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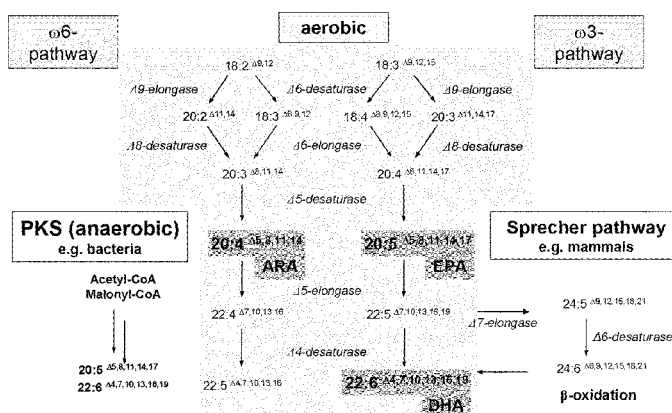
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(54) Title: STABILISING FATTY ACID COMPOSITIONS

Fig. 1



(57) Abstract: Assay method, comprising providing a plant capable of expressing a delta-12 desaturase, wherein said delta-12 desaturase has at least 50% total amino acid sequence identity to at least one of the sequences SEQ ID NO. 328 to 336, and/or at least 59% total amino acid sequence similarity to at least one of the sequences SEQ ID NO. 328 to 336, and wherein the plant is also capable of expressing at least one or more enzymes of unsaturated fatty acid metabolism, of which enzymes at least one is capable of using linoleic acid as a substrate, and of which enzymes at least one is supposedly connected to a plant metabolic property, growing the plant, and measuring said plant metabolic property for said plant.

Stabilising fatty acid compositions

FIELD OF THE INVENTION

5 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.

10 The Sequence Listing, which is a part of the present disclosure, is submitted concurrently with the specification as a text file. The subject matter of the Sequence Listing is incorporated herein in its entirety by reference.

The present invention is concerned with materials and methods for the production of genetically
15 modified plants, particularly where the plants are for the production of at least one unsaturated or polyunsaturated fatty acid. The invention is also concerned with identification of genes conveying an unsaturated fatty acid metabolic property to a plant or plant cell, and generally relates to the field of Delta-12 desaturases.

20 BACKGROUND OF THE INVENTION

For the production of genetically modified plants it is not sufficient to test the effects of nucleic acid sequences in plants under greenhouse conditions. Unfortunately it has frequently been observed that plant metabolic properties differ in unpredictable ways when plants are grown in
25 our field or under greenhouse conditions. Thus, when developing genetically modified plants having altered metabolic properties compared to the corresponding wild-type plant, it is necessary to test such plants in field trials.

However, field trials entail a variety of disadvantages compared to plant growth under greenhouse
30 conditions: for example, field trial plots have frequently been vandalised or devastated by animals, rendering all work of creating the originally planted plants and sending them on the field useless. Further, field trials require completion of Norma Rose procedures of regulatory supervision, making field trials rather cumbersome. Also, the amount of practical work in raising enough plants for a future test, devising a plot layout to plant the plants, and planting and monitoring the plants
35 is more labour intensive than testing plants under greenhouse conditions, particularly as plant maintenance and monitoring work can be highly automated in the greenhouse. Furthermore, growing plants in an automatic greenhouse allows to inspect plant parts that are not readily accessible on a field, for example because on a field the plants are grown too densely or the interesting plant part is growing underground, for example plant roots. Thus it is generally desired
40 to reduce the number of necessary field trials.

This is particularly true in the field of production of polyunsaturated fatty acids. Plants generally cannot produce unsaturated fatty acids of at least 20 carbon atoms in length and comprising at

least two double bonds. Thus, to develop plants for the production of such unsaturated fatty acids, it is necessary to install the whole metabolic process starting from linoleic acid or α -linolenic acid. Even though potentially suitable elongases and desaturase genes are known in the art and have been tested at least in model plants, it is uncertain which combinations of genes and promoters will provide economically satisfactory yields of unsaturated fatty acids in a stable way, particularly under the environmental conditions that change from growth period to growth period. Thus, field trials cannot be avoided when developing plants for the production of unsaturated fatty acids. One of the factors that has to be a certain and by field trials is whether or not the composition of the plant oil will be as expected even under field conditions. It has unfortunately been observed that the composition of plant oils measured in individual plant seeds greatly differs even for seeds obtained from the very same plant, and particularly varies between plants grown under differing conditions. Thus, the composition of a plant oil obtained from harvesting a plurality of plants grown under field conditions cannot always reliably be predicted on the basis of oil composition analyses of individual plant seeds taken from plants grown under greenhouse conditions.

Reproducible production of a specific fatty acid profile is particularly important for commercial canola oil production. There is need to identify ways to reduce the variability in the fatty acid profile of canola oils produced in different environments.

The invention thus and generally aspires to remove or alleviate the above identified shortcomings and to provide materials and methods useful for reducing the number of field trials required for the manufacturing of a marketable plant variety producing unsaturated fatty acids. Further aspects and embodiments of the invention will become apparent below.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Schematic figure of the different enzymatic activities leading to the production of ARA, EPA and DHA

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 Δ -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: Stability of binary plant expression plasmids containing the ColE1/pVS1 origin of replication for plasmid replication in E.coli/Agrobacteria. Left Panel: Stability in Agrobacterium cells by isolating plasmid DNA from Agrobacterium cultures 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 LuCnl promoter while in VC-LLM337-1qcz rc the gene was driven by the VfUSP promoter and was expressed at a lower level than the LuCnl 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(PI_GA)2 driven by the LuCnl 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 LuCnl 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 were 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 genes 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 were 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. [14C]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). In panel D Delta-4 Desaturase (PI), c-d4Des(PI_GA)2, activity was demonstrated by comparison of enzyme activity from yeast microsomes isolated from a strain expressing the c-d4Des(PI_GA)2 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*. [14C]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 (PI) 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(PI_GA)2 genes.

Figure 26: Examples of Desaturase Enzyme Reactions Showing Specificity for Acyl-lipid substrates. [14C]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 a time-course demonstrates the activity of the Delta-4 Desaturase (Tc), c-d4Des(Tc_GA).

Figure 27: Examples of Desaturase Enzyme Reactions Showing Specificity for Acyl-CoA substrates. [14C]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, called nmol 16:1 in H₂O) 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. [¹⁴C]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 R_f's as authentic standards. In the absence of [¹⁴C]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 activity 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 d5E(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*. [¹⁴C]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_GA2) protein relative to microsomes isolated from transgenic B. Napus containing the c-d6Elo(Pp_GA2) gene and the c-d6Elo(Tp_GA2) gene. In panel B the Delta-5 Elongase (Ot), 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 DHGLA 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 levels are represented as %Total Fatty acid vs Growth time (hours). Panel A pertains to samples supplied with DHGLA immediately after induction. Panel B is overnight induction (22hrs) before feeding. Panel C is for cultures supplied with 3X normal DHGLA level. Panel D is for cultures supplied with normal rate of DHGLA (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) + DHGLA B. c-d6Des(Ot_febit) + ALA C. c-d4Des(PI_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 efficiencies of delta-12-desaturation in seed of transgenic Brassica napus and in Brassica napus wildtype seeds. Shown are average conversion efficiencies of various plant populations, as well as the conversion efficiencies observed in a seedbatch 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 efficiencies 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 efficiencies 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 efficiencies 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 efficiencies 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 efficiencies 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 efficiencies 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 efficiencies 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 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 41: The sum of all pathway fatty acids was negatively correlated with seed oil content.

5 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 42: The sum of all pathway fatty acids was negatively correlated with seed oil content.

10 Shown are data of 2 generations of event LBFGKN. For the greenhouse data, one marker corresponds to 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).

15 **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 to 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
20 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,
25 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,
30 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,
35 one marker corresponds to 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).

40 **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 to 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 seedbatch 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 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 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 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 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 to 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 to 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 one 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 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 53: The conversion efficiency of the delta-6 elongase was not 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 54: The conversion efficiency of the delta-6 elongase was not negatively correlated with seed oil content. Shown are data of 2 generations of event LBFGKN. For the greenhouse data, one marker corresponds to 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 negatively correlated with seed oil content. Shown are data of 2 generations of event LBFLFK. For the greenhouse data, one marker corresponds to 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 seedbatch 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 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 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 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 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 to 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 seedbatch 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 to 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 seedbatch 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 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 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 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 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 to 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 seedbatch of one T1 plant, or the analysis of a random selection of T3 seeds representing plots (36 plots) or single plants (60 plants).

5 **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 to 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 seedbatch of one T1 plant, or the analysis of a random selection of T3 seeds
10 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 to one seedbatch of one plant, for the field data, one marker corresponds to
15 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 to one seedbatch of one plant, for the field data, one marker corresponds to
20 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 to 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
25 of one T2 seedbatch 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 to 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
30 of one T2 seedbatch of one T1 plant, or the analysis of a random selection of T3 seeds representing one plot.

35 **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.

40 **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 to 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 seedbatch 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 to 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 seedbatch 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 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 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 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 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 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 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 to 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 seedbatch 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 to 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 seedbatch 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 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.

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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 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.

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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 to 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).

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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 to 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.

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Figure 81: The levels of EPA+DHA (20:5n-3 and 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 in 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).

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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 in 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).

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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 in 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).

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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 in 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).

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Figure 85. 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 an Instant Imager. Duplicate reactions are shown for each enzyme activity in Panels A-C. In Panel A delta-6 desaturase (*Ostreococcus tauri*) activity was demonstrated by the presence of [^{14}C]18:3n-6 ME using membranes isolated from transgenic *Brassica napus*. This desaturase activity was not present in membranes derived from a wild-type (Kumily) *B. napus*. In Panel B delta-5 desaturase (*Thraustochytrium* spp.) activity was demonstrated by the presence of [^{14}C]20:4n-6 ME using membranes isolated from transgenic *Brassica napus*. This desaturase activity was not present in membranes derived from a wild-type (Kumily) *B. napus*. In Panel C omega-3 desaturase activity was demonstrated by the presence of [^{14}C]20:5n-3 ME using membranes isolated from transgenic *Brassica napus*. This desaturase activity was not present in membranes derived from a wild-type (Kumily) *B. napus*.

Figure 86. Delta-12 desaturase (*Phytophthora sojae*), c-d12Des(Ps_GA), substrate preference. During the course of the enzymatic reaction the following lipid pools were isolated: phosphatidylcholine (PC, ■), free fatty acid (FFA, ·), and H₂O (CoA, ○). In Panel A c-d12Des(Ps_GA) enzyme activity is shown using assay conditions to present the fatty acid substrate (18:1(n-9)) in the acyl-phosphatidylcholine form. Desaturated enzymatic product (18:2(n-6)) is found predominantly in the phosphatidylcholine (PC) pool, relative to the free fatty acid (FFA) or H₂O (CoA) pools, indicating c-d12Des(Ps_GA) utilizes 18:1(n-9) attached to phosphatidylcholine as a substrate. In Panel B c-d12Des(Ps_GA) enzyme activity is shown using assay conditions to present the fatty acid substrate (18:1(n-9)) in the acyl-CoA form. Relative to Panel A, desaturated enzymatic product (18:2(n-6)) is not produced in the phosphatidylcholine (PC), free fatty acid (FFA) or H₂O (CoA) pools indicating c-d12Des(Ps_GA) does not utilize 18:1(n-9) bound as an acyl-CoA ester.

Figure 87. Delta-9 desaturase (*Saccharomyces cerevisiae*), d9Des(Sc) substrate preference. During the course of the enzymatic reaction the following lipid pools were isolated: phosphatidylcholine (PC, ■), free fatty acid (FFA, ·), and H₂O (CoA, ○). In Panel A d9Des(Sc) enzyme activity is shown using assay conditions to present the fatty acid substrate (16:0) in the acyl-phosphatidylcholine form. Relative to Panel B, desaturated enzymatic product (16:1(n-7)) is not produced in the phosphatidylcholine (PC), free fatty acid (FFA), or H₂O (CoA) pools indicating d9Des(Sc) does not utilize 18:0 attached to phosphatidylcholine as a substrate. In Panel B d9Des(Sc) enzyme activity is shown using assay conditions to present the fatty acid substrate (16:0) in the acyl-CoA form. Desaturated enzymatic product (16:1(n-7)) is isolated in both the free fatty acid (FFA) and H₂O (CoA) pools, but not the phosphatidylcholine (PC) pool. Furthermore, production of the desaturated enzymatic product (16:1(n-7)) in the H₂O (CoA) pool is linear for the first 60 minutes of the assay as shown by the hashed line ($r^2 = 0.99$). The high levels of [^{14}C]16:1(n-7) detected in the FFA pool likely result from hydrolysis of the desaturated enzymatic product, 16:1(n-7)-CoA, by endogenous thioesterases present in the membrane preparations.

Figure 88. Yield (kg seeds/ha) of canola plants grown in the field in 2014. Plants were either not treated (Yield) or were treated with 2x rate of imidazolinone herbicide (Yield w/herbicide).

Figure 89. EPA plus DHA content in seeds of plants grown in the field with (Imazamox) or without (control) herbicide treatment. *** denotes a significant difference between herbicide treatment and control as calculated by ANOVA, $p < 0.05$.

Figure 90. Oil content in seeds of plants grown in the field with (Imazamox) or without (control) herbicide treatment. *** denotes a significant difference between herbicide treatment and control as calculated by ANOVA, $p < 0.05$.

Figure 91. Protein content in seeds of plants grown in the field with (Imazamox) or without (control) herbicide treatment. *** denotes a significant difference between herbicide treatment and control as calculated by ANOVA, $p < 0.05$.

SUMMARY OF THE INVENTION

The disclosure thus generally provides an assay method, comprising:

- i) providing a plant capable of expressing a delta-12 desaturase, wherein said delta-12 desaturase has at least 50% total amino acid sequence identity to at least one of the sequences SEQ ID NO. 328 to 336, and/or at least 59% total amino acid sequence similarity to at least one of the sequences SEQ ID NO. 328 to 336, and wherein the plant is also capable of expressing at least one or more enzymes of unsaturated fatty acid metabolism, of which enzymes at least one is capable of using linoleic acid as a substrate, and of which enzymes at least one is supposedly connected to a plant metabolic property,
- ii) growing the plant, and
- iii) measuring said plant metabolic property for said plant.

In one aspect, there is provided an assay method for simulating a plant metabolic property of a plant of order Brassicales grown under field conditions, comprising: i) providing a plant of order Brassicales expressing a delta-12 desaturase, wherein said delta-12 desaturase has at least 96% total amino acid sequence identity to SEQ ID NO: 328, and wherein the plant also expresses at least one or more enzymes of unsaturated fatty acid metabolism, of which enzymes at least one is capable of using linoleic acid as a substrate, and of which enzymes at least one is connected to a plant metabolic property, ii) growing the plant under greenhouse conditions, and iii) measuring said plant metabolic property for said plant, wherein the measured metabolic property simulates the plant metabolic property measurement for the plant grown under field conditions; wherein the plant metabolic property is production of polyunsaturated fatty acids.

The disclosure also provides an assay method, comprising:

- i) providing a plant group, wherein the plants of said group are capable of expressing a delta-12 desaturase, wherein said delta-12 desaturase has at least 50% total amino acid sequence identity to at least one of the sequences SEQ ID NO. 328 to 336, and/or at least 59% total amino acid sequence similarity to at least one of the sequences SEQ ID NO. 328 to 336,

and wherein the plants of said group are capable of expressing at least one or more enzymes of unsaturated fatty acid metabolism, of which enzymes at least one is capable of using linoleic acid as a substrate, and of which enzymes at least one is supposedly connected to a plant metabolic property,

- ii) growing the plants of the plant group, and
- iii) measuring said plant metabolic property for plants of said plant group.

Further, the disclosure provides a method of identifying a gene for conveying an unsaturated fatty acid metabolic property to a plant, comprising:

- i) producing a plant comprising said gene,
- ii) performing an assay method according to the invention, wherein said gene is expressed and said plant metabolic property is measured, and
- iii) evaluating said plant for the presence and/or intensity of said unsaturated fatty acid metabolic property.

Also provided is a method of increasing delta-12 desaturase activity in a plant, comprising growing a plant expressing a delta-12 desaturase, wherein said delta-12 desaturase has at least 50% total amino acid sequence identity to at least one of the sequences SEQ ID NO. 328 to 336, and/or at least 59% total amino acid sequence similarity to at least one of the sequences SEQ ID NO. 328 to 336.

Likewise the disclosure provides a method of stabilizing delta-12 desaturase activity in a plant, comprising growing a plant expressing a delta-12 desaturase, wherein said delta-12 desaturase has at least 50% total amino acid sequence identity to at least one of the sequences SEQ ID NO. 328 to 336, and/or at least 59% total amino acid sequence similarity to at least one of the sequences SEQ ID NO. 328 to 336.

Further provided is a method of producing one or more desired unsaturated fatty acids in a plant, comprising growing a plant,

said plant expressing, at least temporarily, a delta-12 desaturase, wherein said delta-12 desaturase has at least 50% total amino acid sequence identity to at least one of the sequences SEQ ID NO. 328 to 336, and/or at least 59% total amino acid sequence similarity to at least one of the sequences SEQ ID NO. 328 to 336, and

said plant expressing one or more further genes to convert linoleic acid to said one or more desired unsaturated fatty acids.

The disclosure also provides a nucleic acid comprising

a) a gene coding for a Delta-12 desaturase, wherein said delta-12 desaturase has at least 50% total amino acid sequence identity to at least one of the sequences SEQ ID NO. 328 to 336, and/or at least 59% total amino acid sequence similarity to at least one of the sequences SEQ ID NO. 328 to 336 and wherein the gene does not code for a Delta-12 desaturase of any of the exact sequences SEQ ID NO. 329 to 336, or

b) a gene coding for a Delta-12 desaturase, wherein said delta-12 desaturase has at least 50% total amino acid sequence identity to at least one of the sequences SEQ ID NO. 328 to

336, and/or at least 59% total amino acid sequence similarity to at least one of the sequences SEQ ID NO. 328 to 336 and wherein the gene is operably linked to an expression control sequence, and wherein the expression control sequence is heterologous to said gene if the gene codes for any of the exact sequences according to SEQ ID NO. 329 to 336.

In the context of the present disclosure is also provided a plant cell comprising a gene coding for a delta-12 desaturase, wherein said delta-12 desaturase has at least 50% total amino acid sequence identity to at least one of the sequences SEQ ID NO. 328 to 336, and/or at least 59% total amino acid sequence similarity to at least one of the sequences SEQ ID NO. 328 to 336.

And the disclosure provides a plant set comprising at least two plant groups,

wherein the plant or plants of each group are capable of expressing a delta-12 desaturase, wherein said delta-12 desaturase has at least 50% total amino acid sequence identity to at least one of the sequences SEQ ID NO. 328 to 336, and/or at least 59% total amino acid sequence similarity to at least one of the sequences SEQ ID NO. 328 to 336,

and wherein the plant or plants of said groups comprise one or more genes coding for at least one or more enzymes of unsaturated fatty acid metabolism, of which enzymes at least one is capable of using linoleic acid as a substrate, and of which enzymes at least one is supposedly connected to a plant metabolic property, and wherein the plants of said groups differ in the expression of at least one of the enzymes of unsaturated fatty acid metabolism.

DETAILED DESCRIPTION OF THE INVENTION

The present invention thus provides an assay method. The assay method of the present invention is particularly suitable for determining the presence and/or intensity of a metabolic property. The metabolic property depends directly or indirectly on the presence or concentration of at least one unsaturated fatty acid in a plant cell, preferably of a fatty acid having at least 18 carbon atoms in length and at least two carbon-carbon double bonds. Thus, the metabolic property preferably is or depends on the production of unsaturated fatty acids and even more polyunsaturated fatty acids in plant cells.

According to the present invention, unsaturated fatty acids preferably are polyunsaturated fatty acids, that is fatty acids comprising at least two, more preferably at least three and even more preferably at least or exactly 4 carbon-carbon double bonds. Unsaturated fatty acids including polyunsaturated fatty acids are generally known to the skilled person, important unsaturated fatty acids are categorised into a omega-3, omega-6 and omega-9 series, without any limitation intended. Unsaturated 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). Unsaturated 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). Unsaturated 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). Unsaturated 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 unsaturated fatty acids are eicosadienoic acid (C20:2d11,14; EDA) and eicosatrienoic acid (20:3d11,14,17; ETrA).

According to the present invention, the metabolic property preferably is the production and particularly preferably the yield of an omega-6 type and/or an omega-3 type unsaturated fatty acid. Such yield is preferably defined as the percentage of said fatty acid relative to the total fatty acids of an extract, preferably of a plant or seed oil. Thus, preferably the assay method of the

present invention entails measuring the amount and/or concentration of an unsaturated fatty acid, preferably of an unsaturated fatty acid having at least 20 carbon atoms length and belonging to the omiga-3 or omega-6 series. The amount and/or concentration is determined on a plant extract, preferably a plant oil or plant lipids. The term "lipids" refers to a complex mixture of molecules comprising compounds such as sterols, waxes, fat soluble vitamins such as tocopherols and carotenoid/retinoids, sphingolipids, phosphoglycerides, glycolipids such as glycosphingolipids, phospholipids such as phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol or diphosphatidylglycerol, monoacylglycerides, diacylglycerides, triacylglycerides or other fatty acid esters such as acetylcoenzyme A esters. "Lipids" can be obtained from biological samples, such as fungi, algae, plants, leaves, seeds, or extracts thereof, by solvent extraction using protocols well known to those skilled in the art (for example, as described in Bligh, E. G., and Dyer, J. J. (1959) *Can J. Biochem. Physiol.* 37: 911-918).

The term "oil" refers to a fatty acid mixture comprising unsaturated and/or saturated fatty acids which are esterified to triglycerides. The oil may further comprise free fatty acids. 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. It is known that most of the fatty acids in plant oil are esterified in triacylglycerides. In addition the oil of the invention may comprise other molecular species, such as monoacylglycerides, diacylglycerides, phospholipids, or any the molecules comprising lipids. Moreover, oil may comprise minor amounts of the polynucleotide or vector of the invention. Such low amounts, however, can be detected only by highly sensitive techniques such as PCR. Oil can be obtained by extraction of lipids from any lipid containing biological tissue and the amount of oil recovered is dependent on the amount of triacylglycerides present in the tissue. Extraction of oil from biological material can be achieved in a variety of ways, including solvent and mechanical extraction. Specifically, extraction of canola oil typically involves both solvent and mechanical extraction, the products of which are combined to form crude oil. The crude canola oil is further purified to remove phospholipids, free fatty acids, pigments and metals, and odiferous compounds by sequential degumming, refining, bleaching, and deodorizing. The final product after these steps is a refined, bleached, and deodorized oil comprising predominantly fatty acids in the form of triglycerides.

The assay method of the present invention comprises the step of providing a plant. According to the present invention, the term "plant" shall mean a plant or part thereof in any developmental stage. Particularly, the term "plant" herein is to be understood to indicate a callus, shoots, root, stem, branch, leaf, flower, pollen and/or seed, and/or any part thereof. The plant can be monocotyledonous or dicotyledonous and preferably is a crop plant. Crop plants include Brassica species, corn, alfalfa, sunflower, soybean, cotton, safflower, peanut, sorghum, wheat, millet and tobacco. The plant preferably is an oil plant. Preferred plants are of order Brassicales, particularly preferred of family Brassicaceae. Even more preferred are plants of tribus Aethionemeae, Alysseae, Alyssopsidae, Anastaticae, Anchonieae, Aphragmeae, Arabideae, Asteae, Biscutelleae, Bivonaeae, Boechereae, Brassiceae, Buniadeae, Calepineae, Camelineae, Cardamineae, Chorisporeae, Cochleariae, Coluteocarpeae, Conringieae, Cremolobeae,

Crucihimalayae, Descurainieae, Dontostemoneae, Erysimeae, Euclidieae, Eudemeae, Eutremeae, Halimolobeae, Heliophileae, Hesperideae, Iberideae, Isatideae, Kernereae, Lepidieae, Malcolmieae, Megacarpaeae, Microlepidieae, Noccaeeae, Notothlaspidieae, Oreophytoneae, Physarieae, Schizopetaleae, Scolioxoneae, Sisymbrieae, Smelowskieae, Stevenieae, Thelypodieae, Thlaspidieae, Turritideae or Yinshanieae. Even more preferred are plants of genus Aethionema, Moriera, Alyssoideae, Alyssum, Aurinia, Berteroa, Bornmuellera, Bornmuellera x Leptoplax, Clastopus, Clypeola, Degenia, Fibigia, Galitzkya, Hormathophylla, Lepidotrichum, Leptoplax, Phyllolepidum, Physocardamum, Physoptychis, Straussiella, Alyssoopsis, Calymmatium, Dielsiocharis, Olimarabidopsis, Anastatica, Cithareloma, Diceratella, Eigia, Eremobium, Farsetia, Lachnocapsa, Lobularia, Malcolmia, Maresia, Morettia, Notoceras, Parolinia, Anchonium, Eremoblastus, Iskandera, Matthiola, Micrantha, Microstigma, Petiniotia, Sterigmostemum, Synstemon, Zerdana, Aphragmus, Lignariella, Abdra, Arabis (rockcress), Arcyosperma, Athysanus, Aubrieta, Baimashania, Botschantzevia, Dendroarabis, Draba, Drabella, Erophila, Pachyneurum, Pseudodraba, Scapiarabis, Schivereckia, Sinoarabis, Tomostima, Asta, Biscutella, Megadenia, Bivonaea, Anelsonia, Boechera, Borodinia, Cusickiella, Nevada, Phoenicaulis, Polyctenium, Sandbergia, Ammosperma, Brassica, Brassica x Raphanus, Cakile, Carrichtera, Ceratocnemum, Coincya, Cordilocarpus, 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, Zilla, Bunias, Calepina, Goldbachia, Leiocarpacea, Spirorhynchus, Camelina, Capsella, Catolobus, Cheesemania, Chrysochamela, Neslia, Noccidium, Pseudoarabidopsis, Aplanodes, Armoracia, Barbarea (winter cress), Cardamine (bittercresses), Iodanthus, Iti, Leavenworthia, Nasturtium, Neobeckia, Ornithocarpa, Planodes, Rorippa (yellowcress), Selenia, Sisymbrella, Chorispora, Diptychocarpus, Litwinowia, Neuroloma, Parrya, Pseudoclausia, Cochlearia, Ionopsidium, Callotlaspi, Coluteocarpus, Eunomia, Kotschyella, Noccaea, Vania, Conringia, Zuvanda, Cremolobus, Menonvillea, Crucihimalaya, Ladakiella, Transberingia, Descurainia, Hornungia, Hymenolobus, Ianhedgea, Pritzelago, Robeschia, Tropidocarpum, Clausia, Dimorphostemon, Dontostemon, Erysimum, Syrenia, Atelantha, Braya, Catenulina, Christolea, Cryptospora, Cymatocarpus, Dichasianthus, Dilophia, Euclidium, Lachnoloma, Leiospora, Lepidostemon, Leptaleum, Neotorularia, Octoceras, Phaeonychium, Pycnoplathopsis, Pycnoplathus, Rhammatophyllum, Shangrilaia, Sisymbriopsis, Solms-laubachia, Spryginia, Streptoloma, Tetracme, Aschersoniodoxa, Brayopsis, Dactylocardamum, Eudema, Onuris, Xerodraba, Chalcanthus, Eutrema, Pegaeophyton, Thellungiella, Exhalimolobos, Halimolobos, Mancoa, Pennellia, Sphaerocardamum, Heliophila (Cape stock), Hesperis, Tchiatchewia, Iberis, Teesdalia, Boreava, Chartoloma, Glastaria, Isatis, Myagrum, Pachypterygium, Sameraria, Schimpera, Tauscheria, Kerneria, Rhizobotrya, Acanthocardamum, Coronopus, Cyphocardamum, Delpinophytum, Lepidium, Lithodraba, Stubendorffia, Winklera, Megacarpaea, Pugionium, Arabidella, Ballantinia, Blennodia, Carinavalva, Cuphonotus, Drabastrum, Geococcus, Harmsiodoxa, Irenepharsus, Menkea, Microlepidium, Pachycladon, Pachymitus, Phlegmatospermum, Scambopus, Stenopetalum, Notothlaspi, Murbeckiella, Oreophyton, Dimorphocarpa, Dithyrea, Lyrocarpa, Nerisyrenia, Paysonia, Physaria (bladderpods), Synthlipsis,

Caulanthus, Mathewsia, Schizopetalon, Sibaropsis, Streptanthella, Werdermannia, Scoliaxon, Lycocarpus, Schoenocrambe, Sisymbrium, Hedinia, Smelowskia, Berteroella, Macropodium, Pseudoturritis, Ptilotrichum, Stevenia, Catadysia, Chaunanthus, Chilocardamum, Chlorocrambe, Coelophragmus, Dictyophragmus, Dryopetalon, Englerocharis, Guillenia, Hesperidanthus, Ivania, Mostacillastrum, Neuontobotrys, Phlebolobium, Polypsecadium, Pringlea, Pterygiosperma, Romanschulzia, Sarcodraba, Sibara, Stanleya, Streptanthus, Thelypodopsis, Thelypodium, Thysanocarpus, Warea, Weberbaueria, Alliaria, Didymophysa, Elburzia, Gagra, Graellsia, Pachyphragma, Parlatoria, Peltaria, Peltariopsis, Pseudocamelina, Pseudovesicaria, Sobolewska, Thlaspi, Turritis, Hilliella, Yinshania. Most preferred are plants of species *Brassica aucheri*, *Brassica balearica*, *Brassica barrelieri*, *Brassica carinata*, *Brassica carinata* x *Brassica napus*, *Brassica carinata* x *Brassica rapa*, *Brassica carinata* x *Brassica juncea*, *Brassica cretica*, *Brassica deflexa*, *Brassica desnottesii*, *Brassica drepanensis*, *Brassica elongata*, *Brassica fruticulosa*, *Brassica gravinae*, *Brassica hilarionis*, *Brassica incana*, *Brassica insularis*, *Brassica juncea*, *Brassica macrocarpa*, *Brassica maurorum*, *Brassica montana*, *Brassica napus*, *Brassica napus* x *Brassica juncea*, *Brassica napus* x *Brassica nigra*, *Brassica nigra*, *Brassica oleracea*, *Brassica oxyrrhina*, *Brassica procumbens*, *Brassica rapa*, *Brassica repanda*, *Brassica rupestris*, *Brassica ruvo*, *Brassica souliei*, *Brassica spinescens*, *Brassica tournefortii* or *Brassica villosa*.

The plant of the assay method of the present invention is capable of expressing a Delta-12 desaturase as defined herein. The plant can be provided by any appropriate means. For example, the plant can be provided by transforming a plant cell with a nucleic acid comprising a gene coding for the Delta-12 desaturase and raising such transformed plant cell to a plant sufficiently developed for measuring the plant metabolic property. According to the invention, a plant can also be provided in the form of an offspring of such transformed plant. Such offspring may be produced vegetatively from material of a parent plant, or may be produced by crossing a plant with another plant, preferably by inbreeding.

The plant is capable of expressing a Delta-12 desaturase. According to the invention, the term "capable of expressing a gene product" means that a cell will produce the gene product provided that the growth conditions of the cell are sufficient for production of said gene product. For example, a plant is capable of expressing a Delta-12 desaturase if a cell of said plant during any developmental stage of said plant will produce the corresponding Delta-12 desaturase. It goes without saying that where expression depends on human intervention, for example the application of an inductor, a plant is likewise considered capable of expressing the Delta-12 desaturase.

According to the invention, the plant is capable of expressing a Delta-12 desaturase, wherein said Delta-12 desaturase has at least 96% total amino acid sequence identity to any of the sequences SEQ ID NO. 328. A Delta-12 desaturase having this desired sequence identity and/or sequence similarity is also called a Delta-12 desaturase of the present invention. A Delta-12 desaturase according to the invention is an enzyme catalysing (at least) the conversion of oleic acid to linoleic acid. For a metabolic pathway for the production of unsaturated and polyunsaturated fatty acids, see for example figure one of WO2006100241. Examples of Delta-12 desaturases referred to herein are:

SEQ ID NO. 328 artificial Delta-12 desaturase sequence

SEQ ID NO. 329	Uniprot G5A275_PHYSP of Phytophthora_sojae
SEQ ID NO. 330	Uniprot H3G9L1_PHYRM of Phytophthora_ramorum
SEQ ID NO. 331	Uniprot G4XUM4_PHYIN of Phytophthora_infestans
SEQ ID NO. 332	Uniprot M4BXW8_HYAAE of Hyaloperonospora_arabidopsidis
5 SEQ ID NO. 333	Uniprot W2PDL4_PHYPN of Phytophthora_parasitica
SEQ ID NO. 334	Uniprot W2LW72_PHYPR of Phytophthora_parasitica
SEQ ID NO. 335	Uniprot W2ZYL2_PHYPR of Phytophthora_parasitica
SEQ ID NO. 336	Uniprot Q6UB74_9STRA of Saprolegnia diclina

10 It has now surprisingly been found that expression of a Delta-12 desaturase of the present invention reduces the difference between fatty acid composition of plants grown under greenhouse and field conditions, respectively. This was unexpected, as plants generally are capable of expressing at least one Delta-12 desaturase even as wild type plants. However, as seen in the accompanying examples, the percentage of linoleic acid (and correspondingly also

15 the concentration of other unsaturated fatty acids metabolically downstream of linoleic acid) differs between wild-type plants grown under greenhouse and field conditions, respectively. However, where plants express a delta-12 desaturases of the present invention, optionally in addition to the one or more type delta-12 desaturase(s), the difference in oil composition between plants raised in the greenhouse and plants raised under field conditions is greatly diminished or

20 even removed. Thus, by making use of the delta-12 desaturases of the present invention it is possible to improve the delta-12 desaturases conversion efficiency in plants grown under greenhouse conditions. The assay method of the present invention hence effectively allows to simulate the influence of field conditions on a plant metabolic property, wherein said metabolic property is directly or indirectly connected to the presence and/or concentration of linoleic acid in

25 a plant cell as described above. Using an assay method of the present invention therefore unexpectedly allows to screen plants for such metabolic properties with higher reliability and prediction accuracy of said plant metabolic property. Effectively the assay method of the present invention enables the skilled person to reduce the number of field trials required for development of a commercially viable plant variety producing unsaturated or even more preferably

30 polyunsaturated fatty acids.

A gene coding for a Delta-12 desaturase of the present invention can be obtained by de novo synthesis. Starting from any of the amino acid sequences SEQ ID NO. 328 to 336, the skilled person can reverse-translate the selected sequence into a nucleic acid sequence and have the

35 sequence synthesised. As described herein, the skilled person can also introduce one or more mutations, including insertions, substitutions and deletions to the amino acid sequence chosen or the corresponding nucleic acid sequence. For reverse translation, the skilled person can and should use nucleic acid codons such as to reflect codon frequency of the plant intended for expression of said Delta-12 desaturase of the present invention. By using any of the amino acid

40 sequences according to SEQ ID NO. 328 to 336 as such or one or more mutations, the person can obtain using routine techniques and standard equipment, a Delta-12 desaturase having the beneficial properties described herein and exhibiting these beneficial properties in numerous plant species.

Instead of starting from any of the amino acid sequences according to SEQ ID NO. 328 to 336, the skilled person may also obtain a Delta-12 desaturase from any organism of class oomycetes, preferably of order Peronosporales, Pythiales or Saprolegniales, particularly preferably of genus Basidiophora, Benua, Bremia, Erapthora, Graminivora, Hyaloperonospora, Novotelnova, 5 Paraperonospora, Perofascia, Peronosclerospora, Peronospora, Phytophthora, Phytopythium, Plasmopara, Plasmoverna, Protobremia, Pseudoperonospora, Salisapiliaceae, Sclerophthora, Sclerospora or Viennotia, or genus Diasporangium, Elongisporangium, Globisporangium, Halophytophthora, Ovatisporangium, Pilasporangium or Pythium, or genus Achlya, Aphanomyces, Aplanes, Aplanopsis, Aquastella, Brevilegnia, Calyptralegnia, Dictyuchus, 10 Geolegnia, Isoachlya, Leptolegnia, Newbya, Plectospira, Protoachlya, Pythiopsis, Saprolegnia, Scoliolegnia or Thraustotheca. Methods for obtaining such Delta-12 desaturase nucleic acid and amino acid sequences are described for example in international publication WO 2006 100 241. Particularly preferably, the skilled person starts with a Delta-12 desaturase nucleic acid sequence obtainable or obtained from any member of the above genera, preferably the genera 15 Phytophthora, Hyaloperonospora or Saprolegnia. Even more preferably, the skilled person starts by using the nucleic acid sequence coding for a Delta-12 desaturase obtainable or obtained from any member of species Phytophthora sojae, Phytophthora parasitica, Phytophthora ramorum or Phytophthora infestans.

20 The amino acid sequence of the Delta-12 desaturase of the present invention may be identical to any of the sequences according to SEQ ID NO. 328 to 336. However, in certain embodiments it is preferred that the amino acid sequence of the Delta-12 desaturase of the present invention is not the sequence according to SEQ ID NO. 328 and/or is not the amino acid sequence according to SEQ ID NO. 329 and/or is not the amino acid sequence according to SEQ ID NO. 330 and/or 25 is not the amino acid sequence according to SEQ ID NO. 331 and/or is not the amino acid sequence according to SEQ ID NO. 332 and/or is not the amino acid sequence according to SEQ ID NO. 333 and/or is not the amino acid sequence according to SEQ ID NO. 334 and/or is not the amino acid sequence according to SEQ ID NO. 335 and/or is not the amino acid sequence according to SEQ ID NO. 336. Where the skilled person for any reason wants to avoid any one 30 or more of the amino acid sequences according to SEQ ID NO. 328 to 336, the skilled person can use any of the remaining sequences of this set of sequences. However, the skilled person can also make up a new amino acid and corresponding nucleic acid sequence by selecting a base sequence from the set of amino acid sequences according to SEQ ID NO. 328 to 336 and introducing one or more mutations (insertions, substitutions and/or deletions) at appropriate 35 positions of the base sequence to obtain a derived sequence. Generally, the skilled person will take into account that the higher the sequence identity and/or similarity between base sequence and derived sequence, the more will the corresponding derived Delta-12 desaturase resemble the Delta-12 desaturase activity that corresponds to the desaturase of the base sequence. Thus, if the skilled person uses a mutated Delta-12 desaturase according to the present invention and 40 such mutated Delta-12 desaturase unexpectedly does not convey the benefits of a Delta-12 desaturase of the present invention, the skilled person should reduce the number of differences of the Delta-12 desaturase sequence to increase resemblance of any of the sequences according to SEQ ID NO. 328 to 336.

For substituting amino acids of a base sequence selected from any of the sequences SEQ ID NO. 328 to 336 without regard to the occurrence of amino acid in other of these sequences, the following applies, wherein letters indicate L amino acids using their common abbreviation and bracketed numbers indicate preference of replacement (higher numbers indicate higher preference): A may be replaced by any amino acid selected from S (1), C (0), G (0), T (0) or V (0). C may be replaced by A (0). D may be replaced by any amino acid selected from E (2), N (1), Q (0) or S (0). E may be replaced by any amino acid selected from D (2), Q (2), K (1), H (0), N (0), R (0) or S (0). F may be replaced by any amino acid selected from Y (3), W (1), I (0), L (0) or M (0). G may be replaced by any amino acid selected from A (0), N (0) or S (0). H may be replaced by any amino acid selected from Y (2), N (1), E (0), Q (0) or R (0). I may be replaced by any amino acid selected from V (3), L (2), M (1) or F (0). K may be replaced by any amino acid selected from R (2), E (1), Q (1), N (0) or S (0). L may be replaced by any amino acid selected from I (2), M (2), V (1) or F (0). M may be replaced by any amino acid selected from L (2), I (1), V (1), F (0) or Q (0). N may be replaced by any amino acid selected from D (1), H (1), S (1), E (0), G (0), K (0), Q (0), R (0) or T (0). Q may be replaced by any amino acid selected from E (2), K (1), R (1), D (0), H (0), M (0), N (0) or S (0). R may be replaced by any amino acid selected from K (2), Q (1), E (0), H (0) or N (0). S may be replaced by any amino acid selected from A (1), N (1), T (1), D (0), E (0), G (0), K (0) or Q (0). T may be replaced by any amino acid selected from S (1), A (0), N (0) or V (0). V may be replaced by any amino acid selected from I (3), L (1), M (1), A (0) or T (0). W may be replaced by any amino acid selected from Y (2) or F (1). Y may be replaced by any amino acid selected from F (3), H (2) or W (2).

According to the invention, sequence identity and similarity are calculated by pairwise alignment of two sequences according to the algorithm of Needleman and Wunsch (J. Mol. Biol. 1970, 48(3), 433-453) using, for amino acid sequence comparisons, a gap opening penalty of 12 and a gap extension penalty of 2 and applying the BLOSUM62 matrix, and using for nucleic acid sequence comparisons a gap opening penalty of 16 and a gap extension penalty of 4. Identity is then calculated as the number of identical positions in the alignment divided by the length of the alignment. Similarity is calculated as the number of positions where amino acids of both sequences have a weight of at least zero or larger in the BLOSUM62 matrix and then dividing this number by the length of the alignment.

The Delta-12 desaturase of the present invention preferably has at least 50% amino acid sequence identity to any of the sequences SEQ ID NO. 328 to 336. Most preferably, the Delta-12 desaturase of the present invention has at least 50% amino acid sequence identity to sequence SEQ ID NO. 329. This desaturase can be shown to be functional in numerous plant species, it is easy to obtain and conveys the benefits of the desaturase of the present invention. Preferably, the Delta-12 desaturase of the present invention has at least 55% amino acid sequence identity to any of the sequences SEQ ID NO. 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338 and 339, wherein identity to SEQ ID NO. 329 is particularly preferred, even more preferably at least 65%, even more preferably at least 72%, even more preferably at least 78%, even more preferably at least 80%, even more preferably at least 82%, even more preferably at least 89%, even more preferably at least 91%, even more preferably at least 96%. The Delta-12 desaturase of the present invention preferably has at least 50% amino acid sequence identity to any of the

sequences SEQ ID NO. 328 to 336. Most preferably, the Delta-12 desaturase of the present invention has at least 50% amino acid sequence identity to sequence SEQ ID NO. 329. This desaturase can be shown to be functional in numerous plant species, it is easy to obtain and conveys the benefits of the desaturase of the present invention. Preferably, the Delta-12 desaturase of the present invention has at least 60% amino acid sequence identity to any of the sequences SEQ ID NO. 1, 2, 3, 4, 5, 6, 7, 8 or 9, where similarity to SEQ ID NO. 329 is particularly preferred, even more preferably at least 73%, even more preferably at least 75%, even more preferably at least 89%, even more preferably at least 95%, even more preferably at least 96%, even more preferably at least 97%, even more preferably at least 98%, even more preferably at least 99%. Preferably, the delta-12 desaturase of the present invention has both the required or preferred minimal identity and the required or preferred minimal similarity. The higher the similarity and identity between the amino acid sequence of the Delta-12 desaturase of the present invention and the amino acid sequence according to SEQ ID NO. 1, 2, 3, 4, 5, 6, 7, 8 or 9, the more reliable will the Delta-12 desaturase of the present invention exhibit Delta-12 desaturase activity in a plant cell and convey the benefits of the present invention.

Preferably, the amino acid sequence of the Delta-12 desaturase of the present invention differs from the amino acid sequences according to any of SEQ ID NO. 328 to 336 only at such one or more positions where at least one of the amino acid sequences SEQ ID NO. 328 to 336 differs from at least one other of the sequences SEQ ID NO. 328 to 336, preferably not allowing any amino acid insertion or deletion. Preferably, the amino acid sequence of the Delta-12 desaturase of the invention can be thought to be the result of exchanging selected amino acids from one chosen base sequence of the sequences SEQ ID NO. 328 to 336 for the corresponding amino acid at the respective positions of any other of the sequences SEQ ID NO. 328 to 336. Also preferably, any mutation should increase the similarity, or, even more preferably, the identity, of the amino acid sequence of the Delta-12 desaturase of the present invention to that of a sequence according to SEQ ID NO. 328 to 336 and reduce the similarity or, even more preferably, the identity, to an amino acid sequence according to SEQ ID NO. 10 to 12.

For the reasons indicated above, the Delta-12 desaturase of the present invention preferably consists of the amino acid sequence SEQ ID NO. 329. Less preferably, the amino acid sequence of the Delta-12 desaturase of the present invention differs from the amino acid sequence according to SEQ ID NO. 329 only at such positions where any of the sequences SEQ ID NO. 338 or 330 to 336 differ from the amino acid sequence of SEQ ID NO. 329. More preferably, the Delta-12 desaturase of the present invention does not differ from the amino acid sequence of SEQ ID NO. 329 by an insertion or deletion and thus only comprises one or more substitutions. Even more preferably, the Delta-12 desaturase of the present invention consists of an amino acid sequence that differs from SEQ ID NO. 329 only by amino acids found at the corresponding position of any of the other amino acid sequence SEQ ID NO. 328 and 330-336.

The plant of the present invention is further capable of expressing at least one or more enzymes of unsaturated fatty acid metabolism. Preferably, such enzymes are capable of using an

unsaturated fatty acid of the omega-6 and/or, more preferably, of the omega-3 series as a substrate. Preferred activities of the enzymes are: desaturase, elongase, ACS, acylglycerol-3-phosphate acyltransferase (AGPAT), choline phosphotransferase (CPT), diacylglycerol acyltransferase (DGAT), glycerol-3-phosphate acyltransferase (GPAT), lysophosphatidate acyltransferase (LPAT), lysophosphatidylcholine acyltransferase (LPCAT), lysophosphatidylethanolamine acyltransferase (LPEAT), lysophospholipid acyltransferase (LPLAT), phosphatidate phosphatase (PAP), phospholipid:diacylglycerol acyltransferase (PDAT), phosphatidylcholine:diacylglycerol choline phosphotransferase (PDCT), particularly Delta-8 desaturase, Delta-6 desaturase, Delta-5 desaturase, Delta-4 desaturase, Delta-9 elongase, Delta-6 elongase, Delta-5 elongase, omega-3 desaturase.

At least one of the enzymes is capable of using linoleic acid as substrate. Such enzymes are known to the skilled person as omega-3 desaturases, Delta-15 desaturases, Delta-9 along gazes and Delta-6 desaturases. It is possible that one or more enzymes of unsaturated fatty acid metabolism can have more than one activity. For example, it is common for omega-3 desaturases to be also Delta-15 desaturases and/or Delta-17 desaturases and/or Delta-19 desaturases. Further preferred enzymes of unsaturated fatty acid metabolic is our Delta-6 along gazes, Delta-8 desaturases, Delta-5 desaturases, Delta-5 elongates this and Delta-4 desaturases. At least one of these enzymes is supposedly connected to a plant metabolic property. Preferably, the metabolic property is the presence and/or concentration of the product of the respective enzyme. Thus, preferably the plant metabolic property is the presence and/or concentration of any of GL a, SDA, EDA, ETrA, the GLA, EDTA, ARA, EPA, DTA, DPA and DHA, wherein particularly preferred are the concentration of ARA, EPA and DHA.

In the assay method of the present invention, the plant is capable of expressing the Delta-12 desaturase of the present invention and at least one more enzyme of unsaturated fatty acid metabolic is and are grown. "Growing" for the present invention means to nurture plant material, preferably a plant can use, embryo or seed, such that cells of said plant material can develop and preferably multiply, such that at least one cell of the developed plant material can be expected to exhibit the plant metabolic property. For example, where the expression of a gene coding for an enzyme of unsaturated fatty acid metabolism, for example a desaturase or elongates, is under the control of a tissue-specific promoter, the plant material is grown such that the corresponding tissue develops.

The plant metabolic property is then measured by any suitable means. For example, the concentration of fatty acids in the form of free fatty acids or in the form of mono-, di- or triglycerides can be measured from extracts of plant material, preferably of plant seeds and most preferably from seed oil.

The assay method of the present invention preferably is not performed only on one plant but on a group of plants. This way, the measured plant metabolic properties will be statistically more significant than measurements taken only on plant material of a single plant, for example a single seed. Even though assay methods of the present invention preferably are performed on plant groups, assay methods of the present invention performed on single plants are also useful and

beneficial. Such methods allow for a fast screening plants and thus are particularly suitable for high throughput evaluation of genes and gene combinations coding for enzymes of unsaturated fatty acid metabolism.

- 5 As indicated above, a preferred assay method of the present invention comprises
- 1) providing a plant group, wherein the plants of said group are capable of expressing a Delta-12 desaturase of the present invention,
and wherein the plants of said group are capable of expressing at least one or more enzymes of unsaturated fatty acid metabolism, of which enzymes at least one is capable of using
 - 10 linoleic acid as a substrate, and of which enzymes at least one is supposedly connected to a plant metabolic property,
 - 2) growing the plants of the plant group, and
 - 3) measuring said plant metabolic property for at least one, preferably at least 2 and even more preferably at least 3, 4, 5, 6 or more plants of said plant group.
- 15 As indicated above, the present invention aspires to and allows to reduce the number of field trials. Thus, the assay method of the present invention (performed on single plants or plant groups) is preferably performed such that the plant or plants are grown under greenhouse conditions. According to the present invention, greenhouse conditions are environmental conditions where intensity can be controlled and adjusted at will, and may also be referred to as
- 20 controlled environment conditions. Greenhouse conditions are generally maintained using standard equipment, for example a germination chamber, a growth cabinet or a greenhouse. Such equipment particularly allows to control more than one environmental condition, preferably light, temperature (of growth material and of air bracket, humidity and growth medium composition, particularly water content, and the concentration of growth regulators like water, nutrients and
- 25 protection agents like bactericides, fungicides, helminthocides and insecticidal agents. Greenhouses, growth cabinets and germination chambers (that is according to the invention equipment for maintaining greenhouse conditions) also allow controlled changes of one or more of the aforementioned environmental conditions, for example in a diurnal rhythm to simulate for example short day or long day light conditions and/or drought or nutrient deficiency conditions.
- 30 For automated greenhouses, such conditions can also be controlled for each individual plant or group of plants. Thus, for example greenhouse conditions allow to subject single or more plants to stress conditions at selected stages of plant development and for a selected duration.

For Brassica species grown in the greenhouse, temperatures can range from 10°C to 30°C, but

35 the preferred conditions include 15-22°C day and 12-20°C night temperatures. Preferably, there is a 3°C difference between night and day. Colder day and night temperatures can be used to slow the growth rate of plants or to assess the effect of temperature on plant growth. A preferred light cycle is 16 hrs light and 8 hrs dark. A shorter day length can be used to delay growth or to delay reproduction. The preferred light intensity is 200-300 micromoles of photons m⁻² s⁻¹, but it

40 can range from 100-1200 micromoles of photons m⁻² s⁻¹. Humidity can range from 20-70%, with the preferred range being 30-50%. Lower humidity is useful to limit disease incidence.

The gene coding for the Delta-12 desaturase of the present invention preferably is operably linked to an expression control sequence to allow constitutive or non-constitutive expression of said

gene. Expression control sequences according to the present invention are known to the skilled person as promoters, transcription factor binding sites and regulatory nucleic acids like for example RNAi. Preferably, the expression control sequence directs expression of the gene in a tissue-specific manner. Where the plant is an oil seed plant, preferably of a Brassica species, expression of the gene preferably is specific to plant seeds in one or more of their developmental stages. According to the present invention, tissue-specific expression does not require the total absence of gene expression in any other tissue. However, tissue-specific expression for a selected tissue means that the maximum amount of mRNA transcript in this tissue is at least 2-fold, preferably at least 5-fold, even more preferably at least 10-fold, even more preferably at least 20-fold, even more preferably at least 50-fold and most preferably at least 100-fold the maximum amount of said mRNA in the other tissues. Furthermore, expression control sequences are known to the skilled person which allow induction or repression of expression by a signal applied by a user, for example application of an inductor like IPTG.

The Delta-12 desaturase of the present invention can be present in the plant or plants of the SA method of the present invention as a single copy gene or in multiple gene copies. It is an advantage of the present invention that even a single copy of the Delta-12 desaturase gene of the present invention can be shown to be sufficient for reducing or even removing differences in oil composition between plants grown under greenhouse and field conditions, and it can also be shown that a single copy of the Delta-12 desaturase gene of the present invention is sufficient for increasing Delta-12 desaturase conversion efficiencies as defined below. This is a considerable advantage, as the production of polyunsaturated fatty acids like EPA and DHA generally requires the introduction of at least 3 genes to provide the activities of a Delta-6 desaturase/Delta-6 elongase, Delta-9 elongase/Delta-8 desaturase, Delta-5 desaturase, Delta-5 elongase and/or Delta-4 desaturase. Thus, establishing a metabolic pathway for production of polyunsaturated fatty acids requires transformation of plants either by very long nucleic acid constructs (for example using a technique known in the art as BiBAC) or multiple transformations using shorter nucleic acid constructs. All of these techniques can be laborious, and labour intensity generally increases the more nucleic acids and/or the longer nucleic acids have to be introduced into a plant. Thus, the possibility of achieving the advantages conferred by the present invention by using only a single gene coding for the Delta-12 desaturase of the present invention allows the skilled person to materialise these benefits with minimal additional work. Also, expression of the Delta-12 desaturase of the present invention does not require functional inactivation of one or more Delta-12 desaturase genes of the plant in question. The skilled person may even decide to prepare one or more plant varieties comprising one or another gene coding for a Delta-12 desaturase of the present invention under the control of a desired expression control sequence, for example a seed-specific promoter. Using such plant varieties, the skilled person can then introduce the genes of unsaturated fatty acid metabolism using normal constructs and without having to increase construct length by an additional Delta-12 desaturase gene and its corresponding expression control sequence.

Where the gene coding for the Delta-12 desaturase of the present invention is under the control of a seed-specific expression control sequence mRNA transcripts of the gene are preferably detectable, for example by qPCR, at least 25 days, more preferably at least 20 days and even more preferably at least 15 days after flowering of the plant. Also preferably, the maximum

expression of the Delta-12 desaturase gene of the present invention is before 40 days after inflorescence, more preferably before 35 days after inflorescence and even more preferably before 33 days after inflorescence. Preferably, the maximum expression, for example as determined by qPCR, is from day 22 to day 35 after inflorescence, even more preferably from day 21 to 31 after inflorescence. To achieve such expression pattern, the SBP promoter or, more preferably, the Napin promoter are particularly preferred. Thus, the present invention allows to materialise the benefits conferred by the use of the Delta-12 desaturase of the present invention without having to express said Delta-12 desaturase constitutively throughout the plant or constitutively throughout seed-development. Constitutive gene expression requires a plant cell to produce a gene product regardless of whether it is beneficial or even necessary or not. Thus, the present invention allows to minimise the stress inflicted upon the plant in question.

