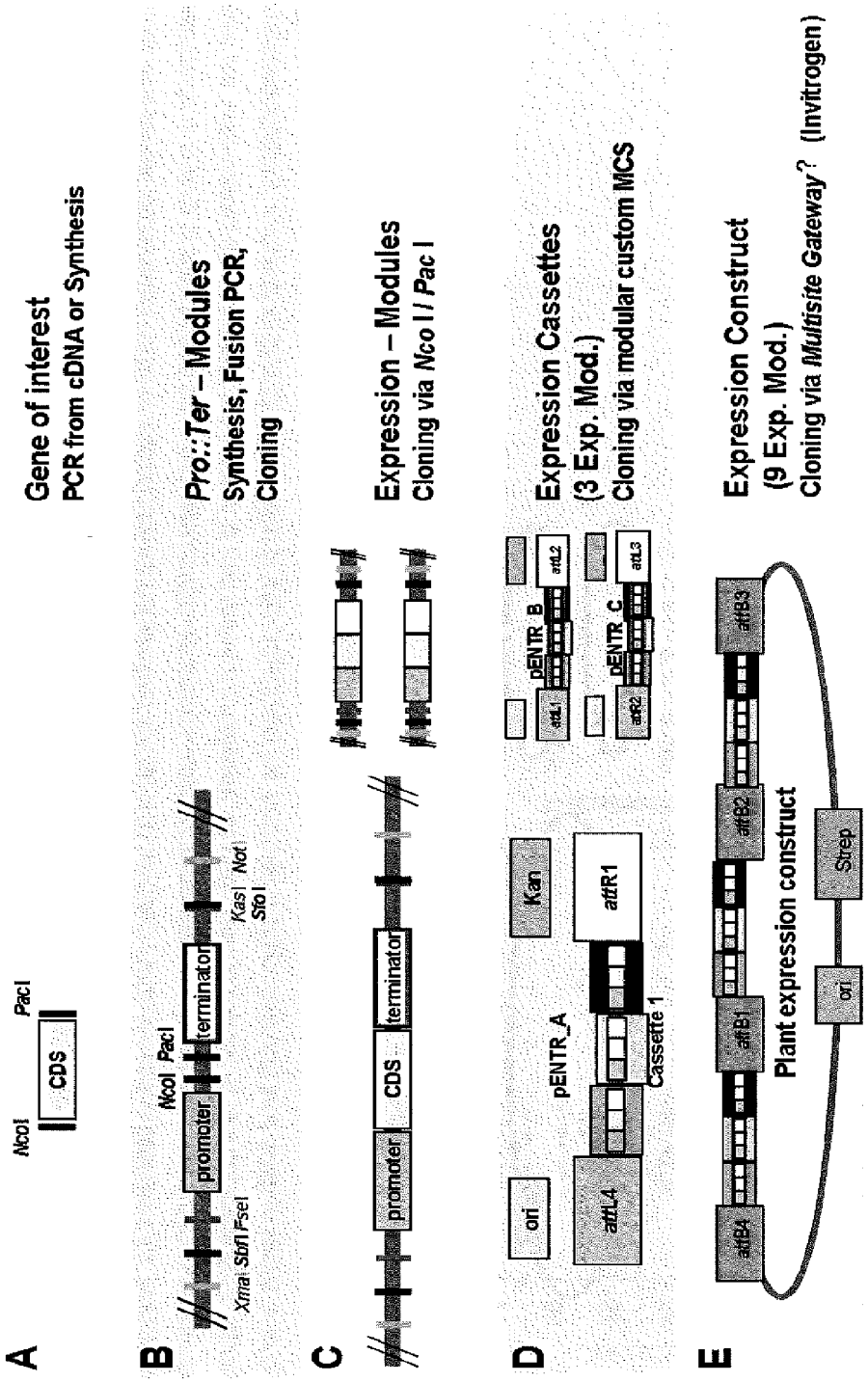


Figure 3: Strategy employed for stepwise buildup of plant expression plasmids of the invention

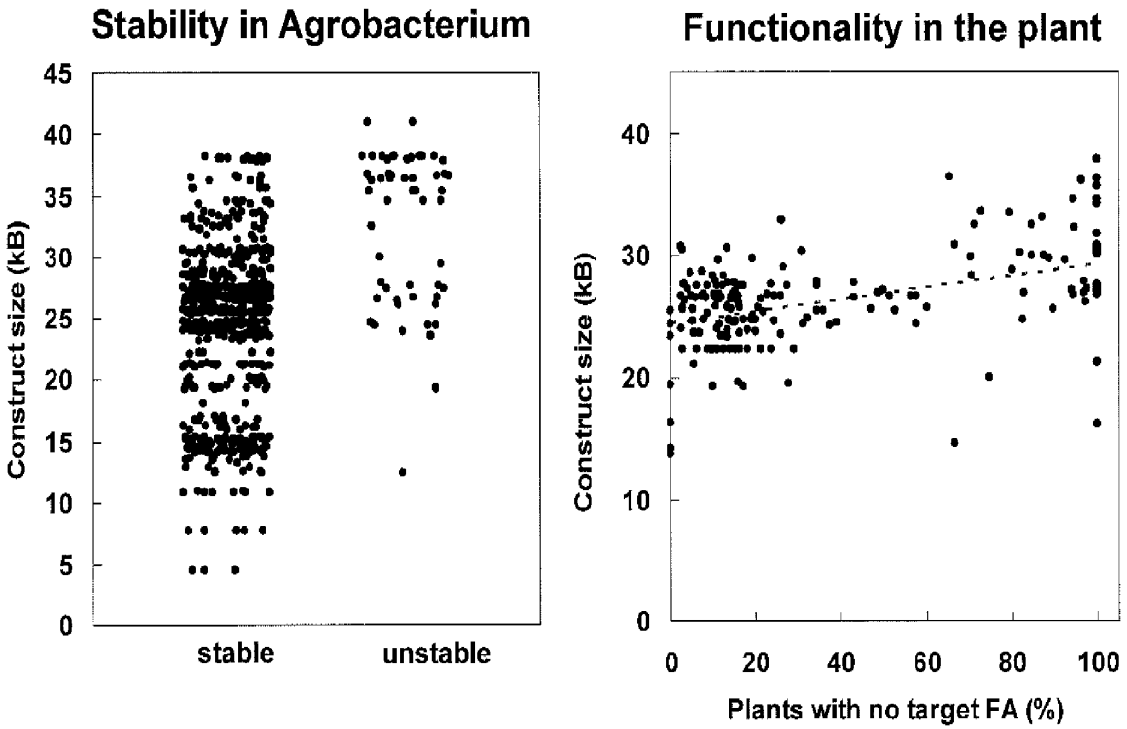


Figure 4: Stabiliy of binary plant expression plasmids containing the ColE1/pVS1 origin of replication for plasmid recplication in *E.coli*/Agrobacteria.Left Panel: Stability in *Agrobacterium* cells by isolating plasmid DNA from *Agrobacterium* cutures prior to usage of this culture for plant transformation, and subjecting the plasmid DNA to a restriction digest. An unexpected restriction pattern indicates disintegration/instability of the plasmid either in *E.coli* or in *Agrobacterium*. Right panel: Under the assumption at least one intact T-DNA from LB to RB was integrated into the plant genome during the transformation process most plants obtained via transformation of a given plasmid are expected to reach the desired trait encoded by the plasmid (here: production of novel fatty acids (FA) in the seeds). The decrease in the percentage of such 'functional' plants indicates instability either in Agrobacteria or during the transfer process into the plant or during the integration process into the genome. As can be seen, the proportion of non functional plants goes sharply up for plasmids above 25,000bp size when ColE1/pVS1 containing plasmids are used.

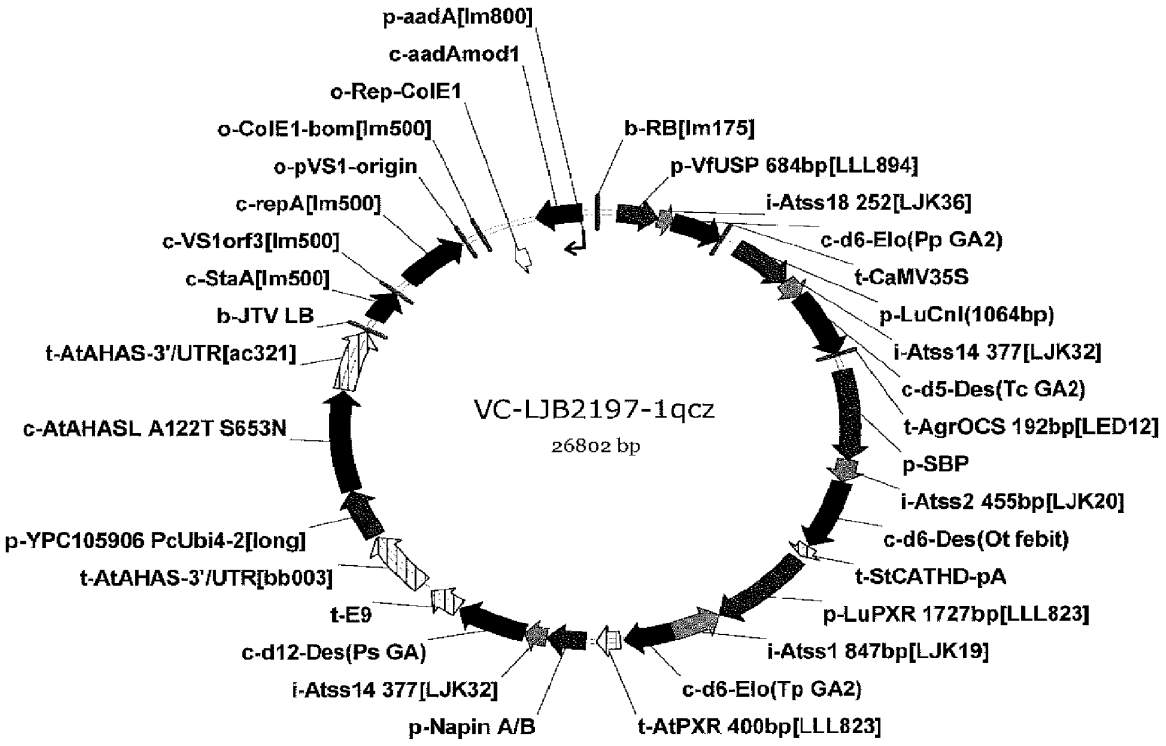


Figure 5: Plasmid map of VC-LJB2197-1qcz indicating the position of genetic elements listed in table 1

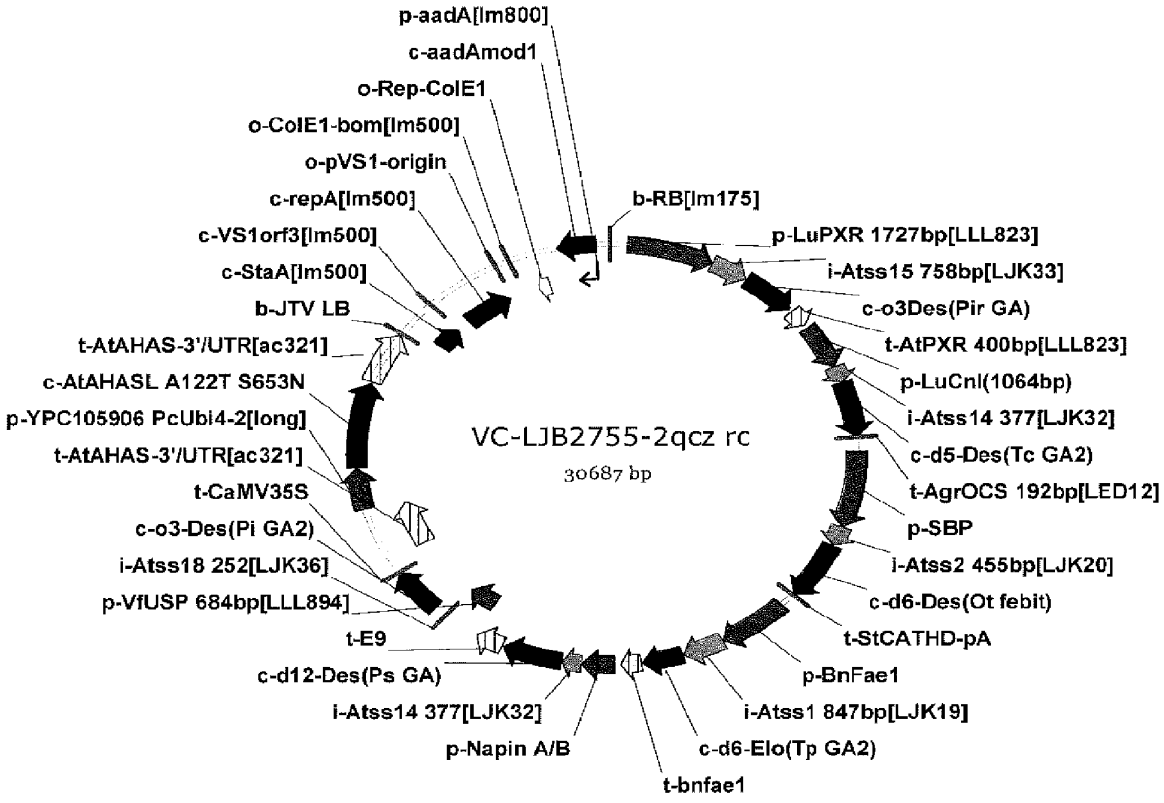


Figure 6: Plasmid map of VC-LJB2755-2qcz rc indicating the position of genetic elements listed in table 2

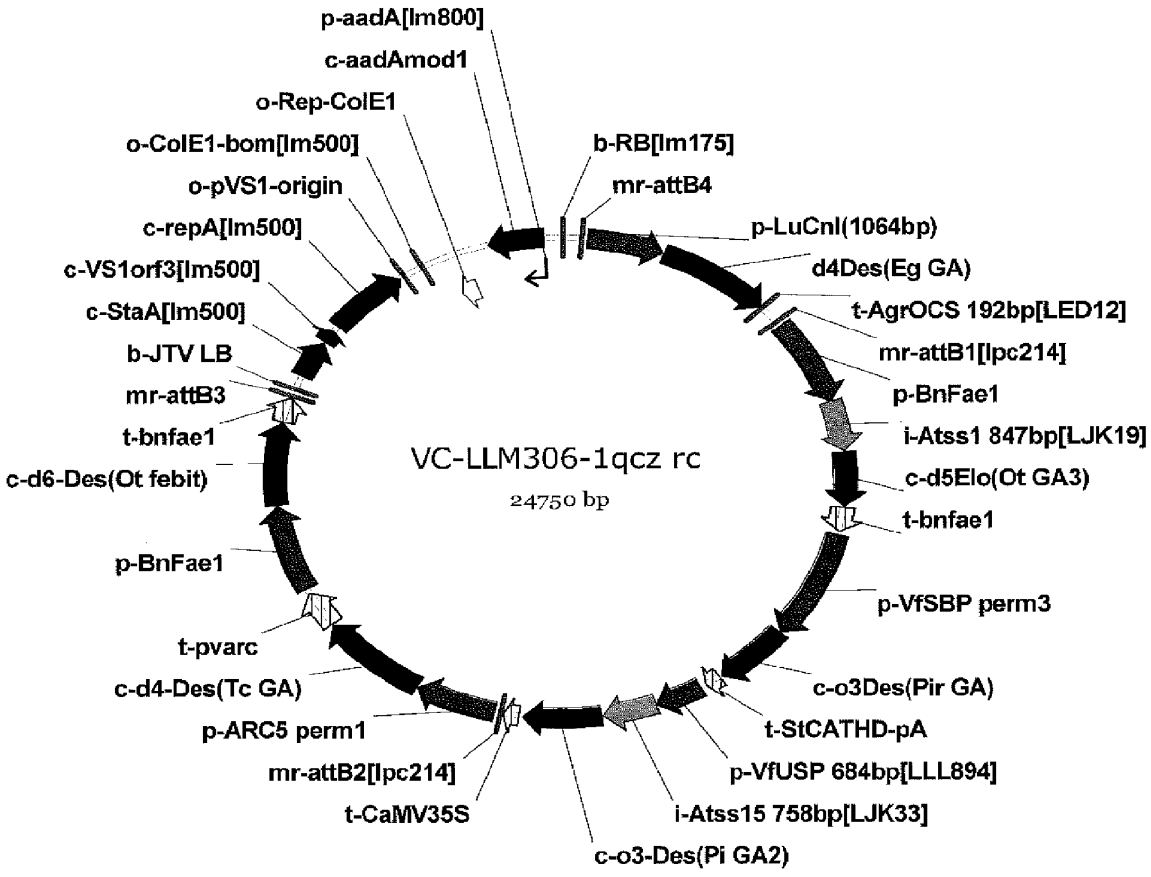


Figure 7: Plasmid map of VC-LLM306-1qcz rc indicating the position of genetic elements listed in table 3

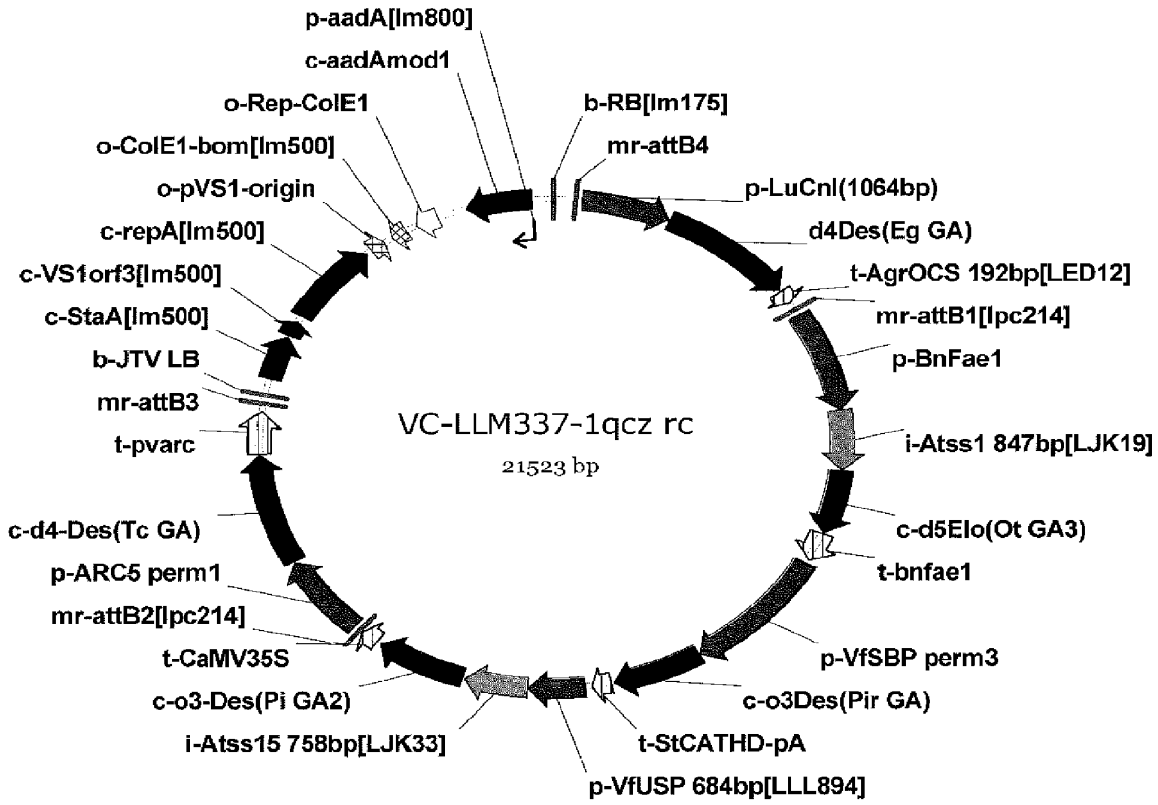


Figure 8: Plasmid map of VC-LLM337-1qcz rc indicating the position of genetic elements listed in table 4

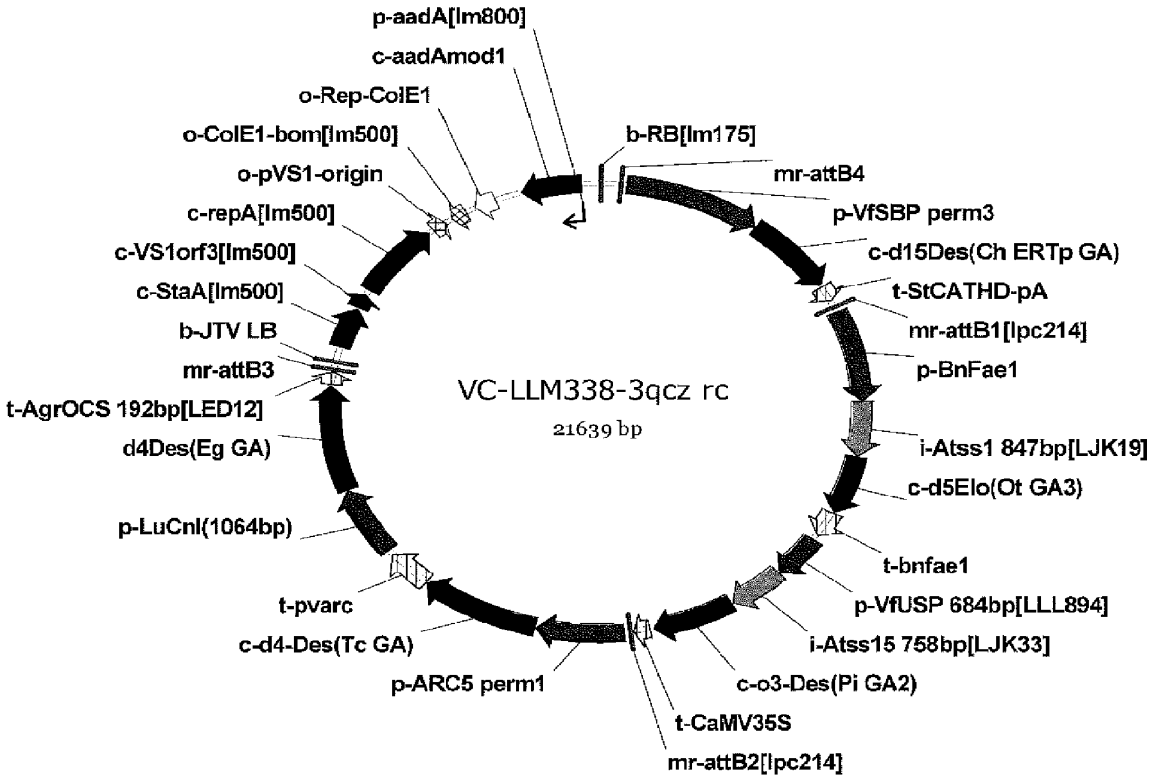


Figure 9: Plasmid map of VC-LLM338-3qcz rc indicating the position of genetic elements listed in table 5

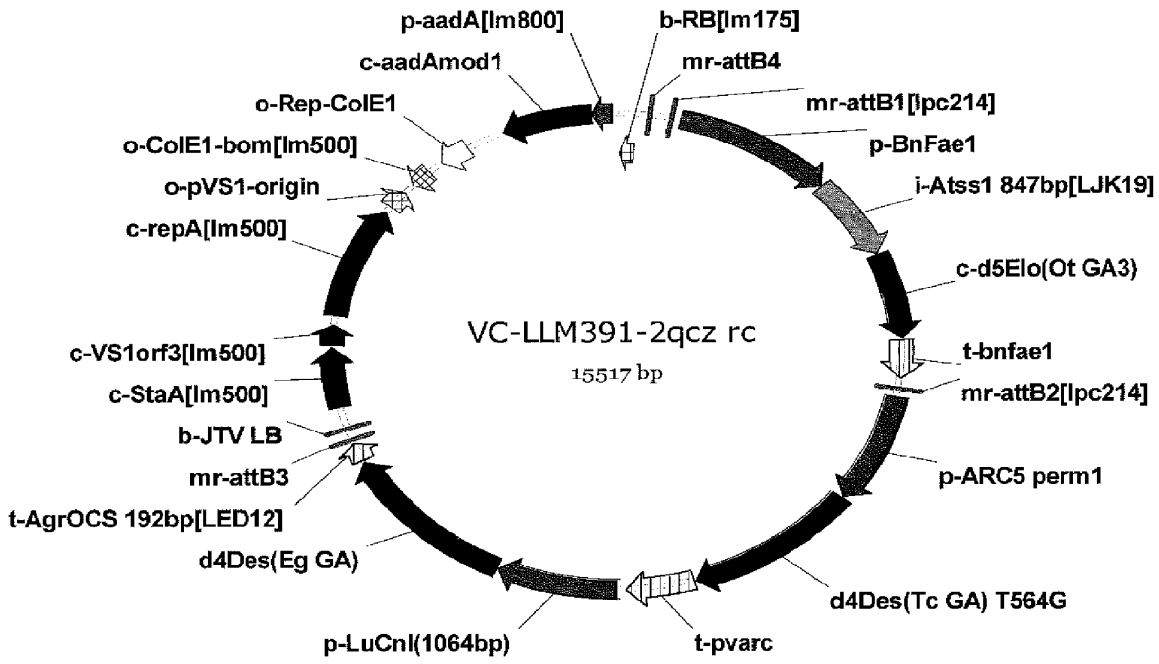


Figure 10: Plasmid map of VC-LLM391-2qcz rc indicating the position of genetic elements listed in table 6

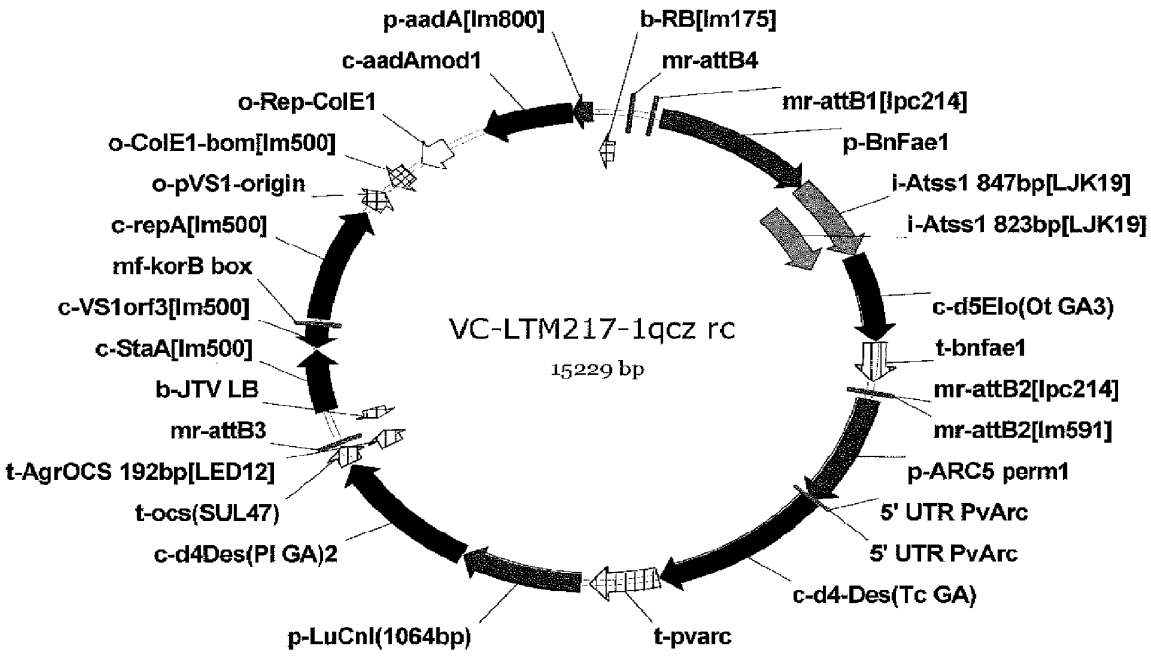


Figure 11: Plasmid map of VC-LTM217-1qcz rc indicating the position of genetic elements listed in table 7

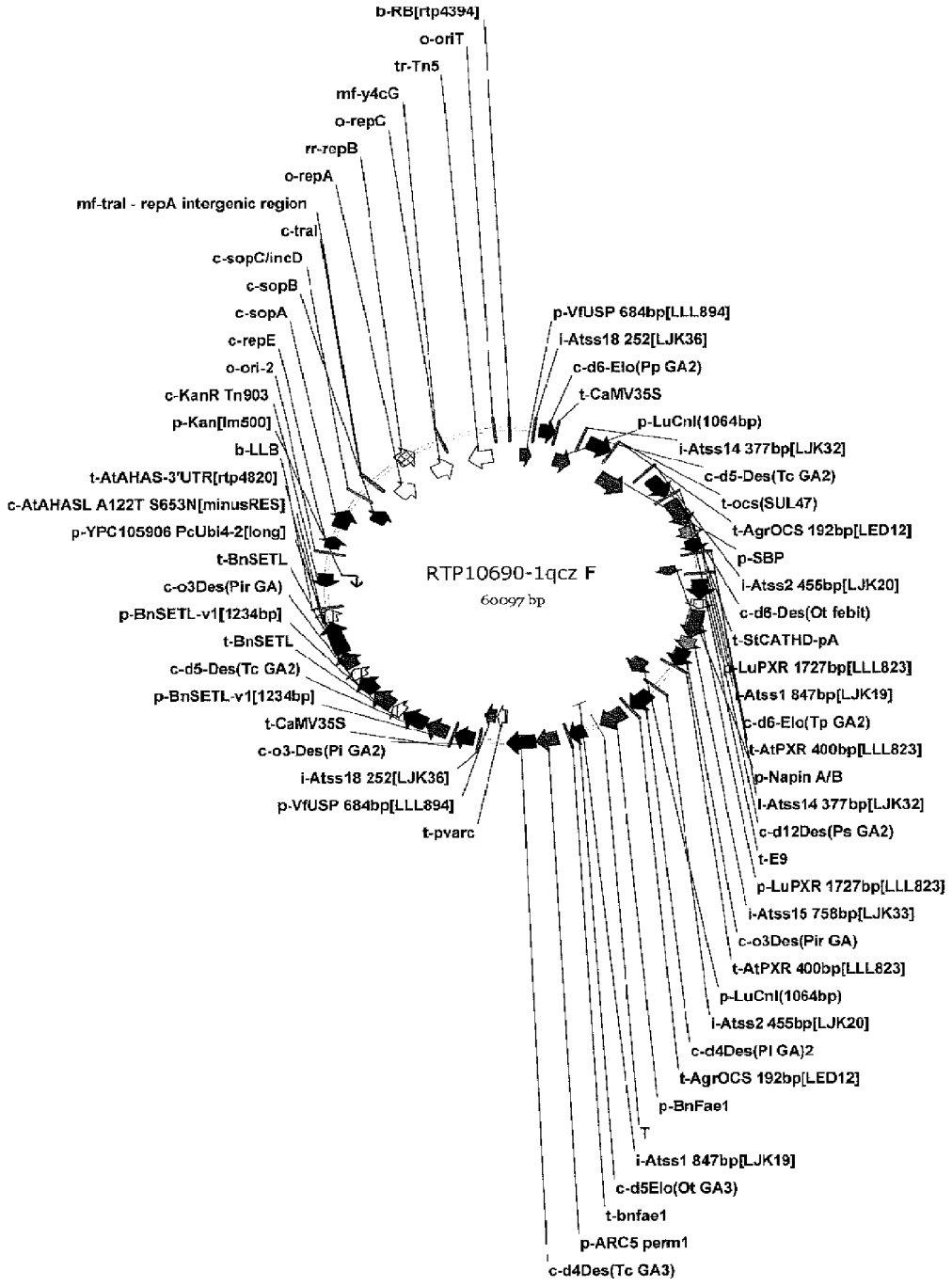


Figure 12: Plasmid map of RTP10690-1qcz_F indicating the position of genetic elements listed in table 8

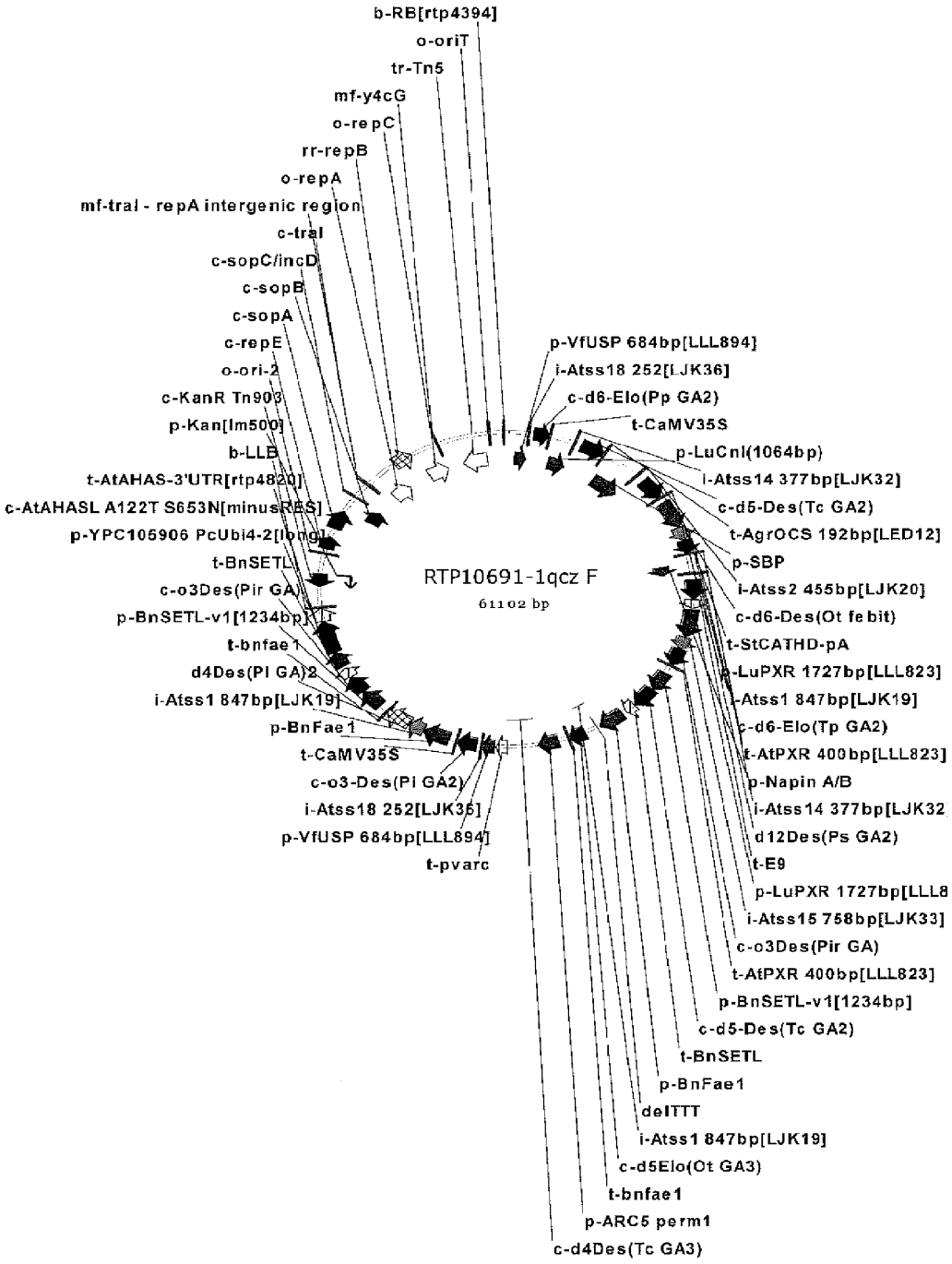


Figure 13: Plasmid map of RTP10691-2qcz indicating the position of genetic elements listed in table 9

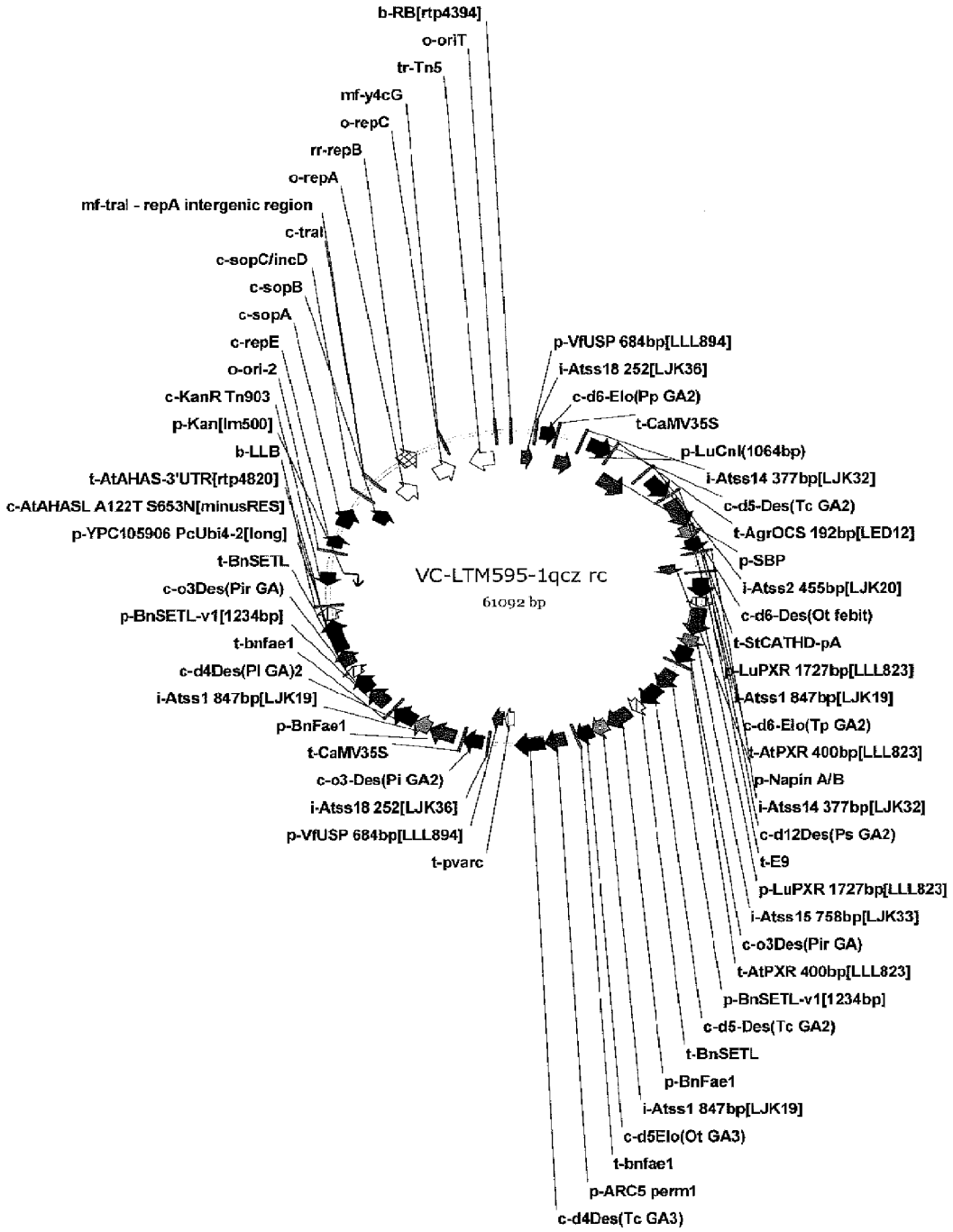


Figure 14: Plasmid map of LTM595-1qcz rc indicating the position of genetic elements listed in table 10

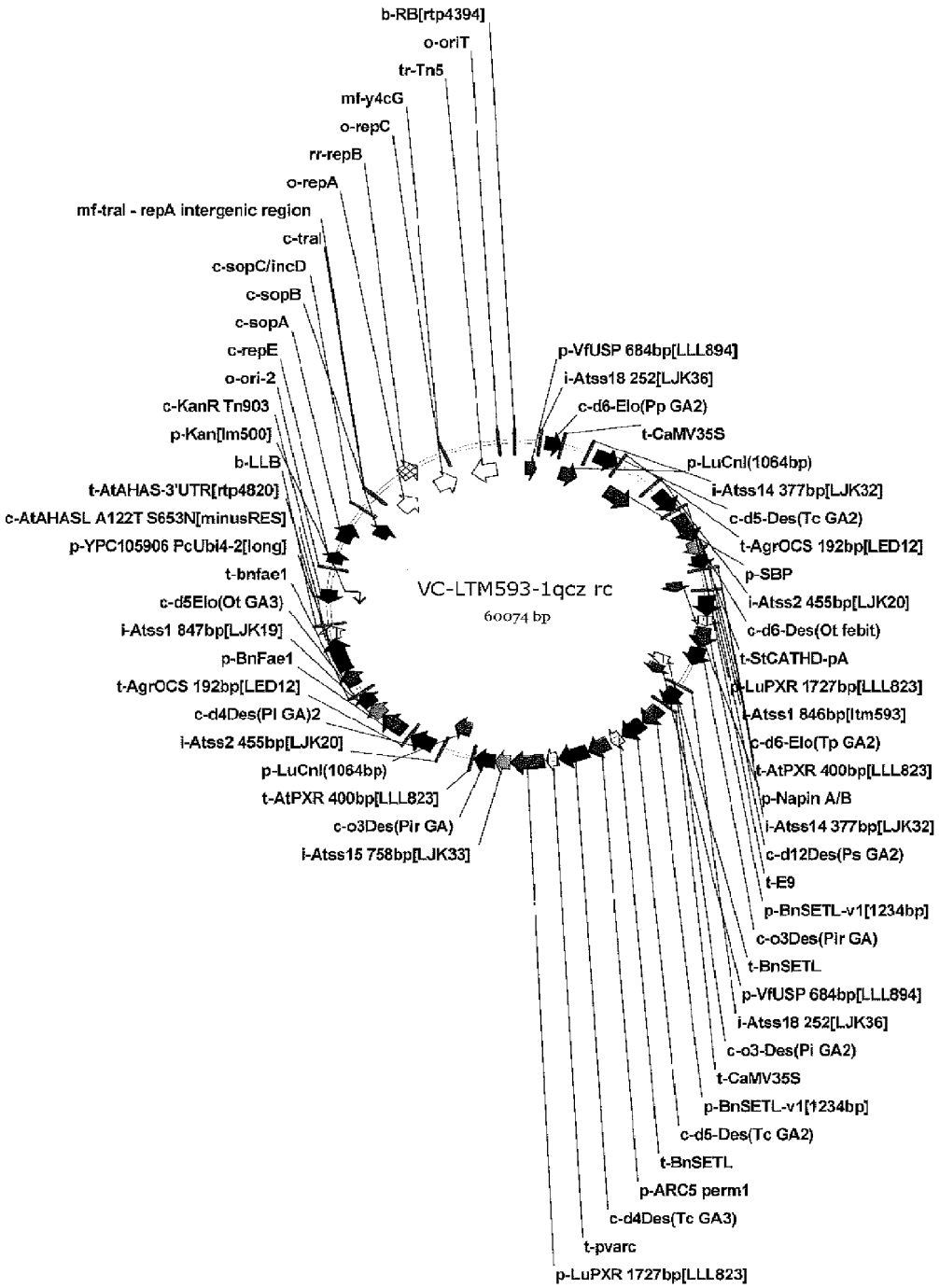


Figure 15: Plasmid map of LTM593-1qcz rc indicating the position of genetic elements listed in table 11

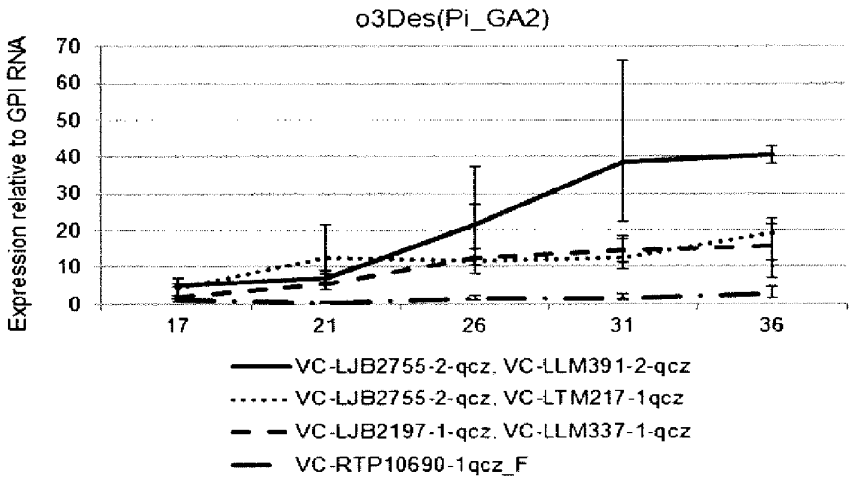


Figure 16: Comparative transcript analysis o3Des(Pi_GA2) driven by the VfUSP promoter during seed development of single copy event of four different construct combinations.

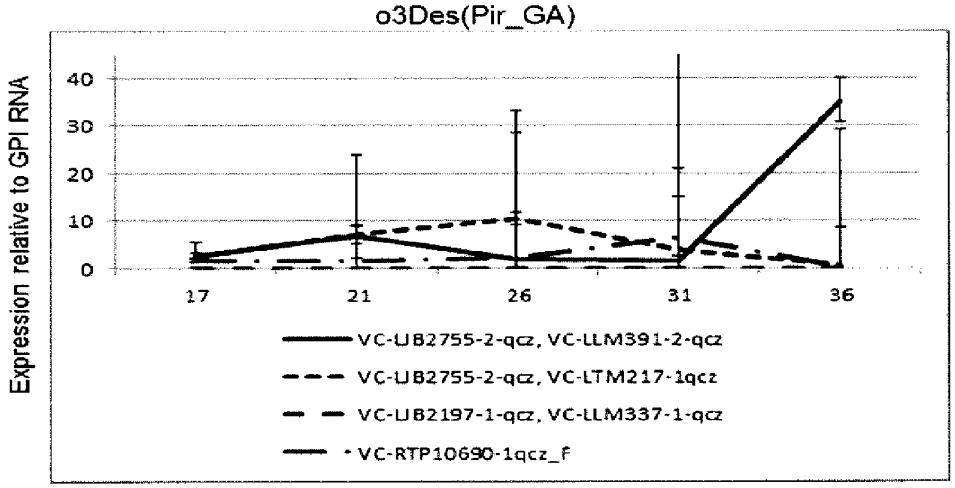


Figure 17: Comparative transcript analysis of o3Des(Pir_GA) during seed development of single copy event of four different construct combinations. In VC-LJB2755-2qcz and VC-RTP10690-1qcz_F the gene was driven by the LuConlinin promoter while in VC-LLM337-1qcz rc the gene was driven by the VfUSP promoter and was expressed at a lower level than the LuConlinin o3Des(Pir_GA) combination.

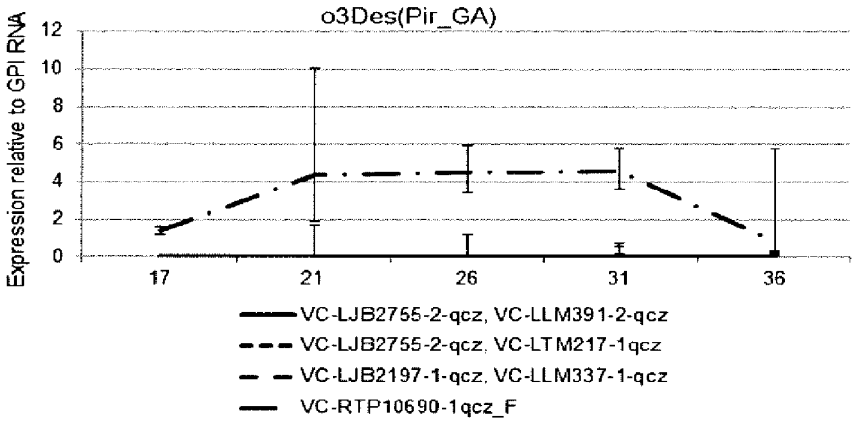


Figure 18: Comparative transcript analysis of o3Des(Pir_GA) driven by the BnSETL promoter during seed development of single copy event of VC-RTP10690-1qcz_F.

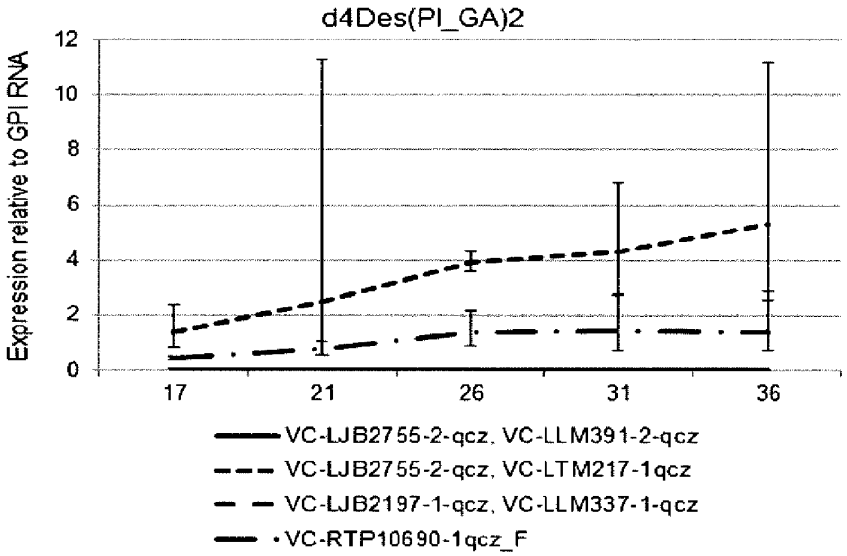


Figure 19: Comparative transcript analysis of d4Des(Pl_GA)2 driven by the LuConlinin promoter during seed development of single copy event from VC-RTP10690-1qcz_F and VC-LTM217-1qcz rc, which was present with VC-LJB2755-1qcz. The other constructs lacked this particular d4Des.

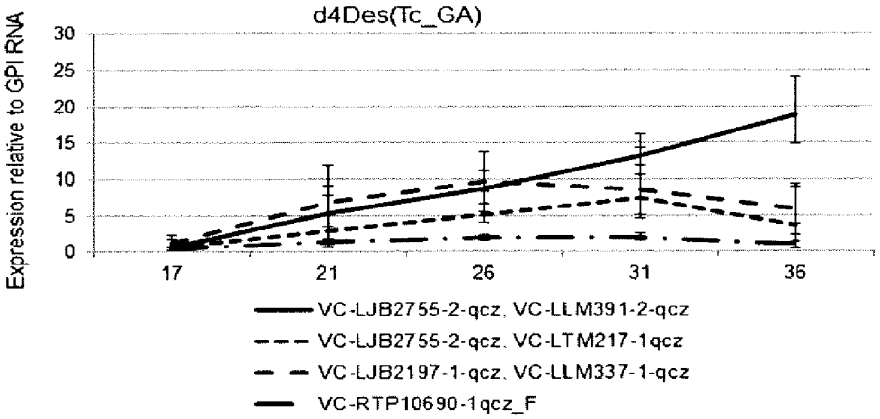


Figure 20: Comparative transcript analysis of d4Des(Tc_GA) driven by the ARC5 promoter during seed development of single copy event of four different construct combinations.

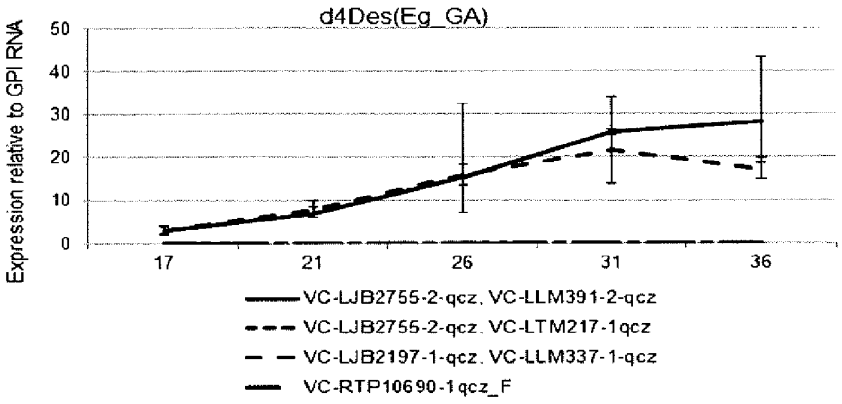


Figure 21: Comparative transcript analysis of d4Des(Eg_GA) driven by the LuConlinin promoter during seed development of single copy event of two different construct combinations; VC-LJB2755-2qcz, VC-LLM391-2qcz rc and VC-LJB2197-1qcz, VC-LLM337-1qcz rc.

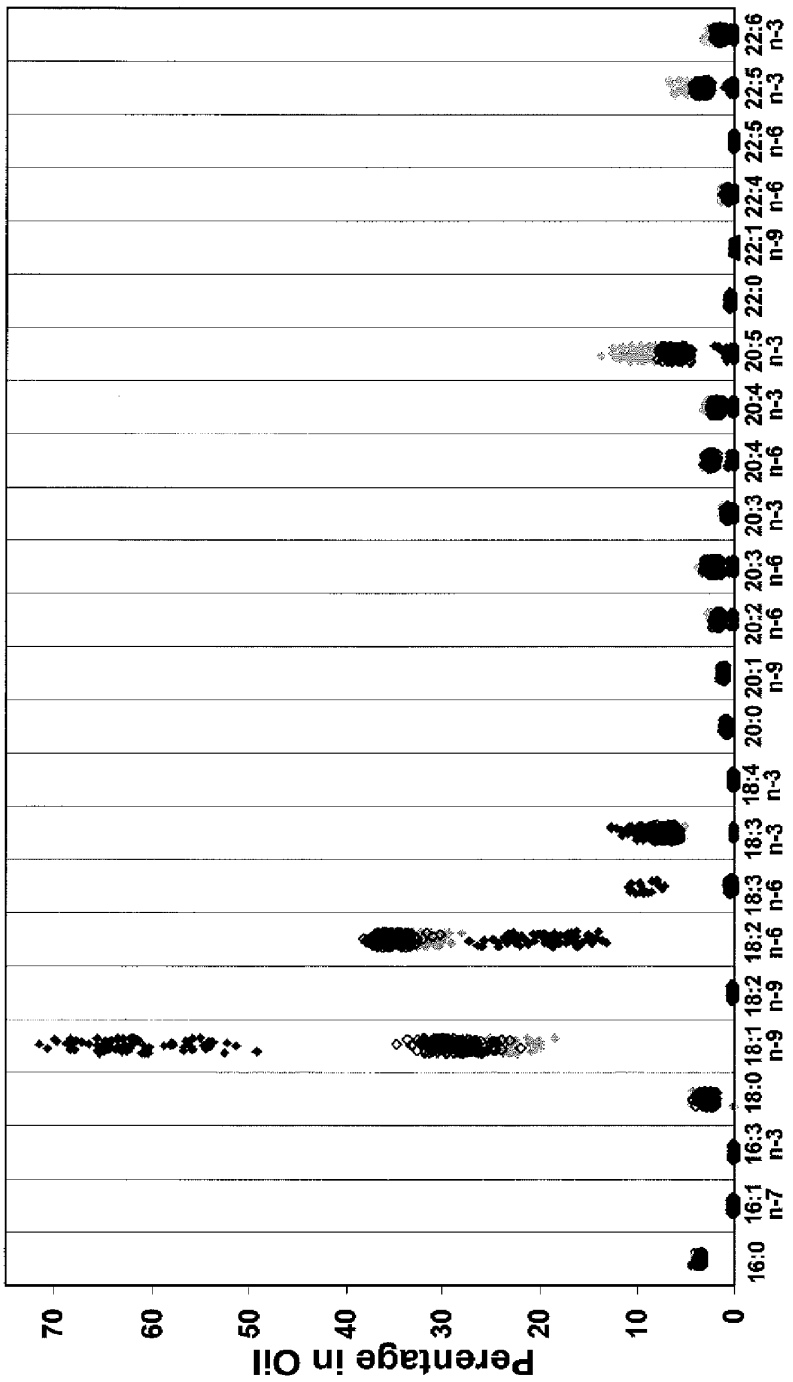


Figure 22: Half Kernel Analysis of segregating T1 seeds of Event LANPMZ. A total of 288 seedlings where analysed. 71 of those seedlings were found to produce no significant amount of VLC-PUFA (dark grey diamonds) while containing >49% Oleic acid and <28% Linoleic acid. 71 seed of 288 seed correspond to 24.65% of the total analysed seed. All remaining seed were capable of producing DHA, indicating the presence of both T-DNA from construct VC-LJB2197-1qcz and VC-LLM337-1qcz rc. Among those seeds producing DHA, one can discriminate a group of 146 seeds showing medium VLC-PUFA levels (open diamonds), and a group of 71 seed showing high VLC-PUFA levels (light grey diamonds). The ratios of these three groups is 71:146:71, which corresponds to the Medelain 1:2:1 ratio (NULL:HETEROZYGOUS:HOMOZYGOUS) expected for a phenotype when all gene conveying this phenotype (in this case the two T-DNAs of plasmid VC-LJB2197-1qcz and VC-LLM337-1qcz rc) integrated into one locus in the genome.

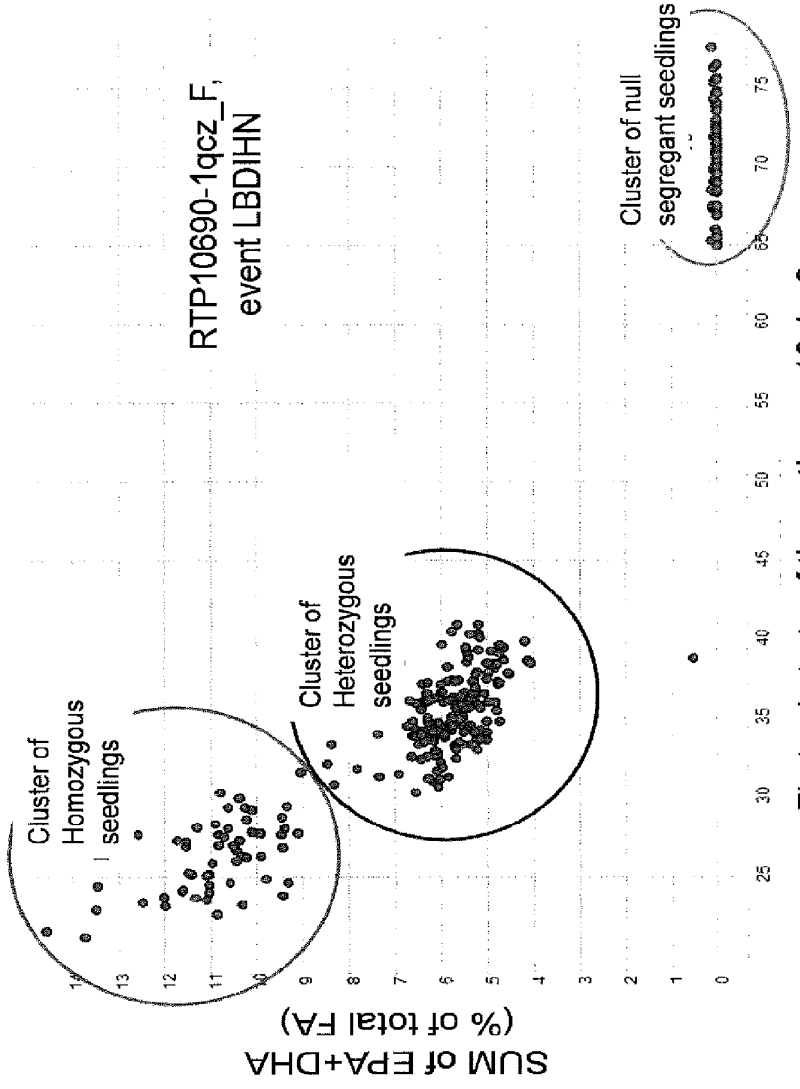


Figure 23: Half Kernel Analysis of segregating T1 seeds of Event LBDIHN. A total of 288 seedlings where analysed. The levels of first substrate fatty acid of the pathway was plotted on the x-axis, the levels of the sum of two products of the pathways (EPA+DHA) was plotted on the y-axis. One can clearly see three clusters, where the ratio of the number of seeds in the these three clusters was 1:2:1 (Homozygous:Heterozygous:Null segregant). This segregation of the phenotype according to the first Mendelian law demonstrates a single locus insertion of the T-DNA of construct RTP10690-1qcz_F into the genome of *B.napus cv Kumily*.

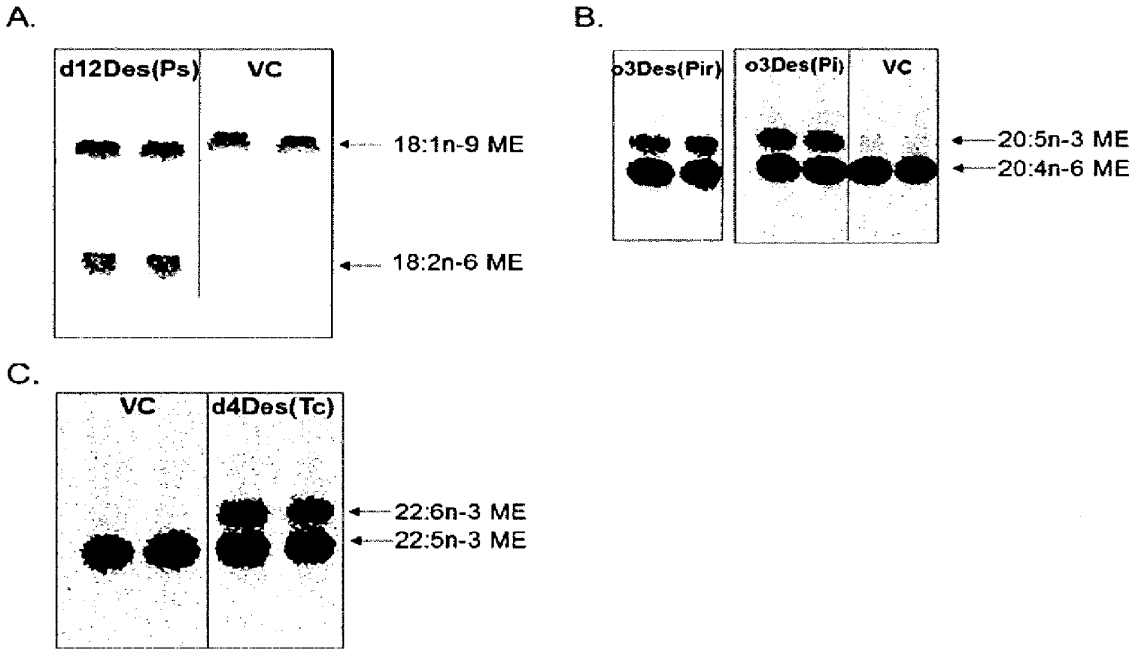


Figure 24: Examples of Desaturase Enzyme Activity Heterologously Expressed in Yeast. [^{14}C]Fatty acid methyl esters (ME's) were isolated from the enzymatic reactions, resolved by TLC as described for each specific enzyme and detected by electronic autoradiography using Instant Imager. In panel A Delta-12 Desaturase (Ps), c-d12Des(Ps_GA), activity was demonstrated by comparison of enzyme activity present in yeast microsomes isolated from a strain expressing the c-d12Des(Ps_GA) protein relative to microsomes isolated from a control strain containing an empty vector (VC). In panel B Omega-3 Desaturase activities, c-o3Des(Pir_GA) and c-o3Des(Pi_GA2), activities were demonstrated by comparison of enzyme activity from yeast microsomes isolated from strains expressing c-o3Des(Pir_GA) protein, c-o3Des(Pi_GA2) protein or an empty vector (VC) control. In panel C Delta-4 Desaturase (Tc), c-d4Des(Tc_GA), activity was demonstrated by comparison of enzyme activity from yeast microsomes isolated from a strain expressing the c-d4Des(Tc_GA) protein relative to microsomes isolated from a control strain containing an empty vector (VC).

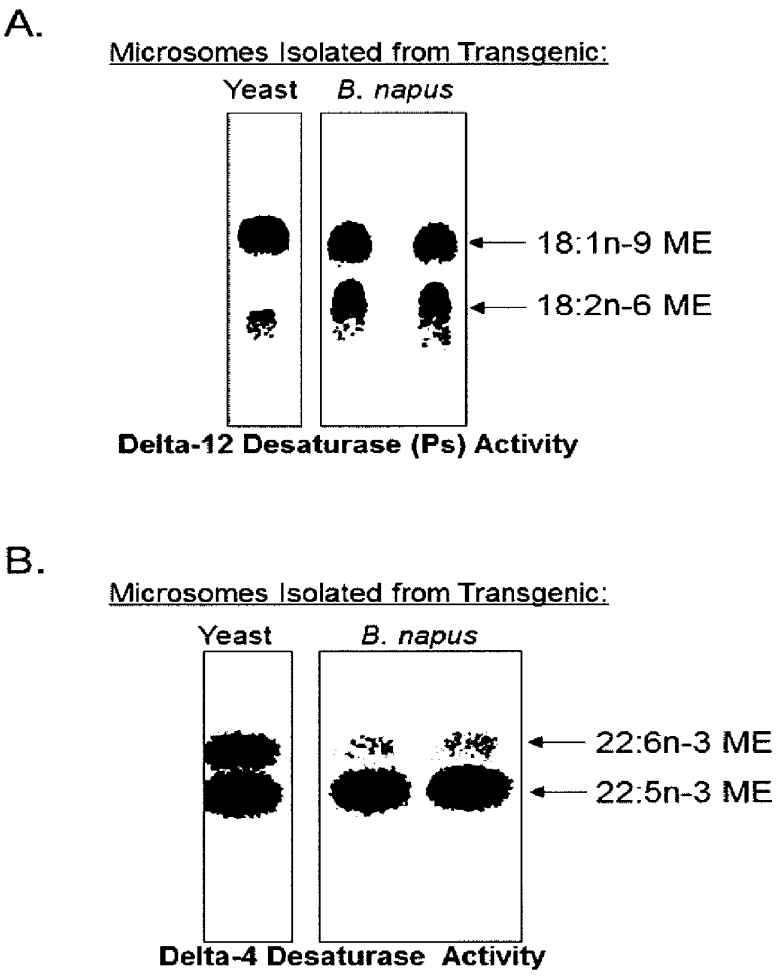
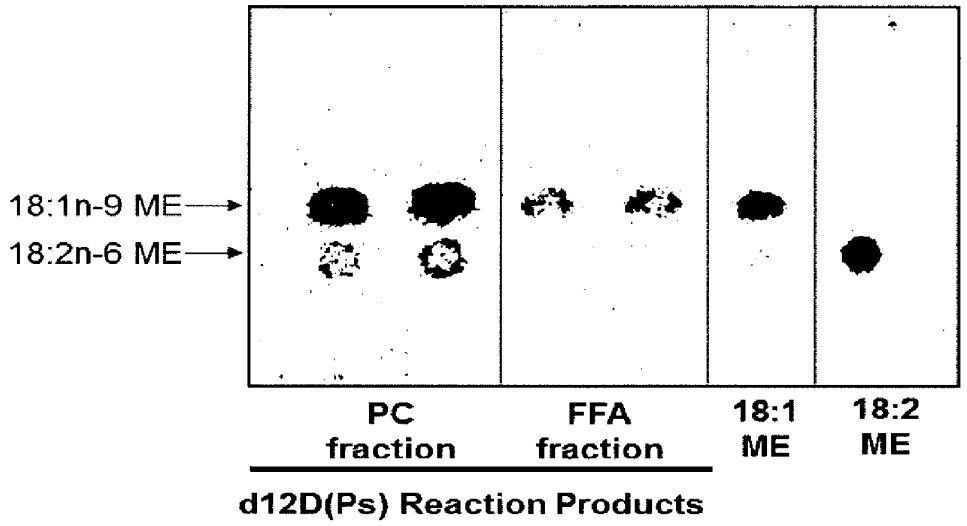
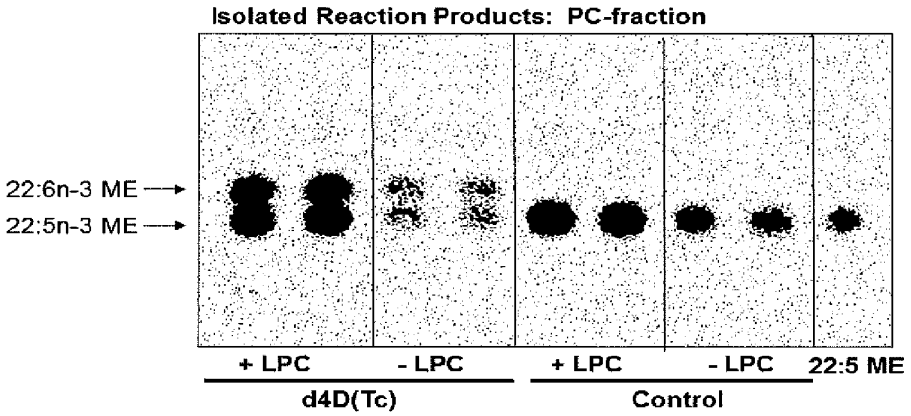


Figure 25: Examples of Desaturase Enzyme Activity in transgenic *Brassica napus*. [^{14}C]Fatty acid methyl esters (ME's) were isolated from the enzymatic reactions, resolved by TLC as described for each specific enzyme and detected by electronic autoradiography using Instant Imager. In panel A Delta-12 Desaturase (Ps), c-d12Des(Ps_GA), activity was demonstrated by comparison of enzyme activity from yeast microsomes isolated from a strain expressing the c-d12Des(Ps_GA) protein relative to microsomes isolated from transgenic *B. napus* containing the *d12Des(Ps_GA2)* gene. In panel B Delta-4 Desaturase (Tc), c-d4Des(Tc_GA), and Delta-4 Desaturase (Pl) activities were demonstrated by comparison of enzyme activity from yeast microsomes isolated from a strain expressing the c-d4Des(Tc_GA) protein relative to microsomes isolated from transgenic *B. napus* containing the *d4Des(Tc_GA3)* and *d4Des(Pl_GA)2* genes.

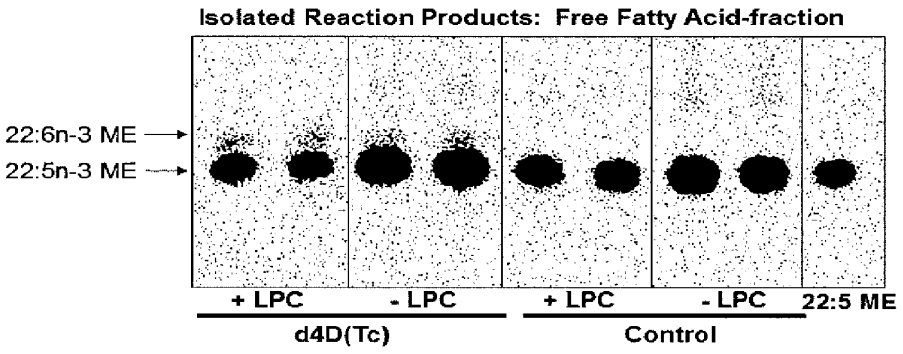
A.


18:1n-9 ME
18:2n-6 ME
PC fraction
FFA fraction
18:1 ME
18:2 ME
d12D(Ps) Reaction Products

B.


Isolated Reaction Products: PC-fraction
22:6n-3 ME
22:5n-3 ME
+ LPC
- LPC
+ LPC
- LPC
22:5 ME
d4D(Tc)
Control

C.


Isolated Reaction Products: Free Fatty Acid-fraction
22:6n-3 ME
22:5n-3 ME
+ LPC
- LPC
+ LPC
- LPC
22:5 ME
d4D(Tc)
Control

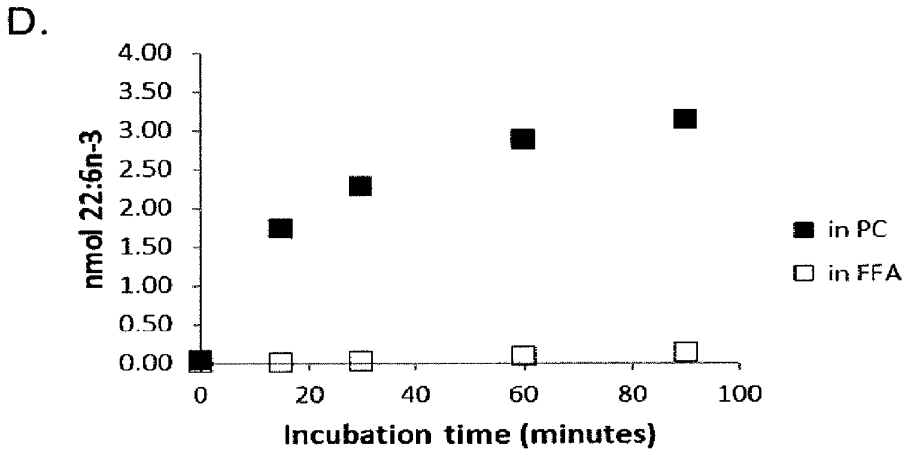
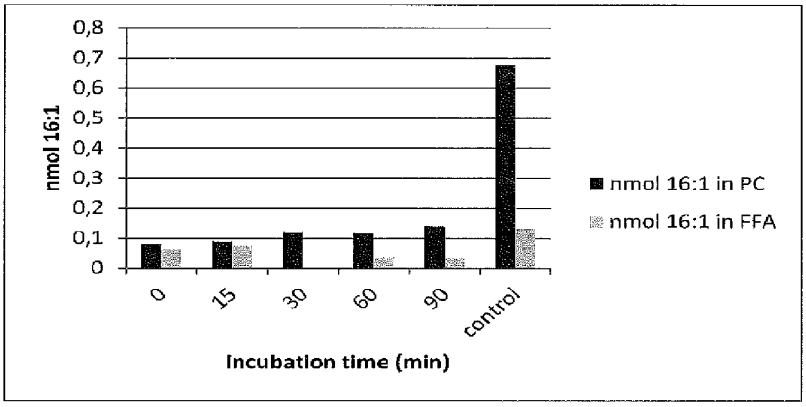
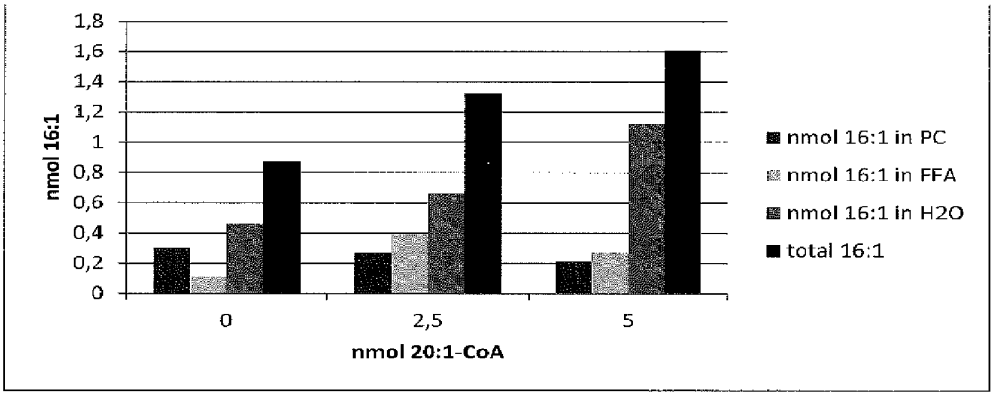


Figure 26: Examples of Desaturase Enzyme Reactions Showing Specificity for Acyl-lipid substrates. [^{14}C]Fatty acid methyl esters (ME's) were isolated from the enzymatic reactions containing microsomes obtained from a yeast strain expressing the protein of interest, resolved by TLC as described for each specific enzyme and detected by electronic autoradiography using Instant Imager. In panel A Delta-12 Desaturase (Ps), c-d12Des(Ps_GA), desaturated enzyme products were only detected in the phosphatidylcholine fraction indicating the enzyme was specific for an acyl-lipid substrate. In panel B and panel C Delta-4 Desaturase (Tc), c-d4Des(Tc_GA), desaturated enzyme products were detected in the phosphatidylcholine fraction indicating the enzyme was specific for an acyl-lipid substrate. In panel D was a time-course demonstrates of the Delta-4 Desaturase (Tc), c-d4Des(Tc_GA).

A.


0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
nmol 16:1
0
15
30
60
90
control
Incubation time (min)
nmol 16:1 in PC
nmol 16:1 in FFA

B.


1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
nmol 16:1
0
2,5
5
nmol 20:1-CoA
nmol 16:1 in PC
nmol 16:1 in FFA
nmol 16:1 in H2O
total 16:1

C.

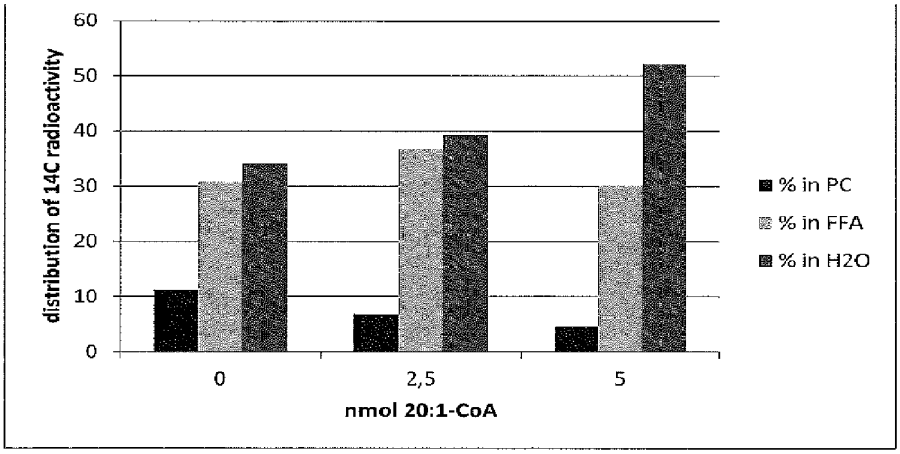

60
50
40
30
20
10
0
distribution of 14C radioactivity
0
2,5
5
nmol 20:1-CoA
% in PC
% in FFA
% in H2O

Figure 27: Examples of Desaturase Enzyme Reactions Showing Specificity for Acyl-CoA substrates. [^{14}C]Fatty acid methyl esters (ME's) were isolated from the enzymatic reactions containing microsomes obtained from a yeast strain expressing the protein of interest, resolved by TLC as described for each specific enzyme and detected by electronic autoradiography using Instant Imager. In panel A PC was in situ labeled with substrate according to the method for determining lipid linked desaturation. Delta-9 Desaturase (Sc), d9D(Sc), desaturated enzyme products were very low in the phosphatidylcholine fraction, except for in the control reaction (none in situ labeled PC), indicating the enzyme cannot desaturate an acyl-lipid substrate. In panel B and C the incubation was done according to the method for determining acyl-CoA linked desaturation. In panel B the amount of radioactivity in the acyl-CoA fraction (MeOH/H2O-phase) was increasing when 20:1-CoA was added to the assay. This indicates that the added 20:1-CoA was competing with the radioactive substrate in formation of PC and free fatty acids. In panel C the amount of desaturated enzyme products was increased in the acyl-CoA fraction when 20:1-CoA was added to the assay, indicating that the desaturation was acyl-CoA linked.

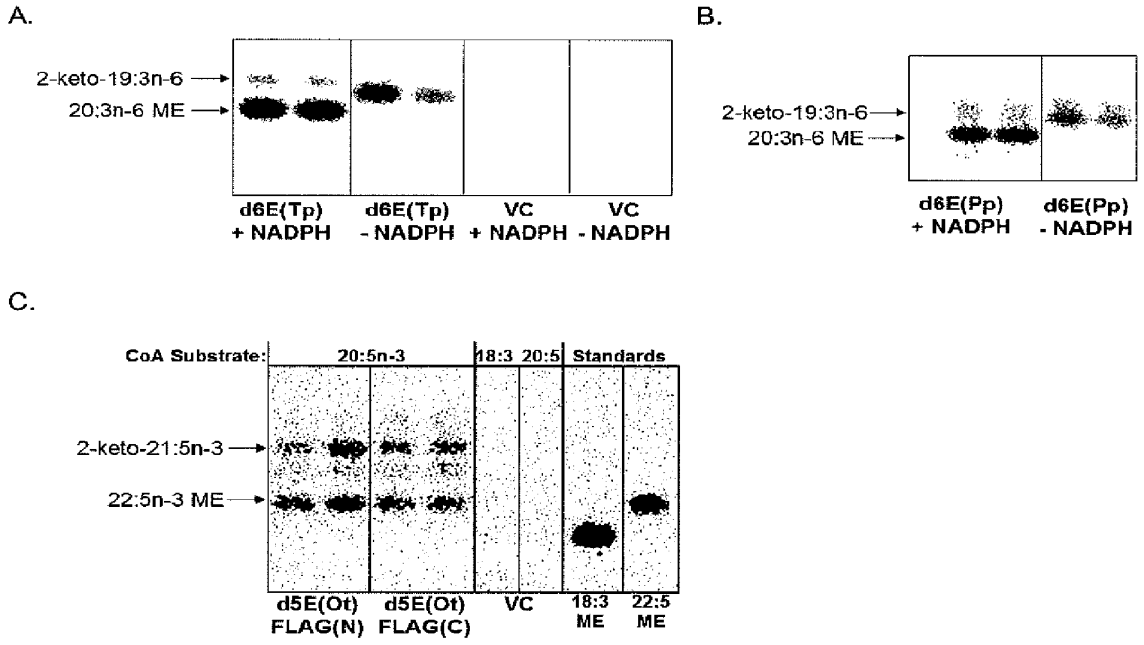


Figure 28: Examples of Elongase Enzyme Activity Heterologously Expressed in Yeast. [^{14}C]Fatty acid methyl esters (ME's) were isolated from the enzymatic reactions, resolved by TLC as described for each specific enzyme and detected by electronic autoradiography using Instant Imager. All FAME's shown had similar Rf's as authentic standards. In the absence of [^{14}C]malonyl-CoA no radioactive fatty acids were observed in any of these elongase reactions. In panel A delta-6 elongase (Tp), c-d6Elo(Tp_GA2), activity was demonstrated by comparison of enzyme activity present in yeast microsomes isolated from a strain expressing the c-d6Elo(Tp_GA2) protein relative to microsomes isolated from a control strain containing an empty vector (VC). In panel B, delta-6 elongase (Pp), c-d6Elo(Pp_GA2), was demonstrated by comparison of enzyme acitivity from yeast microsomes isolated from a strains expressing c-d6Elo(Pp_GA2) protein to microsomes isolated from a control strain containing an empty vector (VC), as shown in panel

A. In panel C, delta-5 elongase (Ot), c-d5Elo(Ot_GA3), activity was demonstrated by comparison of enzyme activity present in yeast microsomes isolated from a strain expressing the d6E(Ot) protein relative to microsomes isolated from a control strain containing an empty vector (VC).

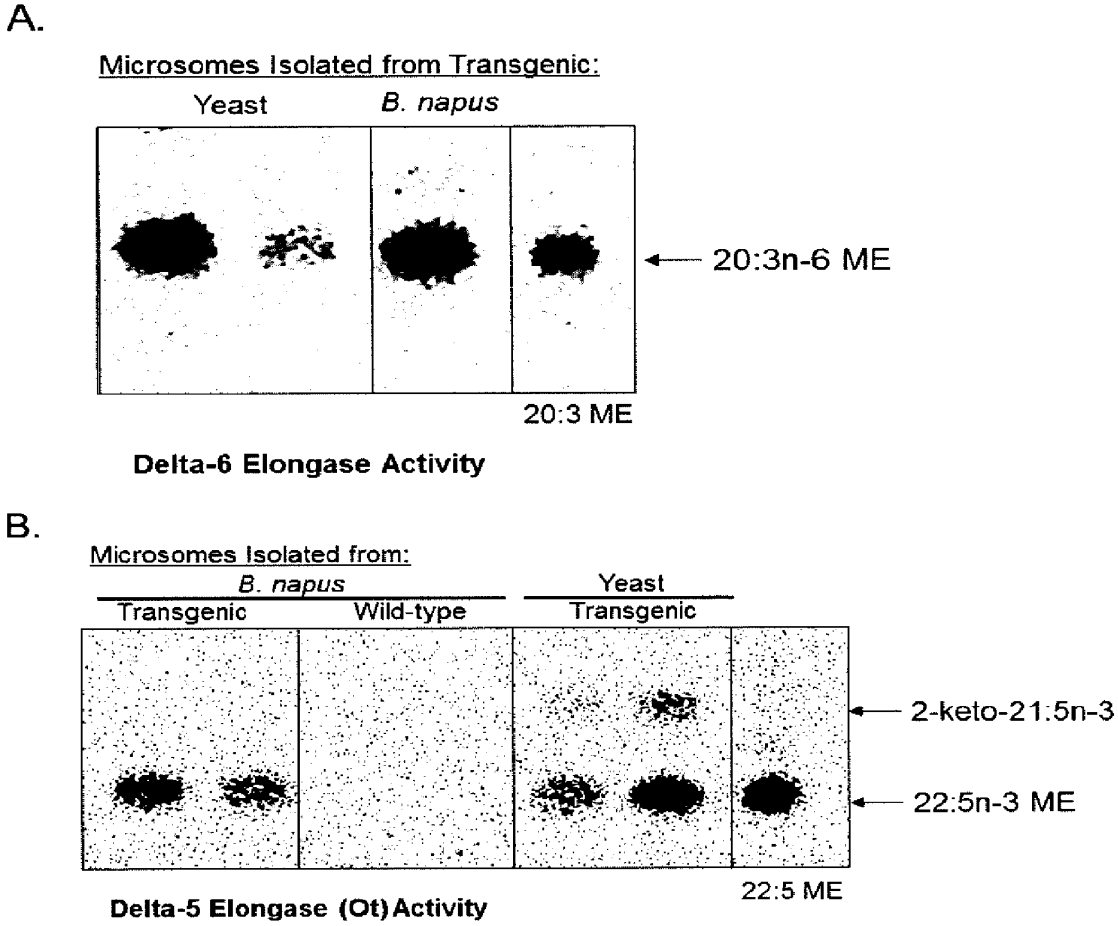


Figure 29: Examples of Elongase Activity in transgenic *Brassica napus*. [^{14}C]Fatty acid methyl esters (ME's) were isolated from the enzymatic reactions, resolved by TLC as described for each specific enzyme and detected by electronic autoradiography using Instant Imager. In panel A Delta-6 Elongase, activity was demonstrated by comparison of enzyme activity from yeast microsomes isolated from a strain expressing the d6E(Pp protein relative to microsomes isolated from transgenic *B. Napus* containing the *d6Elo(Pp_GA2)* gene and the *d6Elo(Tp_GA2)*. In panel B the Delta-5 Elongase (Ot), c-d5Elo(Ot_GA3), activity was demonstrated by comparison of enzyme activity from yeast microsomes isolated from a strain expressing the c-d5Elo(Ot_GA3) protein relative to microsomes isolated from transgenic *B. Napus* containing the *d5Elo(Ot_GA3)* and a wild-type *B. napus* (control).

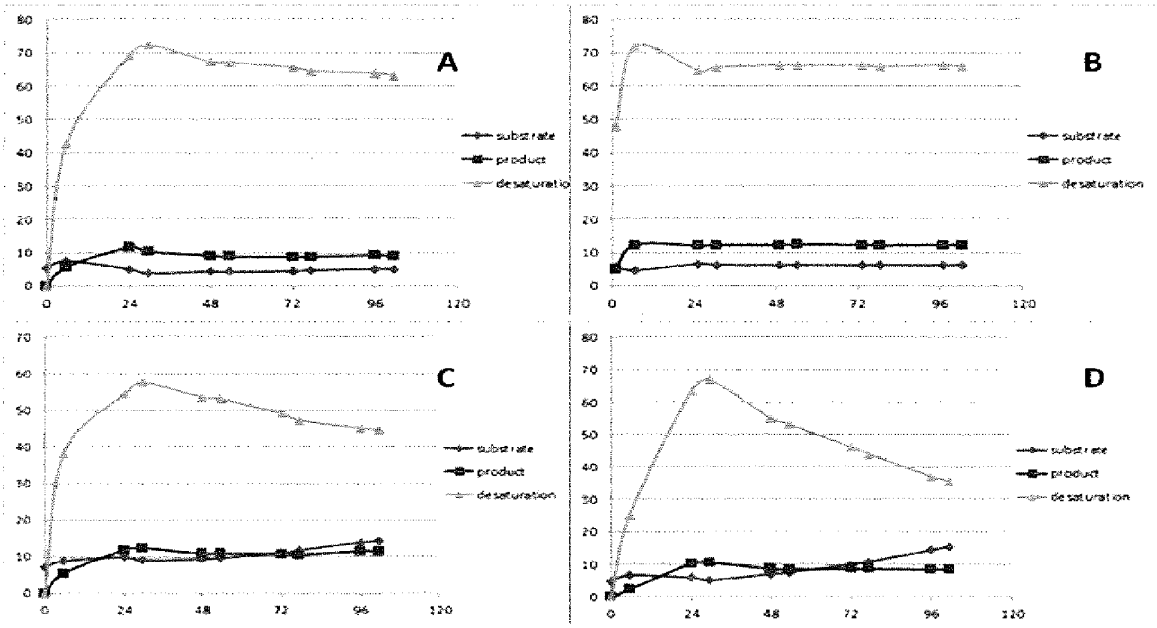


Figure 30: Time course optimization.Yeast cells expressing the c-d5Des(Tc_GA2) were fed with 0.25 mM HGLA and the production of ARA was determined by GC. Samples were collected starting immediately after feeding. In Panels A-D, Desaturation was represented as %Conversion vs Growth Time (hours) and Product and Substrate levesl are represented as %Total Fatty acid vs Growth time (hours). Panel A Samples supplied with HGLA immediately after induction. Panel B overnight induction (22hrs) before feeding. Panel C cultures supplied with 3X normal HGLA level Panel D. cultures supplied with normal rate of HGLA (0.25 mM) daily.

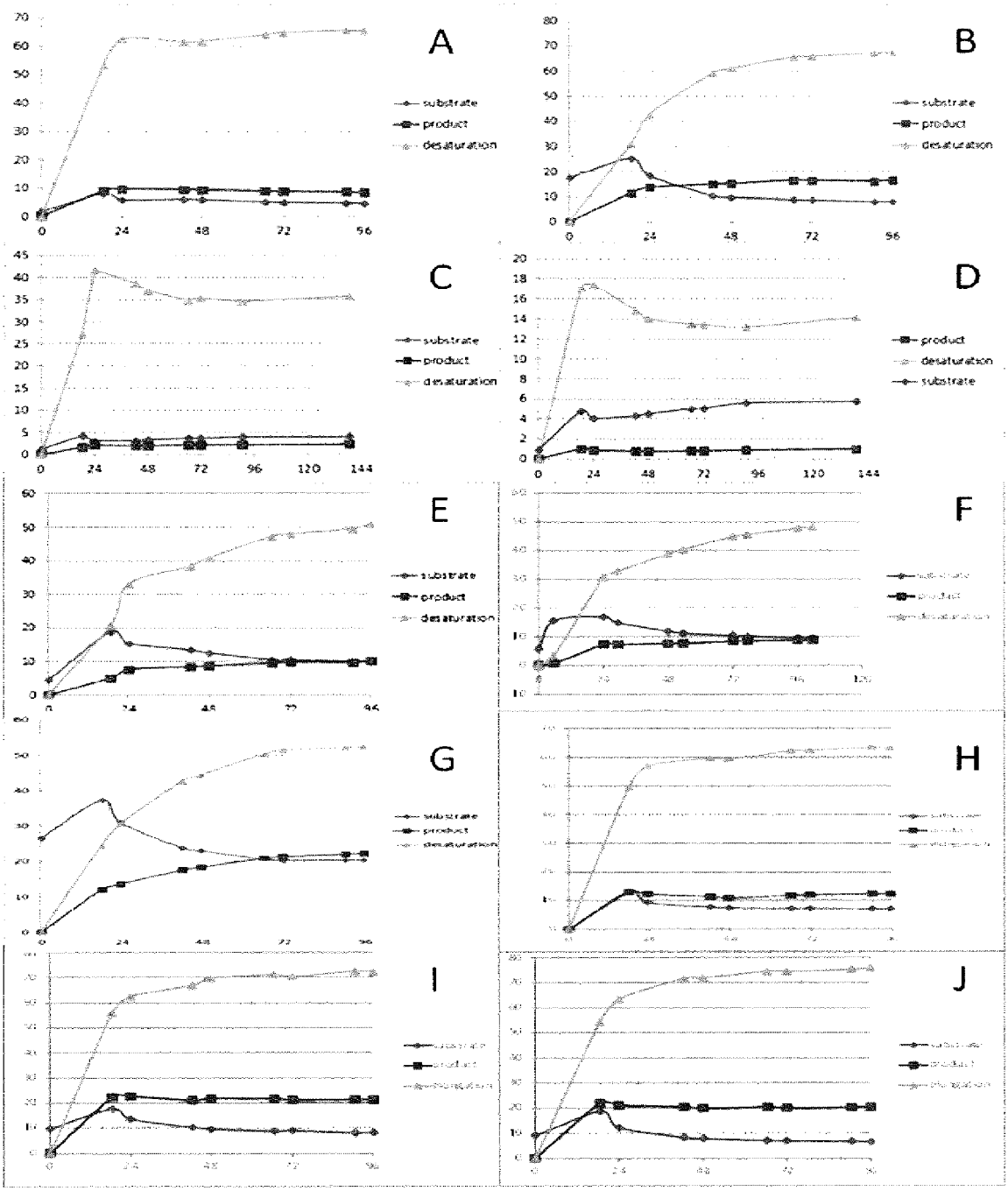


Figure 31: Representative time course graphs for all desaturases and elongases. Yeast cells expressing each enzyme were supplied with 0.25 mM of preferred fatty acid substrate, and fatty acid profiles were obtained by GC at the indicated time points. In Panels A-J, Desaturation and Elongation were represented as %Conversion vs Growth

Time (hours), and Product and Substrate levels were represented as %Total Fatty acid vs Growth time (hours).A. c-d5Des(Tc_GA2) + **HGLA B.** c-d6Des(Ot_fobit) + **ALA C.** c-d4Des(Pl_GA)2 + **DTA** D. c-d4Des(Tc_GA) + **DTA E.** c-o3Des(Pir_GA) + **ARA F.** c-o3Des(Pi_GA2) + **ARA G.** c-d12Des(Ps_GA) + **OA H.** c-d5Elo(Ot_GA3) + **EPA I.** c-d6Elo(Tp_GA2) + **GLA J.** c-d6Elo(Pp_GA2) + **SDA.**

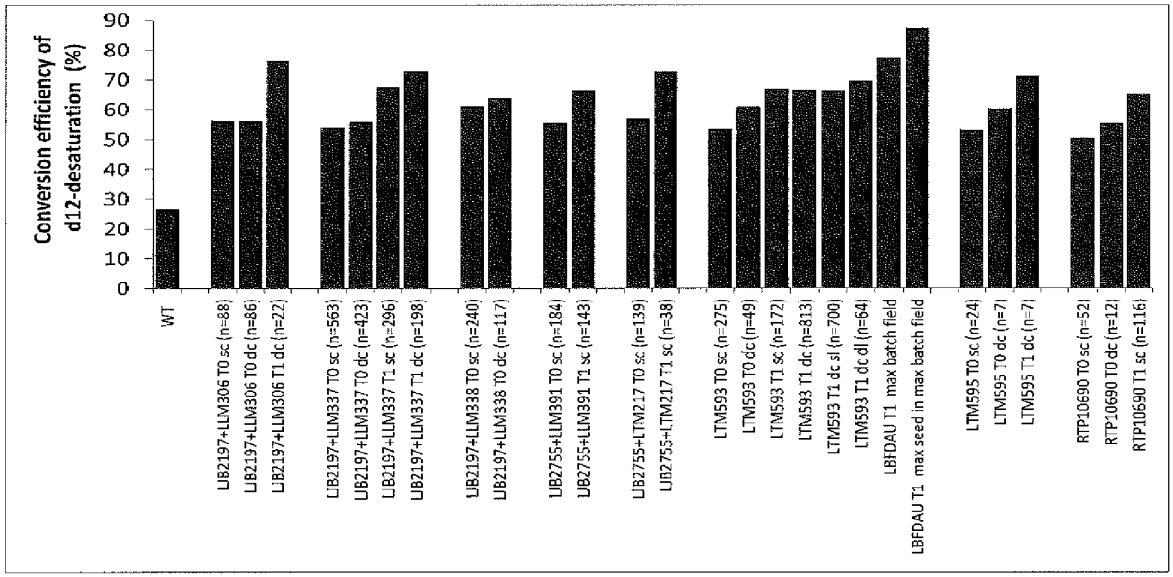


Figure 32: Conversion efficiecies of delta-12-desaturation in seed of transgenic Brassica napus and in Brassica napus wildtype seeds. Shown are average conversien efficiencies of various plant populations, as well as the conversion efficiencies observed in a seedbacth of event LBFDAU having highest EPA+DHA levels, and those efficiencies observed in a single seed of that seedbatch, where this single seed had highest EPA+DHA levels among all 95 measured single seeds. Data were taken from Example 10 to Example 18. T0 and T1 designates the plant generation producing the seeds (all grown in the greenhouse except for the two LBFDAU datapoints)

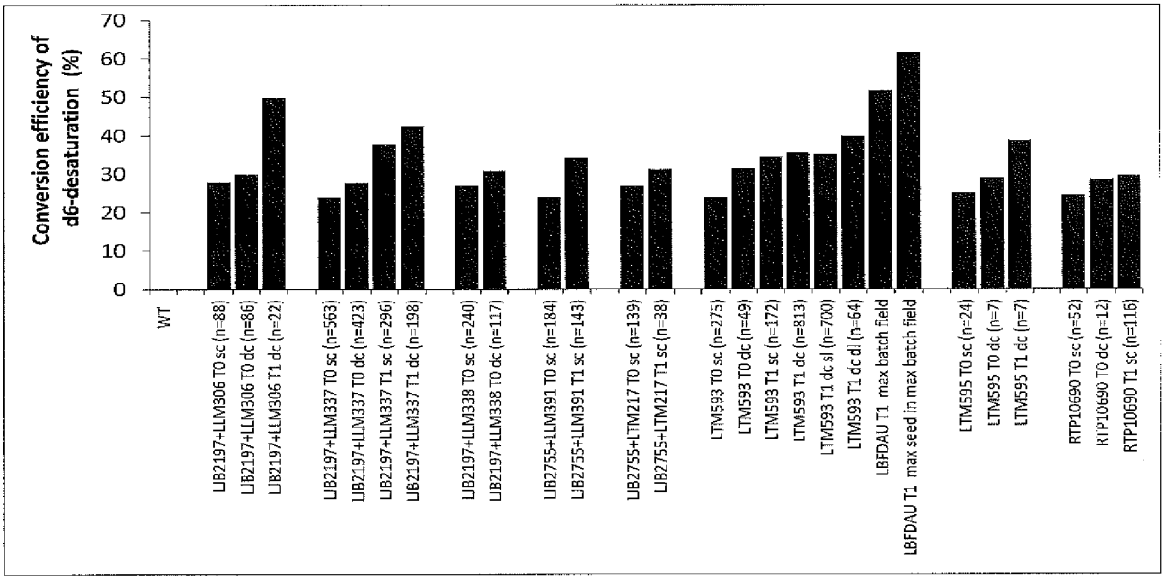


Figure 33: Conversion efficiecies of delta-6-desaturation in seed of transgenic Brassica napus and in Brassica napus wildtype seeds. See caption in Figure 32 for further details.

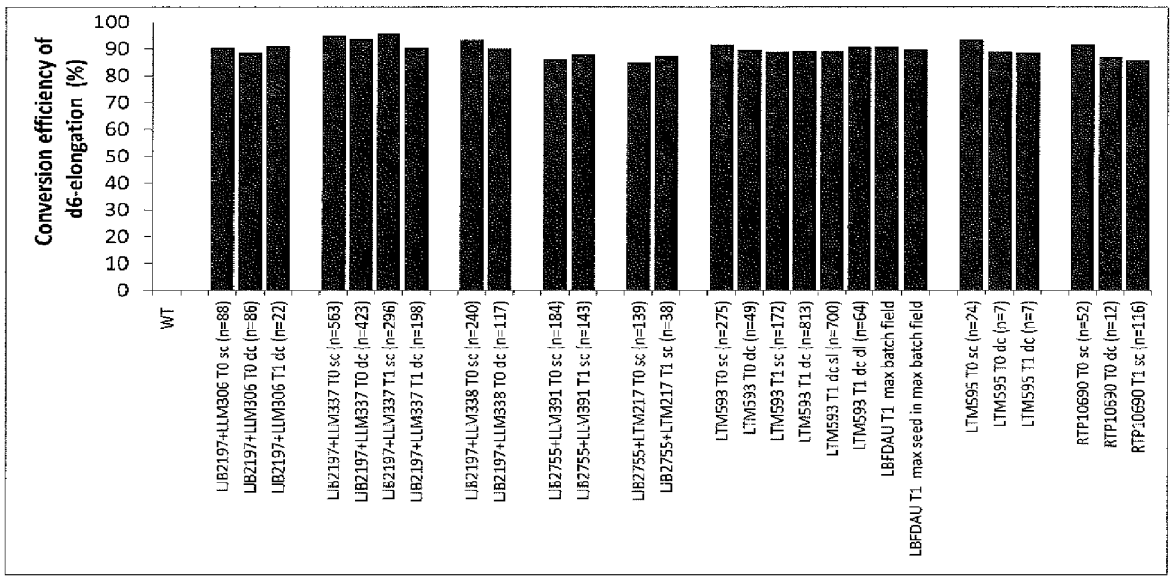


Figure 34: Conversion efficiecies of delta-6-elongation in seed of transgenic Brassica napus and in Brassica napus wildtype seeds. See caption in Figure 32 for further details.

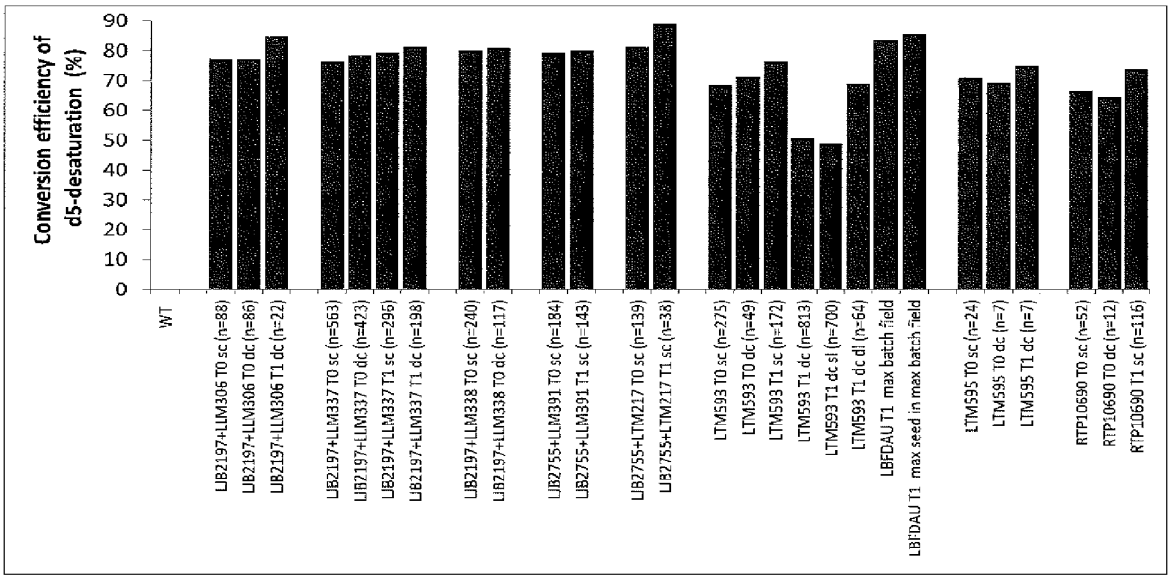


Figure 35: Conversion efficiecies of delta-5-desaturation in seed of transgenic Brassica napus and in Brassica napus wildtype seeds. See caption in Figure 32 for further details.

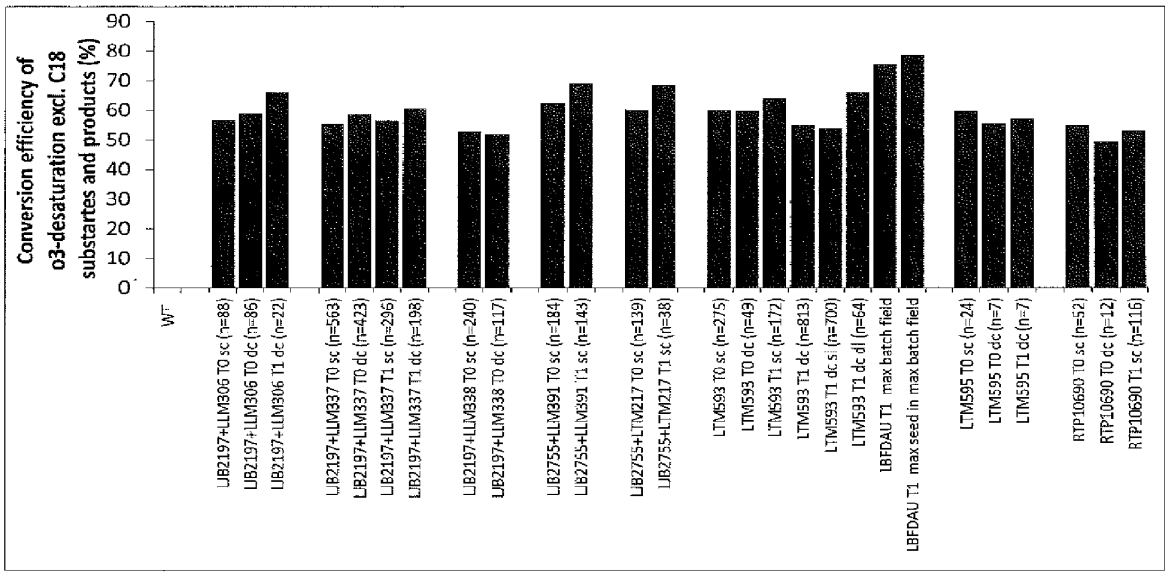


Figure 36: Conversion efficiecies of omega-3 desaturation (excluding C18 fatty acids) in seed of transgenic Brassica napus and in Brassica napus wildtype seeds. See caption in Figure 32 for further details.

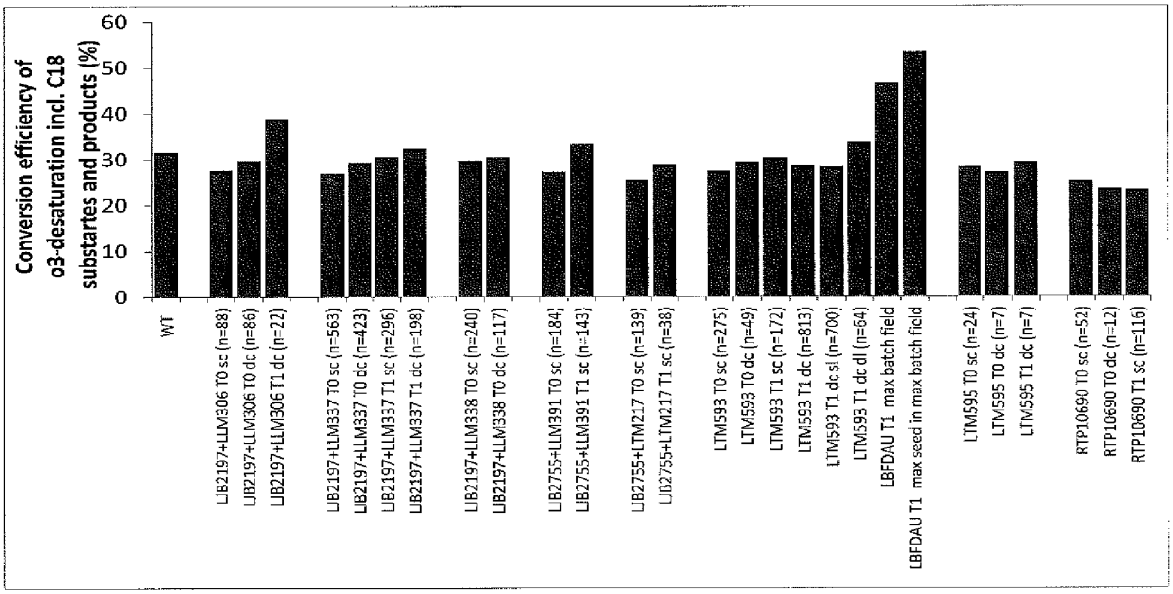


Figure 37: Conversion efficiecies of omega-3 desaturation (including C18 fatty acids) in seed of transgenic Brassica napus and in Brassica napus wildtype seeds. See caption in Figure 32 for further details.

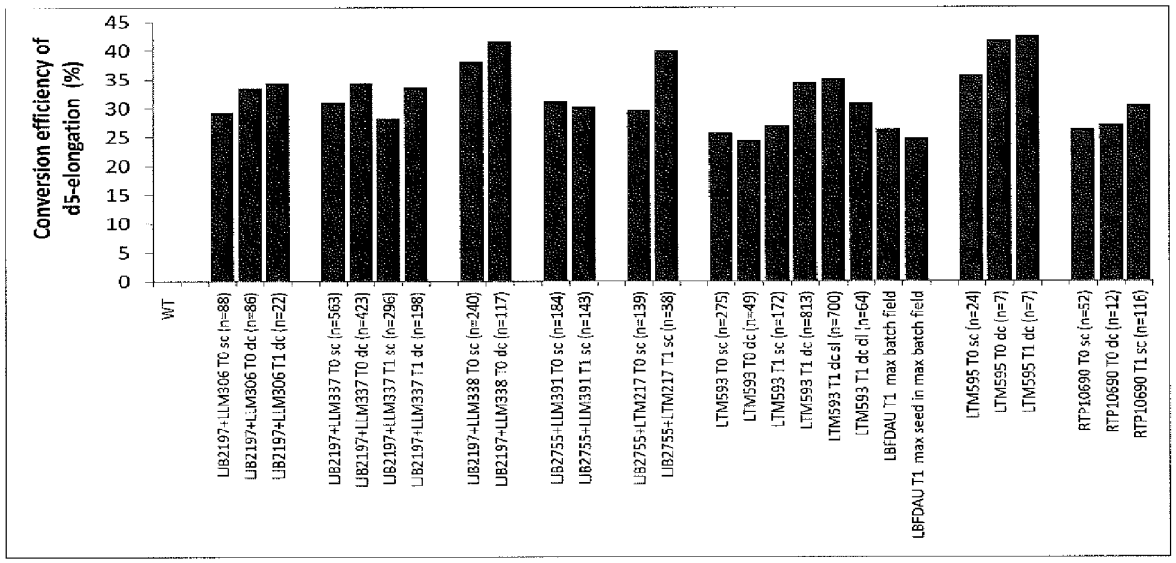


Figure 38: Conversion efficiecies of delta-5-elongation in seed of transgenic Brassica napus and in Brassica napus wildtype seeds. See caption in Figure 32 for further details.

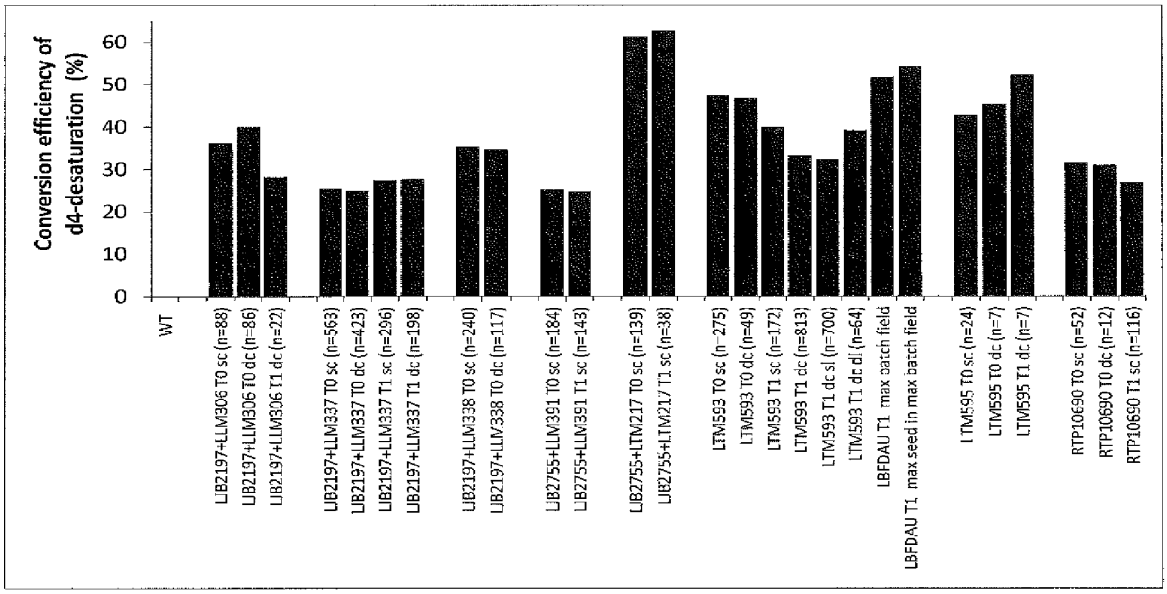


Figure 39: Conversion efficiecies of delta-4-desturation in seed of transgenic *Brassica napus* and in *Brassica napus* wildtype seeds. See caption in Figure 32 for further details.

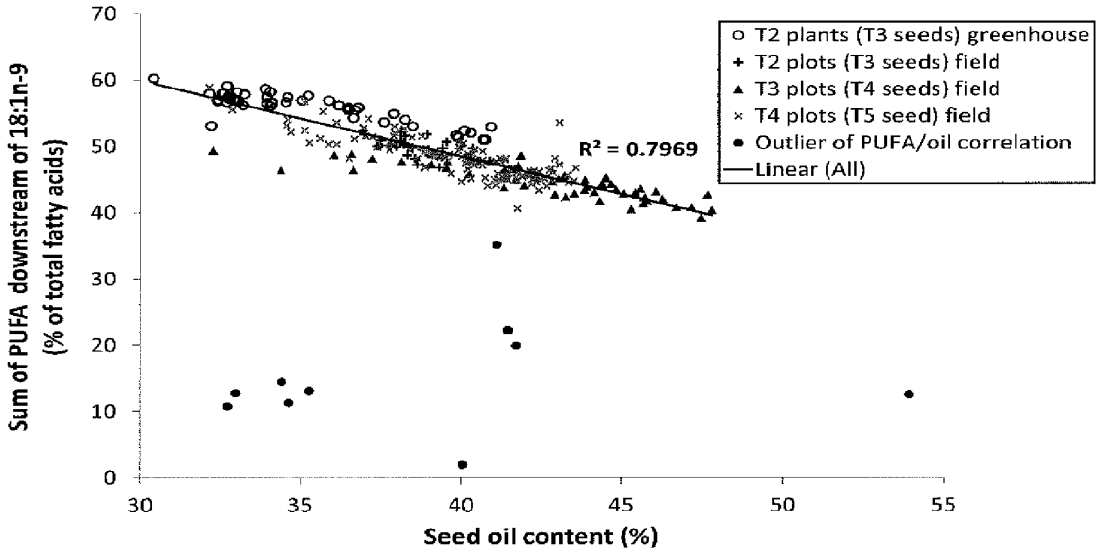


Figure 40: The sum of all pathway fatty acids was negatively correlated with seed oil content. Shown are data of 3 generations of event LANPMZ. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

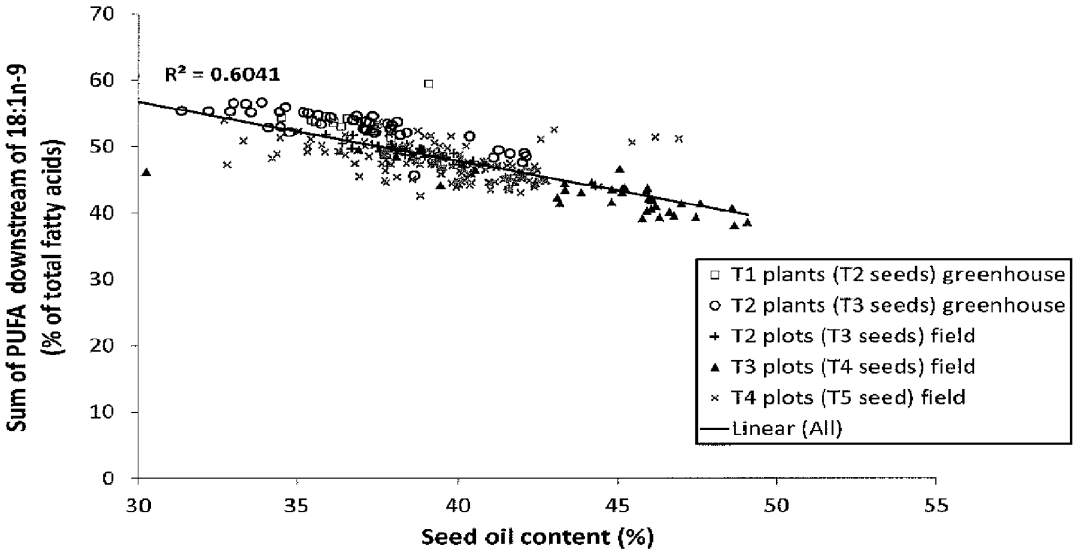


Figure 41: The sum of all pathway fatty acids was negatively correlated with seed oil content. Shown are data of 4 generations of event LAODDN. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

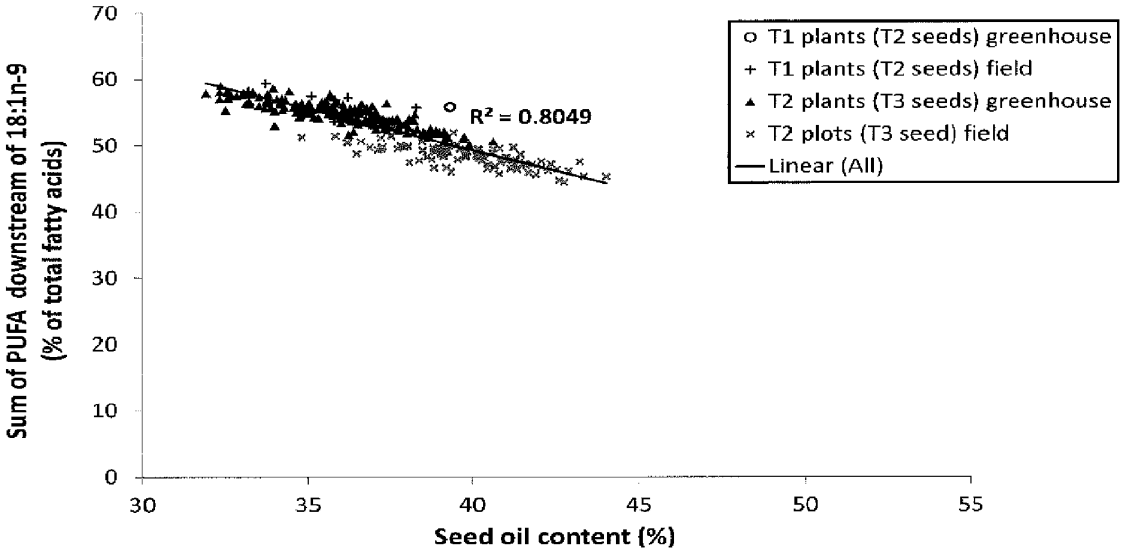


Figure 42: The sum of all pathway fatty acids was negatively correlated with seed oil content. Shown are data of 2 generations of event LBFGKN. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 50 T2 seedbatches, or 182 T3 seedbatches, for the field data, one marker corresponds to an analysis of one T2 seedbtach of one T1 plant, or the analysis of a random selection of T3 seeds representing plots (36 plots) or single plants (60 plants).

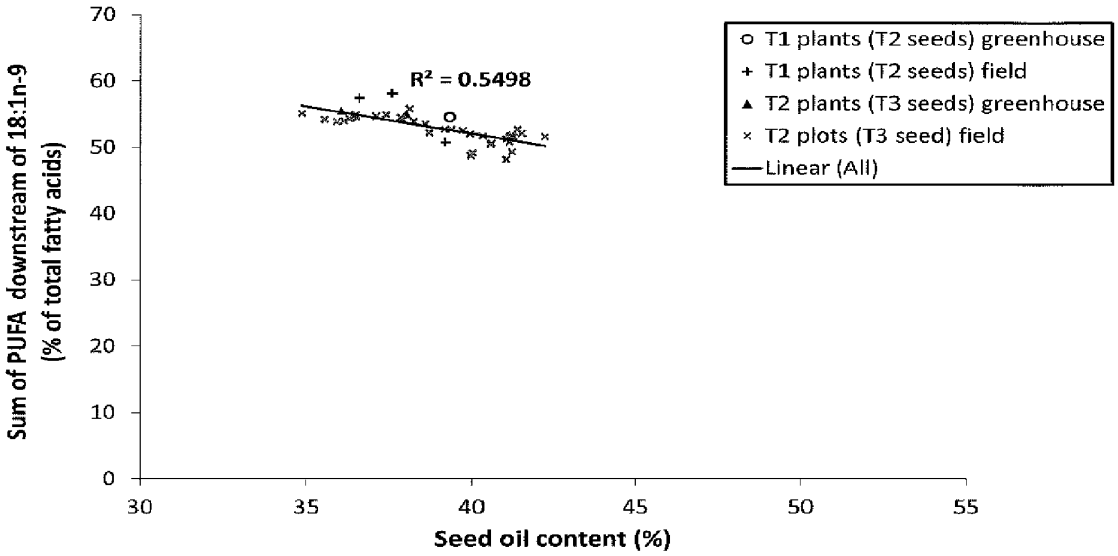


Figure 43: The sum of all pathway fatty acids was negatively correlated with seed oil content. Shown are data of 2 generations of event LBFLFK. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 10 T2 seedbatches, or 195 T3 seedbatches, for the field data, one marker corresponds to an analysis of 1 T2 seedbtach of one T1 plant, or a the analysis of a random selection of T3 seeds representing one plot.