The Delta-12 desaturase of the present invention preferably is expressed in the same plant cell also expressing the other at least one or more enzymes of unsaturated fatty acid metabolism. It is possible but not necessary that the Delta-12 desaturase of the present invention is expressed at the same time as one, some or all of said other genes of unsaturated fatty acid metabolism. For example, the Delta-12 desaturase of the present invention may reach a maximum expression as determined by qPCR during the 1st two thirds of seed development time (preferably, as described above, during days 20 and 31 after inflorescence) with mRNA concentration of said Delta-12 desaturase gene being halved or even less at further stages of seed development, whereas the one or more other genes of unsaturated fatty acid metabolism may start to be or continue to be expressed even after maximal expression of the Delta-12 desaturase gene of the present invention. This is beneficial for reducing unwanted intermediary products in the formation of a polyunsaturated fatty acid. For example, where the skilled person desires to produce EPA and reduce the concentration of intermediates in the final plant oil, expression of the genes coding for Delta-6 desaturase, Delta-6 elongase and Delta-5 desaturase (or Delta-9 elongase, Delta-8 desaturase and Delta-5 desaturase) may be reduced or switched off in this sequence. Thus, the enzymes involved in late steps of product formation have the chance to consume some or all of the required intermediated products without these intermediate products being replenished by the enzymes involved in early steps of product formation. The resulting oil is then enriched in EPA and reduced in content of for example ALA, SDA and/or ETA.

According to the invention there is also provided a method of identifying a gene for conveying an unsaturated fatty acid metabolic property to a plant, comprising:

- i) producing a plant comprising said gene,
- ii) performing an assay method of the present invention, wherein said gene is expressed and said plant metabolic property is measured, and
- iii) evaluating said plant for the presence and/or intensity of said unsaturated fatty acid metabolic property.

As described herein, by using the Delta-12 desaturase of the present invention it is possible to obtain plant metabolic property measurements, preferably plant oil composition data, of plants grown under greenhouse conditions that approximate closely or are identical to plants grown under field conditions.

The invention also provides a method of increasing Delta-12 desaturase activity and/or of stabilising Delta-12 desaturase activity in a plant or part thereof or during developmental stages of a plant or part thereof, preferably during seed development, which methods comprise growing a plant expressing a Delta-12 desaturase of the present invention. As described herein, expression of the Delta-12 desaturase according to the present invention allows to simulate or approximate a plant metabolic property (preferably seed oil composition) obtainable or to be expected under field conditions by merely growing a corresponding plant or plants under greenhouse conditions. Thus, the use of the Delta-12 desaturase according to the present invention effectively reduces the impact of environmental influences (preferably of temperature and/or daily/seasonal temperature variation) on Delta-12 desaturase activity in the plant or plants, thus effectively stabilising or increasing Delta-12 desaturase activity. This also allows to produce unsaturated fatty acids downstream of linoleic acid, for example EPA, DPA and/or DHA, more reliably than without a Delta-12 desaturase of the present invention.

Thus, the invention also provides a method of producing one or more desired unsaturated fatty acids in a plant, comprising growing a plant, said plant expressing, at least temporarily, a Delta-12 desaturase of the present invention and one or more further genes to convert linoleic acid to said one or more desired unsaturated fatty acids. As indicated above, the one or more further genes coding for enzymes for the production of unsaturated fatty acids preferably comprise desaturases and elongases.

The invention also provides a nucleic acid comprising a gene coding for a Delta-12 desaturase of the present invention, wherein the gene does not code for a Delta-12 desaturase of any of the exact sequences SEQ ID NO. 329 to 336. Thus, the present invention provides a nucleic acid comprising a gene coding for a Delta-12 desaturase, wherein said Delta-12 desaturase has at least 50% total amino acid sequence identity to any of the sequences SEQ ID NO. 328 to 336 and/or at least 60% total amino acid sequence similarity to any of the sequences SEQ ID NO. 328 to 336, and wherein the sequence is not any of the sequences SEQ ID NO. 329 to 336. The amino acid sequence according to SEQ ID NO. 328 had not been described in the prior art. The aforementioned nucleic acid of the invention thus for the first time provides you nucleic acid sequences coding for Delta-12 desaturases of the present invention. Corresponding to the preferred Delta-12 desaturase amino acid sequences described above, the present invention provides corresponding nucleic acids comprising a gene coding for such preferred Delta-12 desaturase of the present invention.

The invention also provides a nucleic acid comprising a gene coding for a Delta-12 desaturase of the present invention, wherein the gene is operably linked to an expression control sequence, and wherein the expression control sequence is heterologous to said gene if the gene codes for any of the exact sequences according to SEQ ID NO. 329 to 336. Thus, the invention particularly provides combinations of promoters and genes not found in nature, and particularly not found in any organism of genus *Phytophthora*, *Hyaloperonospora* and *Saprolegnia*.

The nucleic acids of the present invention preferably are expression vectors or transformation constructs useful for transforming a plant cell and causing the Delta-12 desaturase gene of the

present invention to be expressed at least temporarily during plant or plant cell development. Thus, the nucleic acids of the present invention facilitate to materialise the benefits conveyed by the present invention as described herein. Also, the invention provides purified Delta-12 desaturase polypeptides coded by any of the nucleic acids of the present invention.

5

According to the invention, there is also provided a plant cell comprising a gene coding for a Delta-12 desaturase of the present invention. Such plant cells can be obtained, as described above, by transformation of wild-type plant cells or offspring thereof, for example by crossing a plant comprising a gene coding for a Delta-12 desaturase of the invention with a plant not comprising
10 such gene and selecting offspring, preferably seeds, which comprise said gene. This way it is easily possible to transfer the gene coding for a Delta-12 desaturase of the present invention from one germplasm to another. The plant cell of the present invention preferably comprises a gene coding for one of the preferred Delta-12 desaturases of the present invention to materialise the benefits conveyed by such preferred desaturase. Also as described above, the gene coding for
15 the Delta-12 desaturase of the present invention preferably is operably linked to an expression control sequence, and it is particularly preferred that said expression control sequence directs expression to certain tissues and certain times of plant development, for example to developing seed tissue and the above indicated preferred times after flowering.

20

As the present invention provides an assay method which can, as described above, also be used for screening and comparison purposes, the present invention also provides a plant set comprising at least 2 plant groups, each consisting of one or more plants, wherein the plant or plants of each group are capable of expressing a Delta-12 desaturase of the present invention, and wherein the plant or plants of said groups comprise one or more genes coding for at least
25 one or more enzymes of unsaturated fatty acid metabolism, of which enzymes at least one is capable of using linoleic acid as a substrate, and of which enzymes at least one is supposedly connected to a plant metabolic property, and wherein the plant or plants of said groups differ in the expression of at least one of the enzymes of unsaturated fatty acid metabolism. To differ in expression of at least one of the enzymes of unsaturated fatty acid metabolism, one gene present
30 in the plant or plants of one group may be missing in the plant or plants of another group, or may be expressed at different times or in different tissues or in differing intensities. For example, the plants of 2 groups may both comprise a gene coding for a Delta-4 desaturase under the control of identical expression control sequences, but the Delta-4 desaturase nucleic acid sequences are derived from different organisms such that the amino acid sequences of the respective Delta-4
35 desaturases are unique for the plants of each of the groups. Instead of or additional to differing in the genes for Delta-4 desaturases, the groups can also differ in any other nucleic acid sequence coding for an enzyme of unsaturated fatty acid metabolism, included but not limited to omega-3 desaturases, Delta-6 desaturases, Delta-9 elongases, Delta-6 elongases, Delta-8 desaturases, Delta-5 desaturases and Delta-5 elongases.

40

As described above, the present invention allows to reduce the number of field trials for analysing plant metabolic properties, particularly of unsaturated fatty acid metabolism properties. Therefore the plants of the plant set of the present invention preferably are growing under greenhouse

conditions. Even more preferably, at least one plant of at least one group of the plant set of the present invention is within at most 100m distance to a plant of another group.

5 Where plants are grown in an automated greenhouse, in a growth chamber or germination chamber, it is allowable according to the invention to temporarily remove one or more plants from the greenhouse, growth chamber or germination chamber, respectively, for up to 1 hour per day, preferably for not more than 45min, more preferably for not more than 30min and most preferably for not more than 20min per day. Thus, the invention allows to perform analyses done on the plant or plants outside of e.g. a greenhouse or in a separate chamber thereof where environmental
10 conditions can differ from those of the location where the plant or plants are normally grown.

The invention and certain particular aspects thereof is hereinafter described by way of examples. These are intended to describe also additional objects, advantages, and novel features of this invention. They are not intended to limit the scope of the invention or of the claims.
15

EXAMPLES**Example 1: Materials and Methods****A. General Cloning Methods**

5 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

10 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

15 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

20 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.

25 **B. 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

30 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

35 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

40 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.

C. 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 plasmids VC-LJB2197-1qcz, VC-LJB2755-2qcz rc, VC-LLM337-1qcz rc, and VC-LLM391-2qcz rc is given in the Table 1, Table 2, Table 4, and Table 6.

Nomenclature 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[Im175]	148	4	Right border of T-DNA
p-VfUSP_684bp[LLL894]	329	1012	Promoter from <i>UNKNOWN SEED PROTEIN</i> gene <i>USP</i> (accession: X56240) from <i>Vicia faba</i>
i-Atss18_252[LJK36]	1013	1264	i-Atss18_252bp functional intron region; intron with partial 5'UTR, <i>Arabidopsis thaliana</i> , Locus At1g01170, + 37 to + 288 bp (numbering relative to start of transcription) (+ 72 to + 282bp 5'UTR-Intron only)
c-d6El0(Pp_GA2)	1267	2139	<i>Delta-6 ELONGASE</i> from <i>Physcomitrella patens</i>
t-CaMV35S	2140	2355	Terminator CaMV35S from 35S gene from Cauliflower mosaic virus
p-LuCnl(1064bp)	2448	3511	Promoter from <i>CONLININ</i> gene from <i>Linum usitatissimum</i>
i-Atss14_377[LJK32]	3512	3888	i-Atss14_377bp functional intron region; intron with partial 5'UTR, <i>Arabidopsis thaliana</i> , 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	<i>Delta-5 DESATURASE</i> from <i>Thraustochytrium</i> sp. ATCC21685
t-AgrOCS 192bp[LED12]	5212	5403	Terminator from <i>OCTOPINE SYNTHASE</i> gene OCS from <i>Agrobacterium tumefaciens</i>
p-SBP	5539	7337	Promoter from a <i>SUCROSE-BINDING PROTEIN-RELATED</i> gene from <i>Vicia faba</i>
i-Atss2_455bp[LJK20]	7338	7792	i-Atss2_455bp functional intron region; intron with partial 5'UTR, <i>Arabidopsis thaliana</i> , Locus At1g65090, +77 to +531bp (numbering relative to start of transcription) (+113 to + 508bp 5'UTR-Intron only)
c-d6Des(Ot_febit)	7802	9172	<i>Delta-6 DESATURASE</i> from <i>Ostreococcus tauri</i>

Genetic Elements of plasmid VC-LJB2197-1qcz	From	To	Description, Function and Source of Element
t-StCATHD-pA	9200	9434	Terminator from <i>CATHEPSIN D INHIBITOR</i> gene [CATHD] from <i>Solanum tuberosum</i> [Potato]
p-LuPXR 1727bp[LLLL823]	9513	11239	Promoter from <i>PEROXIREDOXIN LIKE PROTEIN</i> gene PXR from <i>Linum usitatissimum</i>
i-Atss1_847bp[LJK19]	11240	12086	i-Atss1_847bp functional intron region; intron with partial 5'UTR, <i>Arabidopsis thaliana</i> , 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	<i>Delta-6 ELONGASE</i> from <i>Thalassiosira pseudonana</i>
t-AtPXR 400bp[LLLL823]	12974	13373	Terminator from <i>PEROXIREDOXIN LIKE PROTEIN</i> gene PXR (At1g48130) from <i>Arabidopsis thaliana</i>
p-Napin A/B	13543	14206	Promoter from <i>napA/B</i> gene (napin, seed storage protein) from <i>Brassica napus</i>
i-Atss14_377[LJK32]	14207	14583	i-Atss14_377bp functional intron region; intron with partial 5'UTR, <i>Arabidopsis thaliana</i> , Locus At5g63190, +166 to +542 bp (numbering relative to start of transcription) (+201 to +542 bp 5'UTR-Intron only)
c-d12Des(Ps_GA)	14590	15786	<i>Delta-12 DESATURASE</i> from <i>Phytophthora sojae</i>
t-E9	15805	16362	Terminator from Small Subunit of RuBisCo <i>rbcS</i> gene (E9) from <i>Pisum sativum</i>
t-AtAHAS-3'/UTR[bb003]	16576	17790	Terminator from <i>AtAHASL [csr1-2]</i> of <i>acetohydroxyacid synthase</i> gene from <i>Arabidopsis</i>
p-PcUbi4-2[long]	17823	18804	Promoter from <i>UBIQUITIN</i> gene <i>UBI4-2</i> with internal intron from <i>Petroselinum crispum</i>
c-AtAHASL_A122T_S653N	18812	20824	<i>ACETOHYDROXYACID SYNTHASE LARGE-SUBUNIT</i> gene/CDS with S653N(<i>csr1-2</i>) mutation and A122T SDM mutation from <i>Arabidopsis</i>
t-AtAHAS-3'/UTR[ac321]	20849	22064	Terminator from <i>AtAHASL [csr1-2]</i> of <i>ACETOHYDROXYACID SYNTHASE</i> gene from <i>Arabidopsis</i>
b-JTV_LB	22232	22105	Left border of T-DNA
c-StaA[Im500]	22338	22967	PVS1 partitioning protein
c-VS1orf3[Im500]	23203	22988	VS1orf3
c-repA[Im500]	23294	24469	pVS1 replication protein [<i>repA</i>] gene/CDS

Genetic Elements of plasmid VC-LJB2197-1qcz	From	To	Description, Function and Source of Element
o-pVS1-origin	24535	24729	broad host-range replication origin of plasmid pVS1 (Genbank: AF133831, Itoh et al.1984)
o-ColE1-bom[Im500]	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 <i>Adenyltransferase</i> [<i>aadA</i>] gene/CDS from SUN100
p-aadA[Im800]	26767	26589	<i>Adenyltransferase</i> [<i>aadA</i>] 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[Im175]	148	4	Right border of T-DNA
p-LuPXR 1727bp[LLL823]	342	2068	Promoter from <i>PEROXIREDOXIN LIKE PROTEIN</i> gene <i>PXR</i> from <i>Linum usitatissimum</i>
i-Atss15_758bp[LJK33]	2069	2826	i-Atss15_758bp functional intron region; intron with partial 5'UTR, <i>Arabidopsis thaliana</i> , 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	<i>Omega-3 DESATURASE</i> from <i>Pythium irregulare</i>
t-AtPXR 400bp[LLL823]	3990	4389	Terminator from <i>PEROXIREDOXIN LIKE PROTEIN</i> gene <i>PXR</i> (At1g48130) from <i>Arabidopsis thaliana</i>
p-LuCnI(1064bp)	4468	5531	Promoter from <i>CONLININ</i> gene from <i>Linum usitatissimum</i>
i-Atss14_377[LJK32]	5532	5908	i-Atss14_377bp functional intron region; intron with partial 5'UTR, <i>Arabidopsis thaliana</i> , 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	<i>Delta-5 DESATURASE</i> from <i>Thraustochytrium</i> sp. ATCC21685
t-AgrOCS 192bp[LED12]	7232	7423	Terminator from <i>OCTOPINE SYNTHASE</i> gene <i>OCS</i> from <i>Agrobacterium tumefaciens</i>
p-SBP	7559	9357	Promoter from a <i>SUCROSE BINDING RELATED-PROTEIN</i> gene from <i>Vicia faba</i>

Genetic Elements of plasmid VC-LJB2755-2qcz rc	From	To	Description, Function and Source of Element
i-Atss2_455bp[LJK20]	9358	9812	i-Atss2_455bp functional intron region; intron with partial 5'UTR, <i>Arabidopsis thaliana</i> , Locus At1g65090, +77 to +531bp (numbering relative to start of transcription) (+113 to +508bp 5'UTR-Intron only)
c-d6Des(Ot_febit)	9822	11192	<i>Delta-6 DESATURASE</i> from <i>Ostreococcus tauri</i>
t-StCATHD-pA	11220	11454	Terminator from <i>CATHEPSIN D INHIBITOR</i> gene [CATHD] from <i>Solanum tuberosum</i> [Potato]
p-BnFae1	11533	12962	Promoter from <i>Beta-KETOACYL-CoA SYNTHASE (FAE1.1)</i> gene from <i>Brassica napus</i>
i-Atss1_847bp[LJK19]	12963	13809	i-Atss1_847bp functional intron region; intron with partial 5'UTR, <i>Arabidopsis thaliana</i> , 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	<i>Delta-6 ELONGASE</i> from <i>Thalassiosira pseudonana</i>
t-bnFae1	14646	15045	Terminator from <i>FATTY ACID ELONGASE (FAE1, At4g34520)</i> gene of <i>Arabidopsis thaliana</i>
p-Napin A/B	15166	15829	Promoter from <i>napA/B</i> gene (napin, seed storage protein) from <i>Brassica napus</i>
i-Atss14_377[LJK32]	15830	16206	i-Atss14_377bp functional intron region; intron with partial 5'UTR, <i>Arabidopsis thaliana</i> , 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	<i>Delta-12 DESATURASE</i> from <i>Phytophthora sojae</i>
t-E9	17428	17985	Terminator from Small Subunit of RuBisCo <i>rbcs</i> gene (E9) from <i>Pisum sativum</i>
p-VfUSP_684bp[LLL894]	18064	18747	Promoter from <i>UNKNOWN SEED PROTEIN</i> gene <i>USP</i> (accession: X56240) from <i>Vicia faba</i>

Genetic Elements of plasmid VC-LJB2755-2qcz rc	From	To	Description, Function and Source of Element
i-Atss18_252[LJK36]	18748	18999	i-Atss18_252bp functional intron region; intron with partial 5'UTR, <i>Arabidopsis thaliana</i> , Locus At1g01170, + 37 to + 288 bp (numbering relative to start of transcription) (+ 72 to + 282bp 5'UTR-Intron only)
c-o3Des(Pi_GA2)	19010	20095	<i>Omega-3-DESATURASE</i> from <i>Phytophthora infestans</i>
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 ACETOHYDROXYACID SYNTHASE gene from <i>Arabidopsis</i>
p-PcUbi4-2[long]	21708	22689	Promoter from UBIQUITIN gene UBI4-2 with internal intron from <i>Petroselinum crispum</i>
c-AtAHASL_A122T_S653N	22697	24709	ACETOHYDROXYACID SYNTHASE LARGE-SUBUNIT gene/CDS with S653N(csr1-2) mutation and A122T SDM mutation from <i>Arabidopsis</i>
t-AtAHAS-3'/UTR[ac321]	24734	25949	Terminator from AtAHASL [csr1-2] of ACETOHYDROXYACID SYNTHASE gene from <i>Arabidopsis</i>
b-JTV_LB	26117	25990	Left border of T-DNA
c-StaA[Im500]	26223	26852	PVS1 partitioning protein
c-VS1orf3[Im500]	27088	26873	VS1orf3
c-repA[Im500]	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[Im500]	28917	28715	pBR322 bom site, partial, from AF234316 pCambia2301
o-Rep-ColE1	29336	29056	pBR322 origin of replication [<i>E. coli</i>] from AF234316 pCambia2301
c-aadAmod1	30473	29682	Codon Optimized <i>Adenyltransferase</i> [aadA] gene/CDS from SUN100
p-aadA[Im800]	30652	30474	<i>Adenyltransferase</i> [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[Im175]	148	4	Right border of T-DNA
p-LuCnl(1064bp)	342	1405	Promoter from <i>CONLININ</i> gene from <i>Linum usitatissimum</i>
c-d4Des(Eg_GA)	1416	3041	<i>Delta-4 DESATURASE</i> from <i>Euglena gracilis</i>
t-AgrOCS 192bp[LED12]	3063	3254	Terminator from <i>OCTOPINE SYNTHASE</i> gene OCS from <i>Agrobacterium tumefaciens</i>
p-BnFae1	3448	4877	Promoter from <i>Beta-KETOACYL-CoA SYNTHASE (FAE1. 1)</i> gene from <i>Brassica napus</i>
i-Atss1_847bp[LJK19]	4878	5724	i-Atss1_847bp functional intron region; intron with partial 5'UTR, <i>Arabidopsis thaliana</i> , 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	<i>Delta-5 ELONGASE</i> from <i>Ostreococcus tauri</i>
t-bnFae1	6651	7050	Terminator from <i>FATTY ACID ELONGASE (FAE1, At4g34520)</i> gene of <i>Arabidopsis thaliana</i>
p-VfSBP_perm3	7099	8897	Promoter derived from a promoter from a <i>SUCROSE-BINDING PROTEIN-RELATED</i> gene from <i>Vicia faba</i>
c-o3Des(Pir_GA)	8901	9992	<i>Omega-3 DESATURASE</i> from <i>Pythium irregulare</i>
t-StCATHD-pA	10023	10257	Terminator from <i>CATHEPSIN D INHIBITOR</i> gene [<i>CATHD</i>] from <i>Solanum tuberosum</i> [Potato]
p-VfUSP_684bp[LLL894]	10331	11014	Promoter from <i>UNKNOWN SEED PROTEIN</i> gene <i>USP</i> (accession: X56240) from <i>Vicia faba</i>

Genetic Elements of plasmid VC-LLM337-1qcz rc	From	To	Description, Function and Source of Element
i-Atss15_758bp[LJK33]	11015	11772	i-Atss15_758bp functional intron region; intron with partial 5'UTR, <i>Arabidopsis thaliana</i> , 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	<i>Omega-3-DESATURASE</i> from <i>Phytophthora infestans</i>
t-CaMV35S	12924	13139	Terminator CaMV35S from 35S gene from Cauliflower mosaic virus
p-ARC5_perm1	13259	14409	Promoter derived from a promoter from <i>ARC/LINE 5</i> gene from <i>Phaseolus vulgaris</i>
c-d4Des(Tc_GA)	14420	15979	<i>Delta-4 DESATURASE</i> from <i>Thraustochytrium</i> sp.
t-pvarc	15993	16592	Terminator of ARC5 gene from <i>Phaseolus vulgaris</i>
b-JTV_LB	16953	16826	Left border of T-DNA
c-StaA[Im500]	17059	17688	PVS1 partitioning protein
c-VS1orf3[Im500]	17924	17709	VS1orf3
c-repA[Im500]	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[Im500]	19753	19551	pBR322 bom site, partial, from AF234316 pCambia2301
o-Rep-ColE1	20172	19892	pBR322 origin of replication [<i>E. coli</i>] from AF234316 pCambia2301
c-aadAmod1	21309	20518	Codon Optimized <i>Adenytransferase</i> [<i>aadA</i>] gene/CDS from SUN100
p-aadA[Im800]	21488	21310	<i>Adenytransferase</i> [<i>aadA</i>] 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) and a left border (nucleotides 10947 to 10820 of VC-LLM391-2qcz 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-LLM391-2qcz rc	From	To	Description, Function and Source of Element
b-RB[Im175]	148	4	Right border of T-DNA
p-BnFae1	540	1969	Promoter from <i>Beta-KETOACYL-CoA SYNTHASE (FAE1.1)</i> gene from <i>Brassica napus</i>
i-Atss1_847bp[LJK19]	1970	2816	i-Atss1_847bp functional intron region; intron with partial 5'UTR, <i>Arabidopsis thaliana</i> , 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	<i>Delta-5 ELONGASE</i> from <i>Ostreococcus tauri</i>
t-bnFae1	3743	4142	Terminator from <i>FATTY ACID ELONGASE (FAE1, At4g34520)</i> gene of <i>Arabidopsis thaliana</i>
p-ARC5perm1	4335	5485	Promoter derived from a promoter from <i>ARCILINE 5</i> gene from <i>Phaseolus vulgaris</i>
c-d4Des(Tc_GA)_T564G	5496	7055	<i>Delta-4 DESATURASE</i> from <i>Thraustochytrium</i> spp.
t-pvarc	7069	7668	Terminator of <i>Arc5</i> gene from <i>Phaseolus vulgaris</i>
p-LuCnl(1064bp)	7747	8810	Promoter from <i>CONLININ</i> gene from <i>Linum usitatissimum</i>
c-d4Des(Eg_GA)	8821	10446	<i>Delta-4 DESATURASE</i> from <i>Euglena gracilis</i>
t-AgrOCS 192bp[LED12]	10468	10659	Terminator from <i>OCTOPINE SYNTHASE</i> gene OCS from <i>Agrobacterium tumefaciens</i>
b-JTV_LB	10947	10820	Left border of T-DNA
c-StaA[Im500]	11053	11682	PVS1 partitioning protein
c-VS1orf3[Im500]	11918	11703	VS1orf3
c-repA[Im500]	12009	13184	pVS1 replication protein [<i>repA</i>] gene/CDS
o-pVS1-origin	13250	13444	broad host-range replication origin of plasmid pVS1 (Genbank: AF133831, Itoh et al.1984)

Genetic Elements of plasmid VC-LLM391-2qcz rc	From	To	Description, Function and Source of Element
o-Cole1-bom[Im500]	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[Im800]	15482	15304	Adenyltransferase [aadA] Spectinomycin Prokaryotic promoter

D. 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 a binary t-plasmid that was used for agrobacteria mediated transformation of plants. 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 plasmid 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-plasmid for plant transformation VC-L TM593-1qcz rc having a total size of ~61.000bp. The structure of the plasmid VC-L TM593-1qcz rc is given in Table 11.

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 to 43695 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 <i>UNKNOWN SEED PROTEIN</i> gene <i>USP</i> (accession: X56240) from <i>Vicia faba</i>
i-Atss18_252[LJK36]	1013	1264	i-Atss18_252bp functional intron region; intron with partial 5' UTR, <i>Arabidopsis thaliana</i> , 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	<i>Delta-6 ELONGASE</i> from <i>Physcomitrella patens</i>
t-CaMV35S	2140	2355	Terminator CaMV35S from 35S gene from Cauliflower mosaic virus
p-LuCnl(1064bp)	2448	3511	Promoter from <i>CONLININ</i> gene from <i>Linum usitatissimum</i>
i-Atss14_377bp[LJK32]	3512	3888	i-Atss14_377bp[LJK32] functional intron region; intron with partial 5'UTR, <i>Arabidopsis thaliana</i> , 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	<i>Delta-5 DESATURASE</i> from <i>Thraustochytrium</i> sp. ATCC21685
t-AgrOCS 192bp[LED12]	5212	5403	Terminator from <i>OCTOPINE SYNTHASE</i> gene <i>OCS</i> from <i>Agrobacterium tumefaciens</i>
p-SBP	5539	7337	Promoter from a <i>SUCROSE-BINDING PROTEIN-RELATED</i> gene from <i>Vicia faba</i>
i-Atss2_455bp[LJK20]	7338	7792	i-Atss2_455bp functional intron region; intron with partial 5'UTR, <i>Arabidopsis thaliana</i> , Locus At1g65090, +77 to +531bp (numbering relative to start of transcription) (+113 to + 508bp 5'UTR-Intron only)
c-d6Des(Ot_febit)	7802	9172	<i>Delta-6 DESATURASE</i> from <i>Ostreococcus tauri</i>

Genetic Elements of plasmid VC-LTM593-1qcz rc	From	To	Description, Function and Source of Element
t-StCATHD-pA	9200	9434	Terminator from <i>CATHEPSIN D INHIBITOR</i> gene [<i>CATHD</i>] from <i>Solanum tuberosum</i> [Potato]
p-LuPXR 1727bp[LLL823]	9513	11239	Promoter from <i>PEROXIREDOXIN LIKE</i> protein gene <i>PXR</i> from <i>Linum usitatissimum</i>
i-Atss1_846bp[ltm593]	11240	12085	i-Atss1_847bp functional intron region; intron with partial 5' UTR, <i>Arabidopsis thaliana</i> , 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-d6Elol(Tp_GA2)	12099	12917	<i>Delta-6 ELONGASE</i> from <i>Thalassiosira pseudonana</i>
t-AtPXR 400bp[LLL823]	12973	13372	Terminator from peroxiredoxin like protein gene <i>PXR</i> (At1g48130) from <i>Arabidopsis thaliana</i>
p-Napin A/B	13542	14205	Promoter from <i>napA/B</i> gene (napin, seed storage protein) from <i>Brassica napus</i>
i-Atss14_377bp[LJK32]	14206	14582	i-Atss14_377bp[LJK32] functional intron region; intron with partial 5' UTR, <i>Arabidopsis thaliana</i> , 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	<i>Delta-12 DESATURASE</i> from <i>Phytophthora sojae</i>
t-E9	15804	16361	Terminator from Small Subunit of RuBisCo <i>rbcS</i> gene (<i>E9</i>) from <i>Pisum sativum</i>
p-BnSETL-v1[1234bp]	16454	17687	<i>SETL-v1 Brassica napus</i> promoter
c-o3Des(Pir_GA)	17690	18781	<i>Omega-3 DESATURASE</i> from <i>Pythium irregulare</i>
t-BnSETL	18803	19416	<i>SETL-v1 Brassica napus</i> terminator
p-VfUSP_684bp[LLL894]	19495	20178	Promoter from <i>UNKNOWN SEED PROTEIN</i> gene <i>USP</i> (accession: X56240) from <i>Vicia faba</i>
i-Atss18_252[LJK36]	20179	20430	i-Atss18_252bp functional intron region; intron with partial 5' UTR, <i>Arabidopsis thaliana</i> , 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	<i>Omega-3-DESATURASE</i> from <i>Phytophthora infestans</i>

Genetic Elements of plasmid VC-LTM593-1qcz rc	From	To	Description, Function and Source of Element
t-CaMV35S	21535	21750	Terminator CaMV35S from 35S gene from Cauliflower mosaic virus
p-BnSETL-v1[1234bp]	21886	23119	SETL-v1 <i>Brassica napus</i> promoter
c-d5Des(Tc_GA2)	23122	24441	Delta-5 DESATURASE from <i>Thraustochytrium</i> sp. ATCC21685
t-BnSETL	24463	25076	SETL-v1 <i>Brassica napus</i> terminator
p-ARC5_perm1	25223	26373	Promoter derived from a promoter from <i>ARCILINE</i> 5 gene from <i>Phaseolus vulgaris</i>
c-d4Des(Tc_GA3)	26384	27943	Delta-4 DESATURASE from <i>Thraustochytrium</i> sp.
t-pvarc	27957	28556	Terminator of ARC5 gene from <i>Phaseolus vulgaris</i>
p-LuPXR 1727bp[LLL823]	28649	30375	Promoter from PEROXIREDOXIN LIKE protein gene PXR from <i>Linum usitatissimum</i>
i-Atss15_758bp[LJK33]	30376	31133	i-Atss15_758bp[LJK33] functional intron region; intron with partial 5'UTR, <i>Arabidopsis thaliana</i> , 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	<i>Omega-3</i> DESATURASE from <i>Pythium irregulare</i>
t-AtPXR 400bp[LLL823]	32297	32696	Terminator from PEROXIREDOXIN LIKE protein gene PXR (At1g48130) from <i>Arabidopsis thaliana</i>
p-LuCnI(1064bp)	32832	33895	Promoter from <i>CONLININ</i> gene from <i>Linum usitatissimum</i>
i-Atss2_455bp[LJK20]	33896	34350	i-Atss2_455bp functional intron region; intron with partial 5'UTR, <i>Arabidopsis thaliana</i> , Locus At1g65090, +77 to +531bp (numbering relative to start of transcription) (+113 to + 508bp 5'UTR-Intron only)
c-d4Des(PI_GA)2	34360	35697	Delta-4 DESATURASE from <i>Pavlova lutheri</i>
t-AgrOCS 192bp[LED12]	35719	35910	Terminator from OCTOPINE SYNTHASE gene OCS from <i>Agrobacterium tumefaciens</i>
p-BnFae1	36104	37533	Promoter from Beta-KETOACYL-CoA SYNTHASE (<i>FAE1.1</i>) gene from <i>Brassica napus</i>
i-Atss1_847bp[LJK19]	37534	38380	i-Atss1_847bp functional intron region; intron with partial 5' UTR, <i>Arabidopsis thaliana</i> , Locus At1g62290 (aspartyl protease family protein), +1 to +847bp

Genetic Elements of plasmid VC-LTM593-1qcz rc	From	To	Description, Function and Source of Element
			(numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTTP6393.
c-d5Elo(Ot_GA3)	38388	39290	<i>Delta-5 ELONGASE</i> from <i>Ostreococcus tauri</i>
t-bnFae1	39307	39706	Terminator from <i>FATTY ACID ELONGASE</i> (<i>FAE1</i> , At4g34520) gene of <i>Arabidopsis thaliana</i>
p-YPC105906_PcUbi4-2[long]	39830	40806	MTX Parsley <i>UBI4-2</i> promoter with internal intron
c-AtAHASL_A122T_S653N[minusRES]	40814	42826	<i>ACETOHYDROXYACID SYNTHASE LARGE-SUBUNIT</i> gene/CDS from <i>Arabidopsis</i> with S653N (<i>csr1-2</i>) mutation and A122T SDM mutation minus restriction sites
t-AtAHAS-3'UTR[rtp4820]	42827	43606	<i>Arabidopsis</i> (dicot) <i>AtAHASL</i> 3' Un-translated Region [trimmed] terminator for <i>ACETOHYDROXYACID SYNTHASE</i> 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[Im500]	45898	45778	Promoter for Kanamycin resistance gene
o-ori-2	47051	47267	ori-2 origin of replication
c-repE	47361	48116	<i>repE</i> gene/CDS
c-sopA	48695	49870	<i>sapA</i> gene/CDS
c-sopB	49870	50841	<i>sopB</i> gene/CDS
c-sopC/incD	50914	51387	<i>incD/sopC</i> partial gene/CDS
c-tral	51890	51949	<i>traI</i> gene/CDS
mf-tral - repA intergenic region	51938	52300	regulatory region of <i>traR</i> dependent quorum sensing regulon - containing 2 <i>tra</i> -boxes (see LI AND FARRAND JOURNAL OF BACTERIOLOGY, Jan. 2000, p. 179-188)
o-repA	52301	53518	<i>Rep-A</i> gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY, Jan. 2000, p. 179..188)

Genetic Elements of plasmid VC-LTM593-1qcz rc	From	To	Description, Function and Source of Element
rr-repB	53748	54758	<i>rep-B</i> gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY, Jan. 2000, p. 179..188)
o-repC	54973	56292	<i>rep-C</i> 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 <i>Rhizobium</i> 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), whereas 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 construct 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 *Physcomitrella 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 exposure of T-DNA internal genes are exposed 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 environment 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[LJK36]	c-d6Elo(Pp_GA2)	t-CaMV35S
PXR/c-o3Des(Pir_GA)	p-LuPXR 1727bp[LLL823]	i-Atss15_758bp[LJK33]	c-o3Des(Pir_GA)	t-AtPXR 400bp[LLL823]
Conlinin/c- d5Des(Tc_GA2)	p-LuCnI(1064bp)	i-Atss14_377[LJK32]	c-d5Des(Tc_GA2)	t-AgrOCS 192bp[LED12]
SBP/c-d6Des(Ot_febit)	p-SBP	i-Atss2_455bp[LJK20]	c-d6Des(Ot_febit)	t-StCATHD-pA
PXR/c-d6Elo(Tp_GA2)	p-LuPXR 1727bp[LLL823]	i-Atss1_847bp[LJK19]	c-d6Elo(Tp_GA2)	t-AtPXR 400bp[LLL823]
Fae/c-d6Elo(Tp_GA2)	p-BnFae1	i-Atss1_847bp[LJK19]	c-d6Elo(Tp_GA2)	t-bnFae1
Napin/c-d12Des(Ps_GA)	p-Napin A/B	i-Atss14_377[LJK32]	c-d12Des(Ps_GA)	t-E9
USP/c-o3Des(Pi_GA2)	p-VfUSP_684bp[LLL894]	i-Atss18_252[LJK36]	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-LuCnI(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(PI_GA)2_var1	p-LuCnI(1064bp)	i-Atss2_455bp[LJK20]	c-d4Des(PI_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[LJK19]	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(PI_GA)2_var2	p-LuCnl(1064bp)	N/A	c-d4Des(PI_GA)2	t-AgrOCS 192bp[LED12]
FAE/c-d4Des(PI_GA)2	p-BnFAE1	i-Atss1_847bp[LJK19]	c-d4Des(PI_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- d6Elo(Pp_GA2)	USP/c- d6Elo(Pp_GA2)	USP/c- d6Elo(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				

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
RB2	RB2	RB2	RB2	RB2				
Conlinin/d4Des(Eg)	Conlinin/d4Des(Eg)				Conlinin/c-d4Des(PI_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	ARC/c-d4Des(Tc_GA)_var1	SETL/c-d5Des(Tc_GA2)			Conlinin/c-d4Des(PI_GA)2_var1
		Conlinin/d4Des(Eg)	Conlinin/d4Des(Eg)	Conlinin/c-d4Des(PI_GA)2_var2		FAE/c-d4Des(PI_GA)2	FAE/c-d4Des(PI_GA)2	FAE/c-d5Elo(Ot_GA3)
					SETL/c-o3Des(Pir_GA)	SETL/c-o3Des(Pir_GA)	SETL/c-o3Des(Pir_GA)	
					UBI/AHAS	UBI/AHAS	UBI/AHAS	UBI/AHAS

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
LB2	LB2	LB2	LB2	LB2	LB	LB	LB	LB

E. 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 <i>Agrobacterium tumefaciens</i> clone 1		Plasmid containing T-DNA 2 harbored by <i>Agrobacterium tumefaciens</i> 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, 0.7% 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 AgNO₃, 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).

F. Procedure for production of transgenic plants using BiBACs

- 10 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}
- 15 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

- 20 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

- 5 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
- 10 transformation success to the strain and construct used (see Table 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 selection plates but where confirmed using qPCR to lack the herbicide resistance marker (%)	69.3	32.0

Table 17: Transformation Efficiencies of the various plasmids and *Agrobacterium* strains used.

- 15 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 selection plates but were confirmed using qPCR to lack the herbicide resistance 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

G. 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.

10 H. 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

I. Non-destructive analysis of lipids in single cotyledons of seedlings

- 5 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
- 10 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 2: 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 enzymes 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 <i>Phytophthora sojae</i>
c-d6Des(Ot_febit)	VC-LJB2197-1qcz	1370	Delta-6 desaturase from <i>Ostreococcus tauri</i>
c-d6Elo(Pp_GA2)	VC-LJB2197-1qcz	872	Delta-6 elongase from <i>Physcomitrella patens</i>
c-d6Elo(Tp_GA2)	VC-LJB2197-1qcz	818	Delta-6 elongase from <i>Thalassiosira pseudonana</i>
c-d5Des(Tc_GA2)	VC-LJB2197-1qcz	1319	Delta-5 desaturase from <i>Thraustochytrium</i> sp. ATCC21685
c-o3Des(Pi_GA2)	VC-LLM337-1qcz rc	1085	Omega-3-desaturase from <i>Phytophthora infestans</i>
c-o3Des(Pir_GA)	VC-LLM337-1qcz rc	1091	Omega-3 desaturase from <i>Pythium irregulare</i>
c-d5Elo(Ot_GA3)	VC-LLM337-1qcz rc	902	Delta-5 elongase from <i>Ostreococcus tauri</i>
c-d4Des(Eg_GA)	VC-LLM337-1qcz rc	1625	Delta-4 desaturase from <i>Euglena gracilis</i>
c-d4Des(Tc_GA)	VC-LLM337-1qcz rc	1559	Delta-4 desaturase from <i>Thraustochytrium</i> sp.

A. 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 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-LJB2197-1qc and VC-LLM337-1qc 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 in the case of multilocus events to select 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-i-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.									
LALHCY (n=15)	c-AHAS	j-t-E9-p3-2	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-	j-t-CaMV_p-LuChl-2	c-d6Elo(Pp_GA)	j-i-Atss18_c-	d6Elo(Pp_GA2)	j-t-PvARC-p3	c-d4Des(Tc_GA)	c-	j-i-Atss15_c-	o3Des(Pl_GA2)	c-o3Des(Pir_GA)	j-p-VfSBPerm3_c-	o3Des(Pir_GA)	j-i-Atss1_c-	d5Elo(Ot_GA3)	c-d4Des(Eg_GA)	
	2.9 (T0: 1.8)	4.4		4.6	3.5	1.9 (T0: 1.1)	2.0	2.9	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			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
LALJKA (n=15)	2 (T0: 1.1)	2.0			2.0	4.1	3.6 (T0: 1.9)	3.8	3.5	3.8 (T0: 2)	(T0: 1.9)		4.0		5.5
LALLTL (n=11)	1.7 (T0: 1.8)	3.5			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	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			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			3.3	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			4.8	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			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			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)		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
LANUCB (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)		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)		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)		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)		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.
sc (n=296)	C-AHAS j-t-F9-p3-2 c-d6Elo(Tp_GA) j-t-StCAT_p2-p- j-t-CaMV_p-LuCl- c-d6Elo(Pp_GA) j-t-Atss18_c- j-t-PVARC-p3 c-d4Des(Tc_GA) c- j-t-Atss15_c- 03Des(Pi_GA2) c-03Des(Pir_GA) j-p- j-t-Atss1_c- c-d4Des(Eg_GA)	1.9 2.1 2.6 2.7 2.4 2.3 1.5 1.6 1.8 1.7
dc (n=198)	C-AHAS j-t-F9-p3-2 c-d6Elo(Tp_GA) j-t-StCAT_p2-p- j-t-CaMV_p-LuCl- c-d6Elo(Pp_GA) j-t-Atss18_c- j-t-PVARC-p3 c-d4Des(Tc_GA) c- j-t-Atss15_c- 03Des(Pi_GA2) c-03Des(Pir_GA) j-p- j-t-Atss1_c- c-d4Des(Eg_GA)	3.4 3.7 4.2 4.8 4.2 4.2 3.2 3.4 3.8 3.6
tc (n=2)	C-AHAS j-t-F9-p3-2 c-d6Elo(Tp_GA) j-t-StCAT_p2-p- j-t-CaMV_p-LuCl- c-d6Elo(Pp_GA) j-t-Atss18_c- j-t-PVARC-p3 c-d4Des(Tc_GA) c- j-t-Atss15_c- 03Des(Pi_GA2) c-03Des(Pir_GA) j-p- j-t-Atss1_c- c-d4Des(Eg_GA)	4.0 5.6 7.6 7.5 7.1 6.3 4.6 4.4 5.9 4.7

Table 42: 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-LLM337-1qcz 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	16:3	18:0	18:1	18:2	18:2	18:3	18:3	18:4	20:1	20:2	20:3	20:3	20:4	20:4	20:5	22:1	22:4	22:5	22:6	22:4	20:2
	16:0	16:1	16:3	18:0	18:1	18:2	18:2	18:3	18:3	18:4	20:1	20:2	20:3	20:3	20:4	20:4	20:5	22:1	22:4	22:5	22:6	22:4	20:2
LALHCY	±	0.3	0 ±	4 ±	23.7	32 ±	±	±	±	0.3	0.8	±	0.2	0.1	±	±	±	0.3	0 ±	±	0 ±	±	±
(n=15)	0.1	± 0	0	0.2	± 1	0.9	0.1	0.4	0.2	± 0	± 0	± 0	± 0	± 0	0.7	0.4	0.8	± 0	0.1	0.3	0	0.2	0.5
LALIAO	5 ±	0.2	0 ±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	0 ±	±	±	±
(n=15)	0.2	± 0	0	0.4	3.4	1.6	0.2	0.9	0.4	0.1	0.1	0.7	0.3	0.4	0.1	1.3	2.5	0.1	0.3	± 1	0	0.4	0.3
LALJKA	±	0.3	0 ±	±	±	±	±	±	±	±	0.6	±	0.1	1.7	1 ±	±	±	0.3	0 ±	±	0 ±	±	±
(n=15)	0.2	± 0	0	0.5	2.2	1.1	0.1	0.5	0.3	0.1	± 0	0.1	± 0	± 1	0.5	0.5	1.5	± 0	0.2	0.9	0	0.4	0.5
LALLTL	5 ±	0.3	0 ±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
(n=11)	0.2	± 0	0	0.3	4.4	1.1	0.2	0.5	0.4	± 0	0.3	0.2	0.1	± 1	0.6	2.4	1.5	± 0	0.4	0.6	0.1	0.4	0.5
LALQA	5.4	0.3	3.8	23.3	±	±	0.5	3.8	±	±	0.7	1.5	0.4	3.5	1.5	6.4	±	±	0.9	2.9	±	1.6	0.3
M	±	±	0 ±	±	±	32.7	±	±	1 ±	0.1	±	±	±	±	±	±	8 ±	0.3	±	±	0 ±	±	±
(n=15)	0.8	0.1	0	0.2	1.4	± 1	0.2	0.3	0.4	± 0	0.1	0.9	0.2	0.8	0.2	1.4	1.3	± 0	0.3	0.7	0	0.4	0.1

LALQDS (n=14)	5 ± 0.3 0.1 ± 0	0 ± 0	3.2 ± 0.4	29.1 ± 2.4	35.7 ± 1.1	0.6 ± 0.1	6.2 ± 0.6	1 ± 0.2	0.8 ± 0.1	0.5 ± 0.2	0.4 ± 0.1	0.2 ± 0.1	1.5 ± 0.2	1 ± 0.2	2.4 ± 0.6	6.7 ± 0.7	0.7 ± 0.1	2.7 ± 0.3	0 ± 0	1 ± 0.2	0.3 ± 0.1
LALRC Q (n=14)	5.1 ± 0.3 0.1 ± 0	4 ± 0	0.5 ± 0	23 ± 1.1	33.2 ± 1.2	1 ± 0.2	3.5 ± 0.5	2.3 ± 0.2	0.8 ± 0.1	0.6 ± 0.1	0.2 ± 0.1	0.2 ± 0	5.1 ± 1.7	± 0.3	4.1 ± 0.7	6.6 ± 0.9	1.9 ± 0.3	2.8 ± 0.4	0 ± 0	1.8 ± 0.3	1.1 ± 0.4
LALWK F (n=14)	5.3 ± 0.3 0.1 ± 0	0 ± 0	0.3 ± 0	26.7 ± 6.7	27.6 ± 3.6	1.3 ± 0.4	3.3 ± 0.3	2.2 ± 0.7	0.8 ± 0.1	0.7 ± 0.1	0.6 ± 0.5	0.2 ± 0.2	2.1 ± 0.9	± 0.3	9.3 ± 2.7	8.8 ± 1.5	0.7 ± 0.3	2.9 ± 0.6	0 ± 0	1.6 ± 0.5	0.1 ± 0.1
LAMABL (n=8)	4.8 ± 0.3 0.2 ± 0	0 ± 0	0.5 ± 0	27.8 ± 6.3	30 ± 3.8	± 0.2	1.4 ± 0.5	2.7 ± 0.7	0.8 ± 0.1	0.7 ± 0.1	0.3 ± 0.1	0.3 ± 0	1.3 ± 0.4	± 0.3	7 ± 0.8	8.6 ± 1.7	1.3 ± 0.3	3.2 ± 0.4	0 ± 0	1.4 ± 0.3	0.1 ± 0.1
LAMCKI (n=10)	5.1 ± 0.2 0.1 ± 0	0 ± 0	0.2 ± 0	26.4 ± 0.7	33.8 ± 0.5	1.1 ± ± 0	4.2 ± 0.2	1.8 ± 0.2	0.8 ± ± 0	0.6 ± ± 0	0.1 ± ± 0	0 ± 0	3.9 ± 0.6	± 0.3	2.9 ± 0.2	6.8 ± 0.6	1.2 ± 0.1	2.7 ± 0.3	0 ± 0	1.3 ± 0.1	0.9 ± 0.1
LAMCL E (n=14)	4.9 ± 0.2 0.2 ± 0	0 ± 0	0.2 ± 0	25.1 ± 2.4	32.2 ± 1.6	1.1 ± 0.3	3.5 ± 0.3	2.4 ± 0.6	0.8 ± ± 0	0.7 ± 0.1	0.4 ± 0.2	0 ± 0.1	2.3 ± 0.5	± 0.3	5.8 ± 1.3	8.4 ± 1.3	1.8 ± 0.4	3.4 ± 0.6	0 ± 0	1.8 ± 0.5	0.1 ± 0.2
LAMCLF (n=9)	5.1 ± 0.3 0.1 ± 0	0 ± 0	0.1 ± 0	24.7 ± 3.5	28.5 ± ± 2	1.1 ± 0.6	2.8 ± 0.5	2 ± 0.6	0.8 ± ± 0	0.6 ± 0.1	1.4 ± 0.7	0.1 ± 0.1	2.8 ± 0.7	± 0.3	9.7 ± 2.2	9.4 ± ± 1	1.5 ± 0.3	2.8 ± 0.5	0 ± 0	1.5 ± 0.3	0 ± 0.1
LAMEU B (n=15)	5 ± 0.3 0.2 ± 0	0 ± 0	0.5 ± 0	28.4 ± 5.6	29.7 ± 2.1	0.9 ± 0.3	3.4 ± 0.6	1.3 ± 0.4	0.9 ± 0.1	0.8 ± 0.1	1 ± 0.4	0.2 ± 0.1	2.7 ± 0.3	± 0.3	8.4 ± 2.9	7.6 ± 1.9	0.5 ± 0.2	2.2 ± 0.7	0 ± 0	1.2 ± 0.5	0.1 ± 0.1

LAMEU 5 ± 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
U (n=13)	0.1	0																					
LAMFJO ± 0.7	0.1	0	3.5 ± 0.7	26.5 ± 1.9	32.9 ± 0.8	0.2 ± 0.1	4.5 ± 0.4	0 ± 0	0.9 ± 0.1	2.2 ± 0.7	1.7 ± 1.9	3.7 ± 0.6	2 ± 2	4.7 ± 1.8	6.2 ± 0.7	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
(n=15)																							
LAMIRY 5 ± 0.3	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 ± 0.2	0.8 ± 0.1	0.7 ± 0.2	0.4 ± 0.1	2.4 ± 0.4	1.4 ± 0.3	4.5 ± 0.9	9.1 ± 2.1	9.1 ± 2.1	0.3 ± 0.1	0 ± 0	0.3 ± 0.2	3 ± 0.6	0 ± 0	1.6 ± 0.4	0.5 ± 0.1
(n=10)																							
LAMJIC ± 0.4	0.1	0	3.1 ± 0.5	28.5 ± 6.6	33.1 ± 3.9	0.6 ± 0.2	5.3 ± 1.2	0.2 ± 0.1	0.8 ± 0.1	0.7 ± 0.3	0.1 ± 0.1	2.6 ± 0.4	1.3 ± 0.3	3.9 ± 1.3	6.2 ± 1.8	6.2 ± 1.8	0.3 ± 0.1	0 ± 0	0.3 ± 0.3	2.6 ± 1	0 ± 0	1.4 ± 0.4	0.4 ± 0.1
(n=9)																							
LAMPJB ± 0.1	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	0.4 ± 0.3	0.2 ± 0.3	2.8 ± 0.4	1.2 ± 0.2	5.5 ± 2.2	5.5 ± 2.2	5.5 ± 2.2	0.4 ± 0.1	0 ± 0	1.5 ± 0.3	3.2 ± 0.4	0 ± 0	0.1 ± 0.3	0.1 ± 0.3
(n=13)																							
LAMQD 5 ± 0.2	0 ± 0	2.7 ± 0.4	26.4 ± 2.2	31.2 ± 1	0.2 ± 0.1	5.2 ± 0.6	0.4 ± 0.1	0.1 ± 0.1	0.7 ± 0.1	1 ± 0.1	1 ± 0.2	3.7 ± 0.4	2.1 ± 0.4	4.7 ± 2	6.7 ± 1.2	6.7 ± 1.2	0.3 ± 0.1	0 ± 0	0.4 ± 0.1	2.7 ± 0.7	0 ± 0	0.8 ± 0.2	1.1 ± 0.1
L (n=14)																							
LAMQN ± 0.3	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 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.2 ± 0.1	4 ± 1.7	± 0.8	2.7 ± 1.1	5.9 ± 1.1	5.9 ± 1.1	0.3 ± 0.1	0 ± 0	1.2 ± 0.2	2.8 ± 0.6	0 ± 0	1.2 ± 0.3	0.4 ± 0.1
E (n=14)																							
LAMQS ± 0.3	0 ± 0	3.4 ± 0.3	24.3 ± 2.7	31.5 ± 1.2	0.8 ± 0.4	4.5 ± 0.7	0.5 ± 0.8	0.3 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.2 ± 0.2	1.9 ± 0.6	1.3 ± 0.5	4.1 ± 1.5	10.6 ± 1.7	10.6 ± 1.7	0.3 ± 0.1	0 ± 0	1.1 ± 0.2	3.6 ± 0.5	0 ± 0	1.8 ± 0.5	0.3 ± 0.3
F (n=14)																							