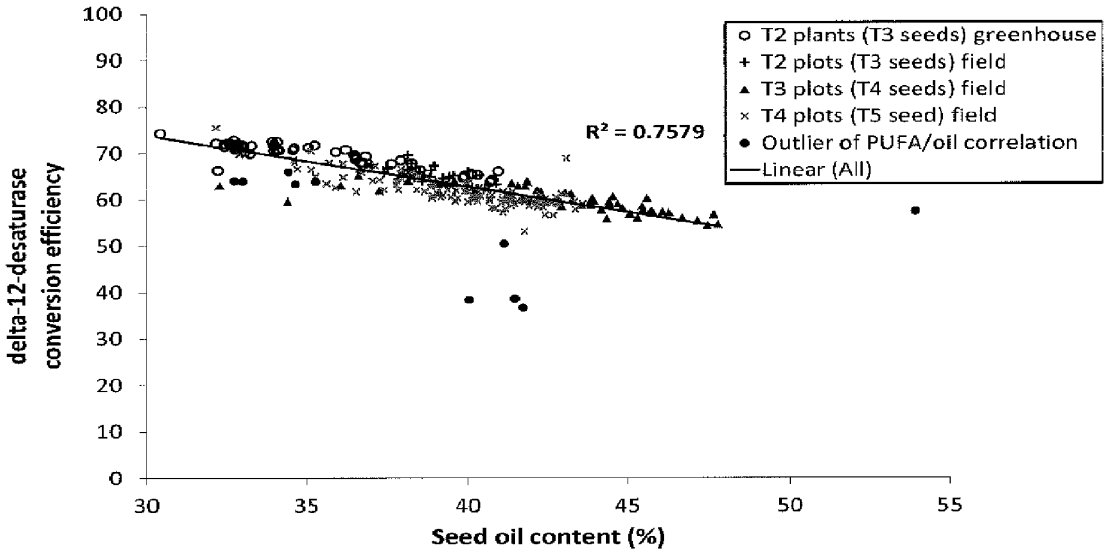


Figure 44: The conversion efficiency of the delta-12-desaturase was negatively correlated with seed oil content. Shown are data of 3 generations of event LANPMZ. For the greenhouse data, one marker corresponds to one seed batch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

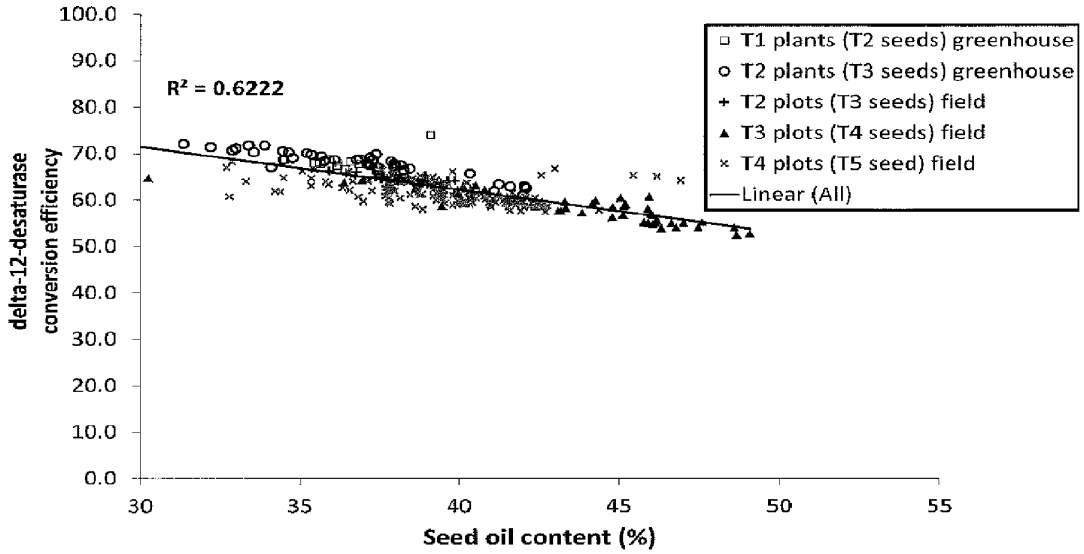


Figure 45: The conversion efficiency of the delta-12-desaturase was negatively correlated with seed oil content. Shown are data of 4 generations of event LAODDN. For the greenhouse data, one marker corresponds to one seed batch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

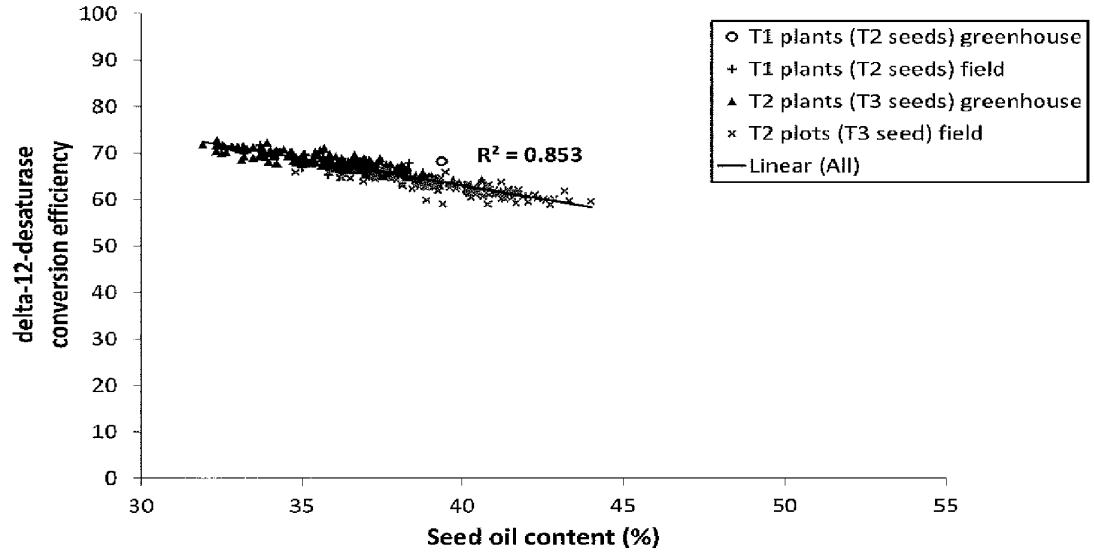


Figure 46: The conversion efficiency of the delta-12-desaturase was negatively correlated with seed oil content. Shown are data of 2 generations of event LBFGKN. For the greenhouse data, one marker

corresponds the analysis of a random selection of seeds representing a bulk of 50 T2 seedbatches, or 182 T3 seedbatches, for the field data, one marker corresponds to an analysis of one T2 seedbtach of one T1 plant, or the analysis of a random selection of T3 seeds representing plots (36 plots) or single plants (60 plants).

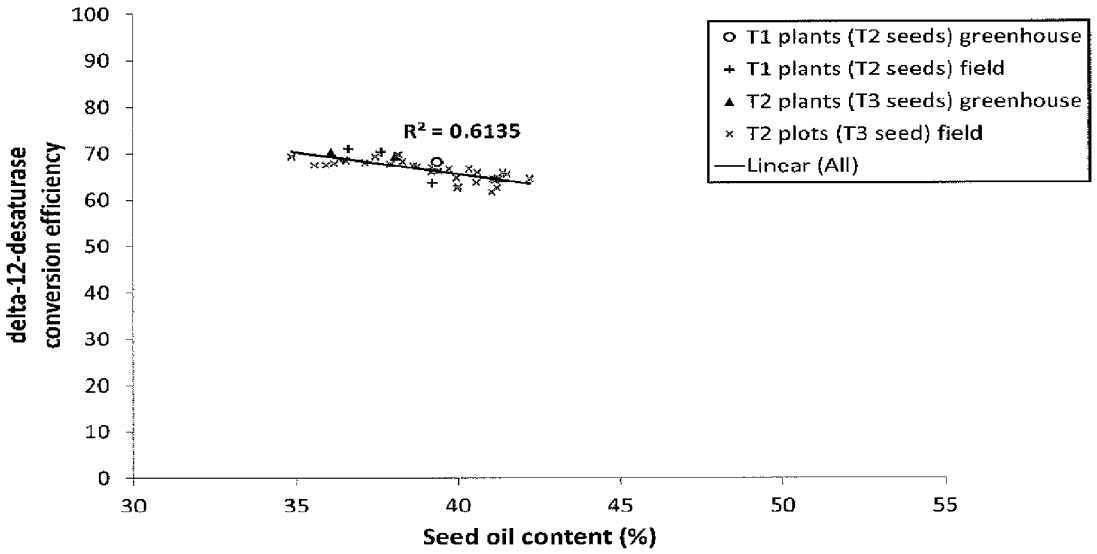


Figure 47: The conversion efficiency of the delta-12-desaturase was negatively correlated with seed oil content. Shown are data of 2 generations of event LBFLFK. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 10 T2 seedbatches, or 195 T3 seedbatches, for the field data, one marker corresponds to an analysis of 1 T2 seedbtach of one T1 plant, or a the analysis of a random selection of T3 seeds representing one plot.

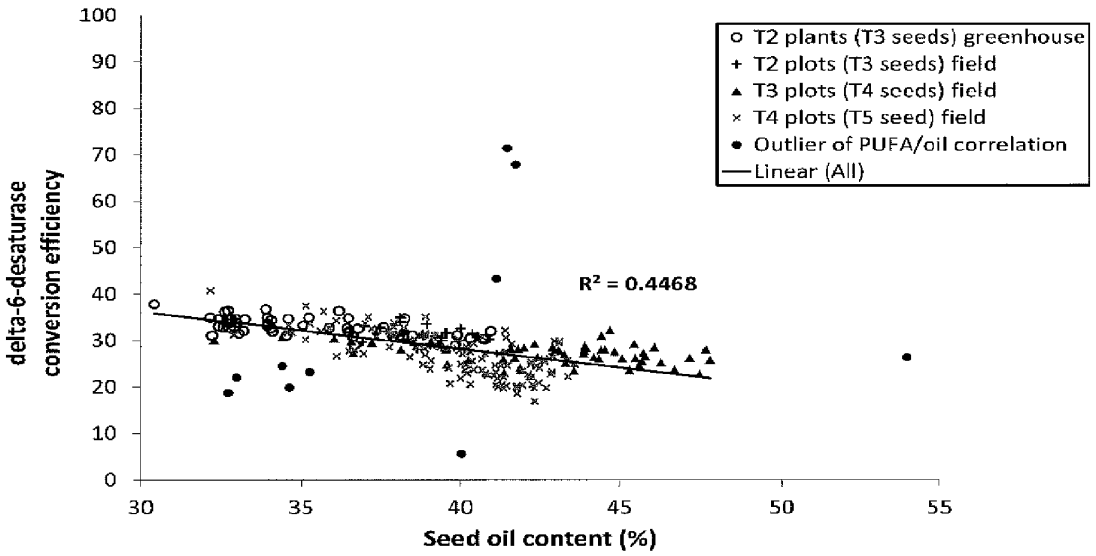


Figure 48: The conversion efficiency of the delta-6-desaturase was negatively correlated with seed oil content. Shown are data of 3 generations of event LANPMZ. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

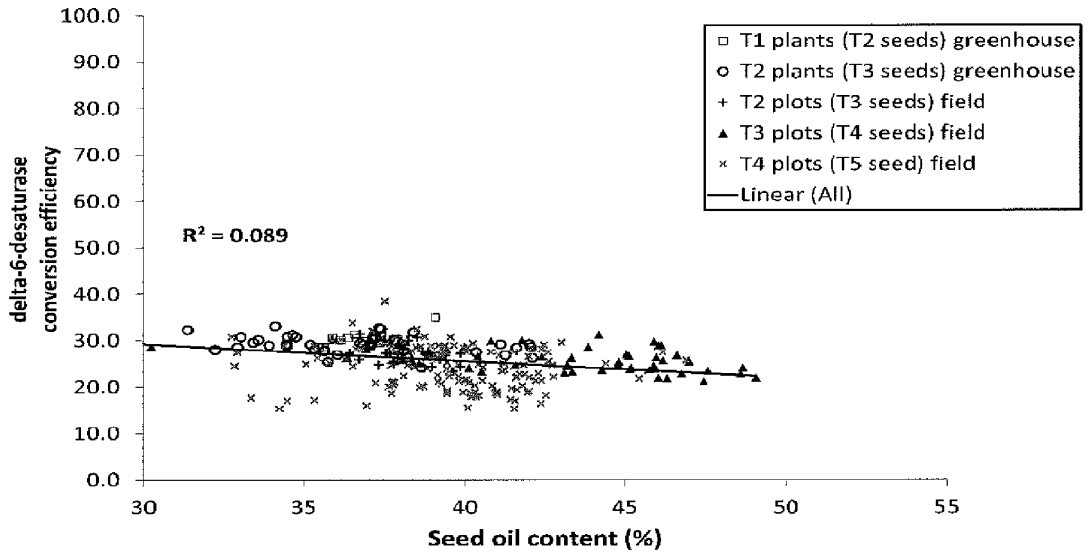


Figure 49: The conversion efficiency of the delta-6-desaturase was negatively correlated with seed oil content. Shown are data of 4 generations of event LAODDN. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

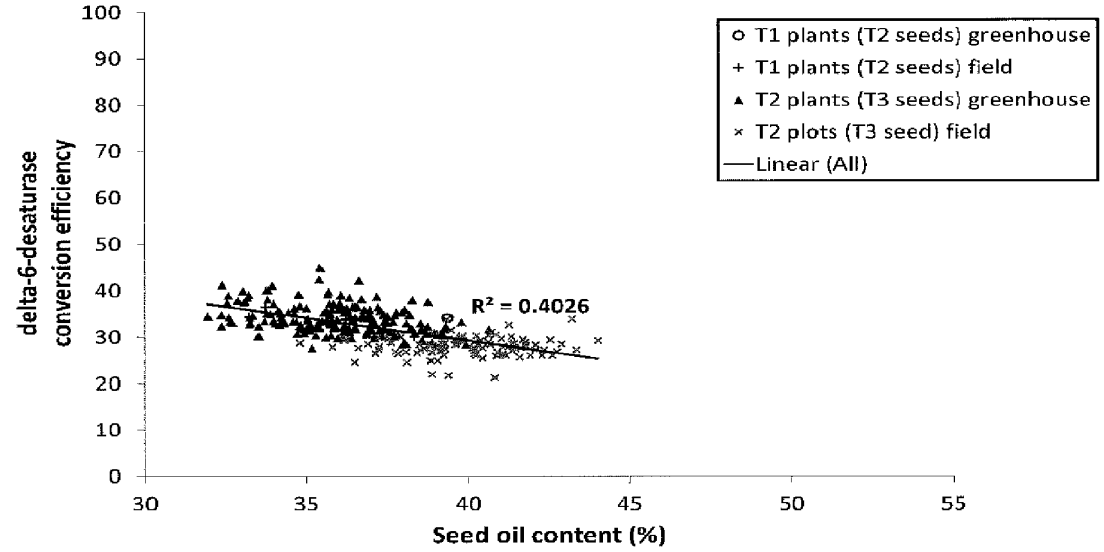


Figure 50: The conversion efficiency of the delta-6-desaturase was negatively correlated with seed oil content. Shown are data of 2 generations of event LBFGKN. For the greenhouse data, one marker

corresponds the analysis of a random selection of seeds representing a bulk of 50 T2 seedbatches, or 182 T3 seedbatches, for the field data, one marker corresponds to an analysis of one T2 seedbtach of one T1 plant, or the analysis of a random selection of T3 seeds representing plots (36 plots) or single plants (60 plants).

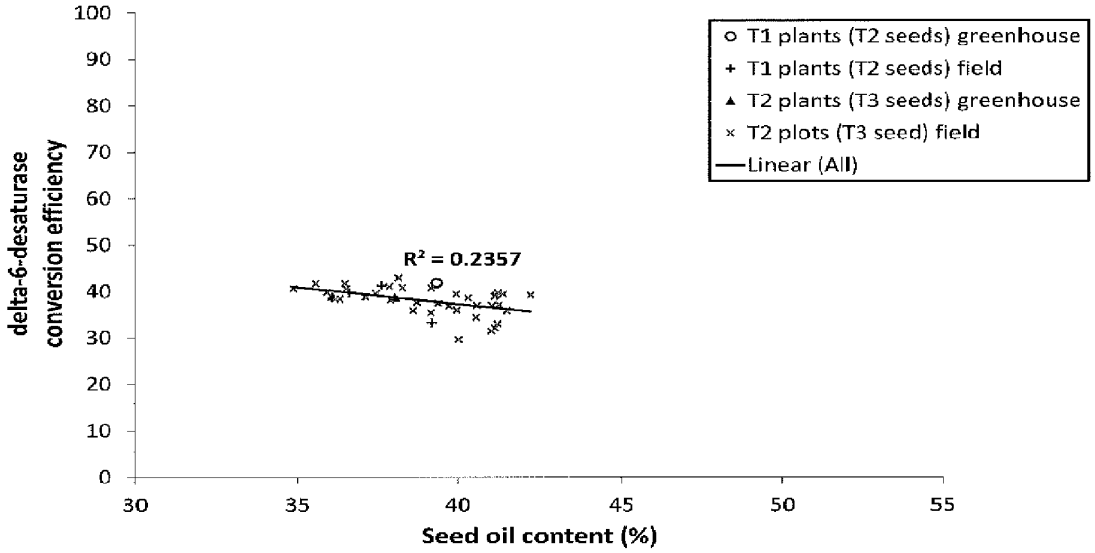


Figure 51: The conversion efficiency of the delta-6-desaturase was negatively correlated with seed oil content. Shown are data of 2 generations of event LBFLFK. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 10 T2 seedbatches, or 195 T3 seedbatches, for the field data, one marker corresponds to an analysis of 1 T2 seedbtach of one T1 plant, or a the analysis of a random selection of T3 seeds representing one plot.

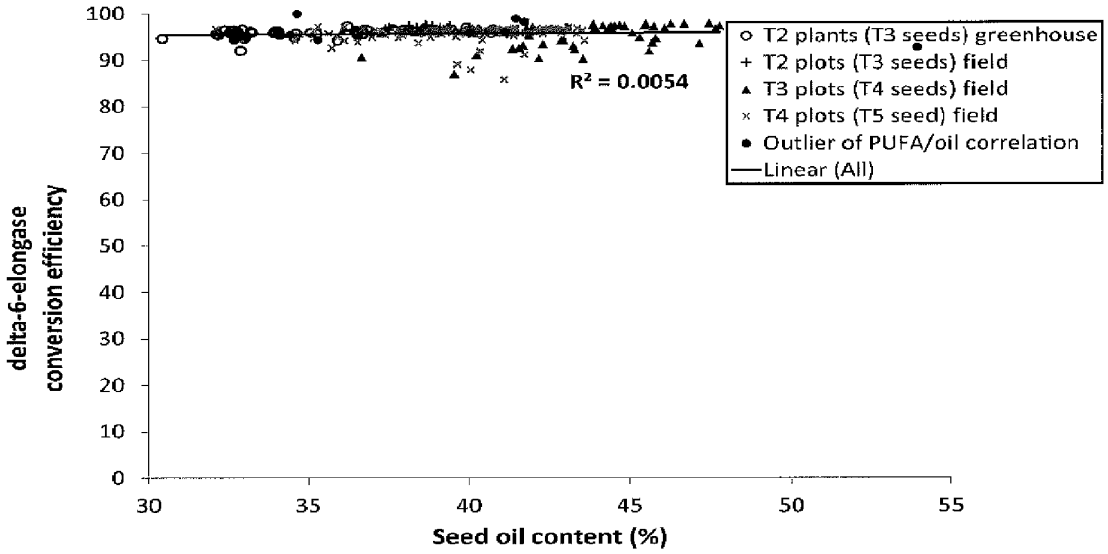


Figure 52: The conversion efficiency of the delta-6 elongase was not correlated with seed oil content. Shown are data of 3 generations of event LANPMZ. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

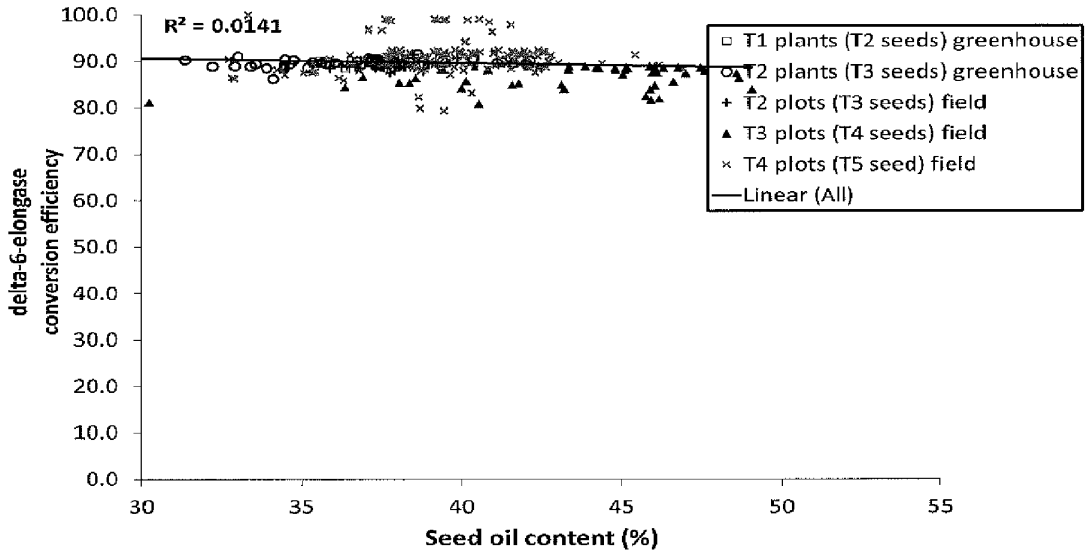


Figure 53: The conversion efficiency of the delta-6 elongase was not correlated with seed oil content. Shown are data of 4 generations of event LAODDN. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

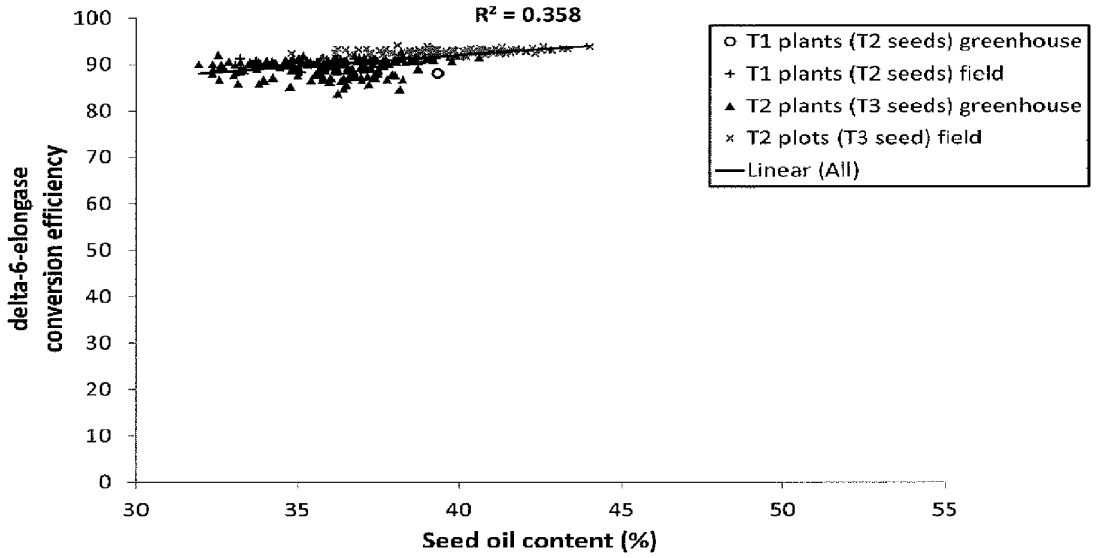


Figure 54: The conversion efficiency of the delta-6 elongase was not correlated with seed oil content. Shown are data of 2 generations of event LBFGKN. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 50 T2 seedbatches, or 182 T3 seedbatches, for the field data, one marker corresponds to an analysis of one T2 seedbtach of one T1 plant, or the analysis of a random selection of T3 seeds representing plots (36 plots) or single plants (60 plants).

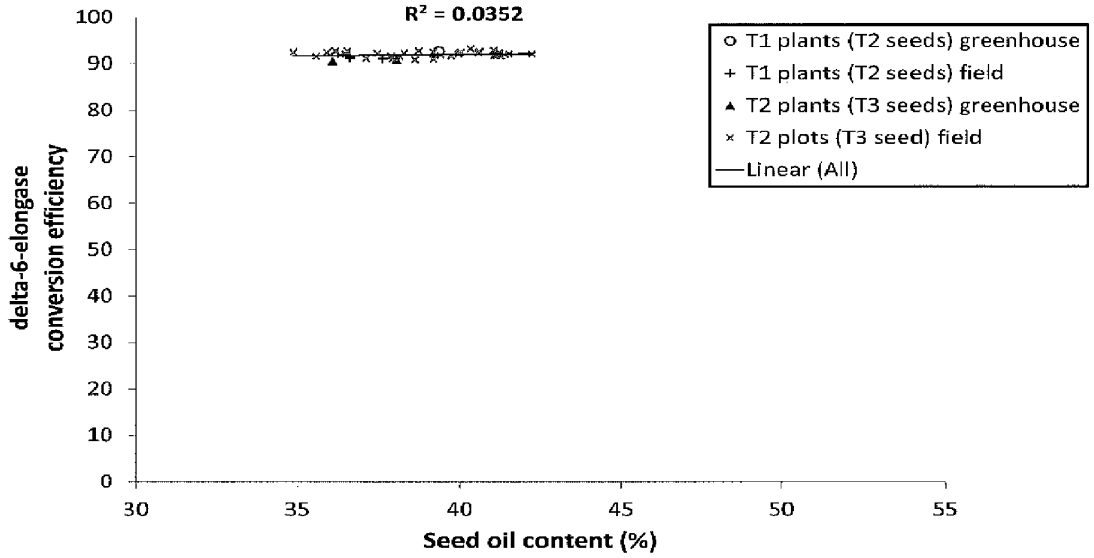


Figure 55: The conversion efficiency of the delta-6 elongase was not correlated with seed oil content. Shown are data of 2 generations of event LBFLFK. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 10 T2 seedbatches, or 195 T3 seedbatches, for the field data, one marker corresponds to an analysis of 1 T2 seedbtach of one T1 plant, or a the analysis of a random selection of T3 seeds representing one plot.

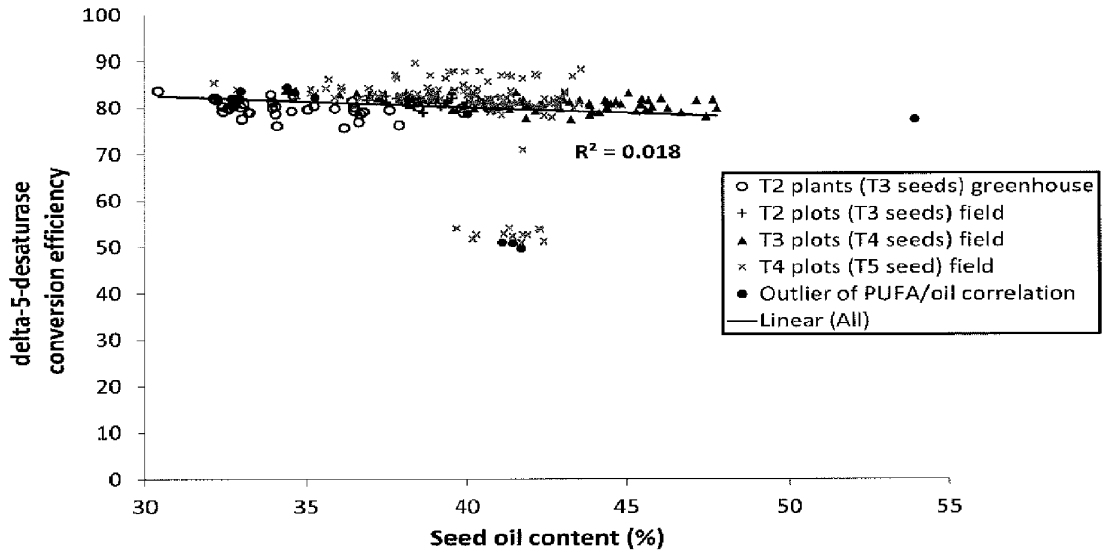


Figure 56: The conversion efficiency of the delta-5-desaturase was not correlated with seed oil content. Shown are data of 3 generations of event LANPMZ. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

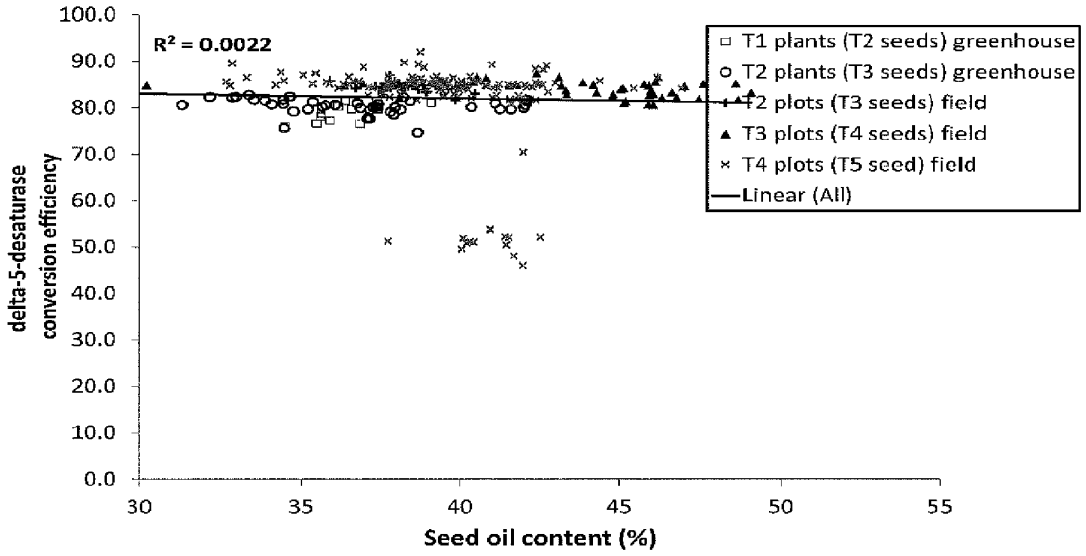


Figure 57: The conversion efficiency of the delta-5-desaturase was not correlated with seed oil content. Shown are data of 4 generations of event LAODDN. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

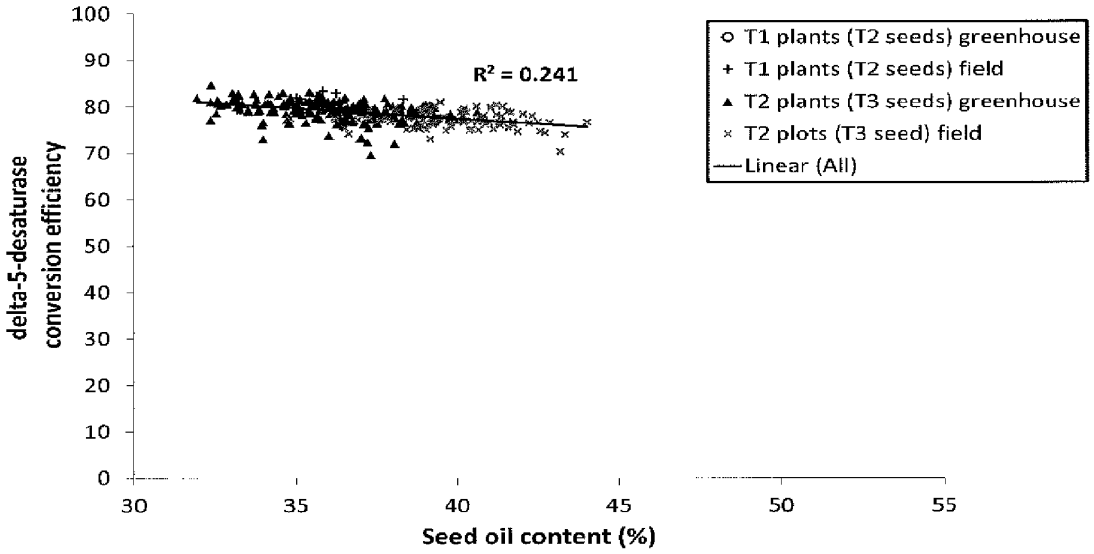


Figure 58: The conversion efficiency of the delta-5-desaturase was not correlated with seed oil content. Shown are data of 2 generations of event LBFGKN. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 50 T2 seedbatches, or 182 T3 seedbatches, for the field data, one marker corresponds to an analysis of one T2 seedbtach of one T1 plant, or the analysis of a random selection of T3 seeds representing plots (36 plots) or single plants (60 plants).

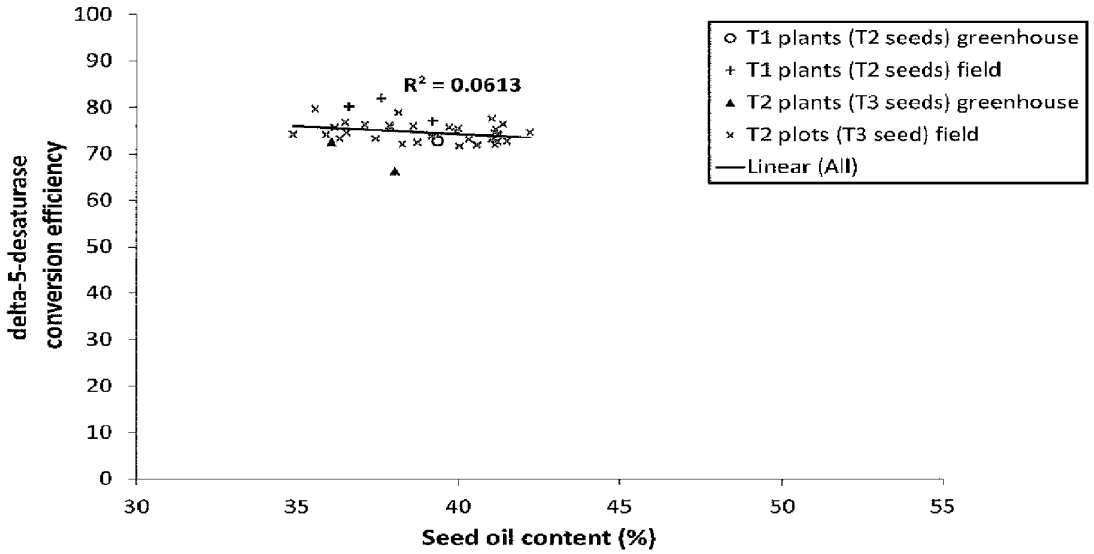


Figure 59: The conversion efficiency of the delta-5-desaturase was not correlated with seed oil content. Shown are data of 2 generations of event LBFLFK. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 10 T2 seedbatches, or 195 T3 seedbatches, for the field data, one marker corresponds to an analysis of 1 T2 seedbtach of one T1 plant, or a the analysis of a random selection of T3 seeds representing one plot.

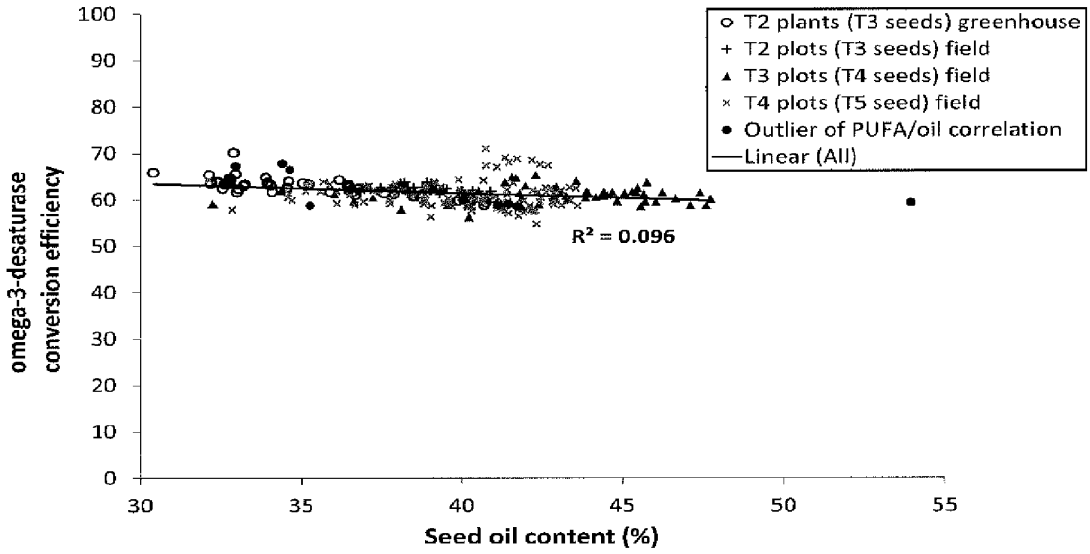


Figure 60: The conversion efficiency of the omega-3-desaturase was not correlated with seed oil content. Shown are data of 3 generations of event LANPMZ. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

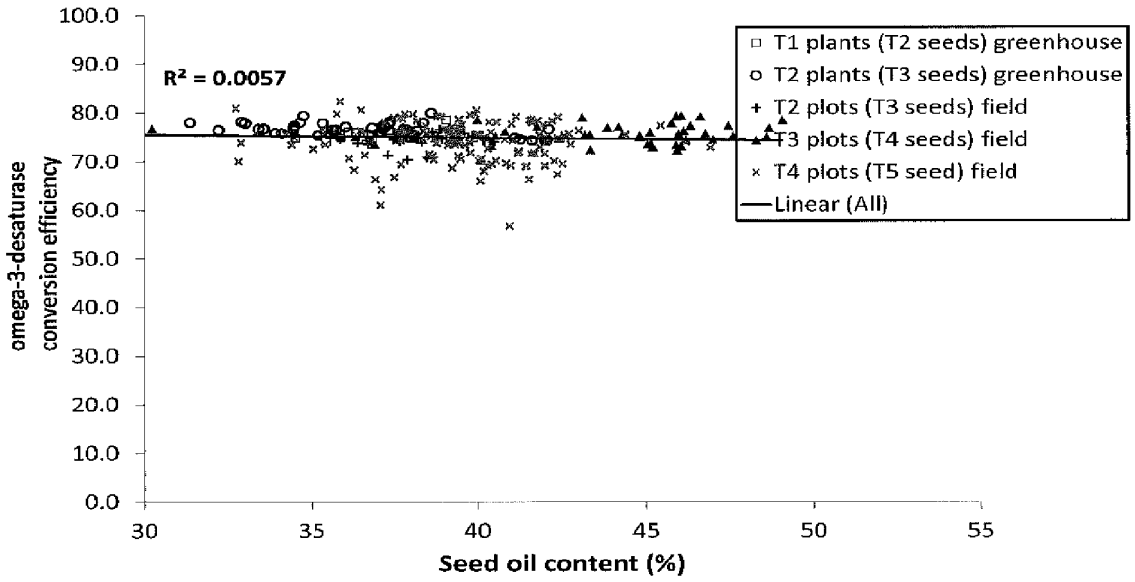


Figure 61: The conversion efficiency of the omega-3-desaturase was not correlated with seed oil content. Shown are data of 4 generations of event LAODDN. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

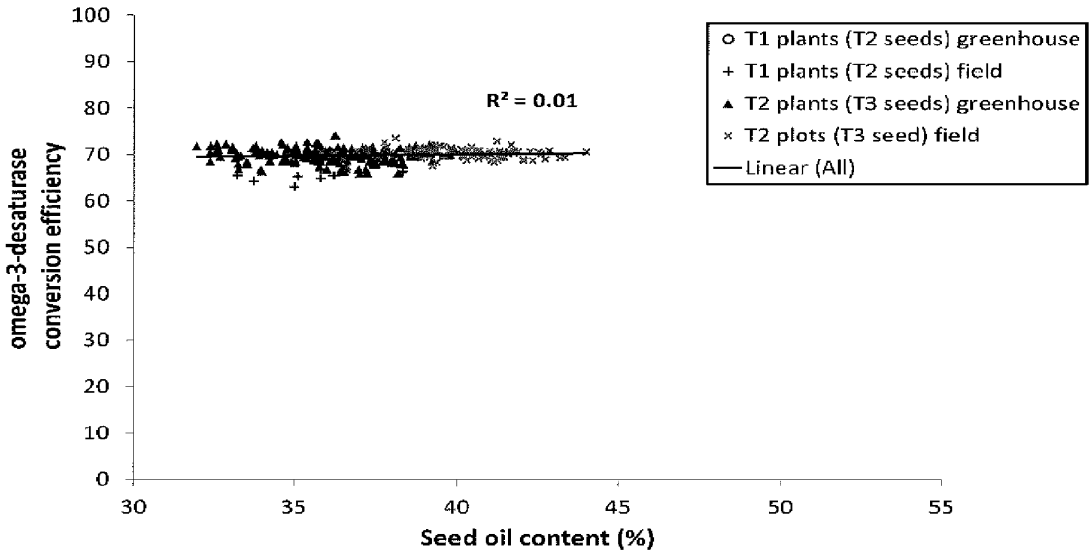


Figure 62: The conversion efficiency of the omega-3-desaturase was not correlated with seed oil content. Shown are data of 2 generations of event LBFGKN. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 50 T2 seedbatches, or 182 T3 seedbatches, for the field data, one marker corresponds to an analysis of one T2 seedbtach of one T1 plant, or the analysis of a random selection of T3 seeds representing plots (36 plots) or single plants (60 plants).

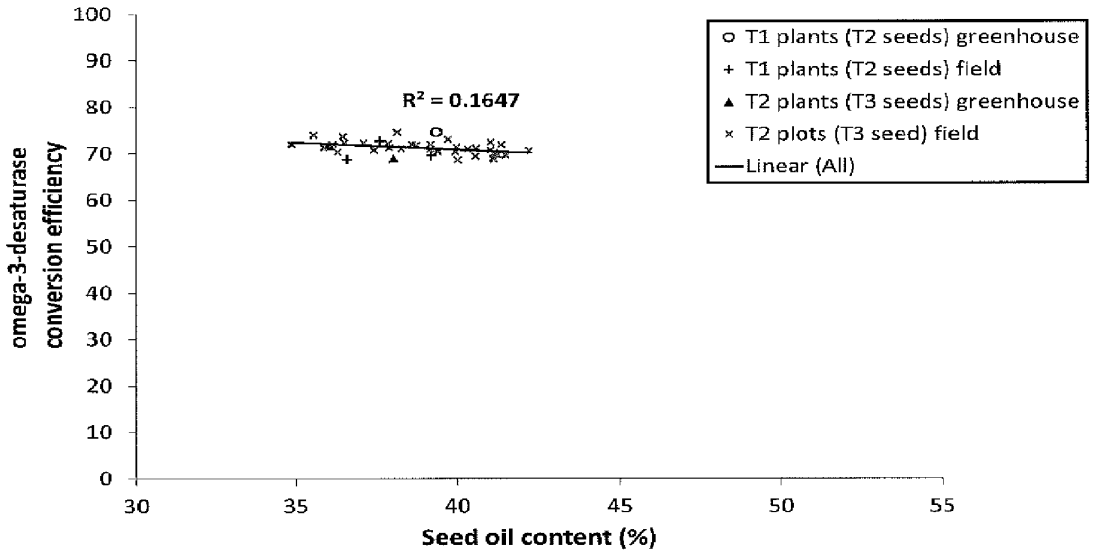


Figure 63: The conversion efficiency of the omega-3-desaturase was not correlated with seed oil content. Shown are data of 2 generations of event LBFLFK. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 10 T2 seedbatches, or 195 T3 seedbatches, for the field data, one marker corresponds to an analysis of 1 T2 seedbtach of one T1 plant, or a the analysis of a random selection of T3 seeds representing one plot.

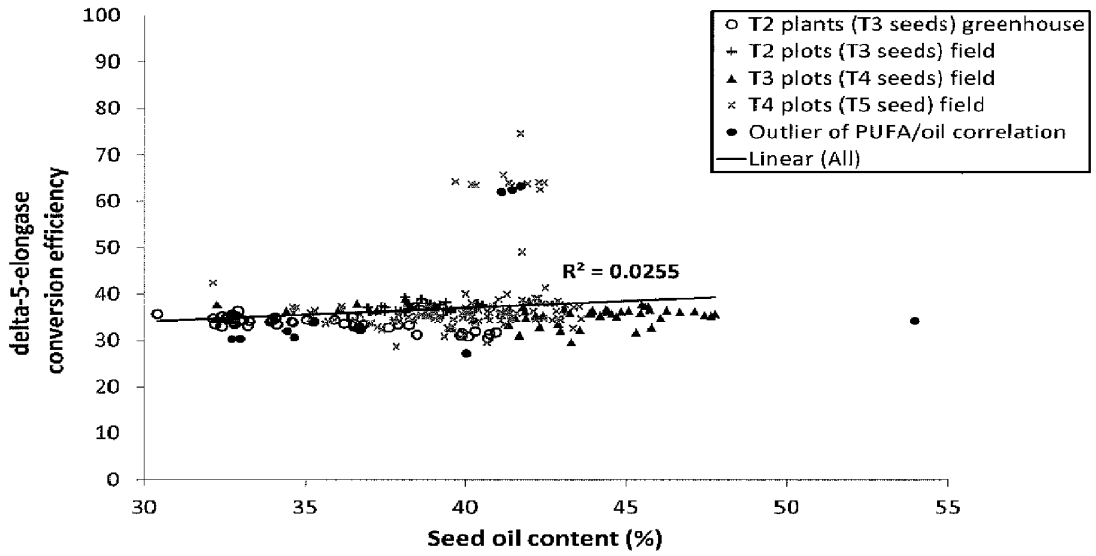


Figure 64: The conversion efficiency of the delta-5-elongase was not correlated with seed oil content. Shown are data of 3 generations of event LANPMZ. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

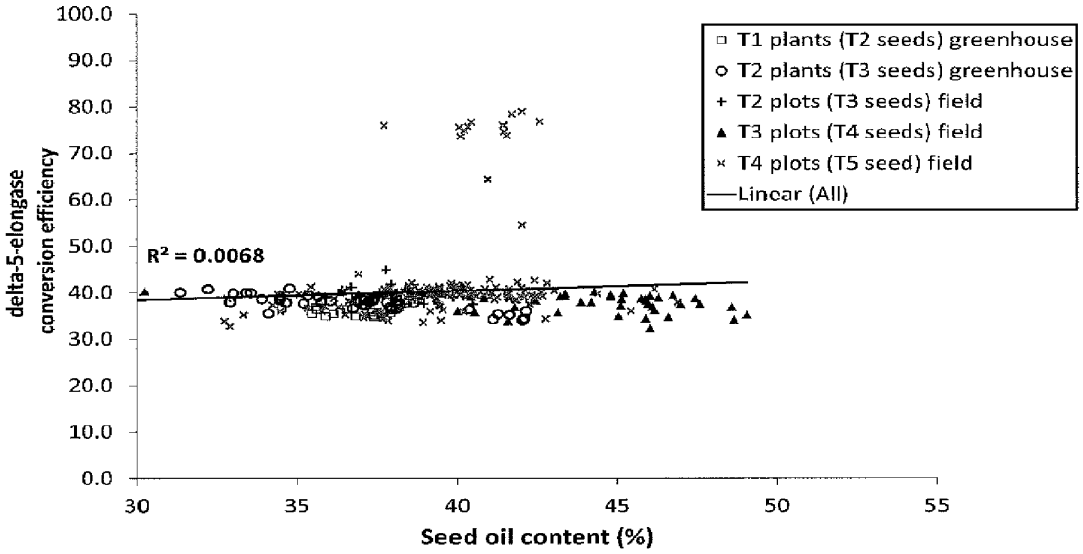


Figure 65: The conversion efficiency of the delta-5-elongase was not correlated with seed oil content. Shown are data of 4 generations of event LAODDN. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

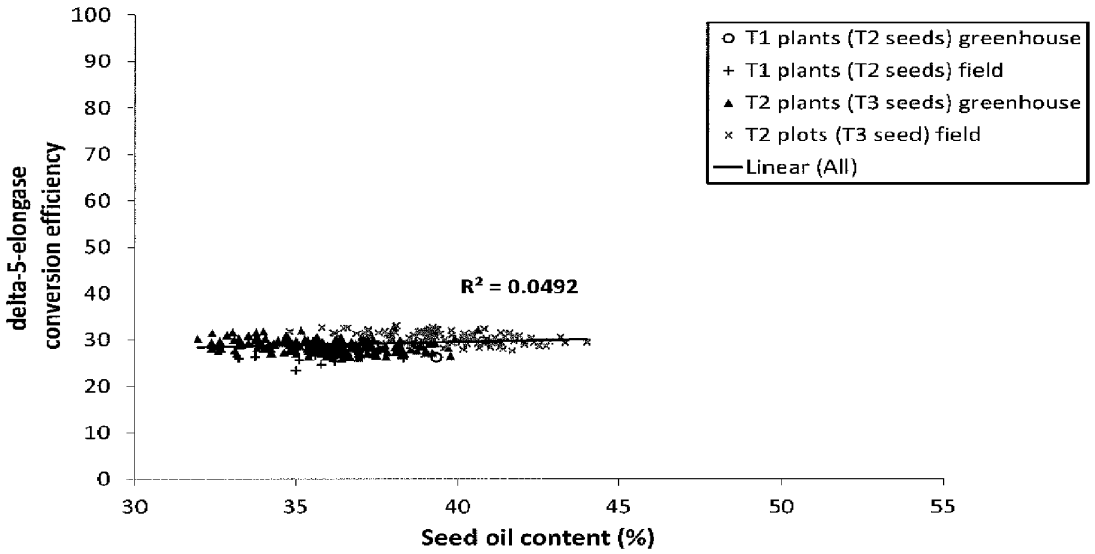


Figure 66: The conversion efficiency of the delta-5-elongase was not correlated with seed oil content. Shown are data of 2 generations of event LBFGKN. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 50 T2 seedbatches, or 182 T3 seedbatches, for the field data, one marker corresponds to an analysis of one T2 seedbtach of one T1 plant, or the analysis of a random selection of T3 seeds representing plots (36 plots) or single plants (60 plants).

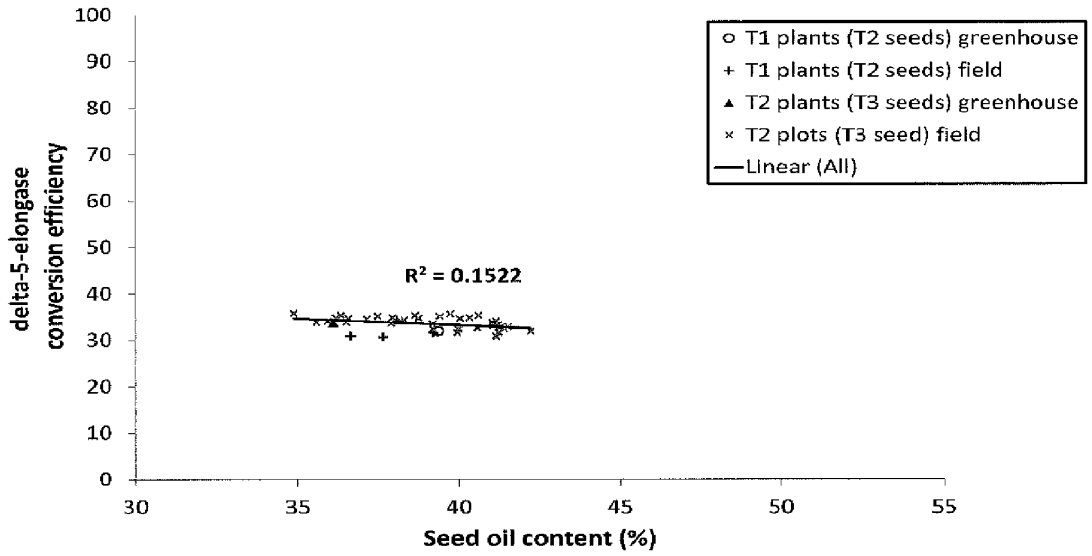


Figure 67: The conversion efficiency of the delta-5-elongase was not correlated with seed oil content. Shown are data of 2 generations of event LBFLFK. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 10 T2 seedbatches, or 195 T3 seedbatches, for the field data, one marker corresponds to an analysis of 1 T2 seedbtach of one T1 plant, or a the analysis of a random selection of T3 seeds representing one plot.

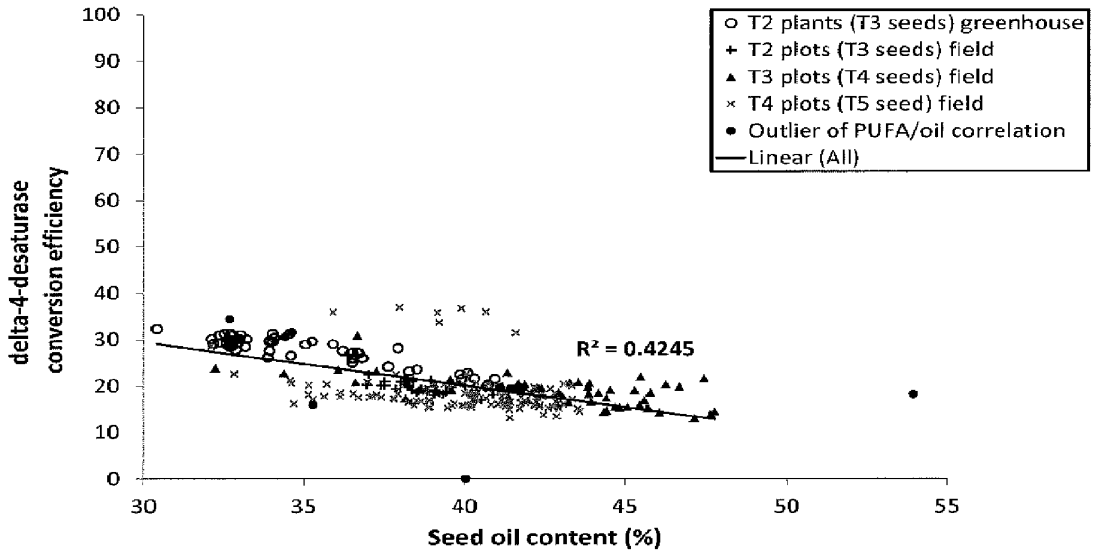


Figure 68: The conversion efficiency of the delta-4-desaturase was negatively correlated with seed oil content. Shown are data of 3 generations of event LANPMZ. For the greenhouse data, one marker corresponds to one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

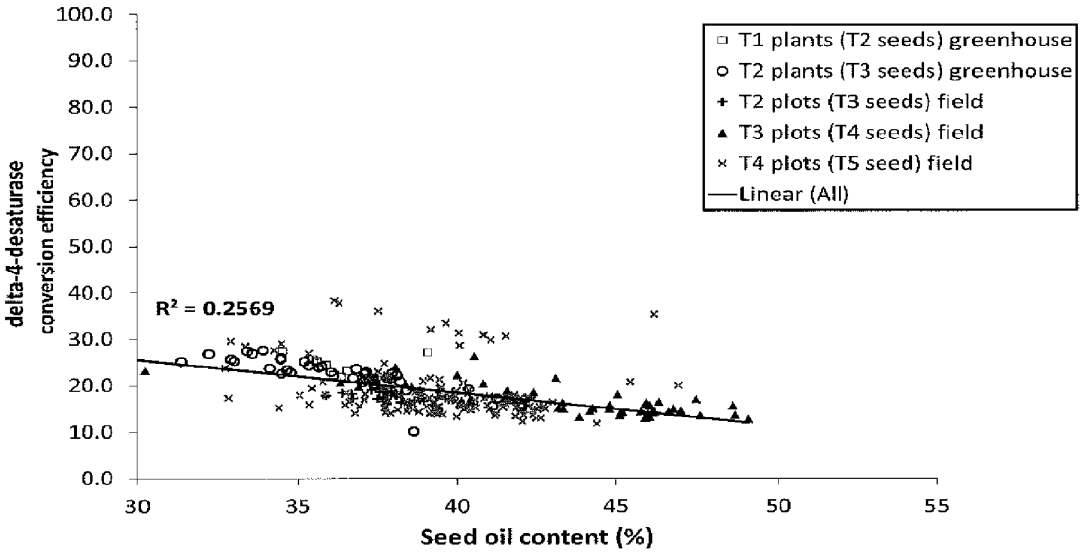


Figure 69: The conversion efficiency of the delta-4-desaturase was negatively correlated with seed oil content. Shown are data of 4 generations of event LAODDN. For the greenhouse data, one marker corresponds to one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

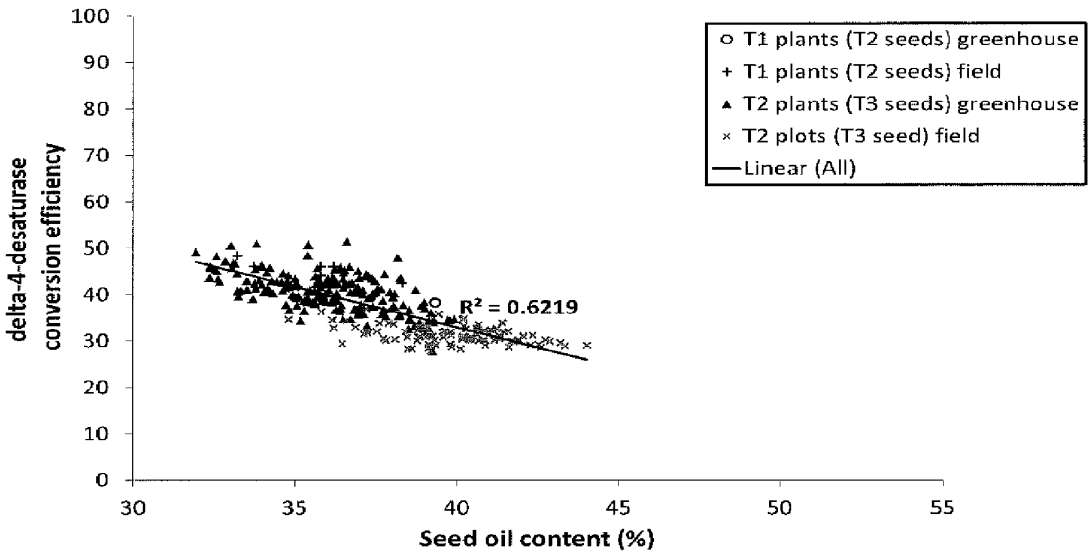


Figure 70: The conversion efficiency of the delta-4-desaturase was negatively correlated with seed oil content. Shown are data of 2 generations of event LBFGKN. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 50 T2 seedbatches, or 182 T3 seedbatches, for the field data, one marker corresponds to an analysis of one T2 seedbtach of one T1 plant, or the analysis of a random selection of T3 seeds representing plots (36 plots) or single plants (60 plants).

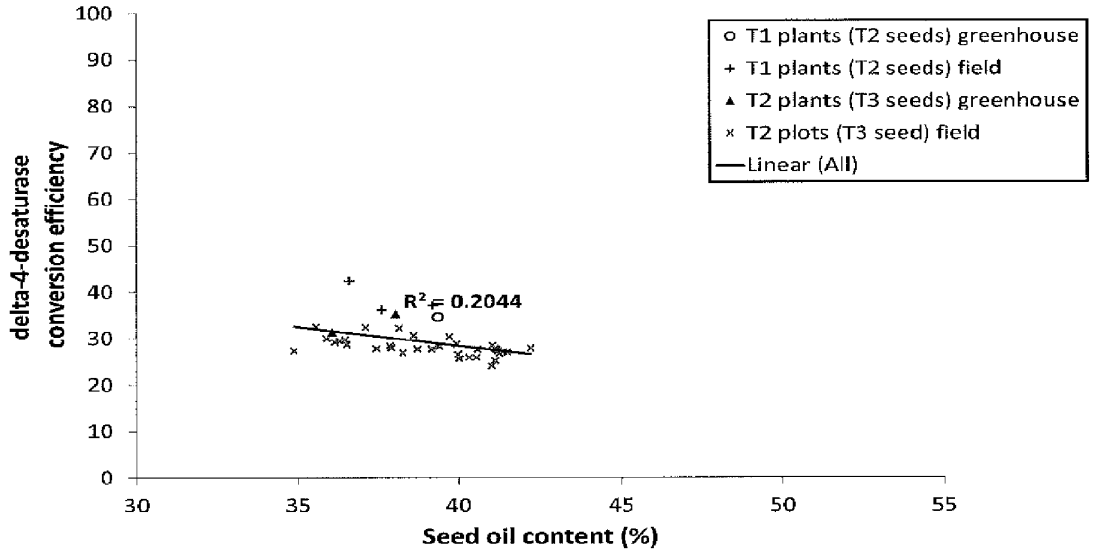


Figure 71: The conversion efficiency of the delta-4-desaturase was negatively correlated with seed oil content. Shown are data of 2 generations of event LBFLFK. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 10 T2 seedbatches, or 195 T3 seedbatches, for the field data, one marker corresponds to an analysis of 1 T2 seedbtach of one T1 plant, or a the analysis of a random selection of T3 seeds representing one plot.