LAMRC	5.5	0.3		4.4	23.9	28.4	1.3	2.7	3.4	0.3		0.6	0.4	0.1	3.7	1.3	5.7	8.7	0.4		1.8	3.3		2.1	0.4	0.4
O	±	±	0 ±	±	±	±	±	±	±	±	1 ±	±	±	±	±	±	±	±	±	0 ±	±	0 ±	±	±	±	
(n=14)	0.6	0.1	0	0.6	6.8	3.1	0.4	0.4	0.8	0.1	0.2	0.1	0.2	0.1	0.8	0.3	1.3	1.7	0.1	0	0.4	0.8	0	0.6	0.2	0.1
	4.8			3.6	23.4	32.7	1.1	3.4	2.3		0.9		0.2	5.4		1.2	3.4	8.2	0.4		3.5		1.8	0.5		
LAMRD	±	0 ±	0 ±	±	±	±	±	±	±	0.2	±	0.7	±	±	0 ±	±	±	±	±	0 ±	2 ±	±	0 ±	±	±	
S (n=15)	0.1	0	0	0.4	1.7	1.3	0.2	0.5	0.3	±0	0.1	±0	0.1	0.9	0.1	0.1	0.8	0.7	0.1	0	0.4	0.3	0	0.3	0.3	
		0.3		3.5		26.2	0.6	3.7		0.2	0.9	0.8	1.6	0.4		1.9	4.6	10.5					1.9	0.3	1.4	
LAMRH	6 ±	±	0 ±	±	26.5	±	±	±	1 ±	±	±	±	±	±	3 ±	±	±	±	0.4	0 ±	1 ±	3.9	0 ±	±	±	
L (n=14)	1	0.1	0	0.5	±6	3.9	0.1	0.8	0.3	0.1	0.1	0.2	0.6	0.1	0.6	0.4	0.8	1.9	±0	0	0.3	±1	0	0.5	0.2	0.4
	4.9			3.6	26.4			4.1		0.2	0.9	0.7		0.5	2.6	1.3	4.4	7.7	0.1		1.3	3.2		1.5	0.2	
LAMRJK	±	0.1	0 ±	±	±	33 ±	1 ±	±	2 ±	±	±	±	0 ±	±	±	±	±	±	±	0 ±	±	±	0 ±	±	±	
(n=15)	0.2	±0	0	0.4	2.9	1	0.2	0.6	0.4	0.1	0.1	0.1	0.1	1.4	1.4	0.3	1.3	1.2	0.2	0	0.4	0.6	0	0.2	0.4	
	4.8			2.7	29.6	32.2	0.5	5.1	0.9		0.8		1.2	0.3	2.8	1.3	4.6				0.9	2.4		1.2	0.4	0.6
LAMRN	±	0.2	0 ±	±	±	±	±	±	±	0.1	±	0.9	±	±	±	±	±	6 ±	0.3	0 ±		±	0 ±	±	±	
Q (n=9)	0.1	±0	0	0.4	3.8	0.9	0.1	0.8	0.2	±0	0.1	±0	0.2	0.1	0.7	0.3	1.3	1.2	±0	0	0.1	0.3	0	0.3	0.2	0.2
	4.9			3.6	24.6	33.4	0.2	4.8	0.4		0.9		2.8			2.4					0.5	2.3		1.3	0.6	
LAMVU	±	0.2	0 ±	±	±	±	±	±	±	0 ±	±	1 ±	±	1 ±	4 ±	±	4 ±	6.7	0.4	0 ±		±	0 ±	±	±	
B (n=15)	0.3	±0	0	0.4	1.9	0.5	0.1	0.4	0.1	0	0.1	0.1	0.5	0.2	0.4	0.3	1.4	±1	±0	0	0.1	0.4	0	0.3	0.2	
	5.1	0.2			23.1	31.7	1.2	4.1	2.4		0.9		0.3		2.5	1.3	4.1	10.3	0.3		1.3	3.6		2.1	0.4	
LAMYD	±	±	0 ±	4 ±	±	±	±	±	±	0.3	±	0.6	±	0 ±	±	±	±	±	±	0 ±	±	±	0 ±	±	±	
P (n=14)	0.5	0.1	0	0.8	2.3	1.1	0.1	0.5	0.3	±0	0.1	±0	0.1	0	0.4	0.3	0.8	1.6	0.1	0	0.3	0.7	0	0.5	0.2	
	4.7	0.2	0 ±	3.4	25 ±	32.7	0.8	4.7	1.5	0.2	0.8	0.7	0.5	0.2	2.3	1.5	3.5	9.2	0.3	0 ±	1.3	3.7	0 ±	2 ±	0.5	0.3
LANBC	±	±	0	±	3.9	±	±	±	±	±	±	±	±	±	±	±	±	±	±0	0	±	±	0	0.6	±	±0
H (n=14)	±	±	0	±																						
	0.8	0.1		0.4		1.5	0.2	0.5	0.5	0.1	0.1	0.1	0.2	0.1	0.4	0.2	0.5	1.8			0.4	0.9		0.1		

LANCE G (n=4)	4.9	0.1	3.3	23.8	33.2	0.9	3.7					0.2	5.5	0.2	0.7	1.5	10.8	0.3		1.2	3.8	1.7	
	±	±	±	±	±	±	2 ±	0.3	0.8	0.7	±	±	±	±	±	±	±	±	0 ±	±	±	0 ±	
	0.1	0	0.2	1.2	0.5	0.1	0.4	0.3	±0	±0	±0	0.1	0.2	0.1	0.1	0.4	0.4	0.1	0	0.1	0.2	0	
LANCO X (n=10)	5.1	0.2	3.1		35.5	0.7	4.1	2.1		0.8			7.2	0.2	0.6	1.6	7.3	0.3		0.9	2.7	1.4	
	±	±	±	25 ±	±	±	±	±	0.3	±	0.7	±	±	±	±	±	±	±	0 ±	±	±	0 ±	
	0.7	0	0.2	2.5	1.3	0.1	0.3	0.3	±0	0.1	±0	0	2.4	0.1	0.1	0.3	1.5	0.1	0	0.4	0.7	0	
LANFEF (n=15)	5.1	0.1	3.5		34.3	0.2	4.5			0.8			2.5	0.8	1.6	3.3	5.5	0.2			2.2	1.1	
	±	±	±	27.2 ±	±	±	±	0.3	0 ±	±	1 ±	±	±	±	±	±	±	±	0 ±	1 ±	±	±	
	0.3	0	0.4	±1	1.1	0.1	0.9	±0	0	0.1	0	0	0.3	0.2	0.4	1.4	0.5	0.1	0	0.1	0.3	0	
LANMG C (n=15)	5.6	0.2		25.1	33.1		4.3	2.1		0.9					7.9	3.6	1.4	3.7		0.7	1.5	0.8	
	±	±	4 ±	±	±	1 ±	±	±	0.2	±	0.7	0.2	0 ±	±	±	±	±	0.3	0 ±	±	±	±	
	0.9	0	0.5	1.9	1.8	0.2	0.3	0.2	±0	0.1	±0	±0	0	1.3	0.7	0.5	1.3	±0	0	0.2	0.6	0	
LANMO M (n=10)	5.4		2.8	26.9	31.8	0.8	4.9	1.4	0.2	0.8	0.7	0.6	0.2		1.2	3.9	8.6			1.2	3.7	1.5	
	±	0.2	±	±	±	±	±	±	±	±	±	±	±	2 ±	±	±	±	0.3	0 ±	±	±	±	
	0.4	±0	0.2	3.1	1.3	0.2	0.5	0.4	0.1	0.1	0.1	0.3	0.1	0.3	0.2	0.5	1.5	±0	0	0.2	0.7	0	
LANPM Z (n=13)	4.8	0.2	3.7	26.2		0.3		0.5			0.9	1.5	0.5			2.7	6.9			0.8	2.8	1.5	
	±	±	±	±	34.4 ±		5 ±	±	0.1	0.9	±	±	±	3 ±	2 ±	±	±	0.4	0 ±	±	±	±	
	0.6	0	0.3	1.4	±1	0.1	0.4	0.1	±0	±0	0.1	0.3	0.2	0.2	0.3	0.3	0.8	±0	0	0.2	0.6	0	
LANTLE (n=15)		0.2	2.4		32.7	0.4	6.1		0.1	0.6	0.8		0.5	2.5	1.7	3.9	7.9			0.7	3.3	1.5	
	5 ±	±	±	26.7 ±	±	±	±	1 ±	±	±	±	1.4	±	±	±	±	±	0 ±	0 ±	±	±	±	
	0.4	0	0.4	±4	1.2	0.3	0.8	0.5	0.1	0.1	0.2	±1	0.5	0.5	0.4	1.4	1.9	0.1	0	0.4	0.8	0	
LANTSP (n=12)	4.9		3.3	35.9		0.8	4.6	1.1	0.2	0.9	0.9	0.8	0.2	2.1	1.2	3.4	6.6				2.5	1.3	
	±	0.2	±	±	26.8 ±	±	±	±	±	±	±	±	±	±	±	±	±	0.4	0 ±	1 ±	±	±	
	0.2	±0	0.3	6.9	±4	0.2	0.9	0.4	0.1	0.1	0.2	0.3	0.1	0.4	0.2	0.9	1.8	±0	0	0.4	0.7	0	

LANUC	5 ± 0.3	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 ± 0.1	0.1 ± 0.7	2.2 ± 0.3	1.1 ± 1.9	5.7 ± 1.4	9.2 ± 0.1	0.2 ± 0	0.9 ± 0.3	3.3 ± 0.5	1.3 ± 0.4	0.6 ± 0.1
B (n=14)	0.5 ± 0	0	0.4 ± 0	4.5 ± 0	2.2 ± 0	0.6 ± 0	0.8 ± 0	1.5 ± 0	0.3 ± 0	0.1 ± 0	0.1 ± 0	0.5 ± 0	0.1 ± 0	0.7 ± 0	0.3 ± 0	1.9 ± 0	1.4 ± 0	0 ± 0	0.3 ± 0	0.5 ± 0	0.4 ± 0	0.5 ± 0
LAOBG	5.2 ± 0.2	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	0.2 ± 0	2.1 ± 0.9	1.1 ± 0.5	3.9 ± 0.6	7.3 ± 1.5	0.2 ± 0	1.1 ± 0.3	2.9 ± 0.6	1.4 ± 0.4	0.1 ± 0.1
Q (n=15)	0.4 ± 0.1	0	0.3 ± 0	7.9 ± 0	4.2 ± 0	0.4 ± 0	0.6 ± 0	0.7 ± 0	0.2 ± 0	0.1 ± 0	0.1 ± 0	0.2 ± 0	0 ± 0	0.9 ± 0	0.5 ± 0	0.6 ± 0	1.5 ± 0.2	0 ± 0	0.3 ± 0	0.6 ± 0	0.4 ± 0.3	0.1 ± 0.1
LAOHL	5.1 ± 0.2	0 ± 0	3.5 ± 0.3	26.5 ± 6.1	2.7 ± 0.2	1.1 ± 0.2	3.7 ± 0.7	2.1 ± 0.4	0.9 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.1 ± 0	0.1 ± 0	4.1 ± 1.6	1.4 ± 0.5	5.3 ± 1.2	6.8 ± 1.2	0.3 ± 0.1	0.8 ± 0.2	2.3 ± 0.4	1.2 ± 0.3	0.3 ± 0.3
R (n=14)	0.2 ± 0	0	0.3 ± 0	6.1 ± 0	2.7 ± 0	0.2 ± 0	0.7 ± 0	0.4 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0 ± 0	1.6 ± 0.5	0.9 ± 0.4	0.8 ± 0.7	1.2 ± 0.1	0 ± 0	0.2 ± 0	0.4 ± 0	0.3 ± 0.3	0.3 ± 0.3
LAOJAT	5.1 ± 0.1	0 ± 0	2.8 ± 0.7	28.7 ± 4	34 ± 1.8	0.6 ± 0.1	5.2 ± 0.8	1.3 ± 0.4	0.1 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.1 ± 0	1.5 ± 0.5	0.9 ± 0.2	4.3 ± 0.7	7.9 ± 1.3	0 ± 0	0.8 ± 0.3	2.9 ± 0.4	1.4 ± 0.3	0.3 ± 0.1
(n=15)	0.5 ± 0.1	0	0.7 ± 0	4 ± 0	1.8 ± 0	0.1 ± 0	0.8 ± 0.5	0.4 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.4 ± 0	0.2 ± 0	0.5 ± 0.2	0.3 ± 0.1	1.6 ± 0.9	0.9 ± 0	0 ± 0	0.1 ± 0	0.6 ± 0	0.3 ± 0.2	0.6 ± 0.6
LAOKLP	4.7 ± 0.2	0 ± 0	3.1 ± 0.2	26 ± 2.2	32.5 ± 1.3	0.2 ± 0.3	5.2 ± 0.5	0.3 ± 0	0 ± 0	0.8 ± 0	1 ± 0	2.5 ± 0.7	1 ± 0	3.5 ± 0.4	2.1 ± 0.3	4.4 ± 1.6	6.5 ± 0.9	0.3 ± 0	0.5 ± 0.1	2.5 ± 0.6	1.2 ± 0.3	1.1 ± 0.6
(n=15)	0.4 ± 0	0	0.2 ± 0	2.2 ± 0	1.3 ± 0	0.5 ± 0	0.5 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0	0.2 ± 0	0.4 ± 0.3	0.3 ± 0.1	1.6 ± 0.9	0.9 ± 0	0 ± 0	0.1 ± 0	0.6 ± 0	0.3 ± 0.2	0.6 ± 0.6
LAOKTE	4.7 ± 0.3	0.1 ± 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	0.9 ± 0.4	2.6 ± 1	1.6 ± 0.6	0.5 ± 0.2
(n=15)	0.3 ± 0.1	0	0.4 ± 0	7.8 ± 0	2.3 ± 0	0.3 ± 0	1.7 ± 0.4	0.6 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.7 ± 0.1	0.2 ± 0	0.8 ± 0.4	0.4 ± 0.2	2.2 ± 0.9	0.1 ± 0	0 ± 0	0.4 ± 0	1 ± 0	0.6 ± 0.2	0.2 ± 0.2
LAPKLS	5.2 ± 0.2	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 ± 0.1	0.7 ± 0.1	0.4 ± 0.3	0.1 ± 0.5	3.1 ± 0.5	1.5 ± 0.4	6 ± 2.3	7.9 ± 1.5	0.2 ± 0.1	0.8 ± 0.3	2.6 ± 0.7	1.4 ± 0.5	0.4 ± 0.2
(n=12)	0.3 ± 0.1	0	0.5 ± 0	1.9 ± 0	0.7 ± 0	0.1 ± 0	0.5 ± 0.4	0.4 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.3	0.1 ± 0.5	0.4 ± 0.2	0.4 ± 0.2	2.3 ± 0.9	1.5 ± 0.1	0 ± 0	0.3 ± 0.3	0.7 ± 0.5	0.3 ± 0.3	0.2 ± 0.2
LAPKX	4.6 ± 0.2	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 ± 0.3	2.3 ± 0.4	1.7 ± 0.2	3.1 ± 0.7	9.8 ± 2	0.3 ± 0	0.9 ± 0.3	3.4 ± 0.7	1.4 ± 0.4	0.5 ± 0.2
M (n=15)	0.3 ± 0	0	0.3 ± 0	2 ± 0	0.9 ± 0	0.2 ± 0	0.5 ± 0.4	0.4 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.7 ± 0.1	0.3 ± 0.3	0.4 ± 0.2	0.2 ± 0.1	0.7 ± 0.3	± 2	± 0	0.3 ± 0.3	0.7 ± 0.4	0.3 ± 0.3	0.2 ± 0.2

LAPKZJ (n=15)	5.1	0.1		3.6	24.6	31.8	0.4	4.7	0.6		0.8	0.9	2.1	0.8	3.4	2.1	4.9	7.7	0.2		0.6	2.8	1.4	0.4	1 ±
	±	±	0 ±	±	±	±	±	±	±	0 ±	±	±	±	±	±	±	±	±	±	0 ±	±	0 ±	±	±	
	0.5	0.1	0	0.4	1.9	1.6	0.2	0.7	0.3	0	0.1	0.2	0.8	0.4	0.5	0.5	2.1	1.1	0.2	0	0.3	0.5	0.3	0.5	0.2
LAPWJ D (n=14)	5.1	0.1		3.6			0.3	4.9	0.5		0.8	0.9	1.6	0.6	3.4	2.2	2.5	6.2	0.2		0.7	2.5	1.3	0.7	0.8
	±	±	0 ±	±	27 ±	34 ±	±	±	±	0 ±	±	±	±	±	±	±	±	±	±	0 ±	±	0 ±	±	±	±
	0.4	0.1	0	0.1	0.9	0.7	0.1	0.2	0.1	0	0.1	0.1	0.4	0.2	0.2	0.2	0.3	0.3	0.2	0	0.1	0.2	0.1	0.1	0.1
LAPWL P (n=14)	4.5				32.5	23.1	0.5	5.1	0.5	0.1	0.8	1.2	2.5	0.9	2.2	1.8	3.5				0.7		1.7	0.2	1.3
	±	0.2	0 ±	3 ±	±	±	±	±	±	±	±	±	±	±	±	±	±	10 ±	0.3	0 ±	±	3.6	0 ±	±	±
	0.4	± 0	0	0.3	6.3	3.7	0.3	0.8	0.3	0.1	0.1	0.2	0.9	0.4	0.4	0.4	0.7	2.3	± 0	0	0.2	± 1	0.6	0.2	0.4
LAQYTA (n=15)	5.6			3.3	24.7	31.5	1.3	4.1	2.1	0.1	0.7		0.2		3.6	1.6	5.8	8.2				2.9	1.4	0.5	0.5
	±	0 ±	0 ±	±	±	±	±	±	±	±	±	0.6	±	0 ±	±	±	±	±	0 ±	0 ±	1 ±	±	±	±	±
	0.2	0	0	0.3	1.3	1.3	0.2	0.5	0.3	0.1	0.1	± 0	0.2	0	1.6	0.8	2.6	1.4	0	0	0.2	0.5	0.3	0.7	0.2
LAQYU T (n=15)	5.5			3.3		33.4			2.2	0.2	0.7	0.6	0.1		3.7	1.5	4.1	7.1	0.1		1.1	2.4	1.3	0.5	0.2
	±	0.2	0 ±	±	26.6	±	1 ±	4 ±	±	±	±	±	±	0 ±	±	±	±	±	±	0 ±	±	±	±	±	±
	0.3	± 0	0	0.3	± 4	1.5	0.2	0.6	0.5	0.1	0.1	0.1	0.1	0	2.1	0.8	1.4	1.5	0.1	0	0.2	0.5	0.3	0.6	0.2
LAQYW Q (n=15)	5.6	0.2		3.9		30.3	1.3	4.1	2.5	0.3	0.8	0.6	0.2		2.3	1.1			0.1		0.8	2.5	1.4	0.2	0.2
	±	±	0 ±	±	26.9	±	±	±	±	±	±	±	±	0 ±	±	±	6.5	7.9	±	0 ±	±	±	±	±	±
	0.2	0.1	0	0.5	± 6	2.1	0.4	0.6	0.8	0.1	0.1	0.1	0.2	0	1.1	0.6	± 3	± 2	0.1	0	0.4	0.7	0.6	0.4	0.2
LAQZM E (n=12)	5.3	0.2		2.5	33.3	24.1	0.5	4.1		0.2	0.7	1.1	1.3	0.6	2.7	1.8	5.6	8.2			0.7		1.6	0.1	1.4
	±	±	0 ±	±	±	±	±	±	0.9	±	±	±	±	±	±	±	±	±	0.3	0 ±	±	3 ±	±	±	±
	0.7	0.1	0	0.2	6.9	4.6	0.1	0.5	± 1	0.1	0.1	0.4	1.1	0.3	0.2	0.3	0.5	1.2	± 0	0	0.3	0.5	0.6	0.1	1.1

Table 43: 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-LJB2197-1qc and VC-LLM337-1qc 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.

[illegible]

LAMCLE (n=1)	4.8	0.2	0.0	3.9	2	22.	31.	9	1.3	3.3	3.1	0.4	0.9	0.6	0.0	0.0	2.0	1.1	3.8	5	0.0	0.0	2.8	4.3	0.0	2.8	0.0	0.0
LAMCLF (n=1)	5.2	0.3	0.0	3.8	0	24.	27.	7	1.7	3.1	3.1	0.3	0.8	0.5	0.7	0.0	1.9	0.9	7.5	2	0.3	0.0	1.3	3.6	0.0	1.9	0.2	0.0
LAMEUB (n=1)	5.4	0.4	0.0	4.7	2	26.	27.	5	1.0	3.4	1.5	0.2	1.0	0.7	1.2	0.3	2.6	1.8	3.8	1	0.3	0.0	0.8	3.6	0.0	2.2	0.3	0.0
LAMEUU (n=1)	10.9	1.2	1.0	2.9	4	16.	27.	3	0.0	8.4	2.3	0.7	1.0	0.7	0.0	0.0	1.1	0.7	4.5	6.6	0.3	0.0	1.4	6.3	0.0	4.5	0.0	0.0
LAMFJO (n=1)	5.5	0.4	0.0	2.4	8	26.	30.	6	0.2	7.3	0.4	0.1	0.6	0.8	2.1	1.0	1.7	1.4	2.8	7.4	0.2	0.0	0.9	4.5	0.0	1.8	0.5	0.5
LAMIRY (n=1)	5.1	0.3	0.0	3.7	0	23.	30.	3	0.7	4.5	1.4	0.2	0.8	0.7	1.1	0.3	2.4	1.6	4.4	6	0.3	0.0	1.1	3.7	0.0	2.0	0.5	0.0
LAMJIC (n=1)	6.3	0.3	0.0	2.9	5	24.	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	2	24.	32.	0	1.1	4.0	2.7	0.4	0.8	0.7	0.0	0.0	2.2	1.1	4.0	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	7	24.	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	4	25.	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	3	20.	30.	2	0.5	3.9	3.6	0.5	0.8	0.6	0.4	0.1	1.3	0.9	6.1	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	8	14.	26.	6	1.0	2.4	4.7	0.5	1.0	0.4	0.3	0.0	3.9	1.5	6.7	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	9	20.	31.	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	7	19.	23.	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	5	25.	31.	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	5	23.	31.	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	9	21.	33.	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	2	19.	29.	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	5	19.	31.	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	4	22.	33.	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	1	22.	33.	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	8	28.	32.	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	2	25.	33.	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	0.0
LANPMZ (n=1)	3.6	0.1	0.0	3.1	5	22.	32.	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	6	19.	32.	5	0.8	4.5	2.2	0.4	0.7	0.6	0.4	0.2	2.3	1.6	4.3	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	0	19.	31.	5	0.5	5.3	1.6	0.3	0.6	0.7	1.4	0.7	3.2	2.5	5.1	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	5	23.	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	7	25.	27.	9	2.1	3.6	4.2	0.6	0.8	0.7	0.1	0.0	1.9	1.1	4.2	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	0	23.	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	7	21.	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	5	22.	34.	1	0.8	5.0	2.3	0.4	0.6	0.6	0.4	0.0	2.1	1.3	4.3	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	9	22.	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	3	21.	29.	8	0.9	4.6	1.9	0.3	0.7	0.6	0.3	0.2	2.3	1.6	5.4	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	0	20.	32.	5	1.1	3.9	3.0	0.4	0.8	0.6	0.0	0.0	3.1	2.0	4.0	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	0	23.	33.	1	0.8	4.3	1.7	0.2	0.9	0.7	0.5	0.0	2.3	1.6	4.3	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	2	23.	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	6	25.	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	2	28.	18.	2	0.5	4.7	0.5	0.2	0.8	1.2	2.9	1.1	1.9	2.1	3.6	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	8	24.	30.	1	1.2	4.3	2.1	0.1	0.7	0.6	0.4	0.0	2.1	1.1	7.5	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	5	21.	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	3	22.	30.	0	2.1	3.6	4.1	0.6	0.8	0.5	0.3	0.0	1.7	1.1	3.8	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	7	21.	30.	7	0.7	3.3	2.7	0.4	0.6	0.6	0.7	0.2	2.7	1.6	6.4	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 fulfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category	16:0	16:1	16:3	18:0	18:1	18:2	18:2	18:3	18:3	18:4	20:0	20:1	20:2	20:3	20:3	20:4	20:4	20:5	22:0	22:1	22:4	22:5	22:5	22:6	22:6	22:4	20:2
y of T1 plants	16:0	n-7	n-3	18:0	n-9	n-6	n-9	n-3	n-3	n-6	n-3	20:0	n-9	n-6	n-3	20:3	n-6	n-3	n-6	n-3	n-9	n-6	n-3	n-6	n-3	n-3	n-9
	4.9	0.2		3.2	28.5		0.5		0.8	0.1	0.8	0.9	1.6	0.7	2.7	1.6	4.6	7.2	0.3		0.7	2.7		1.3	0.3	0.9	
	±	±	0 ±	±	±	31 ±	±	4.7	±	±	±	±	±	±	±	±	±	±	±	0 ±	±	±	0 ±	±	±	±	
sc (n=296)	0.5	0.1	0	0.6	5.4	4	0.3	±1	0.5	0.1	0.1	0.2	0.9	1.2	0.9	0.6	2.3	1.9	0.1	0	0.3	0.7	0	0.4	0.3	0.5	
	5.1	0.2		3.5	23.7	32.2	1.1	4.1	2.3	0.3	0.8	0.6	0.3	0.4		1.6		9.1	0.3		1.4	3.4		1.8	0.6	0.3	
	±	±	0 ±	±	±	±	±	±	±	±	±	±	±	±	3 ±	±	4 ±	±	±	0 ±	±	±	0 ±	±	±	±	
dc (n=198)	0.6	0.1	0	0.6	3.1	2.5	0.3	0.7	0.6	0.1	0.1	0.1	0.2	1.4	2	0.8	1.8	2.8	0.1	0	0.5	1.2	0	0.6	0.7	0.2	
	5.6			4.4		30.2	1.6	3.6	3.1	0.4			0.3	0.1	3.5	1.7	3.4				1.6	3.6		2.2			
	±	0.3	0 ±	±	±	±	±	±	±	±	0.9	0.6	±	±	±	±	±	9.7	0.3	0 ±	±	±	0 ±	±	0.7		
tc (n=2)	0.4	±0	0	0.7	1.5	0.1	0.7	1.2	0.7	0.1	±0	±0	0.1	0.1	1.5	0.4	1.1	±3	±0	0	0.2	1.4	0	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	16:0	16:1	16:3	18:0	18:1	18:2	18:2	18:3	18:3	18:4	20:0	20:1	20:2	20:3	20:3	20:4	20:4	20:5	22:0	22:1	22:4	22:5	22:5	22:6	22:6	22:7	22:8
y of T1	16:0	16:1	16:3	18:0	18:1	18:2	18:2	18:3	18:3	18:4	20:0	20:1	20:2	20:3	20:3	20:4	20:4	20:5	22:0	22:1	22:4	22:5	22:5	22:6	22:6	22:7	22:8
plants	16:0	16:1	16:3	18:0	18:1	18:2	18:2	18:3	18:3	18:4	20:0	20:1	20:2	20:3	20:3	20:4	20:4	20:5	22:0	22:1	22:4	22:5	22:5	22:6	22:6	22:7	22:8
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	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	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	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

- 5 VC-LLM337-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that were 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
LALJKA (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	1.4 ± 1.1	1 ± 0	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	1.1	1.4 ± 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	1.5 ± 0.8	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	1.1	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	1.2	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	1.1 ± 0.3	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	0.3	1.1 ± 0.3	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	0.8	1.2 ± 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	0.8	1.2 ± 0.8	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	1.1 ± 0.3	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	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	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	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	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	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	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	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	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	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	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	127 ± 3.2	4.7 ± 0.3	2.9 ± 0.5
LANMGC (n=15)	64.2 ± 2	1 ± 0	1 ± 0	1 ± 0	2.3 ± 2.2	3 ± 0	3 ± 0	5 ± 0	1.7 ± 1.6	123.7 ± 4	4.4 ± 0.3	3 ± 0.8

LANMOM (n=10)	65.9 ± 3.1	1 ± 0	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 ± 0	1.2 ± 0.4	3 ± 0	3 ± 0	5 ± 0		4 ± 0	124.2 ± 3.4	4.4 ± 0.2	2.4 ± 0.5
LANTLE (n=15)	55.6 ± 2	1 ± 0	1.7	2.5 ± 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	0.8	1.2 ± 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		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		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		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		4 ± 0	125.7 ± 3.2	4.1 ± 0.3	3.8 ± 1.9
LAOKTE (n=15)	62.9 ± 3.7	1 ± 0	1.1	1.7 ± 0.6	2.1 ± 1.1	3 ± 0	3 ± 0	5 ± 0		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	0.8	1.9 ± 0.7	1.3 ± 0.5	3 ± 0	3 ± 0	5 ± 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		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 fulfilling 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

B. 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
5 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
10 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 twentythree
percent, with LALWPA having a DHA level of five percent and an EPA level of eighteen percent
15 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-LJB2197-1qc and VC-LLM337-1qc 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 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-LJB2197-1qc. 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-LLM337-1qc 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-SICAT_p2_p-LuPXR	j-t-CaMV_p-LuChn-2	c-d6Elo(Pp_GA)	j-i-Atss18_c-d6Elo(Pp_GA2)	j-t-PvARC-p3	c-d4Des(Tc_GA)	c-3Des(Pi_GA2_SN	j-i-Atss15_c-3Des(Pi_GA2)	c-3Des(Pir_GA)	j-p-VfSBPerm3_c-3Des(Pir_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	4.0	3.8	4.0	1.8	2.0	2.0	2.0	2.1	2.0	2.1	2.2
LAMADR (n=44)	4.0	3.9	3.8	3.8	4.0	4.6	4.0	3.9	3.4	2.5	3.1	3.0	3.1
LAMQJH (n=30)	4.0	4.0	4.0	4.0	3.9	2.9	4.1	4.1	4.1	4.5	4.0	3.9	4.2
LAMQUI (n=36)	2.2	2.1	4.0	4.0	3.7	3.5	3.6	3.8	1.3	1.5	2.9	5.2	5.5
LAMRUR (n=30)	3.6	3.7	3.4	3.4	3.7	4.1	3.6	3.8	3.6	3.7	3.8	4.3	3.9
LANPSF (n=30)	4.0	4.0	3.8	3.8	3.9	4.1	3.9	4.1	3.5	1.5	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	16:3	18:0	18:1	18:2	18:3	18:4	20:0	20:1	20:2	20:3	20:4	20:5	22:0	22:1	22:4	22:5	22:6	22:7	22:8	22:9
	n-7	n-7	n-3	n-3	n-9	n-6	n-9	n-3	n-3	n-6	n-9	n-6	n-3	n-6	n-3	n-9	n-6	n-3	n-6	n-3	n-6	n-9
LANBC	4.5	0.2	0.1	4 ±	22.2	31.1	1 ±	0.2	0.8	0.6	0.5	0.1	1.8	11.4	0.3	1.7	4.4	0 ±	2.3	0.6	0.4	0.4
H (n=30)	0.6	±0	±0	0.2	1.1	0.7	0.1	±0	±0	±0	0.1	±0	0.1	0.5	±0	0.1	0.2	0	±	±	±	±
LANPM	5 ±	0.2	0 ±	3.6	26.3	35.2	0.4	0.1	0.8	0.8	1.1	0.3	1.5	7.2	0.3	1.3	2.9	0 ±	1.5	0.4	0.5	0.5
Z (n=74)	0.4	±0	0	0.4	2.1	1.6	0.1	0.1	±	±	0.4	0.2	0.2	0.8	0.1	0.1	0.5	0	±	±	±	±
LAOIKC	5.1	0.2	0.1	3.6	22.1	30.8	0.9	0.2	0.8	0.6	0.5	0.1	1.6	10.3	0.3	1.3	4 ±	0 ±	1.8	0.4	0.3	0.3
(n=29)	0.6	±0	±0	0.3	1.7	±1	0.1	±0	0.1	±0	0.1	±0	0.3	0.8	±0	0.1	0.3	0	±	±	±	±
LALHBO	4.4	0.2	0 ±	3.9	19.6	28.8	0.6	0.2	0.9	0.7	0.9	0.3	2 ±	13.4	0.3	1.3	5.7	0 ±	2.7	0.4	0.6	0.6
(n=29)	0.3	0.1	0	0.4	2.3	1.5	0.1	0.1	0.1	±0	0.3	0.1	0.2	1.9	±0	0	0.8	0	±	±	±	±
LALRCH	4.6	0.2	0.1	3.7	22.3	30.1	0.9	0.2	0.8	0.7	0.7	0.2	1.8	11.2	0.3	1.8	4.5	0 ±	2.3	0.6	0.6	0.6
(n=30)	0.2	±0	±0	0.3	1.2	±1	0.1	±0	±0	±0	0.2	0.1	0.2	0.8	±0	0.1	0.4	0	±	±	±	±
LALWP	4.4	0.2	0 ±	3.3	17.3	25.6	1 ±	0.4	0.8	0.7	0.5	0.2	1.6	14.8	0.4	2.1	7.3	0 ±	4 ±	0.5	0.3	0.3
A (n=29)	0.2	0.1	0	0.2	1.6	1.4	0.1	0.1	±0	±0	0.2	±0	0.1	1.5	±0	0	0.2	0	0.7	0.1	0.1	0.1
LALXOL	4.4	0.2	0 ±	4.4	21.4	28.4	0.4	0.3	0.9	0.9	2.5	0.5	1.3	11.6	0.3	0.8	3.5	0 ±	1.8	0.1	1 ±	1 ±
(n=15)	0.3	±0	0	0.6	1.1	1.2	0.1	±0	0.1	0.1	0.5	0.2	0.3	1.3	±0	0	0.8	0	0.4	0.1	0.2	0.2
LALXVM	4.5	0.2	0 ±	3.8	21.3	28.8	0.7	0.2	0.8	0.8	1.5	0.5	1.9	11.7	0.3	1.4	5.1	0 ±	2.3	0.4	0.8	0.8
(n=30)	±1	±0	0	0.4	1.6	1.7	0.2	0.1	±	±	±	±	0.3	1.3	±0	0	0.4	0	±	±	±	±
LALZGS	4.5	0.3	0 ±	3.4	21.8	29.4	0.6	0.2	0.8	0.7	1.3	0.3	1.8	12.1	0.3	1.2	4.7	0 ±	2.1	0.2	0.8	0.8
(n=24)	0.7	±0	0	0.5	1.8	1.4	0.1	±0	0.1	0.1	0.3	0.1	0.3	1.2	±0	0	0.6	0	0.4	0.2	0.2	0.2

LAMAD R (n=44)	3.7 ±	0.2 ± 0	0.1 ± 0	4 ±	22.3 ±	32.5 ±	0.8 ±	4.3 ±	1.9 ±	0.3 ±	0.9 ±	0.7 ±	0.5 ±	2.2 ±	1.4 ±	4.4 ±	10.8 ±	0.3 ±	0 ±	1.6 ±	4.1 ±	0 ±	2.1 ±	0.5 ±	0.3 ±
LAMQJ H (n=30)	4.9 ±	0.3 ± 0	0.1 ± 0	3.8 ±	21.9 ±	30.9 ±	1 ±	4.3 ±	2.2 ±	0.3 ±	0.8 ±	0.6 ±	0.2 ±	3 ±	1.7 ±	3.4 ±	10.7 ±	0.3 ±	0 ±	1.8 ±	4.4 ±	0 ±	2.3 ±	0.7 ±	0.2 ±
LAMQUI (n=36)	3.2 ±	0.1 ± 0	0.1 ± 0	3.1 ±	25.1 ±	26 ±	1.1 ±	4.5 ±	1.5 ±	0.2 ±	0.7 ±	0.8 ±	1.1 ±	2.2 ±	1.3 ±	4.7 ±	11.6 ±	0.2 ±	0 ±	2.4 ±	6.1 ±	0 ±	2.3 ±	0.6 ±	0.7 ±
LAMRU R (n=30)	4.9 ±	0.2 ± 0	0.2 ± 0	3.9 ±	23.4 ±	32 ±	1.1 ±	4 ±	2.3 ±	0.3 ±	0.8 ±	0.7 ±	0.2 ±	2.9 ±	1.5 ±	4 ±	9.4 ±	0.2 ±	0 ±	1.6 ±	3.7 ±	0 ±	1.9 ±	0.6 ±	0.2 ±
LANPSF (n=30)	4.7 ±	0.3 ± 0	0.1 ± 0	3.9 ±	23.2 ±	30.9 ±	0.8 ±	4.5 ±	1.7 ±	0.3 ±	0.8 ±	0.7 ±	0.5 ±	2.2 ±	1.5 ±	3.7 ±	9.9 ±	0.3 ±	0 ±	2.1 ±	4.4 ±	0 ±	2.3 ±	0.7 ±	0.3 ±
WT Kumily (n=46)	5 ±	0.4 ± 0	0.2 ± 0	2.6 ±	66.3 ±	16.8 ±	0 ±	6.1 ±	0 ±	0 ±	0.9 ±	1.2 ±	0 ±	0 ±	0 ±	0 ±	0 ±	0.5 ±	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	16:3	18:	18:	18:	18:	18:	18:	20:	20:	20:	20:	20:	20:	22:	22:	22:	22:	22:	20:						
	16:0 n-7	16:1 n-3	18: n-3	18: 9	18: 6	18: 2n-6	18: 9	18: 3	18: 3n-6	20: 1n-9	20: 2n-6	20: 3n-3	20: 4n-3	20: 5n-6	22: 0	22: 1n-9	22: 4n-6	22: 5n-6	22: 6n-3	22: 3	20: 2n-9						
LANBCH (n=1)	4.6	0.2	0.1	3.9	3	3	1.1	4.0	2.0	0.3	0.8	0.6	0.4	0.1	2.5	1.8	3.3	4	12.	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	6	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	0	4	0.8	3.4	1.9	0.2	0.7	0.5	0.5	0.1	3.0	1.6	6.5	2	12.	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	4	5	0.6	4.8	1.5	0.3	0.9	0.7	1.1	0.4	2.7	2.3	4.3	6	16.	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	5	5	0.8	4.3	1.3	0.2	0.7	0.7	1.2	0.3	2.7	2.0	4.6	3	12.	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	6	2	1.1	4.7	2.8	0.7	0.8	0.7	0.7	0.2	1.6	1.6	5.4	1	18.	0.4	0.0	2.0	0	0.0	5.7	0.5	0.4
LALXOL (n=1)	4.6	0.2	0.0	4.9	0	0	0.5	4.2	0.7	0.2	1.0	0.9	2.3	0.9	2.1	1.9	3.4	0	14.	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	3	9	0.5	4.5	0.8	0.2	0.8	0.9	2.4	0.8	2.7	2.2	4.7	3	14.	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	0	4	0.6	3.7	1.5	0.2	0.6	0.7	1.2	0.3	2.7	1.9	7.0	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	2	3	0.9	3.4	3.1	0.4	0.7	0.7	0.5	0.1	2.6	1.5	5.8	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	7	1	0.7	4.6	2.3	0.4	0.7	0.6	0.4	0.1	2.2	1.5	4.7	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	4	8	1.2	2.4	1.9	0.4	0.7	0.8	1.1	0.3	2.2	1.6	4.0	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	3	0	1.1	3.2	2.7	0.3	0.8	0.6	0.0	0.0	3.0	1.7	4.0	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	8	7	0.8	4.1	2.0	0.3	0.8	0.6	0.5	0.1	2.4	1.4	4.5	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	3	3	1.1	4.0	2.0	0.3	0.8	0.6	0.4	0.1	2.5	1.8	3.3	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: 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), Noli: 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
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	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	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	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	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	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	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	5 ± 0	7.5 ± 1.4	4 ± 0.2	129.2 ± 3.5					
WT Kumily (n=46)													34.9 ± 1.1	32.2 ± 1	

C. 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.

5 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 rcThe 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	16:3	18:0	18:1	18:2	18:2	18:3	18:3	18:4	20:0	20:1	20:2	20:3	20:3	20:4	20:4	20:5	22:0	22:1	22:4	22:5	22:5	22:6	22:6	22:7
	n-7	n-3	n-3	n-9	n-9	n-6	n-9	n-3	n-3	n-3	n-3	n-9	n-6	n-6	n-3	n-3	n-3	n-3	n-3	n-9	n-6	n-3	n-6	n-3	n-3	n-9
LANBCH (n=30)	5.1 ± 0.4 ± 0.2	0	0 ± 0	0.1	2.6 ± 26 ± 1.9	31.3 ± 1 ± 1	0.7 ± 0.1	6.2 ± 0.4	1.2 ± 0.2	0.7 ± 0	0.7 ± 0	0.7 ± 0	0.7 ± 0.1	0.7 ± 0.1	0.2 ± 1.9 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	3.4 ± 8.9 ± 0.8	0.3 ± 0	0 ± 0	1.4 ± 0.2	4.7 ± 0.4	1.5 ± 0.2	0.4 ± 0.2	0.3 ± 0.1	
LANPMZ (n=30)	4.9 ± 0.3 ± 0.1	0	0 ± 0	0.1	2.4 ± 30.7 ± 1.5 ±	32.1 ± 0.8 ± 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 ± 5.8 ± 0.5	0.3 ± 0	0 ± 0	0.8 ± 0.2	3.3 ± 0.1	1 ± 0.1	0 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	
LAOIKC (n=31)	5.2 ± 0.3 ± 0.2	0	0 ± 0	0.1	2.5 ± 26 ± 2.2	31.4 ± 1.1 ± 1.1	0.8 ± 0.1	5.5 ± 0.3	1.4 ± 0.2	0.7 ± 0	0.7 ± 0	0.7 ± 0	0.6 ± 0.1	0.1 ± 0.2	2.4 ± 1.1	1.1 ± 0.1	5.7 ± 0.5	8 ± 0.6	0.2 ± 0	1.1 ± 0.1	4.1 ± 0.1	1.2 ± 0.2	0.1 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	
LALHBO (n=29)	5.1 ± 0.2 ± 0.2	0.1	0 ± 0	0.2	2.5 ± 28 ± 6.4	30.3 ± 2 ± 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 ± 8.5 ± 2.1	0.3 ± 0	0 ± 0	0.9 ± 0.3	4.2 ± 1	1.2 ± 0.3	0 ± 0.1	0.1 ± 0.1	0.4 ± 0.1	
LALWPA (n=30)	5 ± 0.3 ± 0.4	0	0 ± 0	0.2	2.2 ± 21.3 ± 2.7 ±	28.8 ± 1.8 ± 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	2 ± 0.3	0.3 ± 0.2	0.5 ± 0.1	
LALXOL (n=29)	5.1 ± 0.3 ± 0.2	0.1	0 ± 0	0.2	2.6 ± 26.8 ± 1.9 ±	29.7 ± 0.4 ± 0.4	0.4 ± 0.1	6 ± 0.8	0.8 ± 0.2	0.2 ± 0.1	0.7 ± 0	1 ± 0.1	2.1 ± 0.7	0.5 ± 0.2	1.9 ± 0.2	1 ± 0.1	5.5 ± 8.5 ± 1.6	0.3 ± 0	0 ± 0	0.8 ± 0.1	3.8 ± 0.6	1.1 ± 0.2	0 ± 0.2	0 ± 0.2	0.8 ± 0.2	
LALXVM (n=30)	5 ± 0.3 ± 0.3	0	0 ± 0	0.1	2.4 ± 29 ± 4	29.9 ± 1.3 ± 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 ± 7.7 ± 1.3	0.3 ± 0	0 ± 0	0.8 ± 0.2	3.9 ± 0.9	1.1 ± 0.3	0 ± 0.1	0.2 ± 0.1	0.6 ± 0.2	
LALZGS (n=29)	5.1 ± 0.2 ± 0.2	0.1	0 ± 0	0.2	2.6 ± 25.1 ± 1.4 ±	30.6 ± 1.2 ± 1.2	0.6 ± 0.1	5.9 ± 0.4	1 ± 0.1	0.2 ± 0.1	0.7 ± 0	0.8 ± 0	1 ± 0.2	0.3 ± 0.1	2.3 ± 0.1	1.2 ± 0.1	5.7 ± 8.8 ± 0.5	0.2 ± 0.1	0 ± 0	0.9 ± 0.2	4.5 ± 0.1	1.2 ± 0.1	0 ± 0.1	0.1 ± 0.1	0.7 ± 0.1	
LAMADR (n=30)	5.1 ± 0.2 ± 0.1	0.1	0 ± 0	0.2	2.7 ± 25.9 ± 1.2 ±	32 ± 0.1 ± 1	0.7 ± 0.1	5.9 ± 0.4	1.3 ± 0.2	0.2 ± 0	0.7 ± 0	0.8 ± 0	0.7 ± 0.1	0.2 ± 0.1	2.1 ± 0	1 ± 0.1	4.7 ± 8 ± 0.6	0.3 ± 0	0 ± 0	1.3 ± 0.1	4.1 ± 0.3	1.2 ± 0.1	0 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	
LAMQUI (n=30)	4.7 ± 0.3 ± 0.2	0.1	0 ± 0	0.2	2.5 ± 28.8 ± 1.9 ±	29 ± 1.6 ± 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 ± 7.6 ± 0.9	0.2 ± 0	0 ± 0	2.1 ± 0.3	4.6 ± 0.7	1.4 ± 0.2	0 ± 0.2	0.1 ± 0.1	0.5 ± 0.1	

LAMRUR	5.1 ± 0.3 ±		2.6 ±	28.5	32 ±	0.8 ±	5.8 ±	1.3 ±	0.2 ±	0.7 ±	0.7 ±	0.4 ±	0 ±	2.1 ±	1 ±	3.8 ±	7.2 ±	0.2 ±		1.3 ±	3.9 ±		1.2 ±	0.3 ±	0.3 ±
(n=29)	0.3	0	0 ± 0	0.1 ± 2.3	1.9	0.2	0.8	0.2	0	0.1	0.1	0.1	0.1	0.2	0.1	0.6	0.7	0.1	0 ± 0	0.2	0.4	0 ± 0	0.2	0.1	0.1
LANPSF	5.1 ± 0.3 ±		2.6 ±	26.9	31.4	0.7 ±	6.1 ±	1.2 ±	0.2 ±	0.7 ±	0.8 ±	0.7 ±	0.2 ±	1.9 ±	1 ±	3.9 ±	7.6 ±	0.3 ±		1.7 ±	4.2 ±		1.4 ±	0.6 ±	0.4 ±
(n=28)	0.2	0.1	0 ± 0	0.1 ± 1.8	1.4	0.2	0.7	0.3	0	0	0	0.2	0.1	0.2	0.1	0.9	1.1	0	0 ± 0	0.2	0.4	0 ± 0	0.2	0.2	0.1
WT																									
Kumily	5 ±	0.4 ± 0.1 ±	2 ±	56.2	23.3	0 ±	9.5 ±	0.1 ±		0.7 ±	1.1 ±	0.1 ±		0.2 ±	0.1 ±	0.2 ±	0.4 ±	0.3 ±		0 ±	0.2 ±		0 ±		
(n=60)	0.3	0	0.1	0.1 ± 4.2	1.7	0.1	0.7	0.2	0 ± 0	0	0.1	0.1	0 ± 0	0.3	0.3	0.5	1.1	0.1	0 ± 0	0.2	0.6	0 ± 0	0.2	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

		16:1	16:3		18:1	18:2	18:3	18:3	18:4		20:1	20:2	20:3	20:3	20:4	20:4	20:5		22:1	22:4	22:5	22:5	22:6	22:4	20:2	
Event	16:0	n-7	n-3	18:0	n-9	n-6	n-9	n-3	n-3	20:0	n-9	n-6	n-3	n-6	n-3	n-6	n-3	22:0	n-9	n-6	n-3	n-6	n-3	n-3	n-9	
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 were 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	

D. 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

5 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 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 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.
LANPMZ (n=74)	c-AHAS j-t-E9-p3-2 c-d6Elo(Tp_GA) j-t-StCAT_p2-p-LuPXR j-t-CaMV_p-LuChl-2 c-d6Elo(Pp_GA) j-i-Atss18_c-d6Elo(Pp_GA2)	j-t-PvARC-p3 c-d4Des(Tc_GA) c- c3Des(Pl_GA2_SNP) j-i-Atss15_c- c3Des(Pl_GA2) c-03Des(Pir_GA) j-p-VfSBPperm3_c- c3Des(Pir_GA) j-i-Atss1_c- d5Elo(Ot_GA3) c-d4Des(Eg_GA)
	1.9	1.94
	2	2.04
	2.02	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	16:3	18:1	18:2	18:3	18:4	20:1	20:2	20:3	20:4	20:5	22:1	22:4	22:5	22:6	22:7	20:2
	16:0	16:1	16:3	18:1	18:2	18:3	18:4	20:1	20:2	20:3	20:4	20:5	22:1	22:4	22:5	22:6	22:7	20:2
LANPM	5.3	±	0.2	±	±	±	±	0.7	±	±	±	±	0.3	±	±	±	±	±
Z (n=30)	0.3	±0	0	0.8	0.1	0.6	0.3	0.1	0.2	0.1	0.6	1.3	0.1	0.4	0.5	0.6	0.1	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	16:3	18:1	18:2	18:3	18:4	20:1	20:2	20:3	20:4	20:5	22:1	22:4	22:5	22:6	22:7	20:2
	16:0	16:1	16:3	18:1	18:2	18:3	18:4	20:1	20:2	20:3	20:4	20:5	22:1	22:4	22:5	22:6	22:7	20:2
LANPMZ (n=1)	4.9	0.2	0.0	4.0	5	5	3.6	1.6	0.2	1.0	0.7	0.4	0.0	1.7	3.8	0.0	2.5	0.0

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)		8.2 ±	8.7 ±			3 ±	5 ±			5.2 ±	128.5 ±				
	42.4 ± 2	0.8	0.7	9 ± 0	8 ± 0.7	0	0	5 ± 0		0.8	8.5				

E. 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

5 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	22:4 n-3	20:2 n-9
LANPM	5.2	0.3	0.1	2.6	35.6	30.4	0.3	7.1	0.5	0.1	0.6	0.9	1.3	0.4	1.6	2.5	4.5	0.2	0.2	0.5	2.6	0.5	0.3	0.7	0.3	0.5
Z (n=56)	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	1 ±	±	±	±	0 ±	±	0 ±	0	±	±	±
Z (n=56)	0.5	0.1	0.2	0.5	2.7	1.8	0.1	0.5	0.3	0.1	± 0	0.1	0.3	0.1	0.2	0.1	0.3	0.6	0.1	0	0.3	0.3	0	0.2	0.1	0.1
LAOIKC	5.3			2.5	32.6	29.9	0.7	5.9					0.6		2.1	4.6	6.2			0.8	3.3		0.9			0.4
(n=16)	±	0.3	0 ±	±	±	±	±	1 ±	0.2	0.6	0.7	0.7	±	0 ±	±	1 ±	±	±	0.2	0 ±	±	0 ±	0 ±	±	0 ±	±
(n=16)	0.2	± 0	0	0.2	2.2	0.9	0.1	0.4	0.2	± 0	± 0	± 0	0.1	0	0.1	0.1	0.4	0.5	± 0	0	0.1	0.2	0	0.2	0.1	0.1
WT	5.1	0.4	0.1	2.1	59.1	21.3		9.5			0.6							0.1							5.1	0.4
Kumily	±	±	±	±	±	±	0 ±	±	0 ±	0 ±	1 ±	1 ±	0.1	0 ±	0 ±	0 ±	0 ±	±	0.3	0 ±	0 ±	0 ±	0 ±	0 ±	±	±
(n=83)	0.5	0.1	0.1	0.5	1.8	1.5	0.1	0.7	0.1	0	0.1	0.1	± 0	0	0	0.1	0.1	0.1	± 0	0	0.1	0.1	0	0	0.5	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 n-7	16:1 n-3	18: 0	18: 1n-9	18: 2n-6	18: 3n-9	18: 3n-6	18: 4n-3	20: 1n-9	20: 2n-6	20: 3n-3	20: 3n-6	20: 4n-3	20: 4n-6	20: 5n-3	22: 1n-9	22: 4n-6	22: 5n-3	22: 6n-3	22: 4n-3	22: 6n-3	20: 2n-9				
LANPMZ (n=1)	4.9	0.3	0.0	2.3	2	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	6	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 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=56)	43.9 ± 7.8	23.2 ± 3.2
LAOIKC (n=16)	42.6 ± 4	23.1 ± 3
WT Kumily (n=83)	45.3 ± 3.9	

F. 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
- 5 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 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 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	16:3	18:0	18:1	18:2	18:3	18:3	18:4	20:1	20:2	20:3	20:3	20:4	20:4	20:5	22:1	22:4	22:5	22:5	22:6	22:6	22:7	20:2
LANPM	4.5	0.2		2.8	34.7		0.1	6.5	0.6	0.1	0.7	0.9	0.7	0.2	1.7	1.2	2.8	4.3	0.3		0.7	2.8		0.4
Z	±	±	0 ±	±	±	33 ±	±	±	±	±	±	±	±	±	±	±	0 ±	±	±	0 ±	±	±	±	±
(n=124)	0.4	0.1	0	0.4	3.9	4.8	0.2	0.8	0.2	0.1	0.1	0.1	0.3	0.1	0.3	0.6	1.9	0.1	0.1	0	0.4	0.6	0.2	0.2
WT	4.5	0.1				22.5	0.6	7.9	0.1		0.8				0.2				0.2					
Kumily	±	±	0 ±	1.8	60 ±	±	±	±	0 ±	±	1 ±	0.1	0 ±	0 ±	0 ±	0 ±	0 ±	0 ±	0 ±	0 ±	0 ±	0 ±	0 ±	0 ±
(n=56)	0.5	0.1	0	± 1	3.8	3.6	1.5	2.2	0.1	0	0.2	0.4	± 0	0	0.8	0	0.1	0	0.1	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	16:3	18:0	18:1	18:2	18:3	18:3	18:4	20:1	20:2	20:3	20:3	20:4	20:4	20:5	22:1	22:4	22:5	22:5	22:6	22:6	22:7	20:2
LANPMZ (n=1)	5.6	0.3	0.0	2.7	8	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

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

Example 3: 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 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-LLM391-2qcz 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-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 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-LJB2755-2qcz rc	1196	Delta-12 desaturase from <i>Phytophthora sojae</i>
c-d5Des(Tc_GA2)	VC-LJB2755-2qcz rc	1319	Delta-5 desaturase from <i>Thraustochytrium</i> sp. ATCC21685
c-d6Des(Ot_febit)	VC-LJB2755-2qcz rc	1370	Delta-6 desaturase from <i>Ostreococcus tauri</i>
c-d6Elo(Tp_GA2)	VC-LJB2755-2qcz rc	818	Delta-6 elongase from <i>Thalassiosira pseudonana</i>
c-o3Des(Pi_GA2)	VC-LJB2755-2qcz rc	1085	Omega-3-desaturase from <i>Phytophthora infestans</i>
c-o3Des(Pir_GA)	VC-LJB2755-2qcz rc	1091	Omega-3 desaturase from <i>Pythium irregulare</i>
c-d5Elo(Ot_GA3)	VC-LLM391-2qcz rc	902	Delta-5 elongase from <i>Ostreococcus tauri</i>

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-d4Des(Eg_GA)	VC-LLM391-2qcz rc	1625	Delta-4 desaturase from <i>Euglena gracilis</i>
c-d4Des(Tc_GA)_T564 G	VC-LLM391-2qcz rc	1559	Delta-4 desaturase from <i>Thraustochytrium</i> spp.

A. 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.

- 5 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
- 10 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
- 15 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 select 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_j-iAtss15 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-03Des(Pi_GA)	j-i-Atss18_c-03Des(Pi_GA2)	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-i-Atss1_c-d6Elo(Tp_GA2)	c-03Des(Pir_GA)	j-p-LuPXR_j-iAtss15	c-d4Des(Eg_GA)	j-t-PVARC-p-LuCl	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)	
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)		
LAQKQS (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)	
LAPAUJ (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-LJB2755-2qc rc and VC-LLM391-2qc 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.