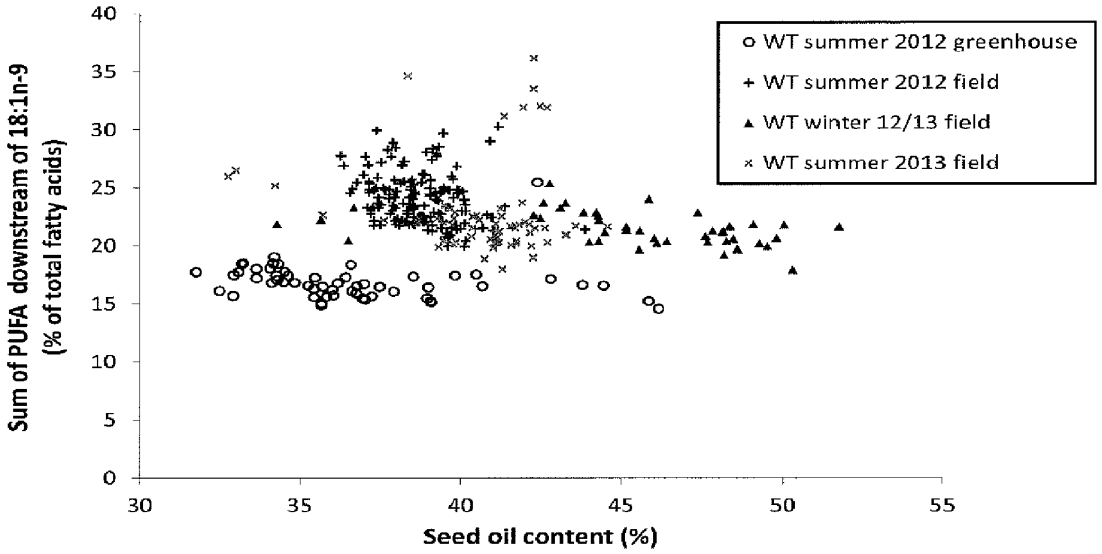


Figure 72: The sum of all pathway fatty acids was not correlated with seed oil content in wildtype canola, but differs between greenhouse and field. Shown are data of three seasons. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

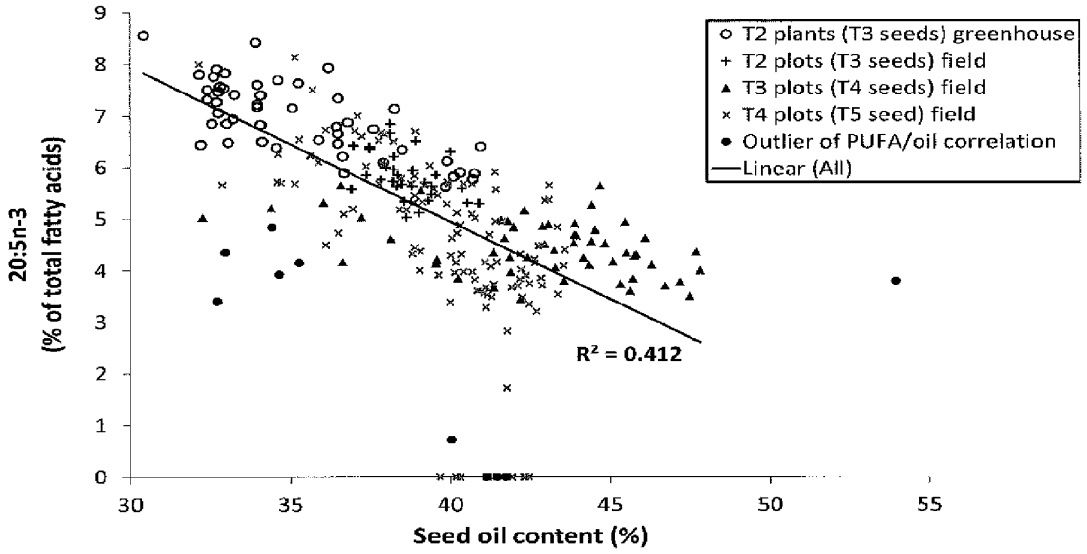


Figure 73: The total fatty acid percentage of 20:5n-3 (EPA) correlated with seed oil content. Shown are data of 3 generations of event LANPMZ. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

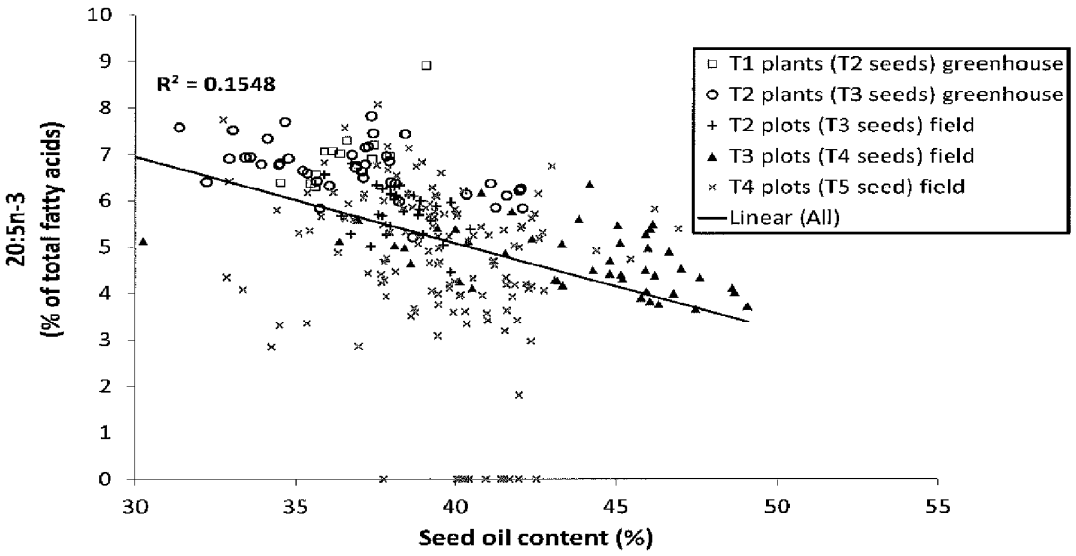


Figure 74: The total fatty acid percentage of 20:5n-3 (EPA) correlated with seed oil content. Shown are data of 4 generations of event LAODDN. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

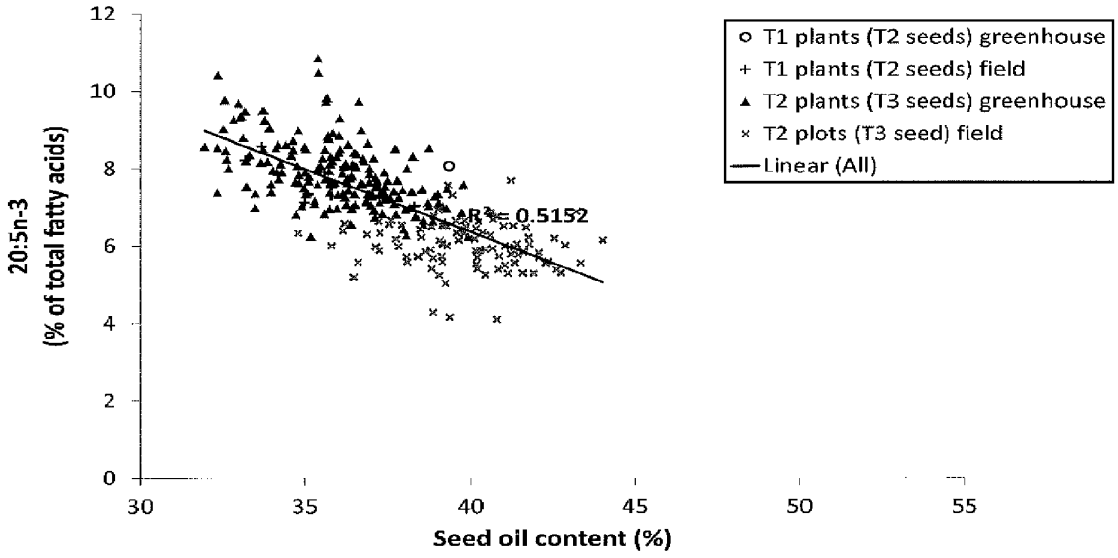


Figure 75: The total fatty acid percentage of 20:5n-3 (EPA) correlated with seed oil content. Shown are data of 2 generations of event LBFGKN. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 50 T2 seedbatches, or 182 T3 seedbatches, for the field data, one marker corresponds to an analysis of one T2 seedbtach of one T1 plant, or the analysis of a random selection of T3 seeds representing plots (36 plots) or single plants (60 plants).

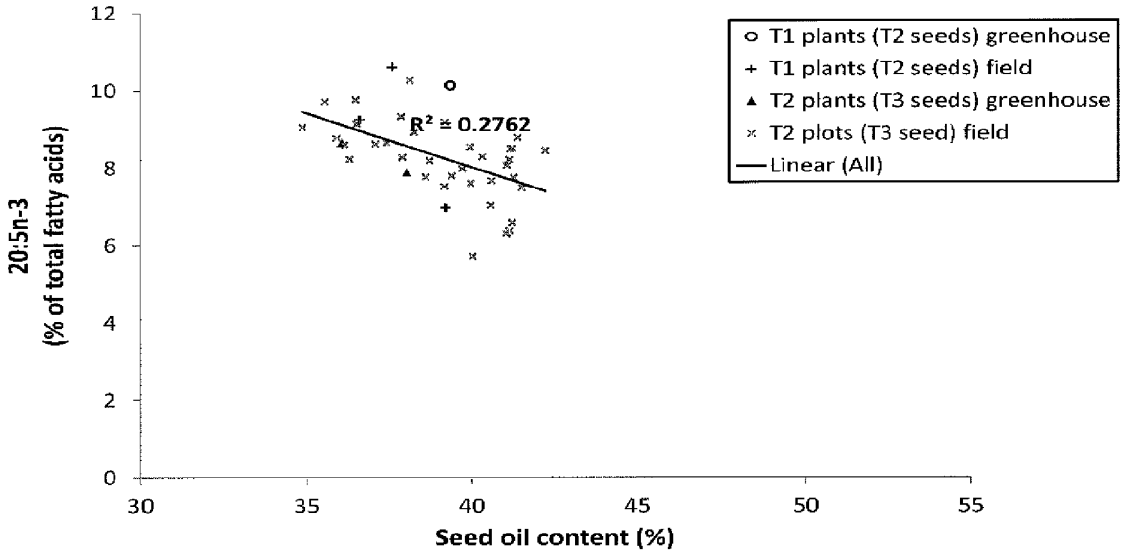


Figure 76: The total fatty acid percentage of 20:5n-3 (EPA) correlated with seed oil content. Shown are data of 2 generations of event LBFLFK. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 10 T2 seedbatches, or 195 T3 seedbatches, for the field data, one marker corresponds to an analysis of 1 T2 seedbtach of one T1 plant, or a the analysis of a random selection of T3 seeds representing one plot.

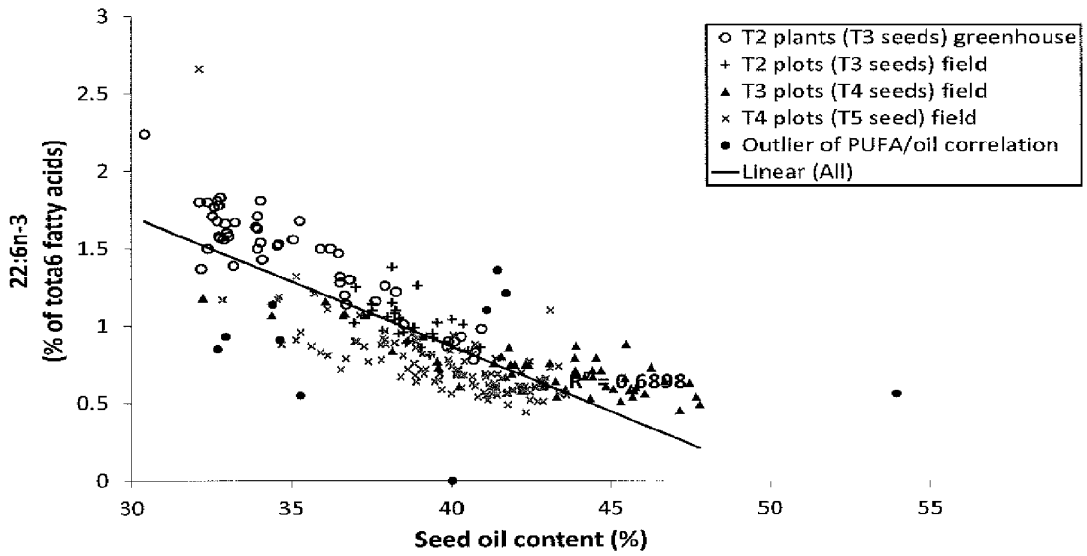


Figure 77: The total fatty acid percentage of 22:6n-3 (DHA) correlated with seed oil content. Shown are data of 3 generations of event LANPMZ. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

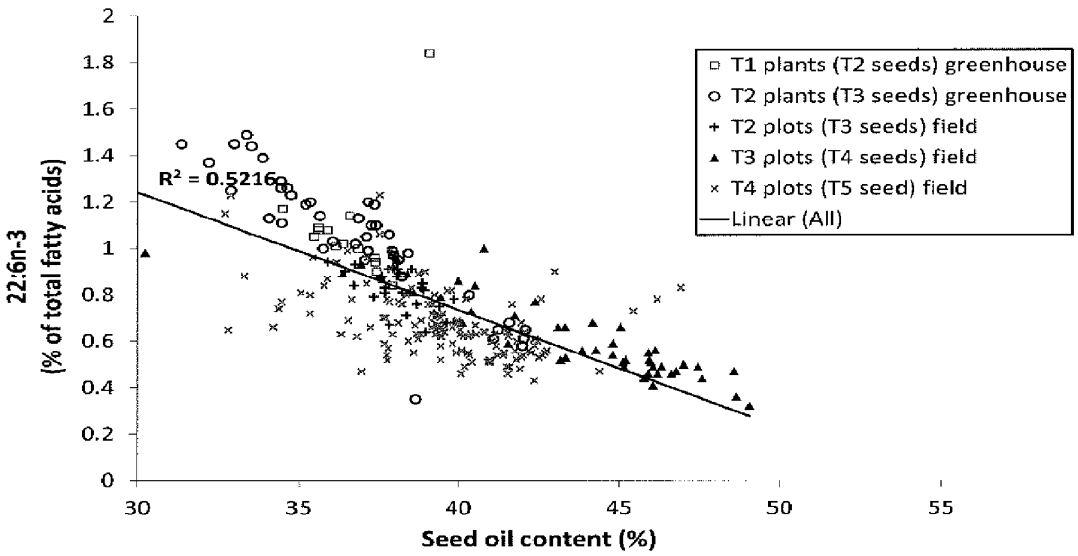


Figure 78: The total fatty acid percentage of 22:6n-3 (DHA) correlated with seed oil content. Shown are data of 4 generations of event LAODDN. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

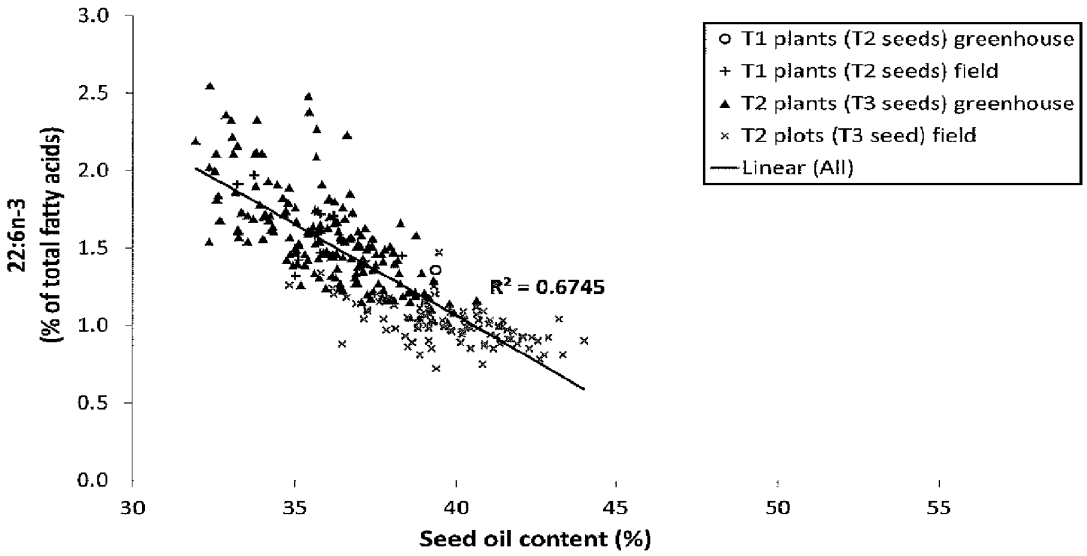


Figure 79: The total fatty acid percentage of 22:6n-3 (DHA) correlated with seed oil content. Shown are data of 2 generations of event LBFGKN. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 50 T2 seedbatches, or 182 T3 seedbatches, for the field data, one marker corresponds to an analysis of one T2 seedbtach of one T1 plant, or the analysis of a random selection of T3 seeds representing plots (36 plots) or single plants (60 plants).

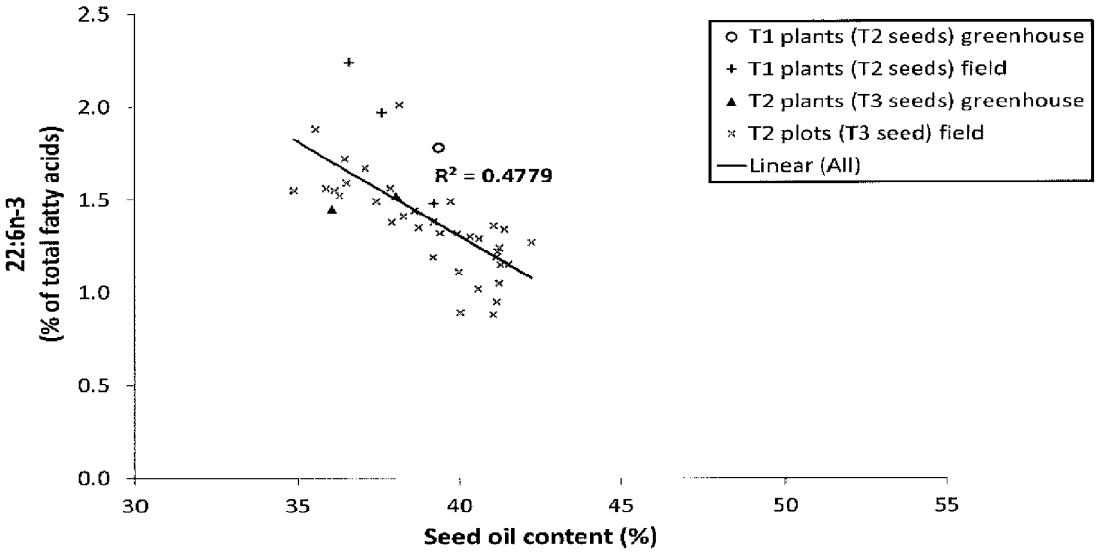


Figure 80: The total fatty acid percentage of 22:6n-3 (DHA) correlated with seed oil content. Shown are data of 2 generations of event LBFLFK. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 10 T2 seedbatches, or 195 T3 seedbatches, for the field data, one marker corresponds to an analysis of 1 T2 seedbtach of one T1 plant, or a the analysis of a random selection of T3 seeds representing one plot.

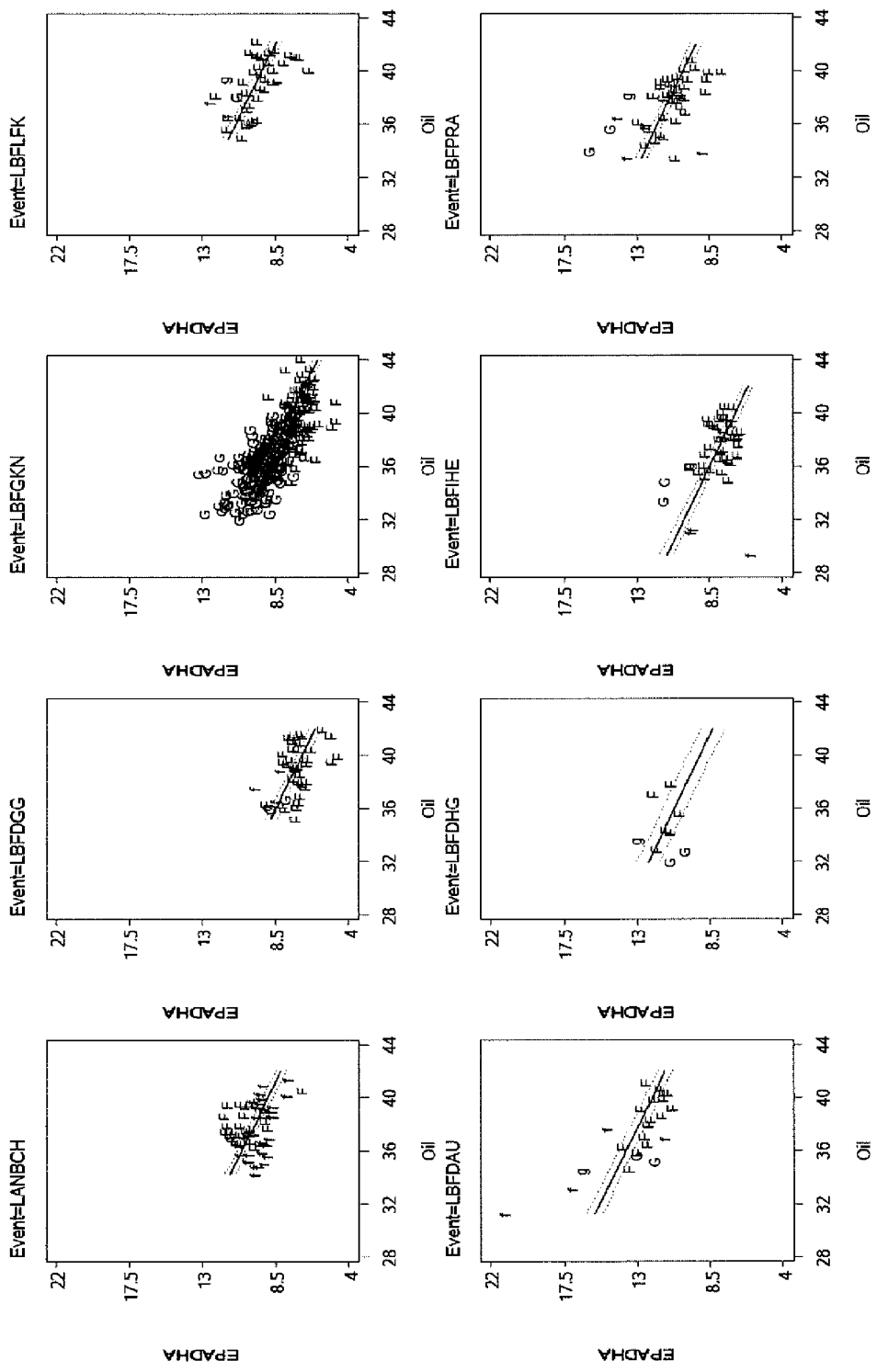


Figure 81: The levels of EPA+DHA (20:5n-3 and 20:6n-3) correlated with seed oil content. Shown are data of homozygous plants (single plant: capital G or F, plots: lower case f, grown greenhouses: G, grown in field trials: f and F). The data are described in more detail in Example 12 (event LANBCH), and example 14 (all other events).

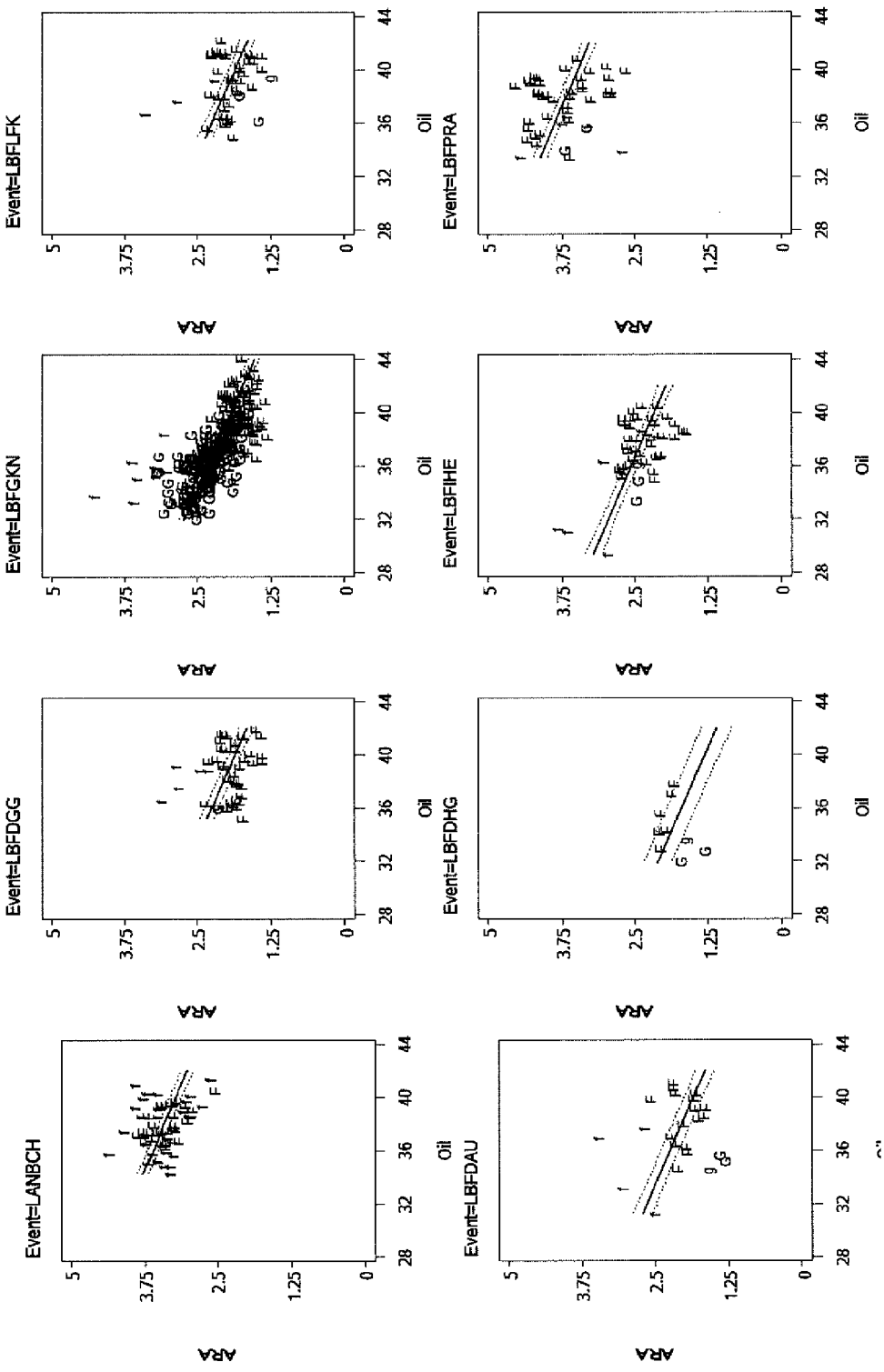


Figure 82: The levels of ARA (20:4n-6) correlated with seed oil content. Shown are data of homozygous plants (single plant: capital G or F, plots: lower case f, grown greenhouses: G, grown in field trials: f and F). The data are described in more detail in Example 12 (event LANBCH), and example 14 (all other events).

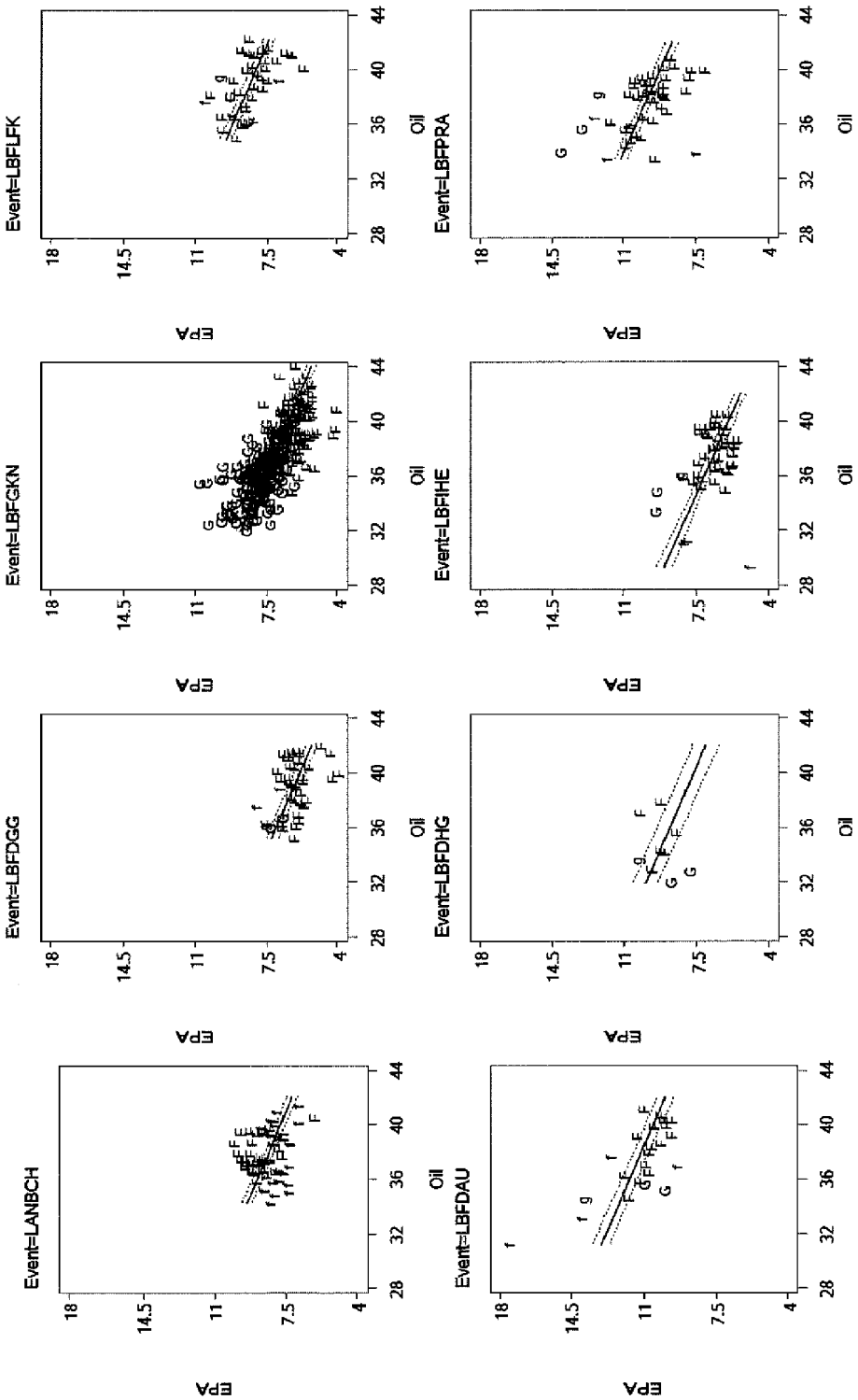


Figure 83: The levels of EPA (20:5n-3) correlated with seed oil content. Shown are data of homozygous plants (single plant: capital G or F, plots: lower case f, grown greenhouses: G, grown in field trials: f and F). The data are described in more detail in Example 12 (event LANBCH), and example 14 (all other events).

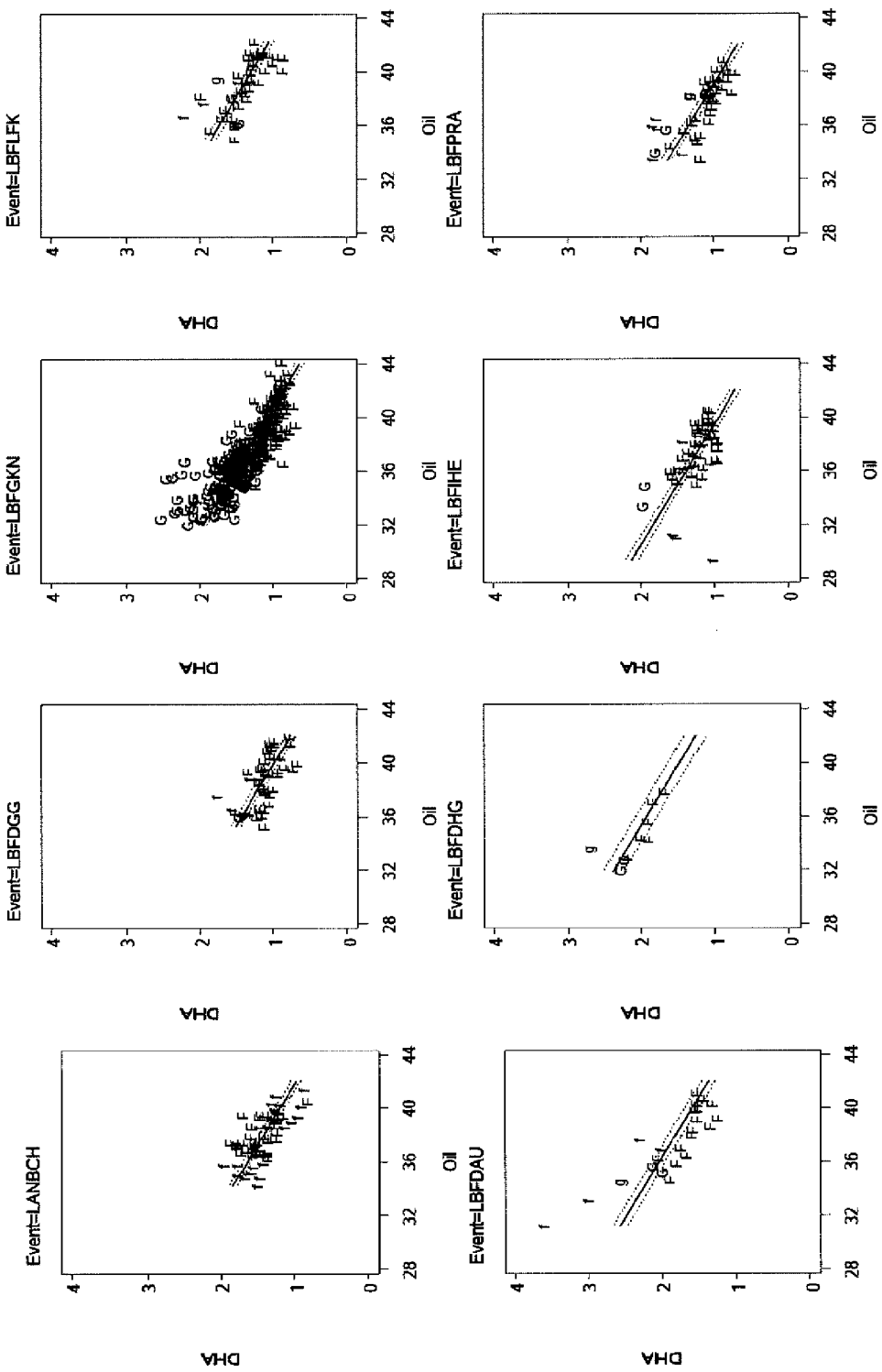


Figure 84: The levels of DHA (22:6n-3) correlated with seed oil content. Shown are data of homozygous plants (single plant: capital G or F, plots: lower case f, grown greenhouses: G, grown in field trials: f and F). The data are described in more detail in Example 12 (event LANBCH), and example 14 (all other events).

Application Data Sheet

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Application Type:	Provisional
Subject Matter:	Utility
Suggested Group Art Unit:	N/A
CD-ROM or CD-R?:	No
Sequence submission?:	Yes
Computer Readable Form (CRF)?:	No
Number of copies of CRF:	0
Title:	MATERIALS AND METHODS FOR PUFA PRODUCTION, AND PUFA-CONTAINING COMPOSITIONS
Attorney Docket Number:	0000077877US02
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Request for Non-Publication?:	No
Total Drawing Sheets:	80
Small Entity?:	No
Petition Included?:	No
Secrecy Order in Parent Appl?:	No

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Representative Information

Representative Customer Number: 29137

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Foreign Priority Information

Applicant Information
Assignee

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Application Number:	62234373
International Application Number:	
Confirmation Number:	4370
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First Named Inventor/Applicant Name:	Toralf Senger
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Application Type:	Provisional

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Payment was successfully received in RAM	$2660

RAM confirmation Number	3475
Deposit Account	503852
Authorized User	DEBNAR, CARMEN

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National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
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<110> BASF Plant Science Company GmbH

<120> MATERIALS AND METHODS FOR PUFA PRODUCTION, AND PUFA-CONTAINING COMPOSITIONS

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<220>
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agattatgta aggctaggta gcgtcacgtg acgttaccta acactagcta gcgtcagctg 120

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actaaggatg acctacccat tctt 204

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<220>
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acagatgaat actagctgtt gttcaca 147

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tattactcac cggtgcgatg ccccccatcg taggtgaagg tggaaattaa tggcgcgcct 180

gatcactgat tagtaactat tacgtaagcc tacgtagcgt cacgtgacgt tagctaacgc 240

tacgtagcct cagctgacgt tacgtaagcc tacgtagcgt cacgtgagct tagctaacgc 300

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gaaatgcgaa tggtaaattg agtaattgaa attcgttatt agggttctaa gctgttttaa 300

cagtcactgg gttaatatct ctcgaatctt gcatggaaaa tgctcttacc attggttttt 360

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actcttgtaa attgaagtat gaaaatatac tgaattgggt attatatgaa tttttctgat 720

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aaactgaagg cgggaaacga caatctgatc actgattagt aactaaggcc tttaattaat 180

ctagaggcgc gccgggcccc ctgcagggag ctcggccggc caatttaaat tgatatcggt 240

acatcgatta cgccaagcta tcaactttgt atagaaaagt tgccatgatt acgccaagct 300

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gcacttgtat cctgcaggtt aggccggcca cacgggcagg acataggg 108

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tatatgatta atgtaattgt ttaatttata ataatagtaa agaattaaac aaaaattata 240

gaaagcatac atattattag caaatcttca ttatttaaaa tcattaatta ctatatatca 300

taatcacatt aggtaattct gtaggttttt atttaagaaa ataatatata ataaatagca 360

accttgctat atcatatgat atcatatagt tggtattaaa tgtttctagt gacatataaa 420

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gggataagac acaacctcct cttatgattg tgtaatatta atagtcctca tataccttca 660

attatttata tgcagtagta gatgcagtat gaagggaaga ggtgtgattc ggagatgcag 720

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cgtcctccac accctgcaac attggctcaa accggtccat tcaagcttgt atccggctcc 1020

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gaacggcaag cccacatcgg aagtcacgtt ttttaatgct atcgaccaca gcccttaccg 1140

agctgcccca ataaagatgg ctcgaagcgg atcatccgca agcttgataa acaagaagaa 1200

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CSIRO Exhibit 1007

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gcctaaccca gaaaatagaa acgattggtt tttatttgag gtaataatgt gccgtggtat 1500

cggcgggaga atactgtggc gttggccaga tgagattctt tagaaagaag tcaaaggttt 1560

ggaagagctg cagcaacaac acatcatcaa aatctgctcc ttctctgaga agacgattgc 1620

catcttctgg gatgcccggt ggcctcaaca aggtctagga gctttggtat gccgagattt 1680

ccatgaagag acaccaggga gacgaggggt ggtggaaggt ttgggggaac attgagagtt 1740

ccgctgctgt tatctcagac tcatcgcata gttcctttaa cttgttatat gctgctactg 1800

tctttgcctg aattactatt ctcaagcaat cctttggttt tttatataat ttgtttctat 1860

tttatctttt gattgcaaca tgttatcaca aaccaaacta atcggtaacg ttattgctta 1920

aacatatcat aagctaagca aatcacacac ctccccaatc catggatatg ttctctcttt 1980

tctcaatcaa tttgtttaca ctcaaacact gatagtttaa actgaaggcg ggaaacgaca 2040

atctgatcac tgattagtaa ctaaggcctt taattaatct agaggcgcgc cgggcccccct 2100

<210> 215
<211> 2065
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 215
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cgttgtgtta tattcagtct aaaacaaggc gcctactacc ggtaattccc gggattagcg 120

gccgctagtc tgtgcgcact tgtatcctgc aggtcaatcg tttaaacact gtacggaccg 180

tggcctaata ggccggtacc caactttatt atacatagtt gataattcac tggccggatg 240

taccgaattc gcggccgcaa gcttgtacac tagtacgcgt caattggcga tcgcagtact 300

taatcagtga tcagtaacta aattcagtac atcaaccgta tttagtttta atttttataa 360

gatttttaca caattataac tttgtttaaa ttaaaatttt attataatat ttttaggtaa 420

ataaaaagtg tggttctaat ggtaaatttc tctgtctgct ccatctatga catcttttgc 480

ggttatcacc tttgctcttt gttagcatca tgtttttttgt aacttttttta attctggttt 540

gtggcagtgg gctccttttc atctgttttg gcaatgacac agctggttgc ttttgaaatc 600

atgctctgtg tttatggctg gtgtgttgtt tatgatacat ttggttgtgt ccctgaatcc 660

ttgtgttgca gttgtttatg atagatttgg ttgtgtccta ctattgtttg cgttcggatt 720

gcgattttgg actggacttg gtttcttagt tgtatttcat ttcgaaaaat tgtaactcgt 780

tatatttttt ttttcttttt gttcttaggg aagaatgatt attgctcatt tatgtgatga 840

gctgaaagaa ctgaaaacaa ttatgtgcat ggtaatattc ctttgcactt gcaaacgcaa 900

taaccgcaag attgaatgat gggtaggtaa attttttttt ttttttttgt ttatggtcat 960

tgcataacat acttttattg gaaattgtct tctcttaaga atgaatgggt ggtttgagac 1020

tattggagat aaaatgattc ttgggagtca gattctttta ccaatatgtg tacacttttt 1080

taagatagat gtttaatgta gtatttttat ttcttatatt gtgtatatac ttattatttg 1140

ttttttcctt aattatatat ttacttattt tatttttttt gtttttatat caaatggtaa 1200

tattacgata gatgtttaat gtaatattta tgtttcttct attctatatt tacttattct 1260

ttatttttttg ctgtaatata ataatggaaa tgataaaaat gtataactat agatttttca 1320

gatagatgtt taatgtaata ttccatttct tatattgtac atttaattat tatttatttt 1380

tcttatatta tatatttatt tattttaatt attttgcctt tcatcaaatg gtaatattat 1440

gatagatgtt taatgtaata tttatttttc ttctattgta tatttacatc tttttatgtt 1500

ttcttataat ttaatataaa aatgtaaaac tatacatttt taaatagatg tttaatgtag 1560

tatttccatt tcttatattg tgtatttact tattttaatt ttcttccttt tatatcaaat 1620

agtaatatta caatagatgt ttaatgtaat atttttgttt cttttattgt atatttactt 1680

tgtttttata atgtattaat gtcgtgtggc atcttttgga agaaggattt tatgttgaga 1740

tgaacattcc atagagcctc aaatagtctc ttttattagt actagaggtt ggtccgccct 1800

acgggcgggt taattacact aaactatttc atattagaat atattttaat tttataaaat 1860

atttgaatat ttactttata ttaaaataat aaatacaatc tatttttatt tctttttatt 1920

ctaagatcat atcataactt ttattaaaat tttttggttt taattataaa gatcaataac 1980

atttttttct gagaaaacaa aattaataaa ttaaagttaa tgaacattta atgatttata 2040

tttttctatt tgatttatat ttcaa 2065

<210> 216
<211> 1500
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 216
atgtatgtgt agattcttgt aattttgcaa taataaaaaa atgtggctta gattaaaaaa 60

aagagttata tatgttgttt ttacattagg tgaggactct gttgtaaaaa aaatgtgatt 120

tgtttttttа tagatgtggt aattttatat atatctcaag gtctcttgtg gattagatag 180

agagtgaatg attaaatgtt tttaaagtgg gctctcctat tgcaataatc taaaaataaa 240

ggtttgtttg ttatacacaa taatttttaa gagagaattt taaacaaaaa atattactcc 300

aatcaaaaat agcattttct aaaaaatatc tcattctcaa ttgatataat ttcttctttt 360

catttgttca ttttatacta cttatttttg gtttaagaaa cttctaaaaa accttcaact 420

gaaaatgctt ttatgcttct aacatttatt tcaaagactt ctgatttatg tttggaacaa 480

atgaaataga gaagcaattc catgaattga agtgattaat gtatgttttg caacaattaa 540

aaatgttgct tgtaagtttg caacaattta aaaataaaaa ggtctaatct gtttttcatt 600

tttcattttt tcacatttag gtgatgagta gttgtagtat aacttaaatg gtggtttgtc 660

ttgttatatt tggttgtcat ttctttattt caagatcttt gatttttttt ttttacaaaa 720

cacatggaat gaattccatg catttgttaa ttgttttgga atttttttttg tactctattt 780

aggtgttagg actctactgt agtatgacct aacatagtgg tgatatgttg atcaaatttt 840

gtgtgacatt tatatgaagg tcttttgatt tatatttagt aaaatgaaat agagagggaa 900

gtttatatga aggtctccag aaattgaagt gactcatgaa tgtttagtta agtaaagatc 960

aatggacttt catcaccaaa tatatatttt ttaaactata caaaaaaaaa tccttttttat 1020

tttaaagaag tttaattata tttaaaaatg atttaaatgt acgtaataat tataaacaga 1080

atattctttt gtagagtgga cctaatgcaa gtaggcaaga tccgacaaaa caagttaaag 1140

acacacccca ccaaaagcaa ccaagagaga cgtctttaat gtactgaatt tagtaactga 1200

aggcgggaaa cgacaatctg atcactgatt agtaactaag gcctttaatt aatctagagg 1260

cgcgccgggc cccctgcagg gagctcggcc ggccaattta aattgatatc ggtacatcga 1320

ttacgccaag ctatcaactt tgtatagaaa agttgccatg attacgccaa gcttggccac 1380

taaggcaaat ttcgcgccct gcagcaaatt tacacattgc cactaaacgt ctaaaccctt 1440

gtaatttgtt tttgttttac tatgtgtgtt atgtatttga tttgcgataa atttttatat 1500

<210> 217
<211> 1948
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 217
cggccgagct ccctgcaggg ggcccggcgc gcctctagat taattaaagg ccttagttac 60

taatcagtga tcagattgtc gtttcccgcc ttcagtttaa actatcagtg ttgctttagt 120

ggtcaatcaa aagatgaaca gtatcttctc gatcgatgat ttctccgatc ccttttggga 180

atcttctccg cctctcgatt cggactcggc caaggccctc acggcggagg aatggaccgt 240

ggagatgttt ttcgaagaga tcgcttcctc cgtgacctct gcgcctgtcg gatccaacaa 300

caacaacaac aacgcgatcg tcggagtttc gtcggcgcag tctcttcctt ccgtctccgg 360

acagaacgat ttcgaggaag acagtcggtt tctccgtcgc gaatcagatg attaccgtcg 420

cgttcttgag aacaagctcg agactgagtg tgcagctact gttgtagctc ttagggtttg 480

tcttctgttt ctctttttttt tatattgcat gtgtaagaag aagaagaaga tctgaatctt 540

aaagctttgt ttgttctctc tgcttgtgtt gtgtaggctg gttctgtgaa gcctgaagat 600

tcgactggtt ctcctgagac tctgtttcaa ccagctctgt ctagtcctct tactcaaggt 660

aataggtgct catcatgcaa attagttttt tacactacat agcttgtctc ttagcttcac 720

catgtttgaa atttttttatg ccaagaaagt gttctaaaaa tcggtctagg cgcctagtcg 780

gcaaatcggg tctgtacgaa atgatttttt caaatcgatt tgaaaatcgg tctagacgtc 840

cgcctaaaca ataattatag cgataaaatt aaggatttgt aatgtagaga tggaaaaaaa 900

aaattttgtt caaatagaga aggaaaatgg ttctttagag aagcttgaaa ccatttcaag 960

tctccttgat atatttttt tgttggagca agtttctaat tttatcataa agtttcaata 1020

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ggtgttagtt caatatcttt ctctttgttt cgagtcttta cacatagtac gtttaatggt 1140

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gaaaaaaact ggtgtaccga tgaagcaggt tactagtgga tcgtctagag attattctga 1260

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ccttgttgtt tgtaggatgc tttcaaatcg agagtcagct aggcggtcta gaaggagaaa 1440

acaggagcaa acatgtgatc tcgaaacaca ggtttctata aatagatcct ctcaacctcc 1500

tattaacatc tccaaggata ttaatagaaa cttaattttt gtgtgtgttt tgataggtta 1560

atgagctaaa aggtgaacac tcctcacttc tcaagcagct tagcaacatg aatcacaagt 1620

atgatgacgc tgccgttggc aatagaatac taaaagccga catcgagaca ctaagagcta 1680

aggtaataac accttctttt ttttcataac cacactaatg ttttgttact ataactaatt 1740

gtatttgatc aaggtgaaaa tggcggaaga gacggttaag agagtaacag gaatgaaccc 1800

tatgcttctc ggtagatcaa atggacataa caacaacaac aatagaatgc cattaactgg 1860

taatagcagg atgggtggtt cttcatgcat cccacctttt caaccgcaat caaatccaaa 1920

catgggggga ctaacaacaa ccattcta 1948

<210> 218
<211> 1600
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 218
caagaccaca agattgcatc tctttgaaag tctcttcagc ccttttgaaa tctccttggc 60

aacaaaatcc attaacaaga gatgagtaag tcaccacgtt tggcttacac ttggtcttac 120

ccatgtctcg aaaaatcttt aaggcggtag tcatatcctg ttgcttcacg tacccatcga 180

tgattgtgga ataagtgaac tcatcaggta caagtctttc ttcggtcatc ctattcatac 240

acatcaatgc ctcattcaac attccggatc tgcaaaaccc ctttatcata gcgttgtggt 300

gcacgacatc aaccttcaca ccctttttcaa ttgagagaga ggaaaccttc tttgcttcct 360

caaaatcacc gcttctgatg aatccatcta tgagggtagc atacacataa gcatccggtg 420

aaattttccg gtctaacatc tccaagaaga ggagcttggc agatgaaaac ccacctctct 480

tacacaaccc actcatcaac atattgtaga tggcagcatc cggtgaaact cctctctcta 540

tcattttacc tttcatctta attgcatcat ccatatgacc tgaagcgaca agaccatgga 600

taagaatccc atacgtaact atatctggtt tgcaacctct ctcagccatc tgcagaagca 660

acttagaagc aatatcatac tctttgactc tgcaataacc ttgtattaga ggggcatagg 720

CSIRO Exhibit 1007

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tgcaaccatt agctactatc caccgtatac tctcagctac ttcaaccttt aaaccatgcc 900

tatacttagc atccataata ttgttcagaa accaaacact gacaactaaa ccccttttctt 960

tcacttcctt caaaagccga tcactcgcca cgaaatcccc tttcttgcag aacccattaa 1020

tcatcgcccc caaaagtctc caagagtcgg cataaacccc ttggacttca actccttgaa 1080

aaccagactc gcgttctcaa catcagccat cttgcagtat ccactaatga tggtgttgta 1140

aaacacaata ttcggaacac aacctttccc ccatcgctct tcaatcagct tcctaccttc 1200

ttctaccttc ccttcgctac acataccttt gagcaatata caagtgctgt aattatcacc 1260

tctctcacgc agttcggatt tcgttaaaat ctaatattct acaaaccctt ttatttttct 1320

tttgaattac cgtcttcatt ggttatatga taacttgata agtaaagctt caataattga 1380

atttgatctg tgtttttttg gccttaatac taaatcctta cataagcttt gttgcttctc 1440

ctcttgtgag ttgagtgtta agttgtaata atggttcact ttcagcttta gaagaaacca 1500

tggaagttgt tgagaggttc tacggagagt tggatggaaa ggtttcccaa ggagtgaacg 1560

ctttgttggg atctttcgga gttgagttga ctgatacccc 1600

<210> 219
<211> 955
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 219
ttttgaacaa gtaaatccaa aaaaaaaaaa aaaaatctca actcaacact aaattatttt 60

aatgtataaa agatgcttaa aacatttggc ttaaaagaaa gaagctaaaa acatagagaa 120

ctcttgtaaa ttgaagtatg aaaatatact gaattgggta ttatatgaat ttttctgatt 180

taggattcac atgatccaaa aaggaaatcc agaagcacta atcagacatt ggaagtagga 240

ttaatcagtg atcagtaact attaaattca attaaccgcg gacatctaca tttttgaatt 300

gaaaaaaaat tggtaattac tctttctttt tctccatatt gaccatcata ctcattgctg 360

atccatgtag atttcccgga catgaagcca tttgtcaacg tcgttgtttg atttcgttaa 420

ggggagagca gtccaagtgt gtttttaatc tcagataggc ctaacatcta gatttatttc 480

agactaaccc aaaaccgaaa cttttgcatt caagtcatct ttcgtgttga ttcgtaattc 540

ttttttttcac attcaacttt ttggtagcag caggttcaga agattcattc ggtttttcaa 600

tgtttcagag taacactaga tttgtttcag attaaaccga aaaatcggat tcgttcagaa 660

gttttatttg gtatgactat gttccggtat gtgatcaata agaaccactt gagttcggtc 720

gagttgtttt tttttttga acttttgtat ctgatcagtt cggatagact cgaaaaagga 780

aaagtaaagt ctaatagaca tcaatatttt gacaaaatat caaaaaatca atatacaata 840

ctaaaagggg atataagcca tggagagggt atccacgtaa gataggaaaa tcaaccaatc 900

agagaaccca aaattttcac atcatctcaa ttatttttcg taaaaaatac aatac 955

<210> 220
<211> 2199
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 220
aaactgaaac tttcaaattc caaagttcga tcagtgtttc taataatcag caaacctgtt 60

gaagaagaca aagtaattgg caaagtaaat tccattattt cataccctct tctttgattc 120

tagtgaagaa gacaaatcaa aatattaatc ttctgctaaa aaactgaaac tttcaagttc 180

caaatttcca aaaaacacat ttctccttaa tagacctttt aacatcaatt caaactacag 240

tccacaagct tttcacatga actggcacaa taaactgcat tcatattctt tggttctagt 300

gaagaagacg catcaaaaac tgtagcagct aaaaaaaaaa ctgaaacttt caagttccaa 360

agttgtacca gtgtttctaa taatgagcaa acctgttgaa agcttttgac ggttttgcca 420

agactcttgc caatctcagg aagcttctta ggtccgaaca gcaacgcagc gactccagcg 480

atcacagcta gctcagggac accgagacca aacagagcat tacaagttag tgcatttctc 540

ctctgctccg gtcggaacgc cactagagaa cgggtgtatg gtcgagtcgt cacagtgaag 600

cagttgtagt agaaggaaga gcgagatgat gagagaggca aagaaactgc tgtgagagaa 660

gaaagagtcg caacatttgt cgccattact gctatcttct ggttctgatg ttatgtgtgt 720

tttctatgag tttttatcca attaaatatt cagaaatgtt atagaaacta aattaatctt 780

cttattgtct caactctaca tgcagtacaa aactcatctc atacgtagaa aagtagaaat 840

taataggaaa actagatcat cactcgcccg ggcggacgga tttttattcc tttgcaaacg 900

cggattgtaa ttttttttatt gtaatgcaaa aattgctata aaacttgtat tttgattttt 960

tttgtgaatt atgaagttaa ttttttttaaa acaattatta cataattgct cagtgaattt 1020

tattatattc taatgtctaa tatattttgt gtattaaaat atgagatcca ccgtaaaaat 1080

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tattcaaaca tgatttttaa atattcaaaa atctagtgtt attaaaattg ttatttgata 1860

aactacccag tcagcatcat cacaccaaaa gttaggcccg aatagtttga aattagaaag 1920

ctcgcaattg aggtctacag gccaaattcg ctcttagccg tacaatatta ctcaccggtg 1980

cgatgccccc catcgtaggt gaaggtggaa attaatggcg cgcctgatca ctgattagta 2040

actattacgt aagcctacgt agcgtcacgt gacgttagct aacgctacgt agcctcagct 2100

gacgttacgt aagcctacgt agcgtcacgt gagcttagct aacgctacct aggctcagct 2160

gacgttacgt aacgctagct agcgtcactc ctgcagcaa 2199

<210> 221
<211> 1748
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 221
aaacattttt tcagattttg aacaagtaaa tccaaaaaaa aaaaaaaaaa tctcaactca 60

acactaaatt attttaatgt ataaaagatg cttaaaacat ttggcttaaa agaaagaagc 120

taaaaacata gagaactctt gtaaattgaa gtatgaaaat atactgaatt gggtattata 180

tgaatttttc tgatttagga ttcacatgat ccaaaaagga aatccagaag cactaatcag 240

acattggaag taggattaat cagtgatcag taactattaa attcaattaa ccgcggacat 300

ctacattttt ttactgctat tctttcatat tagcaactgg ttgattactg aattagtaca 360

gatgtaacta acatatatat tggatattat tattatccaa aagagctatt caacctttta 420

agcatatgta tatacatgtt taattgattt tattagtatt tcttgctctt gttgttttgc 480

ttatattttt gtaatgattt atttatttt agttatttag ttttatcaaa gtggtgatta 540

agagaagcca catgactaat accttgttct ccaacttttt ggttaaaata aatttatcta 600

agatatattt tgatcgattg atatgaatgt tattataaca tttacaattt aaaaaataca 660

gttttttctt tctcatttac attgtttcct ttaggggtga acacttctgg ttattttatc 720

ggttcagttt agattcggtt cgatttggtt aattcagttt tataaatttt tccaactgga 780

gtaaccataa ttaattatgg tttgaatcgg tttaatttac cttaatcaga tccagtttaa 840

ttttttttt aaaaaataat attttataac acatggtgga tataaaaaat gaactatgta 900

aattaaatct aataaaaaac tgaaacacaa tcttaaaaaa ccacaaaagt ttataagaac 960

acaatcaaac caaaataaat agaaaactaa aaacccaaca ttgttaacac agttaatagt 1020

gactttagtt ttagaattta gtatattgta tattgctatt atgtttaatg taaataataa 1080

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gtttaatttc caaatctata acacttgaca aaactcacat cctcgctctt ccttccccca 1440

accctaacaa aaaccttcac gctggggaga aaaaaagcaa caaccttcac cgtcgtctgc 1500

tgctcctcac caccgcgccg tcgaaaattg acctctaccc attactaggt aagcttccca 1560

gacccatcct acatcctcaa ttcactaaac aaacgctaaa gtctctacct ttatctcctc 1620

caatcgtaga tctaaagcta tggctatctc ccaagatctc tacccttccc aagaagacct 1680

cgtctacgag gaagagatcc tccgaaaccc cttcagcctc aagctctggt ggcgctacct 1740

catctcca 1748

<210> 222
<211> 1500
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 222
aaagagaaag caaagtggct tcgccgtgat cgccgagaga ggtcagaagt aaattaaaaa 60

aaaaaaaaaa tcaagagcta aggctcgaga gagatggcgt catcatagac taatcaccgt 120

tagaagatta gtaggaaaaa aaaagatgac acagagacga aagatcttaa cagttgaaca 180

cgtggcggaa aagcgaaatc cgatttaaca gatcggaaaa taaaaaccgg cgagattaaa 240

gaagaaagga gatagagaga agaggtacct tagatccgga tcctgtagat gtggagcagc 300

tcgcttggag actggtgtcc tcgaatccat cagccgaggg agacgcagat ccgccgccgc 360

tccacgagag cggcgtcttc ggactgcctc tagcggaatc aacatccttc ttcaacgaaa 420

cgacaccaaa tcttgacgac cgagaccgcc gttgacggac tccccaccgc aacggaggaa 480

ggttcgtcga tggatcttcc gaaaccaccg tcccagcatg cttcagccgg ataagaagct 540

ccacgaccat ctcgtcgtcc gtcatcgccg ccgccaccca ctcatcgttg accaacatct 600

tagccgctgc cgatgtaact ttcttcatcg acgctaactg atcaaatcta cagctcccgg 660

agaaaaaggg aatcagatac agtatagaag gagcgagaga gaatgaaaga gaagttacag 720

aagagagaga gaaaatgacg aagaaagaca gagggaagtt tgatgtatcg atatagagga 780

gagaaaaaaa gatacatggg tggtgttata tacgaggcta tctaacatta ttcttctttt 840

tatttttatt tttatttta tttttccttg tttcattttc atttatattt tgatttattt 900

tccattttgg aaatatttta atcaaataca atgtgggacc ttcttggaaa gtatatctaa 960

aattatatgt gaagattctc ctaataaaat tatatgtgaa gattctccta ataaacctcc 1020

agtcagcatc atcacaccaa aagttaggcc cgaatagttt gaaattagaa agctcgcaat 1080

tgaggtctac aggccaaatt cgctcttagc cgtacaatat tactcaccgg tgcgatgccc 1140

cccatcgtag gtgaaggtgg aaattaatgg cgcgcctgat cactgattag taactattac 1200

CSIRO Exhibit 1007

gtaagcctac gtagcgtcac gtgacgttag ctaacgctac gtagcctcag ctgacgttac 1260

gtaagcctac gtagcgtcac gtgagcttag ctaacgctac ctaggctcag ctgacgttac 1320

gtaacgctag ctagcgtcac tcctgcagca aatttacaca ttgccactaa acgtctaaac 1380

ccttgtaatt tgtttttgtt ttactatgtg tgttatgtat ttgatttgcg ataaatttt 1440

atatttggta ctaaatttat aacacctttt atgctaacgt ttgccaacac ttagcaattt 1500

<210> 223
<211> 1323
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<220>
<221> misc_feature
<222> (578)..(578)
<223> n is a, c, g, or t

<400> 223
actcttgtaa attgaagtat gaaaatatac tgaattgggt attatatgaa tttttctgat 60

ttaggattca catgatccaa aaaggaaatc cagaagcact aatcagacat tggaagtagg 120

attaatcagt gatcagtaac tattaaattc aattaaccgc ggacatctac atttttgaat 180

tgaaaaaaaa ttggtaatta ctctttcttt ttctccatat tgaccatcat actcattgct 240

gatccatgta gatttcccgg ttctttttct cagactactc actgcccgaa gccactacta 300

ctacacacat tattatctct ctcacatctc tactatttac tcttggattc cgcaaagtaa 360

gtaaaaaaaa aagaaagcaa actctgcttc tcaaatctct ctctacccat ttgctctctc 420

acattttcct ttgaccaaaa ctgaatcttt acttcaagca gcgtaaggtg ctcgctcctt 480

tttgttcctc tgactttggt tttgatatga agttttcatg aaactctttc attacacttg 540

ctttttgaaa aaaaatgatg tagctttcct ggaaaatnta gtttcttttc attatcttgg 600

gatcttgtga tagattaaat gttatgttta cagttcttca tacctggtgg tcaaactctc 660

tttttctttg tttttgtaag ttttaaggtt taatgtttaa agctatttct gtgatgcact 720

gtgattgttt gctaatgctt tactttaatc aatctgatga ttgtttgctt cactcaagtg 780

gtttttataa attagattct ccttttttcat ggaaaaaaat tcatccagga atccaaatag 840

attttatttt tttgcttgtc ttgtctttga gtggagtatt aaatctattt actttatatt 900

aaatttttttt ttatgtaaaa agatatttaa actcaattat gtgattcatg atagtagttc 960

ttcaacgtct actaggtccc ctccttttcac gccaataatg gaggagatga tgaaatttaa 1020

catacaaaat tacagtttaa ccctcttgtg ttccatttag gattaagaat ctggtaggga 1080

cataaaacgt aaatctacca tattgtgtgc tgcttgtgta gtttgtgtag gacccttggt 1140

taccaacctt catgatgata ggttgtgaac attacatagt caagaggagt tccctttttt 1200

tatgacttta ttatagtcaa acaacttgag tagttaacat caatggtttc aatgtcaaga 1260

tatgagttaa gtctttcttt attttgtttg gttttatttg ggtaaaactc cagaaatgaa 1320

agc 1323

<210> 224
<211> 1100
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 224
ttgagttttt gaaatttata acccttttgta aatctaagtt ttaggaaaaa gtcgttgaat 60

tatgaatcgt ttgcttttac cacaagctgc tcgatcaatc ttttagagtt ttcaaatcta 120

aatttaacac aaaaaaatca atttttagtt tttgaattat gtttccattg ctgcgacagc 180

ttaaaaaata atccattttt tatcgtcttc catcatttgt ctccagcttt cggagtggaa 240

atgagttctc ttagactcaa gttggagatg atttcaaatt ggggcgttgg aggaactttc 300

attctttaac agtttataaa gtttgtaaat catttacaaa gtttttaaat catttataaa 360

tgtttataaa ttatttgtaa gagcatataa accatttata aggtttataa aggtatgtaa 420

ataatttatg agggtttata aaatgtttat taagttcata aaaaggtttt ataaagttta 480

gaaacaattt ataacgactt attaatcatt tataaagagt ctataaacgt tttataaagg 540

gtatgtaaat gttttataga cccttataaa gtttgtaaac cctaatagaa ccccgacatg 600

aagtttataa aagaacagtt taggcccgaa tagtttgaaa ttagaaagct cgcaattgag 660

gtctacaggc caaattcgct cttagccgta caatattact caccggtgcg atgcccccca 720

tcgtaggtga aggtggaaat taatggcgcg cctgatcact gattagtaac tattacgtaa 780

gcctacgtag cgtcacgtga cgttagctaa cgctacgtag cctcagctga cgttacgtaa 840

gcctacgtag cgtcacgtga gcttagctaa cgctacctag gctcagctga cgttacgtaa 900

cgctagctag cgtcactcct gcagcaaatt tacacattgc cactaaacgt ctaaaccctt 960

gtaatttgtt tttgttttac tatgtgtgtt atgtatttga tttgcgataa atttttatat 1020

ttggtactaa atttataaca ccttttatgc taacgtttgc caacacttag caatttgcaa 1080

gttgattaat tgattctaaa 1100

<210> 225
<211> 1413
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 225
atatactgaa ttgggtatta tatgaatttt tctgatttag gattcacatg atccaaaaag 60

gaaatccaga agcactaatc agacattgga agtaggatta atcagtgatc agtaactatt 120

aaattcaatt aaccgcggac atctacattt ttgaattgaa aaaaaattgg taattactct 180

ttctttttct ccatattgac catcatactc attgctgatc catgtagatt tcccggacat 240

gaagccattt acaatccatg tccttataaa tggtttatat actcttataa ataatttgct 300
tcttcatgcc tcaagaatat tactactttc aagaatagga aaatctgtgt actaaaaact 360
tcatctcggg ttctatcata ttgtggtact atcagtaggt gactctgtgt ttctatctgc 420
aacataaaag catgaaacta ataagattgt acaattattt tataagtctt tataaatgta 480
aacaatgtaa taaaatctca gacattaaat gaataagttt tgactattat tttataagtc 540
cttgaaagta ttaaattgta ataaacacct caaatatttt gatacttcca gcaatgagga 600
agtcttgagt actagaaacg tcaattcgtg tatgtatcat gtttgacata attaattgtg 660
atctatgcct ttccatttgt aacataaaaa catgaactca ataatttcta tacaataatt 720
tttttaagtt cttatgaaaa tgtaaaagga gttctaaata tctcaagggc attaccatag 780
acaaccttgt ttcgcacaat caaagagtgt gtacatatat gtttgcaata taaaaacacg 840
aaactttgtg attgtgtcaa agtaaataac aaagccttat aaataaaaca tgaccaaaga 900
gttaacctca gaaattgttg cacaaaaacg aagaaatctt ccaatttctt tatgtttgct 960
cataaaagag gccacttgga gatcatttct aataaaaact gaaagaacct cttttgtacc 1020
aaagaacctc ttttgtgtat tatttataaa ctcttataaa attgtatcca ttaataaaag 1080
tttataatta tttataagta tttacaaatt gtagcacaca caagatagca attgatgtag 1140
cttcctcggc taaaacatga tttcctgtaa caataccctt tctagctgat ctcaaggcaa 1200
aagtgaacat ttgtttcatc gcgttacttc ctcctggcat atctagtgaa agagccacgt 1260
ccttcaacaa gggatatatt gcctcaccct ctctgtagac tgtacagaga ccatgcttgt 1320
gatcagaatc aaacaaactc atttcaaggt ttagttttat aaatactact acttacctta 1380
actcttgtgg atttagaagg gaatgagaga agc 1413

<210> 226
<211> 2200
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 226
agctccccag aacaaaataa aaatcgaaac tttgacacta cgaaacgatc aattacgagt 60
gaaaagcgaa aaagaatcga actttaacaa caagagccgt cacaaaggaa gagtgtcaaa 120
ggtaccacag tattggacat cggtggtggc tcgaacaatc gttgagaaga cgaggagaag 180
aggaagaatc gcgattctcg acatcgttga tggaatctcg gaggggtgat cgatgatcaa 240
aagcccaaag gaggttaaac caggagcgga gcagagtaac tttacttctc ttttatattc 300
tcatggaatg taggcgtcat cgtgaccgtt cgtttagcta ggtcattctt gcctttacag 360
taaaacggac ggctagattt cgtttagaga agttatctga tatgaatgat tgcacctgat 420
tatccgtcat aacgaaccat caggattaac caactttgac tgcatacctt cttatttatg 480
acctcttctt ccttattttc ttaaagatat ttatgtaagg agaataaaat ttgaatacaa 540

ttctttttt catgttggca agatctaaca tggattgtgg tatacagatt cactaaaacc 600

tcgtcacttt gtagatagca agaataatgt atagtctaga taacaagaat aatgtacatt 660

tgttcatgaa attatactga tgaattaaga accatgaaag cacatacgtt aagtttcaat 720

ataaataaaa ctatttgtta aaaatagaca tctatttgta ataattagag actaattcat 780

ttacaaactg tataagtgca ttattgtatt attttatttg ttgcgtcatg tatcaatata 840

attaattttc ttccttcggt tgaaaaaata taatctttta ttttagcaaa gggtttaact 900

aaagaaatat aattgctatc aaatataaat aaattataat ctcattaaac tgttcatgtt 960

gtctcaagaa tataccatta aattttattt gtataaggaa ccaaataaca cccgtgaaac 1020

tttacaactt gaaagtaata gtgtactcta agaatgtcaa caaaagttga aatgtaatat 1080

aaataaagaa aagacacgaa gtcaaacatc tatcgaccgg taacagcgtc ggcgacaggt 1140

gtcatcgccg gagacggcaa tggagacgaa cggcatacgg gacacgtagg atgaatcatg 1200

agccaagggt caacgcactt gtcgtggaac aaatggttac aatccggcaa aaccctaatc 1260

atatctgtct tcttgtaatc cgcgagacat atagaacaac acgacgtcgc agtggcatcc 1320

ttttgcaagc tataacccac acgagcctct tcgcacggaa ccttagagca tgcgcaacgg 1380

aggatgaccc cgaatcctta gcgcttaatg gttccattag ttttaatata ataataatat 1440

taagcttaat aaacgagcct ctgccagtca gcatcatcac accaaaagtt aggcccgaat 1500

agtttgaaat tagaaagctc gcaattgagg tctacaggcc aaattcgctc ttagccgtac 1560

aatattactc accggtgcga tgccccccat cgtaggtgaa ggtggaaatt aatggcgcgc 1620

ctgatcactg attagtaact attacgtaag cctacgtagc gtcacgtgac gttagctaac 1680

gctacgtagc ctcagctgac gttacgtaag cctacgtagc gtcacgtgag cttagctaac 1740

gctacctagg ctcagctgac gttacgtaac gctagctagc gtcactcctg cagcaaattt 1800

acacattgcc actaaacgtc taaacccttg taatttgttt ttgttttact atgtgtgtta 1860

tgtatttgat ttgcgataaa tttttatatt tggtactaaa tttataacac cttttatgct 1920

aacgtttgcc aacacttagc aatttgcaag ttgattaatt gattctaaat tatttttgtc 1980

ttctaaatac atatactaat caactggaaa tgtaaatatt tgctaatatt tctactatag 2040

gagaattaaa gtgagtgaat atggtaccac aaggtttgga gatttaattg ttgcaatgct 2100

gcatggatgg catatacacc aaacattcaa taattcttga ggataataat ggtaccacac 2160

aagatttgag gtgcatgaac gtcacgtgga caaaaggttt 2200

<210> 227
<211> 3137
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 227
ctgatttagg attcacatga tccaaaaagg aaatccagaa gcactaatca gacattggaa 60

gtaggattaa tcagtgatca gtaactatta aattcaatta accgcggaca tctacatttt 120

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tgaattgaaa aaaaattggt aattactctt tctttttctc catattgacc atcatactca 180
ttgctgatcc atgtagattt cccggacatg aagccattta caatgagtca aaaaaaagtt 240
gttttgatga gtcactgcta caataatgta agatgataaa actcagacct caatcacaga 300
ggcaaacaac aacactcatt agtcatgatt ttttacccaa acaaaaagat gttatagagc 360
tttaagaaaa aaaaaaatta aaattacaaa tcggtctggt aagagtggta gattgatttt 420
tagaagttta tattccttct ttttgggttc ggtaagaatg atcatcatgc ctgtcttgtc 480
ttttacaatt tccgtaaacg tgcaatgatg tcagattggt aaaagtagct cagctttagc 540
taagcaaatt cgaattagtc aaaattaact aatttaatca tcatatatgc ttccactgat 600
atgttttcac attgcaatat ggctcagctt acttaatatg atgatggtta agaagaaatc 660
gtcaatcata acgagcattt gactagtgcc catgagacct ggtgatatgt tgtttgattg 720
aagcagcaaa aaaaaaaaaa acacctaagc atgtatttta tatatctatg atggaaatta 780
tgcaatccag ggtctagtaa cataactgat gaagctataa aaaaaaaacc atatctaaaa 840
aactatttat acaaaggcaa ataaagtgaa ttgctgctct acatgtcggc cacagatggt 900
acggatccaa tgtctatgga aaatttgatg cacgttagct ccttcttttg cgcgtccagc 960
atcttcattt gcagcgagta tgaaccctat aaagataaaa taaaaagagt agagaatctg 1020
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ggtcaaatac cactaaacta agcctaagac atccaatatg atacaagaga tgcttcaaga 1140
ttaggtagca agaaccaata aagaaaaagc gaatggaact tacaggagga gtgtaaccag 1200
gaagaacttg agaatgtgca accaagaagt ctccggtttc aacaggacaa gttgtctcag 1260
agcaaagttc atgagtctcg gagtgaatgt gccatccaaa gtatgtaact tcaatcacca 1320
gcttcccacc cgtaatccca cgcccttttat taaaagaaaa acacatatca taaacaacac 1380
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agctataggg ttaggagata tattaacctc tttcactttc acttcatact ctgcgttctc 1560
ctctgcaatt aacaaaatac attacactat caaggcagag agctccccag aacaaaataa 1620
aaatcgaaac tttgacacta cgaaacgatc aattacgagt gaaaagcgaa aaagaatcga 1680
actttaacaa caagagccgt cacaaaggaa gagtgtcaaa ggtaccacag tattggacat 1740
cggtggtggc tcgaacaatc gttgagaaga cgaggagaag aggaagaatc gcgattctcg 1800
acatcgttga tggaatctcg gaggggtgat cgatgatcaa aagcccaaag gaggttaaac 1860
caggagcgga gcagagtaac tttacttctc ttttatattc tcatggaatg taggcgtcat 1920
cgtgaccgtt cgtttagcta ggtcattctt gcctttacag taaaacggac ggctagattt 1980
cgtttagaga agttatctga tatgaatgat tgcacctgat tatccgtcat aacgaaccat 2040
caggattaac caactttgac tgcatacctt cttatttatg acctcttctt ccttattttc 2100
ttaaagatat ttatgtaagg agaataaaat ttgaatacaa ttctttttt catgttggca 2160

CSIRO Exhibit 1007

agatctaaca tggattgtgg tatacagatt cactaaaacc tcgtcacttt gtagatagca 2220

agaataatgt atagtctaga taacaagaat aatgtacatt tgttcatgaa attatactga 2280

tgaattaaga accatgaaag cacatacgtt aagtttcaat ataaataaaa ctatttgtta 2340

aaaatagaca tctatttgta ataattagag actaattcat ttacaaactg tataagtgca 2400

ttattgtatt attttatttg ttgcgtcatg tatcaatata attaattttc ttccttcggt 2460

tgaaaaaata taatctttta ttttagcaaa gggtttaact aaagaaatat aattgctatc 2520

aaatataaat aaattataat ctcattaaac tgttcatgtt gtctcaagaa tataccatta 2580

aattttattt gtataaggaa ccaaataaca cccgtgaaac tttacaactt gaaagtaata 2640

gtgtactcta agaatgtcaa caaaagttga aatgtaatat aaataaagaa aagacacgaa 2700

gtcaaacatc tatcgaccgg taacagcgtc ggcgacaggt gtcatcgccg gagacggcaa 2760

tggagacgaa cggcatacgg gacacgtagg atgaatcatg agccaagggt caacgcactt 2820

gtcgtggaac aaatggttac aatccggcaa aaccctaatc atatctgtct tcttgtaatc 2880

cgcgagacat atagaacaac acgacgtcgc agtggcatcc ttttgcaagc tataacccac 2940

acgagcctct tcgcacggaa ccttagagca tgcgcaacgg aggatgaccc cgaatcctta 3000

gcgcttaatg gttccattag ttttaatata ataataatat taagcttaat aaacgtaagg 3060

attggtccat aattaggtgc tggacggact cgtcattgtg ttacgtgtcc gaatttgagt 3120

ggattcgggc cggcttt 3137

<210> 228
<211> 1100
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 228
tatttttgtt catgtcttat tttctttttt cctaatgtaa ctatgagagg cttaaaaact 60

gtaaaatcag caaaacaata tacaattaca gtaaaaaatg tcacatacta agttctatat 120

atgactacaa gtctacaact caactaatca tccacataaa taattagttt tgtcataatt 180

atattatagt aagtacctga agaaaagata aagccatttc tggacaacat catctcgtat 240

tggcatcttt atacgtggac gacaaaatct atcacaataa tagttgctag atatagatac 300

atgaattttg taatatgatt aattaattgg cgcttcataa ctaaaataac taataaaggg 360

taaatgttct taaagtttca taattaatta tgtttcagag tggttgcatt atagtagttt 420

aaaattcaga agtgtacgcg acgagaaaag agatttgctg gtgactattg catcatcttt 480

gacatggaaa aaatcttaga taagaatagt ttgaaattag aaagctcgca attgaggtct 540

accaaaatta gaaattagaa agctcgcaat ccagtcagca tcatcacacc aaaagttagg 600

cccgaatagt ttgaaattag aaagctcgca attgaggtct acaggccaaa ttcgctctta 660

gccgtacaat attactcacc ggtgcgatgc cccccatcgt aggtgaaggt ggaaattaat 720

ggcgcgcctg atcactgatt agtaactatt acgtaagcct acgtagcgtc acgtgacgtt 780

agctaacgct acgtagcctc agctgacgtt acgtaagcct acgtagcgtc acgtgagctt 840

agctaacgct acctaggctc agctgacgtt acgtaacgct agctagcgtc actcctgcag 900

caaatttaca cattgccact aaacgtctaa acccttgtaa tttgtttttg ttttactatg 960

tgtgttatgt atttgatttg cgataaattt ttatatttgg tactaaattt ataacacctt 1020

ttatgctaac gtttgccaac acttagcaat ttgcaagttg attaattgat tctaaattat 1080

ttttgtcttc taaatacata 1100

<210> 229
<211> 811
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 229
aatttttctg atttaggatt cacatgatcc aaaaaggaaa tccagaagca ctaatcagac 60

attggaagta ggattaatca gtgatcagta actattaaat tcaattaacc gcggacatct 120

acatttttga attgaaaaaa aattggtaat tactctttct ttttctccat attgaccatc 180

atactcattg ctgatccatg tagatttccc ggacatgaag ccatttactc tgaccctact 240

ccacaaatat atttttattt ataaaaaggt ggccattgta tactatgtgt gcgtatacag 300

gaataaaaat gtgtcaatgt atatgtaaac tgattccatc ttatatgtaa tgtgcgtgtg 360

taaatgaaga tactagtatc catgtgtcgc ctacttgatt tgttcaactg taactcataa 420

tatctcaaga ttctttcttt tttttctacg aatatcgcaa tctataatac cattaaatta 480

ttgtaacaaa attggttgac atttataaaa tgaaaaagaa gagaagagca tttaaacacg 540

actgatgaaa gtccaatgta gctagataaa ccacgcgtgg tggtcaatgc gttccattcc 600

aaaaggatcc gagttcgaat ccgcaccaca ccagattttc actgcgcgtg gccatgaagc 660

tttcgcattc tcgctcctga gaatggttct ccatttttt tttccagtgt agctagatac 720

cggtctgaat ctaggtttat aatatgctga caatgtaatg ataattaata catcaaaaca 780

tgtgtttctg aaccaaaata aaaacttttt t 811

<210> 230
<211> 2900
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 230
gaaaaacctg catctccaaa aatgttcaaa tggcttaaaa acagagaaaa tgagtggaat 60

attagataga tctaccttta tagaacacac aaaaatacat atctaaaatt attaaatctt 120

cctttaaatg agtggaagat gagaaccatg tgatgaaaaa cctgcaaaac aagataaatt 180

agtaagaaaa acatgagaca gaaacaataa attgatataa agtttgatgt ttataagttc 240

aaagggatta aagagaggtt tgagagtttt agaacgagga acataccatt tttgttgcag 300

ccatttgaga ggagaagaga gaatgtgtaa atgtttttt atataaggag acaaaaattc 360

caataaggtt aaatattttt gatcagaaga cttactagac gacttacttg taagtcgccc 420

agaagacttc aatattttta gcgggaaact aaaatatttt tagcgggagt tagaagaccc 480

taaacataac ccttaaacta aattaactaa ctaaatactt cataaaatca aattaaactt 540

aaaaagtgtt tactatacac agaaataatc acatgtagat ataaatttaa tttttcaaaa 600

aaacatttaa gctttccaaa atctaaccct aagaatacat acaatactac aacatatgtt 660

gccaaaccct agaccaaaga atatcatgat tcactacttt cactcatcta tgttgaaaac 720

aattcaattt tattatatct taatttatat cacttaaaac tgtttataat tacatgattt 780

taattttccg tttatcaaaa tatttttac aaaatttata aattattttt aggatcaact 840

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aagacgtagc ggggatatat tcataaaaat gagttctgtt tttttgtttg gtcacaaggg 960

gctggttgta atttcacaag gcttttggat tacttttgca tttgattcaa gtttgggtat 1020

acttttgcaa tcaaaatcaa gttttgagtc atatttggta aatcgcccta tataaaataa 1080

aattttaaaa agtaatgaat ctacatattt tgtaattttt aaaaaattta gttaacaatt 1140

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tatgtaatat ttttatataa gtaataatgt gaatagaatt tatcaaatca tatgttagaa 1260

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tcggaatttt cggatcgaat cgaaacgaat aacgcatcaa atcaaattta acggataaaa 1500

ccttagtaac acatgcataa accttagtga acttctcaag ctttcgattc tctatcttat 1560

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tactcggtaa gccgagatgt atatccacag ttggagtctt tggatttcat atccagaatt 1860

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atttatatcc atgtacatat atacccttgg ttacaaggac acctacccat acatacgcat 2040

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tgtgattttt gtggttcttt tttttcatct cttacattga ttcagtatgt tgtccaaaaa 2160

aaaaacagtg attcagtatt atatcgagta aattcacaag aacgtagcta caatgtagat 2220

gatttattaa caattttaca agagacaagc aaatgtcgag caatcatatt ctataatatc 2280

aacctaaaag agttaaatcc ataaaattag ttggcaacga gcgatagtat gaaagttagg 2340

CSIRO Exhibit 1007

tgatgacaaa agttgctata ttgcttcaac tatattttca taaatttatt tgtctggatg 2400

aaaaccacaa aattttaaaa atataatttt gattggtaat atgtaaataa cgggatccta 2460

tatttaaacc agtcagcatc atcacaccaa aagttaggcc cgaatagttt gaaattagaa 2520

agctcgcaat tgaggtctac aggccaaatt cgctcttagc cgtacaatat tactcaccgg 2580

tgcgatgccc cccatcgtag gtgaaggtgg aaattaatgg cgcgcctgat cactgattag 2640

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ctgacgttac gtaacgctag ctagcgtcac tcctgcagca aatttacaca ttgccactaa 2820

acgtctaaac ccttgtaatt tgtttttgtt ttactatgtg tgttatgtat ttgatttgcg 2880

ataaattttt atatttggta 2900

<210> 231
<211> 1800
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 231
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tggaagtagg attaatcagt gatcagtaac tattaaattc aattaaccgc ggacatctac 120

atttttgaat tgaaaaaaaa ttggtaatta ctctttcttt ttctccatat tgaccatcat 180

actcattgct gatccatgta gatttcccgg acatgaagcc atttacaatt gaatatatcc 240

tcacatatga aatatatttt ttttttacaa attacaccta ttaaattata cttgatcggt 300

catctgatat atttgaaaga accctatcag ccagctattc ataatttaca taaaagaaaa 360

ttacgtgctt aaaatctctc taaaaaaaaa aaaagacaaa gacatcaaac tgatccatga 420

aagtaaaatg gagtgtattt taattttatc ttcagaccaa tgttatcaat gtagcccata 480

tattaatact aaaacaactt ctgcacaaac acacgaatca aagcctcgtg tttcatcgta 540

gctttagcta aaatttccca aaagcaaatt caatagtatt ttactaggtc aaacccacaa 600

gagaaaaaga aagtcaatcc caaggatcaa gaaatgagaa gtgagaggag aatgctttat 660

tgggtttgct aataactaat aagacatgaa gcagactgaa aacatctggt tttgtccaaa 720

aaagaaggaa gtcagattcc aaaactgcgc acctacattg tttaatactc actcacacat 780

acattcatgt ttttactgtt tatacacagt caataattta tacacagctc catgttttaa 840

tatttaccca tctctctttt gtagtctatc gtagactttc acttgtgtcc ccctcatgcg 900

gcaacatcct cagcaacttg atttactata tacaataata caaatcataa gatatttgtt 960

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agtgtttgtg taaaatgttc gacaagacat tgactaaagc attccgaaca tgtcaacaaa 1200

actacaattc taaaattgca aaaagctgct aaacggtgga atagcattta acacgcattc 1260

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gagcttgtac tagctctatc tttccaaact taactttctt gagtgcttat ttatataaag 1680

catcagtata tggcccaacc caagaaaagc tgaacaaaat tagcaacaat agcaagggac 1740

gaactgcagc tcttcttggt tgtcgtgcct tccaattctc gactttccgt ggaagaacat 1800

<210> 232
<211> 1600
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 232
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atgatatggc agaagaactt gaagagggtg agatacggag tgtagatgag gagcatgagg 120

ctgaagaaga tgatgtgaat gagatggttg cttggagcaa tgatgaggat gaggaccttg 180

gttggcaaac aaatgaacct ataaggataa ataatgagct caaggtacct atccttcagc 240

atttcacatt tcataatcat agctgttagt ggttgagttc tttttattgt ctctaagcct 300

ttgttaactg tttgctaact ccaaaccctt tttaactttt gcaggagctt cctccagttc 360

cacctgtgga tgtcactttg gaaccacatc atgtcatgct tcctgttgga gttgttcttt 420

cggtatcata aaataaactc tacctgtgcg ctattgcttg ctaaacctaa aaccattgtg 480

atttctgatg ttccaggtac tgagcacacc actgacacaa gtaatagtgg agggaatgga 540

aaagcatagt cctttggccg aaggttctat cctatggata agtgaaagaa gaacgccatt 600

gggactggtg gatgagatct ttgcacaagt tgagtgccct tactatagtg tgaggttcaa 660

ttcagaaaac gaagtgccgg agggggttag tgaaggtaca cctgtctcat tcgttgctga 720

gtacgctcag catattctca atatcaagga actgcagaag aaaggttacg atgcatcagg 780

agataacgac gaggaggtag cagaggagct tgagttctca gatgatgaga aagaagcccg 840

aatacataaa aatgcagaag atggaaaaga gagggatgat ggatgaccaa aaagatggta 900

acgcaagaaa caagaagaag aaaaacaatg atctcggaac gtctacaagc aatgactcag 960

gggaatggac agagaaccgt gggtttagtt ctttatcctc caaccgttct gatcctcaaa 1020

tgggtgatcc ggtttcgaac catcagccac gacctcagat ggtgggtttt cctccaccgt 1080

ttggaggaaa atgatcaccg aaccgcgccg tgcgcgggtc gtcggtgagc cagagtttca 1140

CSIRO Exhibit 1007

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agtccacgac gcccgtgatt ttgtagccct ggccgacggc cagcaggtag gccgacaggc 1260

tcatgccggc cgccgccgcc ttttcctcaa tcgctcttcg ttcgtctgga aggcagtaca 1320

ccttgatagg tgggctgccc ttcctggttg gcttggtttc atcagccatc cgcttgccct 1380

catctgttac gccggcggta gccggccagc ctcgcagagc aggattcccg ttgagcaccg 1440

ccaggtgcga ataagggaca gtgaagaagg aacacccgct cgcgggtggg cctacttcac 1500

ctatcctgcc cggctgacgc cgttggatac accaaggaaa gtctacacga acccttttggc 1560

aaaatcctgt atatcgtgcg aaaaaggatg gatataccga 1600

<210> 233
<211> 3284
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 233
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gctaagagcg aatttggcct gtagacctca attgcgagct ttctaatttc aaactattcg 120

ggcctaactt ttggtgtgat gaaagtgtga gaatatttcc atattagaca attctgtaga 180

ttttatttaa tgaaagtgtg agaatatttt tttgtacact atttatcaat ttaataaaaa 240

aatataatat aatttaagat gaaccaacat atttctctaa attctgaatt ttatttttcat 300

ggtgacacgt ggatacgaaa caatgttgta atgtttatcg attaatatat aatgtatgtt 360

atatgtttgt tgtactaaca acaagatttc atacatttca aacgaaaaag agagattgat 420

gtgatcagta ttattgcttt tacaaaacta ttgaaggttc agcgagttct tagtaaagtc 480

gaatccttgt aataatctct gttccaacgg gcaaacatgc agctgttaaa ctatcgacgc 540

cgtttgacct atttttttctt cttctttgtt ttcttcctca tttgtacatg cgtgcaagcc 600

tactgacggt tggatgcctt ggcacgcact tcaacaactc tatatccttc ctggtgaggc 660

tatgtacgct tttaagcttc ttttcttgga cgcccttgag cttcttgctt ggaggtggga 720

gggtgagaag attagccctc ttgctagagc tttgttcgct cctgctgttg cttgtaagct 780

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ctctcacaac ttcgacggtg ttggtagcgt tggacctaag ggatcacttc ctagatcagc 960

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cgatgaaata gggtaattca aaatctagct tgatatgcct tttggttcat tttaaccttc 1200

tgtaaacatt ttttcagatt ttgaacaagt aaatccaaaa aaaaaaaaaa aaatctcaac 1260

CSIRO Exhibit 1007

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agctaaaaac atagagaact cttgtaaatt gaagtatgaa aatatactga attgggtatt 1380
atatgaattt ttctgattta ggattcacat gatccaaaaa ggaaatccag aagcactaat 1440
cagacattgg aagtaggatt aatcagtgat cagtaactat taaattcaat taaccgcgga 1500
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gcttacaaag tgattcaagc tatgcctgag aagccaacgg ctaagacatg gggggctctg 3240
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<210> 234
<211> 1700
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 234
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aaagtcgaat ccttgtaata atctctgttc caacgggcaa acatgcagct gttaaactat 120

cgacgccgtt tgacctattt tttcttcttc tttgttttct tcctcatttg tattactatt 180

ttataagttt tgtgtagcgt tgtaaagctt tgacgctacg tagcgttacc taaggtcacg 240

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tgaggctagc tagcgttagc taacctcacc tgacgctacg tagcgttacg taacgtcacg 360

tgagcctagg tagccttacg taacgtcagg tgacgctacg tagacaatca gtaaattgaa 420

cggagaatat tattcataaa aatacgatag taacgggtga tatattcatt agaatgaacc 480

gaaaccggcg gtaaggatct gagctacaca tgctcaggtt ttttacaacg tgcacaacag 540

aattgaaagc aaatatcatg cgatcatagg cgtctcgcat atctcattaa agcagcaatc 600

aattattcat taatcactac tcagccttac caccttctc agcaccaggt ctagttccca 660

tcttacccat atgctgaagc attgagctaa ggttagatcc aacggtaggg aagtgacggt 720

acttaaaacc gagcttctct atgtgagtcc taaccacagg agctatttga gcgtagtaag 780

agtggtgaag cctagggaac aagtggtgct ctatctgaaa gttaagtcca ccgttaagaa 840

ctccaagcca gtaaccacca acgttagagc tagtctcaac ctgacgttga acgaaagtag 900

ctgatctagg aagtgatccc ttaggtccaa cgctaccaac accgtcgaag ttgtgagaga 960

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gcttacaagc aacagcagga gcgaacaaag ctctagcaag agggctaatc ttctcaccct 1140

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cgttacgtaa cgctagctag cgtcactcct gcagcaaatt tacacattgc cactaaacgt 1680

ctaaaccctt gtaatttgtt 1700

<210> 235

<211> 1532
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 235
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gcatgtgtag ctcagatcct taccgccggt ttcggttcat tctaatgaat atatcacccg 180

ttactatcgt atttttatga ataatattct ccgttcaatt tactgattgt ctacgtagcg 240

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tcaaagcttt acaacgctac acaaaactgc ttgcgtttct tctctcatta cgagaatata 480

ctttttttcaa tattgcttca aaaactttct gtcatgtgaa tttgatttta gatatatgtt 540

taaaaaatac ttcctccccg aaaataagat tttctaaagt gttcgattat taagaattca 600

ttaaatattt ataatttaat ttttctttta ctttattata cattttctaa taacttttta 660

ccaataaaat ttaatcaatt caaatatttt tatttaatgt ttcttaaaag tataaaaaat 720

atcttaatca tatagaaaat atatttttgt ggaacaagta aaaatctaaa acatcttact 780

ttcaggaacg gaggtaatag atagttacat taaaggtctc aacattacta ttttaagtct 840

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cacaaataaa gaagaagaag gtgatatctt tgaggttgat atctcgaaga aaatggggaa 1200

gaaagagcag aaagatcatc attcaacagt agaaagcgac gacaaagtcg aagctgttct 1260

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<210> 236
<211> 1200
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 236
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ttttttgaag tgctggtttt ttatattctc gggttaataa atattgcagg agaagttctg 1140
taacagagct tgtgtcagta gattcttgag aacaaaagga gacaatgcga agaaagcagc 1200

<210> 237
<211> 1600
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 237
aaaagaaata taaaagaata tgaccaaaaa agtaaacgtg agtgagagaa taagaaaatg 60
actacaaaat ataatagcct caattatctt caaaactaag ttgacattta attatgcttt 120
tgcaagatat ttacttttgt tgttcgatca tatttaatga ttattttggt tttgaaacaa 180
atattaacat tatatatatt gtgtctatat tgaactgttg taaattataa acatcaaaat 240
tttaatgtta tcttaattat aatttctaat actagtatat tcaaaaatca aaataaacat 300
attttataaa atagtgccag tacgtagtat gggagataat actagtggct ttataaaggg 360
aaacattgtc tctaaaatct cagataaaat gttaaaacac acttattcac aattatgaag 420
atttgaaata tctgaaattt caaattgatg cacttggtag aaagcaaagg ttcaacgcta 480
agtctacaag gtgtaataat gaagtgaaaa tgctagttta gattaccctt gatatgtgac 540

tgaacatagg gtggagcgtc agtgagtcca tggagtacag aagctaaaca agagacatgg 600

ttaagcacca gaatcaactc gttctccata gagtccagct tttgagatat atgtgaatag 660

ccttgttgca atatacttgt gagtggcagg cgtgatctta ttaacgaaag tccaaattct 720

gaacaaagtt tatatcaagc tacgatggaa atatggaatc cgtatcaaaa tcaactgtac 780

tgtatcatac ggtgcagatt tttagctcga ctctaccacc ttgcgtttac ttttgtgatg 840

aacattgcga ttatatatga ggacctaaat agagggaaaa tgtatgaaga caggatccta 900

agaatgaaaa accagcatcc ccaagatgtg gcaccaagtg ctatcgacca caaactacgc 960

tggacatact ctgatatagt tcgttaagaa atcaaaatgt caacacatat aaataagcag 1020

tcagcatcat cacaccaaaa gttaggcccg aatagtttga aattagaaag ctcgcaattg 1080

aggtctacag gccaaattcg ctcttagccg tacaatatta ctcaccggtg cgatgccccc 1140

catcgtaggt gaaggtggaa attaatggcg cgcctgatca ctgattagta actattacgt 1200

aagcctacgt agcgtcacgt gacgttagct aacgctacgt agcctcagct gacgttacgt 1260

aagcctacgt agcgtcacgt gagcttagct aacgctacct aggctcagct gacgttacgt 1320

aacgctagct agcgtcactc ctgcagcaaa tttacacatt gccactaaac gtctaaaccc 1380

ttgtaatttg tttttgtttt actatgtgtg ttatgtattt gatttgcgat aaatttttat 1440

atttggtact aaatttataa caccttttat gctaacgttt gccaacactt agcaatttgc 1500

aagttgatta attgattcta aattattttt gtcttctaaa tacatatact aatcaactgg 1560

aaatgtaaat atttgctaat atttctacta taggagaatt 1600

<210> 238
<211> 1677
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 238
taattgaaat tcgttattag ggttctaagc tgttttaaca gtcactgggt taatatctct 60

cgaatcttgc atggaaaatg ctcttaccat tggtttttaa ttgaaatgtg ctcatatggg 120

ccgtggtttc caaattaaat aaaactacga tgtcatcgag aagtaaaatc aactgtgtcc 180

acattatcag ttttgtgtat acgatgaaat agggtaattc aaaatctagc ttgatatgcc 240

ttttggttca ttttaacctt ctgtaaacat tttttcagat tttgaacaag taaatccaaa 300

aaaaaaaaaa aaaatctcaa ctcaacacta aattatttta atgtataaaa gatgcttaaa 360

acatttggct taaaagaaag aagctaaaaa catagagaac tcttgtaaat tgaagtatga 420

aaatatactg aattgggtat tatatgaatt tttctgattt aggattcaca tgatccaaaa 480

aggaaatcca gaagcactaa tcagacattg gaagtaggat taatcagtga tcagtaacta 540

ttaaattcaa ttaaccgcgg acatctacat ttttgaattg aaaaaaaatt ggtaattact 600

ctttcttttt ctccatattg accatcatac tcattgtaag acacacagat gaagaagtca 660

aatagctcga cattcctttg gtctggtcca gtgatgtcga ctcacaaagc gaagattgca 720

CSIRO Exhibit 1007

tgaatagact atcatgtgtt tgctatgtat gagtactatg gaatcgaggg atcatttatt 780
tttcatgtca ttgttcttgg aggttgtgtg gagaggcatc accattaatc ttctgaacat 840
cagctatact aatcattaat tggaatcagc gactgcaaat tatggcagag gatgttggaa 900
atagtaccac cacattccta atccactatg ctttccaagc tgcagtaatt tgagttggag 960
acgggagttt tgctcattta ctcaggctct tggacaaaca aatgtgaaat cgcatatctg 1020
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tgaaagcatc taatgtttat agaaaattac caaaaatacc acatttatga aaaattatca 1140
aaaatacaat attcatagta tcacttttca tatttacaat aaccacgttt gttctcaatt 1200
ttaacgaaga acaaacgaca tttataatcc taagataatt ttttctaatt caaaaataat 1260
tttcgatttt caaaaaaaaa attgaaaaaa aaattgaaaa gaaaaattca aaacaaaatt 1320
atatgaaagt tcaaatttga aaaatgataa ttcaaaaaca taaaaaaata tatttttatt 1380
taaataatta tttattatat atatatagac catggagcaa gggggcgtgg gccccggggc 1440
ccaaactttt tttcccatat ataatgtcaa caaggacccg atttttagaa aaaaaaatag 1500
gtataaagga gtccaaaaaa ttcaaattag ttatatatgt atgtaaaaaa atattaaaat 1560
ttatttttgcc caagggccta tagattcatt gggccgaccc tggggttggg gttgtgtgtg 1620
tgtcattcta taattaaccg gcaaaaactc caaaattttt atttaacaaa tgtataa 1677

<210> 239
<211> 5600
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 239
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ttagatgata aattttcatt gtcttgagaa taataaaaaa aaaatctaag gataatatca 120
tagttaaatt tatatgatat ttacttcagt aatattaaaa tattatatac atttttatta 180
tatttggttt agtaatatta aatggattct attttaaatt tcttatgaca atcaaaacca 240
cttagtgtga taatttctga aaaaaattgg caaaaaaatc aaaaatactt atcttattat 300
ttgtagtgat ttttctctct ctcatgttaa aattttgaat gtttaaagtc tttattatct 360
ttaataaata attagattaa atttttaata tataattacc cataatttaa aacaaatttc 420
attaatttta aaatcatcat tatctaaaaa gattatatat tatgttatcc aaaaatattt 480
tacatcataa tattttaaaa taaatataaa tttatgtata ttgttttatg tatatatgaa 540
tgtttttaag tttatttttac ataatcaaat atattttaca aaaataattt ttatcatata 600
taaaatttaa catttaatta attattaaat atttcaaaag tatgaatata acttattctc 660
atggttttta attgataata tatctattta caatttttttg taaaattatt aaacccgcaa 720
gtatggacaa aacacctagt atatatattt ggaacaaaga atacagacaa aacacctagt 780

atatatattt ggaacaaaaa atatacgtac atattttata tacatgaata acttatatat 840

cacttagaaa taggataatc aattgacatt aaactctctt aaattatata ttgtatagaa 900

ctatagatat acgtataaaa tatttataaa agataactac actatatata gaaacagata 960

atgatacatc cacgaaaatt cttctggaaa agaaacagag tggtttcgcg tcagcacacc 1020

tacgttgatc attggaaatt ggaatattga aacacgcttc aaatcaacga ctattaatta 1080

ccaatacacc ctggctttgg ggtgagagtt gatcggttaa ttatccaata catgccgttg 1140

gttaattagg attatataaa aaatcgatca tctattagaa tcgattacgg ttaaataggt 1200

ataaaaatgg agagaattga atcagttata aatttgtttt cagttaaaat atttctatga 1260

tcttcaatcg atttcggtat tttatactca acatggaaaa aatttcaaat gtatttcttc 1320

taaaagcaaa agaatctata aaaactatca ttttatccaa aacaccaaaa tagtctttta 1380

caatctttta cagccttcac ataaacgaaa acaaaagtga acaatttctt tttacagcct 1440

ttacaccaaa aagactacga tgaactatga taaaatttca taatctaaaa acattaatga 1500

ggtaaagact ctctcaaatg ggatattctt cgaaaatttt cataatcgaa cgatatactt 1560

gaatttgcaa ctcatgaccg aaattgtccc aatccataat actctttgac accctatcag 1620

atcccaacgt tgtccctggt ttcgaaacca ccatttcaaa catgaacata tcacaaaata 1680

aacatttaga caccaaatat ctgctaagcg cttacgtaag ctcagctgac gctacctagc 1740

gttagctaac gtcacgtgac gctacctagc gttacgtaac gtcagctgag gctagctagc 1800

tgaattaacg ccgaattaat tcgggggatc tggattttag tactggattt tggttttagg 1860

aattagaaat tttattgata gaagtatttt acaaatacaa atacatacta agggtttctt 1920

atatgctcaa cacatgagcg aaaccctata ggaaccctaa ttcccttatc tgggaactac 1980

tcacacatta ttatggagaa actcgagctt gtcgatcact cggtcttagc tccctttgc 2040

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gagatcatgt agtagaagag gatcttgatg agccattgtg gatatggagc gttggttttc 2160

atatcgtagt aagcttgcac caagttgagc atgaactgga acatctggaa ttgggtgagg 2220

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gcctctcctc ctggagcgtg gtgagcaata gcccaccaga taagggagat agaagagtgg 2400

tggtacacgt ggaggaaaga aatctgtctg gtggatctct tgaggatcat gatcacggta 2460

tccatgaact ccacgtactt ggacatgtag aagaggtaaa cgaggatagc catctccttg 2520

tgctttgggt tataagcgtt tccccacaag gaatatctcc aggtgatagc ttggtaagcg 2580

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caacttgcaa attgctaagt gttggcaaac gttagcataa aaggtgttat aaatttagta 3720
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agggaagatc tgctgctaga gagatgactg ctgaggctaa cggagataag agaaagacca 5040

tcctcattga gggagtgttg tacgatgcta ccaacttcaa acacccagga ggttccatta 5100

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tgatctacag agtggtggag attgtggctt tgttcgcttt gtctttctgg ttgatgtcta 5400

aggcttctcc aacctctttg gttttgggag tggtgatgaa cggaatcgct caaggaagat 5460

gcggatgggt tatgcacgag atgggacacg gatctttcac tggagttatc tggctcgatg 5520

ataggatgtg cgagttcttc tacggagttg gatgtggaat gtctggacac tactggaaga 5580

accagcactc taagcaccac 5600

<210> 240
<211> 1321
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 240
taaagatata ttaggcaatg agtttatgat gttatgttta tatagtttat ttcattttaa 60

attgaaaagc attattttta tcgaaatgaa tctagtatac aatcaatatt tatgtttttt 120

catcagatac tttcctattt tttggcacct ttcatcggac tactgattta tttcaatgtg 180

tatgcatgca tgagcatgag tatacacatg tcttttaaaa tgcatgtaaa gcgtaacgga 240

ccacaaaaga ggatccatac aaatacatct catcgcttcc tctactattc tccgacacac 300

acactgagca tggtgcttaa acactctggt gagttctagt acttctgcta taatgttaaa 360

ttttatatta tatacctact tcctctctct cgctctgtta tgttcgattt cgaaaggatt 420

tcaagatcaa agatgatgag aaaaggtacc ttttcgatat ttaagacaag gaaagaaagg 480

acgaggttga aattttcggg acttggaggg ctaaagtgga agagactgaa tctgaagatg 540

tcgtttctcg aaactttgag atacagaatc atgtctatca ttgaaggaat ggttttggtt 600

tctaagcttg ctttcttctt tctctgttgc ggttgcagat tttaacacgt tagttttttt 660

tttttcgttt ttttgaacgt caacaatgtc ttttttgtac tctttagctc atgtgtaaaa 720

ttctaaattc ttccaataac atacccaaca aattattcgt atctgatttt tatagttttt 780

aacctgttaa tgtaattaat ctaagtgtaa tttttaggct aaatgttaaa ttttatatta 840

aagttttgta acttgaaatt acattcttct tatagcggat aaacagaaaa tgctcttaaa 900

caaatcctga aacaagtaaa aaatacaaca gaaaaatcta acgtttaatt cttaaaacct 960

caaaatcctt atttttacag ctttcaaagt ttaacagctg gaaacctgta gaaaatcaga 1020

cacagcctct caagttttct ggacaataaa tactggtaac gtaagaaaac caattaatga 1080

taccgtcgtt cagtagatag aactgacgat gtgaagatta attgtttctg taatatactg 1140

aatttgaaaa tttatcatca tcatgttaac ggaagttgtc tgtaaaagta gttgattacc 1200

tgttatcgtg taaagtagtt agtaatttct tgcttatttg aaaaatagag aacatttaac 1260

atgtatttt aaataggcac gaccatgcta ctgaacttta tgaaatgctt tggaatctta 1320

t 1321

<210> 241
<211> 100
<212> DNA
<213> Artificial

<220>
<223> amplicon

<400> 241
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tgacactcaa ctaaaatgtt ggattttccc tttcaggtgt 100

<210> 242
<211> 148
<212> DNA
<213> Artificial

<220>
<223> amplicon

<400> 242
cacacacctc cccaatccat ggatatgttc tctcttttct caatcaattt gtttacactc 60

aaacactgat agtttaaact gaaggcggga aacgacaatc tgatcactga ttagtaacta 120

aggcctttaa ttaatctaga ggcgcgcc 148

<210> 243
<211> 147
<212> DNA
<213> Artificial

<220>
<223> amplicon

<400> 243
ggcgcgcctc tagattaatt aaaggcctta gttactaatc agtgatcaga ttgtcgtttc 60

ccgccttcag tttaaactat cagtgttgct ttagtggtca atcaaaagat gaacagtatc 120

ttctcgatcg atgatttctc cgatccc 147

<210> 244
<211> 118
<212> DNA
<213> Artificial

<220>
<223> amplicon

<400> 244
caccaaaagc aaccaagaga gacgtcttta atgtactgaa tttagtaact gaaggcggga 60

aacgacaatc tgatcactga ttagtaacta aggcctttaa ttaatctaga ggcgcgcc 118

<210> 245
<211> 110
<212> DNA
<213> Artificial

<220>
<223> amplicon

<400> 245
gcggacatct acatttttga attgaaaaaa aattggtaat tactctttct ttttctccat 60

attgaccatc atactcattg taagacacac agatgaagaa gtcaaatagc 110

<210> 246
<211> 95
<212> DNA
<213> Artificial

<220>
<223> amplicon

<400> 246
cactgagcat ggtgcttaaa cactctggtg agttctagta cttctgctat aatgttaaat 60

tttatattat atacctactt cctctctctc gctct 95

<210> 247
<211> 101
<212> DNA
<213> Artificial

<220>
<223> amplicon

<400> 247
ctctttcttt ttctccatat tgaccatcat actcattgct gatccatgta gatttcccgg 60

acatgaagcc atttgtcaac gtcgttgttt gatttcgtta a 101

<210> 248
<211> 197
<212> DNA
<213> Artificial

<220>
<223> amplicon

<400> 248
caattaaccg cggacatcta catttttta ctgctattct ttcatattag caactggttg 60

attactgaat tagtacagat gtaactaaca tatatattgg atattattat tatccaaaag 120

agctattcaa ccttttaagc atatgtatat acatgtttaa ttgattttat tagtatttct 180

tgctcttgtt gttttgc 197

<210> 249
<211> 138
<212> DNA
<213> Artificial

<220>

<223> amplicon

<400> 249
aattaaccgc ggacatctac atttttgaat tgaaaaaaaa ttggtaatta ctctttcttt 60

ttctccatat tgaccatcat actcattgct gatccatgta gatttcccgg ttcttttttct 120

cagactactc actgcccg 138

<210> 250
<211> 137
<212> DNA
<213> Artificial

<220>
<223> amplicon

<400> 250
ctctttcttt ttctccatat tgaccatcat actcattgct gatccatgta gatttcccgg 60

acatgaagcc atttacaatc catgtcctta taaatggttt atatactctt ataaataatt 120

tgcttcttca tgcctca 137

<210> 251
<211> 113
<212> DNA
<213> Artificial

<220>
<223> amplicon

<400> 251
ctctttcttt ttctccatat tgaccatcat actcattgct gatccatgta gatttcccgg 60

acatgaagcc atttacaatg agtcaaaaaa aagttgtttt gatgagtcac tgc 113

<210> 252
<211> 160
<212> DNA
<213> Artificial

<220>
<223> amplicon

<400> 252
ctctttcttt ttctccatat tgaccatcat actcattgct gatccatgta gatttcccgg 60

acatgaagcc atttactctg accctactcc acaaatatat ttttatttat aaaaaggtgg 120

ccattgtata ctatgtgtgc gtatacagga ataaaaatgt 160

<210> 253
<211> 169
<212> DNA
<213> Artificial

<220>
<223> amplicon

<400> 253
ccatattgac catcatactc attgctgatc catgtagatt tcccggacat gaagccattt 60

acaattgaat atatcctcac atatgaaata tatttttttt ttacaaatta cacctattaa 120

attatacttg atcggtcatc tgatatattt gaaagaaccc tatcagcca 169

<210> 254
<211> 576
<212> DNA
<213> Artificial

<220>
<223> amplicon

<400> 254
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agtgtgagaa tatttccata ttagacaatt ctgtagattt tatttaatga aagtgtgaga 120

atatttttt gtacactatt tatcaattta ataaaaaaat ataatataat ttaagatgaa 180

ccaacatatt tctctaaatt ctgaatttta ttttcatggt gacacgtgga tacgaaacaa 240

tgttgtaatg tttatcgatt aatatataat gtatgttata tgtttgttgt actaacaaca 300

agatttcata catttcaaac gaaaaagaga gattgatgtg atcagtatta ttgcttttac 360

aaaactattg aaggttcagc gagttcttag taaagtcgaa tccttgtaat aatctctgtt 420

ccaacgggca aacatgcagc tgttaaacta tcgacgccgt ttgacctatt ttttcttctt 480

ctttgttttc ttcctcattt gtacatgcgt gcaagcctac tgacggttgg atgccttggc 540

acgcacttca acaactctat atccttcctg gtgagg 576

<210> 255
<211> 189
<212> DNA
<213> Artificial

<220>
<223> amplicon

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DOCUMENT MADE AVAILABLE UNDER THE PATENT COOPERATION TREATY (PCT)

International application number:		**PCT/EP2015/076630**
International filing date:		**13 November 2015 (13.11.2015)**
Document type:		**Certified copy of priority document**
Document details:	Country/Office:	**US**
	Number:	**62/234,373**
	Filing date:	**29 September 2015 (29.09.2015)**
Date of receipt at the International Bureau:		**24 February 2016 (24.02.2016)**

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a),(b) or (b-*bis*)

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PCT REQUEST

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0	**For receiving Office use only**	
0-1	International Application No.	**PCT/EP2015/076630**
0-2	International Filing Date	**13 NOV 2015 (13.11.2015)**
0-3	Name of receiving Office and "PCT International Application"	**RO/EP**

0-4	**Form PCT/RO/101 PCT Request**	
0-4-1	Prepared Using	PCT Online Filing Version 3.5.000.244e MT/FOP 20141031/0.20.5.20
0-5	**Petition**	
	The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	**Receiving Office (specified by the applicant)**	European Patent Office (EPO) (RO/EP)
0-7	**Applicant's or agent's file reference**	BPS150220PC
I	**Title of Invention**	MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS
II	**Applicant**	
II-1	This person is	Applicant only
II-2	Applicant for	All designated States
II-4	Name	BASF Plant Science Company GmbH
II-5	Address	67056 Ludwigshafen Germany
II-6	State of nationality	DE
II-7	State of residence	DE
III-1	**Applicant and/or inventor**	
III-1-1	This person is	Inventor only
III-1-3	Inventor for	All designated States
III-1-4	Name (LAST, First)	SENGER, Toralf
III-1-5	Address	609 Cheselden Drive Durham, North Carolina 27713 United States of America
III-2	**Applicant and/or inventor**	
III-2-1	This person is	Inventor only
III-2-3	Inventor for	All designated States
III-2-4	Name (LAST, First)	MARTY, Laurent
III-2-5	Address	Neue Stuecker 21 69118 Heidelberg Germany

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III-3	**Applicant and/or inventor**	
III-3-1	This person is	Inventor only
III-3-3	Inventor for	All designated States
III-3-4	Name (LAST, First)	KUNZE, Irene
III-3-5	Address	Mühlenweg 11 06466 Gatersleben Germany
III-4	**Applicant and/or inventor**	
III-4-1	This person is	Inventor only
III-4-3	Inventor for	All designated States
III-4-4	Name (LAST, First)	HAERTEL, Heiko A.
III-4-5	Address	Rudolf-Reusch-Str. 23D 10367 Berlin Germany
III-5	**Applicant and/or inventor**	
III-5-1	This person is	Inventor only
III-5-3	Inventor for	All designated States
III-5-4	Name (LAST, First)	BREMMER, Steven
III-5-5	Address	1604 Kelvington Place Apex, North Carolina 27502 United States of America
III-6	**Applicant and/or inventor**	
III-6-1	This person is	Inventor only
III-6-3	Inventor for	All designated States
III-6-4	Name (LAST, First)	BREAZEALE, Steven
III-6-5	Address	462 Neodak Rd. Apex, North Carolina 27523 United States of America
III-7	**Applicant and/or inventor**	
III-7-1	This person is	Inventor only
III-7-3	Inventor for	All designated States
III-7-4	Name (LAST, First)	BAUER, Jörg
III-7-5	Address	1004 Scholastic Circle Durham, North Carolina 27713 United States of America
III-8	**Applicant and/or inventor**	
III-8-1	This person is	Inventor only
III-8-3	Inventor for	All designated States
III-8-4	Name (LAST, First)	VRINTEN, Patricia
III-8-5	Address	725 310 Stillwater Drive Saskatoon, Saskatchewan S7J 4H5 Canada

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III-9	**Applicant and/or inventor**	
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III-9-4	Name (LAST, First)	STYMNE, Sten
III-9-5	Address	Radmansgatan 1 26132 Landskrona Sweden
III-10	**Applicant and/or inventor**	
III-10-1	This person is	Inventor only
III-10-3	Inventor for	All designated States
III-10-4	Name (LAST, First)	LINDBERG YILMAZ, Jenny
III-10-5	Address	Per Ols väg 9 23737 Bfärred Sweden
III-11	**Applicant and/or inventor**	
III-11-1	This person is	Inventor only
III-11-3	Inventor for	All designated States
III-11-4	Name (LAST, First)	MCELVER, John
III-11-5	Address	5112 Gatewood Dr. Durham, North Carolina 27712 United States of America
III-12	**Applicant and/or inventor**	
III-12-1	This person is	Inventor only
III-12-3	Inventor for	All designated States
III-12-4	Name (LAST, First)	REIN, Dietrich
III-12-5	Address	Flottwellstrasse 3a 10785 Berlin Germany
III-13	**Applicant and/or inventor**	
III-13-1	This person is	Inventor only
III-13-3	Inventor for	All designated States
III-13-4	Name (LAST, First)	ANDRE, Carl
III-13-5	Address	1012 Winona Rd Raleigh, North Carolina 27609 United States of America

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IV-1	**Agent or common representative; or address for correspondence**	
	The person identified below is hereby/ has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	Agent
IV-1-1	Name	Herzog Fiesser & Partner Patentanwälte PartG mbB
IV-1-2	Address	Dudenstraße 46 68167 Mannheim Germany
IV-1-3	Telephone No.	0621-391813400
IV-1-4	Facsimile No.	0621-391813444
IV-1-5	e-mail	mailman@hfp-ip.de
IV-1-5(a)	E-mail authorization The receiving Office, the International Searching Authority, the International Bureau and the International Preliminary Examining Authority are authorized to use this e-mail address, if the Office or Authority so wishes, to send notifications issued in respect of this international application:	as advance copies followed by paper notifications
IV-1-6	Agent's registration No.	446
V	**DESIGNATIONS**	
V-1	**The filing of this request constitutes under Rule 4.9(a), the designation of all Contracting States bound by the PCT on the international filing date, for the grant of every kind of protection available and, where applicable, for the grant of both regional and national patents.**	
VI-1	**Priority claim of earlier national application**	
VI-1-1	Filing date	14 November 2014 (14.11.2014)
VI-1-2	Number	62/079,622
VI-1-3	Country or Member of WTO	US
VI-2	**Priority claim of earlier national application**	
VI-2-1	Filing date	29 September 2015 (29.09.2015)
VI-2-2	Number	62/234,373
VI-2-3	Country or Member of WTO	US

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VI-3	**Incorporation by reference :** where an element of the international application referred to in Article 11(1)(iii)(d) or (e) or a part of the description, claims or drawings referred to in Rule 20.5(a) is not otherwise contained in this international application but is completely contained in an earlier application whose priority is claimed on the date on which one or more elements referred to in Article 11(1)(iii) were first received by the receiving Office, that element or part is, subject to confirmation under Rule 20.6, incorporated by reference in this international application for the purposes of Rule 20.6.		
VII-1	**International Searching Authority Chosen**	European Patent Office (EPO) (ISA/EP)	
VIII	**Declarations**	Number of declarations	
VIII-1	Declaration as to the identity of the inventor	–	
VIII-2	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	–	
VIII-3	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	–	
VIII-4	Declaration of inventorship (only for the purposes of the designation of the United States of America)	–	
VIII-5	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	–	
IX	**Check list**	Number of sheets	Electronic file(s) attached
IX-1	Request (including declaration sheets)	6	✓
IX-2	Description	20	✓
IX-3	Claims	2	✓
IX-4	Abstract	1	✓
IX-5	Drawings	0	–
IX-7	TOTAL	29	

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	Accompanying Items	Paper document(s) attached	Electronic file(s) attached
IX-8	Fee calculation sheet	–	✓
IX-15	Separate indications concerning deposited microorganism or other biological material	–	✓
IX-19	Other	Pre-conversion archive	✓
IX-19	Other	separate indications concerning deposited microor-ganisms or othe	✓
IX-20	**Figure of the drawings which should accompany the abstract**		
IX-21	**Language of filing of the international application**	English	
X-1	**Signature of applicant, agent or common representative**	/Dr. Alexander Dick, European Patent Attorney/	
X-1-1	Name	Herzog Fiesser & Partner Patentanwälte PartG mbB	
X-1-2	Name of signatory	Dr. Alexander Dick	
X-1-3	Capacity (if such capacity is not obvious from reading the request)	(Representative)	

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10-1	**Date of actual receipt of the purported international application**	**13 NOV 2015 (13.11.2015)**
10-2	**Drawings:**	
10-2-1	Received	
10-2-2	Not received	
10-3	**Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application**	
10-4	**Date of timely receipt of the required corrections under PCT Article 11(2)**	
10-5	**International Searching Authority**	ISA/EP
10-6	**Transmittal of search copy delayed until search fee is paid**	

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11-1	**Date of receipt of the record copy by the International Bureau**	

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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62/079,622 14 November 2014 (14.11.2014) US
62/234,373 29 September 2015 (29.09.2015) US

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(74) Agent: **HERZOG FIESSER & PARTNER PATENTANWÄLTE PARTG MBB**; Dudenstraße 46, 68167 Mannheim (DE).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *with amended claims (Art. 19(1))*

— *with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description (Rules 13bis.4(d)(i) and 48.2(a)(viii))*

Date of publication of the amended claims: 21 July 2016

(54) Title: MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS

(57) Abstract: The present invention generally is concerned with the modification of plant lipids containing PUFAs. In this context, the invention is particularly concerned with plants and plant materials for such modifications, wherein the plants preferably are oil-seed plants. Regarding plant parts, the invention is particularly concerned with seeds of such plants and preferably seeds of oilseed plants. The invention is also concerned with plant positions obtainable or obtained by the modification method of the invention, and with full stuff of feedstuff comprising such liquid compositions.