Category of T1 plants	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_j-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.									
	c-AHAS	c-03Des(Pl_GA)	j-i-Atss18_c-03Des(Pl_GA2)	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-i-Atss1_c-d6Elo(Tp_GA2)	c-03Des(Pir_GA)	j-p-LuPXR_j-Atss15	c-d4Des(Eg_GA)	j-i-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	16:3	18:0	18:1	18:2	18:3	18:4	20:1	20:2	20:3	20:4	20:5	22:1	22:4	22:5	22:6	22:7	20:2
	n-7	n-3	n-3	n-9	n-9	n-6	n-3	n-3	n-9	n-6	n-3	n-6	n-3	n-9	n-6	n-3	n-3	n-3	n-9
LAPCTC (n=11)	5.1			28.2	28.9	1.3		0.8	0.9	0.7		2.2	1.8	1.2	10.9	0.6	3.5		0.2
	±	0.3	0 ±	±	±	±	5 ±	±	±	±	0.2	0.1	±	±	±	±	±	0 ±	±
	0.2	±0	0	5.5	1.6	0.2	0.2	0.5	0.1	0.1	±0	0.4	0.5	0.4	2.1	0.2	1.1	0	0.1
LAPCS	5.1			27.9	28.8	1.3	4.9	3.2	0.9	0.7		2.1	1.7	1.3	11.4	0.6			0.2
	±	0.3	0 ±	±	±	±	±	±	±	±	0.1	±	±	±	±	±	±	0 ±	±
C (n=11)	0.1	±0	0	4.2	2.3	0.2	0.4	0.6	0.2	0.1	0.1	±0	0.4	0.5	0.3	0.1	0.3	0.1	0.1
LAPYTJ	5.9	0.3		3.3	26.5	0.9	4.9	2.1	0.4	0.8		2.2	1.3	2.9	8.4	0.9	2.8		0.1
	±	±	0 ±	±	±	±	±	±	±	0.6	0 ±	0 ±	±	±	±	±	±	0 ±	±
S (n=15)	0.3	0.1	0	1.9	1.6	0.2	0.7	0.3	0.1	0.1	0	0	0.3	0.3	0.5	0	0.3	0.5	0.1
LAQKQ	5.3	0.3		3.2	29.7	1.1	5.3	2.6	0.6	0.9	0.7	1.7	1.3	1.1	8.8	0.5	2.4		0.1
	±	±	0 ±	±	±	±	±	±	±	±	±	0 ±	±	±	±	±	±	0 ±	±
S (n=15)	0.3	0.1	0	0.6	±8	0.3	0.5	0.6	0.1	0.2	0.1	0	0.6	0.5	0.2	0.2	0.3	0.6	0.1
LAPARV	5.3			3.5	26.5	0.7	5.4	1.9	0.4	0.9		1.9	1.4	1.7	8.4	0.6	2.4		
	±	0.3	0 ±	±	±	±	±	±	±	0.7	0 ±	0 ±	±	±	±	±	±	0 ±	±
S (n=15)	0.2	±0	0	0.3	1.9	1.2	0.3	0.2	0.1	0.1	±0	0	0.3	0.2	0.3	0.2	0.5	0	0.2
APCMY	6.2	0.3		2.4	26.7	0.5	6.5	1.9	0.4	0.7	0.1		1.8	1.6		0.6	2.6		
	±	±	0 ±	±	±	±	±	±	±	1 ±	±	0 ±	±	±	±	±	±	0 ±	±
S (n=15)	1.3	0.1	0	1.4	8.6	14.5	0.3	1.4	0.4	0.2	0.1	0	0.5	0.4	0.4	0.3	0.8	0	0.2
LAPBO	5.1	0.2		2.9	27.3	34.9	5.6	1.9		0.8			1.4	1.6	7.8		3.1		
	±	±	0 ±	±	±	±	±	±	0.4	±	0.7	0 ±	±	±	±	0.3	±	0 ±	±
S (n=15)	0.6	0.1	0	0.2	2.6	0.9	0.6	0.2	±0	0.1	±0	0	0.3	0.2	0.1	±0	0.4	0.7	±0

LAPAW A (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	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	± 0.2
LAPBY W (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	1.9 ± 0.2	1.4 ± 0.2	1.6 ± 0.1	7.9 ± 0.8	0.4 ±0	0 ± 0	0.5 ± 0.2	2 ± 0.4	0 ± 0	1 ± 0.2	± 0.2
LAPQE P (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	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	± 0.1
LAODD N (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	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	± 0.1
LAPAX (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	0.8 ± 0.1	0.7 ± 0.1	0.1 ± 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	± 0.1
LAPZOJ (n=10)	5.6 ± 0.6	0.3 ±0	0 ± 0	34.1 ± 3 ±	33.5 ± 6.8	33.5 ± 2.8	0.6 ± 0.2	6.5 ± 0.6	1.3 ± 0.3	0.3 ±0	0.8 ± 0.1	0.7 ± 0.1	0 ± 0	1.6 ± 0.4	1.1 ± 0.3	1.1 ± 0.2	5.5 ± 1.5	0.2 ±0	0 ± 0	0.4 ± 0.2	2.2 ± 0.6	0 ± 0	0.8 ± 0.3	± 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	16:3	18:	18:	18:	18:	18:	18:	18:	18:	20:	20:	20:	20:	20:	20:	20:	22:	22:	22:	22:	22:	22:	20:	
	16:0 n-7	18:	18:	18:	18:	18:	18:	18:	18:	18:	18:	20:	20:	20:	20:	20:	20:	20:	22:	22:	22:	22:	22:	22:	20:	
LAPCTC (n=1)	5.1	0.3	0.0	2.7	2	7	1.3	4.8	2.6	0.5	0.8	0.6	0.3	0.1	2.4	1.9	1.9	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	8	8	1.7	5.2	3.5	1.0	0.8	0.6	0.3	0.1	2.5	2.7	1.0	1	0.3	0.0	0.6	5.3	0.0	1.9	0.4	0.3
LAPYTYJ (n=1)	5.5	0.3	0.0	3.0	0	1	1.1	4.9	2.4	0.5	0.8	0.6	0.0	0.0	2.4	1.7	2.8	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	4	3	1.4	4.7	3.9	0.9	0.8	0.6	0.0	0.0	2.2	1.8	1.5	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	3	2	0.6	5.6	2.0	0.5	0.8	0.6	0.1	0.0	2.0	1.5	2.3	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	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	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	5	7	0.7	6.7	2.3	0.6	0.7	0.7	0.1	0.1	2.2	1.8	2.0	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	2	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	4	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	6	9	1.5	5.7	1.3	0.4	0.7	0.8	0.3	0.1	1.9	1.6	1.0	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	1	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
LAPAU (n=1)	5.5	0.3	0.0	3.1	6	6	0.7	5.4	1.9	0.4	0.8	0.6	0.1	0.0	2.4	1.7	2.3	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	8	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 fulfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Categor y of T1 plants	16:1 16:3		18:1 18:2		18:2 18:3		18:3 18:4		20:1 20:2		20:2 20:3		20:3 20:4		20:4 20:5		22:1 22:4		22:4 22:5		22:5 22:6		22:6 22:7	
	16:0 n-7	16:3 n-3	18:0 n-9	18:2 n-6	18:3 n-9	18:3 n-3	18:4 n-3	20:0 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 n-9	22:4 n-6	22:5 n-3	22:6 n-6	22:6 n-3	22:7 n-3	22:7 n-9			
sc (n=143)	5.4	0.3	2.9	29.6	32.8	0.8	5.6	1.9	0.4	0.8	0.7	0.1		1.5	1.5	0.3		0.6	2.6		1.1	0.4	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																																				
y of T1																																				
plants	16:0	n-7	16:3	18:0	n-9	18:2	n-6	18:3	n-9	18:3	n-3	18:3	n-6	18:4	20:0	n-9	20:1	n-6	20:2	n-3	20:3	n-6	20:4	n-3	20:4	n-6	20:5	22:0	n-9	22:1	22:4	22:5	22:6	22:6	22:7	20:2
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	0.0	0.4	3.3	0.0	1.0	0.2	0.3									

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 were 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), Noli: 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
LAPAUJ (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 fulfilling 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), 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	67.9 ±	1 ±	1.1 ±	1 ±	1.7 ±	3 ±	3 ±	5 ±	1.1 ±	3.9 ±	127.9 ±	3.6 ±	3.5 ±	36 ±	28.5 ±
for oil and protein)	3	0	0.5	0.2	1.1	0	0	0	0.7	0.3	4	0.4	1.1	1.4	0.8

B. 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.

- 5 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 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. 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-03Des(Pl_GA)	j-i-Atss18_c-03Des(Pl_GA2)	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-i-Atss1_c-d6Elo(Tp_GA2)	c-03Des(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)
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 were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1	16:2	16:3	18:0	18:1	18:2	18:3	18:4	20:0	20:1	20:2	20:3	20:4	20:5	22:0	22:1	22:4	22:5	22:6	22:7	22:8	22:9
LAPBO	5.3	0.3	0.2	0.2	3.4	26.8	35.6	0.6	6.1	1.6	0.4	0.8	0.1	1.8	1.4	1.4	0.3		3.2	1.3	0.6	0.1	0.1
W	±	±	±	±	±	±	±	±	±	±	±	±	0 ±	±	±	±	0 ±	1 ±	±	±	±	±	±
(n=54)	0.3	0.1	0.1	0.1	0.2	1.9	2.1	0.1	0.1	0.2	0.1	0.1	0	0.3	0.3	0.1	0	0.1	0.5	0	0.3	0.2	0.1
LAODD	5.4	0.3	0.2	0.2	3.5	27.7	35.4	0.6	6.5	1.4	0.8	0.1	0.1	1.8	1.4	0.3	0.8	0.8		1.1	0.6	0.1	0.1
N (n=63)	0.4	0.1	0.1	0.1	0.4	2.4	1.8	0.1	0.8	0.2	±0	0.1	0.1	0.2	0.2	0.1	0	0.1	0.3	0	0.3	0.1	0.1
WT					2.6	66.3	16.8		6.1														
Kumily	5 ±	0.4	0.2	±	±	±	0 ±	±	0 ±	0 ±	0.9	1.2	0 ±	0 ±	0 ±	0.5	0 ±	0 ±	0 ±	0 ±	0 ±	0 ±	0 ±
(n=46)	0.1	±0	±0	±0	0.1	1.7	1.3	0	0.4	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	16:3	18:	18:	18:	18:	18:	18:	20:	20:	20:	20:	20:	20:	20:	20:	22:	22:	22:	22:	22:	22:	20:
			n-7	0	9	6	9	3	6	3	0	9	6	3	0	9	6	3	0	9	6	3	6	3
LAPBOW					24.	34.																		
(n=1)	5.7	0.3	0.2	3.3	1	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
LAODDN					25.	34.																		
(n=1)	5.3	0.3	0.2	3.1	1	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

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	34.8 ±	
(n=54)	2.9	30.8 ± 2.3
LAODDN		
(n=63)	36.6 ± 3	28.6 ± 2.4
WT Kumily	34.9 ±	32.2 ± 1
(n=46)	1.1	

C. 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-

- 5 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
- 10 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	16:3	18:0	18:1	18:2	18:3	18:4	20:0	20:1	20:2	20:3	20:3	20:4	20:4	20:5	22:0	22:1	22:4	22:5	22:5	22:6	22:4	22:2
	n-7	n-3	n-3	n-9	n-9	n-6	n-3	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-3	n-9	n-6	n-3	n-6	n-3	n-3	n-9
LAPBOW (n=31)	5.3	0.4		2.4	31.9		0.6	7.7	1.4	0.4			0.1	1.5	0.9	1.6	5.5	0.2		0.9	3.1		0.8	0.4
	±	±	0 ±	±	±	33.3	±	±	±	±	0 ±	±	±	±	±	±	±	0 ±	±	±	0 ±	±	±	±
	0.2	0.1	0	0.1	1.6	±1	0.1	0.5	0.2	0.1	±0	0	0.1	0.1	0.1	0.2	0.5	0.1	0	0.1	0.3	0	0.1	0.1
LAODDN (n=31)	5.1			2.5	32.1	33.4	0.6	7.9	1.3	0.4			0.2	0.1	0.8	1.4	5.7			0.8	3.1		0.8	0.4
	±	0.4	0.1	±	±	±	±	±	±	±	0.7	±	±	±	±	±	±	0.3	0 ±	±	±	0 ±	±	0.1
	0.2	±0	±0	0.2	2.3	1.4	0.1	0.5	0.2	0.1	±0	0.1	0.1	0.1	0.1	0.2	0.7	±0	0	0.1	0.4	0	0.1	±0
WT			0.1		56.2	23.3		9.5	0.1		1.1	0.1		0.2	0.1	0.2	0.4	0.3			0.2			
Kumily (n=60)	5 ±	0.4	±	2 ±	±	±	0 ±	±	0 ±	0.7	±	±	±	±	±	±	±	±	0 ±	±	±	0 ±	0 ±	
	0.3	±0	0.1	0.1	4.2	1.7	0.1	0.7	0.2	0	0.1	0.1	0	0.3	0.3	0.5	1.1	0.1	0	0.2	0.6	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: 1n- 7	16: 3n- 3	18: 0	18: 1n- 9	18: 2n- 6	18: 3n- 9	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: 6n- 3	22: 4n- 3	20: 2	20: 9						
LAPBOW (n=1)	5.5	0.4	0.1	2.3	3	30. 32.	3	9	0.6	0.7	0.6	0.5	1.8	7.6	3n- 3	4n- 3	0.6	0.7	1.5	0.4	0.6	0.6	0.1	0.0	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	1	30. 33.	3	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	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 were 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=60)	38.7 ± 1.1	

D. 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.

- 5 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 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. 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.																	
LAODDN (n=30)	1.92	c-AHAS	c-03Des(Pi_GA)	j-i-Atss18_c-	j-i-Atss14_c-	c-d6Elo(Tp_GA)	j-i-Atss1_c-	c-03Des(Pir_GA)	j-p-LuPXR_i-Atss15	c-d4Des(Eg_GA)	j-t-PVARC-p-LuChl	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)	1.9		
										1.8							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	16:3	18:0	18:1	18:2	18:3	18:4	20:0	20:1	20:2	20:3	20:3	20:4	20:4	20:5	22:0	22:1	22:4	22:5	22:5	22:6	22:7	20:2
LAODD	±	0.3	0 ±	±	±	±	±	±	±	±	±	0 ±	±	±	±	±	±	0 ±	1 ±	±	0 ±	±	±	0 ±
N (n=30)	0.5	± 0	0	0.7	2.5	4.1	5.1	0.7	0.3	0.1	0.1	0	0.2	0.5	0.2	0.8	0.2	0	0.3	0.4	0	0.4	0.1	0

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	16:1	16:3	18:0	18:1	18:2	18:3	18:4	20:0	20:1	20:2	20:3	20:3	20:4	20:4	20:5	22:0	22:1	22:4	22:4	22:5	22:5	22:6	22:6	22:7	20:2
LAODD (n=1)	5.2	0.2	0.0	3.7	2	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 were 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)

Event	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	Nol	PH
LAODDN	43.5 ±										
(n=30)	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

E. 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	16:3	18:0	18:1	18:2	18:3	18:4	20:0	20:1	20:2	20:3	20:4	20:5	22:0	22:1	22:4	22:5	22:6	22:7	22:8	22:9
LAPBOW (n=16)	5.3	0.4	0.2	2.4	36.1	31.3	7.9	1.3	0.4	0.6	0.7	0.1	0.8	1.4	4.5	0.2	0.2	0.7	2.7	0.7	0.3	0.1
LAODDN (n=47)	5.5	0.5	0.2	2.7	36.8	30.3	7.9	1.3	0.4	0.6	0.7	0.1	0.8	1.2	4.7	0.2	0.2	0.4	2.6	0.6	0.2	0.1
WT	5.1	0.4	0.1	2.1	59.1	21.3	9.5	0.6	0.1	0.1	0.1	0.1	0.1	0.2	0.7	0.7	0.3	0.3	0.3	0.2	0.1	0.1
Kumily (n=83)	0.5	0.1	0.1	0.5	1.8	1.5	0.7	0.1	0	0.1	1	0.1	0.1	0.1	0.1	0.3	0.1	0.1	0.1	0	0.5	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: 1n- 7	16: 3n- 3	18: 1n- 9	18: 2n- 6	18: 3n- 3	18: 4n- 3	20: 1n- 9	20: 2n- 6	20: 3n- 3	20: 4n- 3	20: 5n- 3	20: 6n- 3	22: 1n- 9	22: 4n- 6	22: 5n- 3	22: 6n- 3	22: 4n- 3	22: 6n- 3	20: 2n- 9						
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
WT Kumily (n=83)	45.3 ± 3.9	

F. 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.

5 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	16:3	18:0	18:1	18:2	18:3	18:4	20:0	20:1	20:2	20:3	20:4	20:5	22:0	22:1	22:4	22:5	22:6	22:7	22:8	22:9	
LAODDN (n=142)	4.7	0.2		2.8	34.8	33.8	0.3	1.1	0.2	0.7	0.8	0.1	1.2	1.1	1.4	4.5	0.3	0.5	2.9	0.1	0.7	0.1	0.1
	±	±	0 ±	±	±	±	7.7	±	±	±	±	±	±	±	±	0 ±	±	±	±	±	±	±	
	0.4	0.1	0	0.4	3.5	4.8	0.3	0.4	0.2	0.1	0.1	0.1	1.2	0.4	1.9	0.1	0	0.4	0.5	0.2	0.2	0.1	
WT	4.5	0.1				22.5	0.6	7.9	0.1	0.8			0.2		0.2								
Kumily (n=56)	±	±	0 ±	1.8	60 ±	±	±	0 ±	±	±	1 ±	0 ±	0 ±	0 ±	±	0 ±	0 ±	0 ±	0 ±	0 ±	0 ±	0 ±	
	0.5	0.1	0	± 1	3.8	3.6	1.5	2.2	0.1	0	0.2	0	0.8	0	0	0.1	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	16:3	18:0	18:1	18:2	18:3	18:4	20:0	20:1	20:2	20:3	20:4	20:5	22:0	22:1	22:4	22:5	22:6	22:7	22:8	22:9
LAODDN (n=1)	4.5	0.2	0.0	2.6	3	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	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

Example 4: 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 enzymes for EPA and DHA synthesis	Length (bp)	Enzymatic function and source of encoded protein
c-d12Des(Ps_GA2)	1197	Delta-12 desaturase from <i>Phytophthora sojae</i>
c-d6Des(Ot_febit)	1371	Delta-6 desaturase from <i>Ostreococcus tauri</i>
c-d6Elo(Pp_GA2)	873	Delta-6 elongase from <i>Physcomitrella patens</i>
c-d6Elo(Tp_GA2)	819	Delta-6 elongase from <i>Thalassiosira pseudonana</i>
2 copies of c-d5Des(Tc_GA2)	1320	Delta-5 desaturase from <i>Thraustochytrium</i> sp. ATCC21685
c-o3Des(Pi_GA2)	1086	Omega-3-desaturase from <i>Phytophthora infestans</i>
2 copies of c-o3Des(Pir_GA)	1092	Omega-3 desaturase from <i>Pythium irregulare</i>
c-d5Elo(Ot_GA3)	903	Delta-5 elongase from <i>Ostreococcus tauri</i>
c-d4Des(PI_GA)2	1338	Delta-4 desaturase from <i>Pavlova lutheri</i>
c-d4Des(Tc_GA3)	1560	Delta-4 desaturase from <i>Thraustochytrium</i> sp.

Fatty acid Profile in selected T1 seed batches

A. 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

- 5 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
- 10 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
- 15 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
- 20 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
- 25 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 were measured per event. The T1 plants underwent a selection from 250 segregating T1 seedlings, using zygosity 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 one 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 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-L TM593-1qcz 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-03Des(Pl_GA)2	J-p-BnSETL-v1_c-03Des(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=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
LBFBV (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
LBFPC (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
LBFHQ (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
LBFABZ (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
LBFKWK (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
LBFGLZ (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
LBFIMG (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
LBFNR (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
LBFGLJA (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
LBFIU (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: 1.8)	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: 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: 2)	(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: 2)	(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: 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: 2)	(T0: 3.5)	(T0: 1.8)		(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: 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: 1)	(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: 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: 1)	(T0: 3.6)	(T0: 1.7)		(T0: 1.5)	(T0: 2)	(T0: 1.6)	(T0: 3.6)	(T0: 1.5)	partial double copy
LBFPZPJ (n=0)	(T0: 2)		(T0: 1.8)	(T0: 1.9)	(T0: 1)	(T0: 1)	(T0: 3.5)	(T0: 1)		(T0: 2.3)	(T0: 3)	(T0: 2.4)	(T0: 3.5)	(T0: 2.4)	partial double copy
LBFNSS (n=0)	(T0: 1.1)		(T0: 1)	(T0: 1.2)	(T0: 1.1)	(T0: 1)	(T0: 2.5)	(T0: 1.8)		(T0: 2.3)	(T0: 2.5)	(T0: 2)	(T0: 2.5)	(T0: 2)	partial double copy
LPGAOR (n=0)	(T0: 1.1)		(T0: 1.8)	(T0: 2)	(T0: 1.7)	(T0: 1)	(T0: 2.9)	(T0: 1.8)		(T0: 1.8)	(T0: 1.1)	(T0: 1)	(T0: 2.9)	(T0: 1.1)	partial double copy
LBZOE (n=0)	(T0: 1)		(T0: 1)	(T0: 1.1)	(T0: 1.1)	(T0: 1)	(T0: 2.8)	(T0: 1)		(T0: 1.1)	(T0: 2.1)	(T0: 1.7)	(T0: 2.8)	(T0: 1.8)	partial double copy

LBFDJS (n=0)	(T0: 1)		(T0: 1.2)	(T0: 1)	(T0: 1)	(T0: 2.9)	(T0: 1.2)				(T0: 0.9)	(T0: 2.2)	(T0: 1.9)	(T0: 2.9)	(T0: 1.9)	partial double copy
LBFDKC (n=0)	(T0: 1)		(T0: 1)	(T0: 1.2)	(T0: 1.8)	(T0: 2.5)	(T0: 2.2)				(T0: 1.7)	(T0: 1.6)	(T0: 1.4)	(T0: 2.5)	(T0: 0.6)	partial double copy
LBFDKA (n=5)	4.2 (T0: 1.6)	3.8	5.4 (T0: 2.6)	5.2 (T0: 3.1)	(T0: 2.4)	10.1 (T0: 5.6)	4.8 (T0: 3.1)	6	5.7	5.7 (T0: 3.3)	5.7 (T0: 3.9)	5.9 (T0: 2.9)	10.1 (T0: 5.6)	5.7 (T0: 3.5)	5.7 (T0: 3.5)	partial triple copy
LBFLGH (n=0)	(T0: 0.9)		(T0: 1)	(T0: 1.1)	(T0: 1.1)	(T0: 2.9)	(T0: 2.3)				(T0: 2.5)	(T0: 3)	(T0: 2.1)	(T0: 2.9)	(T0: 2.5)	partial triple copy
LBFNUS (n=0)	(T0: 1.1)		(T0: 1)	(T0: 0.9)	(T0: 1.4)	(T0: 3.9)	(T0: 2.2)				(T0: 2.3)	(T0: 3.1)	(T0: 2.3)	(T0: 3.9)	(T0: 3.3)	partial triple copy
LBFLCH (n=0)	(T0: 1.8)		(T0: 2.1)	(T0: 2.1)	(T0: 2.3)	(T0: 5)	(T0: 3.1)				(T0: 2.3)	(T0: 5.3)	(T0: 3.3)	(T0: 5)	(T0: 3.4)	partial triple copy
LBFZMI (n=0)	(T0: 3.3)		(T0: 2.1)	(T0: 2)	(T0: 1.5)	(T0: 4.6)	(T0: 2.2)				(T0: 1.7)	(T0: 1.9)	(T0: 2.9)	(T0: 4.6)	(T0: 2.6)	partial triple copy
LBFIDT (n=7)	3.8 (T0: 1.5)	4	4.3 (T0: 2.2)	4.2 (T0: 1.9)	(T0: 1.5)	8.5 (T0: 4.5)	4 (T0: 2.5)	4.3	4.3	4.3 (T0: 2.5)	3.8 (T0: 3.8)	4.2 (T0: 2.4)	8.5 (T0: 4.5)	3.9 (T0: 2.4)	3.9 (T0: 2.4)	partial triple copy
LBFAZW (n=0)	(T0: 1.8)	(T0: 2)	(T0: 2.1)	(T0: 1.8)	(T0: 1.4)	(T0: 4.5)	(T0: 1.7)				(T0: 2.5)	(T0: 3.3)	(T0: 2.4)	(T0: 4.5)	(T0: 2.5)	partial triple copy
LBFBBI (n=0)	(T0: 2)	(T0: 2)	(T0: 1.9)	(T0: 1.5)	(T0: 1.6)	(T0: 4.2)	(T0: 1.9)				(T0: 1.9)	(T0: 2.7)	(T0: 1.9)	(T0: 4.2)	(T0: 1.7)	partial triple copy
LBFAZW (n=0)	(T0: 1.8)	(T0: 2)	(T0: 2.1)	(T0: 1.8)	(T0: 1.4)	(T0: 4.5)	(T0: 1.7)				(T0: 2.5)	(T0: 3.3)	(T0: 2.4)	(T0: 4.5)	(T0: 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 (arithmetically 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, LFPFNC), while other positions on the T-DNA clearly indicate a double copy insertion that was either inserted in one locus (e.g. event LBFNC), 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 regions 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 copies, tested using Chi-Square test
LBFDDG	073 : 122 : 053 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A07 : 076 : 119 : 053 : 000 : 000 : 000 : : 000 : 000 : 000 : 000 : 000 : 000	073 : 123 : 052 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFCKN	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
LBFHIE	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
LBFLLI	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
LBFNPF	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
LBFBVA	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 : 117 : 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 : 041 : 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
LBFBAF	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
LBFPRF	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 : 000	A13 : 019 : 040 : 060 : 067 : 044 : 013 : 003 : 000 : 000 : 000 : 000 : 000 : 000	019 : 040 : 060 : 072 : 039 : 013 : 003 : 000 : 000 : 000 : 000 : 000 : 000	double locus, 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 : 000	007 : 031 : 044 : 017 : 003 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFDJG	026 : 078 : 088 : 036 : 014 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 029 : 072 : 085 : 035 : 017 : 001 : 000 : 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 : 015 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A11 : 018 : 061 : 096 : 059 : 015 : 000 : 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 : 015 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 016 : 058 : 087 : 067 : 016 : 000 : 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 : 014 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 008 : 058 : 094 : 062 : 012 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	008 : 058 : 095 : 061 : 012 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFDHG	022 : 064 : 084 : 037 : 004 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A07 : 023 : 062 : 090 : 033 : 005 : 000 : 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 : 000	059 : 126 : 051 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFBAB	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 : 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 : 051 : 073 : 118 : 157 : 149 : 036 : 023 : 002 : 001 : 000 : 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 : 011 : 030 : 023 : 008 : 000 : 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 : 001 : 000 : 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 : 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 : 000	029 : 146 : 214 : 136 : 055 : 007 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFLGC	064 : 121 : 057 : 005 : 002 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 013 : 034 : 059 : 060 : 049 : 026 : 006 : 000 : 002 : 000 : 000 : 000 : 000	013 : 068 : 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 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	064 : 156 : 232 : 110 : 022 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus

LBFZPJ	060 : 000 : 129 : 000 : 059 : 000 : 001 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 013 : 032 : 042 : 066 : 059 : 029 : 008 : 000 : 000 : 000 : 000 : 000 : 000	013 : 032 : 044 : 065 : 049 : 036 : 010 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFNSS	155 : 359 : 142 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 044 : 094 : 127 : 183 : 113 : 058 : 033 : 003 : 002 : 000 : 000 : 000 : 000	042 : 163 : 268 : 141 : 039 : 003 : 001 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBGAOR	149 : 302 : 144 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A04 : 040 : 158 : 274 : 103 : 019 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	161 : 310 : 120 : 003 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFZOE	026 : 060 : 035 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 010 : 025 : 051 : 026 : 008 : 002 : 000 : 000 : 000 : 000 : 000 : 000 : 000	010 : 025 : 049 : 031 : 006 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFDJS	048 : 131 : 060 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 008 : 043 : 090 : 074 : 026 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	008 : 043 : 089 : 075 : 025 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFDKC	001 : 109 : 133 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A07 : 001 : 000 : 109 : 008 : 126 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	001 : 108 : 132 : 004 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	no wildtypes
LBFDKA	015 : 072 : 101 : 052 : 008 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 001 : 012 : 041 : 054 : 076 : 045 : 018 : 001 : 000 : 000 : 000 : 000 : 000	001 : 012 : 041 : 053 : 069 : 050 : 019 : 003 : 000 : 000 : 000 : 000 : 000	triple locus
LBFLGH	061 : 137 : 051 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 015 : 034 : 052 : 068 : 039 : 026 : 012 : 002 : 001 : 000 : 000 : 000 : 000	014 : 033 : 053 : 060 : 045 : 030 : 012 : 001 : 000 : 000 : 000 : 000 : 000	triple locus
LBFNUS	054 : 137 : 058 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 003 : 016 : 048 : 054 : 034 : 008 : 003 : 000 : 000 : 000 : 000 : 000 : 000	004 : 020 : 071 : 081 : 060 : 010 : 003 : 000 : 000 : 000 : 000 : 000 : 000	triple locus
LBFLCH	019 : 077 : 090 : 052 : 011 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 019 : 039 : 013 : 006 : 050 : 050 : 021 : 008 : 019 : 013 : 009 : 002 : 000	019 : 039 : 013 : 041 : 063 : 027 : 026 : 013 : 007 : 000 : 000 : 000 : 000	triple locus
LBFZMI	017 : 016 : 036 : 038 : 023 : 027 : 005 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 017 : 043 : 061 : 033 : 008 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	007 : 015 : 039 : 051 : 034 : 014 : 002 : 000 : 000 : 000 : 000 : 000 : 000	triple locus
LBFIDT	069 : 000 : 131 : 000 : 045 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 015 : 033 : 058 : 064 : 048 : 015 : 012 : 002 : 000 : 000 : 000 : 000 : 000	003 : 018 : 050 : 056 : 061 : 031 : 019 : 008 : 000 : 000 : 000 : 000 : 000	triple locus, isolation of one locus

LBFAZW	013 : 065 : 109 : 041 : 006 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 004 : 021 : 073 : 086 : 040 : 010 : 001 : 000 : 000 : 000 : 000 : 000 : 000	003 : 018 : 069 : 079 : 048 : 013 : 002 : 000 : 001 : 000 : 000 : 000 : 000	trippe! locus
	005 : 057 : 139 : 040 : 004 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 009 : 030 : 074 : 066 : 051 : 014 : 001 : 000 : 000 : 000 : 000 : 000 : 000	005 : 065 : 127 : 044 : 004 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	trippe! locus
LBFAZW	013 : 065 : 109 : 041 : 006 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 004 : 021 : 073 : 086 : 040 : 010 : 001 : 000 : 000 : 000 : 000 : 000 : 000	003 : 018 : 069 : 079 : 048 : 013 : 002 : 000 : 001 : 000 : 000 : 000 : 000	trippe! locus

Table 138: Fatty acid profiles of T2 seeds harvested from T1 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 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	16:3	18:0	18:1	18:2	18:2	18:3	18:3	18:4	20:0	20:1	20:2	20:3	20:3	20:4	20:4	20:5	22:0	22:1	22:4	22:5	22:5	22:6	22:4	22:4	20:2
	16:0	16:1	16:3	18:0	18:1	18:2	18:2	18:3	18:3	18:4	20:0	20:1	20:2	20:3	20:3	20:4	20:4	20:5	22:0	22:1	22:4	22:5	22:5	22:6	22:4	22:4	20:2
LBFDGG (n=50)	4.8 ± 0.2 ± 0.2 0	0 ± 0 0 0 ± 0	2.9 ± 0.3 ± 1.5 ± 3 ± 28.3 35.2	2.9 ± 0.3 ± 1.5 ± 3 ± 28.3 35.2	28.3 ± 1.5 ± 1 28.3 35.2	35.6 ± 0.8 ± 1 35.6 0.8 ± 1	0.8 ± 0.1 0.1 0.1	1.9 ± 0.4 ± 0.1 0.2 0.1	4.5 ± 0.3 0.3 0.3	2 ± 0.5 ± 0.3 ± 0.2 2 0.3 0.2	0.8 ± 0.1 0 0	0.7 ± 0.1 0 0	0.1 ± 0.1 0 0	0.1 ± 0.1 0 0	2.7 ± 1.5 ± 0.2 0.2 0.1	2.2 ± 0.3 0.3 0.3	7.7 ± 0.4 ± 0.8 0.8	0.4 ± 0.4 ± 0 0	0 ± 0 0 ± 0	0.1 ± 0.1 0 ± 0	0.3 ± 0.1 0.3 ± 0.1	1.9 ± 0.2 1.9 ± 0.2	0.2 ± 0.3 0 0.3	1.4 ± 0.3 ± 0.2 ± 0.3 0.1 0.1	0.3 ± 0.1 0.3 ± 0.1	0.3 ± 0.1 0.3 ± 0.1	0.2 ± 0.2 ± 0.2 ± 0.2 ±
LBFGKN (n=50)	4.6 ± 0.1 ± 0.3 0	0 ± 0 0 0 ± 0	3 ± 0.2 ± 1.6 ± 0.7 3 ± 28.3 35.2	3 ± 0.2 ± 1.6 ± 0.7 3 ± 28.3 35.2	28.3 ± 1.6 ± 0.7 28.3 35.2	35.2 ± 0.8 ± 1 35.2 0.8 ± 1	0.8 ± 0.1 0.1 0.1	1.9 ± 0.4 ± 0.1 0.2 0.1	4.5 ± 0.3 0.3 0.3	2 ± 0.5 ± 0.3 ± 0.2 2 0.3 0.2	0.8 ± 0.1 0 0	0.7 ± 0.1 0 0	0.1 ± 0.1 0 0	0.1 ± 0.1 0 0	2.8 ± 1.4 ± 0.2 0.2 0.1	2.3 ± 0.9 0.2 0.2	8.1 ± 0.4 ± 0.9 0.9	0.4 ± 0.4 ± 0 0	0 ± 0 0 ± 0	0.3 ± 0.1 0.3 ± 0.1	1.9 ± 0.2 1.9 ± 0.2	0.2 ± 0.3 0 0.3	1.4 ± 0.2 ± 0.3 0.3 0 0	1.4 ± 0.2 ± 0.3 1.4 ± 0.2 ± 0.3	1.4 ± 0.2 ± 0.3 1.4 ± 0.2 ± 0.3	1.4 ± 0.2 ± 0.3 1.4 ± 0.2 ± 0.3	0.2 ± 0.2 ± 0 0
LBFIHE (n=34)	5.4 ± 0.2 ± 0.9 0.1	0 ± 0 0 0 ± 0	3.1 ± 0.4 ± 2.1 ± 1.4 3.1 ± 26.6 34.7	3.1 ± 0.4 ± 2.1 ± 1.4 3.1 ± 26.6 34.7	26.6 ± 2.1 ± 1.4 26.6 34.7	34.7 ± 0.9 ± 1.4 34.7 0.9 ± 1.4	0.9 ± 0.2 0.2 0.2	2.6 ± 0.3 ± 0.4 0.9 0.1	3.9 ± 0.3 0.4 0.4	2.6 ± 0.3 ± 0.4 0.9 0.1	0.8 ± 0.1 0.1 0.1	0.9 ± 0.1 0.1 0.1	0.2 ± 0.1 0.1 0.1	0.1 ± 0.1 0 0	3.1 ± 1.5 ± 0.2 0.3 0.2	2.5 ± 0.8 0.4 0.4	8.4 ± 0.4 ± 1 1	0.4 ± 0.4 ± 0 0	0 ± 0 0 ± 0	0.3 ± 0.1 0.3 ± 0.1	1.6 ± 0.1 0.2 0.2	0.2 ± 0.3 0 0.3	1.5 ± 0.2 ± 0.3 0.3 0.1 0.1	1.5 ± 0.2 ± 0.3 1.5 ± 0.2 ± 0.3	1.5 ± 0.2 ± 0.3 1.5 ± 0.2 ± 0.3	1.5 ± 0.2 ± 0.3 1.5 ± 0.2 ± 0.3	0.3 ± 0.3 ± 0.3 ± 0.3 ±
LBFLDI (n=60)	6.6 ± 0.3 ± 1.2 0.1	0 ± 0 0 0 ± 0	2.2 ± 0.2 ± 3.3 2.2 ± 31.1 30 ± 1.9	2.2 ± 0.2 ± 3.3 2.2 ± 31.1 30 ± 1.9	31.1 ± 3.3 ± 1.9 31.1 30 ± 1.9	30 ± 0.7 ± 1.9 30 0.7 ± 1.9	0.7 ± 0.2 0.2 0.2	1.1 ± 0.2 ± 0.3 0.3 0.1	2.6 ± 0.3 0.6 0.6	1.1 ± 0.2 ± 0.3 0.3 0.1	0.8 ± 0.1 0.1 0.1	0.8 ± 0.1 0.1 0.1	0.4 ± 0.1 0.3 0.3	0.2 ± 0.1 0.1 0.1	2.6 ± 1.6 ± 0.2 0.2 0.2	1.7 ± 0.8 0.2 0.2	8.2 ± 0.4 ± 1.7 1.7	0.4 ± 0.4 ± 0 0	0 ± 0 0 ± 0	0.3 ± 0.1 0.3 ± 0.1	2.4 ± 0.1 0.5 0.5	0.1 ± 0.2 0.1 0.1	0.2 ± 0.3 0 0.3	1.4 ± 0.3 ± 0.4 0.3 0 0.3	1.4 ± 0.3 ± 0.4 1.4 ± 0.3 ± 0.4	1.4 ± 0.3 ± 0.4 1.4 ± 0.3 ± 0.4	0.4 ± 0.4 ± 0.4 ± 0.4 ±
LBFPNF (n=52)	5 ± 0.2 ± 0.2 0	0 ± 0 0 0 ± 0	2.5 ± 0.2 ± 1.3 ± 1.1 2.5 ± 30.5 27.8	2.5 ± 0.2 ± 1.3 ± 1.1 2.5 ± 30.5 27.8	30.5 ± 1.3 ± 1.1 30.5 27.8	27.8 ± 1.8 ± 1.1 27.8 1.8 ± 1.1	1.8 ± 0.2 0.2 0.2	2.9 ± 0.4 ± 0.3 0.3 0.1	4.5 ± 0.4 0.4 0.4	2.9 ± 0.4 ± 0.3 0.3 0.1	0.8 ± 0.1 0.1 0.1	0.7 ± 0.1 0.1 0.1	0.2 ± 0.1 0 0	0.1 ± 0.1 0 0	3.5 ± 1.5 ± 0.2 0.3 0.3	2.5 ± 0.8 0.4 0.4	9.8 ± 0.4 ± 0.8 0.8	0.4 ± 0.4 ± 0 0	0 ± 0 0 ± 0	0.3 ± 0.1 0.3 ± 0.1	2.7 ± 0.1 0.3 0.3	0.1 ± 0.2 0 0.1	1.2 ± 0.3 ± 0.1 1.2 ± 0.3 ± 0.1	1.2 ± 0.3 ± 0.1 1.2 ± 0.3 ± 0.1	1.2 ± 0.3 ± 0.1 1.2 ± 0.3 ± 0.1	1.2 ± 0.3 ± 0.1 1.2 ± 0.3 ± 0.1	0.2 ± 0.2 ± 0.2 ± 0.2 ±
LBFNSQ (n=51)	5 ± 0.1 ± 0.4 0	0 ± 0 0 0 ± 0	2.7 ± 0.5 ± 1.6 ± 1.9 2.7 ± 26.9 35.7	2.7 ± 0.5 ± 1.6 ± 1.9 2.7 ± 26.9 35.7	26.9 ± 1.6 ± 1.9 26.9 35.7	35.7 ± 0.8 ± 1.9 35.7 0.8 ± 1.9	0.8 ± 0.2 0.2 0.2	1.5 ± 0.3 ± 0.2 0.2 0.1	5.4 ± 0.6 0.6 0.6	1.5 ± 0.3 ± 0.2 0.2 0.1	0.8 ± 0.1 0.1 0.1	0.4 ± 0.1 0.4 0.4	0.2 ± 0.1 0.1 0.1	0.1 ± 0.1 0 0	4.3 ± 2.2 ± 0.6 0.3 0.3	1.6 ± 0.8 0.4 0.4	6.8 ± 0.4 ± 1.4 1.4	0.4 ± 0.4 ± 0 0	0 ± 0 0 ± 0	0.3 ± 0.1 0.3 ± 0.1	1.7 ± 0.1 0.3 0.3	0.1 ± 0.2 0 0.3	1.3 ± 0.6 ± 0.3 0.3 0.2 0.3	1.3 ± 0.6 ± 0.3 1.3 ± 0.6 ± 0.3	1.3 ± 0.6 ± 0.3 1.3 ± 0.6 ± 0.3	1.3 ± 0.6 ± 0.3 1.3 ± 0.6 ± 0.3	0.5 ± 0.5 ± 0.5 ± 0.5 ±
LBFDGL (n=57)	4.9 ± 0.1 ± 0.1 0	0 ± 0 0 0 ± 0	3 ± 0.4 ± 2.2 ± 1.9 3 ± 26.9 36.3	3 ± 0.4 ± 2.2 ± 1.9 3 ± 26.9 36.3	26.9 ± 2.2 ± 1.9 26.9 36.3	36.3 ± 0.6 ± 1.9 36.3 0.6 ± 1.9	0.6 ± 0.2 0.2 0.2	1.5 ± 0.3 ± 0.7 0.8 0.1	5.8 ± 0.7 0.7 0.7	1.5 ± 0.3 ± 0.7 0.8 0.1	0.8 ± 0.1 0.1 0.1	0.7 ± 0.1 0 0	0.2 ± 0.1 0 0	0.1 ± 0.1 0 0	4.7 ± 3 ± 0.7 0.5 0.5	3 ± 0.8 0.3 0.3	5.5 ± 0.4 ± 1.8 1.8	0.4 ± 0.4 ± 0 0	0 ± 0 0 ± 0	0.3 ± 0.1 0.3 ± 0.1	1.4 ± 0.1 0.3 0.3	0.1 ± 0.2 0 0.4	1 ± 0.9 ± 0.4 1 ± 0.9 ± 0.4	1 ± 0.9 ± 0.4 1 ± 0.9 ± 0.4	1 ± 0.9 ± 0.4 1 ± 0.9 ± 0.4	1 ± 0.9 ± 0.4 1 ± 0.9 ± 0.4	0.2 ± 0.2 ± 0.2 ± 0.2 ±
LBFIEF (n=6)	5 ± 0.1 ± 0.2 0	0 ± 0 0 0 ± 0	3.4 ± 0.4 ± 2.9 ± 2.1 3.4 ± 27.6 36.2	3.4 ± 0.4 ± 2.9 ± 2.1 3.4 ± 27.6 36.2	27.6 ± 2.9 ± 2.1 27.6 36.2	36.2 ± 0.6 ± 2.1 36.2 0.6 ± 2.1	0.6 ± 0.1 0.1 0.1	1.5 ± 0.4 ± 0.2 0.2 0.1	5.7 ± 0.5 0.5 0.5	1.5 ± 0.4 ± 0.2 0.2 0.1	1 ± 0.1 0.1 0.1	0.7 ± 0.1 0 0	0.2 ± 0.1 0 0	0.1 ± 0.1 0 0	4.2 ± 2.5 ± 0.3 0.3 0.3	0.7 ± 0.3 0.3 0.3	5.2 ± 0.4 ± 0.8 0.8	0.4 ± 0.4 ± 0 0	0 ± 0 0 ± 0	0.3 ± 0.1 0.3 ± 0.1	1.6 ± 0.1 0.3 0.3	0.1 ± 0.2 0 0.4	1 ± 0.9 ± 0.4 1 ± 0.9 ± 0.4	1 ± 0.9 ± 0.4 1 ± 0.9 ± 0.4	1 ± 0.9 ± 0.4 1 ± 0.9 ± 0.4	1 ± 0.9 ± 0.4 1 ± 0.9 ± 0.4	0.2 ± 0.2 ± 0.2 ± 0.2 ±
LBFBVAV (n=50)	5.1 ± 0.2 ± 0.2 0	0 ± 0 0 0 ± 0	2.6 ± 0.3 ± 1.3 ± 1.4 2.6 ± 29.5 27.2	2.6 ± 0.3 ± 1.3 ± 1.4 2.6 ± 29.5 27.2	29.5 ± 1.3 ± 1.4 29.5 27.2	27.2 ± 1.6 ± 1.4 27.2 1.6 ± 1.4	1.6 ± 0.2 0.2 0.2	1.6 ± 0.3 ± 0.1 0.3 0.1	5.3 ± 0.3 0.3 0.3	1.6 ± 0.3 ± 0.1 0.3 0.1	0.8 ± 0.1 0.2 0.2	0.7 ± 0.1 0 0	0.1 ± 0.1 0 0	0.1 ± 0.1 0 0	10.9 ± 6.5 ± 0.7 0.5 0.5	0.1 ± 0.7 0.1 0.1	1.8 ± 0.4 ± 0.7 0.7	0.4 ± 0.4 ± 0 0	0 ± 0 0 ± 0	0.3 ± 0.1 0.3 ± 0.1	0.8 ± 0.1 0.3 0.3	0.1 ± 0.2 0 0.5	0.3 ± 0.4 0.1 0.1	0.3 ± 0.4 0.3 ± 0.4	0.3 ± 0.4 0.3 ± 0.4	0.3 ± 0.4 0.3 ± 0.4	0.7 ± 0.7 ± 0.7 ± 0.7 ±
LBFPNC (n=32)	4.8 ± 0.1 ± 0.3 0	0 ± 0 0 0 ± 0	3.9 ± 0.7 ± 1.7 ± 1 3.9 ± 26.7 35.6	3.9 ± 0.7 ± 1.7 ± 1 3.9 ± 26.7 35.6	26.7 ± 1.7 ± 1 26.7 35.6	35.6 ± 0.8 ± 1 35.6 0.8 ± 1	0.8 ± 0.1 0.1 0.1	1.7 ± 0.2 ± 0.3 0.3 0.1	3.3 ± 0.3 0.5 0.5	1.7 ± 0.2 ± 0.3 0.3 0.1	1.1 ± 0.2 0.2 0.2	0.7 ± 0.1 0 0	0.2 ± 0.1 0 0	0.1 ± 0.1 0 0	4.9 ± 2 ± 0.9 0.9 0.9	2 ± 0.4 0.7 0.7	6.9 ± 0.4 ± 1.6 1.6	0.4 ± 0.4 ± 0.1 0.1	0 ± 0 0 ± 0	0.3 ± 0.1 0.3 ± 0.1	1.9 ± 0.1 0.5 0.5	0.1 ± 0.2 0 0.2	0.9 ± 0.4 ± 0.2 0.2 0.2	0.9 ± 0.4 ± 0.2 0.9 ± 0.4 ± 0.2	0.9 ± 0.4 ± 0.2 0.9 ± 0.4 ± 0.2	0.9 ± 0.4 ± 0.2 0.9 ± 0.4 ± 0.2	0.4 ± 0.4 ± 0.4 ± 0.4 ±
LBFGHQ (n=46)	5.1 ± 0.1 ± 0.2 0.1	0 ± 0 0 0 ± 0	2.4 ± 0.1 ± 1.3 2.4 ± 30.7 35 ± 1.7	2.4 ± 0.1 ± 1.3 2.4 ± 30.7 35 ± 1.7	30.7 ± 1.3 ± 1.7 30.7 35 ± 1.7	35 ± 0.5 ± 1.7 35 0.5 ± 1.7	0.5 ± 0.1 0.1 0.1	1.3 ± 0.4 ± 0.6 0.2 0.2	7.7 ± 0.5 0.6 0.6	1.3 ± 0.4 ± 0.6 0.2 0.2	0.9 ± 0.1 0.3 0.3	0.8 ± 0.1 0.1 0.1	0.1 ± 0.1 0 0	0.1 ± 0.1 0 0	2.5 ± 1.7 ± 0.3 0.3 0.3	0.6 ± 0.1 0.1 0.1	4.9 ± 0.4 ± 0.7 0.7	0.4 ± 0.4 ± 0 0	0 ± 0 0 ± 0	0.3 ± 0.1 0.3 ± 0.1	1.9 ± 0.2 0.3 0.3	0.1 ± 0.2 0 0.4	1 ± 0.8 ± 0.3 1 ± 0.8 ± 0.3	1 ± 0.8 ± 0.3 1 ± 0.8 ± 0.3	1 ± 0.8 ± 0.3 1 ± 0.8 ± 0.3	1 ± 0.8 ± 0.3 1 ± 0.8 ± 0.3	0.3 ± 0.3 ± 0.3 ± 0.3 ±
LBFAZB (n=49)	4.9 ± 0.1 ± 0.1 0	0 ± 0 0 0 ± 0	3.1 ± 0.2 ± 1.6 ± 1.1 3.1 ± 28.4 34.1	3.1 ± 0.2 ± 1.6 ± 1.1 3.1 ± 28.4 34.1	28.4 ± 1.6 ± 1.1 28.4 34.1	34.1 ± 1 ± 1.1 34.1 1 ± 1.1	1 ± 0.1 0.1 0.1	1.9 ± 0.3 ± 0.4 0.3 0.1	4.7 ± 0.4 0.4 0.4	1.9 ± 0.3 ± 0.4 0.3 0.1	0.8 ± 0.1 0.1 0.1	0.6 ± 0.1 0.1 0.1	0.1 ± 0.1 0.1 0.1	0.1 ± 0.1 0 0	6.3 ± 3.3 ± 0.7 0.3 0.3	0.8 ± 0.2 0.2 0.2	4.5 ± 0.3 ± 0.9 0.9	0.3 ± 0.3 ± 0 0	0 ± 0 0 ± 0	0.4 ± 0.1 0.2 0.2	1.4 ± 0.1 0.2 0.2	0.1 ± 0.2 0 0.3	0.9 ± 1.5 ± 0.2 0.9 ± 1.5 ± 0.2	0.9 ± 1.5 ± 0.2 0.9 ± 1.5 ± 0.2	0.9 ± 1.5 ± 0.2 0.9 ± 1.5 ± 0.2	0.9 ± 1.5 ± 0.2 0.9 ± 1.5 ± 0.2	0.2 ± 0.2 ± 0.2 ± 0.2 ±

LBFQKW (n=72)	5.1 ± 0.2 ±		2.5 ±	30.1	32.1	1.1 ±	6.1 ±	1.7 ±	0.2 ±	0.8 ±	0.7 ±	0.1 ±	0.1 ±	6 ±	3.3 ±	0.5 ±	4.1 ±	0.4 ±		0.4 ±	1.5 ±	0.1 ±	0.8 ±	1.7 ±	0.3 ±
	0.2	0	0 ± 0	0.2 ± 1.3	1.6	0.1	0.4	0.3	0.1	0.1	0	0	0	0.7	0.4	0.1	0.8	0	0 ± 0	0.1	0.3	0	0.2	0.3	0.1
LBFNRU (n=58)	5 ± 0.2 ±		2.6 ±	31.8	29.2	1 ±	7.1 ±	1.2 ±	0.2 ±	0.8 ±	0.8 ±	0.1 ±	0.1 ±	5.9 ±	3.6 ±	0.4 ±	4.2 ±	0.4 ±		0.4 ±	1.7 ±	0.1 ±	0.9 ±	1.9 ±	0.3 ±
	0.2	0	0 ± 0	0.2 ± 2.5	1.9	0.1	0.5	0.2	0	0.1	0	0	0	0.7	0.5	0.1	0.9	0	0 ± 0	0	0.4	0	0.3	0.4	0.1
LBFQIZ (n=43)	4.9 ± 0.2 ±		2.6 ±	30.5	30 ±	1 ±	6.2 ±	1.3 ±	0.2 ±	0.8 ±	0.7 ±	0.2 ±	0.2 ±	5.7 ±	3.5 ±	0.6 ±	5.3 ±	0.4 ±		0.4 ±	2.1 ±	0.1 ±	1 ±	1.7 ±	0.4 ±
	0.6	0.1	0 ± 0	0.4 ± 3	2.6	0.2	0.8	0.2	0	0.1	0.1	0	0	0.8	0.5	0.1	1.1	0	0 ± 0	0.1	0.4	0	0.2	0.4	0.1
LBFQIGM (n=56)	4.8 ± 0.2 ±		2.3 ±	31.9	30.6	1 ±	6.3 ±	1.3 ±	0.2 ±	0.7 ±	0.8 ±	0.1 ±	0.1 ±	5 ±	2.5 ±	1 ±	5.4 ±	0.4 ±		0.4 ±	2 ±	0.4 ±	1 ±	1.2 ±	0.3 ±
	0.4	0	0 ± 0	0.2 ± 2.7	2.7	0.1	0.7	0.2	0	0.1	0.1	0	0	0.7	0.3	0.2	1	0	0 ± 0	0.1	0.5	2.2	0.2	0.2	0.1
LBFNR	5 ± 0.2 ±		2.8 ±	31.4	30 ±	1.1 ±	7 ±	1.3 ±	0.3 ±	0.9 ±	0.8 ±	0.1 ±	0.1 ±	5.3 ±	3.5 ±	0.4 ±	4.4 ±	0.4 ±		0.3 ±	1.7 ±		0.8 ±	1.7 ±	0.3 ±
(n=61)	0.2	0	0 ± 0	0.4 ± 1.5	2.1	0.1	0.6	0.2	0	0.1	0.1	0	0	1.2	0.3	0.1	1.1	0	0 ± 0	0	0.4	0 ± 0	0.2	0.3	0.1
LBFNTK (n=69)	5.1 ± 0.2 ±		2.5 ±	29.6	29 ±	1.3 ±	6.4 ±	1.5 ±	0.1 ±	0.8 ±	0.8 ±	0.1 ±	0.1 ±	6.7 ±	3.8 ±	0.6 ±	5 ±	0.4 ±		0.5 ±	2 ±	0.1 ±	0.9 ±	1.8 ±	0.5 ±
	0.4	0	0 ± 0	0.2 ± 1.4	1.8	0.2	0.6	0.3	0.1	0.1	0	0	0	0.8	0.5	0.2	0.9	0	0 ± 0	0.1	0.4	0	0.2	0.3	0.1
LBFQJA (n=42)	5.2 ± 0.2 ±		3.1 ±	27.7	34.5	1.9 ±	3.3 ±	5.8 ±	0.5 ±	0.8 ±	0.7 ±	0.1 ±	0.1 ±	4.7 ±	1.5 ±	1.3 ±	3.8 ±	0.3 ±		0.7 ±	1.4 ±	0.1 ±	0.8 ±	1.2 ±	0.1 ±
	0.2	0	0 ± 0	0.2 ± 0.8	0.8	0.2	0.2	0.9	0.2	0	0.1	0	0	0.6	0.2	0.2	0.6	0	0 ± 0	0.1	0.2	0	0.2	0.2	0
LBFIFV (n=58)	4.8 ± 0.1 ±		3.2 ±	27.6	37.3	0.8 ±	3.7 ±	1.8 ±	0.2 ±	0.9 ±	0.7 ±	0.1 ±	0.1 ±	6.3 ±	2.5 ±	1.3 ±	4.2 ±	0.4 ±		0.4 ±	1.1 ±	0.1 ±	1.1 ±	1 ±	0.2 ±
	0.4	0	0 ± 0	0.3 ± 1.5	0.9	0.1	0.2	0.3	0	0.1	0.2	0	0	0.6	0.2	0.2	0.7	0	0 ± 0	0.1	0.2	0	0.3	0.2	0
LBFLEF (n=52)	4.7 ± 0.1 ±		3.1 ±	28.7	33.9	1 ±	4.4 ±	1.9 ±	0.3 ±	0.9 ±	0.7 ±	0.1 ±	0.1 ±	5.9 ±	2.9 ±	1.1 ±	5.3 ±	0.4 ±		0.5 ±	1.7 ±		1 ±	1.1 ±	0.2 ±
	0.1	0	0 ± 0	0.2 ± 2	1.1	0.1	0.3	0.5	0	0	0	0	0	0.6	0.3	0.2	0.7	0	0 ± 0	0.1	0.2	0 ± 0	0.2	0.2	0.1
LBFLLD (n=44)	4.8 ± 0.1 ±		3.4 ±	28 ±	34.6	1 ±	4.2 ±	2.2 ±	0.3 ±	0.9 ±	0.7 ±	0.1 ±	0.1 ±	5.2 ±	2.5 ±	1.2 ±	5.5 ±	0.4 ±		0.6 ±	1.9 ±	0.1 ±	0.8 ±	1 ±	0.2 ±
	0.1	0	0 ± 0	0.3 ± 1	1	0.1	0.2	0.5	0	0.1	0	0	0	0.6	0.4	0.3	0.7	0	0 ± 0	0.1	0.2	0	0.2	0.2	0
LBFNQW (n=51)	4.8 ± 0.1 ±		3.2 ±	27.6	33.5	1 ±	4.2 ±	2 ±	0.3 ±	1.3 ±	0.9 ±	0.2 ±	0.1 ±	5.3 ±	2.7 ±	1.1 ±	6.2 ±	0.4 ±		0.5 ±	2 ±	0.1 ±	0.9 ±	0.8 ±	0.5 ±
	0.2	0	0 ± 0	0.3 ± 1.5	1.1	0.1	0.3	0.3	0	0.3	0.1	0.1	0.1	0.5	0.4	0.2	1	0	0 ± 0	0.1	0.3	0	0.2	0.4	0.1
LBFBAF (n=19)	4.8 ± 0.1 ±		3 ±	26 ±	33.8	1.2 ±	3.4 ±	2.4 ±	0.3 ±	0.8 ±	0.7 ±	0.2 ±	0.1 ±	7.4 ±	2.9 ±	2.1 ±	6.2 ±	0.3 ±		0.3 ±	1.7 ±	0.1 ±	1.1 ±	0.6 ±	0.4 ±
	0.1	0	0 ± 0	0.2 ± 0.9	1	0.1	0.2	0.3	0	0	0	0	0	0.7	0.3	0.3	0.8	0	0 ± 0	0	0.2	0	0.1	0.1	0.1
LBFDAU (n=10)	5.2 ± 0.2 ±		3.3 ±	20.5	31.8	0.9 ±	4.1 ±	2.5 ±	0.3 ±	0.9 ±	0.6 ±	0.2 ±	0.1 ±	4.2 ±	3.1 ±	1.6 ±	13.9	0.4 ±		0.2 ±	2.3 ±	0.1 ±	2.6 ±	0.4 ±	0.3 ±
	1	0	0 ± 0	0.3 ± 0.9	0.5	0.1	0.2	0.2	0	0.1	0.1	0	0	0.2	0.3	0.3	1	0	0 ± 0	0	0.2	0	0.4	0.1	0