WO 2016/075325 A4

CSIRO Exhibit 1007

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PATENT ASSIGNMENT COVER SHEET

Electronic Version v1.1
Stylesheet Version v1.2

EPAS ID: PAT4514337

SUBMISSION TYPE:	NEW ASSIGNMENT
NATURE OF CONVEYANCE:	ASSIGNMENT

CONVEYING PARTY DATA

Name	Execution Date
TORALF SENGER	04/27/2017
CARL ANDRE	04/28/2017

RECEIVING PARTY DATA

Name:	BASF PLANT SCIENCE LP
Street Address:	26 DAVIS DRIVE
City:	RALEIGH
State/Country:	NORTH CAROLINA
Postal Code:	27709

PROPERTY NUMBERS Total: 1

Property Type	Number
Application Number:	15526162

CORRESPONDENCE DATA

Fax Number: (312)474-0448

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ATTORNEY DOCKET NUMBER:	32471/H150220
NAME OF SUBMITTER:	JOSHUA T. ELLIOTT
SIGNATURE:	/Joshua T. Elliott/
DATE SIGNED:	07/21/2017
	This document serves as an Oath/Declaration (37 CFR 1.63).

Total Attachments: 2
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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1. 76) AND ASSIGNMENT

Title of Invention	

As the below named inventor, I hereby declare that:

This declaration is directed to: [ ] The attached application, or

[x] PCT international application number PCT/EP2015/076630 filed on 13 Nov 2015

The above-identified application was made or authorized to be made by me. I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

WHEREAS,
BASF Plant Science LP
26 Davis Drive
27709 Raleigh
United States of America

(hereinafter referred to as "ASSIGNEE(S)")
is desirous of acquiring the entire right, title and interest in and to said invention and in and to any Letters Patent that may be granted therefore in the United States and its territorial possessions and in any and all foreign countries;

NOW, THEREFORE, for good and valuable consideration the receipt and sufficiency of which is hereby acknowledged, I, by these presents do assign and transfer unto said ASSIGNEE(S), the full and exclusive right to the said invention in the United States and its territorial possessions and in all foreign countries (including the right to claim priority under the terms of the International Convention and other relevant International Treaties and Arrangements from the aforesaid application) and the entire right, title and interest in and to any and all Letters Patent which may be granted therefor in the United States and its territorial possessions and in any and all foreign countries and in and to any and all divisions, reissues, continuations, substitutions and renewals thereof.

I further declare that I either have the obligation to assign or have already assigned the full and exclusive rights in and to any inventions made during my employment to said ASSIGNEE based on my employment contract with said ASSIGNEE, and/or that said ASSIGNEE has obtained full and exclusive rights in and to any such inventions based on applicable national law, and that by this Assignment such transfer of right, title and interest in such inventions to said ASSIGNEE is herewith confirmed or restated.

I hereby authorize and request the Patent Office Officials in the United States and its territorial possessions and any and all foreign countries to issue any and all of said Letters Patent, when granted, to said ASSIGNEE(S) as the assignee of my entire right, title and interest in and to the same, for the sole use and behoof of said ASSIGNEE(S), its (his) successors and assigns, to the full end of the term for which said Letters Patent may be granted, as fully and entirely as the same would have been held by me had this Assignment and sale not been made.

Further, I agree that I will communicate to said ASSIGNEE(S) or its (his) representatives any facts known to me respecting said invention, and testify in any legal proceeding, sign all lawful papers, execute all divisional, continuation, substitute, renewal and reissue applications, execute all necessary assignment papers to cause any and all of said Letters Patent to be issued to said ASSIGNEE(S), make all rightful oaths, and, generally do everything possible to aid said ASSIGNEE(S), its (his) successors and assigns, to obtain and enforce proper protection for said invention in the United States and its territorial possessions and in any and all foreign countries.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

LEGAL NAME OF INVENTOR
Inventor: Toralf Senger

Date: Apr 27, 2017

Signature: T. Senger

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1. 76) AND ASSIGNMENT

Title of Invention	

As the below named inventor, I hereby declare that:

This declaration is directed to: [ ] The attached application, or

[x] PCT international application number PCT/EP2015/076630 filed on 13 Nov 2015

The above-identified application was made or authorized to be made by me. I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

WHEREAS,
BASF Plant Science LP
26 Davis Drive
27709 Raleigh
United States of America

(hereinafter referred to as "ASSIGNEE(S)")
is desirous of acquiring the entire right, title and interest in and to said invention and in and to any Letters Patent that may be granted therefore in the United States and its territorial possessions and in any and all foreign countries;

NOW, THEREFORE, for good and valuable consideration the receipt and sufficiency of which is hereby acknowledged, I, by these presents do assign and transfer unto said ASSIGNEE(S), the full and exclusive right to the said invention in the United States and its territorial possessions and in all foreign countries (including the right to claim priority under the terms of the International Convention and other relevant International Treaties and Arrangements from the aforesaid application) and the entire right, title and interest in and to any and all Letters Patent which may be granted therefor in the United States and its territorial possessions and in any and all foreign countries and in and to any and all divisions, reissues, continuations, substitutions and renewals thereof.

I further declare that I either have the obligation to assign or have already assigned the full and exclusive rights in and to any inventions made during my employment to said ASSIGNEE based on my employment contract with said ASSIGNEE, and/or that said ASSIGNEE has obtained full and exclusive rights in and to any such inventions based on applicable national law, and that by this Assignment such transfer of right, title and interest in such inventions to said ASSIGNEE is herewith confirmed or restated.

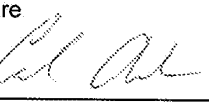
I hereby authorize and request the Patent Office Officials in the United States and its territorial possessions and any and all foreign countries to issue any and all of said Letters Patent, when granted, to said ASSIGNEE(S) as the assignee of my entire right, title and interest in and to the same, for the sole use and behoof of said ASSIGNEE(S), its (his) successors and assigns, to the full end of the term for which said Letters Patent may be granted, as fully and entirely as the same would have been held by me had this Assignment and sale not been made.

Further, I agree that I will communicate to said ASSIGNEE(S) or its (his) representatives any facts known to me respecting said invention, and testify in any legal proceeding, sign all lawful papers, execute all divisional, continuation, substitute, renewal and reissue applications, execute all necessary assignment papers to cause any and all of said Letters Patent to be issued to said ASSIGNEE(S), make all rightful oaths, and, generally do everything possible to aid said ASSIGNEE(S), its (his) successors and assigns, to obtain and enforce proper protection for said invention in the United States and its territorial possessions and in any and all foreign countries.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

LEGAL NAME OF INVENTOR
Inventor: Carl Andre

Date: April 28, 2017

Signature: 

PTO/SB/08a (07-09)
Approved for use through 07/31/2016. OMB 0651-0031
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Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	2

Complete if Known	
Application Number	15/526,162-Conf. #1033
Filing Date	May 11, 2017
First Named Inventor	Toralf Senger
Art Unit	
Examiner Name	
Attorney Docket Number	32471/H150220

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] Number[4] Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
		WO-2005/083093-A2	09-09-2005	Basf Plant Science Gmbh		
		WO-2006/012325-A1	02-02-2006	Du Pont		
		WO-2010/023202-A2	03-04-2010	Basf Plant Science Gmbh		
		WO-2011/006948-A1	01-20-2011	Basf Plant Science Co Gmbh		
		WO-2013/153404-A1	10-17-2013	Rothamsted Res Ltd		

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5]Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

PTO/SB/08b (07-09)
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Substitute for form 1449/PTO				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	15/526,162-Conf. #1033
				Filing Date	May 11, 2017
				First Named Inventor	Toralf Senger
(Use as many sheets as necessary)				Art Unit	
				Examiner Name	
Sheet	2	of	2	Attorney Docket Number	32471/H150220

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
		Browse et al., Fatty acid composition of leaf lipids determined after combined digestion and fatty acid methyl ester formation from fresh tissue, Anal. Biochem., 152(1):141-5 (1986).	
		Cahoon et al., Biosynthetic origin of conjugated double bonds: production of fatty acid components of high-value drying oils in transgenic soybean embryos, Proc. Natl. Acad. Sci. USA, 96(22):12935-40 (1999).	
		Datar et al. Cell and Cell Debris Removal: Centrifugation and Crossflow Filtration, pp. 472-503 IN: Rehm et al. (eds.), Biotechnology, Second, Completely Revised Edition, Volume 3 (Bioprocessing) edited by Stephanopoulos, Weinheim, Germany: VCH (1993).	
		Fujiwara et al., Seed-specific repression of GUS activity in tobacco plants by antisense RNA, Plant Mol. Biol., 20(6):1059-69 (1992).	
		Hull et al., Analysis of the promoter of an abscisic acid responsive late embryogenesis abundant gene of Arabidopsis thaliana, Plant Sci., 14:181-92 (1996).	
		International Preliminary Report on Patentability, International Application No. PCT/EP2015/076630, dated May 16, 2017.	
		International Search Report and Written Opinion, International Application No. PCT/EP2015/076630, mailed March 7, 2016.	
		Livak et al., Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods, 25(4):402-8 (2001).	
		Vilardell et al., Regulation of the rab17 gene promoter in transgenic Arabidopsis wild-type, ABA-deficient and ABA-insensitive mutants, Plant Mol. Biol., 24(4):561-9 (1994).	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

Electronic Acknowledgement Receipt

EFS ID:	29918594
Application Number:	15526162
International Application Number:	
Confirmation Number:	1033
Title of Invention:	MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS
First Named Inventor/Applicant Name:	Toralf Senger
Customer Number:	4743
Filer:	Joshua Thomas Elliott/Audrey Nagelberg
Filer Authorized By:	Joshua Thomas Elliott
Attorney Docket Number:	32471/H150220
Receipt Date:	28-JUL-2017
Filing Date:	
Time Stamp:	11:59:44
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Foreign Reference	WO2005083093A2.PDF	21001826 e7cbbc53ebf9a6c5ea46aef868a058fac38d3924	no	463

Warnings:

CSIRO Exhibit 1007

Information:					
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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: July 28, 2017
Electronic Signature for Joshua T. Elliott: /Joshua T. Elliott/

Docket No.: 32471/H150220
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Toralf Senger et al.

Application No.: 15/526,162 | Confirmation No.: 1033

Filed: May 11, 2017 | Art Unit: 0

For: MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS | Examiner: Not Yet Assigned

INFORMATION DISCLOSURE STATEMENT (IDS)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Pursuant to 37 C.F.R. §§ 1.56, 1.97, and 1.98, the attention of the United States Patent and Trademark Office is hereby directed to the references listed on the attached PTO/SB/08. It is respectfully requested that the information be expressly considered during the prosecution of the above-identified application, and that the references be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

This Information Disclosure Statement is filed before the mailing date of a first Office Action on the merits (37 C.F.R. § 1.97(b)(3)).

In accordance with 37 C.F.R. § 1.98(a)(2)(ii), copies of U.S. patents and U.S. patent application publications are not submitted. Submitted herewith are copies of foreign patents and non-patent literature in accordance with 37 C.F.R. § 1.98(a)(2).

In accordance with 37 C.F.R. § 1.97(g), the filing of this Information Disclosure Statement shall not be construed as a representation that a search has been made. In accordance with 37 C.F.R. § 1.97(h), the filing of this Information Disclosure Statement shall not be construed to be an admission that the information cited in this

Information Disclosure Statement is, or is considered to be, material to the patentability as defined in 37 C.F.R. § 1.56(b).

It is submitted that the Information Disclosure Statement is in compliance with 37 C.F.R. § 1.98, and the Examiner is respectfully requested to consider the listed references.

Applicant believes no fee is due with this response. However, if a fee is due, please charge our Deposit Account No. 13-2855, under Order No. 32471/H150220 from which the undersigned is authorized to draw.

Dated: July 28, 2017

Respectfully submitted,

Electronic signature: /Joshua T. Elliott/
Joshua T. Elliott
Registration No.: 43,603
MARSHALL, GERSTEIN & BORUN LLP
233 S. Wacker Drive
6300 Willis Tower
Chicago, Illinois 60606-6357
(312) 474-6300
Attorney for Applicant

Electronic Acknowledgement Receipt	
EFS ID:	31196166
Application Number:	15526162
International Application Number:	
Confirmation Number:	1033
Title of Invention:	MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS
First Named Inventor/Applicant Name:	Toralf Senger
Customer Number:	4743
Filer:	Joshua Thomas Elliott/Audrey Nagelberg
Filer Authorized By:	Joshua Thomas Elliott
Attorney Docket Number:	32471/H150220
Receipt Date:	12-DEC-2017
Filing Date:	
Time Stamp:	10:14:27
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Foreign Reference	WO0159128A2.PDF	7402212 4c9b5eb4a2a6d520626d149c3a70999359e976ff	no	135

Warnings:

Information:					
2	Foreign Reference	WO0226946A2.PDF	4992504 c4126b93d93ed691833f341ee0f9ac6bb6da2fff	no	98
Warnings:					
Information:					
3	Foreign Reference	WO02052024A2.PDF	1559662 7e89e9d277f61dc6b5f82e01ab89d99a846cd006	no	31
Warnings:					
Information:					
4	Foreign Reference	WO03078639A2.PDF	2067036 00a34d8a2f085c73509287d2f2837585c11a82d9	no	50
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5	Foreign Reference	WO03089452A2.PDF	5854347 a45e567346c93fc0a0e5f8648103d41777e93953	no	116
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Warnings:					
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7	Foreign Reference	WO2004071467A2.PDF	7235479 9450e867643ee1cf81506e27d330471533189cfb	no	132
Warnings:					
Information:					
8	Foreign Reference	WO2004087902A2.PDF	17539145 f5dccce615352e49739dc40445a488016469506a	no	270
Warnings:					
Information:					
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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	9

Complete if Known	
Application Number	15/526,162-Conf. #1033
Filing Date	May 11, 2017
First Named Inventor	Toralf Senger
Art Unit	
Examiner Name	
Attorney Docket Number	32471/H150220

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		US-5,627,289	05-06-1997	Jeromin et al.	
		US-6,303,849	10-16-2001	Potts et al.	
		US-6,689,880	02-10-2004	Chen et al.	
		US-6,733,974	05-11-2004	Feazel	
		US-6,740,488	05-25-2004	Rangwala et al.	
		US-6,818,807	11-16-2004	Trolinder et al.	
		US-6,825,400	11-30-2004	Behr et al.	
		US-6,893,826	05-17-2005	Hillyard et al.	
		US-6,900,014	05-31-2005	Weston et al.	
		US-7,423,198	09-09-2008	Yao et al.	
		US-8,999,411	04-07-2015	Froman et al.	

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		WO-01/059128-A2	08-16-2001	Basf Ag		
		WO-02/26946-A2	04-04-2002	Bioriginal Food & Science Corp		
		WO-2002/052024-A2	07-04-2002	Biogemma Uk Ltd		
		WO-2003/078639-A2	09-25-2003	Univ York		
		WO-2003/089452-A2	10-30-2003	Sangamo Biosciences, Inc		
		WO-2003/093482-A2	11-13-2003	Basf Plant Science Gmbh		

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Complete if Known	
Application Number	15/526,162-Conf. #1033
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First Named Inventor	Toralf Senger
Art Unit	
Examiner Name	
Attorney Docket Number	32471/H150220

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		WO-2004/071467-A2	08-26-2004	Du Pont		
		WO-2004/087902-A2	10-14-2004	Univ Bristol		
		WO-2004/090123-A2	10-21-2004	Basf Plant Science Gmbh		
		WO-2005/007845-A2	01-27-2005	Univ York		
		WO-2005/012316-A2	02-10-2005	Basf Plant Science Gmbh		
		WO-2005/083053-A2	09-09-2005	Basf Plant Science Gmbh		

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet	3	of	9

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		WO-2006/008099-A2	01-26-2006	Basf Plant Science Gmbh		
		WO-2006/069710-A1	07-06-2006	Basf Plant Science Gmbh		
		WO-2006/100241-A2	09-28-2006	Basf Plant Science Gmbh		
		WO-2007/096387-A1	08-30-2007	Basf Plant Science		
		WO-2008/022963-A2	02-28-2008	Basf Plant Science Gmbh		
		WO-2009/111263-A1	09-11-2009	Monsanto Technology Llc		

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet	4	of	9

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Filing Date	May 11, 2017
First Named Inventor	Toralf Senger
Art Unit	
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Attorney Docket Number	32471/H150220

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		WO-2010/066703-A2	06-17-2010	Basf Plant Science Gmbh		
		WO-2013/185184-A2	12-19-2013	Commw Scient Ind Res Org		
		WO-2015/089587-A1	06-25-2015	Commw Scient Ind Res Org		

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Substitute for form 1449/PTO				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	15/526,162-Conf. #1033
				Filing Date	May 11, 2017
				First Named Inventor	Toralf Senger
(Use as many sheets as necessary)				Art Unit	
				Examiner Name	
Sheet	5	of	9	Attorney Docket Number	32471/H150220

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
		ABIDI et al., "Effect of Genetic Modification on the Distribution of Minor Constituents in Canola Oil", Journal of the American Oil Chemists' Society, Vol. 76, Issue 4, pp. 463-7 (April 1999).	
		ARONDEL, et al., "Map-based Cloning of a Gene Controlling Omega-3 Fatty Acid Desaturation in Arabidopsisc", Science Vol. 258, Issue 5086, November 20, 1992, pp. 1353-1355.	
		BAI, et al., "X-ray Structure of a Mammalian Stearoyl-CoA Desaturase", Nature, August 2015, Vol. 524, pp. 252-256.	
		BLIGH, et al., "A Rapid Method of Total Lipid Extraction and Purification", Canadian Journal of Biochemistry and Physiology, Vol. 37, Issue 1, 1959, pp. 911-917.	
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		DE BLOCK, et al., "Transformation of Brassica Napus and Brassica Oleracea Using Agrobacterium Tumefaciens and the Expression of the Bar and Neo Genes in the Transgenic Plants", Plant Physiol., v.91(2):694-701 (1989).	
		DOLDE, et al., "Tocopherols in Breeding Lines and Effects of Planting Location, Fatty Acid Composition, and Temperature During Development", JAOCS, 76:349-55 (March 1999).	
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Examiner Signature		Date Considered	

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Substitute for form 1449/PTO				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				**Application Number**	15/526,162-Conf. #1033
				Filing Date	May 11, 2017
				First Named Inventor	Toralf Senger
(Use as many sheets as necessary)				Art Unit	
				Examiner Name	
Sheet	6	of	9	Attorney Docket Number	32471/H150220

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
		DOMERGUE, et al., "In Vivo Characterization of the First Acyl-CoA Δ6-Desaturase from a Member of the Plant Kingdom, the Microalga Ostreococcus Tauri", Biochem. J., 389(Pt. 2):483-90 (2005).	
		DUBOS, et al., "Integrating Bioinformatic Resources to Predict Transcription Factors Interacting with Cis-Sequences Conserved in Co-Regulated Genes", BMC Genomics, 15:317 (2014).	
		FOCKS, et al., "Wrinkled1: A Novel, Low-Seed-Oil Mutant of Arabidopsis with a Deficiency in the Seed-Specific Regulation of Carbohydrate Metabolism", Plant Physiol., 118(1):91-101 (1998).	
		GRIFFITHS, et al., "Delta 6- and Delta 12-desaturase Activities and Phosphatidic Acid Formation in Microsomal Preparations from the Developing Cotyledons of Common Borage (Borango officinalis), Biochem. J., 252(3):641-7 (1988).	
		International Preliminary Report on Patentability, International Application No. PCT/EP2015/076596, issued May 16, 2017.	
		International Preliminary Report on Patentability, PCT Application No. PCT/EP2015/076608, completed February 28, 2017.	
		International Preliminary Report on Patentability, PCT application no. PCT/EP2015/076605, dated May 16, 2017.	
		International Search Report and Written Opinion for PCT Patent Application No. PCT/EP2015/076596, Issued on March 11, 2016, 15 pages.	
		International Search Report and Written Opinion for PCT Patent Application No. PCT/EP2015/076605, Issued on February 24, 2016, 13 pages.	
		International Search Report and Written Opinion for PCT Patent Application No. PCT/EP2015/076608, Issued on March 9, 2016, 13 pages.	

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(Use as many sheets as necessary)				Art Unit	
				Examiner Name	
Sheet	7	of	9	Attorney Docket Number	32471/H150220

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		JAIN, et al., "Identification of a Novel Lysophospholipid Acyltransferase in Saccharomyces Cerevisiae", J. Biol. Chem., 282(42):30562-9 (2007).	
		KARGIOTIDOU, et al., "Low Temperature and Light Regulate Delta 12 Fatty Acid Desaturases (FAD2) at a Transcriptional Level in Cotton (Gossypium Hirsutum)", J. Exp. Bot., 49(8):2043-56 (2008).	
		KNUTZON, et al., "Identification of Delta5-dehydratase from Mortierella Alpina by Heterologous Expression in Bakers' Yeast and Canola", J. Biol. Chem., 273(45):29360-6 (1998).	
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		O'MALLEY, et al., "An Adapter Ligation-Mediated Pcr Method for High-Throughput Mapping of T-DNA Inserts in the Arabidopsis Genome", Nature Protocols, Vol. 2, Issue 11, 2007, pp. 2910-7.	
		OKAYASU, et al., "Purification and Partial Characterization of Linoleoyl-CoA Desaturase from Rat Liver Microsomes", Archives of Biochemistry and Biophysics, 206(1):21-8 (1981).	
		OKULEY, et al., "Arabidopsis FAD2 Gene Encodes the Enzyme that is Essential for Polyunsaturated Lipid Synthesis", The Plant Cell Online, Vol. 6, Issue 1, Jan 1994, pp. 147-158.	
		PAUL, et al., "Members of the Arabidopsis FAE1-like 3-Ketoacyl-CoA Synthase Gene Family Substitute for the Elop Proteins of Saccharomyces Cerevisiae", J. Biol. Chem., 281(14):9018-29 (2006).	
		QI, et al., "Production of Very Long Chain Polyunsaturated Omega-3 and Omega-6 Fatty Acids in Plants", Nature Biotechnology, Vol. 22, Issue 6, Jun 2004, pp. 739-745.	

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(Use as many sheets as necessary)				Art Unit	
				Examiner Name	
Sheet	8	of	9	Attorney Docket Number	32471/H150220

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
		QUEK, et al., "Commercial Extraction of Vitamin E from Food Sources" The Encyclopedia of Vitamin E, Eds. Preedy, et al., CABI Publishers, Oxford, U.K., 2007, pp. 140-152.	
		RIEKHOF, et al., "Lysophosphatidylcholine Metabolism in Saccharomyces Cerevisiae The Role of P-Type Atpases in Transport and in Broad Specificity Acyltransferase in Acylation", J. Biol. Chem., 282(51):36853-61 (2007).	
		RUIZ-LOPEZ, et al., "Successful High-level Accumulation of Fish Oil Omega-3 Long-Chain Polyunsaturated Fatty Acids in a Transgenic Oilseed Crop",Plant J., 77(2):198-208 (2014).	
		RUUSKA, et al., "Contrapuntal Networks of Gene Expression during Arabidopsis Seed Filling", The Plant Cell Online, Vol. 14, Issue 6, Jun 2002, pp. 1191-1206.	
		RYCHLIK, et al, "A computer program for choosing optimal oligonudeotides for filter hybridization, sequencing and in vitro amplification of DNA", Nucleic Acids Research, 17(21):8543-51 (1989).	
		SARKAR, et al., "Specificity Determinants for the Abscisic Acid Response Element", FEBS Open Bio, Volume 3, Issue 1, January 01, 2013, pp. 101-105.	
		SHANKLIN, et al., "Desaturation and Related Modifications of Fatty Acids1", Annual Review of Plant Physiology and Plant Molecular Biology, Vol. 49, June 1998, pp. 611-641.	
		SHANKLIN, et al., "Stearoyl-acyl-carrier-protein desaturase from Higher Plants is Structurally Unrelated to the Animal and Fungal Homologs", Proc. Natl. Acad. Sci. USA, 88(6):2510-4 (1991).	
		STRITTMATTER et al., "Purification and Properties of Rat Liver Microsomal Stearyl Coenzyme A Desaturase", Proc. Natl. Acad. Sci. USA, 71(11):4565-9 (1974).	
		STYMNE, et al., "Biosynthesis of γ-linolenic Acid in Cotyledons and Microsomal Preparations of the Developing Seeds of Common Borage (Borago Officinalis)", Biochem. J., 240(2):385-93 (1986).	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

PTO/SB/08b (07-09)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Substitute for form 1449/PTO				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				**Application Number**	15/526,162-Conf. #1033
				Filing Date	May 11, 2017
				First Named Inventor	Toralf Senger
(Use as many sheets as necessary)				Art Unit	
				Examiner Name	
Sheet	9	of	9	Attorney Docket Number	32471/H150220

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
		TAMAKI, et al., "LPT1 Encodes a Membrane-bound O-Acyltransferase Involved in the Acylation of Lysophospholipids in the Yeast Saccharomyces Cerevisiae", J. Biol. Chem., 282(47):34288-98 (2007).	
		TANG, et al., "Oleate Desaturase Enzymes of Soybean: Evidence of Regulation Through Differential Stability and Phosphorylation", Plant J., 44(3):433-46 (2005).	
		WANG, et al., "Crystal Structure of Human Stearoyl-Coenzyme a Desaturase in Complex with Substrate", Nature Structural & Molecular Biology, Vol. 22, 2015, pp. 581 - 585.	
		WU, et al., "Stepwise Engineering to Produce High Yields of Very Long-Chain Polyunsaturated Fatty Acids in Plants", Nature Biotechnology, Vol. 23, Issue 8, 2005, pp. 1013-1017.	
		XIAO, et al., "Characterization of the Promoter and 5'-UTR Intron of Oleic Acid Desaturase (FAD2) Gene in Brassica Napus", Gene, Vol. 545, Issue 1, July 2014, pp. 45-55.	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

Electronic Acknowledgement Receipt	
EFS ID:	31196131
Application Number:	15526162
International Application Number:	
Confirmation Number:	1033
Title of Invention:	MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS
First Named Inventor/Applicant Name:	Toralf Senger
Customer Number:	4743
Filer:	Joshua Thomas Elliott/Audrey Nagelberg
Filer Authorized By:	Joshua Thomas Elliott
Attorney Docket Number:	32471/H150220
Receipt Date:	12-DEC-2017
Filing Date:	
Time Stamp:	10:11:45
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Foreign Reference	WO2004090123A2.PDF	3116999 d5529efc5352b11b2fe908336fe67f2df70a49d9	no	54

Warnings:

CSIRO Exhibit 1007

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: December 12, 2017
Electronic Signature for Joshua T. Elliott: /Joshua T. Elliott/

Docket No.: 32471/H150220
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Toralf Senger et al.

Application No.: 15/526,162 | Confirmation No.: 1033

Filed: May 11, 2017 | Art Unit: 0

For: MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS | Examiner: Not Yet Assigned

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT (IDS)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Pursuant to 37 C.F.R. §§ 1.56, 1.97, and 1.98, the attention of the United States Patent and Trademark Office is hereby directed to the references listed on the attached PTO/SB/08. It is respectfully requested that the information be expressly considered during the prosecution of the above-identified application, and that the references be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

This Information Disclosure Statement is filed before the mailing date of a first Office Action on the merits (37 C.F.R. § 1.97(b)(3)).

In accordance with 37 C.F.R. § 1.98(a)(2)(ii), copies of U.S. patents and U.S. patent application publications are not submitted. Submitted herewith are copies of foreign patents and non-patent literature in accordance with 37 C.F.R. § 1.98(a)(2).

In accordance with 37 C.F.R. § 1.97(g), the filing of this Information Disclosure Statement shall not be construed as a representation that a search has been made. In accordance with 37 C.F.R. § 1.97(h), the filing of this Information Disclosure Statement shall not be construed to be an admission that the information cited in this

Information Disclosure Statement is, or is considered to be, material to the patentability as defined in 37 C.F.R. § 1.56(b).

It is submitted that the Information Disclosure Statement is in compliance with 37 C.F.R. § 1.98, and the Examiner is respectfully requested to consider the listed references.

Applicant believes no fee is due with this response. However, if a fee is due, please charge our Deposit Account No. 13-2855, under Order No. 32471/H150220 from which the undersigned is authorized to draw.

Dated: December 12, 2017

Respectfully submitted,

Electronic signature: /Joshua T. Elliott/
Joshua T. Elliott
Registration No.: 43,603
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UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
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U.S. APPLICATION NUMBER NO.	FIRST NAMED INVENTOR	ATTY. DOCKET NO.
15/526,162	Toralf SENGER	32471/H150220

4743
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INTERNATIONAL APPLICATION NO.	
PCT/EP2015/076630	
I.A. FILING DATE	PRIORITY DATE
11/13/2015	11/14/2014

CONFIRMATION NO. 1033
371 ACCEPTANCE LETTER

OC000000100687597

Date Mailed: 07/03/2018

NOTICE OF ACCEPTANCE OF APPLICATION UNDER 35 U.S.C 371 AND 37 CFR 1.495

The applicant is hereby advised that the United States Patent and Trademark Office, in its capacity as a Designated / Elected Office (37 CFR 1.495), has ACCEPTED the above identified international application for national patentability examination in the United States Patent and Trademark Office.

The United States Application Number assigned to the application is shown above. A Filing Receipt will be issued for the present application in due course. **THE DATE APPEARING ON THE FILING RECEIPT AS THE "FILING DATE or 371(c) DATE" IS THE DATE ON WHICH THE LAST OF THE 35 U.S.C. 371 (c)(1) and (c)(2) REQUIREMENTS HAS BEEN RECEIVED IN THE OFFICE. THIS DATE IS SHOWN BELOW.** The filing date of the above identified application is the international filing date of the international application (Article 11(3) and 35 U.S.C. 363)

05/11/2017
DATE OF RECEIPT OF 35 U.S.C.
371(c)(1) and (c)(2) REQUIREMENTS

The following items have been received:

- Copy of the International Application filed on 05/11/2017
- Copy of the International Search Report filed on 05/11/2017
- Copy of Article 19 Amendments filed on 05/11/2017
- Preliminary Amendments filed on 05/11/2017
- Information Disclosure Statements filed on 07/28/2017
- Sequence Listing txt file (EFS or physical medium) filed on 05/11/2017
- Inventor's Oath or Declaration filed on 05/11/2017
- U.S. Basic National Fees filed on 05/11/2017
- Assignment filed on 07/21/2017
- Authorize Access to Search Results filed on 05/11/2017
- Priority Documents filed on 05/11/2017
- Authorization to Permit Access filed on 05/11/2017
- Application Data Sheet (37 CFR 1.76) filed on 05/11/2017

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.495(c).

FORM PCT/DO/EO/903 (371 Acceptance Notice)

- Properly executed inventor's oath or declaration for the following inventor(s) has not been submitted: **Toralf SENGER** and **Carl ANDRE**

Applicant is reminded that any communications to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)

SHAKEEL AHMED

Telephone: (703) 756-1423

FORM PCT/DO/EO/903 (371 Acceptance Notice)

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
15/526,162	05/11/2017		1620	32471/H150220	12	3

CONFIRMATION NO. 1033

FILING RECEIPT

4743
MARSHALL, GERSTEIN & BORUN LLP
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CHICAGO, IL 60606-6357

OC000000100687596

Date Mailed: 07/03/2018

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Toralf SENGER, Durham, NC;
Carl ANDRE, Raleigh, NC;

Applicant(s)

BASF PLANT SCIENCE COMPANT GMBH, Ludwigshafan, GERMANY;

Power of Attorney:
Joshua Elliott--43603

Domestic Priority data as claimed by applicant

This application is a 371 of PCT/EP2015/076630 11/13/2015
which claims benefit of 62/234,373 09/29/2015
and claims benefit of 62/079,622 11/14/2014

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 07/02/2018

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 15/526,162**

Projected Publication Date: 10/11/2018

Non-Publication Request: No

Early Publication Request: No
Title

MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS

Preliminary Class

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

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For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

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Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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NOT GRANTED

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 15/526,162

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	300
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	520
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	760
TOTAL CLAIMS (37 CFR 1.16(i))	12 minus 20 =	*			OR	x 100 =	0.00
INDEPENDENT CLAIMS (37 CFR 1.16(h))	3 minus 3 =	*				x 460 =	0.00
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						0.00
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	1580

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)	OR	RATE($)	ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

CSIRO Exhibit 1007

MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET

Substitute for Form PTO-1360
(For use with Form PTO/SB/06)

Application Number	Filing Date
15526162	

Applicant(s) Toralf SENGER

* May be used for additional claims or amendments

CLAIMS	AS FILED		AFTER FIRST AMENDMENT		AFTER SECOND AMENDMENT	
	Indep	Depend	Indep	Depend	Indep	Depend
1	1		1			
2	1		1			
3	1		1			
4		1		1		
5		3		1		
6		(1)		1		
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10		(1)		1		
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Total Indep	3		3		0	
Total Depend	11	↵	9	↵	0	↵
Total Claims	14		12		0	

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	Indep	Depend	Indep	Depend	Indep	Depend
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CSIRO Exhibit 1007

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
15/526,162	05/11/2017	Toralf SENGER	32471/H150220

CONFIRMATION NO. 1033

4743
MARSHALL, GERSTEIN & BORUN LLP
233 SOUTH WACKER DRIVE
6300 WILLIS TOWER
CHICAGO, IL 60606-6357

PUBLICATION NOTICE



Title:MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS

Publication No.US-2018-0291390-A1
Publication Date:10/11/2018

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently http://www.uspto.gov/patft/.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Public Records Division. The Public Records Division can be reached by telephone at (571) 272-3150 or (800) 972-6382, by facsimile at (571) 273-3250, by mail addressed to the United States Patent and Trademark Office, Public Records Division, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently https://portal.uspto.gov/pair/PublicPair. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

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Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

Docket No.: 32471/H150220
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Toralf Senger et al.

Application No.: 15/526,162 Confirmation No.: 1033

Filed: May 11, 2017 Art Unit: Not Yet Assigned

For: MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS Examiner: Not Yet Assigned

REQUEST FOR CORRECTED FILING RECEIPT

MS Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Applicant hereby requests that a corrected Filing Receipt be issued in the above-identified patent application. The official Filing Receipt received by Applicant, a copy of which is attached hereto, has errors in the Applicant:

"BASF PLANT SCIENCE COMPANT GMBH, Ludwigshafan, GERMANY;" should be -- BASF PLANT SCIENCE COMPANY GMBH, Ludwigshafen, GERMANY; --

Applicant additionally requests that all pertinent U.S. Patent and Trademark Office records relating to the subject application be changed to reflect this correction.

Applicant believes no fee is due with this submission. However, if a fee is due, please charge our Deposit Account No. 13-2855, under Order No. 32471/H150220 from which the undersigned is authorized to draw.

Dated: November 13, 2018

Respectfully submitted,

Electronic signature: /Joshua T. Elliott/
Joshua T. Elliott
Registration No.: 43,603
MARSHALL, GERSTEIN & BORUN LLP
233 S. Wacker Drive
6300 Willis Tower
Chicago, Illinois 60606-6357
(312) 474-6300
Attorney for Applicant

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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P.O. Box 1450
Alexandria, Virginia 22313-1450
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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
15/526,162	05/11/2017		1620	32471/H150220	12	3

CONFIRMATION NO. 1033

4743
MARSHALL, GERSTEIN & BORUN LLP
233 SOUTH WACKER DRIVE
6300 WILLIS TOWER
CHICAGO, IL 60606-6357

FILING RECEIPT

OC000000100687596

Date Mailed: 07/03/2018

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Toralf SENGER, Durham, NC;
Carl ANDRE, Raleigh, NC;

Applicant(s)

~~BASF PLANT SCIENCE COMPANT GMBH, Ludwigshafan, GERMANY;~~
BASF PLANT SCIENCE COMPANY GMBH, Ludwigshafen, GERMANY;

Power of Attorney:
Joshua Elliott--43603

Domestic Priority data as claimed by applicant

This application is a 371 of PCT/EP2015/076630 11/13/2015
which claims benefit of 62/234,373 09/29/2015
and claims benefit of 62/079,622 11/14/2014

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

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If Required, Foreign Filing License Granted: 07/02/2018

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 15/526,162**

Projected Publication Date: 10/11/2018

Non-Publication Request: No

Early Publication Request: No
Title

MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS

Preliminary Class

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Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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Electronic Acknowledgement Receipt	
EFS ID:	34296615
Application Number:	15526162
International Application Number:	
Confirmation Number:	1033
Title of Invention:	MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS
First Named Inventor/Applicant Name:	Toralf SENGER
Customer Number:	4743
Filer:	Joshua Thomas Elliott/Natalie Abila
Filer Authorized By:	Joshua Thomas Elliott
Attorney Docket Number:	32471/H150220
Receipt Date:	13-NOV-2018
Filing Date:	11-MAY-2017
Time Stamp:	17:10:54
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Corrected Filing Receipt	Request_for_Corrected_Filing_Receipt.PDF	15802 4ae2928c32cfe35c23d49811fd55e25ad68c6fa8	no	2

Warnings:

Information:					
2	Request for Corrected Filing Receipt	Marked_Up_Filing_Receipt.PDF	273404 884382bf5b1876ac537a73e92cc4dcdb923684ff	no	3
Warnings:					
Information:					
			Total Files Size (in bytes):	289206	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
15/526,162	05/11/2017	1655	1620	32471/H150220	12	3

CONFIRMATION NO. 1033

CORRECTED FILING RECEIPT

4743
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233 SOUTH WACKER DRIVE
6300 WILLIS TOWER
CHICAGO, IL 60606-6357

OC000000105846124

Date Mailed: 11/16/2018

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Toralf SENGER, Durham, NC;
Carl ANDRE, Raleigh, NC;

Applicant(s)

BASF PLANT SCIENCE COMPANY GMBH, Ludwigshafen, GERMANY;

Power of Attorney:
Joshua Elliott--43603

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Permission to Access Search Results: Yes

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The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 15/526,162**

Projected Publication Date: Not Applicable

Non-Publication Request: No

Early Publication Request: No
Title

MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS

Preliminary Class

424

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

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Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

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The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1. 76) AND ASSIGNMENT

Title of Invention	

As the below named inventor, I hereby declare that:

This declaration is directed to: [ ] The attached application, or

[x] PCT international application number PCT/EP2015/076630 filed on 13 Nov 2015

The above-identified application was made or authorized to be made by me. I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

WHEREAS,
BASF Plant Science LP
26 Davis Drive
27709 Raleigh
United States of America

(hereinafter referred to as "ASSIGNEE(S)")
is desirous of acquiring the entire right, title and interest in and to said invention and in and to any Letters Patent that may be granted therefore in the United States and its territorial possessions and in any and all foreign countries;

NOW, THEREFORE, for good and valuable consideration the receipt and sufficiency of which is hereby acknowledged, I, by these presents do assign and transfer unto said ASSIGNEE(S), the full and exclusive right to the said invention in the United States and its territorial possessions and in all foreign countries (including the right to claim priority under the terms of the International Convention and other relevant International Treaties and Arrangements from the aforesaid application) and the entire right, title and interest in and to any and all Letters Patent which may be granted therefor in the United States and its territorial possessions and in any and all foreign countries and in and to any and all divisions, reissues, continuations, substitutions and renewals thereof.

I further declare that I either have the obligation to assign or have already assigned the full and exclusive rights in and to any inventions made during my employment to said ASSIGNEE based on my employment contract with said ASSIGNEE, and/or that said ASSIGNEE has obtained full and exclusive rights in and to any such inventions based on applicable national law, and that by this Assignment such transfer of right, title and interest in such inventions to said ASSIGNEE is herewith confirmed or restated.

I hereby authorize and request the Patent Office Officials in the United States and its territorial possessions and any and all foreign countries to issue any and all of said Letters Patent, when granted, to said ASSIGNEE(S) as the assignee of my entire right, title and interest in and to the same, for the sole use and behoof of said ASSIGNEE(S), its (his) successors and assigns, to the full end of the term for which said Letters Patent may be granted, as fully and entirely as the same would have been held by me had this Assignment and sale not been made.

Further, I agree that I will communicate to said ASSIGNEE(S) or its (his) representatives any facts known to me respecting said invention, and testify in any legal proceeding, sign all lawful papers, execute all divisional, continuation, substitute, renewal and reissue applications, execute all necessary assignment papers to cause any and all of said Letters Patent to be issued to said ASSIGNEE(S), make all rightful oaths, and, generally do everything possible to aid said ASSIGNEE(S), its (his) successors and assigns, to obtain and enforce proper protection for said invention in the United States and its territorial possessions and in any and all foreign countries.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

LEGAL NAME OF INVENTOR
Inventor: Toralf Senger

Date: Apr 27, 2017

Signature: T. Senger

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1. 76) AND ASSIGNMENT

Title of Invention	

As the below named inventor, I hereby declare that:

This declaration is directed to: [ ] The attached application, or

[x] PCT international application number PCT/EP2015/076630 filed on 13 Nov 2015

The above-identified application was made or authorized to be made by me. I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

WHEREAS,
BASF Plant Science LP
26 Davis Drive
27709 Raleigh
United States of America

(hereinafter referred to as "ASSIGNEE(S)")
is desirous of acquiring the entire right, title and interest in and to said invention and in and to any Letters Patent that may be granted therefore in the United States and its territorial possessions and in any and all foreign countries;

NOW, THEREFORE, for good and valuable consideration the receipt and sufficiency of which is hereby acknowledged, I, by these presents do assign and transfer unto said ASSIGNEE(S), the full and exclusive right to the said invention in the United States and its territorial possessions and in all foreign countries (including the right to claim priority under the terms of the International Convention and other relevant International Treaties and Arrangements from the aforesaid application) and the entire right, title and interest in and to any and all Letters Patent which may be granted therefor in the United States and its territorial possessions and in any and all foreign countries and in and to any and all divisions, reissues, continuations, substitutions and renewals thereof.

I further declare that I either have the obligation to assign or have already assigned the full and exclusive rights in and to any inventions made during my employment to said ASSIGNEE based on my employment contract with said ASSIGNEE, and/or that said ASSIGNEE has obtained full and exclusive rights in and to any such inventions based on applicable national law, and that by this Assignment such transfer of right, title and interest in such inventions to said ASSIGNEE is herewith confirmed or restated.

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Further, I agree that I will communicate to said ASSIGNEE(S) or its (his) representatives any facts known to me respecting said invention, and testify in any legal proceeding, sign all lawful papers, execute all divisional, continuation, substitute, renewal and reissue applications, execute all necessary assignment papers to cause any and all of said Letters Patent to be issued to said ASSIGNEE(S), make all rightful oaths, and, generally do everything possible to aid said ASSIGNEE(S), its (his) successors and assigns, to obtain and enforce proper protection for said invention in the United States and its territorial possessions and in any and all foreign countries.

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LEGAL NAME OF INVENTOR
Inventor: Carl Andre

Date: Apl 28, 2017

Signature: 

Electronic Acknowledgement Receipt	
EFS ID:	35358452
Application Number:	15526162
International Application Number:	
Confirmation Number:	1033
Title of Invention:	MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS
First Named Inventor/Applicant Name:	Toralf SENGER
Customer Number:	4743
Filer:	Joshua Thomas Elliott/Natalie Abila
Filer Authorized By:	Joshua Thomas Elliott
Attorney Docket Number:	32471/H150220
Receipt Date:	08-MAR-2019
Filing Date:	11-MAY-2017
Time Stamp:	15:28:59
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Oath or Declaration filed	H150220_Declaration.PDF	3524121 a29360fd814ed8ce7d18d800033f54a6105aa766	no	2

Warnings:

Information:	
Total Files Size (in bytes):	3524121

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

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www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/526,162	05/11/2017	Toralf SENGER	32471/H150220	1033

4743 7590 09/06/2019

MARSHALL, GERSTEIN & BORUN LLP
233 SOUTH WACKER DRIVE
6300 WILLIS TOWER
CHICAGO, IL 60606-6357

EXAMINER	
DAVIS, DEBORAH A	

ART UNIT	PAPER NUMBER
1655	

NOTIFICATION DATE	DELIVERY MODE
09/06/2019	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

mgbdocket@marshallip.com

Office Action Summary	Application No. 15/526,162	Applicant(s) SENGER et al.	
	Examiner DEBORAH A DAVIS	Art Unit 1655	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 2 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b)☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 1-12 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☐ Claim(s) _____ is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☑ Claim(s) 1-12 are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

DETAILED ACTION

Notice of Pre-AIA or AIA Status

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Election/Restrictions

Restriction is required under 35 U.S.C. 121 and 372.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In accordance with 37 CFR 1.499, applicant is required, in reply to this action, to elect a single invention to which the claims must be restricted.

Group I, claim(s) 1-9, drawn to an extracted *Brassica napus* plant lipid composition comprising EPA, DHA, and optionally ARA in their respective amounts.

Group II, claim(s) 10, drawn to a method of altering plant lipids composition, comprising the step of growing a *Brassica napus* plant according to claim 2.

Group III, claim(s) 11-12, drawn to a method of altering plant lipids composition, comprising the step of growing a *Brassica napus* plant according to claim 2.

Groups I-III lack unity of invention because even though the inventions of these groups require the technical feature of and extract of *Brassica napus* plant lipid, this technical feature is not a special technical feature as it does not make a contribution over the prior art in view of the various anticipatory (X) references that teach such a limitation containing *Brassica napus* plant lipid extract. Therefore, the

shared./corresponding technical feature is not a contribution over the prior art and, thus, the claims lack unity and are properly restrictable.

REQUIREMENT FOR UNITY OF INVENTION

As provided in 37 CFR 1.475(a), a national stage application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive concept ("requirement of unity of invention"). Where a group of inventions is claimed in a national stage application, the requirement of unity of invention shall be fulfilled only when there is a technical relationship among those inventions involving one or more of the same or corresponding special technical features. The expression "special technical features" shall mean those technical features that define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art.

The determination whether a group of inventions is so linked as to form a single general inventive concept shall be made without regard to whether the inventions are claimed in separate claims or as alternatives within a single claim. See 37 CFR 1.475(e).

When Claims Are Directed to Multiple Categories of Inventions:

As provided in 37 CFR 1.475 (b), a national stage application containing claims to different categories of invention will be considered to have unity of invention if the claims are drawn only to one of the following combinations of categories:

(1) A product and a process specially adapted for the manufacture of said product; or

(2) A product and a process of use of said product; or

(3) A product, a process specially adapted for the manufacture of the said product, and a use of the said product; or

(4) A process and an apparatus or means specifically designed for carrying out the said process; or

(5) A product, a process specially adapted for the manufacture of the said product, and an apparatus or means specifically designed for carrying out the said process.

Otherwise, unity of invention might not be present. See 37 CFR 1.475 (c).

The examiner has required restriction between product or apparatus claims and process claims. Where applicant elects claims directed to the product/apparatus, and all product/apparatus claims are subsequently found allowable, withdrawn process claims that include all the limitations of the allowable product/apparatus claims should be considered for rejoinder. All claims directed to a nonelected process invention must include all the limitations of an allowable product/apparatus claim for that process invention to be rejoined.

In the event of rejoinder, the requirement for restriction between the product/apparatus claims and the rejoined process claims will be withdrawn, and the rejoined process claims will be fully examined for patentability in accordance with 37 CFR 1.104. Thus, to be allowable, the rejoined claims must meet all criteria for patentability including the requirements of 35 U.S.C. 101, 102, 103 and 112. Until all claims to the elected product/apparatus are found allowable, an otherwise proper restriction requirement between product/apparatus claims and process claims may be

maintained. Withdrawn process claims that are not commensurate in scope with an allowable product/apparatus claim will not be rejoined. See MPEP § 821.04. Additionally, in order for rejoinder to occur, applicant is advised that the process claims should be amended during prosecution to require the limitations of the product/apparatus claims. **Failure to do so may result in no rejoinder.** Further, note that the prohibition against double patenting rejections of 35 U.S.C. 121 does not apply where the restriction requirement is withdrawn by the examiner before the patent issues. See MPEP § 804.01.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to DEBORAH A DAVIS whose telephone number is (571)272-0818. The examiner can normally be reached on 9-5 M-S (MiddayFlex).

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Terry Mckelvey can be reached on (571) 272-0775. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only.

For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/DEBORAH A DAVIS/
Examiner, Art Unit 1655

/MICHAEL BARKER/
Primary Examiner, Art Unit 1655

Docket No.: 32471/H150220
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Toralf Senger et al.

Application No.: 15/526,162 | Confirmation No.: 1033

Filed: May 11, 2017 | Art Unit: 1655

For: MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS | Examiner: D. A. Davis

RESPONSE TO RESTRICTION REQUIREMENT
AND AMENDMENT UNDER 37 C.F.R. § 1.115

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

INTRODUCTORY COMMENTS

Prior to examination on the merits, please amend the above-identified U.S. patent application as follows:

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 5 of this paper.

AMENDMENTS TO THE CLAIMS

1. (Original) Extracted *Brassica napus* plant lipid composition comprising eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and optionally arachidonic acid (ARA), wherein

a) the content of EPA is at least 5% higher than of ARA, and/or

b) the sum of contents of EPA+DHA is at least 7% higher than ARA and/or

c) the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%.

2. (Original) *Brassica napus* plant or part therof, comprising lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and optionally arachidonic acid (ARA), wherein

a) the content of EPA is at least 5% higher than of ARA, and/or

b) the sum of contents of EPA+DHA is at least 7% higher than ARA and/or

c) the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%.

3. (Previously presented) *Brassica napus* plant or part thereof, comprising lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), wherein, when the Brassica napus plant or part thereof is grown, the content of ARA decreases at least 0.5% by weight of total lipids.

4. (Original) *Brassica napus* plant or part thereof according to claim 3, wherein the content of EPA increases at least 1.0% by weight of total lipids.

5. (Previously presented) *Brassica napus* plant according to claim 2, comprising a nucleic acid comprising

a) a Delta-5 elongase gene under the control of a promoter such that expression of the Delta-5 elongase gene is maintained or increased in late stage seed development, and/or

b) a Delta-5 desaturase gene under the control of a promoter such that expression of the Delta-5 desaturase gene is reduced or prevented in late stage seed development.

6. (Previously presented) Seed of a *Brassica napus* plant according to claim 2.

7. (Previously presented) *Brassica napus* plant lipid composition obtainable or obtained by a process comprising the steps of

a) growing a Brassica napus plant according to claim 2 at least until the lipids content of ARA has decreased, and

b) harvesting the plant or a part thereof and

c) extracting lipids composition from the harvested material to obtain said lipid composition.

8. (Original) *Brassica napus* plant lipid composition according to claim 7, wherein the process further comprises degumming, deodorising, bleaching, decolourising, drying, winterizing and/or fractionating of the extracted lipids to obtain said lipid composition.

9. (Previously presented) Foodstuff or feedstuff comprising a *Brassica napus* plant lipid composition according to claim 1.

10. (Previously presented) Method of altering plant lipids composition, comprising the step of growing a *Brassica napus* plant according to claim 2 to produce lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), wherein the step of growing and lipids production is continued until the content of ARA has decreased.

11. (Previously presented) Method of producing a *Brassica napus* plant lipid composition, comprising the steps of

a) growing Brassica napus plants according to claim 2,

b) harvesting the plants or a part thereof when the lipids content of ARA has decreased.

12. (Previously presented) Method according to claim 10, wherein ARA decreases by at least 0.5 wt.-%.

13. (New) The extracted *Brassica napus* plant lipid composition of claim 1, wherein the content of EPA is at least 5% higher than of ARA.

14. (New) The extracted *Brassica napus* plant lipid composition of claim 1, wherein the sum of contents of EPA+DHA is at least 7% higher than ARA.

15. (New) The extracted *Brassica napus* plant lipid composition of claim 1, wherein the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%.

16. (New) The *Brassica napus* plant or part therof of claim 2, wherein the content of EPA is at least 5% higher than of ARA.

17. (New) The *Brassica napus* plant or part therof of claim 2, wherein the sum of contents of EPA+DHA is at least 7% higher than ARA.

18. (New) The *Brassica napus* plant or part therof of claim 2, wherein the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%.

REMARKS

Upon entry of this amendment, claims 1-18 will be pending. New claims 13-18 have been added. New claims 13, 14, and 15 depend from independent claim 1 and require subparts (a), (b), and (c), respectively, of claim 1. New claims 16, 17, and 18 depend from independent claim 2 and require subparts (a), (b), and (c), respectively, of claim 2. In view thereof, no new matter has been added.

In response to the restriction requirement set forth in the Office Action mailed September 6, 2019, applicant hereby provisionally elects **Group I**, claims 1-9, for continued examination, with traverse. The claims encompassing the elected Group are thus claims 1-9 and new claims 13-18.

Applicant respectfully requests that Groups II (claim 10) and III (claims 11-12) be rejoined with Group I for examination. First, Groups II and III include claims that are ultimately dependent from claims of Group I. Applicant respectfully asserts that the examination of Groups II and III along with Group I would not present a search or examination burden, as the Groups are related (as the claims of Groups II and III all include the recitations of either claim 1 or claim 2 from Group I). In addition, the Office Action does not specifically point out the special technical feature of claims 1 and 2 in the prior art.

In view thereof, Applicant respectfully requests that the restriction requirement be withdrawn. If the restriction requirement is not withdrawn, Applicant requests that all withdrawn process claims be rejoined when the elected product claims are found allowable.

The Director is hereby authorized to charge any additional fees which may be required with respect to this communication, or credit any overpayment, to Deposit Account No. 13-2855, under Order No. 32471/H150220.

Dated: November 6, 2019

Respectfully submitted,

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Electronic Acknowledgement Receipt	
EFS ID:	37672760
Application Number:	15526162
International Application Number:	
Confirmation Number:	1033
Title of Invention:	MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS
First Named Inventor/Applicant Name:	Toralf SENGER
Customer Number:	4743
Filer:	Joshua Thomas Elliott/Rebecca Walsh
Filer Authorized By:	Joshua Thomas Elliott
Attorney Docket Number:	32471/H150220
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		Preliminary_Amendment_Under_37_CFR1.PDF	30772 1a8dc982e3421ae922d398b71b7a30adfafae6cc	yes	5

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Response to Election / Restriction Filed	1	1
	Claims	2	4
	Applicant Arguments/Remarks Made in an Amendment	5	5
Warnings:			
Information:			
	Total Files Size (in bytes):	30772	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (09-11)
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/526,162	Filing Date 05/11/2017	☐ To be Mailed

ENTITY: ☑ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $80 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $420 =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).				
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))					
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT	11/06/2019	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 19	Minus	** 20	= 0		x $100 =	0
	Independent (37 CFR 1.16(h))	* 3	Minus	*** 3	= 0		x $460 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	0

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.	SLIE
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".	/HELENA L PAYTON/
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".	
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.	

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/526,162	05/11/2017	Toralf SENGER	32471/H150220	1033

4743 7590 03/05/2020

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EXAMINER	
DAVIS, DEBORAH A	

ART UNIT	PAPER NUMBER
1655	

NOTIFICATION DATE	DELIVERY MODE
03/05/2020	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

mgbdocket@marshallip.com

Office Action Summary	Application No. 15/526,162	Applicant(s) SENGER et al.	
	Examiner DEBORAH A DAVIS	Art Unit 1655	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 06 November 2019.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 1-18 is/are pending in the application.

5a) Of the above claim(s) 10-12 is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 1-9 and 13-18 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

Notice of Pre-AIA or AIA Status

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

DETAILED ACTION

Election/Restrictions

Applicant's election with traverse of Group I, claims 1-9 and 13-18 newly added in the reply filed on 06 November 2019 is acknowledged. The traversal is on the ground(s) that there is not a serious burden to examine all groups I, II, and III. This is not found persuasive because burden is not the only criteria restriction is based on alone, but also includes the criteria of patentable independent or distinct inventions. Further, a reference that anticipates one group may not necessarily anticipate or even make obvious the other. Classification is illustrative only and do not represent all the classes and subclasses which must be searched for each invention; nor is the search limited to issued US patents, but rather includes foreign patents and applications as well as Non-patented literature of various Data Bases searches. Upon allowance of the composition claims, method claims will be automatically considered for rejoinder.

The requirement is still deemed proper and is therefore made FINAL.

Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Claims 1-4, 6-9, 13-18 are rejected under 35 U.S.C. 101 because the claimed invention is directed to a judicial exception (i.e., a law of nature, a natural phenomenon, or an abstract idea) without significantly more. Claim(s) and claims dependent thereon) are directed to a composition comprising natural products. The claim(s) does/do not include additional elements that are sufficient to amount to significantly more than the judicial exception.

Effective January 7, 2019, subject matter eligibility determinations under 35 U.S.C. § 101 follow the procedure explained in the Federal Register notice titled ***2019 Revised Patent Subject Matter Eligibility Guidance*** (Federal Register, Vol. 84, No.4, 50-57), which is found at: https://www.govinfo.gov/content/pkg/FR-2019-01-07/pdf/2018-28282.pdf. Applicants are kindly asked to review this guidance as well as MPEP 2106.

The statutory categories of invention under 35 U.S.C. 101 are processes, machines, manufactures, and compositions of matter. However, certain members of these categories constitute judicial exceptions, i.e., the courts have determined that these entities are not patentable subject matter. These judicial exceptions include abstract ideas, laws of nature, and natural phenomena. The Office released guidance on December 16, 2014 for the examination of claims reciting natural products under 35 U.S.C. 101 in light of the recent Supreme Court decisions in *Association for Molecular Pathology v. Myriad Genetics, Inc.* (569 U.S. ___, 133 S. Ct. 2107, 2116, 106 USPQ2d 1972 (2013)) and *Mayo Collaborative Services v. Prometheus Laboratories* (566 U.S. ___, 132 S. Ct. 1289, 101 USPQ2d 1961 (2012)), *Diamond v. Chakrabarty*, 447 U.S.

303 (1980)) and *Funk Brothers Seed Co. v. Kalo Inoculant Co.* - 333 U.S. 127 (1948)).

(*inter alia*). See eg. MPEP 2106.04(b)

The Supreme Court has explained that the judicial exceptions reflect the Court's view that abstract ideas, laws of nature, and natural phenomena are "the basic tools of scientific and technological work", and are thus excluded from patentability because "monopolization of those tools through the grant of a patent might tend to impede innovation more than it would tend to promote it." *Alice Corp.*, 134 S. Ct. at 2354, 110 USPQ2d at 1980 (quoting *Myriad,* 133 S. Ct. at 2116, 106 USPQ2d at 1978 and *Mayo Collaborative Servs. v. Prometheus Labs. Inc.*, 566 U.S. 66, 71, 101 USPQ2d 1961, 1965 (2012)). The Supreme Court's concern that drives this "exclusionary principle" is pre-emption. *Alice Corp.*, 134 S. Ct. at 2354, 110 USPQ2d at 1980. The Court has held that a claim may not preempt abstract ideas, laws of nature, or natural phenomena; i.e., one may not patent every "substantial practical application" of an abstract idea, law of nature, or natural phenomenon, even if the judicial exception is narrow.