LBFPR	4.9 ± 0.2 ±		2.6 ±	25.4	31 ±	1.3 ±	5.4 ±	2 ±	0.2 ±	0.8 ±	0.1 ±	0.1 ±	2.9 ±	1.6 ±	3 ±	12.3	0.4 ±		0.2 ±	2.7 ±		1.4 ±	0.1 ±	0.4 ±
(n=16)	0.2	0	0 ± 0	0.6 ± 2.2	1.3	0.2	0.8	0.4	0.1	0.1	0	0	0.6	0.3	0.3	± 1.4	0	0 ± 0	0	0.2	0 ± 0	0.2	0	0.1
LBFIFU	4.7 ± 0.1 ±		3.8 ±	25.9	34.7	1.1 ±	3.6 ±	2.7 ±	0.3 ±	0.9 ±	0.7 ±	0.1 ±	4.2 ±	1.7 ±	2.7 ±	8 ±	0.3 ±		0.4 ±	2 ±	0.1 ±	1.3 ±	0.3 ±	0.2 ±
(n=11)	0.2	0	0 ± 0	0.9 ± 2.6	± 1.3	0.3	0.6	0.9	0.1	0.1	0	0	0.6	0.3	0.5	1.8	0	0 ± 0	0.1	0.3	0.1	0.4	0.2	0
LBFDDK	5.1 ± 0.2 ±		3 ±	26.1	35.6	0.6 ±	4.8 ±	1.3 ±	0.2 ±	0.9 ±	0.8 ±	0.3 ±	5.1 ±	3 ±	0.9 ±	5.7 ±	0.4 ±		0.5 ±	2.2 ±	0.1 ±	1.1 ±	1.4 ±	0.3 ±
(n=2)	0.2	0	0 ± 0	0.9 ± 3.9	± 0.6	0	0.7	0.4	0.1	0.2	0	0	1.6	1.3	0.4	0.7	0	0 ± 0	0.1	0.5	0	0.2	0.7	0
LBFDDJG	4.4 ± 0.1 ±		3.4 ±	27.3	33.1	1.1 ±	3.6 ±	2.1 ±	0.4 ±	0.9 ±	0.6 ±	0.1 ±	6.1 ±	2.6 ±	1.6 ±	6.8 ±	0.4 ±		0.4 ±	1.7 ±	0.1 ±	1.3 ±	1 ±	0.4 ±
(n=12)	0.1	0	0 ± 0	0.3 ± 1.4	± 1.9	0.2	0.1	0.4	0.1	0.1	0.2	0	0 ± 0	1.1	0.4	1.4	0	0 ± 0	0.1	0.2	0	0.4	0.4	0.1
LBFDFK	5.1 ± 0.2 ±		2.5 ±	28.7	29 ±	1.2 ±	5.6 ±	1.5 ±	0.2 ±	0.8 ±	0.7 ±	0.1 ±	3.8 ±	2.4 ±	1.3 ±	9.9 ±	0.4 ±		0.5 ±	3 ±	0.1 ±	1.8 ±	0.6 ±	0.3 ±
(n=15)	0.3	0	0 ± 0	0.1 ± 1.6	1.7	0.2	0.4	0.2	0	0	0	0	0.3	0.1	0.1	0.8	0.1	0 ± 0	0	0.3	0	0.2	0.1	0.1
LBFDFG	4.9 ± 0.1 ±		3.6 ±	25.8	34.1	0.9 ±	4.4 ±	1.9 ±	0.3 ±	0.9 ±	0.6 ±	0.1 ±	3.7 ±	2.2 ±	1.4 ±	9 ±	0.3 ±		0.4 ±	2.5 ±	0.1 ±	1.6 ±	0.6 ±	0.2 ±
(n=15)	0.3	0	0 ± 0	0.3 ± 1.4	± 0.8	0.1	0.2	0.2	0	0	0	0	0.5	0.3	0.2	0.7	0	0 ± 0	0.1	0.2	0	0.3	0.2	0
LBFDFQM	5.2 ± 0.1 ±		3.8 ±	26.3	31.6	1.2 ±	4.3 ±	1.8 ±	0.2 ±	1.1 ±	0.9 ±	0.2 ±	4.8 ±	3 ±	1.1 ±	8.2 ±	0.4 ±		0.4 ±	2.2 ±	0.1 ±	1.5 ±	0.8 ±	0.5 ±
(n=12)	0.3	0	0 ± 0	0.4 ± 1.2	± 1	0.1	0.4	0.2	0	0.1	0.1	0.1	0.6	0.4	0.2	1.1	0	0 ± 0	0.1	0.2	0	0.2	0.1	0.2
LBFDFHG	5.4 ± 0.2 ±		2.3 ±	24 ±	34 ±	1 ±	4.1 ±	2.7 ±	0.3 ±	0.7 ±	0.7 ±	0.1 ±	3 ±	1.7 ±	1.7 ±	10.4	0.4 ±		0.6 ±	2.4 ±	0.3 ±	2.7 ±	0.5 ±	0.2 ±
(n=5)	0.1	0	0 ± 0	0.2 ± 0.6	0.7	0.1	0.2	0.1	0	0	0	0	0.1	0.1	0.1	± 0.8	0	0 ± 0	0.1	0.1	0	0.2	0	0
LBFDFKA	5.1 ± 0.1 ±		3.7 ±	24.2	29.7	1.5 ±	4.4 ±	1.8 ±	0.2 ±	1.1 ±	0.9 ±	0.2 ±	7.6 ±	5.1 ±	0.6 ±	6.6 ±	0.4 ±		0.3 ±	2 ±	0.1 ±	1.3 ±	1.8 ±	0.9 ±
(n=5)	0.3	0	0 ± 0	0.4 ± 2.8	± 1.1	0.3	0.3	0.1	0	0.1	0.1	0.1	0.6	0.8	0.1	1	0	0 ± 0	0.1	0.3	0	0.5	0.2	0
LBFIDT	5 ± 0.2 ±		2.6 ±	27 ±	30.8	1.1 ±	6.2 ±	1.8 ±	0.4 ±	0.8 ±	0.7 ±	0.1 ±	4.5 ±	3 ±	1 ±	8.2 ±	0.4 ±		0.5 ±	2.6 ±	0.1 ±	1.7 ±	1 ±	0.3 ±
(n=7)	0.1	0	0 ± 0	0.1 ± 1.7	± 1.2	0.2	0.4	0.2	0.1	0	0	0	1.3	0.9	0.2	0.6	0	0 ± 0	0.1	0.2	0	0.2	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	16:1 16:3 18:										16:0 n-7 n-3 0 9 6 9 3 6 3 0									

LBFQHQ (n=1)	5.4	0.2	0.0	2.3	28.	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
LBFQZB (n=1)	5.1	0.1	0.0	3.1	26.	30.	6	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
LBFQKW (n=1)	4.4	0.2	0.0	2.0	26.	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.	26.	2	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
LBFQIZ (n=1)	5.2	0.2	0.0	2.3	27.	31.	3	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
LBFQIM (n=1)	4.9	0.1	0.0	2.5	28.	29.	9	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.	26.	3	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.	27.	0	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
LBFQJA (n=1)	5.2	0.2	0.0	3.0	27.	33.	1	2.1	3.4	5.2	0.6	0.8	0.8	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.	34.	6	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
LBFLEL (n=1)	5.1	0.2	0.0	2.8	22.	32.	8	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
LBFLEL (n=1)	4.7	0.1	0.0	3.4	26.	33.	9	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.	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
LFBFAP (n=1)	4.9	0.1	0.0	3.2	24.	31.	5	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.	31.	7	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
LBFPPRA (n=1)	4.8	0.1	0.0	3.2	21.	28.	9	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
LBFIFU (n=1)	4.8	0.1	0.0	3.2	22.	34.	4	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.	35.	9	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.	32.	4	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.	29.	2	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
LBFLCG (n=1)	5.0	0.2	0.0	4.4	22.	34.	6	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
LBFPQM (n=1)	5.1	0.2	0.0	3.3	25.	30.	7	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
LBFDHG (n=1)	5.4	0.2	0.0	2.1	23.	33.	9	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
LBFDKA (n=1)	5.6	0.2	0.0	3.2	20.	30.	9	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.	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 category "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 zygosity analysis listed in Table 137 revealed 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 zygosity analysis listed in Table 137 revealed a double locus insertion of both copies. The number of T1 plants fulfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Categor	16:0		16:1		18:0		18:1		18:2		18:3		18:3		18:4		20:0		20:1		20:2		20:3		20:3		20:4		20:4		20:5		22:0		22:1		22:4		22:5		22:6		22:7																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
	n-7	n-3	n-3	n-9	n-6	n-9	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	n-3

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	16:3	18:0	18:1	18:2	18:2	18:3	18:3	18:3	18:4	20:0	20:1	20:2	20:3	20:3	20:4	20:4	20:5	22:0	22:1	22:4	22:5	22:5	22:6	22:6	22:7	20:2
	n-7	n-7	n-3	n-3	n-9	n-6	n-9	n-3	n-6	n-3	n-3	n-3	n-9	n-6	n-3	n-3	n-6	n-6	n-3	n-3	n-9	n-6	n-3	n-6	n-3	n-3	n-9	
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 were 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), Noli: 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
LBFGKN (n=50)	43.3 ± 1.7	9 ± 0	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	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	8.9 ± 0.7	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	8.8 ± 0.5	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 ± 1.3	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	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	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
LBFBAB (n=50)	46.6 ± 1.6	9 ± 0	7.4 ± 0.8	9 ± 0	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	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	9 ± 0	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	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	9 ± 0	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	8.9 ± 0.7	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	9 ± 0	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
LFIGM (n=56)	36.1 ± 1.3	9 ± 0	6.7 ± 0.8	9 ± 0.3	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	9 ± 0	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	9 ± 0	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	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
LBFIIV (n=58)	42.4 ± 0.6	9 ± 0	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	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	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	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	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	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	7.4 ± 0.8	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
LBFIIV (n=11)	46.8 ± 6.4	9 ± 0	8.7 ± 0.9	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	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	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	8.7 ± 1.3	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	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	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	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	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 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=177)	41.5 ± 5.2	9 ± 0	0.7	9 ± 0.4	1.5	0	0.5	4.8 ± 0.4	8 ± 1.5	5.1 ± 1	109.1 ± 10.6	3.9 ± 0.6	1.4	37 ± 1.4	29.5 ± 0.9
dc (n=781)	43.4 ± 4.4	0.3	1.1	9 ± 0.3	0.8	0	0.9	4.5 ± 0.5	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	0.3	8 ± 1.1	9 ± 0.2	0.8	0	4.6 ± 1	4.4 ± 0.5	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	0.8	0.6	8.4 ± 1	0	5 ± 0.3	4.8 ± 0.4	1.1	4.8 ± 1.1	110.8 ± 14.3	3.5 ± 0.7	0.9	36.6 ± 2.7	28.9 ± 1.7

B. 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.

5 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.

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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 were measured per event. The T1 plants underwent a selection from ~80 segregating T1 seedlings, using zygosity 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. Homozygosity 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	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Pl_GA)2	j-p-LuPXR_j-i-Atss15	j-p-PvARC5_t-BnSETL	c-d5Des(Tc_GA)	j-i-Atss18_c-03Des(Pl_GA2)	j-p-BnSETL-v1_c-03Des(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
LBFIHE (n=8)		2.1 (T0: 1)									1.9 (T0: 1.2)			1.9 (T0: 1.1)	single copy
LBFLDI (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

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-LTM593-1qc 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	16:3	18:0	18:1	18:2	18:2	18:3	18:3	18:4	20:0	20:1	20:2	20:3	20:3	20:4	20:4	20:5	22:0	22:1	22:4	22:5	22:5	22:6	22:4	20:2
	n-7	n-3	n-3	n-9	n-9	n-6	n-9	n-3	n-6	n-3	n-9	n-9	n-6	n-3	n-3	n-6	n-3	n-3	n-3	n-9	n-6	n-3	n-6	n-3	n-3	n-9
LBFDDG (n=5)	4.4 ± 0.2	0	0	0.1 ± 2.4	32.3 ± 1.9	34.4 ± 1.6	0	0.6	1.5 ± 0.1	0.4 ± 0.1	0	0	0	0	0	1.1 ± 2.7	6.9 ± 0.3	0	0.3 ± 0.3	0	0 ± 0	0	0.2	0	1.4 ± 0.3	0.1 ± 0
LBFCKN (n=12)	4.8 ± 0.2	0	0	0.3 ± 2.6	27.8 ± 1.9	36 ± 1.3	0.1	0.7	1.5 ± 0.2	0.4 ± 0.1	0	0	0.1	0	0	1.1 ± 3.3	8 ± 0.8	0.3 ± 0.1	0.3 ± 0.1	0 ± 0	0.4 ± 0.1	0	0.3	0	1.8 ± 0.4	0.2 ± 0
LBFHIE (n=8)	4.9 ± 0.3	0.1	0	0.4 ± 2.4	30.8 ± 3.1	36.1 ± 1.2	0.1	1.3	1.5 ± 0.5	0.3 ± 0.1	0	0.1	0	0	0	0.9 ± 2.8	6.4 ± 1.1	0.3 ± 0.1	0.3 ± 0.1	0 ± 0	0.5 ± 0.1	0	0.3	0	1.3 ± 0.2	0.1 ± 0
LBFLLI (n=13)	4.5 ± 0.2	0.1	0	0.6 ± 2.3	30 ± 5.9	35.4 ± 6.1	0.1	0.8	1.3 ± 0.3	0.3 ± 0.1	0	0.1	0.1	0	0	0.8 ± 2.9	6.4 ± 1.6	0.3 ± 0.1	0.3 ± 0.1	0 ± 0	0.4 ± 0.1	0	0.3	0	1.3 ± 0.4	0.1 ± 0
LBFNNF (n=9)	4.7 ± 0.2	0	0	0.3 ± 2.4	29.2 ± 1.8	31.7 ± 1	0.1	0.6	2.6 ± 0.6	0.6 ± 0.1	0	0	0	0	0	1.2 ± 4.2	8.4 ± 1.1	0.3 ± 0.1	0	0 ± 0	0.5 ± 0.1	0	0.4	0	1.5 ± 0.3	0.1 ± 0
LBFDAU (n=4)	4.6 ± 0.2	0	0	0.3 ± 2.3	24.3 ± 3.7	31.2 ± 2.5	0.1	0.6	2.2 ± 0.5	0.4 ± 0.1	0	0.1	0	0	0	1.7 ± 2.9	13.4 ± 3.4	0.3 ± 0.1	0	0 ± 0	0.3 ± 0.1	0	0.7	0	2.7 ± 0.7	0.2 ± 0
LBFPPA (n=5)	4.8 ± 0.2	0	0	0.4 ± 2.5	25.1 ± 0.9	34.5 ± 2.4	0.2	0.7	2.2 ± 0.4	0.4 ± 0.1	0	0	0	0	0	1.3 ± 3.6	10.7 ± 1.9	0.3 ± 0.1	0	0 ± 0	0.5 ± 0.1	0	0.3	0	1.7 ± 0.2	0.2 ± 0.1
LBFLLK (n=4)	5.1 ± 0.2	0.1	0	0.4 ± 2.1	27 ± 4.3	32.3 ± 0.5	0.1	0.8	1.9 ± 0.5	0.4 ± 0.1	0	0.1	0	0	0	1.7 ± 2.7	9.5 ± 1.8	0.3 ± 0.1	0	0 ± 0	0.5 ± 0.1	0	0.4	0	2.1 ± 0.5	0.2 ± 0
LBFLLC (n=3)	4.8 ± 0.2	0	0	0.3 ± 3	26.4 ± 1.3	33.3 ± 1	0	0.7	2 ± 0.2	0.5 ± 0.1	0	0	0	0	0	1.2 ± 2.9	9.9 ± 0.9	0.3 ± 0.1	0	0 ± 0	0.7 ± 0.1	0	0.3	0	2.3 ± 0.5	0.2 ± 0
LBFPPM (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: 1n-7		16: 3n-3	18:0	18:1 n-9		18:2 n-6	18:2n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:1 n-9	20:2 n-6	20:3 n-3	20: 3n-6	20: 4n-6	20: 5n-3	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	22:6 n-6	22:7 n-3	22:7 n-6		
	0	1	3		6	5	5.74		1.69	0.45	0.62		0.74	0.12	0.05	5	2.2		1.38	3	8.02		0.29		0	0.35	2.21
LBFDGG (n=1)	4.5	0.2	1	0.1	2.4	6	33.4	0.71	5.74	1.69	0.45	0.62	0.12	0.05	5	2.2	1.38	3	8.02	0.29	0	0.35	2.21	0.1	1.79	0	0.13
LBFGKN (n=1)	4.5	0.2	0.0	0.0	2.28	8	34.2	0.65	4.99	1.73	0.42	0.61	0.69	0.23	0.1	2.4	3.3	4	9.74	0.31	0	0.48	2.27	0.17	2.31	0	0.23
LBFIHE (n=1)	4.8	0.2	0.0	0.0	2.95	26.2	38.0	0.52	3.19	2.12	0.22	0.78	0.69	0.15	0	2.7	3.6	7	8.16	0.35	0	0.59	1.72	0.12	1.55	0	0.09
LBFLDI (n=1)	4.8	0.2	0.0	0.0	2.51	27.6	35.7	0.54	4.15	1.66	0.26	0.7	0.69	0.29	0.1	3	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	0.2	0.1	0.1	2.11	9	31.1	0.91	5.04	1.72	0.68	0.67	0.7	0.11	0.04	2.8	1.34	6	10.24	0.36	0	0.54	3.24	0.14	2.09	0.15	0.12
LBFDAU (n=1)	4.8	0.2	0.1	0.1	1.94	7	28.2	0.98	5.23	2.78	0.5	0.63	0.62	0.13	0.13	8	2.3	1	17.57	0.36	0	0.29	3.16	0.09	3.62	0	0.25
LBFPRA (n=1)	4.7	0.1	0.1	0.1	1.92	6	32.2	1.21	4.87	2.09	0.44	0.51	0.66	0.13	0.07	4	1.29	7	12.46	0.28	0	0.44	2.58	0.08	1.8	0.08	0.26
LBFLFK (n=1)	4.9	0.2	0.1	0.1	2.34	3	22.4	0.9	4.55	2.5	0.42	0.66	0.62	0.13	0.07	6	2.18	7	11.06	0.3	0	0.76	3.2	0.24	2.66	0.46	0.21
LBFLCG (n=1)	5.0	0.2	0.1	0.1	2.65	8	32.2	0.74	5.16	2.3	0.46	0.69	0.65	0.14	0.09	4	1.36	5	10.87	0.31	0	0.69	2.92	0.22	2.82	0.21	0.15
LBFPQM (n=1)	5.0	0.2	0.1	0.1	2.67	7	30.8	1.02	4.34	2.31	0.45	0.69	0.63	0.12	0.07	3.4	1.75	8	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: 1n-3	16: 3n-3	18:0	18:1 n-9	18:2 n-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: 3n-6	20:4 n-3	20: 4n-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	22:4 n-3	20:2 n-9
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
LBFDAU seed																										

LBFDAU seed	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																										
LBFDAU seed	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																										
LBFDAU seed	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																										
LBFDAU seed	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																										
LBFDAU seed	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																										
LBFDAU seed	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																										
LBFDAU seed	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																										
LBFDAU seed	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																										
LBFDAU seed	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																										
LBFDAU seed	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																										
LBFDAU seed	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																										
LBFDAU seed	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																										
LBFDAU seed	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

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LBFDAU seed	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
49																										
LBFDAU seed	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
50																										
LBFDAU seed	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
70																										
LBFDAU seed	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
71																										
LBFDAU seed	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
75																										
LBFDAU seed	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
76																										
LBFDAU seed	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
77																										
LBFDAU seed	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
78																										
LBFDAU seed	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
79																										
LBFDAU seed	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
80																										
LBFDAU seed	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
81																										
LBFDAU seed	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
82																										
LBFDAU seed	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
83																										

[illegible]

C. 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

- 5 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 were measured per event. For each event, T2 seedbatches of two homozygous T1 plants where selected for seeding. Comparison of the results with Table 135 confirms homozygosity 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	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Pl_GA)2	j-p-LuPXR_j-Atss15	j-p-PvARC5_t-BnSETL	c-d5Des(Tc_GA)	j-i-Atss18_c-03Des(Pl_GA2)	c-03Des(Pl_GA)	j-p-BnSETL-v1_c-03DES(Plr_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)	j-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. 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	16:3	18:0	18:1	18:2	18:2	18:3	18:3	18:4	20:0	20:1	20:2	20:3	20:3	20:4	20:4	20:5		22:1	22:4	22:5	22:5	22:6	22:4	20:2
	n-7	n-7	n-3	n-3	n-9	n-6	n-9	n-3	n-6	n-3	20:0	n-9	n-6	n-3	n-6	n-3	n-6	n-3	n-3	n-9	n-6	n-3	n-6	n-3	n-3	n-9
LBFDAU (n=218)	4.8 ± 0.2 ±			4.2 ± 23 ±	31.5	1.1 ± 4.6 ±	2.2 ± 0.4 ±	1 ± 0.1	0.1	0.1	0.7 ± 0.1 ±	0.1 ± 0.1 ±	0.1 ± 0.1 ±	0.1 ± 4.5 ±	3.2 ± 1.4 ±	10.5	0.4 ±				0.4 ± 2.3 ±	0.2 ± 2.1 ±	2.1 ± 0.6 ±	0.4 ±		
	0.2	0	0 ± 0.3	1.6 ± 1.2 ±	0.4	0.4	0.1	0.1	0	0	0	0	0	0.8	0.6	0.3	± 1.4	0	0 ± 0.1	0.3	0.1	0.4	0.2	0.1		
LBFDGG (n=200)	5 ± 0.2 ±			3.2 ± 30.4	34.1	0.8 ± 5 ±	1.6 ± 0.3 ±	0.9 ± 0.7 ±	0.1 ± 0.1 ±	2 ± 0.4 ±	7 ± 0.4 ±										0.4 ± 2 ±	0.1 ± 1.3 ±	0.3 ± 0.1 ±			
	0.2	0	0 ± 0.3	± 2.2 ± 1.6 ±	0.1	0.5	0.2	0	0.1	0	0	0	0	0.2	0.2	0.3	0.8	0	0 ± 0.1	0.2	0	0.3	0	0	0	
LBFGKN (n=182)	4.8 ± 0.2 ±			3.6 ± 28.3	34.6	0.7 ± 4.7 ±	1.8 ± 0.3 ±	0.9 ± 0.7 ±	0.2 ± 0.1 ±	2.4 ± 1.3 ±	7.9 ± 0.4 ±										0.4 ± 2 ±	0.1 ± 1.6 ±	0.3 ± 0.2 ±			
	0.2	0	0 ± 0.4	± 1.9 ± 1.6 ±	0.2	0.5	0.4	0.2	0.1	0	0	0	0.1	0	0.3	0.2	0.3	0.9	0	0 ± 0.1	0.2	0.1	0.3	0.1	0.1	
LBFIHE (n=157)	4.9 ± 0.2 ±			3.7 ± 25.9	32.9	0.8 ± 4.7 ±	2.4 ± 0.3 ±	0.8 ± 0.7 ±	0.2 ± 0.1 ±	2.7 ± 1.7 ±	2.5 ± 9.6 ±	0.4 ±									0.4 ± 2.2 ±	0.2 ± 2 ±	2 ± 0.3 ±	0.3 ±		
	0.3	0	0 ± 0.6	± 1.9 ± 1.8 ±	0.1	0.6	0.7	0.1	0.1	0	0	0	0.1	0.1	0.3	0.3	0.4	1.3	0	0 ± 0.1	0.3	0.1	0.5	0.1	0.1	
LBFLDI (n=229)	5.9 ± 0.3 ±	0 ±	2.9 ± 27.8	32.5	0.7 ± 4.7 ±	1.7 ± 0.4 ±	0.8 ± 0.6 ±	0.1 ± 0.1 ±	0.1 ± 0.1 ±	2.4 ± 1.4 ±	2.4 ± 9.2 ±	0.3 ±									0.5 ± 2.6 ±	0.1 ± 1.7 ±	0.3 ± 0.2 ±			
	1	0.1	0.3	± 2.5 ± 1.8 ±	0.1	0.5	0.3	0.1	0.1	0.1	0.1	0	0	0	0.3	0.2	0.4	1.5	0	0 ± 0.1	0.4	0	0.4	0.1	0	
LBFPRA (n=196)	5 ± 0.2 ±			3.9 ± 23.2	30.9	1.1 ± 3.8 ±	2.6 ± 0.3 ±	0.9 ± 0.6 ±	0.1 ± 0.1 ±	3 ± 1.7 ±	3.5 ± 13.5	0.4 ±									0.3 ± 2.6 ±	0.1 ± 1.7 ±	0.1 ± 0.3 ±			
	0.2	0	0 ± 0.7	± 2 ± 1.9 ±	0.2	0.6	0.5	0.1	0.1	0	0	0	0	0	0.4	0.2	0.4	± 2	0	0 ± 0.1	0.3	0.1	0.3	0	0.1	
LBFDHG (n=177)	5.7 ± 0.3 ±			3.2 ± 24.1	34.5	0.8 ± 4.4 ±	2.5 ± 0.3 ±	0.9 ± 0.7 ±	0.1 ± 0.1 ±	3.1 ± 1.9 ±	1.5 ± 8.5 ±	0.4 ±									0.8 ± 2.5 ±	0.2 ± 2.4 ±	0.8 ± 0.2 ±			
	0.2	0	0 ± 0.5	± 0.8 ± 1.4 ±	0.1	0.3	0.5	0.1	0.1	0	0	0	0	0	0.6	0.4	0.3	1.2	0	0 ± 0.1	0.3	0.1	0.3	0.2	0.1	
LBFLFK (n=195)	5 ± 0.2 ±	0.1 ± 4 ±	25.9	31.9	1 ± 4.7 ±	1.8 ± 0.4 ±	1 ± 0.7 ±	0.1 ± 0.1 ±	0.1 ± 0.1 ±	4 ± 2.4 ±	1.4 ± 8.3 ±	0.4 ±									0.6 ± 2.7 ±	0.2 ± 1.5 ±	0.8 ± 0.3 ±			
	0.1	0	0.1	0.4	± 1.8 ± 1.3 ±	0.2	0.4	0.3	0.1	0.1	0	0	0	0	0.9	0.5	0.3	1	0	0 ± 0.1	0.5	0	0.4	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.

		16:1	16:3		18:1	18:2	18:2	18:3	18:3	18:4		20:1	20:2	20:3	20:3	20:3	20:4	20:4	20:5		22:1	22:4	22:5	22:5	22:6	22:6	22:4	20:2
Event	16:0	n-7	n-3	18:0	n-9	n-6	n-9	n-3	n-3	n-3	20:0	n-9	n-6	n-3	n-6	n-3	n-3	n-6	n-3	22:0	n-9	n-6	n-3	n-6	n-3	n-3	n-3	n-9
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	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	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	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	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	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	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	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	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 were 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	

D. 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 means instead of means were used in the statistical analysis. Common letters in the Table 154, Table 155 and Table 156 indicate 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 statistically 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 5 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 :1 16 :0	16 :3 n- 7	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 :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 :1 n- 9	22 :4 n- 6	22 :5 n- 3	22 :5 n- 6	22 :6 n- 3	22 :4 n- 3	20 :2 n- 9
LBFD AU (n=16)	4. 7 ± 0. 1	0. 0 ± ± 0	2. 7 ± 0. 1	28 .6 ± 1. 5	29 .2 ± 0. 7	1 1 ± 0. 1	6 1 ± 0. 3	1. 0 ± 0. 0	0. 0 ± ± 0	0. 0 ± ± 0	0. 0 ± ± 0	3 3 ± 0. 3	2 2 ± 0. 2	2 2 ± 0. 2	10 .7 ± 0. 7	0. 0 ± ± 0	0. 0 ± ± 0	2. 9 ± 0. 2	0. 0 ± ± 0	1. 6 ± 0. 2	0. 3 ± 0. 2	0. 0 ± ± 1
LBFD GG (n=36)	4. 7 ± 0. 1	0. 0 ± ± 0	2. 5 ± 0. 2	34 .2 ± 1. 9	32 .3 ± 0. 2	0. 6 ± 0. 1	1. 7 ± 0. 5	1. 2 ± 0. 1	0. 0 ± ± 0	0. 0 ± ± 0	0. 0 ± ± 0	0. 0 ± ± 0	2 3 ± 0. 3	1 9 ± 0. 2	6. 1 ± 0. 7	0. 0 ± ± 0	0. 0 ± ± 0	2. 1 ± 0. 2	0. 0 ± ± 0	1. 1 ± 0. 2	0. 2 ± 0. 1	0. 0 ± ± 0
LBFG KN (n=36 +60)	4. 6 ± 0. 2	0. 0 ± ± 0	2. 6 ± 0. 2	33 .7 ± 1. 7	32 .8 ± 0. 4	0. 6 ± 0. 1	7. 5 ± 0. 6	0. 9 ± 0. 1	0. 0 ± ± 0	0. 8 ± 0. 1	0. 0 ± ± 0	0. 0 ± ± 0	1 2 ± 0. 3	1. 8 ± 0. 1	1. 6 ± 0. 2	0. 0 ± ± 0	0. 0 ± ± 0	0. 3 ± 0. 1	1 1 ± 0. 2	0. 0 ± ± 0	1 0 ± ± 0	0. 0 ± ± 0
LBFIH E (n=36)	4. 8 ± 0. 2	0. 0 ± ± 0	2. 6 ± 0. 2	31 .2 ± 1. 7	33 .9 ± 0. 2	0. 6 ± 0. 1	6. 7 ± 0. 7	1. 3 ± 0. 2	0. 0 ± ± 0	0. 7 ± 0. 1	0. 0 ± ± 0	0. 0 ± ± 0	1 2 ± 0. 2	1. 4 ± 0. 1	2. 7 ± 0. 3	6. 0 ± ± 0	0. 0 ± ± 0	0. 3 ± 0. 1	1. 9 ± 0. 2	0. 0 ± ± 0	1. 2 ± 0. 2	0. 2 ± ± 0

LBFL	4.			2.	33	32	0.	6.			0.					1.		6.				2.		1.		
DI	9	0.		5	.4	.7	6	8	1	0.	7	0.	0.	0.	2	2	2	2	0.		0.	1	0.	1	0.	0.
(n=36)	±	3	0	±	±	±	±	±	±	2	±	8	2	1	±	±	±	±	3	0	3	±	1	±	2	2
	0.	±	±	0.	1.	1.	0.	0.	0.	±	0.	±	±	±	0.	0.	0.	0.	±	±	±	0.	±	0.	±	±
	2	0	0	2	7	2	1	6	1	0	1	0	0	0	3	2	2	7	0	0	0	2	0	2	0	0
LBFL	4.			2.			0.	6.	1.		0.				4.	2.	1.	6.			0.	2.		1.		
T	6	0.		7	30	29	9	5	6	0.	7	0.	0.	0.	4	6	5	8	0.		6	9	0.	2	1	0.
(n=32)	±	2	0	±	±	.9	±	±	±	3	±	7	1	1	±	±	±	±	3	0	±	±	1	±	±	3
	0.	±	±	0.	1.	±	0.	0.	0.	±	0.	±	±	±	0.	0.	0.	0.	±	±	0.	0.	±	0.	0.	±
	1	0	0	2	4	1	1	5	1	0	1	0	0	0	4	2	2	7	0	0	1	3	0	2	1	0
LBFL	4.			2.	28	32	0.	5.	1.	0.					2.	1.	3.					2.		1.		0.
RA	8	0.		6	.4	.7	8	7	6	3	0.	0.	0.	0.	3	2	8	9.	0.		0.	4	0.	1	0.	2
(n=36)	±	2	0	±	±	±	±	±	±	±	7	8	2	1	±	±	±	6	3	0	3	±	1	±	1	±
	0.	±	±	0.	2.	1.	0.	0.	0.	0.	±	±	±	±	0.	0.	0.	±	±	±	±	0.	±	0.	±	0.
	2	0	0	2	1	4	1	4	2	1	0	0	0	0	3	2	5	1	0	0	0	3	0	2	0	1
LBFL	5.			2.	28		0.		1.									9.				3.		1.	0.	
HD	2	0.		4	.2	29	9	6.	6	0.	0.	0.	0.	0.	2.	1.	2	6	0.		0.	5	0.	9	5	0.
(n=4)	±	2	0	±	±	.5	±	5	±	3	6	7	2	1	9	7	±	±	3	0	6	±	2	±	±	3
	0.	±	±	0.	1.	±	0.	±	0.	±	±	±	±	±	±	±	0.	0.	±	±	±	0.	±	0.	0.	±
	1	0	0	1	4	1	2	0	1	0	0	0	0	0	0	0	1	4	0	0	0	1	0	2	1	0
LBFL	4.			2.	30	30	0.	6.	1.	0.					3.	1.	1.					3.		1.	0.	0.
FK	7	0.		6	.1	.2	9	2	5	3	0.	0.	0.	0.	3	9	9	8.	0.		0.	2	0.	4	5	3
(n=36)	±	2	0	±	±	±	±	±	±	±	6	8	1	1	±	±	±	2	3	0	5	±	1	±	±	±
	0.	±	±	0.	1.	1.	0.	0.	0.	0.	±	±	±	±	0.	0.	0.	±	±	±	±	0.	±	0.	0.	0.
	2	0	0	2	9	1	1	4	2	1	0	0	0	0	3	2	2	1	0	0	0	4	0	3	1	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 n- 7	16 n- 3	18 :0	18 n- 9	18 n- 6	18 n- 9	18 n- 3	18 n- 6	18 n- 3	20 :0	20 n- 9	20 n- 6	20 n- 3	20 n- 6	20 n- 3	20 n- 6	20 n- 3	22 :0	22 n- 9	22 n- 6	22 n- 3	22 n- 6	22 n- 3	22 n- 6	20 :0
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
LBFDG G (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	6. 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
LBFLDI (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	0. 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
LBFDH G (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- VC-LTM593-1qcz rc. The events are indicated in the first column, along with the number of field plots that were 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 Height: 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	Sta nd		Vigo r		First Flow er		Last Flowe r		Mat urity		Plan t Heig ht		Lod ging		Sha tter		Agr on Sco re		Mois ture		TWK		Oil		Prot ein	
LBFD AU (n=16)	2 3 . 7	. a	. 5 b c d	. b c d	4 3 . 8	. b c d e	7 7 . 3	a b c d e	9 4 . 5	. a 5	1 1 5 b		2 . a b	3 b	1 . . 5	a 7	4 . a b	9 . 0	a a	3 . 5	a b c d	3 8 . 5	b c	2 6 . 6	. c	
LBFD GG (n=36)	2 3 . 0	. a	. 5 b c d	. b c d	4 4 . 0	. b c d	7 7 . 1	b c d	9 4 . 3	. a	1 1 6 2	. . . b	2 . . 3	b	1 . . 6	a 2	4 . a b	8 . 9	a a	3 . 8	a b c a	3 8 . 8	a b c	2 6 . 3	. c	
LBFD HG (n=4)	2 3 . 4	. .	. 5 . 8	. . .	4 3 . 9	. . .	7 7 . 2	. . .	1 1 5 9		1 1 5 9		2 . . 3		1 . . 5	4 . . 4	9 . 0	. . .	3 . 7	. . .	3 9 . 3	a b c	2 6 . 5	. c		
LBFG KN (n=36)	2 2 . 8	. a	. 6 b c d	. b c d	4 3 . 3	. c d e	7 6 . 5	c d e	9 4 . 2	. a	1 1 8 b	. . . b	2 . a b	1 . . 8	a a	4 . a b	8 . 5	a a	3 . 7	a b	3 6 . 7	. c	2 6 . 5	. c		
LBFI HE (n=36)	2 1 . 7	. a	. 5 . c d	. c d	4 5 . 7	. . . b	7 9 . 7	a a b	9 4 . 7	. a	1 1 5 2	. . . b	2 . . 3	1 . . b	4 . a a	1 b	8 . 7	a a	3 . 4	b c d	3 9 . 8	a b	2 6 . 5	. c		
LBFL FK (n=36)	2 2 . 5	. a	. 5 . d	. . d	4 5 . 6	. b c	7 8 . 0	a b c	9 4 . 3	. a	1 1 8 4	. . . b	2 . a b	1 . . 5	a a	4 . a b	8 . 6	a a	3 . 7	a a	3 8 . 3	b c	2 7 . 1	. c		

Event	Stand		Vigor		First Flow-er		Last Flow-er		Mat-ur-ity		Plan-t Heig-ht		Lod-ging		Sha-tter		Agr-on Sco-re		Mois-ture		TWK		Oil		Prot-ein	
LBFP RA (n=36)	2 2 .	a b c	6 b c	3 d	4 4 .	b b c	7 8 .	a b c	9 4 .	a b c	1 2 1.	b a b	2 2 .	a b b	1 1 .	a b a	4 4 .	a b a	8 8 .	a b a	3 3 .	a b c	3 9 .	a b c	2 6 .	c
Topa s	2 4 .	a b c	5 d	2 d	4 8 .	a b c	8 0 .	a b c	9 5 .	a b c	1 3 9.	a b b	3 3 .	a b b	2 2 .	a b a	4 4 .	a b a	7 7 .	b b c	3 3 .	c d	3 7 .	c d	2 6 .	c
Kumil y	2 8 .	a b c	6 a b	9 b	4 3 .	c d e	7 6 .	c d e	9 4 .	a b c	1 1 9.	b a b	2 2 .	a b a	1 1 .	a b a	4 4 .	a b a	7 7 .	b b c	3 3 .	a b c	3 9 .	a b c	2 8 .	a
Contr ol 1	2 8 .	a b c	7 a b	7 a	4 2 .	c d e	7 3 .	e f g	8 9 .	b c d	1 2 1.	b a b	3 3 .	a b a	2 2 .	a b a	3 3 .	b b c	7 7 .	b b c	3 3 .	d e f	3 9 .	a b c	2 7 .	c
Contr ol 2	2 5 .	a b c	6 a b	6 c	4 0 .	d e f	7 4 .	e f g	9 0 .	b c d	1 1 9.	b a b	3 3 .	a b a	2 2 .	a b a	3 3 .	b b c	7 7 .	b b c	3 3 .	d e f	4 0 .	a b c	2 7 .	b c

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- 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 harvested from 4 different geographic regions. Alpha-Tocopherol (mg/100g seed), Beta-Tocopherol (mg/100g seed), Delta-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	
LB FD AU	13.3	a b	0.25	a	0.58	a	2.95	a	4.37	a	0.64	c d	11.0	b c d	2.3	B	4.5	a b	9.4	a	11.8	ab	14.5	a
LB FD GG	14.1	a b	0.23	a	0.45	b c d	2.56	b	4.04	a b c	0.69	b c d	13.1	a b	2.3	B	4.3	a b	9.2	a	10.9	cd ef	13.6	a b
LB FG KN	12.9	b	0.23	a	0.52	a b c	2.69	a b	4.06	a b c	0.70	b c	11.3	b c	2.3	b	4.4	a b	9.3	a	11.0	bc def	13.5	a b
LB FIH E	13.2	a b	0.23	a	0.45	b c d	2.0	c d	3.59	c d e	0.65	c d	15.7	a	2.2	b	4.7	a b	9.1	a	10.8	cd ef	13.4	a b
LB FL FK	12.5	b	0.23	a	0.52	a b c	2.57	b	3.89	a b c	0.68	b c d	12.6	a b c	2.3	b	4.3	b	9.6	a	11.3	ab cde	13.7	a b
LB FP RA	13.6	a b	0.22	a	0.47	b c d	2.49	b c	3.92	a b c	0.62	d	10.9	b c d	2.2	b	4.3	a b	9.6	a	12.0	a	14.6	a
Tepas	14.7	a b	0.25	a	0.36	d	1.66	e	3.19	e	0.69	b c d	7.6	d	2.7	a	4.8	a	9.0	a	10.5	ef	12.9	b
Kumily	12.3	b	0.	a	0.54	a b	2.44	b c	3.75	b c d	0.	a	11.0	b c d	2.2	b	4.3	b	9.1	a	11.1	ab c	14	a

Event	Alpha-Tocopherol	Beta-Tocopherol	Delta-Tocopherol	Gamma-Tocopherol	Tocopherols (VitE)	Sinapine	Glucosinolate	Phytate	Ash	Cru de Fiber	ADF	NDF
		23				78					.5	.3
Control 1	16.6a	0.25a	0.43cd	2.41bc	4.14ab	0.73a	9.5cd	2.3b	4.6ab	8.6a	10.2f	12.8b
Control 2	12.0b	0.20a	0.45cd	2.08d	3.35de	0.72a	11.9bc	2.3b	4.4ab	9.2a	10.6df	13.8ab

Table 156: Herbicide 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 applied at a 2x rate of 70 g imazamox /ha. Brassica napus cv Kumily, which is the non-transgenic 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
LBFPRA	2	a	1	ab	1	a
Topas	1	a	1	b	1	a

Event	IMI Injury 7 DAT		IMI Injury 14 DAT		IMI Injury 21 DAT	
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.

% Injury	1-7 Scale	Category	Injury Symptoms	Growth Rates and Recovery Effects
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 5: Environmental effects on VLC-PUFA levels. Correlation between oil content, VLC-PUFA levels, and delta-12-desaturase conversion efficiency

It was consistently observed that comparable populations yield higher VLC-PUFA levels in the greenhouse compared to the field. This trend usually coincides 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 (*i.e.* delta-12-desaturase conversion efficiency, see Figure 2). The same has been done for event LAODDN (event described in example 13), and is plotted Figure 41. This analysis was also done for two events described in example 18, namely for event LBFGKN (Figure 42), and for event LBFLFK (Figure 43). For all transgenic events, a negative correlation was observed between oil content and delta-12-desaturase conversion efficiency. This correlation was consistent across all plants, whether grown in the greenhouse or in the field.

The same analysis for wildtype Kumily (Figure 72) revealed no such correlation between delta-12-desaturase conversion efficiency and seed oil content. In addition, Figure 72 shows a difference between greenhouse grown and field grown wildtype plants, in that field grown wildtype plants have higher delta-12-desaturase conversion efficiencies compared to greenhouse grown wildtype plants, regardless of oil content. Consequently, comparing Figure 72 with Figure 40 through Figure 43, it can be concluded that the introduction of a transgeneic delta-12-desaturase on a non-native, seed-specific promoter reduced the effect of the environment on delta-12-desaturase conversion efficiency. Surprisingly, this effect is dominant over the endogenous delta-12-desaturase and results in more consistent VLC-PUFA production across environmental conditions.

Example 6: 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 OD₆₀₀ = 0.2 (in SD-medium-uracil+raffinose+galactose). After 24 hours at 30 °C the culture (typically OD₆₀₀ = 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 [¹⁴C]-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 [¹⁴C]-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:H₂O (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:CHCl₃ (2:1) and CHCl₃ (500 µl). The chloroform phase was recovered, dried under N₂(g) and fatty acids were methylated by addition of 2 ml MeOH containing 2% H₂SO₄ and incubation of 30 minutes at 90 °C. FAMES were extracted by addition of 2 ml H₂O and 2 ml hexane and separated by AgNO₃-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

5 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 [¹⁴C]18:1n-9-CoA, 16:0-lysphosphatidylcholine (LPC), and NADH membranes containing c-d12Des(Ps_GA) form an [¹⁴C]18:2-fatty acid that can be isolated as a methyl ester and resolves on AgNO₃-TLC
10 [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
15 isolated 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 [¹⁴C]acyl-CoA's which may include, but are not limited to: [¹⁴C]18:2n-6-CoA, [¹⁴C]20:3n-6-CoA, [¹⁴C]20:4n-6-CoA, [¹⁴C]22:5n-3-CoA.

20 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 [¹⁴C]acyl-CoA in the general assay described above. [¹⁴C]Acyl-CoA's may include, but are not limited to: [¹⁴C]18:1n-9-CoA,
25 [¹⁴C]18:2n-6-CoA, [¹⁴C]20:3n-6-CoA, [¹⁴C]20:4n-6-CoA, [¹⁴C]22:5n-3-CoA. Isolated fatty acid methyl esters derived from enzymatic substrates and products can be resolved using AgNO₃-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 "Desaturase Headgroup (CoA vs PC) Preference", as suggested in previous reports (Domergue et al. (2005)
30 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 [¹⁴C]acyl-CoA
35 general assay as described above. [¹⁴C]Acyl-CoA's may include, but are not limited to: [¹⁴C]18:1n-9-CoA, [¹⁴C]18:2n-6-CoA, [¹⁴C]20:3n-6-CoA, [¹⁴C]20:4n-6-CoA, [¹⁴C]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-

5 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 [¹⁴C]acyl-CoA in the general assay described above. [¹⁴C]Acyl-CoA's may include, but are not limited to: [¹⁴C]18:1n-9-CoA, [¹⁴C]18:2n-6-CoA, [¹⁴C]20:3n-6-CoA, [¹⁴C]20:4n-6-CoA, [¹⁴C]22:5n-3-CoA. Isolated fatty acid methyl esters derived from enzymatic substrates and products can be resolved using
10 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 [¹⁴C]acyl-CoA in the general assay
15 described above. [¹⁴C]Acyl-CoA's may include, but are not limited to: [¹⁴C]18:1n-9-CoA, [¹⁴C]18:2n-6-CoA, [¹⁴C]20:3n-6-CoA, [¹⁴C]20:4n-6-CoA, [¹⁴C]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.

20 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 [¹⁴C]acyl-CoA in the general assay described above. [¹⁴C]Acyl-CoA's may include, but are not limited to: [¹⁴C]18:1n-9-CoA, [¹⁴C]18:2n-6-CoA, [¹⁴C]20:3n-6-CoA, [¹⁴C]20:4n-6-CoA, [¹⁴C]22:5n-3-CoA. Isolated fatty acid
25 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(PI_GA)2 Enzyme Activity: c-d4Des(PI_GA)2

enzyme activity and substrate specificity can be demonstrated in microsomes isolated from a yeast strain expressing the c-d4Des(PI_GA)2 protein using an [¹⁴C]acyl-CoA in the general assay
30 described above. [¹⁴C]Acyl-CoA's may include, but are not limited to: [¹⁴C]18:1n-9-CoA, [¹⁴C]18:2n-6-CoA, [¹⁴C]20:3n-6-CoA, [¹⁴C]20:4n-6-CoA, [¹⁴C]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.

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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 [¹⁴C]acyl-CoA in the general assay described above. [¹⁴C]Acyl-CoA's may include, but are not limited to: [¹⁴C]18:1n-9-CoA, [¹⁴C]18:2n-6-CoA, [¹⁴C]20:3n-6-CoA, [¹⁴C]20:4n-6-CoA, [¹⁴C]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 separated 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 "Desaturase Enzyme Activity in Microsomes Isolated from Transgenic Yeast" for microsomes isolated from yeast expression strains.

In summary in "Desaturase Enzyme Activity in Microsomes Isolated from Transgenic Yeast" 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) a transgenic *B. napus* event containing genes encoding omega-3 desaturase proteins from both *Phytophthora infestans* (c-o3Des(Pi_GA2)) and *Pythium irregulare* (c-o3Des(Pir_GA)) that contains at least one enzyme, localized to the microsomes, capable of desaturating arachidonic acid (20:4n-6) to form eicosapentaenoic acid (20:5n-3) (Figure 85, Panel C), (5) 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, (6) gene c-d4Des(PI_GA)2 encodes a delta-4 desaturase protein from *Pavlova lutheri* (c-d4Des(PI_GA)2 that desaturates docosapentaenoic acid (22:5n-3) to form docosahexaenoic acid (22:6n-3) in microsomes isolated from a transgenic yeast (Figure 24, panel D), (7) 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(PI_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), (8) a transgenic *B. napus* event containing the gene encoding a delta-6 desaturase protein from *Ostreococcus tauri* (c-d6Des(Ot_febit), capable of desaturating linoleic acid (18:2n-6) to form gamma-linolenic acid (18:3n-6) (Figure 85, Panel A), and (9) a transgenic *B. napus* event containing the gene encoding a delta-5 desaturase protein from *Thraustachytrium* ssp. (c-d5Des(Tc_GA2), capable of desaturating dihomo-gamma-linolenic acid (20:3n-6) to form arachidonic acid (20:4n-6) (Figure 85, Panel B). 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 and Figure 85).

Using the methods described in "Desaturase Enzyme Activity in Microsomes Isolated from Transgenic Yeast" 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, "Desaturase Enzyme Activity in Microsomes Isolated from Transgenic Yeast" 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, "Desaturase Activity in Microsomes Isolated from Transgenic *Brassica napus*". 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 CoA, phospholipid and neutral lipid pools over a growth time course. However, highly active endogenous 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 backbone utilized by the desaturase of interest.

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 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 lysophosphatidyl choline acyl transferase (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. Furthermore, the assays testing for desaturation of acyl-phospholipid substrates, described in Demonstration of Phosphatidylcholine Specificity, can be complemented by testing in an assay developed to monitor desaturation of the substrate in the acyl-CoA form. Specifically, we have devised a strategy, described in Demonstration of acyl-CoA Specificity, in which the substrate to be tested remains in its acyl-CoA form and is not incorporated into phospholipids (e.g. phosphatidylcholine) by lysophosphatidyl choline acyl transferase (LPCAT). By comparing the relative desaturase activity, observed in assays where the substrate is in the acyl-phospholipid form compared to the acyl-CoA form, the actual backbone (e.g. phosphatidylcholine or CoA) covalently bound to the desaturated fatty acid product can be determined.

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 "Desaturase Enzyme Activity in Microsomes Isolated from Transgenic Yeast", but after a pre-incubation in the presence 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 analysis of the aqueous phase.

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 μ l 0.15 M acetic acid and 1 ml MeOH:CHCl₃ (1:1). Part (about 10%) of the CHCl₃ 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 [CHCl₃: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% H₂SO₄ at 90 °C for 30 minutes. The methyl esters were extracted in hexane and analyzed as described above for the respective enzymes (Example 21, "Desaturase Enzyme Activity in Microsomes Isolated from Transgenic Yeast"). 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:H₂O (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 (0.7 ml), 1.4 ml of MeOH) and CHCl₃ (1.9 ml). The chloroform phase was recovered, dried under N₂(g) and fatty acids were methylated by addition of 2 ml MeOH containing 2% H₂SO₄ and incubation of 30 minutes at 90 °C. FAMES were extracted by addition of 2 ml H₂O and 2 ml hexane and separated by AgNO₃-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 "Desaturase Enzyme Activity in Microsomes Isolated from Transgenic Yeast" can be further characterized to establish the backbone of the oleic acid substrate. In the desaturase assay described in "Desaturase Headgroup (CoA vs PC) Preference" 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. However, a preincubation with 20:1n-9-CoA results in PC saturated with 20:1n-9, thus precluding incorporation of [¹⁴C]-18:1n-9 into PC (described in "Demonstration of Acyl-CoA Specificity"). 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 and Figure 86, Panel A). Furthermore, the d12Des(Ps_GA) activity was negligible in the assay for demonstration of acyl-CoA specificity (Figure 86, Panel B), showing

that 18:1n-9-acyl-CoA is not a preferred substrate for the delta-12 desaturase (*Phytophthora sojae*). In conclusion, delta-12 desaturase (*Phytophthora sojae*) clearly desaturates 18:1n-9 covalently bound to PC, but not an 18:1n-9-acyl CoA substrate.