While preemption is the concern underlying the judicial exceptions, it is not a standalone test for determining eligibility. *Rapid Litig. Mgmt. v. CellzDirect, Inc.*, 827 F.3d 1042, 1052, 119 USPQ2d 1370, 1376 (Fed. Cir. 2016). Instead, questions of preemption are inherent in and resolved by the two-part framework from *Alice Corp. and Mayo* (the *Alice/Mayo* test referred to by the Office as Steps 2A and 2B). It is necessary to evaluate eligibility using the *Alice/Mayo* test, because while a preemptive claim may be ineligible, the absence of complete preemption does not demonstrate that a claim is eligible.

Products of Nature: When a law of nature or natural phenomenon is claimed as a physical product, the courts have often referred to the exception as a "product of nature". Products of nature are considered to be an exception because they tie up the use of naturally occurring things, but they have been labeled as both laws of nature and natural phenomena. See *Myriad* 133 S. Ct. at 2116-17, 106 USPQ2d at 1979 (claims to isolated DNA held ineligible because they "claim naturally occurring phenomena" and are "squarely within the law of nature exception"); *Funk Bros. Seed Co. v. Kalo Inoculant Co.*, 333 U.S. 127, 130, 76 USPQ 280, 281 (1948) (claims to bacterial mixtures held ineligible as "manifestations of laws of nature" and "phenomena of nature"). Step 2A of the Office's eligibility analysis uses the terms "law of nature" and "natural phenomenon" as inclusive of "products of nature".

It is important to keep in mind that product of nature exceptions include both naturally occurring products and non-naturally occurring products that lack markedly different characteristics from any naturally occurring counterpart. Instead, the key to the eligibility of all non-naturally occurring products is whether they possess markedly different characteristics from any naturally occurring counterpart.

When a claim recites a nature-based product limitation, examiners use the markedly different characteristics analysis discussed in MPEP § 2106.04(c) to evaluate the nature-based product limitation and determine the answer to Step 2A. Nature-based products, as used herein, include both eligible and ineligible products and merely refer to the types of products subject to the markedly different characteristics analysis used to identify product of nature exceptions.

The Markedly Different Characteristics Analysis

The markedly different characteristics analysis is part of Step 2A, because the courts use this analysis to identify product of nature exceptions. If the claim includes a nature-based product that has markedly different characteristics, then the claim does not recite a product of nature exception and is eligible. If the claim includes a nature-based product that does not exhibit markedly different characteristics from its closest naturally occurring counterpart in its natural state, then the claim is directed to a "product of nature" exception (Step 2A: YES), and requires further analysis in Step 2B to determine whether any additional elements in the claim add significantly more to the exception.

Nature-based Product Claim Analysis

Where the claim is to a nature-based product by itself, the markedly different characteristics analysis should be applied to the entire product. Where the claim is to a nature-based product produced by combining multiple components, the markedly different characteristics analysis should be applied to the resultant nature-based combination, rather than its component parts. Where the claim is to a nature-based product in combination with non-nature based elements, the markedly different characteristics analysis should be applied only to the nature-based product limitation. For a product-by-process claims, the analysis turns on whether the nature-based product in the claim has markedly different characteristics from its naturally occurring counterpart.

The markedly different characteristics analysis compares the nature-based product limitation to its naturally occurring counterpart in its natural state. Markedly different characteristics can be expressed as the product's structure, function, and/or other properties, and are evaluated based on what is recited in the claim on a case-by-case basis. If the analysis indicates that a nature-based product limitation does not exhibit markedly different characteristics, then that limitation is a product of nature exception. If the analysis indicates that a nature-based product limitation does have markedly different characteristics, then that limitation is not a product of nature exception. Because the markedly different characteristics analysis compares the nature-based product limitation to its naturally occurring counterpart in its natural state, the first step in the analysis is to select the appropriate counterpart(s) to the nature-based product. When there are multiple counterparts to the nature-based product, the comparison should be made to the closest naturally occurring counterpart. When the nature-based product is a combination produced from multiple components, the closest counterpart may be the individual nature-based components of the combination. Because there is no counterpart mixture in nature, the closest counterparts to the claimed mixture are the individual components of the mixture, *i.e.*, each naturally occurring species by itself. *See, e.g.*, *Funk Bros.*, 333 U.S. at 130, 76 USPQ at 281 (comparing claimed mixture of bacterial species to each species as it occurs in nature).

Markedly changed characteristics can include structural, functional, chemical changes. In order to show a marked difference, a characteristic must be changed as compared to nature, and cannot be an inherent or innate characteristic of the naturally occurring counterpart or an incidental change in a characteristic of the naturally occurring counterpart. *Myriad,* 133 S. Ct. at 2111, 106 USPQ2d at 1974-75. Thus, in order to be markedly different, applicant must have caused the claimed product to possess at least one characteristic that is different from that of the counterpart. If there is no change in any characteristic, the claimed product lacks markedly different characteristics, and is a product of nature exception.

The claims 1-4, 6-9, and 13-18 are directed to an extracted *Brassica napus* plant lipid composition comprising eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA) including their various percentage of each EPA, DHA and ARA which would appear to be inherent within the *Brassica napus* plant lipids. The claim 6 is drawn to a *Brassica napus* plant seed. Claims 7-8 are drawn to a product-by-

process of *Brassica napus* plant lipid. With respect to claim 9, a foodstuff or feedstuff comprising a *Brassica napus* plant lipid composition would also read on a natural product because it could be consumed. The *Brassica napus* plant lipids and seed read on a product of nature that are not markedly different from its closest naturally-occurring counterpart because there is no indication that their preparation has caused the nature-based product to have any characteristics that are markedly different from the closest naturally-occurring product and the claims do not include additional elements that are sufficient to amount to significantly more than the judicial exception. It is noted, as discussed above, the broadest reasonable interpretation (BRI) of the claims that are being examined and if the claims scope includes any scope without any markedly different characteristics from the natural components existing in nature, then the claims are properly rejected under the present 35 USC 101 guidelines. The rationale for this determination is explained below:

Step 1: Determine if the claims are directed to one of the four statutory categories of patentable subject matter identified by 35 U.S.C. 101: a process, machine, manufacture or composition of matter. YES, the claims are directed to a composition comprising a *Brassica napus* plant lipid and the *Brassica napus* plant seed, which is a statutory category within at least one of the four categories of patent eligible subject matter.

Step 2A: PRONG ONE: Evaluate whether the claim recites a Judicial Exception (e.g., law of nature, natural phenomenon, or an abstract idea). YES, the claims are product claims reciting something that appears to be a nature-based product (i.e., composition comprising *Brassica napus* plant lipid composition comprising EPA, DHA,

and ARA and its percentages and a *Brassica napus* plant seed) which is not markedly different from the closest naturally-occurring counterpart (i.e., the individual nature-based products).

Note: with respect to extracts of natural products such as plants, the closest naturally-occurring counterpart is always the same compounds found in the extract, present in the non-isolated form in the source plant material. Extracts that are made simply by separating the extracted components from the non-extracted components, is a partitioning process that absent any specific chemical modification, merely separates the compounds leaving their activities unchanged.

There is no indication that extracting *Brassica napus* plant lipids and *Brassica napus* plant seed would be markedly different from what occurs in nature, since the lipids of EPA, ARA, and DHA are lipids found within the plant of *Brassica napus*.

Nor is there any difference in functional characteristics. To show a marked difference, the characteristic(s) must be changed as compared it closest natural-occurring counterpart. For example, an assertion of changed functionality must be accompanied with evidence of a comparison of the claimed composition with its closest naturally-occurring counterpart and should apply to the full scope of the claim.

PRONG TWO: Evaluate whether the judicial exception is integrated into a practical application. **The claims are directed to a composition, not its practical use such as a particular treatment or prophylaxis for a disease or medical condition.**

Thus the cited claims are directed to a judicial exception to patentable subject matter.

Step 2b: Determine whether the claim directed to a judicial exception provides an inventive concept. For example, the claims may recite additional elements that amount to significantly more than the judicial exception. In the instant case, NO, the claims are directed to *Brassica napus* plant lipid composition and a *Brassica napus* plant seed without any other components that could add significantly more to the exception. No other specific limitations other than what is well-understood, routine and conventional in the field at a high level of generality have been added to the claimed nature-based product.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent for a claimed invention may not be obtained, notwithstanding that the claimed invention is not identically disclosed as set forth in section 102, if the differences between the claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains. Patentability shall not be negated by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103 are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 1-9 and 13-18 is/are rejected under 35 U.S.C. 103 as being unpatentable over WIPO Publication (WO 2013/153404).

The claims are drawn to a *Brassica napus* plant lipid composition comprising eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and optionally arachidonic acid (ARA).

The cited WIPO reference beneficially teach lipid composition comprising T2 seeds from *Camelina sativa*. *Camelina sativa* is a relative of the common oil seed *Brassica napus* (known as canola). The lipid composition of T2 oil seeds comprise EPA, DHA and ARA. The EPA can be in a percentage of 8.8%, the DHA can be in a percentage of 7.2 %, and the ARA can be in a percentage of 1.7 % (see e.g. Table 5, page 46). Therefore, the content of EPA would be at least 5% higher than ARA and ARA is less than 4%. The content of EPA is more than 7% and the content of DHA is more than 2% (see e.g. Table 5, page 46). The *Camelina sativa* seeds are collected from plants grown in controlled conditions. The ARA T2 seeds of ARA can be present in an amount of 1.2 to 1.7 which would leave a decrease in content of at least 0.5% (see page e.g. 37 and Table 5). The EPA content of T2 seeds can increase in content by at least 1.0% of total lipids (see e.g. Table 5). The T2 seeds were grown and were the lipid composition was obtain by pressing the oil from the seed or by solvent extraction (see e.g. page 37, paragraphs 1-2). The seed oil can be useful for biofuel for feed. With respect to claim 5, all plants contain nucleic acids. The WIPO reference teach Delta-5 desaturase gene within the seeds of *Camelina sativa* comprising seed specific promoters at mid-stage development to express significant amounts of polyunsaturated fatty acids that can include DHA and DPA (see e.g. page 44, paragraph 4, page 47,

Example 4) . Therefore, one skilled in the art would reasonably expect that DHA and DPA would be expressed in even higher levels in late stage seed development if the expression of Delta-5 desaturase gene is maintained or increased.

The cited reference does not teach the particular *Brassica napus* plant species from which the oil is produced.

It would have been obvious to one skilled in the art at the time the invention was filed substitute *Camelina sativa* plant species for *Brassica Napus* plant species to extract oil lipids in light of its teaching that Camelina seeds are a relative of the common oil seed *Brassica Napus* (known as canola) and its oil content is comparable to *Brassica napus* (see e.g. page 20, paragraph 1). With respect to claims 7-8, please note that the patentability of a product does not depend on its method of production. If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is unpatentable even though the prior product was made by a different process." In re Thorpe, 777 F.2d 695, 698, 227 USPQ 964, 966 (Fed. Cir. 1985) (citations omitted) (see e.g. MPEP 2105). The cited WIPO reference teach lipid compositions comprising EPA, DHA and ARA in the percentages claims and therefore would be the same lipid composition or obvious as instantly claimed.

From the teachings of the reference, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the reference, especially in the absence of the evidence to the contrary.

Conclusion

No claim is allowed.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure: (WO 2010/023202) The WIPO Publication teach *Brassica carinata* plants that have been transformed to express polyunsaturated fatty acids of EPA and ARA (see e.g. Example 4).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to DEBORAH A DAVIS whose telephone number is (571)272-0818. The examiner can normally be reached on 9-5 M-S (Midday Flex).

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Terry Mckelvey can be reached on (571) 272-0775. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see https://ppair-my.uspto.gov/pair/PrivatePair. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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/DEBORAH A DAVIS/
Examiner, Art Unit 1655

/MICHAEL BARKER/
Primary Examiner, Art Unit 1655

Notice of References Cited	Application/Control No. 15/526,162	Applicant(s)/Patent Under Reexamination SENGER et al.	
	Examiner DEBORAH A DAVIS	Art Unit 1655	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
	A					
	B					
	C					
	D					
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	F					
	G					
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	J					
	K					
	L					
	M					

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*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
*	N	WO-2013153404-A1	10-2013	WO	HASLAM RICHARD	C12N9/1029
*	O	WO-2010023202-A2	03-2010	WO	WU GUOHAI	C12N9/00
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
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Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/526,162	SENGER et al.
	Examiner	Art Unit
	DEBORAH A DAVIS	1655

CPC - Searched*		
Symbol	Date	Examiner
A61K 36/31	02/04/2020	D.D.

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner
424	755	02/03/2020	D.D.
424	776	02/04/2020	D.D.

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
PALM INVENTOR SEARCH - ODP; EAST NAME SEARCH; EAST SEARCH; GOOGLE SCHOLAR SEARCH, IDS	02/03/2020	D.D.

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

Bibliographic Data

Application No: 15/526,162

Foreign Priority claimed: ○ Yes ◉ No

35 USC 119 (a-d) conditions met: ☐ Yes ☐ No ☐ Met After Allowance

Verified and Acknowledged: /DEBORAH A DAVIS/ (Examiner's Signature) | (Initials)

Title: MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS

FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
05/11/2017	424	1655	32471/H150220
RULE			

APPLICANTS

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INVENTORS

Toralf SENGER Durham, NC, UNITED STATES

Carl ANDRE Raleigh, NC, UNITED STATES

CONTINUING DATA

This application is a 371 of PCT/EP2015/076630 11/13/2015

PCT/EP2015/076630 has PRO of 62234373 09/29/2015

PCT/EP2015/076630 has PRO of 62079622 11/14/2014

FOREIGN APPLICATIONS

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Complete if Known	
Application Number	15/526,162-Conf. #1033
Filing Date	May 11, 2017
First Named Inventor	Toralf Senger
Art Unit	
Examiner Name	
Attorney Docket Number	32471/H150220

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		WO-2006/012325-A1	02-02-2006	Du Pont		
		WO-2010/023202-A2	03-04-2010	Basf Plant Science Gmbh		
		WO-2011/006948-A1	01-20-2011	Basf Plant Science Co Gmbh		
		WO-2013/153404-A1	10-17-2013	Rothamsted Res Ltd		

Examiner Signature	/DEBORAH A DAVIS/	Date Considered	02/04/2020

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Art Unit	
Examiner Name	

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
		Browse et al., Fatty acid composition of leaf lipids determined after combined digestion and fatty acid methyl ester formation from fresh tissue, Anal. Biochem., 152(1):141-5 (1986).	
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		International Preliminary Report on Patentability, International Application No. PCT/EP2015/076630, dated May 16, 2017.	
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		Livak et al., Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods, 25(4):402-8 (2001).	
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EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S30	4996	(mustard seed or brassica or rape seed) and (EPA or DHA or ARA)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	NEAR	OFF	2020/02/03 10:58
S31	646	(mustard seed or brassica or rape seed) same (EPA or DHA or ARA)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	NEAR	OFF	2020/02/03 10:59
S32	304	(mustard seed or brassica napus or rape seed) same (EPA or DHA or ARA)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	NEAR	OFF	2020/02/03 11:00
S33	36	(mustard seed or brassica napus or rape seed) same (EPA and DHA and ARA)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	NEAR	OFF	2020/02/03 11:03
S34	212	(mustard seed or brassica napus or rape seed) same5 (EPA and DHA and ARA)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	NEAR	OFF	2020/02/03 11:06
S35	18	Toralf Senger.in. and (mustard seed or brassica napus or rape seed) same5 (EPA and DHA and ARA)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	NEAR	OFF	2020/02/03 11:44
S36	3	("20110039010").PN.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/02/03 11:58
S37	1	S36 and (DHA and ARA and EPA)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	NEAR	OFF	2020/02/03 11:59
S38	1	S36 and (DHA and ARA and EPA) and brassica napus	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	NEAR	OFF	2020/02/03 12:15
S39	1	S36 and (DHA and ARA and EPA) and brassica napus and elongase	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	NEAR	OFF	2020/02/03 12:50
S40	1	S36 and (DHA and ARA and EPA) and brassica napus and elongase and promoter	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	NEAR	OFF	2020/02/03 12:53

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Sheet 1 of 9

Complete if Known	
Application Number	15/526,162-Conf. #1033
Filing Date	May 11, 2017
First Named Inventor	Toralf Senger
Art Unit	
Examiner Name	
Attorney Docket Number	32471/H150220

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
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		WO-2003/078639-A2	09-25-2003	Univ York		
		WO-2003/089452-A2	10-30-2003	Sangamo Biosciences, Inc		
		WO-2003/093482-A2	11-13-2003	Basf Plant Science Gmbh		

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		WO-2004/090123-A2	10-21-2004	Basf Plant Science Gmbh		
		WO-2005/007845-A2	01-27-2005	Univ York		
		WO-2005/012316-A2	02-10-2005	Basf Plant Science Gmbh		
		WO-2005/083053-A2	09-09-2005	Basf Plant Science Gmbh		

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		WO-2006/069710-A1	07-06-2006	Basf Plant Science Gmbh		
		WO-2006/100241-A2	09-28-2006	Basf Plant Science Gmbh		
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		WO-2008/022963-A2	02-28-2008	Basf Plant Science Gmbh		
		WO-2009/111263-A1	09-11-2009	Monsanto Technology Llc		

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		WO-2013/185184-A2	12-19-2013	Commw Scient Ind Res Org		
		WO-2015/089587-A1	06-25-2015	Commw Scient Ind Res Org		

Examiner Signature	/DEBORAH A DAVIS/	Date Considered	02/04/2020

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				Examiner Name	
Sheet	5	of	9	Attorney Docket Number	32471/H150220

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Sheet	6	of	9	Attorney Docket Number	32471/H150220

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Application Number	15/526,162-Conf. #1033
Filing Date	May 11, 2017
First Named Inventor	Toralf Senger
Art Unit	
Examiner Name	

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		QUEK, et al., "Commercial Extraction of Vitamin E from Food Sources" The Encyclopedia of Vitamin E, Eds. Preedy, et al., CABI Publishers, Oxford, U.K., 2007, pp. 140-152.	
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Application Number:	15526162
International Application Number:	
Confirmation Number:	1033
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First Named Inventor/Applicant Name:	Toralf SENGER
Customer Number:	4743
Filer:	Joshua Thomas Elliott/Rebecca Walsh
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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/AIA/22 (03-13)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)	Docket Number (Optional) 32471/H150220
Application Number 15/526,162-Conf. #1033	Filed May 11, 2017
For MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS	
Art Unit 1655	Examiner D. A. Davis

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

	Fee	Small Entity Fee	Micro Entity Fee	
☐ One month (37 CFR 1.17(a)(1))	$200	$100	$50	$
☐ Two months (37 CFR 1.17(a)(2))	$600	$300	$150	$
☒ Three months (37 CFR 1.17(a)(3))	$1,400	$700	$350	$ 1,400.00
☐ Four months (37 CFR 1.17(a)(4))	$2,200	$1,100	$550	$
☐ Five months (37 CFR 1.17(a)(5))	$3,000	$1,500	$750	$

☐ Applicant asserts small entity status. See 37 CFR 1.27.

☐ Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

☐ A check in the amount of the fee is enclosed.

☒ Payment by credit card. ~~Form PTO-2038 is attached.~~

☐ The Director has already been authorized to charge fees in this application to a Deposit Account.

☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 13-2855 .

☒ Payment made via EFS-Web.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

☐ applicant.

☒ attorney or agent of record. Registration number 43,603 .

☐ attorney or agent acting under 37 CFR 1.34. Registration number _____.

/Joshua T. Elliott/ — Signature
September 4, 2020 — Date

Joshua T. Elliott — Typed or printed name
(312) 474-6300 — Telephone Number

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

☐ * Total of 1 forms are submitted.

Electronic Patent Application Fee Transmittal	
Application Number:	15526162
Filing Date:	11-May-2017
Title of Invention:	MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS
First Named Inventor/Applicant Name:	Toralf SENGER
Filer:	Joshua Thomas Elliott/Rebecca Walsh
Attorney Docket Number:	32471/H150220
Filed as Large Entity	

Filing Fees for U.S. National Stage under 35 USC 371

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 3 months with $0 paid	1253	1	1400	1400
Miscellaneous:				
SUBMISSION- INFORMATION DISCLOSURE STMT	1806	1	240	240
		Total in USD ($)		**1640**

Electronic Acknowledgement Receipt	
EFS ID:	40477447
Application Number:	15526162
International Application Number:	
Confirmation Number:	1033
Title of Invention:	MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS
First Named Inventor/Applicant Name:	Toralf SENGER
Customer Number:	4743
Filer:	Joshua Thomas Elliott/Rebecca Walsh
Filer Authorized By:	Joshua Thomas Elliott
Attorney Docket Number:	32471/H150220
Receipt Date:	04-SEP-2020
Filing Date:	11-MAY-2017
Time Stamp:	13:42:07
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$1640
RAM confirmation Number	E202094D42364613
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	Mendelpt1.pdf	12301546 aac348053c3db2e885fbbcf87a63b8584f14a192	no	38
Warnings:					
Information:					
2	Non Patent Literature	Mendelpt2.pdf	14239856 d2b1bcf2715c58340bdeafe58bdb6275b4cecd51	no	38
Warnings:					
Information:					
3	Non Patent Literature	Schwender779.PDF	174533 b5d058672c329bd6aaa89a242a900f808fb0eca6	no	4
Warnings:					
Information:					
4	Non Patent Literature	Shrestha_et_al_2019_IJMS_o3Des_MoA.PDF	855030 ad9654a1bc5d12646b11ca109bda095f8a0b522a	no	13
Warnings:					
Information:					
5	Non Patent Literature	Smith76.PDF	1037850 968df00ffd2e81588cbd8a09d3682899822e28bb	no	10
Warnings:					
Information:					
6	Non Patent Literature	Spector.PDF	521495 1001e9d8c2230d026fd88e1847811c6154198507	no	3
Warnings:					
Information:					

7	Non Patent Literature	Stymne305.PDF	1422966 8621d72d6f343e97a0d303d09f354781c45f1998	no	10
Warnings:					
Information:					
8	Non Patent Literature	SanchezGarcia.PDF	179230 79448cab7e1a594daf3461a065a88c75732a4f20	no	8
Warnings:					
Information:					
9	Non Patent Literature	Tudzynski.PDF	454982 ac62676b8b4edfecd195afd1686878faefd037a2	no	13
Warnings:					
Information:					
10	Non Patent Literature	Tumaney47.PDF	742557 6e38f3227340dcb0286a9e2d3c633115db66c6e2	no	10
Warnings:					
Information:					
11	Non Patent Literature	Wachter.PDF	9546134 16cf650525ba87fbc896e5734be94770669da482	no	16
Warnings:					
Information:					
12	Non Patent Literature	Wang10255.PDF	694126 023758725f85e172c7b386ed4eea399a802e86cb	no	8
Warnings:					
Information:					
13	Non Patent Literature	Wijesundra.PDF	292100 91d276efb6ec5bbc0142115620e52e4e10f15752	no	4
Warnings:					
Information:					

14	Non Patent Literature	Yamashita.PDF	604601 2dc70f16ca1a6193fe9d0a8f927eaff1ac30bd64	no	9
Warnings:					
Information:					
15	Transmittal Letter	Information_Disclosure_Statement.PDF	17867 8424a8e4c70f9842514f5be3f290d426447bdfec	no	2
Warnings:					
Information:					
16	Foreign Reference	CN101400798Apt1.pdf	18655809 b5b48b3283073f552853b7d7825ad3f5b338ecf3	no	232
Warnings:					
Information:					
17	Foreign Reference	CN101400798Apt2.pdf	12168976 8e69a2f6e9d5c684aabed5c9c44540dc76a6bedc	no	232
Warnings:					
Information:					
18		Amendment_in_Response_to_Non-Final_Office_Action_Under_37 CFR1.PDF	50620 a9fbc561057ba470e71e8783b7813ed43c1841bc	yes	9

Multipart Description/PDF files in .zip description

Document Description	Start	End
Applicant Arguments/Remarks Made in an Amendment	5	9
Claims	2	4
Amendment/Req. Reconsideration-After Non-Final Reject	1	1

Warnings:					
Information:					
19	Extension of Time	Two_Month_Request_for_Extension_of_Time_Under_37_CFR_1136a.PDF	22682 4c89f1a8f523ac3b652ea7d35997c24ec6b45ad2	no	1
Warnings:					

Information:					
20	Non Patent Literature	TRUSKA141.PDF	909115 c65501ab5711233689c33c9e4cce12023ca08d78	no	7
Warnings:					
Information:					
21	Fee Worksheet (SB06)	fee-info.pdf	32801 bb32c11cf3f44fc09741620f5f937ff165a3a404	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	74924876		

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New Applications Under 35 U.S.C. 111

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New International Application Filed with the USPTO as a Receiving Office

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Docket No.: 32471/H150220
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Toralf Senger et al.

Application No.: 15/526,162 | Confirmation No.: 1033

Filed: May 11, 2017 | Art Unit: 1655

For: MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS | Examiner: D. A. Davis

INFORMATION DISCLOSURE STATEMENT (IDS)

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Pursuant to 37 C.F.R. §§ 1.56, 1.97, and 1.98, the attention of the United States Patent and Trademark Office is hereby directed to the references listed on the attached PTO/SB/08. It is respectfully requested that the information be expressly considered during the prosecution of the above-identified application, and that the references be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

This Information Disclosure Statement is filed after the mailing date of a first Office Action on the merits, but before the mailing date of any of a Final Action under 37 C.F.R. § 1.113, a Notice of Allowance under 37 C.F.R. § 1.311 or an action that otherwise closes prosecution in this application (37 C.F.R. § 1.97(c)).

In accordance with 37 C.F.R. § 1.98(a)(2)(ii), copies of U.S. patents and U.S. patent application publications are not submitted. Submitted herewith are copies of foreign patents and non-patent literature in accordance with 37 C.F.R. § 1.98(a)(2).

In accordance with 37 C.F.R. § 1.97(g), the filing of this Information Disclosure Statement shall not be construed as a representation that a search has been made. In accordance with 37 C.F.R. § 1.97(h), the filing of this Information Disclosure

Statement shall not be construed to be an admission that the information cited in this Information Disclosure Statement is, or is considered to be, material to patentability as defined in 37 C.F.R. § 1.56(b).

It is submitted that the Information Disclosure Statement is in compliance with 37 C.F.R. § 1.98, and the Examiner is respectfully requested to consider the listed references.

Please charge our Credit Card in the amount of $240.00 covering the fee set forth in 37 C.F.R. § 1.17(p). The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 13-2855, under Order No. 32471/H150220.

Dated: September 4, 2020

Respectfully submitted,

Electronic signature: /Joshua T. Elliott/
Joshua T. Elliott
Registration No.: 43,603
MARSHALL, GERSTEIN & BORUN LLP
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Attorney for Applicant

Docket No.: 32471/H150220
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Toralf Senger et al.

Application No.: 15/526,162 | Confirmation No.: 1033

Filed: May 11, 2017 | Art Unit: 1655

For: MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS | Examiner: D. A. Davis

AMENDMENT IN RESPONSE TO NON-FINAL OFFICE ACTION UNDER 37 C.F.R. § 1.111

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

INTRODUCTORY COMMENTS

In response to the Office Action dated March 5, 2020, please amend the above-identified U.S. patent application as follows:

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 5 of this paper.

AMENDMENTS TO THE CLAIMS

1. (Cancelled).

2. (Currently amended) *Brassica napus* plant or part thereof~~therof~~, comprising lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and optionally arachidonic acid (ARA), wherein

a) the content of EPA is at least 5% higher than of ARA, and/or

b) the sum of contents of EPA+DHA is at least 7% higher than ARA and/or

c) the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%, and

wherein the Brassica napus plant or part thereof comprises a Delta-5 elongase gene under the control of a promoter such that expression of the Delta-5 elongase gene is maintained or increased in late stage seed development.

3. (Currently amended) Brassica napus plant ~~or part thereof,~~ comprising seeds that comprise lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), wherein, when the Brassica napus plant or part thereof is grown, the content of ARA in the lipids of the seeds decreases at least 0.5% by weight of total lipids and the content of EPA and/or DHA in the lipids of the seeds increases during late stage seed development.

4. (Currently amended) Brassica napus plant ~~or part thereof~~ according to claim 3, wherein the content of EPA in the lipids of the seeds increases at least 1.0% by weight of total lipids during late stage seed development.

5. (Currently amended) Brassica napus plant according to claim 3[[2]], comprising a nucleic acid comprising

a) a Delta-5 elongase gene under the control of a promoter such that expression of the Delta-5 elongase gene is maintained or increased in late stage seed development, and/or

b) a Delta-5 desaturase gene under the control of a promoter such that expression of the Delta-5 desaturase gene is reduced or prevented in late stage seed development.

6. (Previously presented) Seed of a *Brassica napus* plant according to claim 2.

7. (Currently amended) An extracted *Brassica napus* seed~~plant~~ lipid composition obtainable or obtained by a process comprising the steps of

a) growing a Brassica napus plant producing arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the seeds,~~according to claim 2 at least until the lipids content of ARA has decreased, and~~

b) determining the peak of ARA content in the lipids of the developing seeds of the Brassica napus plant of a),

c) harvesting seeds of the plant when, after the peak of ARA content, the ARA content has decreased and the EPA and/or DHA content in the lipids has increased in the lipids of the developing seeds, and~~harvesting the plant or a part thereof and~~

[[c]]d) extracting a lipid~~lipids~~ composition from the harvested seeds of c)~~material~~ to obtain said lipid composition.

8. (Currently amended) The extracted *Brassica napus* seed~~plant~~ lipid composition according to claim 7, wherein the process further comprises degumming, deodorising, bleaching, decolourising, drying, winterizing and/or fractionating of the extracted lipids to obtain said lipid composition.

9. (Currently amended) Foodstuff or feedstuff comprising the extracted~~a~~ *Brassica napus* seed~~plant~~ lipid composition according to claim 7[[1]].

10. (Withdrawn) Method of altering plant lipids composition, comprising the step of growing a *Brassica napus* plant according to claim 2 to produce lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), wherein the step of growing and lipids production is continued until the content of ARA has decreased.

11. (Withdrawn) Method of producing a *Brassica napus* plant lipid composition, comprising the steps of

a) growing Brassica napus plants according to claim 2,

b) harvesting the plants or a part thereof when the lipids content of ARA has decreased.

12. (Withdrawn) Method according to claim 10, wherein ARA decreases by at least 0.5 wt.-%.

13. (Currently amended) The extracted *Brassica napus* seed~~plant~~ lipid composition of claim 7[[1]], wherein the content of EPA is at least 5% higher than of ARA.

14. (Currently amended) The extracted *Brassica napus* seed~~plant~~ lipid composition of claim 7[[1]], wherein the sum of contents of EPA+DHA is at least 7% higher than ARA.

15. (Currently amended) The extracted *Brassica napus* seed~~plant~~ lipid composition of claim 7[[1]], wherein the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%.

16. (Currently amended) The *Brassica napus* plant or part thereof~~therof~~ of claim 2, wherein the content of EPA is at least 5% higher than of ARA.

17. (Currently amended) The *Brassica napus* plant or part thereof~~therof~~ of claim 2, wherein the sum of contents of EPA+DHA is at least 7% higher than ARA.

18. (Currently amended) The *Brassica napus* plant or part thereof~~therof~~ of claim 2, wherein the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%.

REMARKS

Upon entry of this amendment, claims 2-18 will be pending in the present application. Claims 2-5, 7-9, and 13-18 have been amended. Claim 1 has been cancelled. Claims 10-12 have been withdrawn.

Claim 2 has been amended to incorporate the subject matter of claim 5 (part (a)). Claim 3 has been amended to recite that the "the content of EPA and/or DHA in the lipids of the seeds increases during late stage seed development," support for which can be found in the application as filed (see, e.g., paragraphs [0040], [0058] of the application publication). Claim 7 has been amended to recite an extracted Brassica napus seed lipid composition obtainable or obtained by a process comprising the steps of

a) growing a Brassica napus plant producing arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the seeds,

b) determining the peak of ARA content in the lipids of the developing seeds of the Brassica napus plant of a),

c) harvesting seeds of the plant when, after the peak of ARA content, the ARA content has decreased and the EPA and/or DHA content in the lipids has increased in the lipids of the developing seeds, and

d) extracting a lipids composition from the harvested seeds of c)~~material~~ to obtain said lipid composition.

Support for the amendments to claim 7 can be found in the application as filed (see, e.g., paragraphs [0066] and [0067] of the application publication).

Reconsideration of the objections and rejections is respectfully requested in view of the foregoing amendments and remarks that follow.

Claim Rejections – 35 USC §101

Claims 1-4, 6-9, and 13-18 were rejected as being directed to a judicial exception. The Office Action indicates that the claims are directed a composition comprising natural products. Applicant respectfully traverses based on the amended claims.

Independent claim 2 has been amended to incorporate a portion of the subject matter of claim 5 (part (a)) (which was not rejected under §101). In view thereof, claim 2 (and claims depending therefrom) are not directed to natural products.

Independent claim 3 recites that the Brassica napus plant comprises seeds that comprise EPA, DHA, and ARA, wherein when grown, the content of ARA in the lipids of the seeds decreases at least 0.5% by weight of total lipids and the content of EPA and/or DHA in the lipids of the seeds increases during late stage seed development. The claimed plant is not naturally-occurring and, as such claimed subject matter is not possible in naturally-occurring products (see, e.g., paragraph [0003], stating that "… wild-type plants lack the required enzymes to produce polyunsaturated fatty acids, particularly EPA and DHA, in sufficient quantities"). As such claim 3 (and claims depending therefrom) are not directed to natural products.

Independent claim 7 has been amended to recite that the Brassica napus seed lipid composition is obtained or obtainable by a process of (a) growing a Brassica napus plant producing arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the seeds, and (c) harvesting seeds of the plant when, after the peak of ARA content, the ARA content has decreased and the EPA and/or DHA content in the lipids has increased in the lipids of the developing seeds. As with claim 3, the Brassica napus plant and thus the Brassica napus seed lipid composition is not naturally-occurring and, as such claimed subject matter is not possible in naturally-occurring products (see, e.g., paragraph [0003], stating that "… wild-type plants lack the required enzymes to produce polyunsaturated fatty acids, particularly EPA and DHA, in sufficient quantities"). As such claim 7 (and claims depending therefrom) are not directed to natural products.

In view of the above, Applicant respectfully requests that these rejections be withdrawn.

Claim Rejections – 35 USC §103

Claims 1-9 and 13-18 were rejected as being unpatentable as obvious over WO 2013/153404. Applicant respectfully traverses based on the amended claims.

A. Independent Claim 2

Claim 2 has been amended to recite that the Brassica napus plant or part thereof comprises a Delta-5 elongase gene under the control of a promoter such that expression of the Delta-5 elongase gene is maintained or increased in late stage seed development.

With respect to claim 2, WO 2013/153404 provides no teaching or suggestion to provide a Brassica napus plant with a Delta-5 elongase gene under the control of a promoter such that expression of the Delta-5 elongase gene is maintained or increased in late stage seed development. As shown in Figure 2 of WO 2013/153404, the delta-5 elongase in WO 2013/153404 was driven by the conlinin1 promoter. As shown in Truska et al.[1], the conlinin1 promoter has very little activity in 40-day old seeds (see, e.g., Figure 2b). As such, independent claim 2 (and claims 6, 16, 17, and 18 depending therefrom) are not obvious over WO 2013/153404.

In view thereof, Applicant respectfully requests the rejections of claims 2, 6, and 16-18 be withdrawn.

B. Independent Claims 3 and 7

Independent claim 3 recites that the Brassica napus plant comprises seeds that comprise EPA, DHA, and ARA, wherein when grown, the content of ARA in the lipids of the seeds decreases at least 0.5% by weight of total lipids and the content of EPA and/or DHA in the lipids of the seeds increases during the same period.

Independent claim 7 has been amended to recite that the Brassica napus seed lipid composition is obtained or obtainable by a process of (a) growing a Brassica napus plant producing arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the seeds, and (c) harvesting seeds of the plant when, after the peak of ARA content, the ARA content has decreased and the EPA and/or DHA content in the lipids has increased in the lipids of the developing seeds.

[1] "Molecular analysis of flax 2S storage protein conlinin and seed specific activity of its promoter," Plant Physiology and Biochemistry 41 (2003) 141-147.

The Office Action indicates that "[t]he WIPO reference teach Delta-5 desaturase gene within the seeds of *Camelina sativa* comprising seed specific promoters at mid-stage development to express significant amounts of polyunsaturated fatty acids that can include DHA and DPA …" and that "[t]herefore, one skilled in the art would reasonably expect that DHA and DPA would be expressed in even higher levels in late stage seed development if the expression of Delta-5 desaturase gene is maintained or increased." *Office Action at p. 10-11.*

Applicant respectfully disagrees. As indicated above, Figure 2 of WO 2013/153404 shows that the delta-5 elongase in WO 2013/153404 was driven by the conlinin1 promoter. As also discussed above, a person of skill in the art would have recognized that conlinin1 has very little activity in 40-day old seeds (see Truska et al.).

In view thereof, a person of skill in the art would not have expected the content of EPA and/or DHA in the lipids of seeds to increase during late stage seed development, let alone that the content of EPA and/or DHA in the lipids of seeds would **increase** while the content of ARA in the lipids of the seeds would **decrease** during late stage seed development.

There is simply no teaching, suggestion, or motivation that would have led a person of skill in the art to modify the conlinin1 promoter to achieve the recited increase in EPA and/or DHA and decrease in ARA during late stage seed development, let alone to modify WO 2013/153404 by using Brassica napus and then also modifying the promoter with a reasonable expectation of achieving the results achieved by the presently claimed inventions.

Thus, the Brassica napus plant of claim 3 (and claims dependent thereon) and the extracted Brassica napus seed lipid composition of claim 7 (and claims dependent thereon) would not have been obvious over the cited art.

In view thereof, Applicant respectfully requests that the rejections be withdrawn.

Conclusion

In view of the above, Applicant believes the claims are in condition for allowance and requests notification of the same. Should the Office have any questions regarding this response or the application, the Office is invited to contact the undersigned at the telephone number indicated below.

The Director is hereby authorized to charge any additional fees which may be required with respect to this communication, or credit any overpayment, to Deposit Account No. 13-2855, under Order No. 32471/H150220.

Dated: September 4, 2020

Respectfully submitted,

Electronic signature: /Joshua T. Elliott/
Joshua T. Elliott
Registration No.: 43,603
MARSHALL, GERSTEIN & BORUN LLP
233 S. Wacker Drive
6300 Willis Tower
Chicago, Illinois 60606-6357
(312) 474-6300
Attorney for Applicant

PTO/SB/08a (07-09)
Approved for use through 11/30/2020. OMB 0651-0031
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Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 8

Complete if Known	
Application Number	15/526,162-Conf. #1033
Filing Date	May 11, 2017
First Named Inventor	Toralf Senger
Art Unit	1655
Examiner Name	D. A. Davis
Attorney Docket Number	32471/H150220

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		US-2015/0299676-A1	10-22-2015	Walsh et al.	
		US-2016/0369290	12-22-2016	Cirpus et al.	

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Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
		AU-2011289381-A1	01-24-2013	Du Pont		
		WO-93/10241-A1	05-27-1993	Calgene, Inc		
		WO-94/13814-A1	06-23-1994	Nickerson Biocem Ltd		
		WO-95/27791-A1	10-19-1995	Calgene Inc		
		WO-96/24674-A1	08-15-1996	Gene Shears Pty Ltd		
		WO-98/55632-A1	12-10-1998	Calgene LLC		

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Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 2 of 8

Complete if Known	
Application Number	15/526,162-Conf. #1033
Filing Date	May 11, 2017
First Named Inventor	Toralf Senger
Art Unit	1655
Examiner Name	D. A. Davis
Attorney Docket Number	32471/H150220

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		WO-98/55631-A1	12-10-1998	Calgene LLC		
		WO-99/64616-A2	12-16-1999	Abbott Lab		
		WO-00/18889-A2	04-06-2000	Calgene LLC		
		WO-2006/024509-A2	03-09-2006	Basf Plant Science GmbH		
		WO-2011/161093-A1	12-29-2011	Basf Plant Science Co GmbH		
		WO-2013/049227-A2	04-04-2013	Geneart AG		
		CN-101400798-A (corresponds to US-2016/0369290)	04-01-2009	Basf Plant Science GmbH		X

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				Examiner Name	D. A. Davis
Sheet	3	of	8	Attorney Docket Number	32471/H150220

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
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				Examiner Name	D. A. Davis
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Electronic Acknowledgement Receipt	
EFS ID:	40477631
Application Number:	15526162
International Application Number:	
Confirmation Number:	1033
Title of Invention:	MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS
First Named Inventor/Applicant Name:	Toralf SENGER
Customer Number:	4743
Filer:	Joshua Thomas Elliott/Rebecca Walsh
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Attorney Docket Number:	32471/H150220
Receipt Date:	04-SEP-2020
Filing Date:	11-MAY-2017
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Application Type:	U.S. National Stage under 35 USC 371

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National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/526,162	05/11/2017	Toralf SENGER	32471/H150220	1033

4743 7590 12/08/2020

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DAVIS, DEBORAH A	

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Office Action Summary	Application No. 15/526,162	Applicant(s) SENGER et al.	
	Examiner DEBORAH A DAVIS	Art Unit 1655	AIA (FITF) Status Yes

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Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 04/09/2020.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☑ This action is **FINAL.** 2b) ☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 2-18 is/are pending in the application.

5a) Of the above claim(s) 10-12 is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 1-9 and 13-18 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

DETAILED ACTION

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Applicants' amendment filed 04 September 2020 has been received and entered. Currently, claims 2-18 are pending. Claims 10-12 are withdrawn. Claims 2-9 and 13-18 are currently amended and under consideration for examination. All other claims not mentioned are cancelled.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent for a claimed invention may not be obtained, notwithstanding that the claimed invention is not identically disclosed as set forth in section 102, if the differences between the claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains. Patentability shall not be negated by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103 are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 1-9 and 13-18 stand rejected under 35 U.S.C. 103 as being unpatentable over WIPO Publication (WO 2013/153404) for reasons of record and restated below:

The claims are drawn to a *Brassica napus* plant lipid composition comprising eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and optionally arachidonic acid (ARA).

The cited WIPO reference beneficially teach lipid composition comprising T2 seeds from *Camelina sativa*. *Camelina sativa* is a relative of the common oil seed *Brassica napus* (known as canola). The lipid composition of T2 oil seeds comprise EPA, DHA and ARA. The EPA can be in a percentage of 8.8%, the DHA can be in a percentage of 7.2 %, and the ARA can be in a percentage of 1.7 % (see e.g. Table 5, page 46). Therefore, the content of EPA would be at least 5% higher than ARA and ARA is less than 4%. The content of EPA is more than 7% and the content of DHA is more than 2% (see e.g. Table 5, page 46). The *Camelina sativa* seeds are collected from plants grown in controlled conditions. The ARA T2 seeds of ARA can be present in an amount of 1.2 to 1.7 which would leave a decrease in content of at least 0.5% (see page e.g. 37 and Table 5). The EPA content of T2 seeds can increase in content by at least 1.0% of total lipids (see e.g. Table 5). The T2 seeds were grown and were the lipid composition was obtain by pressing the oil from the seed or by solvent extraction (see e.g. page 37, paragraphs 1-2). The seed oil can be useful for biofuel for feed. With respect to claim 5, all plants contain nucleic acids. The WIPO reference teach Delta-5 desaturase gene within the seeds of *Camelina sativa* comprising seed specific promoters at mid-stage development to express significant amounts of polyunsaturated fatty acids that can include DHA and DPA (see e.g. page 44, paragraph 4, page 47, Example 4) . Therefore, one skilled in the art would reasonably expect that DHA and

DPA would be expressed in even higher levels in late stage seed development if the expression of Delta-5 desaturase gene is maintained or increased.

The cited reference does not teach the particular *Brassica napus* plant species from which the oil is produced.

It would have been obvious to one skilled in the art at the time the invention was filed to substitute *Camelina sativa* plant species for *Brassica Napus* plant species to extract oil lipids in light of its teaching that Camelina seeds are a relative of the common oil seed *Brassica Napus* (known as canola) and its oil content is comparable to *Brassica napus* (see e.g. page 20, paragraph 1). With respect to claims 7-8, please note that the patentability of a product does not depend on its method of production. If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is unpatentable even though the prior product was made by a different process." In re Thorpe, 777 F.2d 695, 698, 227 USPQ 964, 966 (Fed. Cir. 1985) (citations omitted) (see e.g. MPEP 2105). The cited WIPO reference teach lipid compositions comprising EPA, DHA and ARA in the percentages claims and therefore would be the same lipid composition or obvious as instantly claimed.

From the teachings of the reference, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the reference, especially in the absence of the evidence to the contrary.

Response to Arguments

Applicant's arguments filed 04 September 2020 have been fully considered but they are not persuasive of error.

35 USC 101 Rejection - Withdrawn

Applicant's arguments/remarks of page 6 that wild type plants lac the required enzymes to produce polyunsaturated fatty acids and therefore not naturally occurring has been fully considered and found persuasive. Therefore, this rejection has been withdrawn.

35 USC 103(a) Rejection – Maintained

Applicants argue that claim 2 of the cited WIPO reference '404 provides no teaching or suggestion to provide a *Brassica napus* plant with a Delta-5 elongase gene under the control of a promoter such that expression of the Delta-5 elongase gene is maintained or increased in late stage seed development. Applicants argue that as shown in Figure 2 of the WIPO '404 reference, the delta-5 elongase was driven by the conlinin1 promoter. As shown in Truska et al. the conlinin1 promoter has very little activity in 40-day old seeds (see, e.g., Figure 2b). Applicants conclude that, as such, independent claim 2 (and claims 6, 16, 17, and 18 depending therefrom) are not obvious over the cited WIPO '404 reference.

In response, the instant claims do not recite a particular promoter. The WIPO '404 reference also discloses the use of other promoters (see page 35-36). With respect to the Truksa et al reference as evidence, this reference teaches protein conlinin as a

seed specific promoter for flax seed proteins. This teaching is a stark contrast from promoting Delta 5 elongase gene expression of EPA + DPA. Further, the Examiner is of the opinion that it would have been obvious to one skilled in the art at the time the invention was filed substitute *Camelina sativa* plant species for *Brassica Napus* plant species to extract oil lipids in light of its teaching that Camelina seeds are a relative of the common oil seed *Brassica Napus* (known as canola) and its oil content is comparable to *Brassica napus* (see e.g. page 20, paragraph 1) with an expectation of success.

Conclusion

No claim is allowed.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to DEBORAH A DAVIS whose telephone number is (571)272-0818. The examiner can normally be reached on 9-5 M-S (Midday Flex).

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Terry Mckelvey can be reached on (571) 272-0775. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see https://ppair-my.uspto.gov/pair/PrivatePair. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/DEBORAH A DAVIS/
Examiner, Art Unit 1655

/MICHAEL BARKER/
Primary Examiner, Art Unit 1655

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/526,162	SENGER et al.
	Examiner	Art Unit
	DEBORAH A DAVIS	1655

CPC - Searched*		
Symbol	Date	Examiner
A61K 36/31	02/04/2020	D.D.

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner
424	755	02/03/2020	D.D.
424	776	02/04/2020	D.D.

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
PALM INVENTOR SEARCH - ODP; EAST NAME SEARCH; EAST SEARCH; GOOGLE SCHOLAR SEARCH, IDS	02/03/2020	D.D.
UPDATED SEARCH ABOVE	12/01/2020	D.D.

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	8

Complete if Known	
Application Number	15/526,162-Conf. #1033
Filing Date	May 11, 2017
First Named Inventor	Toralf Senger
Art Unit	1655
Examiner Name	D. A. Davis
Attorney Docket Number	32471/H150220

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		US-2015/0299676-A1	10-22-2015	Walsh et al.	
		US-2016/0369290	12-22-2016	Cirpus et al.	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
		AU-2011289381-A1	01-24-2013	Du Pont		
		WO-93/10241-A1	05-27-1993	Calgene, Inc		
		WO-94/13814-A1	06-23-1994	Nickerson Biocem Ltd		
		WO-95/27791-A1	10-19-1995	Calgene Inc		
		WO-96/24674-A1	08-15-1996	Gene Shears Pty Ltd		
		WO-98/55632-A1	12-10-1998	Calgene LLC		

Examiner Signature	/DEBORAH A DAVIS/	Date Considered	12/02/2020

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		WO-98/55631-A1	12-10-1998	Calgene LLC		
		WO-99/64616-A2	12-16-1999	Abbott Lab		
		WO-00/18889-A2	04-06-2000	Calgene LLC		
		WO-2006/024509-A2	03-09-2006	Basf Plant Science GmbH		
		WO-2011/161093-A1	12-29-2011	Basf Plant Science Co GmbH		
		WO-2013/049227-A2	04-04-2013	Geneart AG		
		CN-101400798-A (corresponds to US-2016/0369290)	04-01-2009	Basf Plant Science GmbH		x

Examiner Signature	/DEBORAH A DAVIS/	Date Considered	12/02/2020

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		AKERMOUN et al., Complex lipid biosynthesis: phospholipid synthesis, Biochemical Society Transactions 28: 713-5 (2000).	
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Examiner Signature	/DEBORAH A DAVIS/	Date Considered	12/02/2020

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First Named Inventor	Toralf Senger
Art Unit	1655
Examiner Name	D. A. Davis
Attorney Docket Number	32471/H150220

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		CERTIK et al., Desaturase-defective fungal mutants: useful tools for the regulation and overproduction of polyunsaturated fatty acids, Trends in Biotechnology, vol. 16, no. 12, 1 December 1998, pages 500-505.	
		DEAL et al., Histone variants and modifications in plant gene regulation, Curr. Opin. Plant Biol., 14(2):116-22 (April 2011).	
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		HAMILTON, A binary-BAC system for plant transformation with high-molecular-weight DNA, Gene, 200(1-2):107-16 (October 1997).	

Examiner Signature	/DEBORAH A DAVIS/	Date Considered	12/02/2020

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Attorney Docket Number	32471/H150220

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		HATTORI et al., Experimentally determined sequence requirement of ACGT-containing abscisic acid response element, Plant Cell Physiol., 43(1):136-40 (January 2002).	
		HE et al, Agrobacterium-Mediated Transformation of Large DNA Fragments Using a BIBAC Vector System in Rice, Plant Molecular Biology Reporter, vol. 28, no. 4, 2 March 2010, pages 613-619.	
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Examiner Signature	/DEBORAH A DAVIS/	Date Considered	12/02/2020

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NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
		LOPEZ et al., Identification of novel motif patterns to decipher the promoter architecture of co-expressed genes in Arabidopsis thaliana, BMC Syst. Biol., 7 Suppl 3:S10 (October 2013).	
		LOWENTHAL et al., Quantitative bottom-up proteomics depends on digestion conditions, Anal. Chem., 86(1):551-8 (January 2014).	
		MACHENS et al., Identification of a novel type of WRKY transcription factor binding site in elicitor-responsive cis-sequences from Arabidopsis thaliana, Plant Mol. Biol., 84(4-5):371-85 (2014).	
		MAKRIYANNIS et al., Design and study of peptide-ligand affinity chromatography adsorbents: application to the case of trypsin purification from bovine pancreas, Biotechnol. Bioeng., 53(1):49-57 (January 1997).	
		MANTLE et al., Differentiation of Claviceps purpurea in axenic culture, J. Gen. Microbiol., 93(2):321-34 (April 1976).	
		MEGGENDORFER et al., Functional nuclear topography of transcriptionally inducible extra-chromosomal transgene clusters, CHromosome Res., 18(4):401-17 (June 2010).	
		MENDEL, *Versuche über Pflanzenhybriden* Verhandlungen des naturforschenden Vereines in Brünn, Bd. IV für das Jahr, 1865 Abhandlungen:3-47 (1866).	
		MEY et al., The biotrophic, non-appressorium-forming grass pathogen Claviceps purpurea needs a Fus3/Pmk1 homologous mitogen-activated protein kinase for colonization of rye ovarian tissue, Mol. Plant Microbe Interact., 15(4):303-12 (April 2002).	
		Meyer et al., Novel fatty acid elongases and their use for the reconstitution of docosahexaenoic acid biosynthesis, Journal of Lipid Research, 45:1899-1909 (2004).	
		MUINO et al., Structural determinants of DNA recognition by plant MADS-domain transcription factors, Nucleic Acids Res., 42(4):2138-46 (February 2014).	

Examiner Signature	/DEBORAH A DAVIS/	Date Considered	12/02/2020

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /D.A.D/

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	7	of	8		

Complete if Known	
Application Number	15/526,162-Conf. #1033
Filing Date	May 11, 2017
First Named Inventor	Toralf Senger
Art Unit	1655
Examiner Name	D. A. Davis
Attorney Docket Number	32471/H150220

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
		MURASHIGE et al., A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. Physiologia Plantarum 15, 3:473-497 (1962).	
		NAKAGAWA et al., Diversity of preferred nucleotide sequences around the translation initiation codon in eukaryote genomes, Nucleic Acids Res., 36(3):861-71 (February 2008).	
		NISHIKATA et al., Database construction for PromoterCAD: synthetic promoter design for mammals and plants, ACS Synth. Biol., 3(3):192-6 (March 2014).	
		PARKER et al., Local DNA topography correlates with functional noncoding regions of the human genome, Science, 324(5925):389-92 (April 2009).	
		PETRIE et al., Metabolic engineering Camelina sativa with fish oil-like levels of DHA, PLoS One, 9(1):e85061 (January 2014).	
		POTTS et al., Inheritance of fatty acid composition in Brassica juncea, Proceedings of the 10th International Rapeseed Congress, September 26, 1999,	
		PROC et al., A quantitative study of the effects of chaotropic agents, surfactants, and solvents on the digestion efficiency of human plasma proteins by trypsin, J> Proteome Res., 9(10):5422-37 (October 2010).	
		RAMAMOORTHY et al., Length and sequence dependent accumulation of simple sequence repeats in vertebrates: potential role in genome organization and regulation, Gene, 551(2):167-75 (November 2014).	
		Schwender et al., "Rubisco without the Calvin cycle improves the carbon efficiency of developing green seeds", Nature, 432:779-82 (2004).	
		Shrestha et al., Int. J. Mol. Sci., Comparison of the substrate preferences of ω3 fatty acid desaturases for long chain polyunsaturated fatty acids, 20:3058 (2019).	

Examiner Signature	/DEBORAH A DAVIS/	Date Considered	12/02/2020

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[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /D.A.D/

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Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	8	of	8

Complete if Known	
Application Number	15/526,162-Conf. #1033
Filing Date	May 11, 2017
First Named Inventor	Toralf Senger
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Examiner Name	D. A. Davis
Attorney Docket Number	32471/H150220

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		SMITH et al., Measurement of protein using bicinchoninic acid, Anal. Biochem., 150(1):76-85 (October 1985).	
		SPECTOR, Essentiality of fatty acids, Lipids, 34 Suppl: S1-3 (1999).	
		STYMNE et al., Evidence for the reversibility of the acyl-CoA:lysophosphatidylcholine acyltransferase in microsomal preparations from developing safflower (Carthamus tinctorius L.) cotyledons and rat liver, Biochem. J., 233(2):305-14 (1984).	
		SÁNCHEZ-GARCÍA et al., Differential temperature regulation of three sunflower microsomal oleate desaturase (FAD2) isoforms overexpressed in *Saccharomyces cerevisia,* Eur. J. Lipid Sci. Tech., 106:583-590 (2004).	
		TRUKSA et al., Molecular analysis of flax 2S storage protein conlinin and seed specific activity of its promoter, Plant Physiol. and Biochem., 41:141-7 (2003).	
		TUDZYNSKI et al., Biotechnology and genetics of ergot alkaloids, Appl. Microbiol. Biotechnol., 57(5-6):593-605 (December 2001).	
		TUMANEY et al., Synthesis of azidophospholipids and labeling of lysophosphatidylcholine acyltransferase from developing soybean cotyledons, Biochim. Biophys. Acta, 1439(1):47-56 (July 1999).	
		WACHTER et al., Synthetic CpG islands reveal DNA sequence determinants of chromatin structure, Elife, 3:e03397 (September 2014).	
		Wang et al., ω3 fatty acid desaturases from microorganisms: structure, function, evolution, and biotechnological use, App. Microbiol., 97:10255-62 (2013).	
		WIJESUNDRA, The influence of triacylglycerol structure on the oxidative stability of polyunsaturated oils, Lipid Technology, 20:199-202 (2008).	
		YAMASHITA et al., ATP-independent fatty acyl-coenzyme A synthesis from phospholipid: coenzyme A-dependent transacylation activity toward lysophosphatidic acid catalyzed by acyl-coenzyme A:lysophosphatidic acid acyltransferase, J. Biol. Chem., 276(29):26745-52 (July 2001).	

Examiner Signature	/DEBORAH A DAVIS/	Date Considered	12/02/2020

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[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /D.A.D/

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S30	4996	(mustard seed or brassica or rape seed) and (EPA or DHA or ARA)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	NEAR	OFF	2020/02/03 10:58
S31	646	(mustard seed or brassica or rape seed) same (EPA or DHA or ARA)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	NEAR	OFF	2020/02/03 10:59
S32	304	(mustard seed or brassica napus or rape seed) same (EPA or DHA or ARA)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	NEAR	OFF	2020/02/03 11:00
S33	36	(mustard seed or brassica napus or rape seed) same (EPA and DHA and ARA)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	NEAR	OFF	2020/02/03 11:03
S34	212	(mustard seed or brassica napus or rape seed) same5 (EPA and DHA and ARA)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	NEAR	OFF	2020/02/03 11:06
S35	18	Toralf Senger.in. and (mustard seed or brassica napus or rape seed) same5 (EPA and DHA and ARA)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	NEAR	OFF	2020/02/03 11:44
S36	3	("20110039010").PN.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2020/02/03 11:58
S37	1	S36 and (DHA and ARA and EPA)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	NEAR	OFF	2020/02/03 11:59
S38	1	S36 and (DHA and ARA and EPA) and brassica napus	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	NEAR	OFF	2020/02/03 12:15
S39	1	S36 and (DHA and ARA and EPA) and brassica napus and elongase	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	NEAR	OFF	2020/02/03 12:50
S40	1	S36 and (DHA and ARA and EPA) and brassica napus and elongase and promoter	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	NEAR	OFF	2020/02/03 12:53

2/5/2020 4:23:18 PM
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Request for Continued Examination (RCE) Transmittal

Address to:
Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Application Number	15/526,162-Conf. #1033
Filing Date	May 11, 2017
First Named Inventor	Toralf Senger
Art Unit	1655
Examiner Name	D. A. Davis
Attorney Docket Number	32471/H150220

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. See Instruction Sheet for RCEs (not to be submitted to the USPTO) on page 2.

1. Submission required under 37 CFR 1.114 Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).
   - a. [ ] Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.
     - i. [ ] Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____
     - ii. [ ] Other _____
   - b. [X] Enclosed
     - i. [X] Amendment/Reply
     - ii. [ ] Affidavit(s)/ Declaration(s)
     - iii. [ ] Information Disclosure Statement (IDS)
     - iv. [ ] Other _____

2. Miscellaneous
   - a. [ ] Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of _____ months. (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)
   - b. [ ] Other _____

3. Fees The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.
   - a. [X] The Director is hereby authorized to charge the following fees, any underpayment of fees, or credit any overpayments, to Deposit Account No. 13-2855 .
     - i. [X] RCE fee required under 37 CFR 1.17(e)
     - ii. [ ] Extension of time fee (37 CFR 1.136 and 1.17)
     - iii. [ ] Other _____
   - b. [ ] Check in the amount of $ _____ enclosed
   - c. [ ] Payment by credit card (Form PTO-2038 enclosed)
   - d. [X] Payment by EFS-Web

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

Signature	/Joshua T. Elliott/	Date	May 10, 2021
Name (Print/Type)	Joshua T. Elliott	Registration No.	43,603

CERTIFICATE OF MAILING OR TRANSMISSION

I hereby certify that this correspondence is being EFS-Web transmitted to the United States Patent and Trademark Office (USPTO), deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Mail Stop RCE, Commissioner for Patents, P. O. Box 1450, Alexandria, VA 22313-1450 or facsimile transmitted to the USPTO on the date shown below.

Signature	/Joshua T. Elliott/		
Name (Print/Type)	Joshua T. Elliott	Date	May 10, 2021

Electronic Patent Application Fee Transmittal	
Application Number:	15526162
Filing Date:	11-May-2017
Title of Invention:	MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS
First Named Inventor/Applicant Name:	Toralf SENGER
Filer:	Joshua Thomas Elliott/Rebecca Walsh
Attorney Docket Number:	32471/H150220
Filed as Large Entity	

Filing Fees for U.S. National Stage under 35 USC 371

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 2 months with $0 paid	1252	1	640	640
Miscellaneous:				
RCE- 1ST REQUEST	1801	1	1360	1360
		Total in USD ($)		**2000**

Electronic Acknowledgement Receipt	
EFS ID:	42678054
Application Number:	15526162
International Application Number:	
Confirmation Number:	1033
Title of Invention:	MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS
First Named Inventor/Applicant Name:	Toralf SENGER
Customer Number:	4743
Filer:	Joshua Thomas Elliott/Rebecca Walsh
Filer Authorized By:	Joshua Thomas Elliott
Attorney Docket Number:	32471/H150220
Receipt Date:	10-MAY-2021
Filing Date:	11-MAY-2017
Time Stamp:	13:20:35
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$2000
RAM confirmation Number	E202150D21201769
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Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		Amendment_After_Final_Action_Under_37CFR1.PDF	37322 f241884ebb30c2172687a316d4eecd59f0a2cf3f	yes	6

Multipart Description/PDF files in .zip description

Document Description	Start	End
Applicant Arguments/Remarks Made in an Amendment	5	6
Claims	2	4
Response After Final Action	1	1

Warnings:

Information:

2	Extension of Time	Two_Month_Request_for_Extension_of_Time_Under_37_CFR_1136a.PDF	22654 71927ae0c0f52163d1b219492298f127ee595090	no	1

Warnings:

Information:

3	Request for Continued Examination (RCE)	Request_for_Continued_Examination_Transmittal.PDF	27064 b769fe214d4311e627db1da69d90485aa02c696c	no	1

Warnings:

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Information:

4	Fee Worksheet (SB06)	fee-info.pdf	32647 7543223f0b098c5778d841bb9fde87f81b5dac58	no	2

Warnings:

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Total Files Size (in bytes):	119687

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Docket No.: 32471/H150220
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Toralf Senger et al.

Application No.: 15/526,162 | Confirmation No.: 1033

Filed: May 11, 2017 | Art Unit: 1655

For: MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS | Examiner: D. A. Davis

AMENDMENT AFTER FINAL ACTION UNDER 37 C.F.R. § 1.116

MS AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

INTRODUCTORY COMMENTS

In response to the Office Action dated December 8, 2020, please amend the above-identified U.S. patent application as follows:

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 5 of this paper.

AMENDMENTS TO THE CLAIMS

1. (Cancelled).

2. (Currently amended) *Brassica napus* plant or part thereof, comprising lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and optionally arachidonic acid (ARA), wherein

a) the content of EPA is at least 5% higher than of ARA, and/or

b) the sum of contents of EPA+DHA is at least 7% higher than ARA and/or

c) the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%, and

wherein the Brassica napus plant or part thereof comprises a Delta-5 elongase gene under the control of a FAE promoter ~~such that expression of the Delta-5 elongase gene is maintained or increased in late stage seed development~~.

3. (Currently amended) Brassica napus plant comprising seeds that comprise lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), wherein, when the Brassica napus plant or part thereof is grown, the content of ARA in the lipids of the seeds decreases at least 0.5% by weight of total lipids and the content of EPA and/or DHA in the lipids of the seeds increases ~~during late stage seed development~~, wherein the seeds comprise a Delta-5 elongase gene under the control of a FAE promoter.

4. (Previously presented) Brassica napus plant according to claim 3, wherein the content of EPA in the lipids of the seeds increases at least 1.0% by weight of total lipids during late stage seed development.

5. (Cancelled).

6. (Previously presented) Seed of a *Brassica napus* plant according to claim 2.

7. (Currently amended) An extracted *Brassica napus* seed lipid composition obtainable or obtained by a process comprising the steps of

a) growing a Brassica napus plant producing arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the seeds, wherein the

Brassica napus plant comprises a Delta-5 elongase gene under the control of a FAE promoter,

b) determining the peak of ARA content in the lipids of the developing seeds of the Brassica napus plant of a),

c) harvesting seeds of the plant when, after the peak of ARA content, the ARA content has decreased and the EPA and/or DHA content in the lipids has increased in the lipids of the developing seeds, and

d) extracting a lipid composition from the harvested seeds of c) to obtain said lipid composition.

8. (Cancelled)

9. (Cancelled)

10. (Withdrawn) Method of altering plant lipids composition, comprising the step of growing a *Brassica napus* plant according to claim 2 to produce lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), wherein the step of growing and lipids production is continued until the content of ARA has decreased.

11. (Withdrawn) Method of producing a *Brassica napus* plant lipid composition, comprising the steps of

a) growing Brassica napus plants according to claim 2,

b) harvesting the plants or a part thereof when the lipids content of ARA has decreased.

12. (Withdrawn) Method according to claim 10, wherein ARA decreases by at least 0.5 wt.-%.

13. (Previously presented) The extracted *Brassica napus* seed lipid composition of claim 7, wherein the content of EPA is at least 5% higher than of ARA.

14. (Previously presented) The extracted *Brassica napus* seed lipid composition of claim 7, wherein the sum of contents of EPA+DHA is at least 7% higher than ARA.

15. (Previously presented) The extracted *Brassica napus* seed lipid composition of claim 7, wherein the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%.

16. (Previously presented) The *Brassica napus* plant or part thereof of claim 2, wherein the content of EPA is at least 5% higher than of ARA.

17. (Previously presented) The *Brassica napus* plant or part thereof of claim 2, wherein the sum of contents of EPA+DHA is at least 7% higher than ARA.

18. (Previously presented) The *Brassica napus* plant or part thereof of claim 2, wherein the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%.

19. (New) The *Brassica napus* plant or part thereof of claim 2, wherein expression of the Delta-5 elongase gene is maintained or increased in late stage seed development.

20. (New) The *Brassica napus* plant of claim 3, wherein, when the Brassica napus plant or part thereof is grown, the content of ARA in the lipids of the seeds decreases at least 0.5% by weight of total lipids and the content of EPA and/or DHA in the lipids of the seeds increases during late stage seed development.

REMARKS

Upon entry of this amendment, claims 2-4, 6-7, and 10-20 will be pending in the present application. Claims 2, 3, and 7 have been amended as described herein. Claims 5, 8, and 9 have been cancelled. New dependent claims 19 and 20 have been added. Claims 10-12 have been withdrawn. No new matter has been added.

Claim 2, 3, and 7 have been amended to recite that the Brassica napus plant, part thereof, or seeds thereof comprise a Delta-5 elongase gene under the control of a **FAE** promoter, support for which is found throughout U.S. Provisional Patent Applications 62/079,622 and 62/234,373, which were incorporated by reference in the current application (see, e.g., Application No. 62/079,622 at pages 62, 63, 67, 75, 76, 80 and Application No. 62/234,373 at page 68).

Reconsideration of the rejections is respectfully requested in view of the foregoing amendments and remarks that follow.

Claim Rejections – 35 USC §103

Claims 1-9 and 13-18 were rejected as being unpatentable as obvious over WO 2013/153404. Applicant respectfully traverses based on the amended claims.

With respect to Applicant's prior argument related to the delta-5 elongase in WO 2013/153404 being driven by the conlinin1 promoter, the Office Action states that "[i]n response, the instant claims do not recite a particular promoter. The WIPO '404 reference also discloses the use of other promoters (see page 35-36). …." *Office Action at p. 5-6.*

Independent claims 2, 3, and 7 now recite that the Brassica napus plant or part thereof or seeds comprise a Delta-5 elongase gene under the control of a **FAE promoter**.

The cited prior art does not teach or suggest using a FAE promoter, let alone using such a promoter in a Brassica napus plant to drive expression of a Delta-5 elongase gene. Moreover, there is simply no teaching or suggestion that using a FAE promoter to drive expression of a Delta-5 elongase gene in a Brassica napus plant would result in the claimed lipid profile, which was surprising. In view thereof, a person of ordinary skill in the art would not have expected the content of EPA and/or DHA in the lipids of seeds would

increase while the content of ARA in the lipids of the seeds would **decrease**, resulting in the surprising lipid profile.

There is simply no teaching, suggestion, or motivation that would have led a person of skill in the art to modify the conlinin1 promoter of WO 2013/153404 to a FAE promoter, let alone to modify WO 2013/153404 to use a FAE promoter to drive a Delta-5 elongase gene in *Brassica napus*.

Thus, the subject matter recited in the pending claims would not have been obvious over the cited art. In view thereof, Applicant respectfully requests that the rejections be withdrawn.

Conclusion

In view of the above, Applicant believes the claims are in condition for allowance and requests notification of the same. Should the Office have any questions regarding this response or the application, the Office is invited to contact the undersigned at the telephone number indicated below.

The Director is hereby authorized to charge any additional fees which may be required with respect to this communication, or credit any overpayment, to Deposit Account No. 13-2855, under Order No. 32471/H150220.

Dated: May 10, 2021

Respectfully submitted,

Electronic signature: /Joshua T. Elliott/
Joshua T. Elliott
Registration No.: 43,603
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Attorney for Applicant

PTO/AIA/22 (10-20)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)	Docket Number (Optional) 32471/H150220
Application Number 15/526,162-Conf. #1033	Filed May 11, 2017
For MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS	
Art Unit 1655	Examiner D. A. Davis

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

	Fee	Small Entity Fee	Micro Entity Fee	
☐ One month (37 CFR 1.17(a)(1))	$220	$110	$55	$
☒ Two months (37 CFR 1.17(a)(2))	$640	$320	$160	$ 640.00
☐ Three months (37 CFR 1.17(a)(3))	$1,480	$740	$370	$
☐ Four months (37 CFR 1.17(a)(4))	$2,320	$1,160	$580	$
☐ Five months (37 CFR 1.17(a)(5))	$3,160	$1,580	$790	$

☐ Applicant asserts small entity status. See 37 CFR 1.27.

☐ Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

☐ A check in the amount of the fee is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☐ The Director has already been authorized to charge fees in this application to a Deposit Account.

☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 13-2855 .

☒ Payment made via EFS-Web.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

☐ applicant.

☒ attorney or agent of record. Registration number 43,603 .

☐ attorney or agent acting under 37 CFR 1.34. Registration number _____ .

/Joshua T. Elliott/ — Signature
May 10, 2021 — Date

Joshua T. Elliott — Typed or printed name
(312) 474-6300 — Telephone Number

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

☐ * Total of 1 forms are submitted.

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/526,162	Filing Date 05/11/2017	☐ To be Mailed

ENTITY: ☑ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $80 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $420 =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).				
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))					
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
AMENDMENT	Total (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
AMENDMENT	Total (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.	SLIE
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".	/DEIDRE C KING-WOODWARD/
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".	
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.	

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/526,162	05/11/2017	Toralf SENGER	32471/H150220	1033

4743 7590 06/03/2021

MARSHALL, GERSTEIN & BORUN LLP
233 SOUTH WACKER DRIVE
6300 WILLIS TOWER
CHICAGO, IL 60606-6357

EXAMINER	
MI, QIUWEN	

ART UNIT	PAPER NUMBER
1655	

NOTIFICATION DATE	DELIVERY MODE
06/03/2021	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

mgbdocket@marshallip.com

Office Action Summary	**Application No.** 15/526,162	**Applicant(s)** SENGER et al.	
	Examiner QIUWEN MI	**Art Unit** 1655	**AIA (FITF) Status** Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 5/10/2021.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 2-4,6-7 and 10-20 is/are pending in the application.

5a) Of the above claim(s) 10-12 is/are withdrawn from consideration.

6) ☑ Claim(s) 2-4,6 and 16-20 is/are allowed.

7) ☑ Claim(s) 7 and 13-15 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

Notice of Pre-AIA or AIA Status

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

DETAILED ACTION

CONTINUED EXAMINATIONS

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 5/10/2021 has been entered.

Claims 2-4, 6, 7, and 10-20 are pending. Claims 10-12 are withdrawn.

Claims 12-4, 6, 7, and 13-20 are examined on the merits.

Claim Rejections –35 USC § 102/103

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a)(1) the claimed invention was patented, described in a printed publication, or in public use, on sale, or otherwise available to the public before the effective filing date of the claimed invention.
>
> (a)(2) the claimed invention was described in a patent issued under section 151, or in an application for patent published or deemed published under section 122(b), in which the patent or application, as the case may be, names another inventor and was effectively filed before the effective filing date of the claimed invention.

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 7, and 13-15 are newly rejected under 35 USC § 102 (a)(2) as being anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over Iassonova et al (US 2014/0220215 A1).

Iassonova et al teach in some embodiments, the second fat including both EPA and DHA may be derived from a vegetable-sourced oil, such as, for example, a rapeseed oil. In some embodiments, the rapeseed oil is a canola oil that includes at least 2 wt %, at least 3 wt %, at least 5 wt %, at least 7 wt %, at least 10 wt %, at least 13 wt %, at least 15 wt %, or at least 20 wt % VLC Omega-3 PUFAs. In some embodiments, such canola oil includes at least 2 wt %, at least 3 wt %, at least 5 wt %, at least 7 wt %, at least 10 wt %, at least 13 wt %, at least 15 wt %, or at least 20 wt % combined DHA and EPA [0022] (since the composition doesn't contain ARA, thus claims 13 and 14 are met; . Iassonova et al teach methods of preparing rapeseed that has been modified to produce VLC omega-3 PUFAs are known to those of skill in the relevant arts and are described, for example, in U.S. Pat. No. 7,544,859 (Heinz et al.), U.S. patent application Ser. No. 10/566,944 (Zank et al.), U.S. Pat. No. 7,777,098 (Cirpus et al.), U.S. patent application Ser. No. 12/768,227 (Cirpus et al.), U.S. patent application Ser. No. 10/590,457

(Cirpus et al.), U.S. Pat. No. 8,049,064 (Cirpus et al.), Ser. No. 12/438,373 (Bauer et al.), and International Patent Application No. PCT/CA2007/001218 (Meesaptodsuk et al.), the entireties of which are incorporated herein by reference [0020]. Iassonova et al teach in one implementation, food products of the disclosure contain at least 16 mg of VLC omega-3 PUFA (preferably DHA and/or EPA) (since the combination of DHA and EPA is 20%, and it could be "and/or", thus claim 15 is met), desirably at least 320 mg of VLC omega-3 PUFA (preferably DHA and/or EPA), per 50 g of the food product [0036].

As discussed above, the cited references disclose EPA and DHA may be derived from a vegetable-sourced oil, such as, for example, a rapeseed oil. Although the cited reference does not teach the claimed process in claim 7, the extracted lipid composition comprising EPA and/or DHA from the reference is not patentably distinct from the claimed extracts from claims 7, and 13-15. Consequently, the claimed method appears to be anticipated by the references.

In the alternative, even if the claimed method is not identical to the referenced method with regard to some unidentified characteristics, the differences between that which is disclosed and that which is claimed are considered to be so slight that the referenced method is likely to inherently possess the same characteristics of the claimed method particularly in view of the similar characteristics which they have been shown to share. Thus, the claimed method would have been obvious to those of ordinary skill in the art with the meaning of U.S.C. 103.

Accordingly, the claimed invention as a whole was at least *prima facie* obvious, if not anticipated by the reference, especially in the absence of sufficient, clear, and convincing evidence to the contrary.

With respect to the art rejection above, please note that Patent and Trademark Office is not equipped to conduct experimentation in order to determine whether Applicants' method

differs and, if so, to what extent, from that of discussed references. Therefore, with the showing of the references, the burden of establishing non-obviousness by objective evidence is shifted to the Applicants.

Applicant is noted that the process steps in claim 7 are broad and can include additional processing steps to result in a product that might be the same as a product made by a different process. What is being claimed is merely a "lipid composition" that is extracted from the harvested seeds of Brassica napus. The process present in the product by process is open; there can be additional steps as a part of extracting from the harvested seeds. Could it not include a step of purifying to a single lipid as a part of "extracting a lipid composition" as claimed? The claims read on a combination of purified EPA and DHA. Would that purified lipid be any different from the same purified lipid made by a different process? As long it could make EPA/DHA, any genetically modified Brassica napus seeds will do, it doesn't have to contain delta-5 elongase gene under the control of a FAE promoter.

Applicant's arguments have been fully considered and are persuasive. Therefore, the rejection has been withdrawn. However, upon further consideration, a new ground(s) of rejection is made in view of Iassonova et al.

Conclusion

Claims 2-4, 6, and 16-20 are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to QIUWEN MI whose telephone number is (571)272-5984. The examiner can normally be reached on Monday-Friday 8:30 am to 5:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Terry McKelvey can be reached on 571-272-0775. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Qiuwen Mi/

Primary Examiner, Art Unit 1655

Notice of References Cited	Application/Control No. 15/526,162	Applicant(s)/Patent Under Reexamination SENGER et al.	
	Examiner QIUWEN MI	Art Unit 1655	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-20140220215-A1	08-2014	lassonova; Diliara	A23D9/00	426/541
	B					
	C					
	D					
	E					
	F					
	G					
	H					
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/526,162	SENGER et al.
	Examiner	Art Unit
	QIUWEN MI	1655

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
west, stn	05/28/2021	QM
inventor name search, palm	05/28/2021	QM

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

/QIUWEN MI/ Primary Examiner, Art Unit 1655	

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/526,162	SENGER et al.
	Examiner	**Art Unit**
	QIUWEN MI	1655

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

CLAIMS										
☐ Claims renumbered in the same order as presented by applicant						☐ CPA		☐ T.D.		☐ R.1.47
CLAIM		**DATE**								
Final	Original	05/28/2021								
	1	-								
	2	=								
	3	=								
	4	=								
	5	-								
	6	=								
	7	✓								
	8	-								
	9	-								
	10	N								
	11	N								
	12	N								
	13	✓								
	14	✓								
	15	✓								
	16	=								
	17	=								
	18	=								
	19	=								
	20	=								

Refine Search

Search Results

Term	Documents
(17 AND 5).PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD,FPRS.	0
(L17 and l5).PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD,FPRS.	0

Database: [redacted]

Search Type: (•) **Prior Art** ( ) **Interference**

Search: L18 Refine Search

Order By: (•) **Date** ( ) **Hits**

Recall Text Clear Interrupt

Search History

DATE: Friday, May 28, 2021 Purge Queries Printable Copy Create Case

Order By	Set Name Side by Side	Query	Hit Count	Set Name Result Set	Set Name Grid	Set Name Classification
Prior Art Searches						
	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD,FPRS; PLUR=YES; OP=ADJ					
Date	L18	L17 and l5	0	L18	L18	L18
Date	L17	L16 and transgenic	0	L17	L17	L17
	DB=PGPB; PLUR=YES; OP=ADJ					
Date	L16	20140220215	2	L16	L16	L16
	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD,FPRS; PLUR=YES; OP=ADJ					
Date	L15	L14 not @pd>20141114	30	L15	L15	L15
Date	L14	l2 near20 l3 near20 rapeseed oil	55	L14	L14	L14
Date	L13	L12 not @pd>20141114	184	L13	L13	L13

Date	L12	l2 same l3 same rapeseed oil	342	L12	L12	L12
Date	L11	L10 not l8	37	L11	L11	L11
Date	L10	L9 not @pd>20141114	50	L10	L10	L10
Date	L9	L4 same extract$	148	L9	L9	L9
Date	L8	L7 not @pd>20141114	13	L8	L8	L8
Date	L7	L6 same extract$	31	L7	L7	L7
Date	L6	L5 same l4	186	L6	L6	L6
Date	L5	arachidonic acid or ARA	128642	L5	L5	L5
Date	L4	L3 same l2 same l1	599	L4	L4	L4
Date	L3	decosahexaenoic acid or DHA	39287	L3	L3	L3
Date	L2	eicosapentaenoic acid or EPA	94760	L2	L2	L2
Date	L1	brassica napus or brassic$ nap$ or rapeseed	115549	L1	L1	L1

END OF SEARCH HISTORY

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BIB DATA SHEET

CONFIRMATION NO. 1033

SERIAL NUMBER	FILING or 371(c) DATE RULE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
15/526,162	05/11/2017	424	1655	32471/H150220

APPLICANTS

BASF PLANT SCIENCE COMPANY GMBH, Ludwigshafen, GERMANY;

INVENTORS

Toralf SENGER, Durham, NC;
Carl ANDRE, Raleigh, NC;

**** CONTINUING DATA ***************************

This application is a 371 of PCT/EP2015/076630 11/13/2015
which claims benefit of 62/234,373 09/29/2015
and claims benefit of 62/079,622 11/14/2014

**** FOREIGN APPLICATIONS ***************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****

07/02/2018

Foreign Priority claimed ❑ Yes ☑ No 35 USC 119(a-d) conditions met ❑ Yes ☑ No Verified and Acknowledged /DEBORAH A DAVIS/ Examiner's Signature	❑ Met after Allowance Initials	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
		NC	0	12	3

ADDRESS

MARSHALL, GERSTEIN & BORUN LLP
233 SOUTH WACKER DRIVE
6300 WILLIS TOWER
CHICAGO, IL 60606-6357
UNITED STATES

TITLE

MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS

FILING FEE RECEIVED		
1620	FEES: Authority has been given in Paper No._____ to charge/credit DEPOSIT ACCOUNT No._____ for following:	❑ All Fees ❑ 1.16 Fees (Filing) ❑ 1.17 Fees (Processing Ext. of time) ❑ 1.18 Fees (Issue) ❑ Other _____ ❑ Credit

BIB (Rev. 05/07).

FILE 'MEDLINE, AGRICOLA, CABA, BIOSIS, CAPLUS, EMBASE' ENTERED AT

12:39:37 ON 28 MAY 2021

L1 274 S (DELTA-5 OR DELTA 5) (2A) ELONGASE

L2 14 S FAE (2A) PROMOTER

L3 170643 S BRASSICA NAPUS OR BRASSIC? NAP? OR RAPESEED

L4 0 S L1 AND L2 AND L3

L5 33 S L1 AND L3

L6 12 S L5 NOT PY>2014

L7 5 DUP REM L6 (7 DUPLICATES REMOVED)

L8 7 S L2 AND L3

L9 4 S L8 NOT PY>2014

L10 2 DUP REM L9 (2 DUPLICATES REMOVED)

L11 175148 S EICOSAPENTAENOIC ACID OR EPA

L12 103657 S DECOSAHEXAENOIC ACID OR DHA

L13 678 S L3 AND L11 AND L12

L14 3 S L13 AND L1

L15 418 S L13 NOT PY>2014

L16 1 S L14 NOT PY>2014

L17 1 S L13 AND L2

L18 1 S L17 NOT PY>2014

L19 8 S LBFLFK OR LBFDAU OR PTA-121703

L20 5 S L3 AND L19

L21 0 S L20 NOT PY>2014

Freeform Search

Database:

Search Type: (•) Prior Art ( ) Interference

Term: LBFLFK or LBFDAU or PTA-121703

Recall Text

Display: 20 **Documents in Display Format:** - **Starting with Number** 1

Order By: (•) Date ( ) Hits

Generate: ( ) Hit List (•) Hit Count ( ) Side by Side ( ) Image

Search Clear Interrupt

Search History

DATE: Friday, May 28, 2021 Purge Queries Printable Copy Create Case

Order By	Set Name Side by Side	Query	Hit Count	Set Name Result Set	Set Name Grid	Set Name Classification
Prior Art Searches						
	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD,FPRS; PLUR=YES; OP=ADJ					
Date	L24	LBFLFK or LBFDAU or PTA-121703	18	L24	L24	L24
Date	L23	L22 and l19	237	L23	L23	L23
	DB=PGPB,USPT,USOC; PLUR=YES; OP=ADJ					
Date	L22	A61k$/$.cpc.	638139	L22	L22	L22
	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD,FPRS; PLUR=YES; OP=ADJ					
Date	L21	L19 same l3	0	L21	L21	L21
Date	L20	L19 same l2	3	L20	L20	L20
Date	L19	L1 same l4 same l5	599	L19	L19	L19
Date	L18	l1 same l2 same l3	0	L18	L18	L18
Date	L17	l16 not @pd>20141114	4	L17	L17	L17

Date	L16	l1 same l3	5	L16	L16	L16
Date	L15	l7 not @pd>20141114	6	L15	L15	L15
Date	L14	11033593.pn.	2	L14	L14	L14
Date	L13	10731169.pn.	2	L13	L13	L13
Date	L12	9428757.pn.	4	L12	L12	L12
Date	L11	8822662.pn.	7	L11	L11	L11
Date	L10	8853383.pn.	4	L10	L10	L10
Date	L9	8455035.pn.	2	L9	L9	L9
Date	L8	8785163.pn.	2	L8	L8	L8
Date	L7	L1 same l2	21	L7	L7	L7
Date	L6	L2 same l3	0	L6	L6	L6
Date	L5	decosahexaenoic acid or DHA	39287	L5	L5	L5
Date	L4	eicosapentaenoic acid or EPA	94760	L4	L4	L4
Date	L3	FAE near2 promoter	39	L3	L3	L3
Date	L2	(delta-5 or delta 5) near2 elongase	295	L2	L2	L2
Date	L1	brassica napus or brassic$ nap$ or rapeseed	115549	L1	L1	L1

END OF SEARCH HISTORY

Docket No.: 32471/H150220
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Toralf Senger et al.

Application No.: 15/526,162 | Confirmation No.: 1033

Filed: May 11, 2017 | Art Unit: 1655

For: MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS | Examiner: Quiwen Mi

AMENDMENT AFTER NON-FINAL ACTION UNDER 37 C.F.R. § 1.116

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

INTRODUCTORY COMMENTS

In response to the Office Action dated December 8, 2020, please amend the above-identified U.S. patent application as follows:

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 4 of this paper.

AMENDMENTS TO THE CLAIMS

1. (Cancelled).

2. (Previously presented) *Brassica napus* plant or part thereof, comprising lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and optionally arachidonic acid (ARA), wherein

a) the content of EPA is at least 5% higher than of ARA, and/or

b) the sum of contents of EPA+DHA is at least 7% higher than ARA and/or

c) the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%, and

wherein the Brassica napus plant or part thereof comprises a Delta-5 elongase gene under the control of a FAE promoter.

3. (Previously presented) Brassica napus plant comprising seeds that comprise lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), wherein, when the Brassica napus plant or part thereof is grown, the content of ARA in the lipids of the seeds decreases at least 0.5% by weight of total lipids and the content of EPA and/or DHA in the lipids of the seeds increases, wherein the seeds comprise a Delta-5 elongase gene under the control of a FAE promoter.

4. (Previously presented) Brassica napus plant according to claim 3, wherein the content of EPA in the lipids of the seeds increases at least 1.0% by weight of total lipids during late stage seed development.

5. (Cancelled).

6. (Previously presented) Seed of a *Brassica napus* plant according to claim 2.

7. (Cancelled)

8. (Cancelled)

9. (Cancelled)

10. (Withdrawn) Method of altering plant lipids composition, comprising the step of growing a *Brassica napus* plant according to claim 2 to produce lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), wherein the step of growing and lipids production is continued until the content of ARA has decreased.

11. (Withdrawn) Method of producing a *Brassica napus* plant lipid composition, comprising the steps of

a) growing Brassica napus plants according to claim 2,

b) harvesting the plants or a part thereof when the lipids content of ARA has decreased.

12. (Withdrawn) Method according to claim 10, wherein ARA decreases by at least 0.5 wt.-%.

13. (Cancelled)

14. (Cancelled)

15. (Cancelled)

16. (Previously presented) The *Brassica napus* plant or part thereof of claim 2, wherein the content of EPA is at least 5% higher than of ARA.

17. (Previously presented) The *Brassica napus* plant or part thereof of claim 2, wherein the sum of contents of EPA+DHA is at least 7% higher than ARA.

18. (Previously presented) The *Brassica napus* plant or part thereof of claim 2, wherein the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%.

19. (Previously presented) The *Brassica napus* plant or part thereof of claim 2, wherein expression of the Delta-5 elongase gene is maintained or increased in late stage seed development.

20. (Previously presented) The *Brassica napus* plant of claim 3, wherein, when the Brassica napus plant or part thereof is grown, the content of ARA in the lipids of the seeds decreases at least 0.5% by weight of total lipids and the content of EPA and/or DHA in the lipids of the seeds increases during late stage seed development.

REMARKS

Upon entry of this amendment, claims 2-4, 6, 10-12, and 16-20 will be pending in the present application. Claims 7 and 13-15 have been cancelled. No claims have been amended. No new matter has been added.

Claims 2-4, 6, and 16-20 have been allowed.

Claims 10-12 were previously withdrawn.

Reconsideration of the rejections is respectfully requested in view of the foregoing amendments and remarks that follow.

Claim Rejections – 35 USC §103

Claims 7 and 13-15 were rejected as being unpatentable as anticipated by or, in the alternative, obvious over US 2014/0220215.

Applicant respectfully disagrees with the rejection. However, in an effort to advance prosecution only, and not for any reason related to patentability, claims 7 and 13-15 have been cancelled. Applicant reserves the right to pursue the cancelled subject matter in further prosecution, including in continuing applications.

Rejoinder of claims

Method claims 10-12 all ultimately depend on claim 2 and thus include all of the recitations of claim 2, which has been allowed. As such, Applicant respectfully requests that withdrawn method claims 10-12 be rejoined and allowed.

Conclusion

In view of the above, Applicant believes the claims are in condition for allowance and requests notification of the same. Should the Office have any questions regarding this response or the application, the Office is invited to contact the undersigned at the telephone number indicated below.

The Director is hereby authorized to charge any additional fees which may be required with respect to this communication, or credit any overpayment, to Deposit Account No. 13-2855, under Order No. 32471/H150220.

Dated: October 4, 2021

Respectfully submitted,

Electronic signature: /Joshua T. Elliott/
Joshua T. Elliott
Registration No.: 43,603
MARSHALL, GERSTEIN & BORUN LLP
233 S. Wacker Drive
6300 Willis Tower
Chicago, Illinois 60606-6357
(312) 474-6300
Attorney for Applicant

Electronic Patent Application Fee Transmittal	
Application Number:	15526162
Filing Date:	11-May-2017
Title of Invention:	MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS
First Named Inventor/Applicant Name:	Toralf SENGER
Filer:	Joshua Thomas Elliott/Rebecca Walsh
Attorney Docket Number:	32471/H150220
Filed as Large Entity	

Filing Fees for U.S. National Stage under 35 USC 371

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 1 month with $0 paid	1251	1	220	220
Miscellaneous:				
		Total in USD ($)		**220**

Electronic Acknowledgement Receipt	
EFS ID:	43939394
Application Number:	15526162
International Application Number:	
Confirmation Number:	1033
Title of Invention:	MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS
First Named Inventor/Applicant Name:	Toralf SENGER
Customer Number:	4743
Filer:	Joshua Thomas Elliott/Rebecca Walsh
Filer Authorized By:	Joshua Thomas Elliott
Attorney Docket Number:	32471/H150220
Receipt Date:	04-OCT-2021
Filing Date:	11-MAY-2017
Time Stamp:	14:39:27
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$220
RAM confirmation Number	E202104E41190230
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Extension of Time	One_Month_Request_for_Extension_of_Time_Under_37_CFR_1136a.PDF	22767 0acc8e7cb6acc2b93ce47be13b41c25d0cd30c46	no	1
Warnings:					
Information:					
2		Amendment.PDF	30090 2dae3638df6d97ef0a0cad28dd8279a146e31c72	yes	5

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Amendment/Req. Reconsideration-After Non-Final Reject	1	1
	Claims	2	3
	Applicant Arguments/Remarks Made in an Amendment	4	5

Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	38394 9f848d870ec38e39051cff5bd56f51077d0b0867	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	91251		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/AIA/22 (10-20)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)	Docket Number (Optional) 32471/H150220
Application Number 15/526,162-Conf. #1033	Filed May 11, 2017
For MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS	
Art Unit 1655	Examiner Qiuwen Mi

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

	Fee	Small Entity Fee	Micro Entity Fee	
[x] One month (37 CFR 1.17(a)(1))	$220	$110	$55	$ 220.00
[ ] Two months (37 CFR 1.17(a)(2))	$640	$320	$160	$
[ ] Three months (37 CFR 1.17(a)(3))	$1,480	$740	$370	$
[ ] Four months (37 CFR 1.17(a)(4))	$2,320	$1,160	$580	$
[ ] Five months (37 CFR 1.17(a)(5))	$3,160	$1,580	$790	$

[ ] Applicant asserts small entity status. See 37 CFR 1.27.

[ ] Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

[ ] A check in the amount of the fee is enclosed.

[ ] Payment by credit card. Form PTO-2038 is attached.

[ ] The Director has already been authorized to charge fees in this application to a Deposit Account.

[x] The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 13-2855 .

[x] Payment made via EFS-Web.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

[ ] applicant.

[x] attorney or agent of record. Registration number 43,603 .

[ ] attorney or agent acting under 37 CFR 1.34. Registration number _____ .

/Joshua T. Elliott/	October 4, 2021
Signature	Date
Joshua T. Elliott	(312) 474-6300
Typed or printed name	Telephone Number

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

[ ] * Total of 1 forms are submitted.

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/526,162	Filing Date 05/11/2017	☐ To be Mailed

ENTITY: ☑ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $80 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $420 =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).				
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))					
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT	10/04/2021	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 19	Minus	** 20	= 0		x $100 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	*** 3	= 0		x $480 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	0

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.	LIE
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".	/MARSHA R RICHARDS/
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".	
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.	

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
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www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

4743 7590 10/20/2021

MARSHALL, GERSTEIN & BORUN LLP
233 SOUTH WACKER DRIVE
6300 WILLIS TOWER
CHICAGO, IL 60606-6357

EXAMINER	
MI, QIUWEN	
ART UNIT	PAPER NUMBER
1655	

DATE MAILED: 10/20/2021

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/526,162	05/11/2017	Toralf SENGER	32471/H150220	1033

TITLE OF INVENTION: MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1200	$0.00	$0.00	$1200	01/20/2022

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PTOL-85 (Rev. 02/11)

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

4743 7590 10/20/2021
MARSHALL, GERSTEIN & BORUN LLP
233 SOUTH WACKER DRIVE
6300 WILLIS TOWER
CHICAGO, IL 60606-6357

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/526,162	05/11/2017	Toralf SENGER	32471/H150220	1033

TITLE OF INVENTION: MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1200	$0.00	$0.00	$1200	01/20/2022

EXAMINER	ART UNIT	CLASS-SUBCLASS
MI, QIUWEN	1655	424-755000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-09 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1_____
2_____
3_____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. Fees submitted: ❑Issue Fee ❑Publication Fee (if required) ❑Advance Order - # of Copies _____

4b. Method of Payment: *(Please first reapply any previously paid fee shown above)*

❑ Electronic Payment via EFS-Web ❑ Enclosed check ❑ Non-electronic payment by credit card (Attach form PTO-2038)

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29

❑ Applicant asserting small entity status. See 37 CFR 1.27

❑ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/526,162	05/11/2017	Toralf SENGER	32471/H150220	1033

4743 7590 10/20/2021

MARSHALL, GERSTEIN & BORUN LLP
233 SOUTH WACKER DRIVE
6300 WILLIS TOWER
CHICAGO, IL 60606-6357

EXAMINER	
MI, QIUWEN	

ART UNIT	PAPER NUMBER
1655	

DATE MAILED: 10/20/2021

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 15/526,162	Applicant(s) SENGER et al.	
	Examiner QIUWEN MI	Art Unit 1655	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☑ This communication is responsive to the claim amenment filed on 10/4/2021.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☑ The allowed claim(s) is/are 2-4,6,10-12 and 16-20 . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some* c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☑ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 7/28/17; 12/12/17.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. _____.
5. ☑ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/QIUWEN MI/
Primary Examiner, Art Unit 1655

Notice of Pre-AIA or AIA Status

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Election/Restrictions

Claim 2, directed to an allowable product. Pursuant to the procedures set forth in MPEP § 821.04(B), claims 10-12, directed to the process of making or using an allowable product, previously withdrawn from consideration as a result of a restriction requirement, claims 10-12 are hereby rejoined and fully examined for patentability under 37 CFR 1.104.

Because all claims previously withdrawn from consideration under 37 CFR 1.142 have been rejoined, **the restriction requirement as set forth in the Office action mailed on 9/6/2019 is hereby withdrawn**. In view of the withdrawal of the restriction requirement as to the rejoined inventions, applicant(s) are advised that if any claim presented in a continuation or divisional application is anticipated by, or includes all the limitations of, a claim that is allowable in the present application, such claim may be subject to provisional statutory and/or nonstatutory double patenting rejections over the claims of the instant application. Once the restriction requirement is withdrawn, the provisions of 35 U.S.C. 121 are no longer applicable. See *In re Ziegler*, 443 F.2d 1211, 1215, 170 USPQ 129, 131-32 (CCPA 1971). See also MPEP § 804.01.

EXAMINER'S COMMENT

IN THE CLAIMS:

Claims 2-4, 6, 10-12, and 16-20 are allowed.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Any inquiry concerning this communication or earlier communications from the examiner should be directed to QIUWEN MI whose telephone number is (571)272-5984. The examiner can normally be reached on Monday-Friday 8:30 am to 5:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Terry McKelvey can be reached on 571-272-0775. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Qiuwen Mi/

Primary Examiner, Art Unit 1655

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/526,162	SENGER et al.
	Examiner	Art Unit
	QIUWEN MI	1655

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
west, stn	10/04/2021	QM
inventor name search, palm	10/04/2021	QM
google search	10/04/2021	QM
interference text search	10/04/2021	QM

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner
	interference text search (USPatfull + PGPub): see attachment	10/04/2021	/Q. M./

/QIUWEN MI/ Primary Examiner, Art Unit 1655	

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/526,162	SENGER et al.
	Examiner	**Art Unit**
	QIUWEN MI	1655

CPC						
Symbol					**Type**	**Version**
A61K	/	36	/	31	F	2013-01-01
C12Q	/	1	/	6895	I	2013-01-01
C12N	/	15	/	8247	A	2013-01-01
C12N	/	9	/	0071	A	2013-01-01
C12N	/	9	/	93	A	2013-01-01
C12Y	/	114	/	19	A	2013-01-01
C12Y	/	114	/	19003	A	2013-01-01
C12Y	/	602	/	01003	A	2013-01-01
C12Y	/	114	/	19006	A	2013-01-01
A61K	/	31	/	202	A	2013-01-01

CPC Combination Sets								
Symbol					**Type**	**Set**	**Ranking**	**Version**
	/		/					

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	12	
/QIUWEN MI/ Primary Examiner, Art Unit 1655	04 October 2021	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	none

U.S. Patent and Trademark Office — Part of Paper No.: 20211004

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/526,162	SENGER et al.
	Examiner	**Art Unit**
	QIUWEN MI	1655

INTERNATIONAL CLASSIFICATION					
CLAIMED					
A61K	/	36	/	00	

NON-CLAIMED					
	/		/		

US ORIGINAL CLASSIFICATION	
CLASS	**SUBCLASS**

CROSS REFERENCES(S)						
CLASS	**SUBCLASS (ONE SUBCLASS PER BLOCK)**					

NONE (Assistant Examiner) (Date)	**Total Claims Allowed:** 12	
/QIUWEN MI/ Primary Examiner, Art Unit 1655 (Primary Examiner) 04 October 2021 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure none

U.S. Patent and Trademark Office Part of Paper No.: 20211004

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/526,162	SENGER et al.
	Examiner	**Art Unit**
	QIUWEN MI	1655

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIMS

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	2	7	18												
2	3	8	19												
3	4	9	20												
4	6														
10	10														
11	11														
12	12														
5	16														
6	17														

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	12	
/QIUWEN MI/ Primary Examiner, Art Unit 1655	04 October 2021	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	none

U.S. Patent and Trademark Office — Part of Paper No.: 20211004

Refine Search

Search Results

Term	Documents
(27 SAME 26 SAME 25).PGPB,USPT.	0
(L27 same l26 same l25).PGPB,USPT.	0

Database:

Search Type: ○ **Prior Art** ◉ **Interference**

Search: L28 Refine Search

Order By: ◉ **Date** ○ **Hits**

Recall Text Clear Interrupt

Search History

DATE: Friday, May 28, 2021 Purge Queries Printable Copy Create Case

Order By	Set Name Side by Side	Query	Hit Count	Set Name Result Set	Set Name Grid	Set Name Classification
Interference Searches						
	DB=PGPB,USPT; PLUR=YES; OP=ADJ					
Date	L28	L27 same l26 same l25	0	L28	L28	L28
Date	L27	FAE near2 promoter.clm.	5	L27	L27	L27
Date	L26	(delta-5 or delta 5) near2 elongase.clm.	27	L26	L26	L26
Date	L25	(brassica napus or brassic$ nap$ or rapeseed).clm.	7244	L25	L25	L25
Prior Art Searches						
	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD,FPRS; PLUR=YES; OP=ADJ					

Date	L24	LBFLFK or LBFDAU or PTA-121703	18	L24	L24	L24
Date	L23	L22 and l19	237	L23	L23	L23
	DB=PGPB,USPT,USOC; PLUR=YES; OP=ADJ					
Date	L22	A61k$/$.cpc.	638139	L22	L22	L22
	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD,FPRS; PLUR=YES; OP=ADJ					
Date	L21	L19 same l3	0	L21	L21	L21
Date	L20	L19 same l2	3	L20	L20	L20
Date	L19	L1 same l4 same l5	599	L19	L19	L19
Date	L18	l1 same l2 same l3	0	L18	L18	L18
Date	L17	l16 not @pd>20141114	4	L17	L17	L17
Date	L16	l1 same l3	5	L16	L16	L16
Date	L15	l7 not @pd>20141114	6	L15	L15	L15
Date	L14	11033593.pn.	2	L14	L14	L14
Date	L13	10731169.pn.	2	L13	L13	L13
Date	L12	9428757.pn.	4	L12	L12	L12
Date	L11	8822662.pn.	7	L11	L11	L11
Date	L10	8853383.pn.	4	L10	L10	L10
Date	L9	8455035.pn.	2	L9	L9	L9
Date	L8	8785163.pn.	2	L8	L8	L8
Date	L7	L1 same l2	21	L7	L7	L7
Date	L6	L2 same l3	0	L6	L6	L6
Date	L5	decosahexaenoic acid or DHA	39287	L5	L5	L5
Date	L4	eicosapentaenoic acid or EPA	94760	L4	L4	L4
Date	L3	FAE near2 promoter	39	L3	L3	L3
Date	L2	(delta-5 or delta 5) near2 elongase	295	L2	L2	L2
Date	L1	brassica napus or brassic$ nap$ or rapeseed	115549	L1	L1	L1

END OF SEARCH HISTORY

Web Search History

date, time	web site	search string
5/28/2021 12:47:39 PM	Google	(brassica napus or brassic$ nap$ or rapeseed) and (delta-5 elongase) and (FAE promoter) and (eicosapentaenoic acid or EPA) and (decosahexaenoic acid or DHA)

PTO/SB/08a (07-09)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 9

Complete if Known	
Application Number	15/526,162-Conf. #1033
Filing Date	May 11, 2017
First Named Inventor	Toralf Senger
Art Unit	
Examiner Name	
Attorney Docket Number	32471/H150220

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		US-5,627,289	05-06-1997	Jeromin et al.	
		US-6,303,849	10-16-2001	Potts et al.	
		US-6,689,880	02-10-2004	Chen et al.	
		US-6,733,974	05-11-2004	Feazel	
		US-6,740,488	05-25-2004	Rangwala et al.	
		US-6,818,807	11-16-2004	Trolinder et al.	
		US-6,825,400	11-30-2004	Behr et al.	
		US-6,893,826	05-17-2005	Hillyard et al.	
		US-6,900,014	05-31-2005	Weston et al.	
		US-7,423,198	09-09-2008	Yao et al.	
		US-8,999,411	04-07-2015	Froman et al.	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] Number[4] Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
		WO-01/059128-A2	08-16-2001	Basf Ag		
		WO-02/26946-A2	04-04-2002	Bioriginal Food & Science Corp		
		WO-2002/052024-A2	07-04-2002	Biogemma Uk Ltd		
		WO-2003/078639-A2	09-25-2003	Univ York		
		WO-2003/089452-A2	10-30-2003	Sangamo Biosciences, Inc		
		WO-2003/093482-A2	11-13-2003	Basf Plant Science Gmbh		

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5]Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /Q.M/

PTO/SB/08a (07-09)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	2	of	9

Complete if Known	
Application Number	15/526,162-Conf. #1033
Filing Date	May 11, 2017
First Named Inventor	Toralf Senger
Art Unit	
Examiner Name	
Attorney Docket Number	32471/H150220

U. S. PATENT DOCUMENTS

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FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] Number[4] Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
		WO-2004/071467-A2	08-26-2004	Du Pont		
		WO-2004/087902-A2	10-14-2004	Univ Bristol		
		WO-2004/090123-A2	10-21-2004	Basf Plant Science Gmbh		
		WO-2005/007845-A2	01-27-2005	Univ York		
		WO-2005/012316-A2	02-10-2005	Basf Plant Science Gmbh		
		WO-2005/083053-A2	09-09-2005	Basf Plant Science Gmbh		

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5]Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /Q.M/

PTO/SB/08a (07-09)
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	3	of	9

Complete if Known	
Application Number	15/526,162-Conf. #1033
Filing Date	May 11, 2017
First Named Inventor	Toralf Senger
Art Unit	
Examiner Name	
Attorney Docket Number	32471/H150220

U. S. PATENT DOCUMENTS

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		WO-2006/008099-A2	01-26-2006	Basf Plant Science Gmbh		
		WO-2006/069710-A1	07-06-2006	Basf Plant Science Gmbh		
		WO-2006/100241-A2	09-28-2006	Basf Plant Science Gmbh		
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		WO-2008/022963-A2	02-28-2008	Basf Plant Science Gmbh		
		WO-2009/111263-A1	09-11-2009	Monsanto Technology Llc		

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet	4	of	9

Complete if Known	
Application Number	15/526,162-Conf. #1033
Filing Date	May 11, 2017
First Named Inventor	Toralf Senger
Art Unit	
Examiner Name	
Attorney Docket Number	32471/H150220

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		WO-2010/066703-A2	06-17-2010	Basf Plant Science Gmbh		
		WO-2013/185184-A2	12-19-2013	Commw Scient Ind Res Org		
		WO-2015/089587-A1	06-25-2015	Commw Scient Ind Res Org		

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		TANG, et al., "Oleate Desaturase Enzymes of Soybean: Evidence of Regulation Through Differential Stability and Phosphorylation", Plant J., 44(3):433-46 (2005).	
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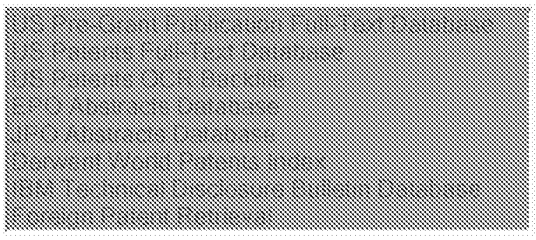
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Term	Documents
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Database: 

Search Type: (•) **Prior Art** ( ) **Interference**

Search: L14 [Refine Search]

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Order By	Set Name Side by Side	Query	Hit Count	Set Name Result Set	Set Name Grid	Set Name Classification
Prior Art Searches						
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Date	L10	L9 same (l4 or l5)	93	L10	L10	L10
Date	L9	l6 same l7	19311	L9	L9	L9

Date	L8	L3 same l4 same l5	0	L8	L8	L8
Date	L7	decosahexaenoic acid or DHA	40378	L7	L7	L7
Date	L6	eicosapentaenoic acid or EPA	96813	L6	L6	L6
Date	L5	FAE near2 promoter	40	L5	L5	L5
Date	L4	(delta-5 or delta 5) near2 elongase	296	L4	L4	L4
Date	L3	brassica napus or brassic$ nap$ or rapeseed	118453	L3	L3	L3
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L3 15 S FAE (2A) PROMOTER

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L5 106082 S DECOSAHEXAENOIC ACID OR DHA

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		WO-2010/023202-A2	03-04-2010	Basf Plant Science Gmbh		
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		WO-2013/153404-A1	10-17-2013	Rothamsted Res Ltd		

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		Hull et al., Analysis of the promoter of an abscisic acid responsive late embryogenesis abundant gene of Arabidopsis thaliana, Plant Sci., 14:181-92 (1996).	
		International Preliminary Report on Patentability, International Application No. PCT/EP2015/076630, dated May 16, 2017.	
		International Search Report and Written Opinion, International Application No. PCT/EP2015/076630, mailed March 7, 2016.	
		Livak et al., Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods, 25(4):402-8 (2001).	
		Vilardell et al., Regulation of the rab17 gene promoter in transgenic Arabidopsis wild-type, ABA-deficient and ABA-insensitive mutants, Plant Mol. Biol., 24(4):561-9 (1994).	

Examiner Signature	/QIUWEN MI/	Date Considered	10/04/2021

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /Q.M/

Docket No.: 32471/H150220
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of: Toralf Senger et al.	Allowed: October 20, 2021
Application No.: 15/526,162	Confirmation No.: 1033
Filed: May 11, 2017	Art Unit: 1655
For: MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS	Examiner: Qiuwen Mi

AMENDMENT AFTER ALLOWANCE UNDER 37 C.F.R. § 1.312

MS Issue Fee
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

INTRODUCTORY COMMENTS

Prior to issuance of the patent, applicant respectfully requests entry of this amendment under 37 C.F.R. 1.312 for the above-captioned patent application.

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 5 of this paper.

AMENDMENTS TO THE CLAIMS

1. (Cancelled).

2. (Currently amended) *Brassica napus* plant or part thereof, comprising seeds that comprise lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and optionally arachidonic acid (ARA), wherein

a) the content of EPA is at least 5% higher than of ARA, and/or

b) the sum of contents of EPA+DHA is at least 7% higher than ARA and/or

c) the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%, and

wherein the Brassica napus plant or part thereof comprises a Delta-5 elongase gene under the control of a FAE promoter.

3. (Previously presented) Brassica napus plant comprising seeds that comprise lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), wherein, when the Brassica napus plant or part thereof is grown, the content of ARA in the lipids of the seeds decreases at least 0.5% by weight of total lipids and the content of EPA and/or DHA in the lipids of the seeds increases, wherein the seeds comprise a Delta-5 elongase gene under the control of a FAE promoter.

4. (Previously presented) Brassica napus plant according to claim 3, wherein the content of EPA in the lipids of the seeds increases at least 1.0% by weight of total lipids during late stage seed development.

5. (Cancelled).

6. (Previously presented) Seed of a *Brassica napus* plant according to claim 2.

7. (Cancelled)

8. (Cancelled)

9. (Cancelled)

10. (Currently amended) Method of altering plant seed lipids composition, comprising the step of growing a *Brassica napus* plant according to claim 2 to produce seeds that comprise lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), wherein the step of growing and lipids production is continued until the content of ARA in the lipids of the seeds has decreased.

11. (Currently amended) Method of producing a *Brassica napus* plant seed lipid composition, comprising the steps of

a) growing Brassica napus plants according to claim 2,

b) harvesting the plants or a part thereof when the lipids content of ARA in the lipids of the seeds has decreased.

12. (Currently amended) Method according to claim 10, wherein ARA in the lipids of the seeds decreases by at least 0.5 wt.-%.

13. (Cancelled)

14. (Cancelled)

15. (Cancelled)

16. (Currently amended) The *Brassica napus* plant or part thereof of claim 2, wherein the content of EPA is at least 5% higher than of ARA in the lipids of the seeds.

17. (Currently amended) The *Brassica napus* plant or part thereof of claim 2, wherein the sum of contents of EPA+DHA is at least 7% higher than ARA in the lipids of the seeds.

18. (Currently amended) The *Brassica napus* plant or part thereof of claim 2, wherein the content of ARA in the lipids of the seeds is less than 4% and the content of EPA in the lipids of the seeds is more than 7% and the content of DHA in the lipids of the seeds is more than 2%.

19. (Previously presented) The *Brassica napus* plant or part thereof of claim 2, wherein expression of the Delta-5 elongase gene is maintained or increased in late stage seed development.

20. (Previously presented) The *Brassica napus* plant of claim 3, wherein, when the Brassica napus plant or part thereof is grown, the content of ARA in the lipids of the seeds decreases at least 0.5% by weight of total lipids and the content of EPA and/or DHA in the lipids of the seeds increases during late stage seed development.

REMARKS

Claims 2-4, 6, 10-12 and 16-20 are pending and have been allowed. Applicant respectfully requests that this amendment be entered, which makes minor amendments to claims 2, 10-12, and 16-18. Independent claim 2 has been amended to recite that the claimed plant or part thereof comprises seeds that comprise lipids. Dependent claims 10-12 and 16-18 have been amended to recite seed lipids and that the specified EPA, DHA, and/or ARA is in the lipids of the seeds. Support for the amendments are found throughout the application as filed (see, e.g., Abstract and Example 5). No new matter has been added.

In the event the U.S. Patent and Trademark Office determines that an extension is required, applicant petitions for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to Deposit Account No. 13-2855 referencing docket no. 32471/H150220/US.

Dated: January 18, 2022

Respectfully submitted,

By /Joshua T. Elliott/
Joshua T. Elliott
Registration No.: 43,603
MARSHALL, GERSTEIN & BORUN LLP
233 S. Wacker Drive
6300 Willis Tower
Chicago, Illinois 60606-6357
(312) 474-6300
Attorney for Applicant

Electronic Acknowledgement Receipt	
EFS ID:	44766473
Application Number:	15526162
International Application Number:	
Confirmation Number:	1033
Title of Invention:	MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS
First Named Inventor/Applicant Name:	Toralf SENGER
Customer Number:	4743
Filer:	Joshua Thomas Elliott/Karin Krahn
Filer Authorized By:	Joshua Thomas Elliott
Attorney Docket Number:	32471/H150220
Receipt Date:	18-JAN-2022
Filing Date:	11-MAY-2017
Time Stamp:	13:50:41
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		Amendment_After_Allowance_Under_37CFR1.PDF	30431 70a03d0a213726f82d70d33b24b37d96018bc327	yes	5

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Amendment after Notice of Allowance (Rule 312)	1	1
	Claims	2	4
	Applicant Arguments/Remarks Made in an Amendment	5	5
Warnings:			
Information:			
	Total Files Size (in bytes):	30431	

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Examiner-Initiated Interview Summary	**Application No.** 15/526,162	**Applicant(s)** SENGER et al.		
	Examiner QIUWEN MI	**Art Unit** 1655	**AIA (First Inventor to File) Status** Yes	**Page** 1 of 1

All Participants (applicant, applicants representative, PTO personnel)	**Title**	**Type**
QIUWEN MI	Primary Examiner	Telephonic
Joshua T. Elliott	Attorney of Record	

Date of Interview: 20 January 2022

Issues Discussed:

Proposed Amendment(s)

Applicant has agreed with the proposed claim amendment by canceling claim 6.

/QIUWEN MI/ Primary Examiner, Art Unit 1655	

Applicant is reminded that a complete written statement as to the substance of the interview must be made of record in the application file. It is the applicants responsibility to provide the written statement, unless the interview was initiated by the Examiner and the Examiner has indicated that a written summary will be provided. See MPEP 713.04
Please further see:
MPEP 713.04
Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews, paragraph (b)
37 CFR § 1.2 Business to be transacted in writing

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/526,162	SENGER et al.
	Examiner	**Art Unit**
	QIUWEN MI	1655

CPC						
Symbol					**Type**	**Version**
A61K	/	36	/	31	F	2013-01-01
C12Q	/	1	/	6895	I	2013-01-01
C12N	/	15	/	8247	A	2013-01-01
C12N	/	9	/	0071	A	2013-01-01
C12N	/	9	/	93	A	2013-01-01
C12Y	/	114	/	19	A	2013-01-01
C12Y	/	114	/	19003	A	2013-01-01
C12Y	/	602	/	01003	A	2013-01-01
C12Y	/	114	/	19006	A	2013-01-01
A61K	/	31	/	202	A	2013-01-01

CPC Combination Sets							
Symbol					**Type**	**Set**	**Ranking**	**Version**
	/		/					

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	11	
/QIUWEN MI/ Primary Examiner, Art Unit 1655	20 January 2022	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	none

U.S. Patent and Trademark Office — Part of Paper No.: 20220120

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/526,162	SENGER et al.
	Examiner	**Art Unit**
	QIUWEN MI	1655

INTERNATIONAL CLASSIFICATION					
CLAIMED					
A61K	/	36	/	00	
NON-CLAIMED					
	/		/		

US ORIGINAL CLASSIFICATION	
CLASS	**SUBCLASS**

CROSS REFERENCES(S)						
CLASS	**SUBCLASS (ONE SUBCLASS PER BLOCK)**					

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 11	
/QIUWEN MI/ Primary Examiner, Art Unit 1655 (Primary Examiner)	20 January 2022 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure none

U.S. Patent and Trademark Office — Part of Paper No.: 20220120

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/526,162	SENGER et al.
	Examiner	**Art Unit**
	QIUWEN MI	1655

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIMS

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	2	8	19												
9	3	11	20												
10	4														
2	10														
3	11														
4	12														
5	16														
6	17														
7	18														

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 11	
/QIUWEN MI/ Primary Examiner, Art Unit 1655 (Primary Examiner)	20 January 2022 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure none

U.S. Patent and Trademark Office Part of Paper No.: 20220120

Response to Rule 312 Communication	Application No.	Applicant(s)	
	15/526,162	SENGER et al.	
	Examiner	Art Unit	AIA (FITF) Status
	QIUWEN MI	1655	Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

1. ☑ The amendment filed on 18 January 2022 under 37 CFR 1.312 has been considered, and has been:

a) ☐ entered.

b) ☐ entered as directed to matters of form not affecting the scope of the invention.

c) ☐ disapproved because the amendment was filed after the payment of the issue fee.
Any amendment filed after the date the issue fee is paid must be accompanied by a petition under 37 CFR 1.313(c)(1) and the required fee to withdraw the application from issue.

d) ☐ disapproved. See explanation below.

e) ☑ entered in part. See explanation below.

Entered with the cancellation of claim 6, as it does not further limit claim 2.

/QIUWEN MI/
Primary Examiner, Art Unit 1655

PART B–FEE(S) TRANSMITTAL

Complete and send this form, together with the applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

By fax, send to: (571) 273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

MARSHALL, GERSTEIN & BORUN LLP
233 S. Wacker Drive
6300 Willis Tower
Chicago, Illinois 60606-6357

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

Joshua T. Elliott	(Typed or printed name)
/Joshua T. Elliott/	(Signature)
January 20, 2022	(Date)

APPLICATION NO	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO	CONFIRMATION NO
15/526,162	05/11/2017	Toralf Senger	32471/H150220	1033

TITLE OF INVENTION: MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS

APPLN: TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1,200.00			$1,200.00	01/20/2022

EXAMINER	ART UNIT	CLASS-SUBCLASS
Mi, Qiuwen	1655	

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363)

☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-09 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1. MARSHALL, GERSTEIN & BORUN LLP
2.
3.

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: BASF PLANT SCIENCE COMPANY GMBH

(B) RESIDENCE: (CITY and STATE or COUNTRY): Ludwigshafen, Germany

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. Fees Submitted: ☒ Issue Fee ☐ Publication Fee (if required) ☐ Advance Order - # of Copies _____

4b. Method of Payment (*Please first reapply any previously paid fee shown above*):

☒ Electronic Payment via EFS-Web ☐ Enclosed check ☐ Non-electronic payment by credit card (Attach form PTO-2038)

☒ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. 13-2855

5. Change of Entity Status (from status indicated above)

☐ Applicant certifying micro entity status. See 37 CFR 1.29. NOTE: Absent a valid Certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

☐ Applicant asserting small entity status. See 37 CFR 1.27. NOTE: If the application was previously under micro entity status, checking this box will be taken as a notification of loss of entitlement to micro entity status.

☐ Applicant changing to regular undiscounted fee status. NOTE: Checking this box will be taken as a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature /Joshua T. Elliott/ Date January 20, 2022

Typed or printed name Joshua T. Elliott Registration No. 43,603

Electronic Patent Application Fee Transmittal

Application Number:	15526162
Filing Date:	11-May-2017
Title of Invention:	MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS
First Named Inventor/Applicant Name:	Toralf SENGER
Filer:	Joshua Thomas Elliott/Rebecca Walsh
Attorney Docket Number:	32471/H150220

Filed as Large Entity

Filing Fees for U.S. National Stage under 35 USC 371

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
UTILITY APPL ISSUE FEE	1501	1	1200	1200

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**1200**

Electronic Acknowledgement Receipt	
EFS ID:	44790756
Application Number:	15526162
International Application Number:	
Confirmation Number:	1033
Title of Invention:	MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS
First Named Inventor/Applicant Name:	Toralf SENGER
Customer Number:	4743
Filer:	Joshua Thomas Elliott/Rebecca Walsh
Filer Authorized By:	Joshua Thomas Elliott
Attorney Docket Number:	32471/H150220
Receipt Date:	20-JAN-2022
Filing Date:	11-MAY-2017
Time Stamp:	13:48:58
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$1200
RAM confirmation Number	E20221JD50004519
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	Issue_Fee_Transmittal.PDF	36641 acb8a24f8ffd87651dee391c8e8a966a0cc40d16	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	37990 4f8aad12459fb20da7c5042599d7de0d3e2c5cf1	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	74631		

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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www.uspto.gov

APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/526,162	03/01/2022	11260095	32471/H150220	1033

4743 7590 02/09/2022

MARSHALL, GERSTEIN & BORUN LLP
233 SOUTH WACKER DRIVE
6300 WILLIS TOWER
CHICAGO, IL 60606-6357

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 681 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

INVENTOR(s) (Please see PAIR WEB site http://pair.uspto.gov for additional inventors):

Toralf SENGER, Durham, NC;
Carl ANDRE, Raleigh, NC;

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

BASF PLANT SCIENCE COMPANY GMBH, Ludwigshafen, GERMANY;

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