5 Delta-4 desaturase (*Thraustochytrium* ssp.), c-d4Des(Tc_GA) Substrate Preference: The c-d4Des(Tc_GA) enzyme activity demonstrated in "Desaturase Enzyme Activity in Microsomes Isolated from Transgenic Yeast" can be further characterized to establish the backbone of the docosopentaenoic acid substrate. In the desaturase assay described in "Desaturase Headgroup (CoA vs PC) Preference" without additional 16:0-lysphosphatidylcholine (LPC), desaturation was
10 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 [¹⁴C]22:5n-3 from CoA to 16:0-LPC
15 generating a [¹⁴C]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 "Desaturase Enzyme Activity in Microsomes Isolated from Transgenic Yeast". The microsomal fraction of the yeast strain expressing the enzyme of interest was pre-incubated with 10 nmol 20:1n-9-CoA (50 μM) and 0.5 mM DTNB
20 (5,5'-dithiobis-(2-nitrobenzoic acid) for 10 min before addition of NADH and [¹⁴C]labelled acyl-CoA substrate. The preincubation with 20:1n-9-CoA minimizes the incorporation of [¹⁴C]labelled substrate into PC. DTNB prevents the reverse reaction of LPCAT and thereby the entering of acyl-CoA into PC via acyl exchange. 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
25 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:CHCl₃ (1:1). Part (about 10%) of the CHCl₃ 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 [CHCl₃:MeOH:acetic
30 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% H₂SO₄ at 90 °C for 30 minutes.

The methyl esters were extracted in hexane and analyzed as described above for the respective enzymes "Desaturase Enzyme Activity in Microsomes Isolated from Transgenic Yeast". 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:H₂O (1:4) and incubated for 20 minutes at 90 °C. Fatty acids were extracted by addition of 3 M HCl (0.7 ml), 1.4 ml of MeOH and CHCl₃ (1.9 ml). The chloroform phase was recovered, dried under N₂(g) and fatty acids were methylated by addition of 2 ml MeOH containing 2% H₂SO₄ and incubation of 30 minutes at 90 °C. FAMES were extracted by addition of 2 ml H₂O and 2 ml hexane and separated by AgNO₃-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 and DTNB addition) leads to the desaturated product in the H₂O-phase (or product in any of the lipid pools PC/FFA/H₂O since PC-dependent enzymes cannot be active if the substrate is not incorporated into PC (see Figure 86, Panel B), it can be concluded that the enzyme was acyl-CoA dependent (see Figure 27 and Figure 87, Panels A and B). Similarly if a desaturase demonstrates activity in the PC-specific assay, but not in the assay where the substrate is presented as an acyl-CoA, then it can be concluded the enzyme utilizes a fatty acid covalently attached to phosphatidylcholine as a substrate.

Delta-9 desaturase (*Saccharomyces cerevisiae*), d9Des(Sc) Substrate Preference:

Analysis of the [¹⁴C]-distribution during of the d9Des(Sc) reaction, in the assay for demonstration of acyl-CoA dependency, shows that greater than 95% of the radioactivity (substrate and product) is present in the H₂O (CoA) and FFA-pools (data not shown), indicating incorporation into PC was insignificant. During the reaction, product (16:1n-9) in the acyl-CoA pool increases linearly up to 60 minutes, showing that the enzyme preferentially converts 16:0 covalently bound to CoA (Figure 87, Panel B). The amount of 16:1n-9 in the H₂O fraction then levels out or slightly decreases, while the 16:1n-9 in the FFA pool increases, due to degradation of acyl-CoA by thioesterases present in the isolated membranes.

In the assay for demonstrating PC specificity, the d9Des(Sc) showed no activity (Figure 87, Panel A), which indicates that when 16:0 fatty acid is attached to PC (or a FFA) it is not a preferred substrate.

The clear presence of desaturase activity in the "Acyl-CoA Specific" assay compared to the absence of activity in the "Phosphatidylcholine Specific" assay demonstrates that the delta-9 desaturase (*Saccharomyces cerevisiae*) utilizes 16:0 covalently attached to Coenzyme A. Interestingly, recent crystal structures of both the human and mouse stearyl-coenzyme A desaturases have been reported with bound stearyl-CoA confirming that this desaturase utilizes a coenzyme A substrate (Wang et al (2015) Nat Struct Mol Bio 22: 581-585 and Bai et al (2015) Nature 524: 252-257).

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 embodiment of the invention 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*, *Pseudomonas aeruginosa*, *Bacillus thuringiensis*), mammalian tissue (e.g. liver) and plant tissue (e.g. leaves, 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 "Desaturase Enzyme Activity in Microsomes Isolated from Transgenic Yeast". Isolation of microsomes containing expressed elongases was generally as described above in "Desaturase Enzyme Activity in Microsomes Isolated from Transgenic Yeast" 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 MgCl₂, 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 CaCl₂, protease inhibitor). The protein concentrations in the microsomes were measured according to the BCA method. Resuspended microsomes were aliquoted and frozen in N₂(l) and stored at -80 °C.

In the elongase assay [¹⁴C]-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 [¹⁴C]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:CHCl₃ (2:1) and CHCl₃ (250 µl). The chloroform phase was recovered, dried under N₂(g), and fatty acids were methylated by addition of 2 ml MeOH containing 2% H₂SO₄ and incubation of 30 minutes at 90 °C. FAMES were extracted by addition of 2 ml H₂O 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 MgCl₂, 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 [¹⁴C]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.

5 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.

10 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 in 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.

25 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 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 "Elongase Activity" 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*.

It is to be understood that, if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art, in Australia or any other country.

In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express language or necessary implication, the word "comprise" or variations such as "comprises" or "comprising" is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

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Claims

1. An assay method for simulating a metabolic property of a plant of order Brassicales grown under field conditions, comprising:
 - i) providing a transgenic plant of order Brassicales expressing a delta-12 desaturase, wherein said delta-12 desaturase has at least 96% total amino acid sequence identity to SEQ ID NO: 328,
and wherein the plant also expresses at least one or more enzymes of unsaturated fatty acid metabolism, of which enzymes at least one is capable of using linoleic acid as a substrate, and of which enzymes at least one is connected to a plant metabolic property,
 - ii) growing the plant under greenhouse conditions, and
 - iii) measuring said plant metabolic property for said plant, wherein the measured metabolic property simulates the metabolic property measurement for the plant grown under field conditions;
- 15 wherein the plant metabolic property is production of polyunsaturated fatty acids.
2. An assay method according to claim 1, wherein the plant is of family Brassicaceae.
3. An assay method according to claim 1 or 2, wherein the Delta-12 desaturase is obtained
10 from or identical to a Delta-12 desaturase of an organism of class oomycetes.
4. An assay method according to any one of claims 1 to 3, wherein the delta-12 desaturase expressed by the transgenic plant has 100% total amino acid sequence identity to SEQ ID NO. 328.

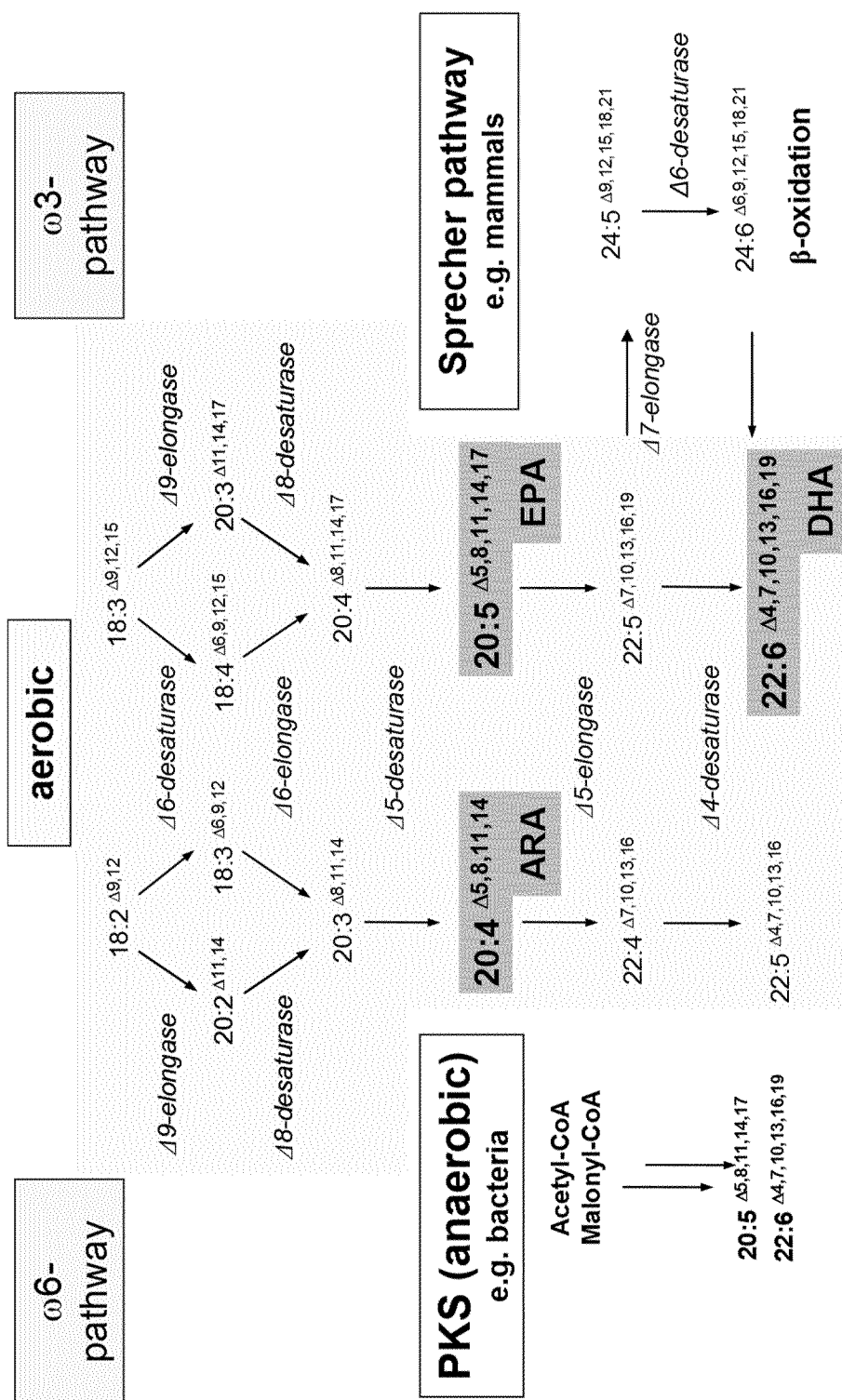


Fig. 1

Fig. 2

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	18:2n-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									
	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 List of all o3Des Fatty acid products		18:2n-6	18:3n-6	20:2n-6	20:3n-6	20:4n-6	22:4n-6	22:5n-6		
			18:3n-3	18:4n-3	20:3n-3	20:4n-3	20:5n-3	22:5n-3	22:6n-3		

Fig. 3

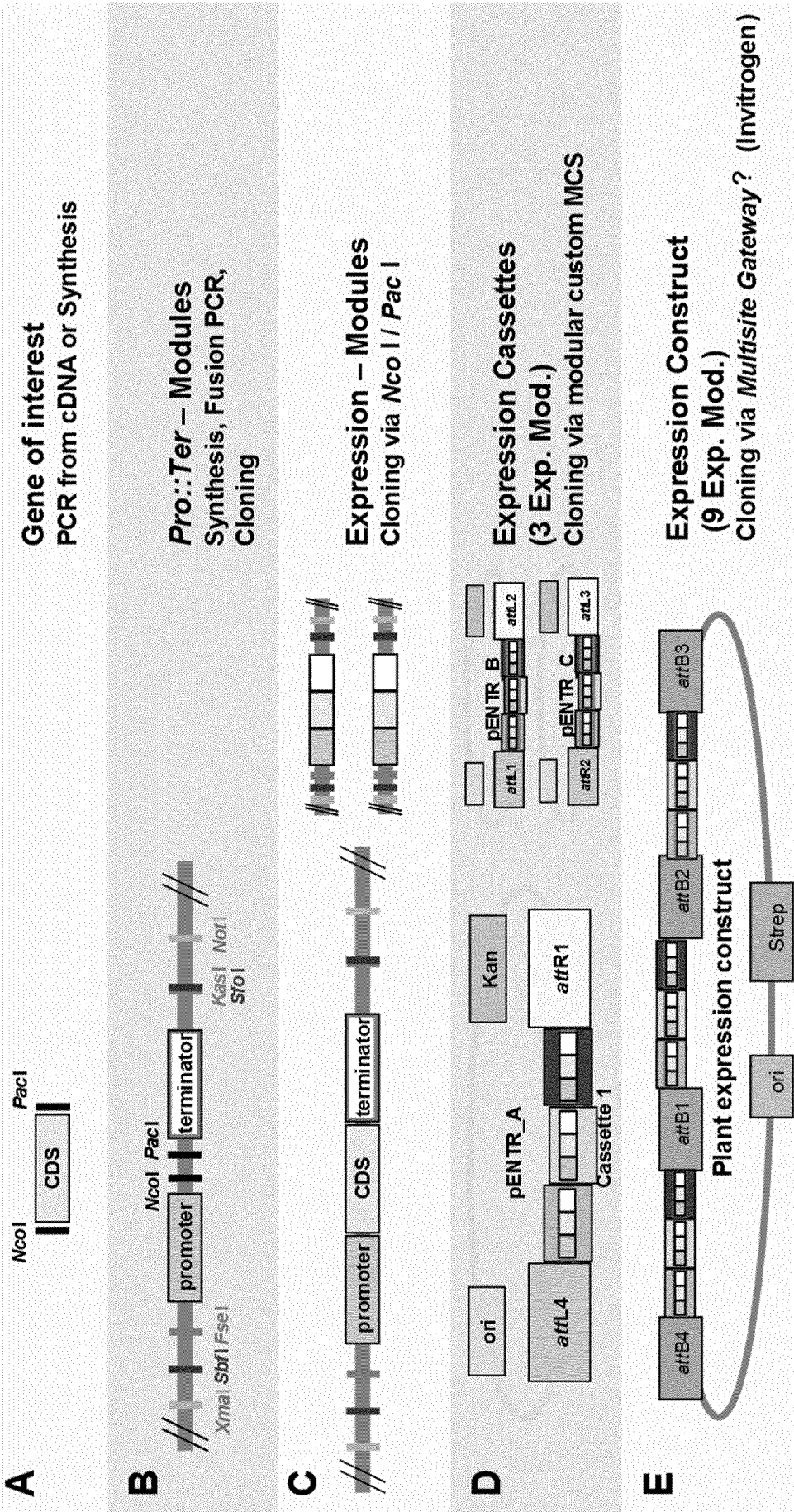


Fig. 4

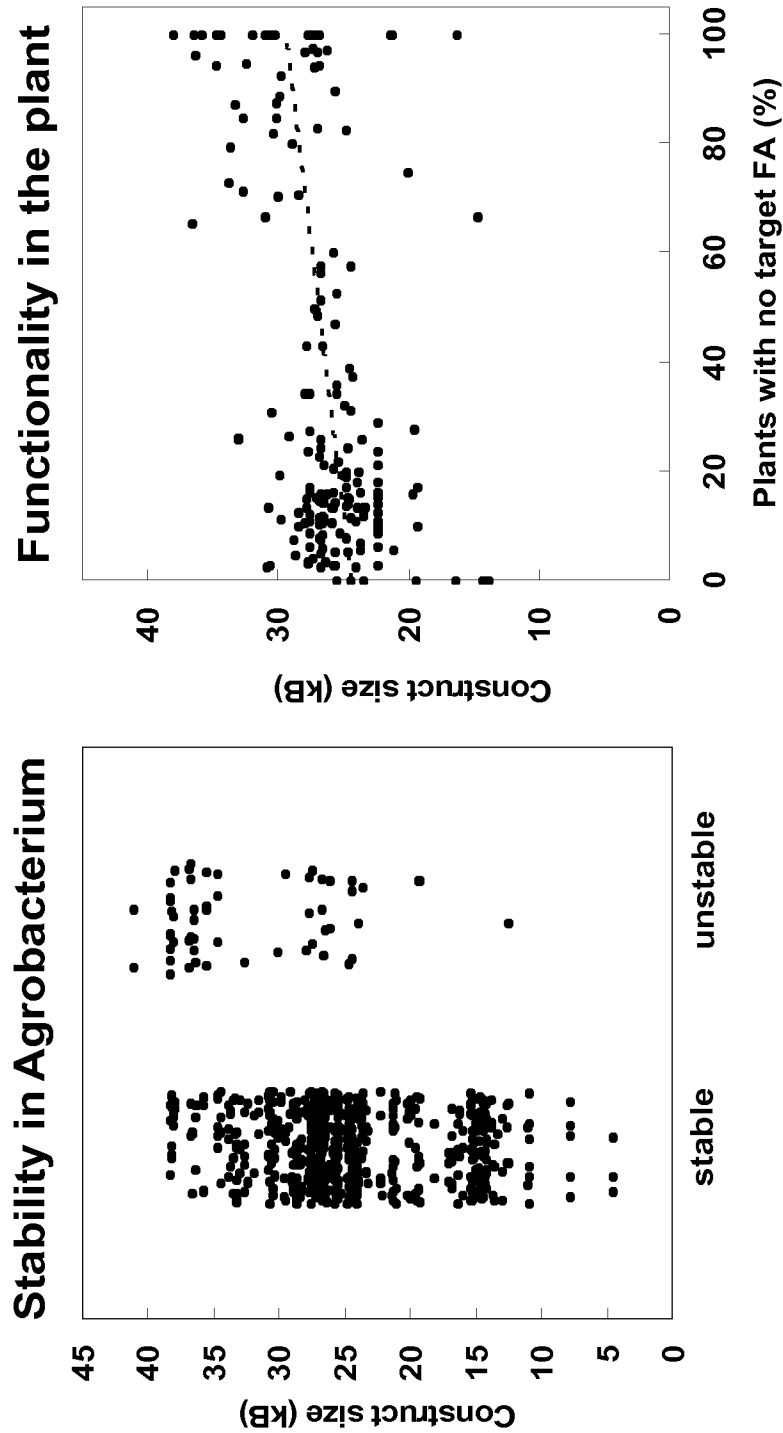


Fig. 5

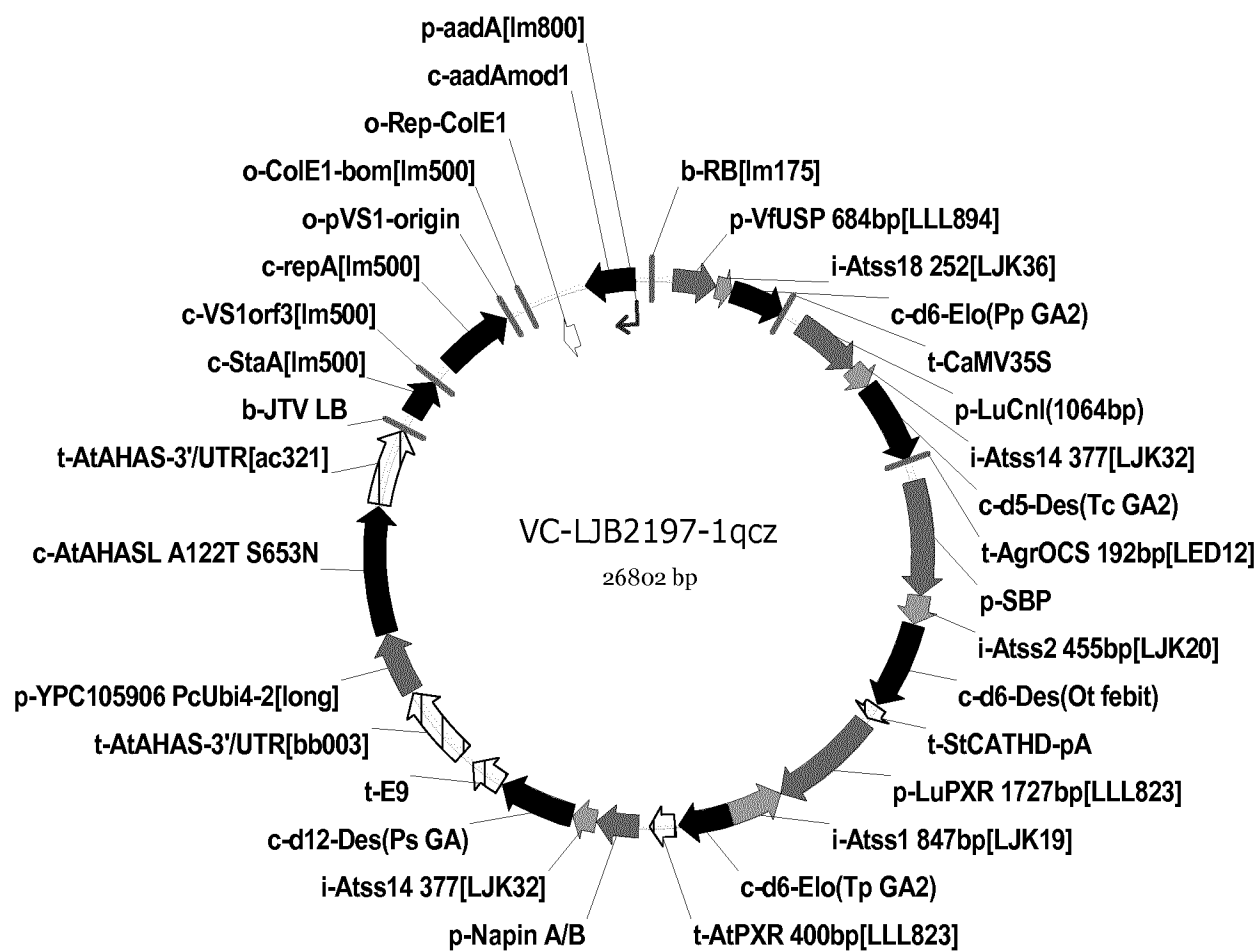


Fig. 6

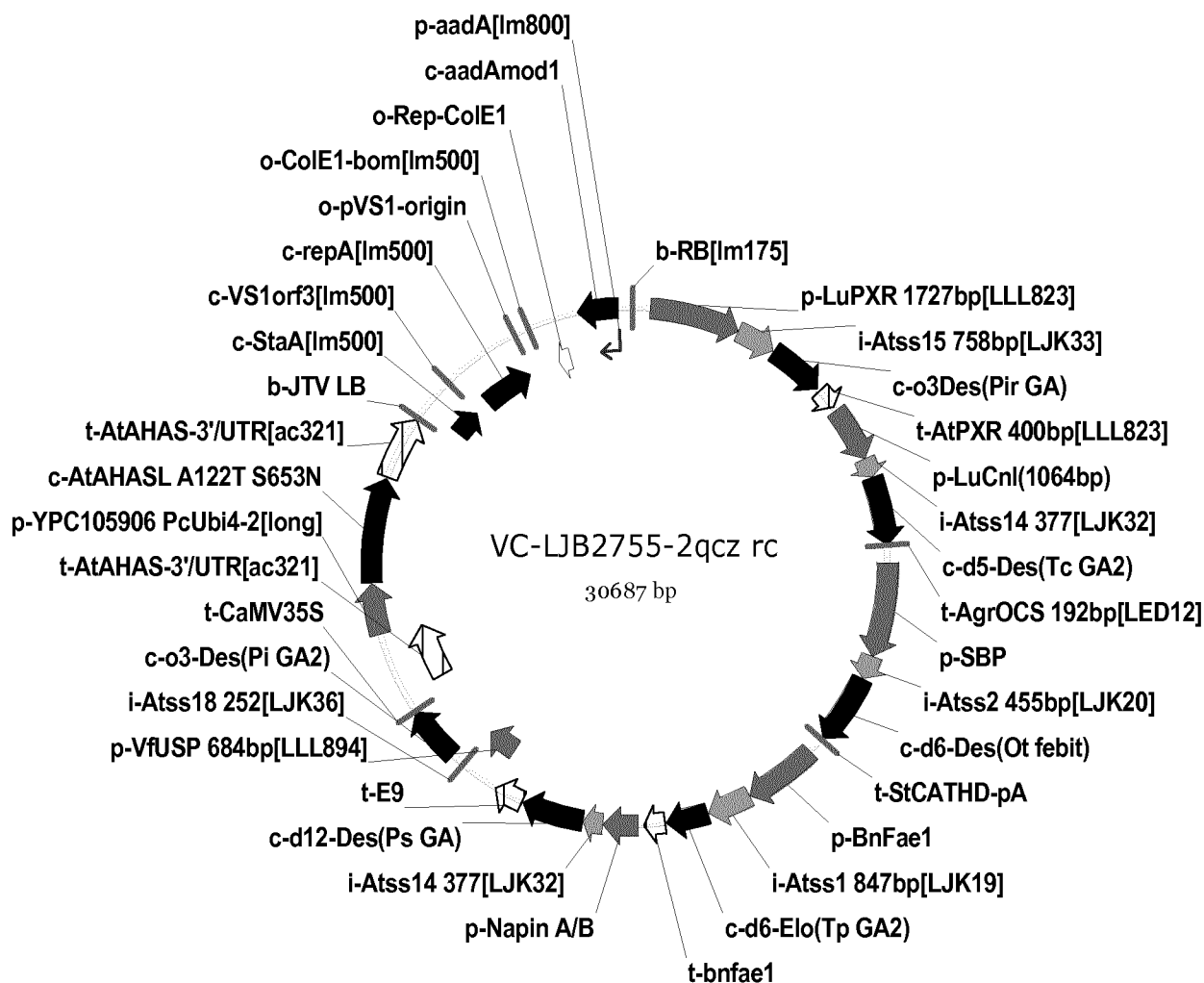


Fig. 7

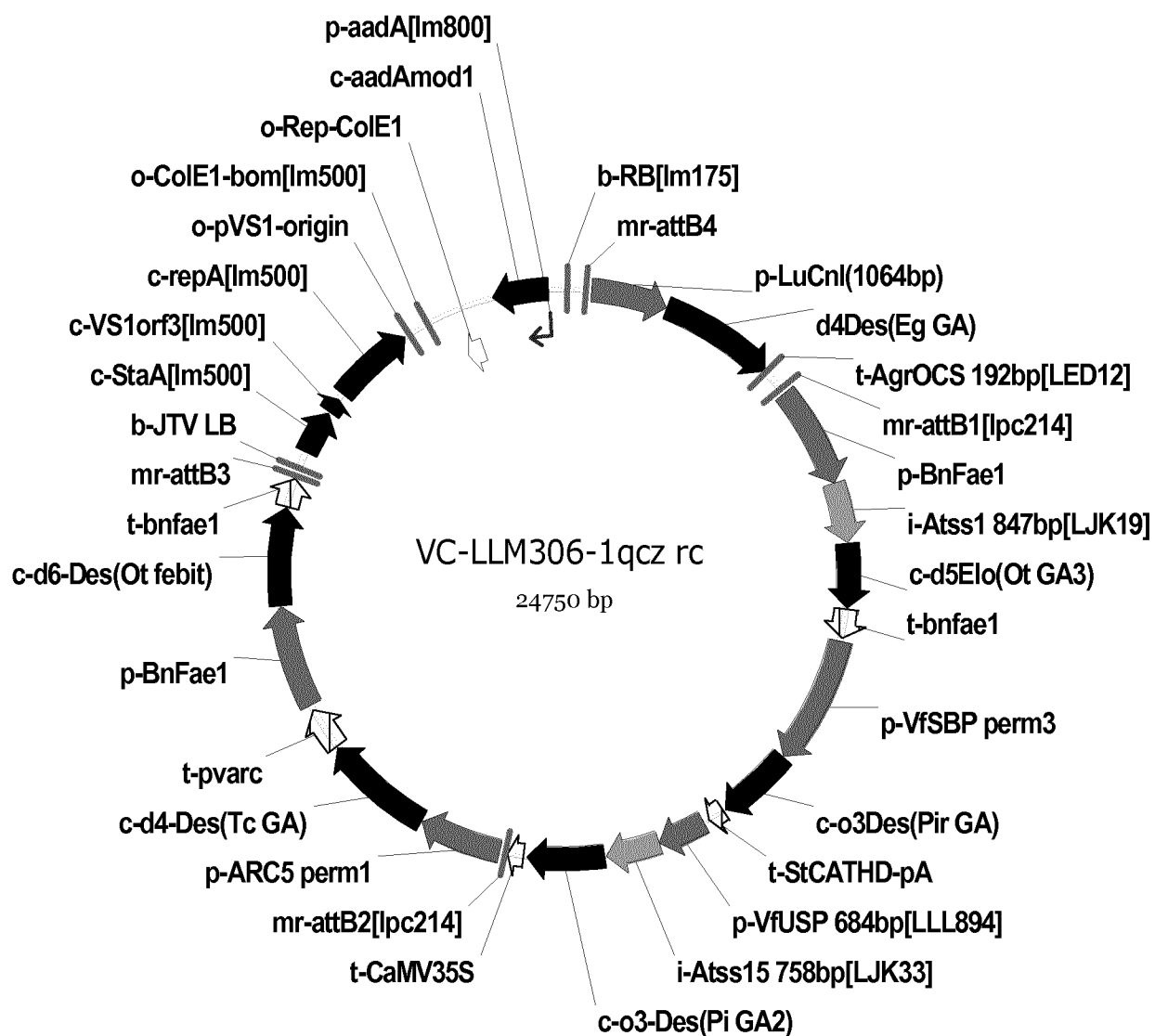


Fig. 8

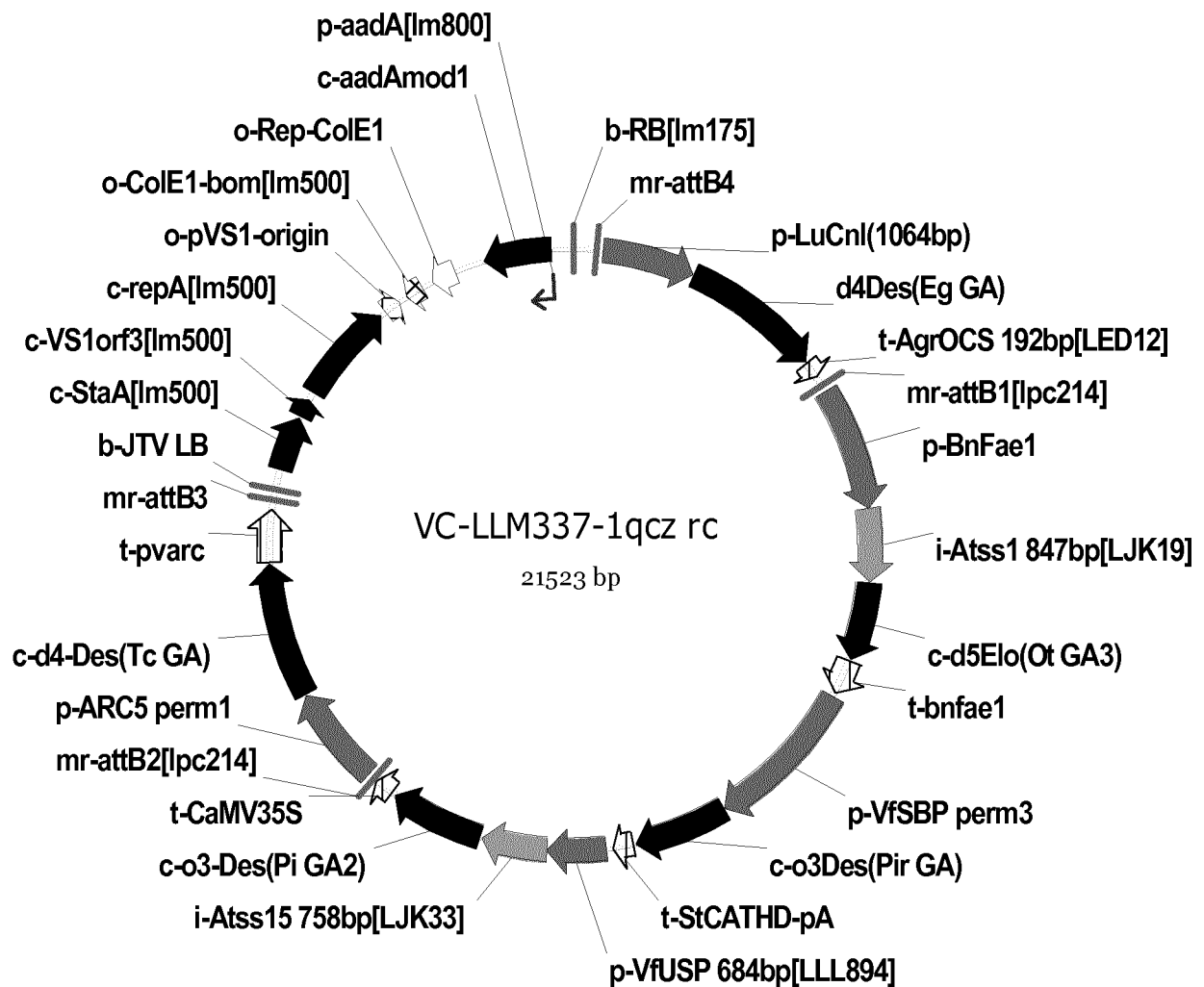


Fig. 9

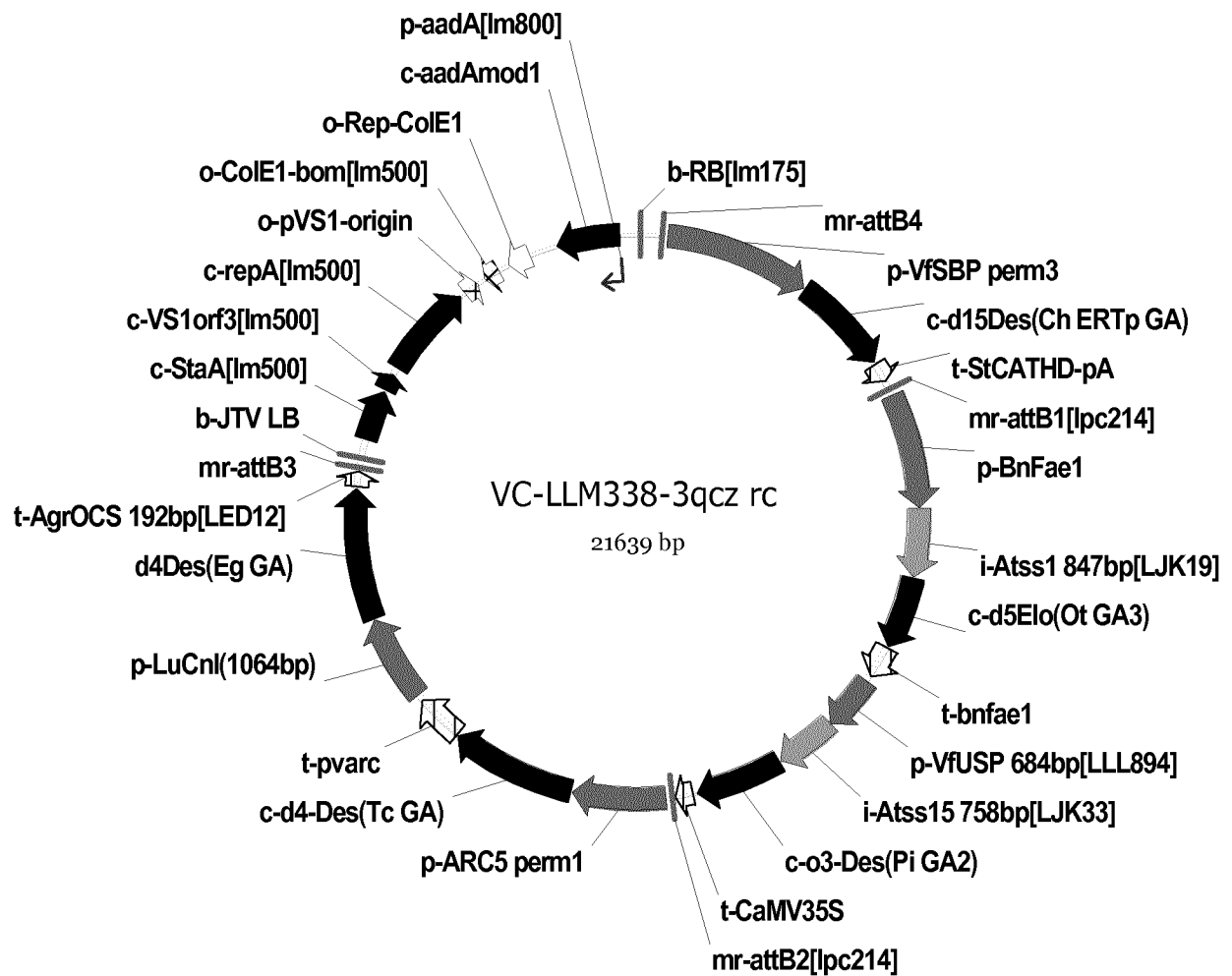


Fig. 10

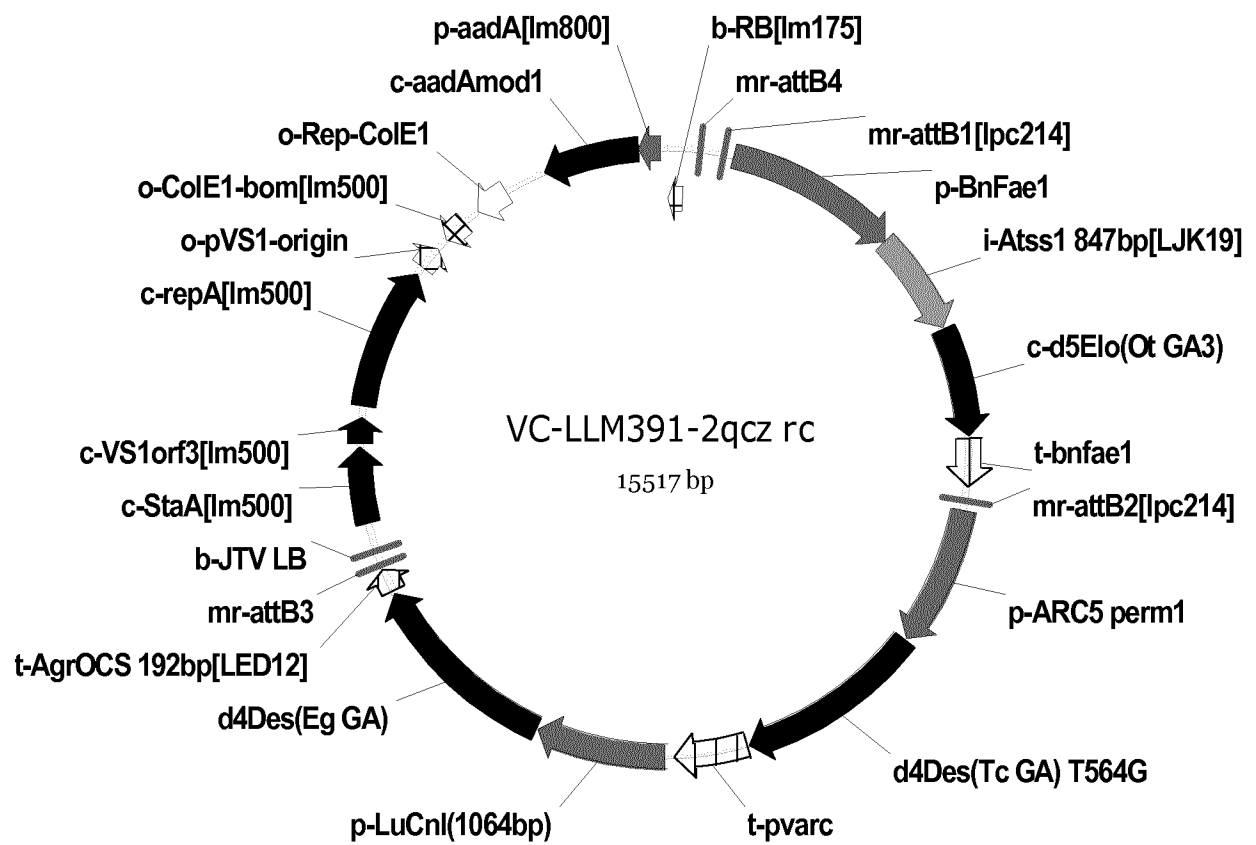


Fig. 11

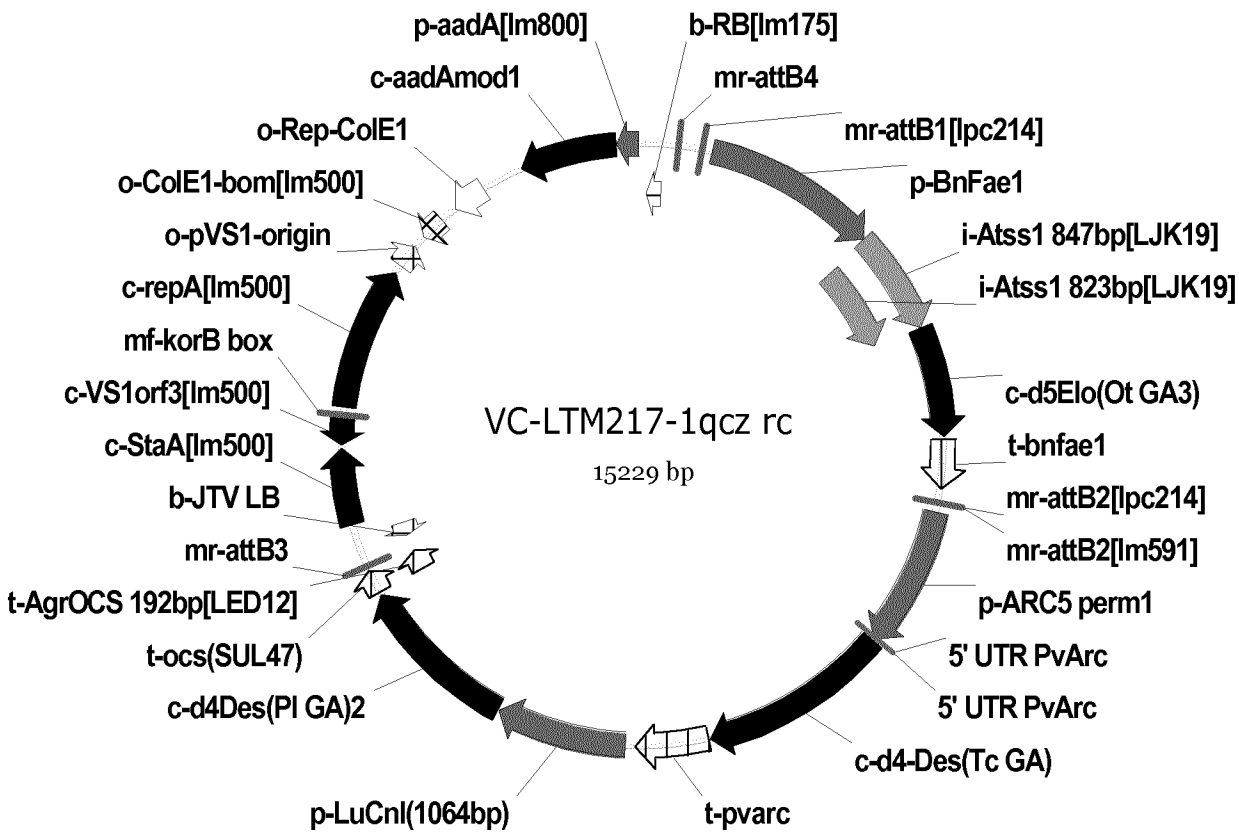


Fig 12

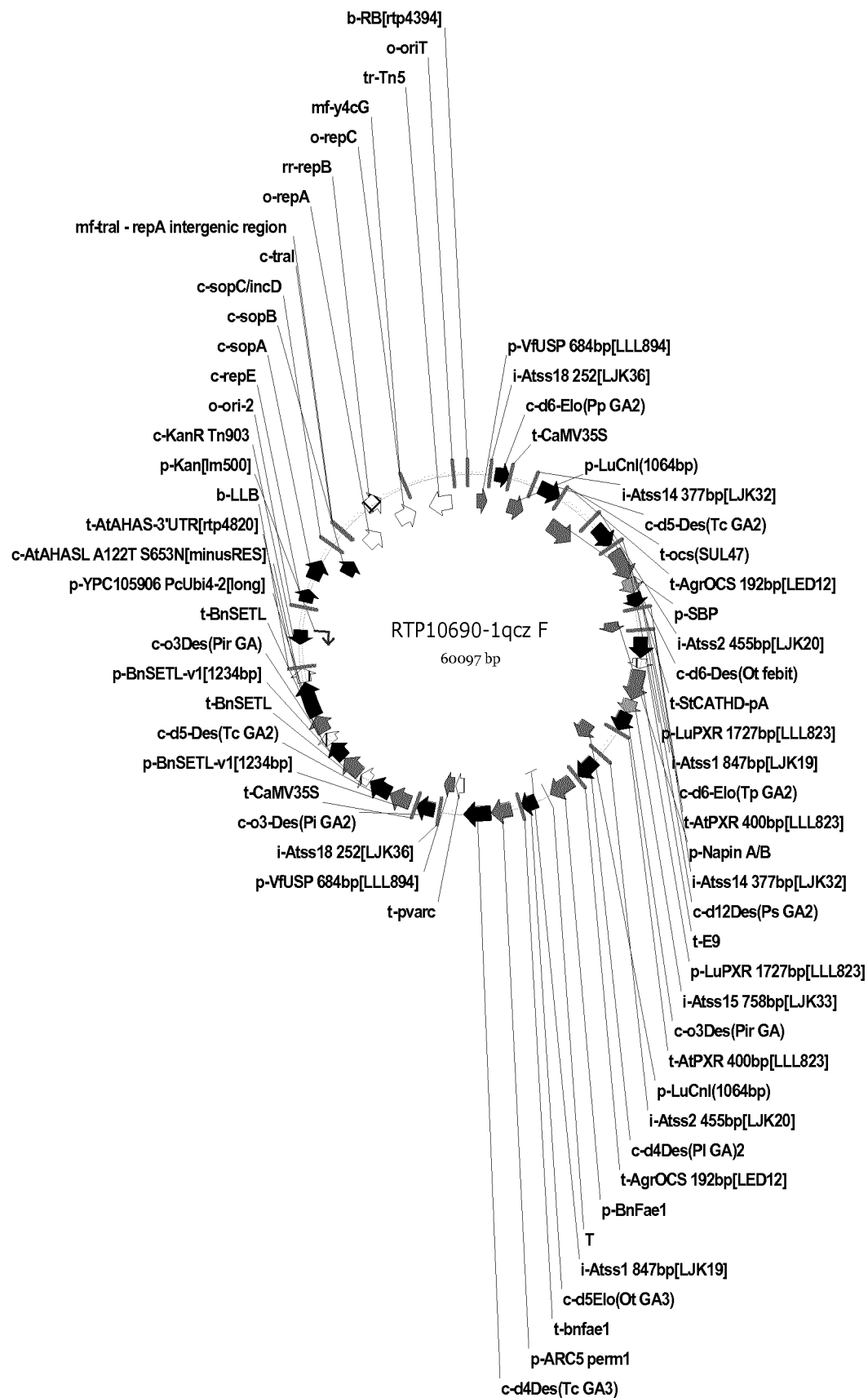


Fig. 13

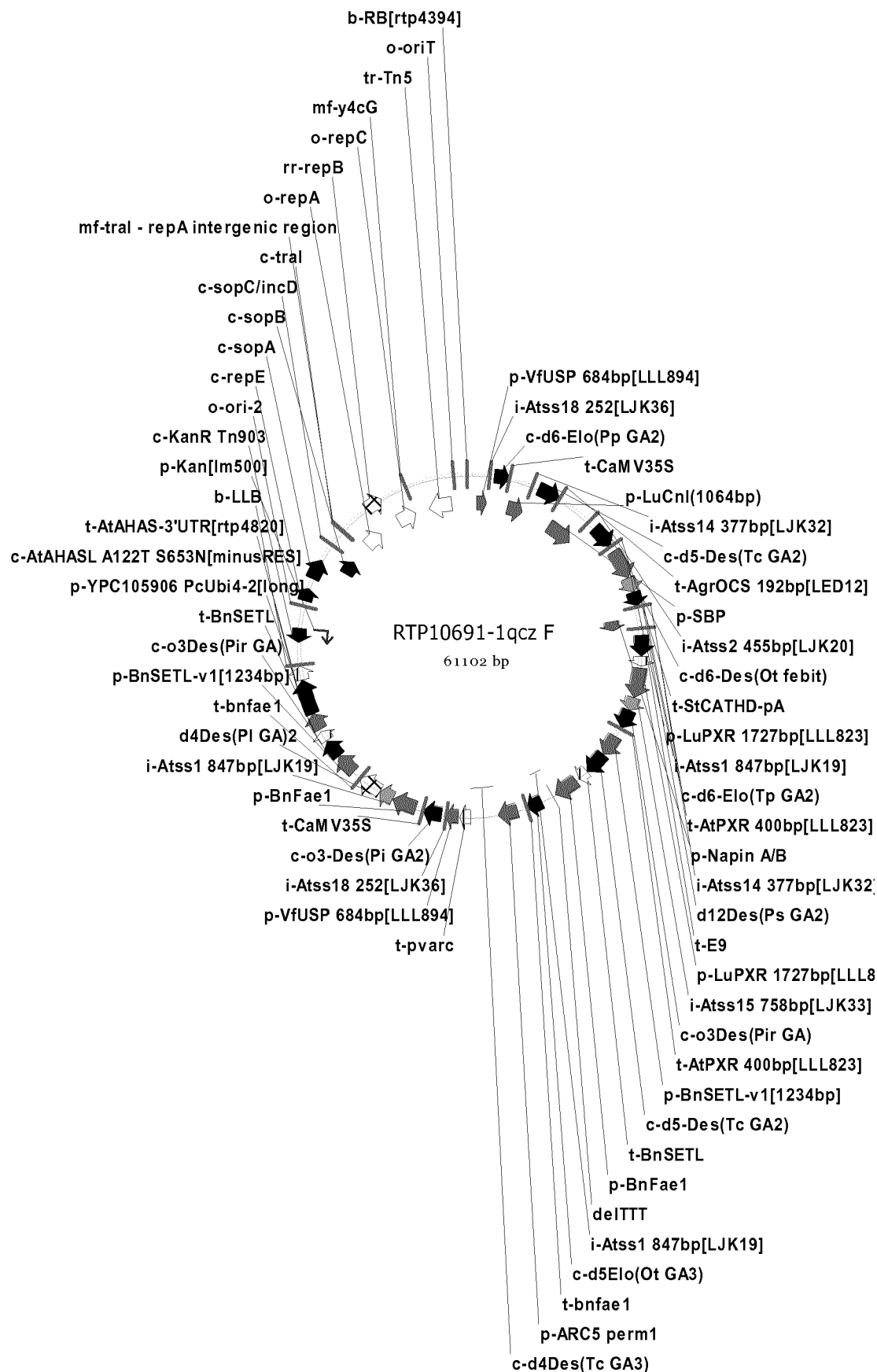


Fig. 14

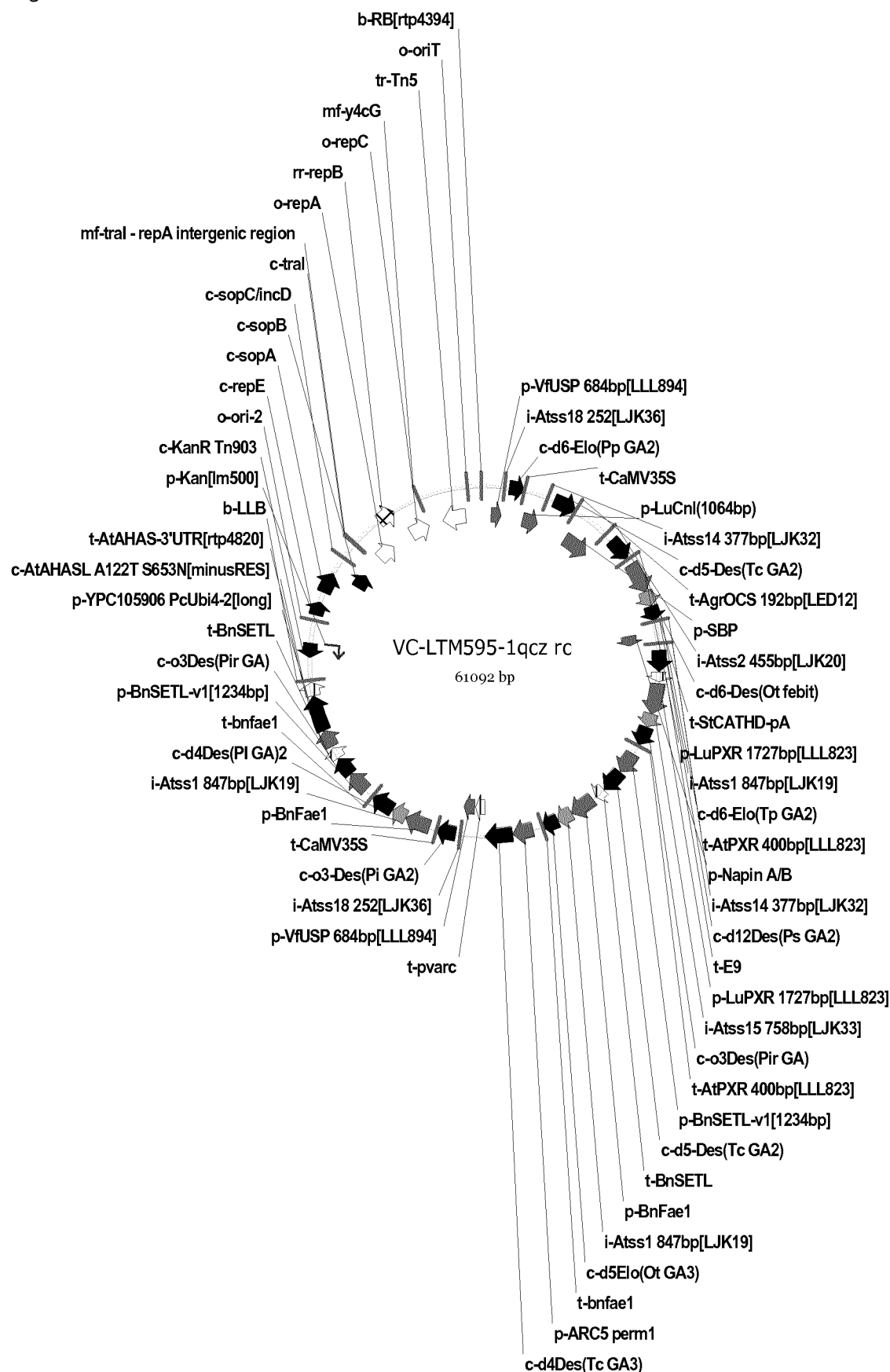


Fig. 15

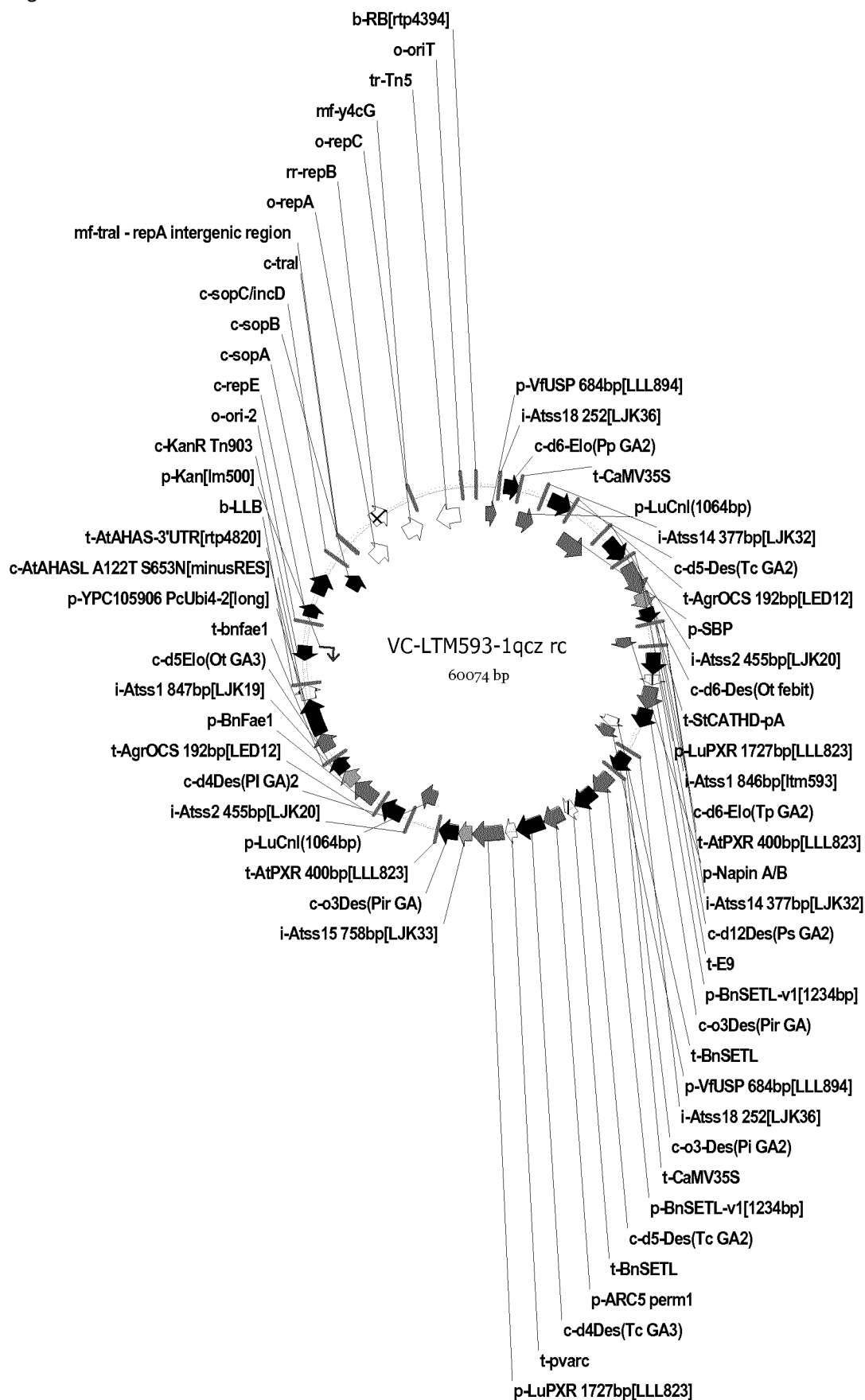


Fig. 16

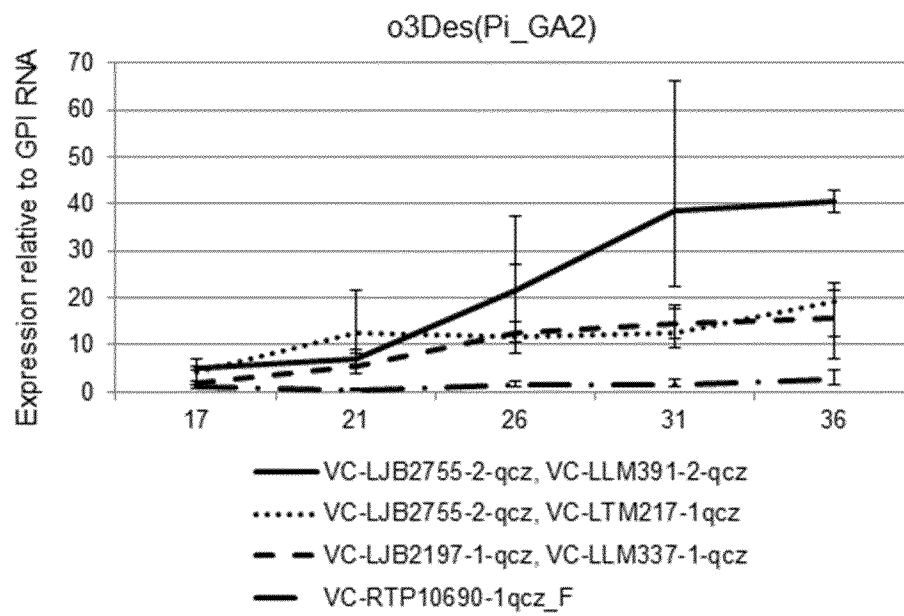


Fig. 17

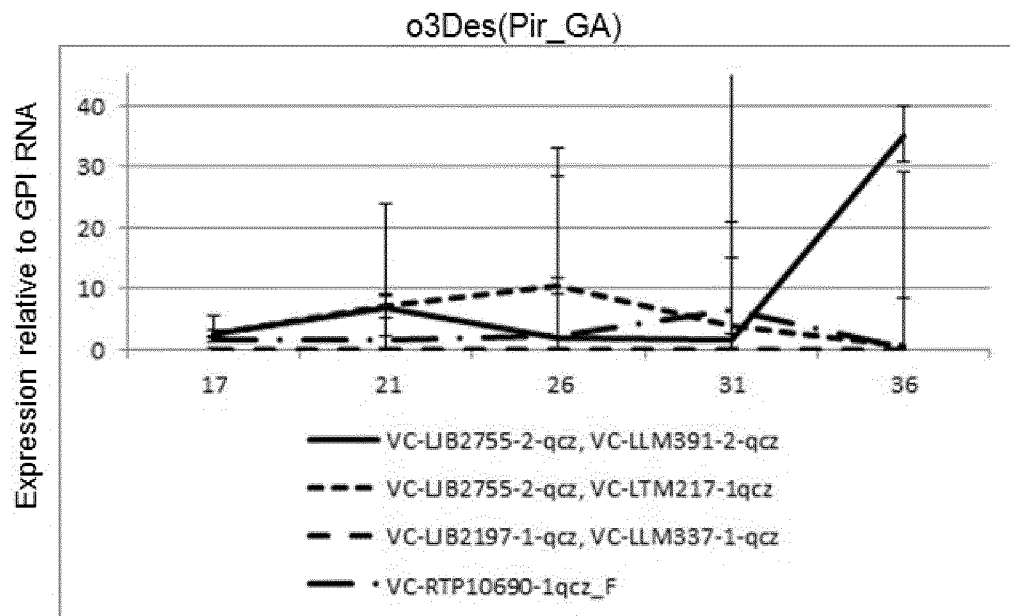


Fig. 18

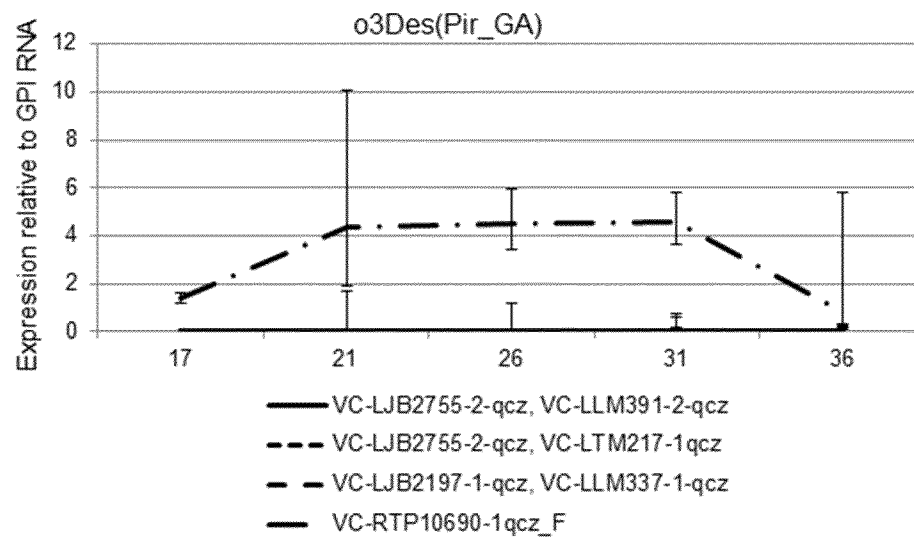


Fig. 19

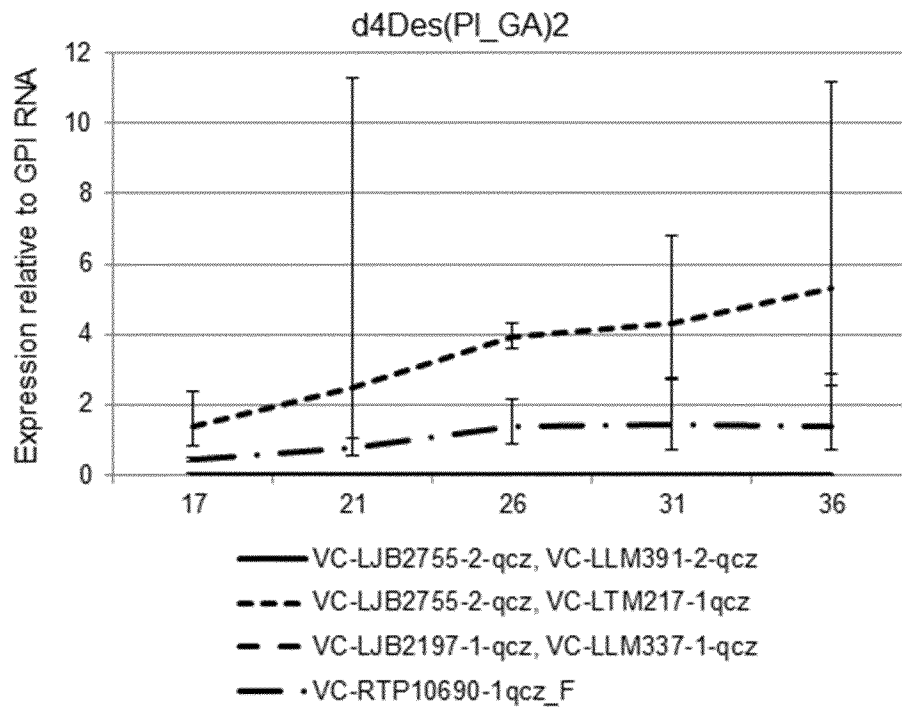


Fig. 20

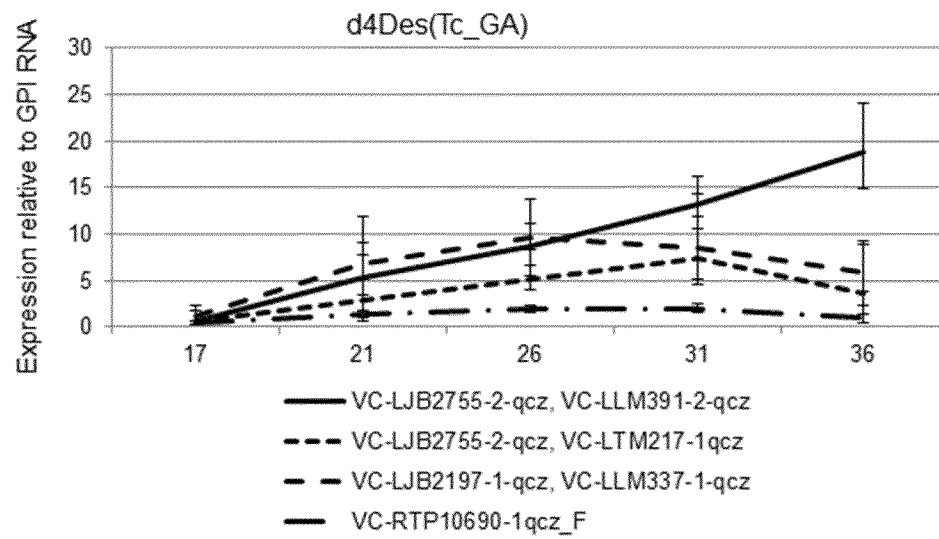


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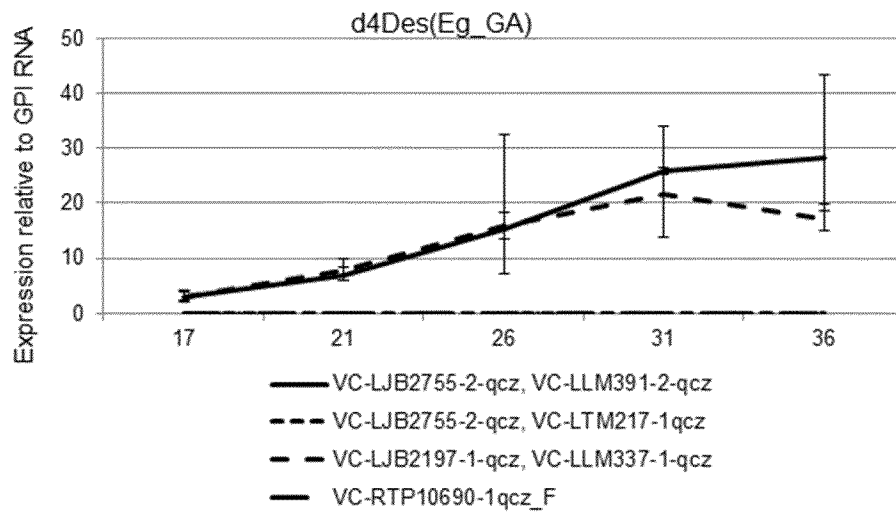


Fig. 22

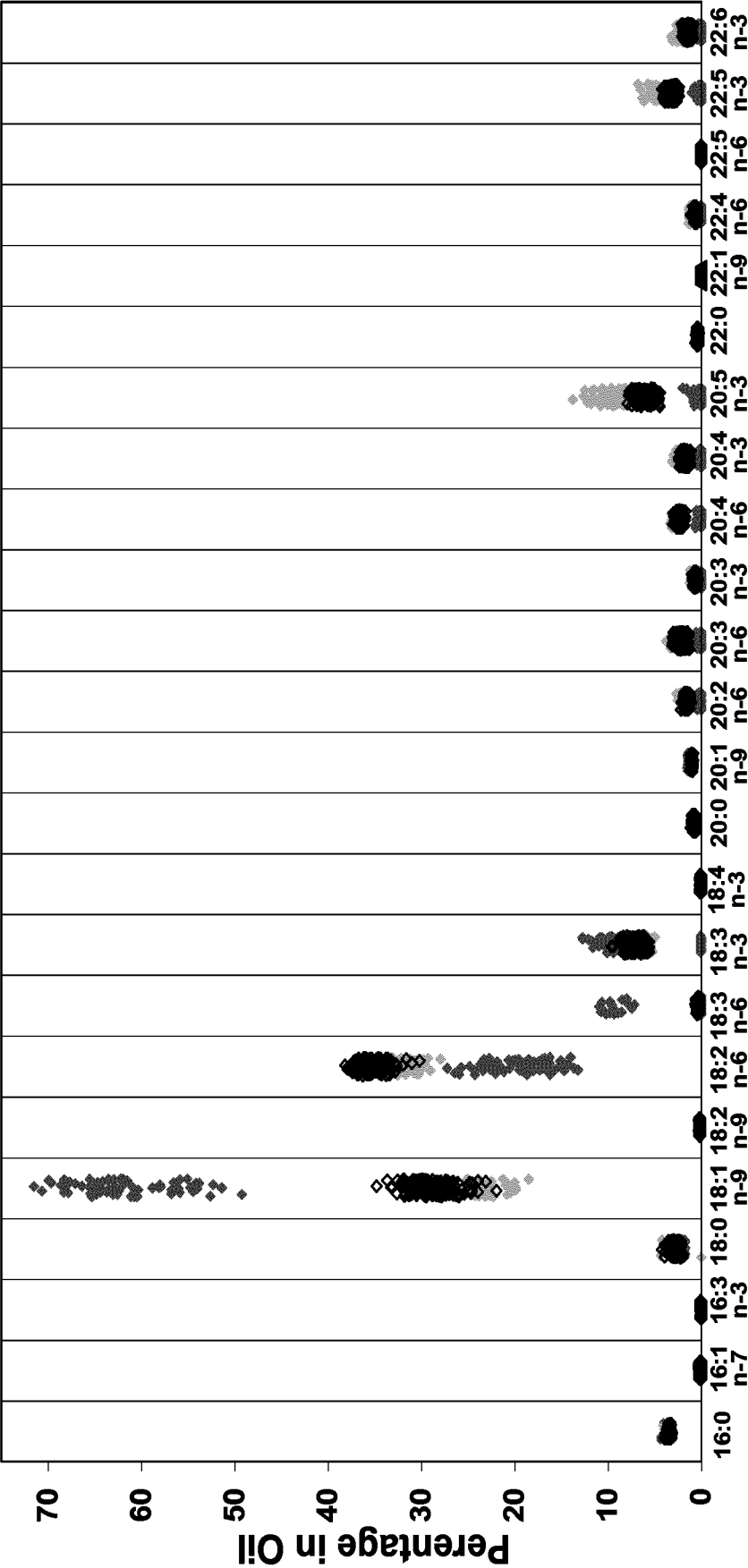


Fig. 23

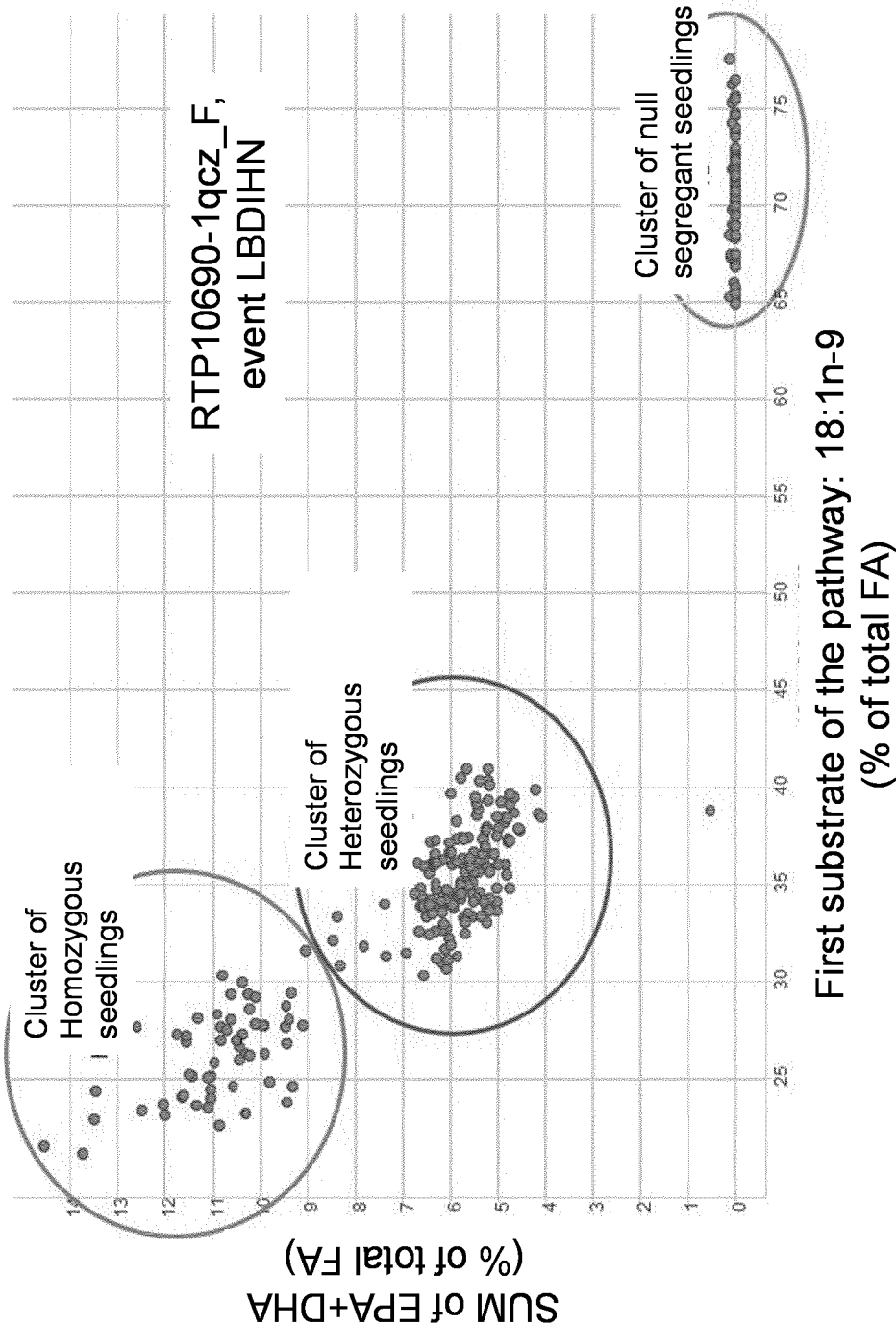


Fig. 24

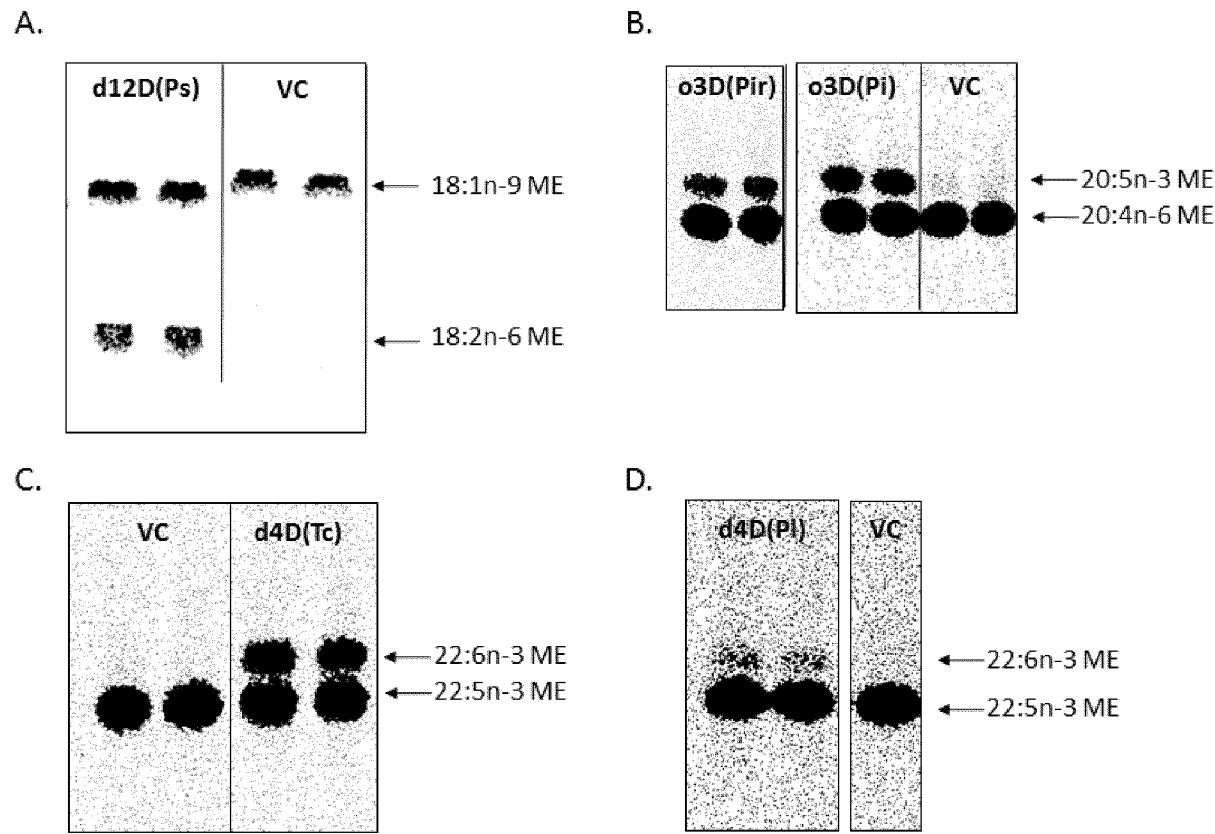
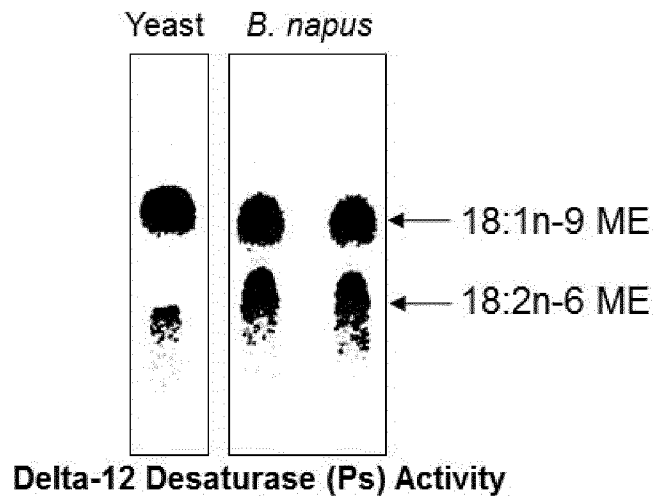


Fig. 25

A.

Microsomes Isolated from Transgenic:

B.

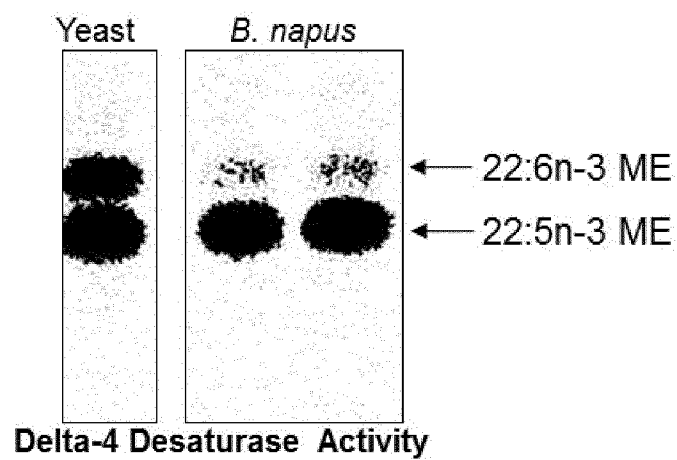
Microsomes Isolated from Transgenic:

Fig. 26

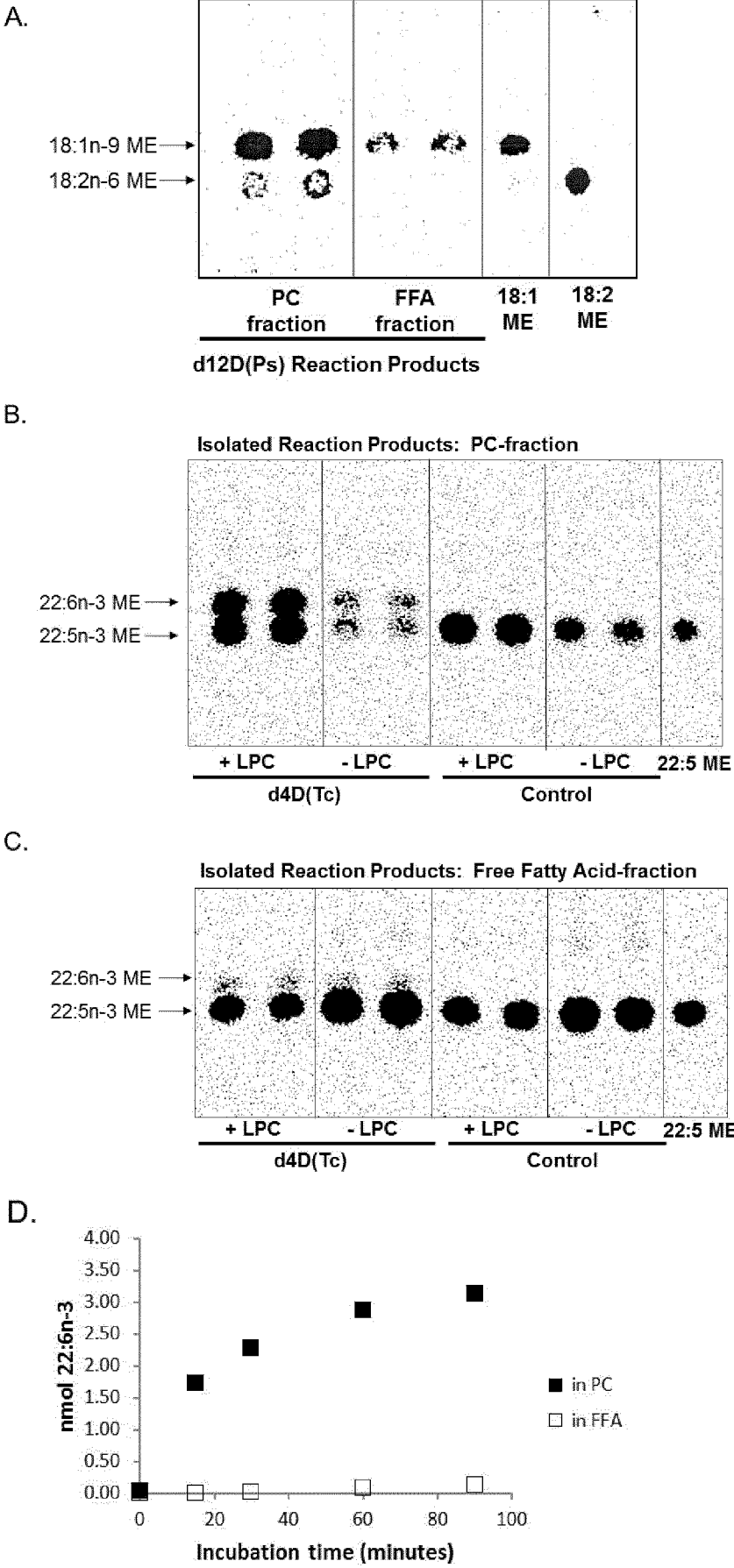
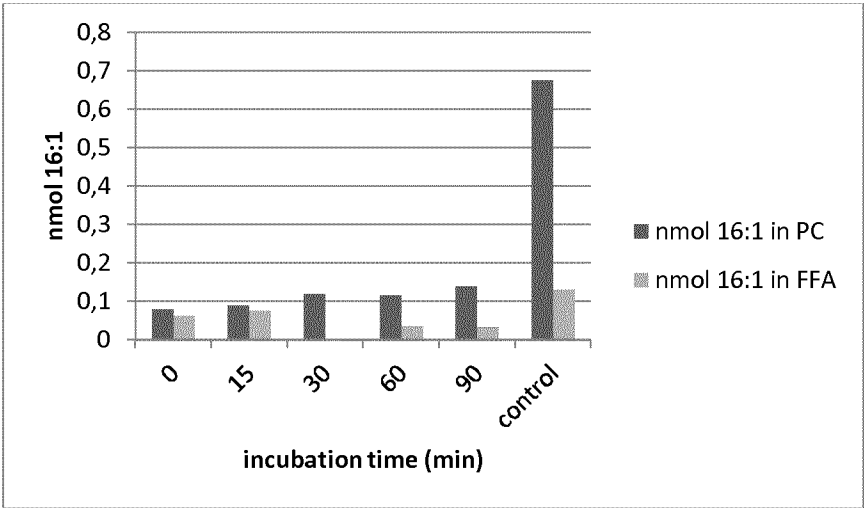
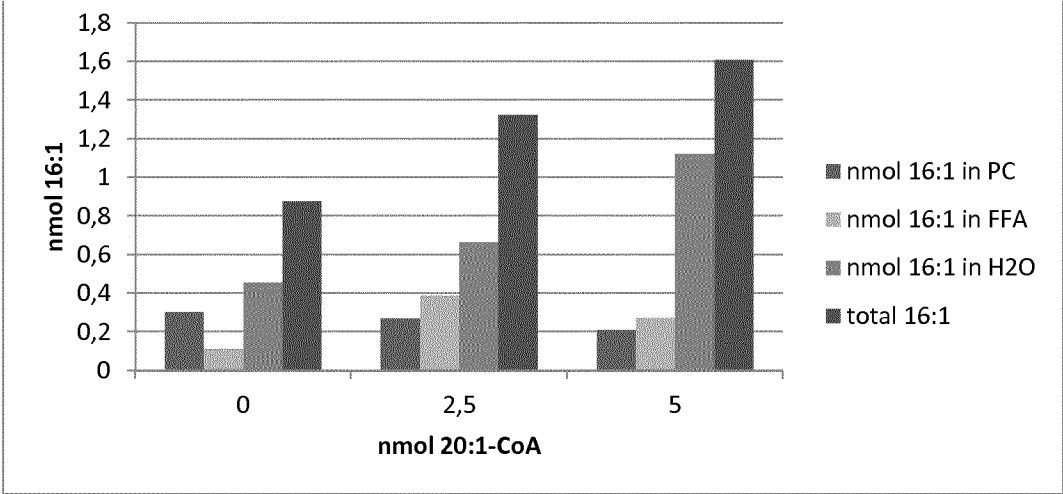


Fig. 27

A.



B.



C.

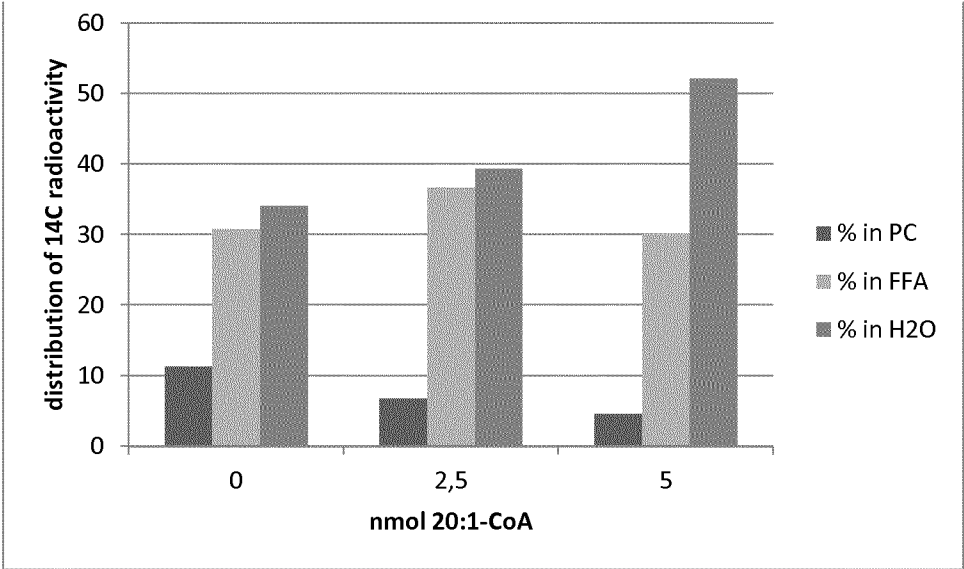


Fig. 28

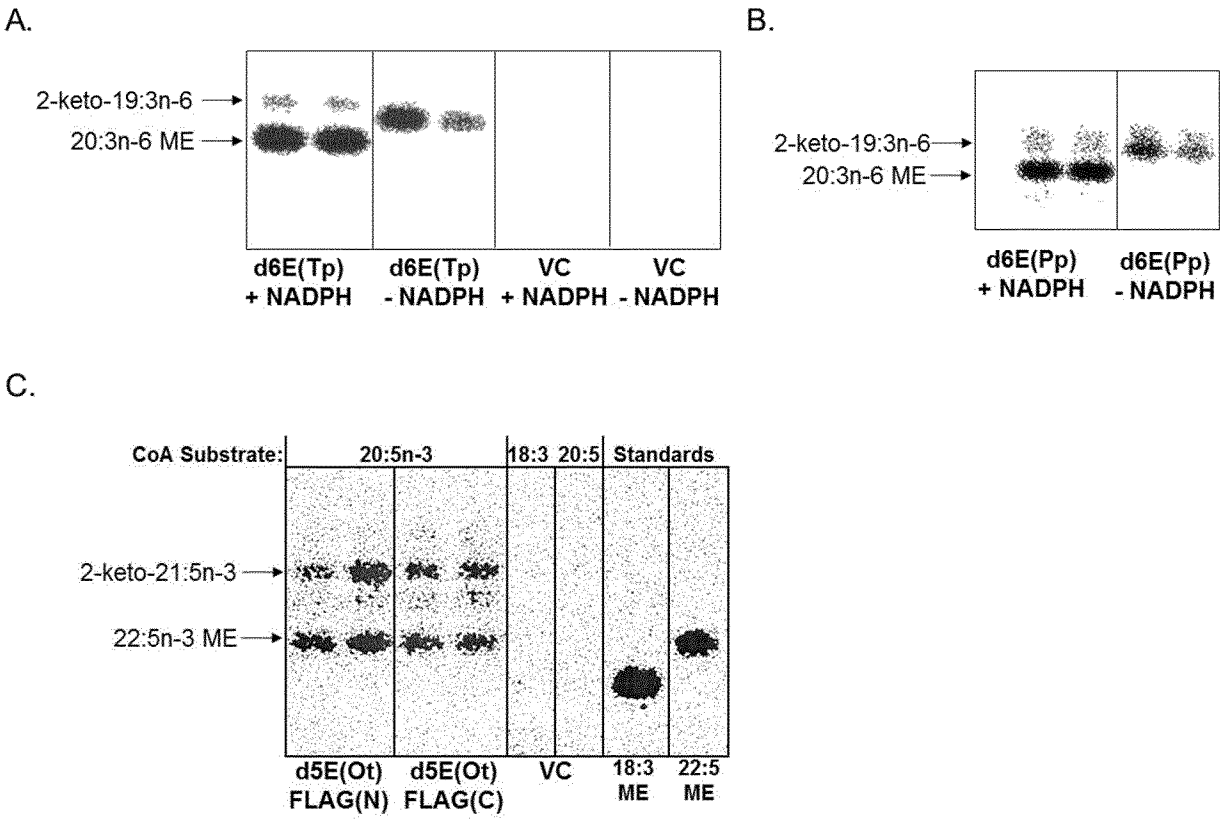
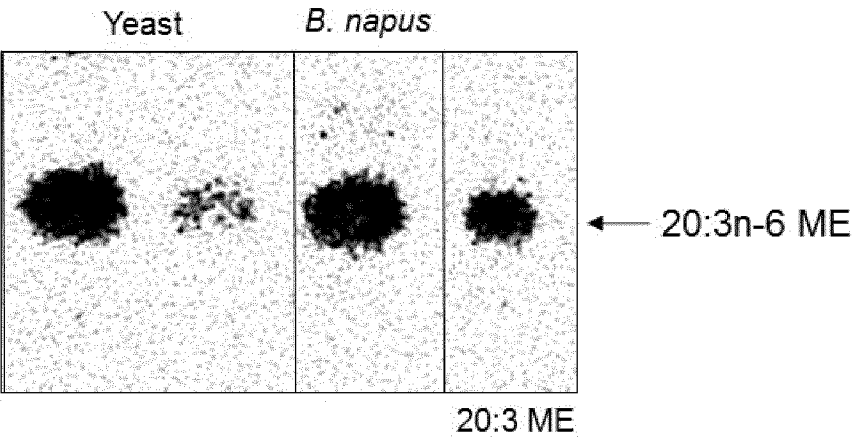


Fig. 29

A.

Microsomes Isolated from Transgenic:



Delta-6 Elongase Activity

B.

Microsomes Isolated from:

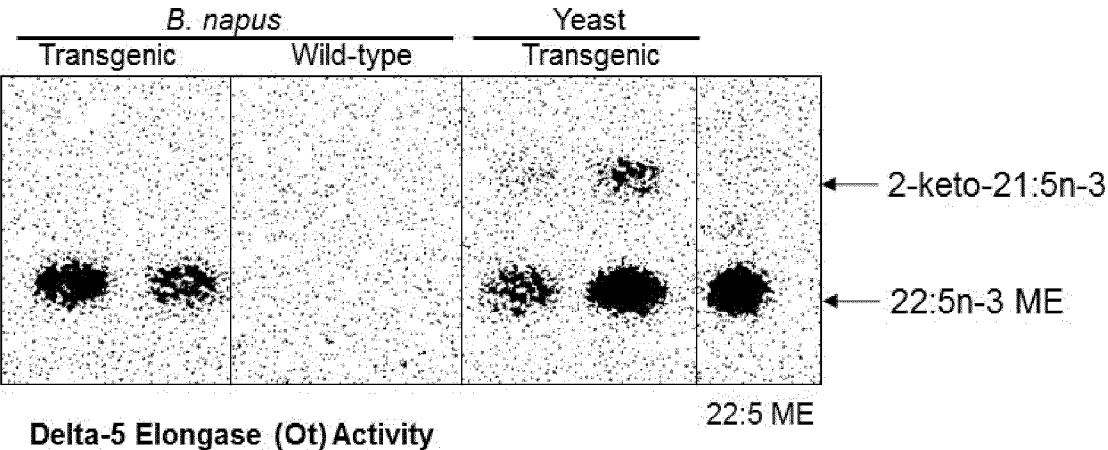


Fig. 30

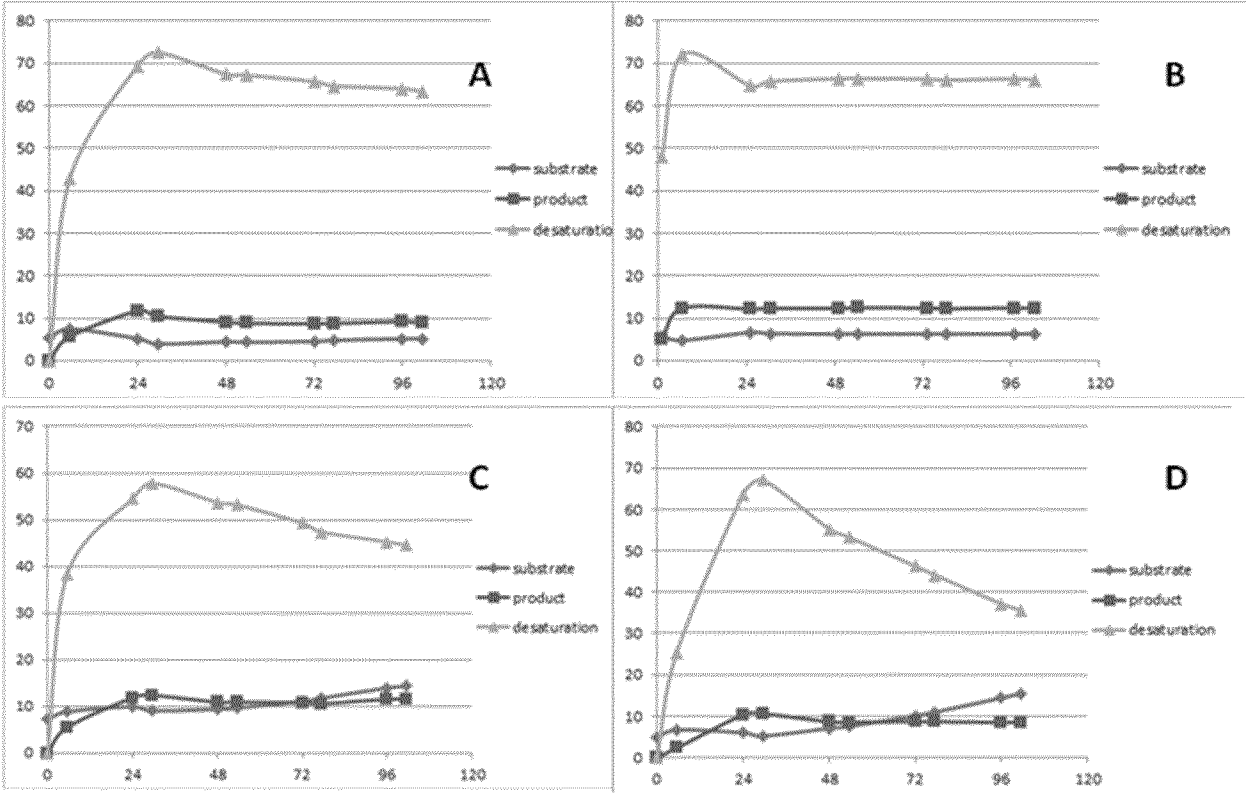


Fig. 31

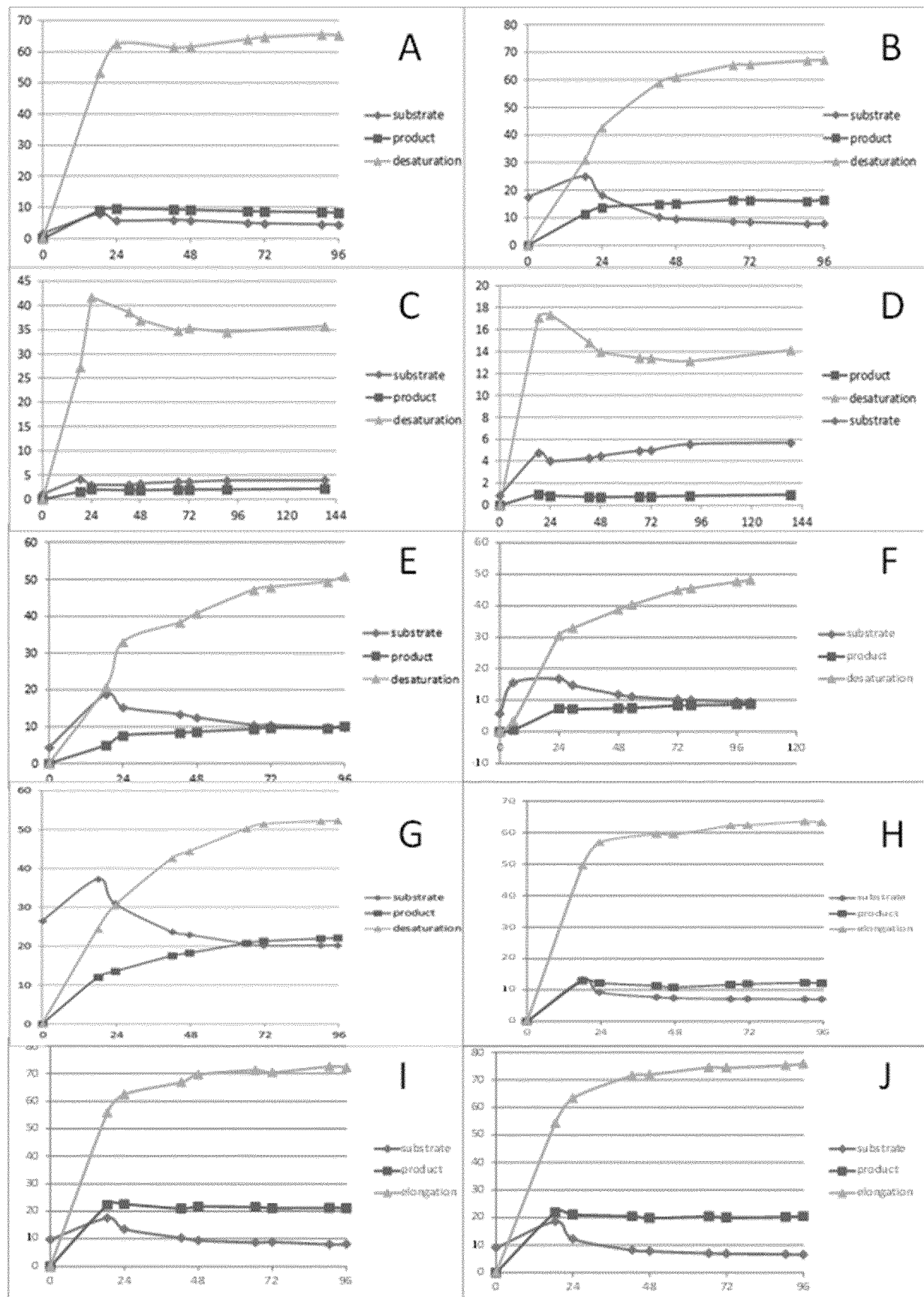


Fig. 32

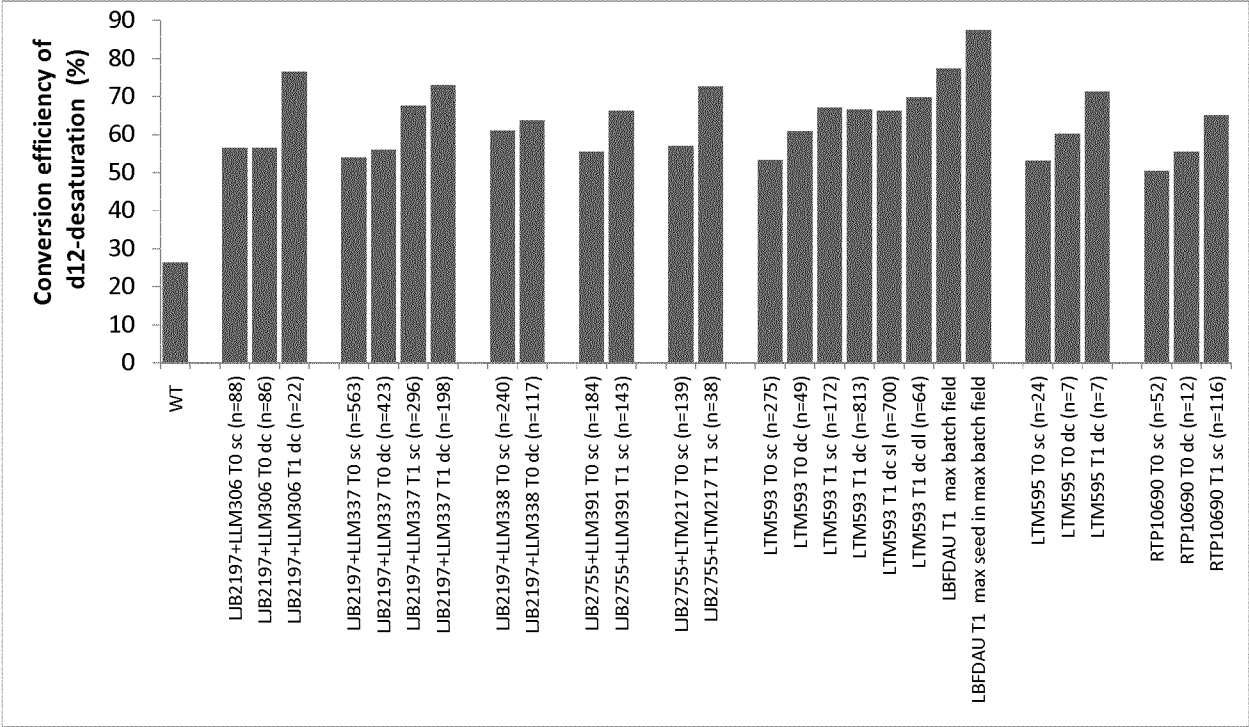


Fig. 33

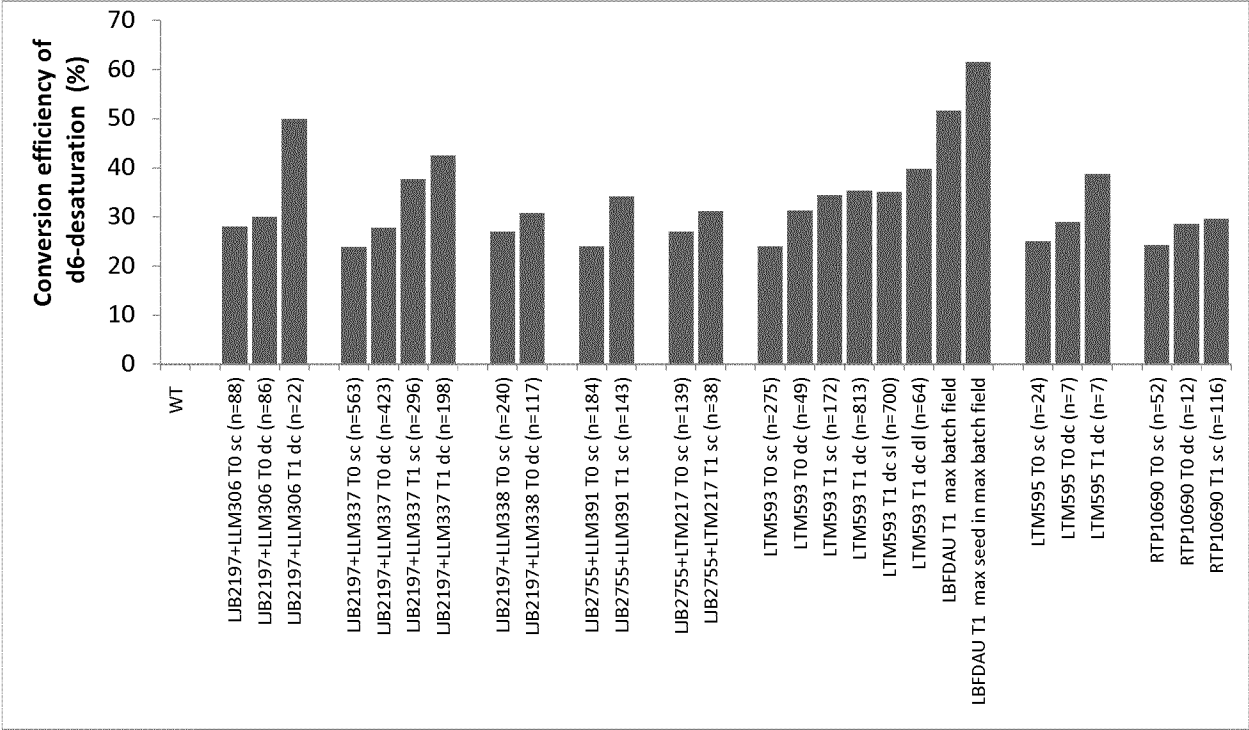
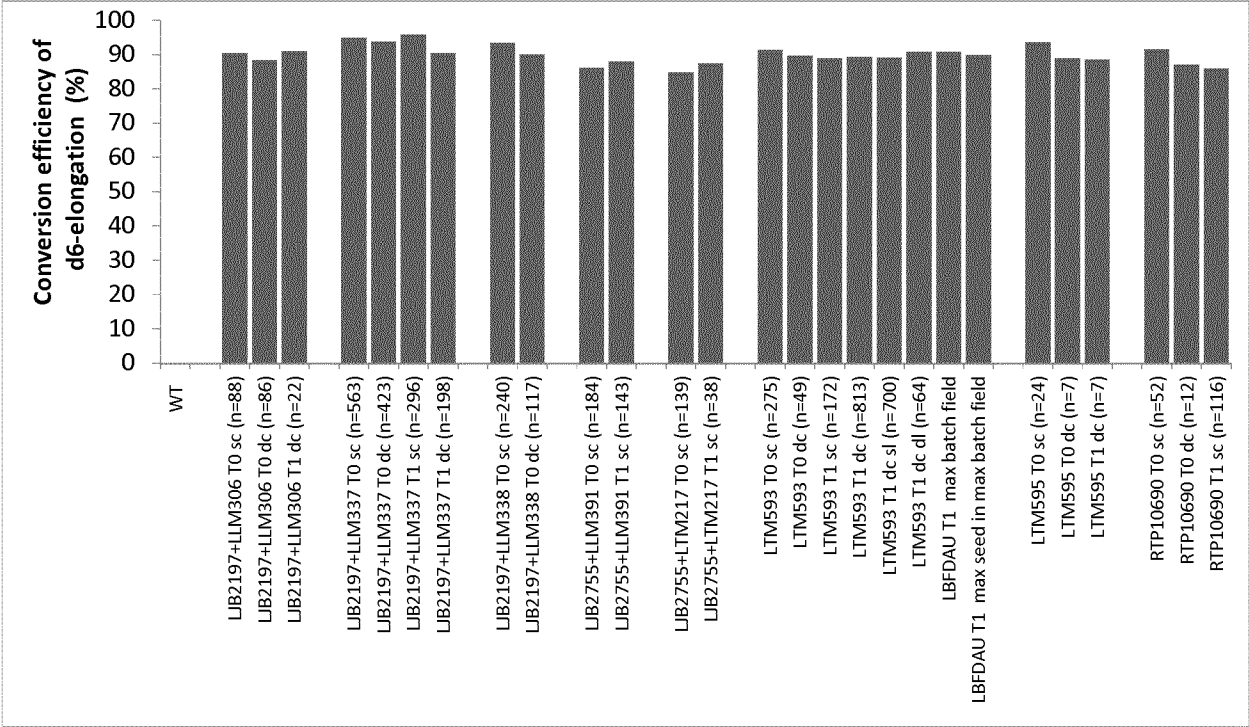
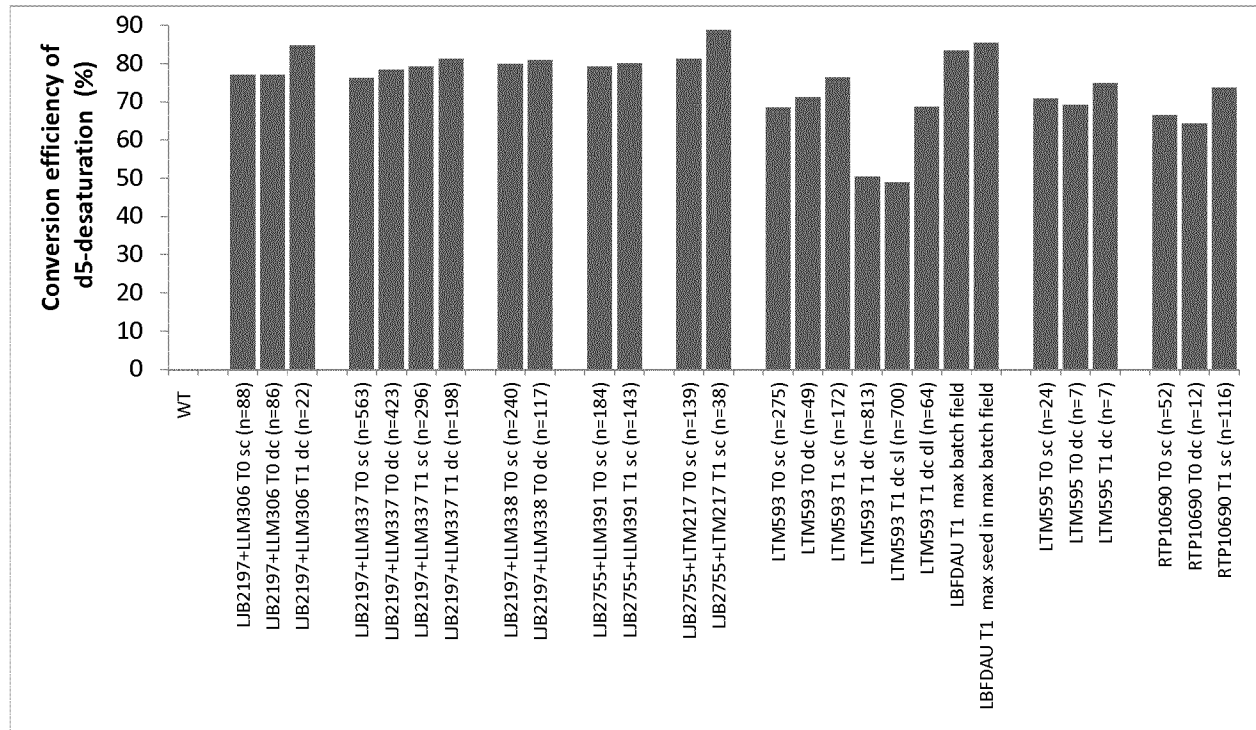


Fig. 34





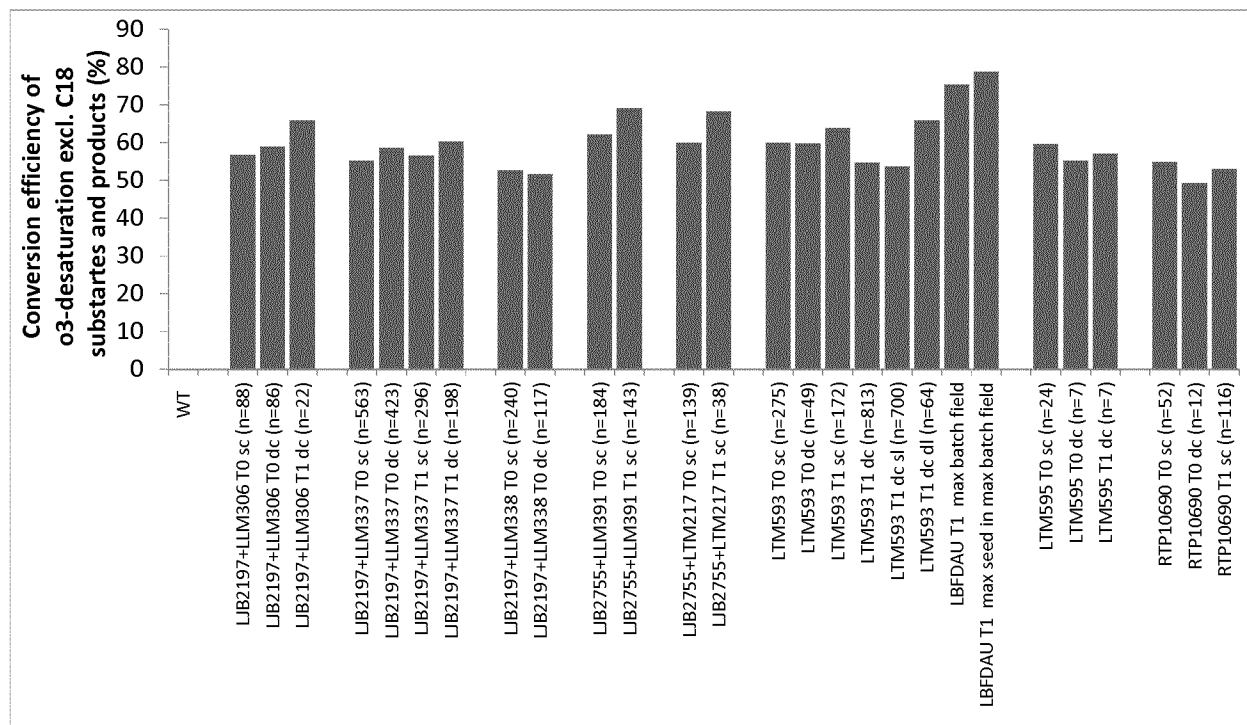
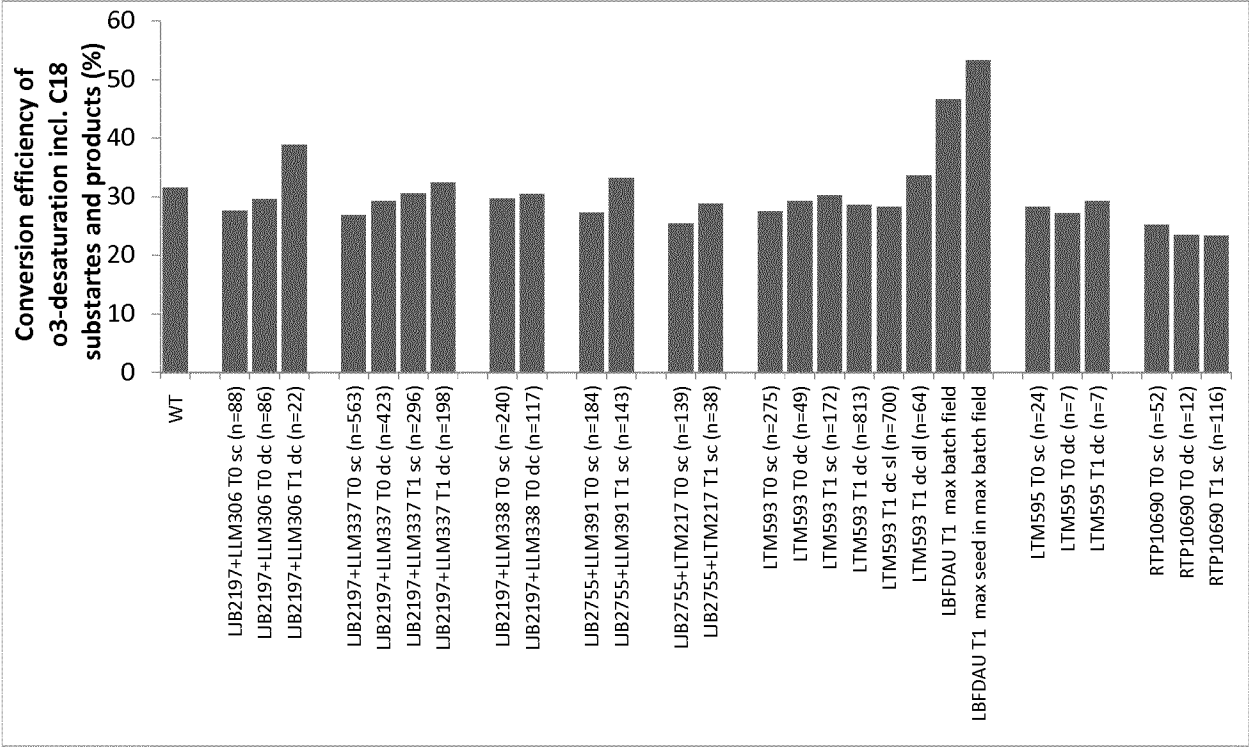


Fig. 37



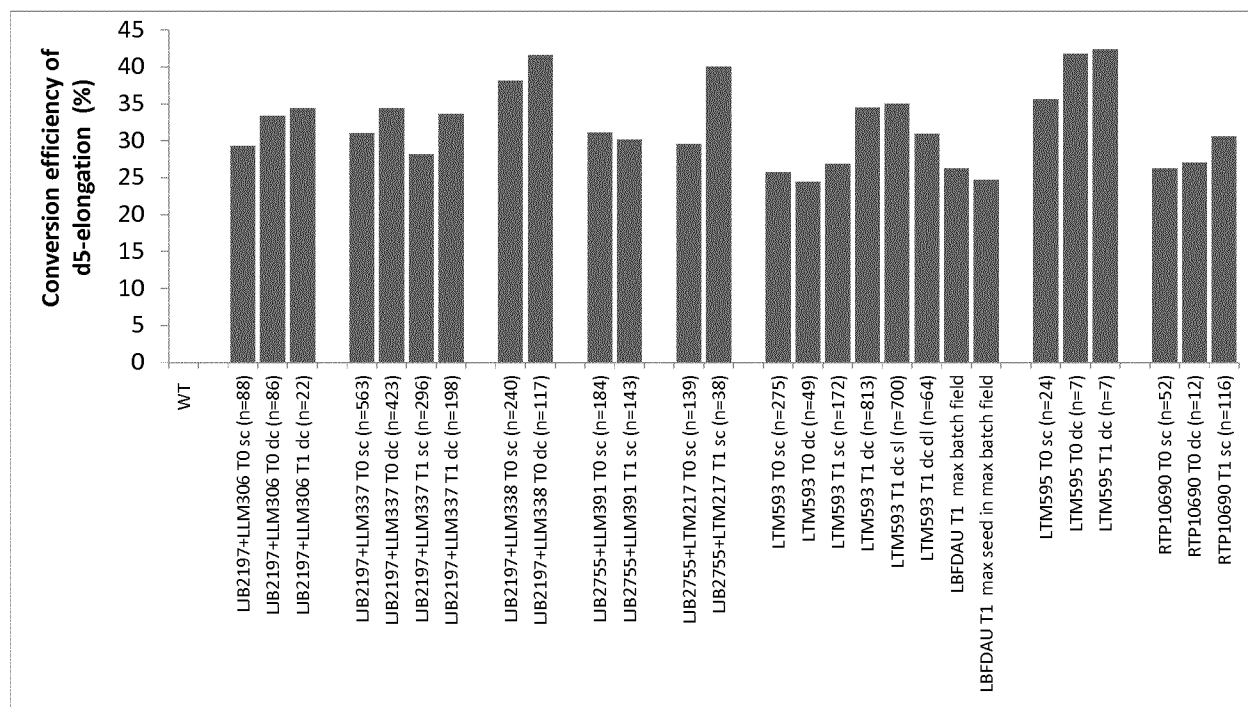


Fig. 39

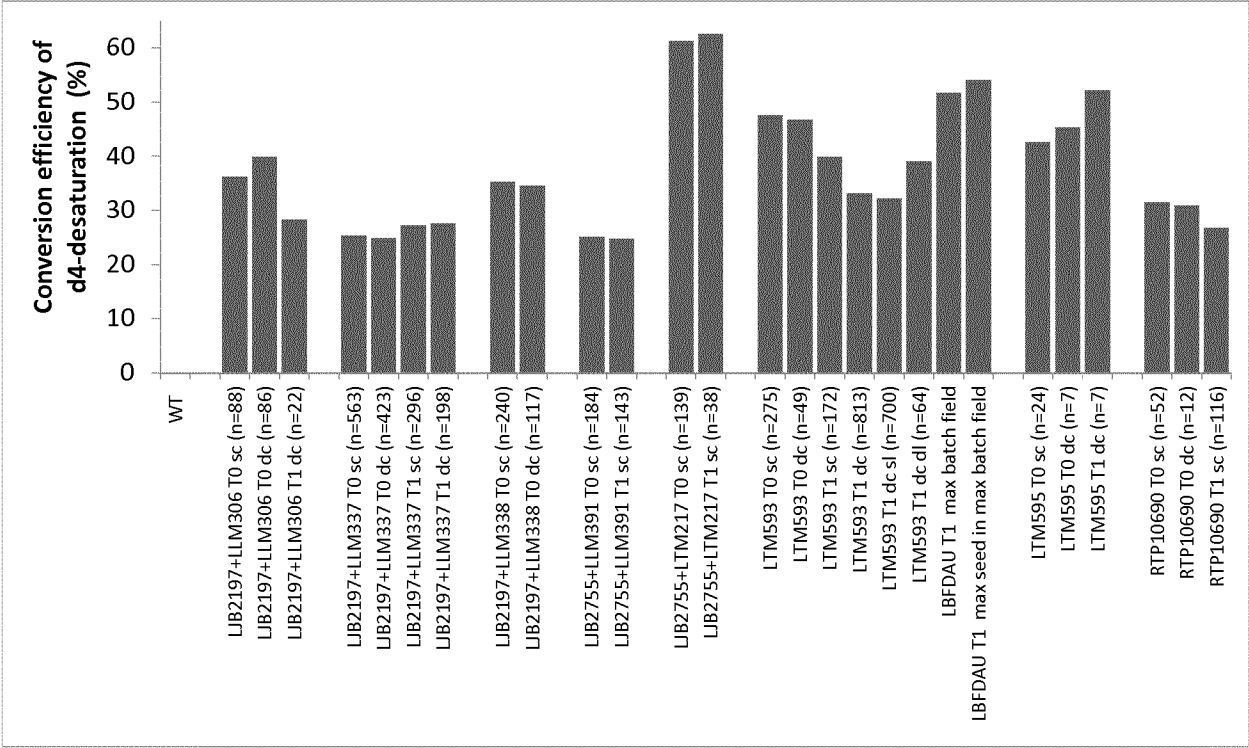


Fig. 40

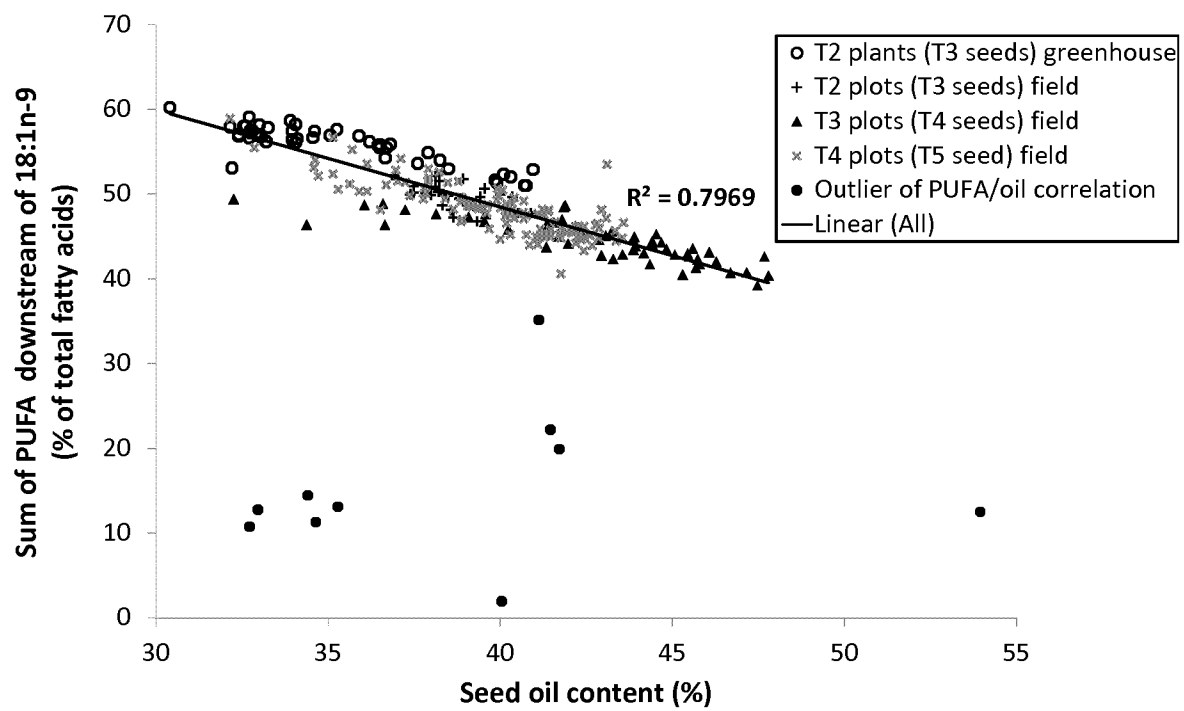


Fig. 41

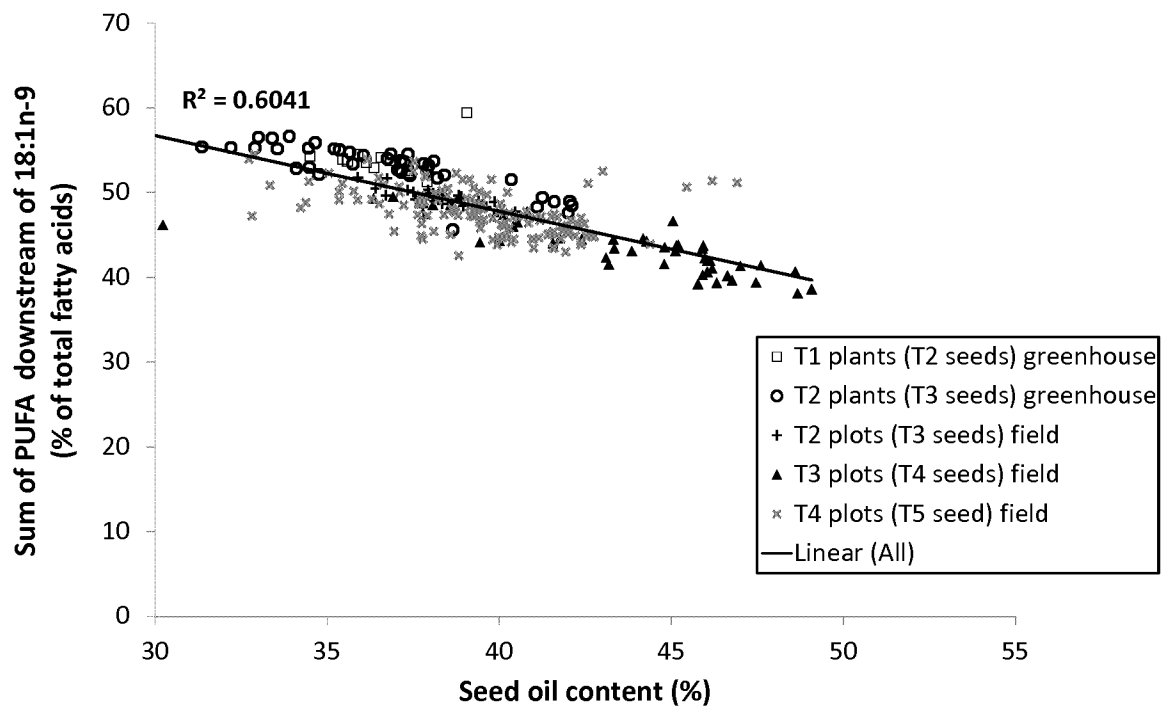


Fig. 42

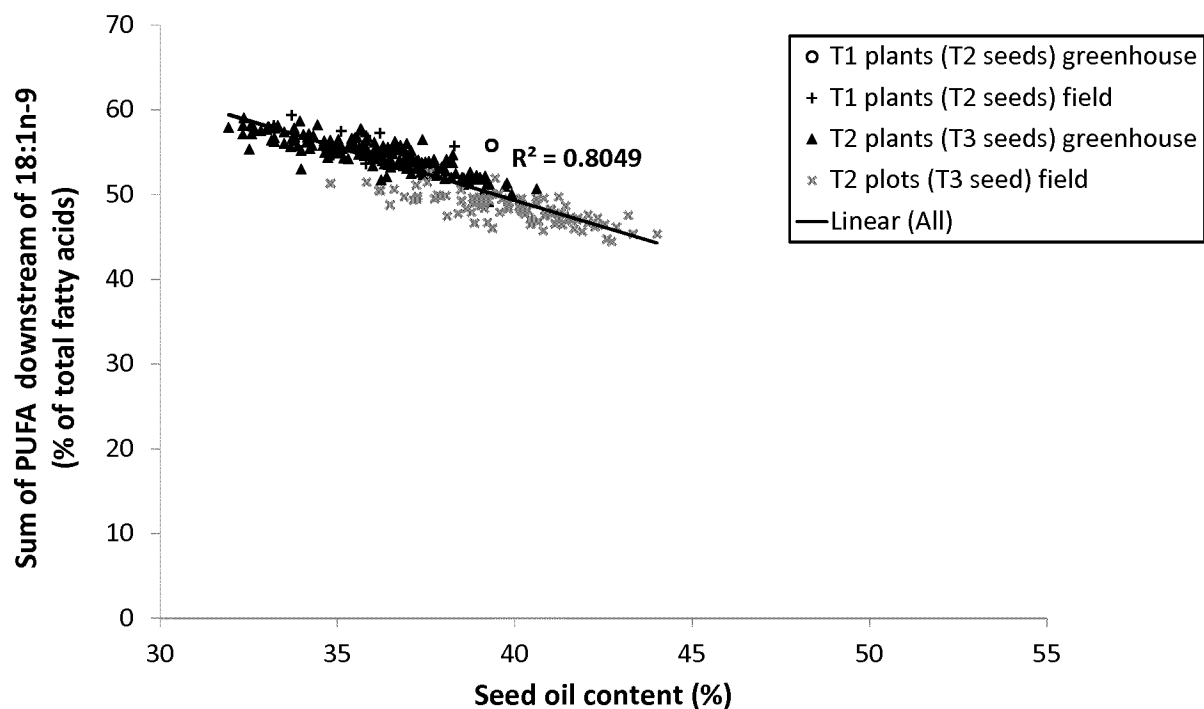


Fig. 43

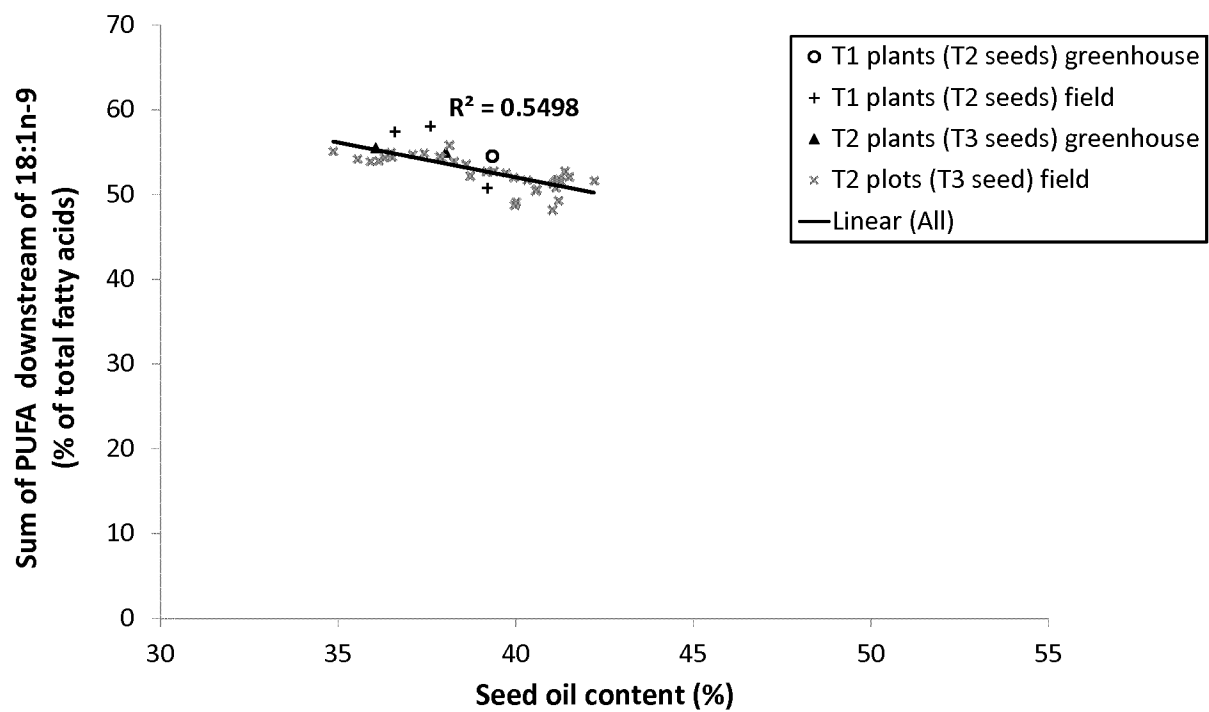


Fig. 44

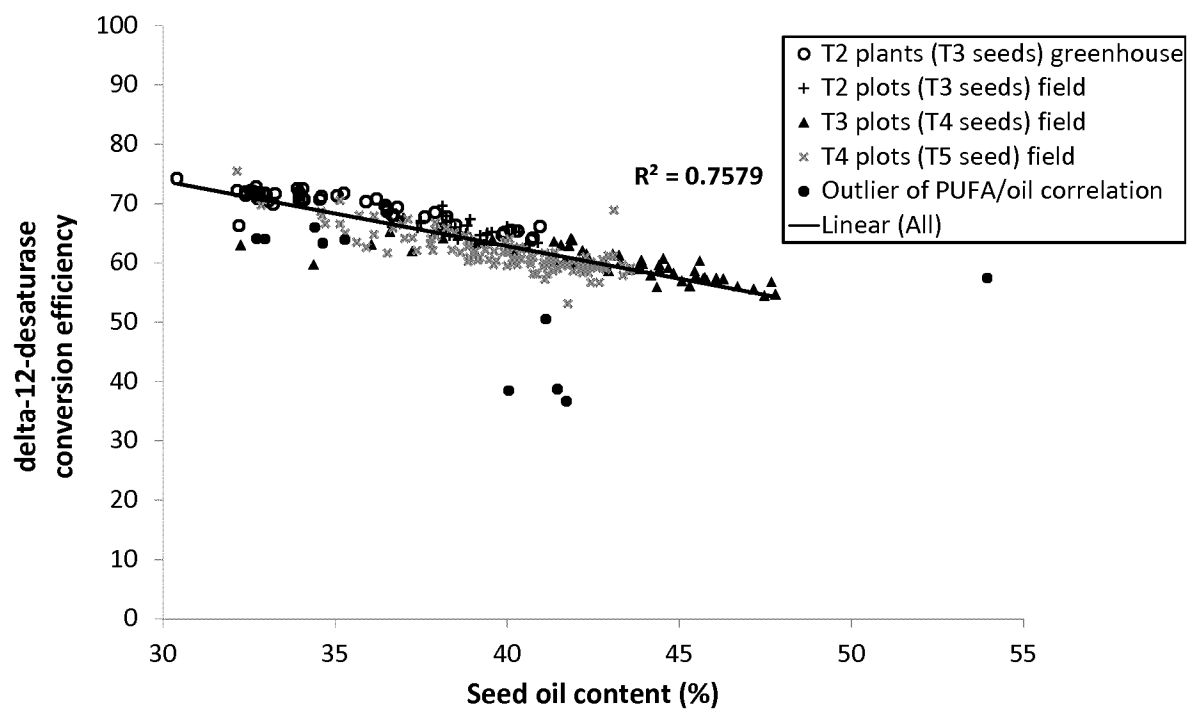


Fig. 45

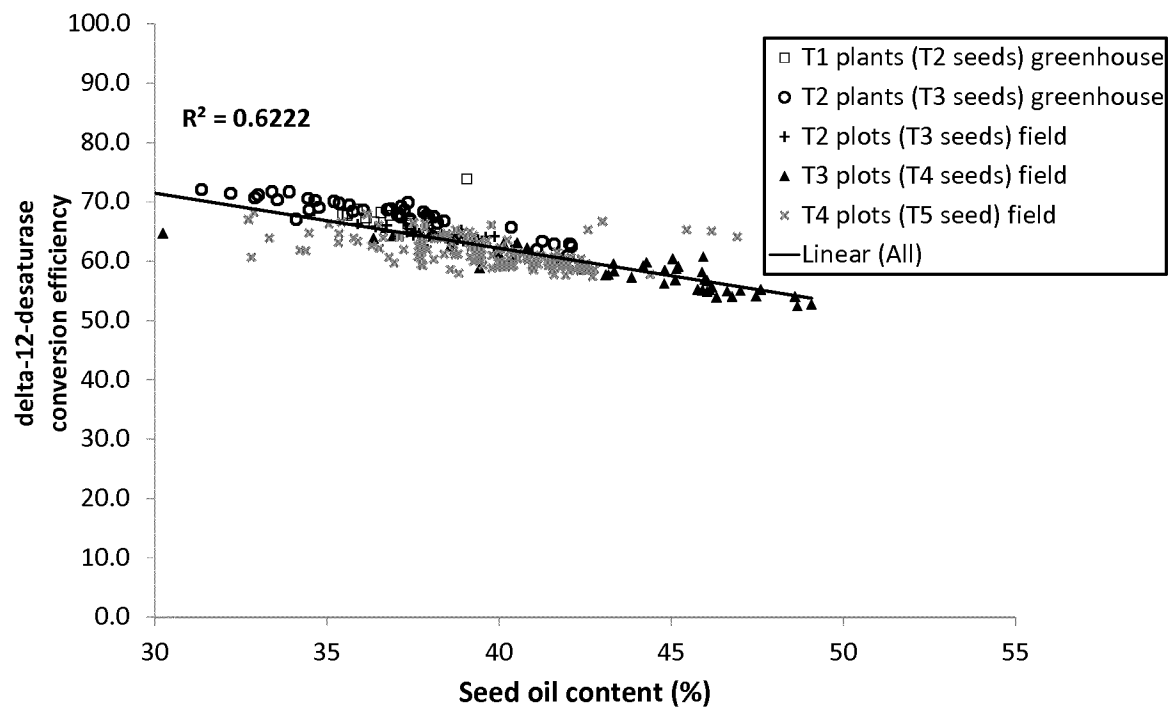


Fig. 46

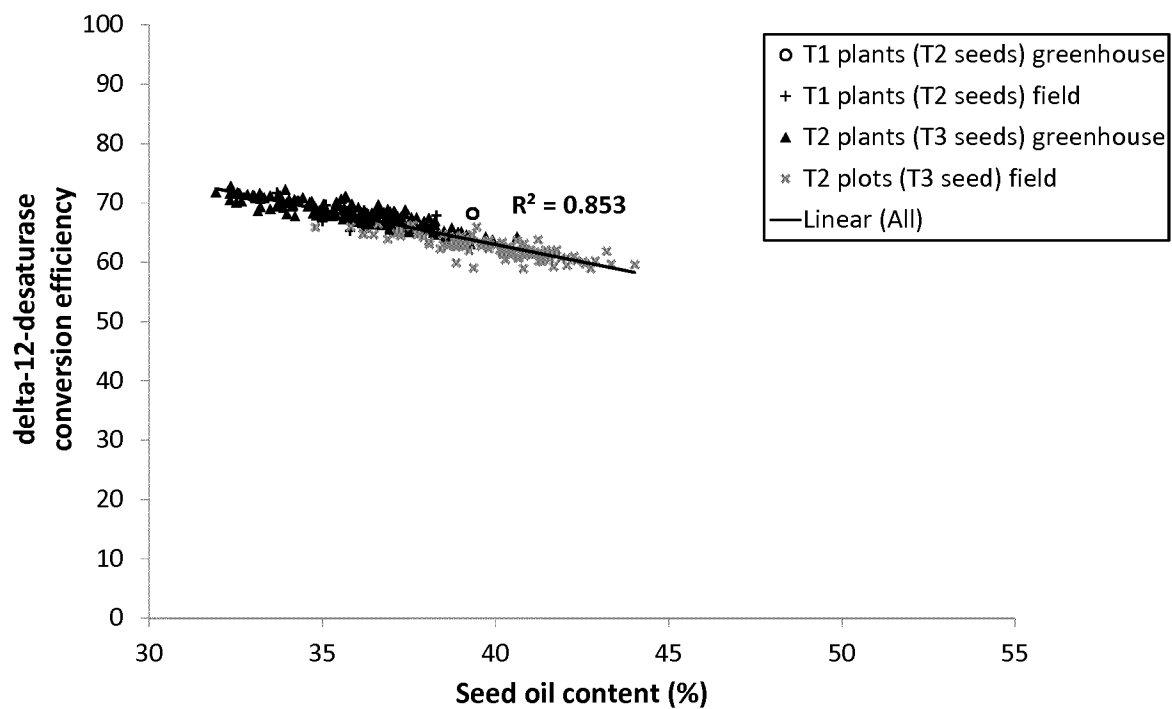


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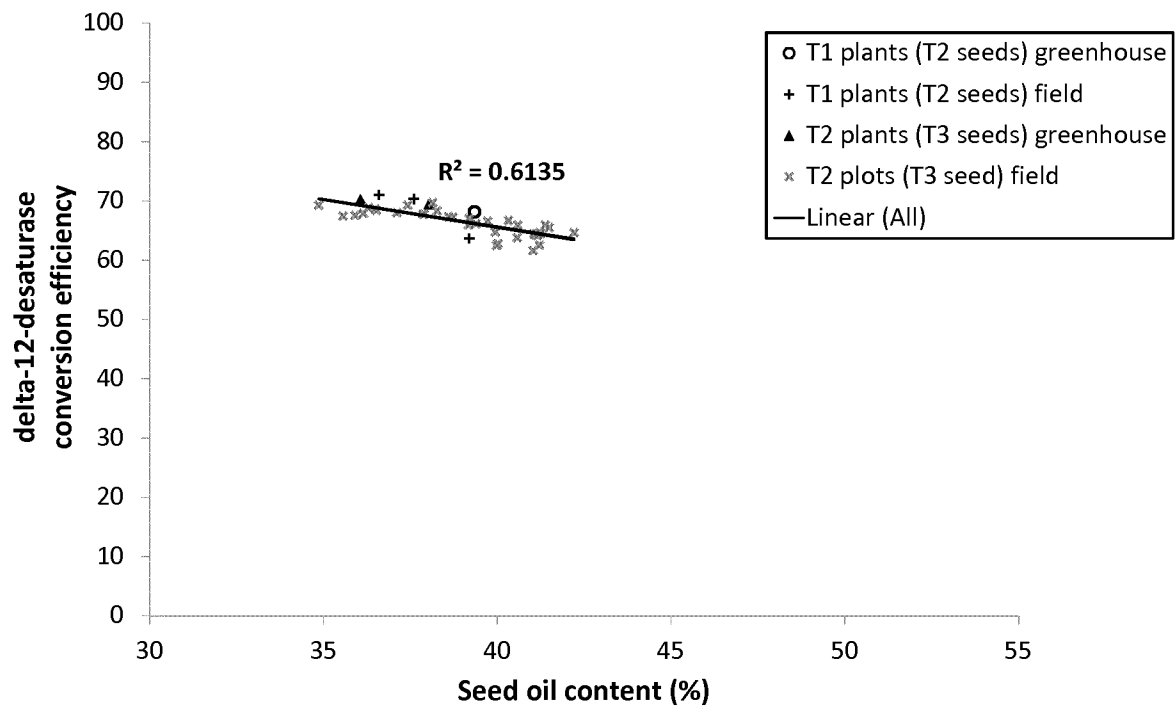


Fig. 48

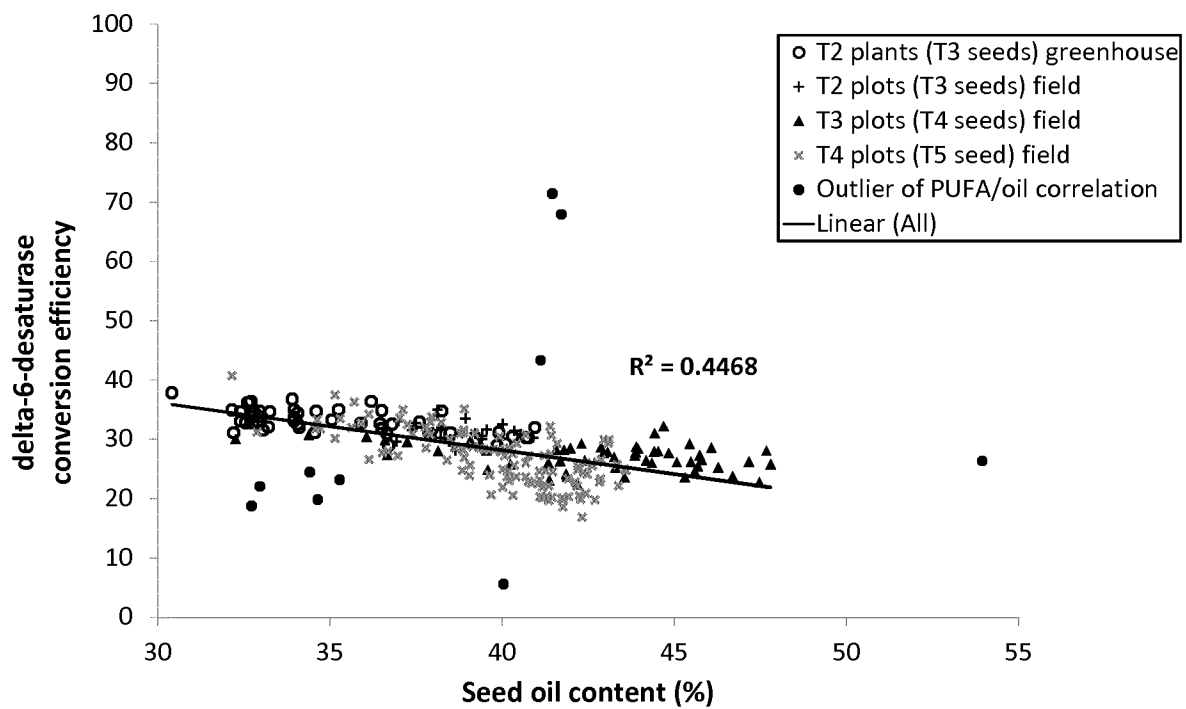


Fig. 49

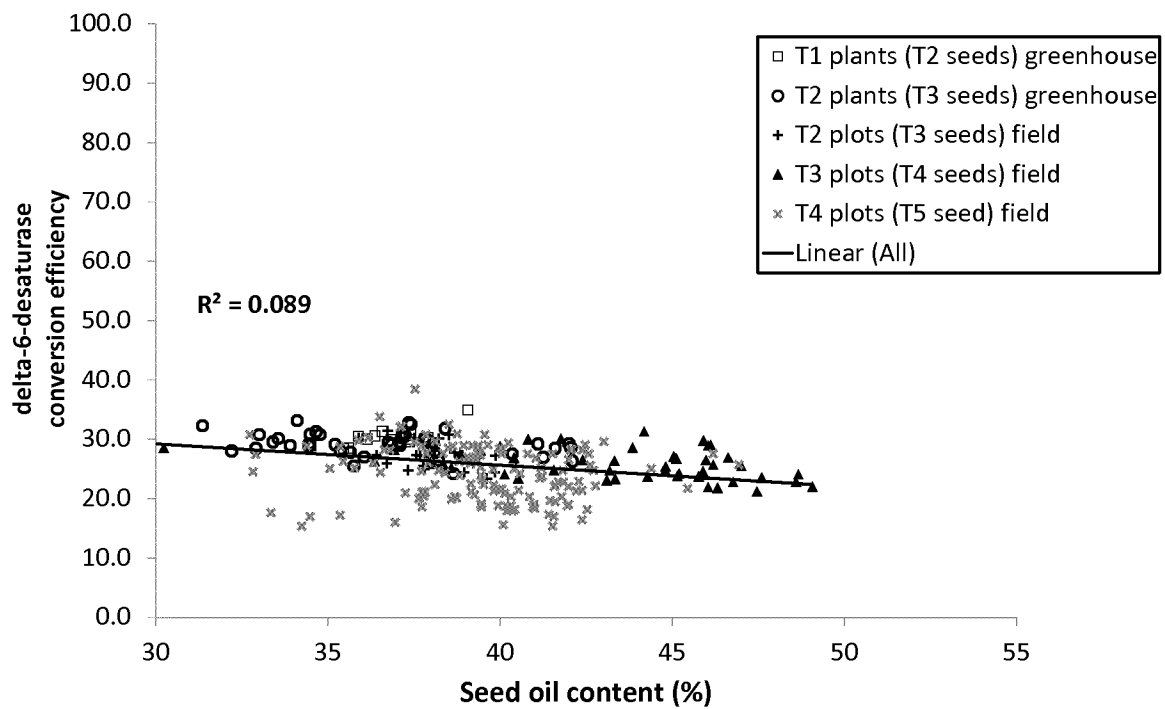


Fig. 50

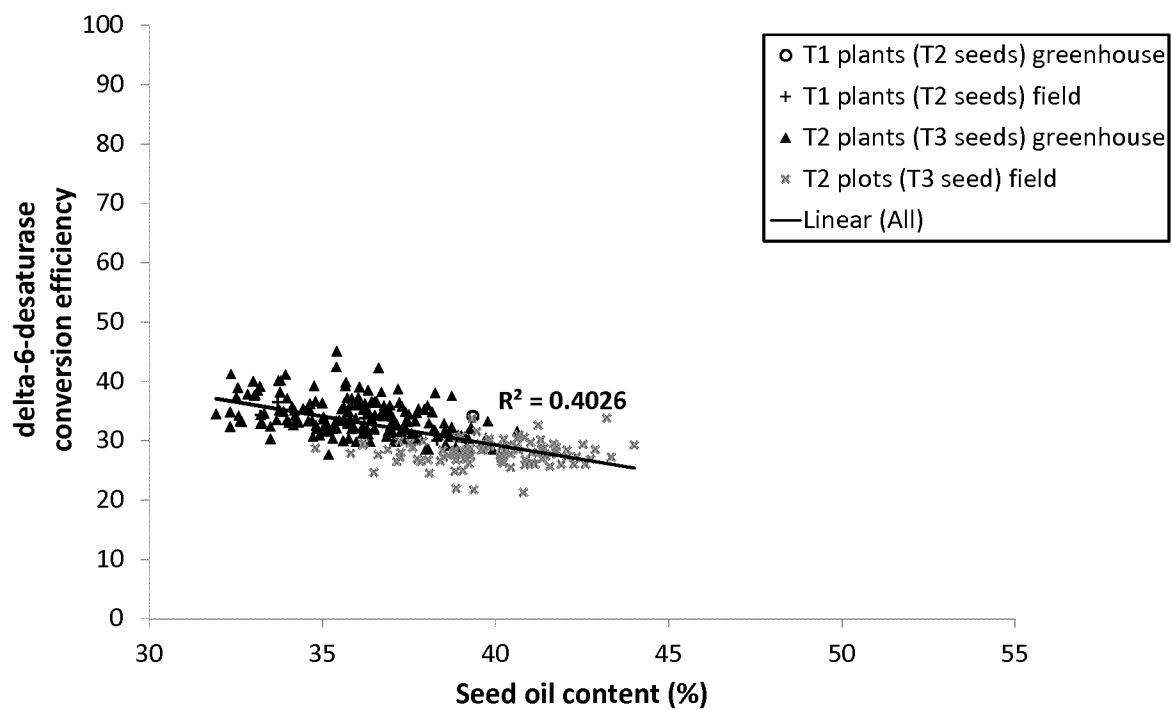


Fig. 51

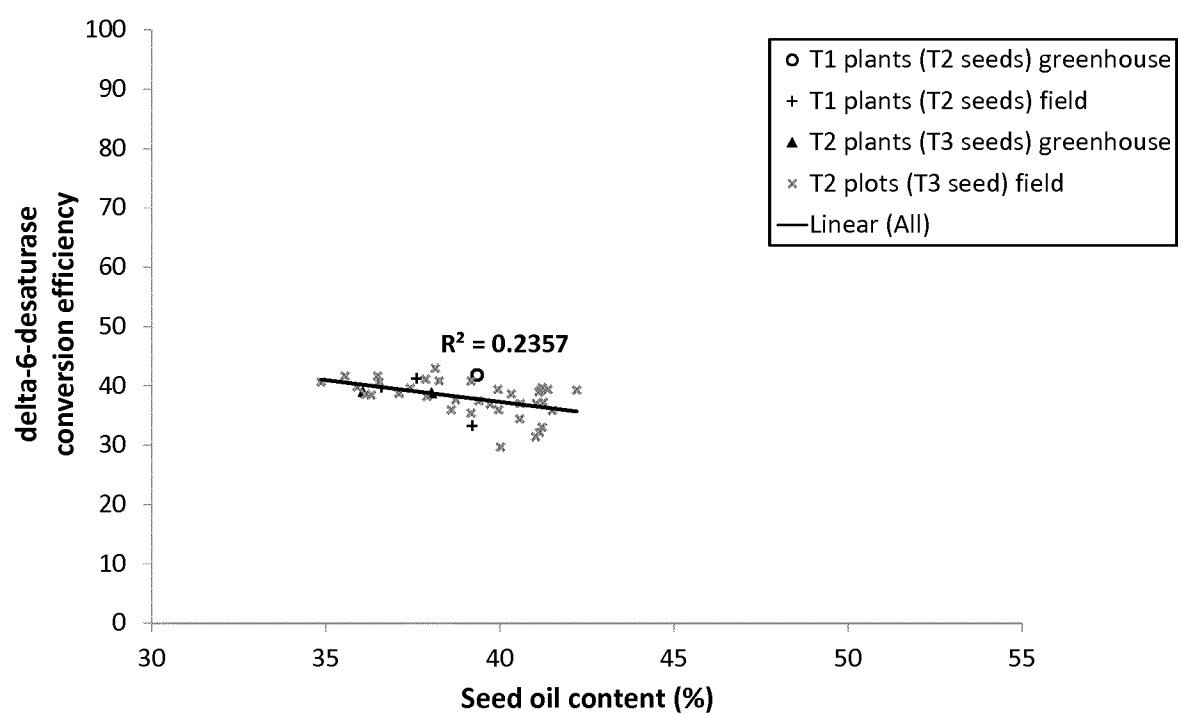


Fig. 52

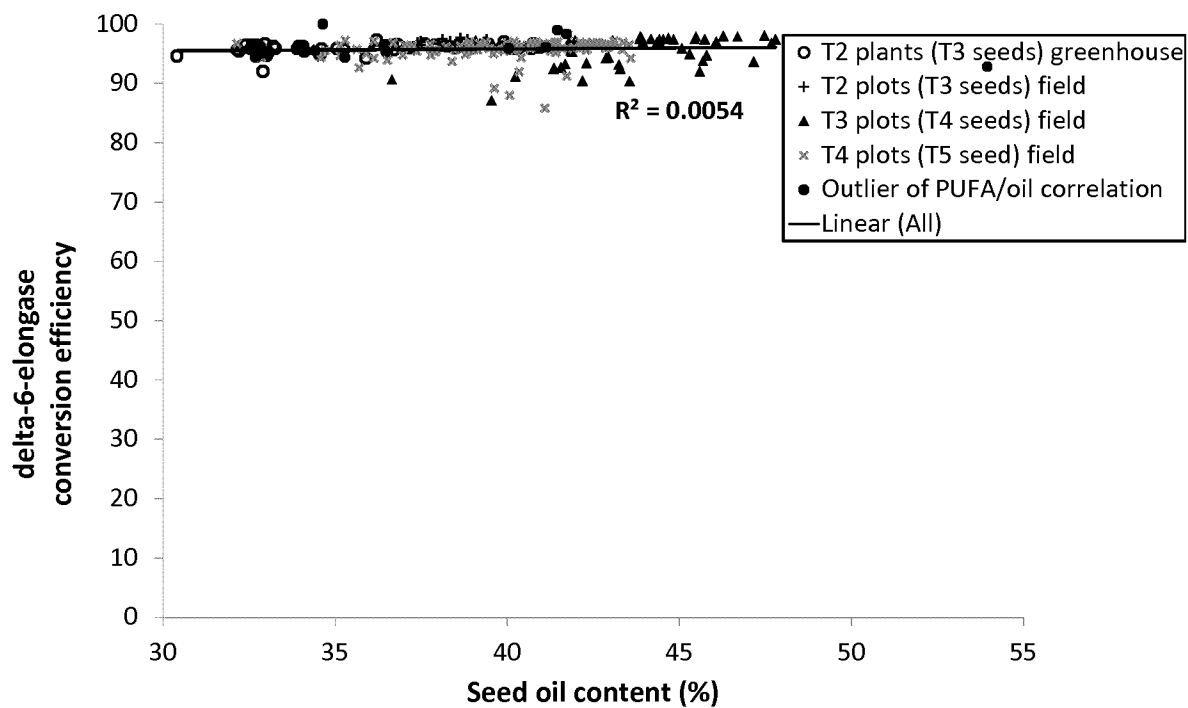


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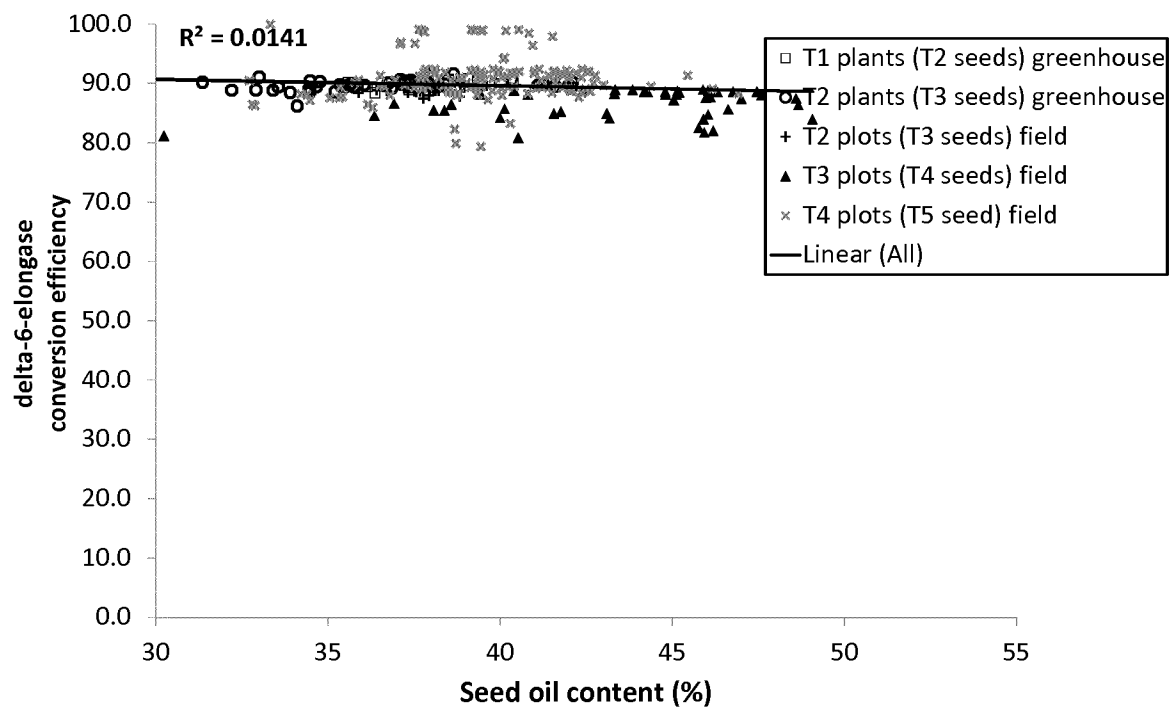


Fig. 54

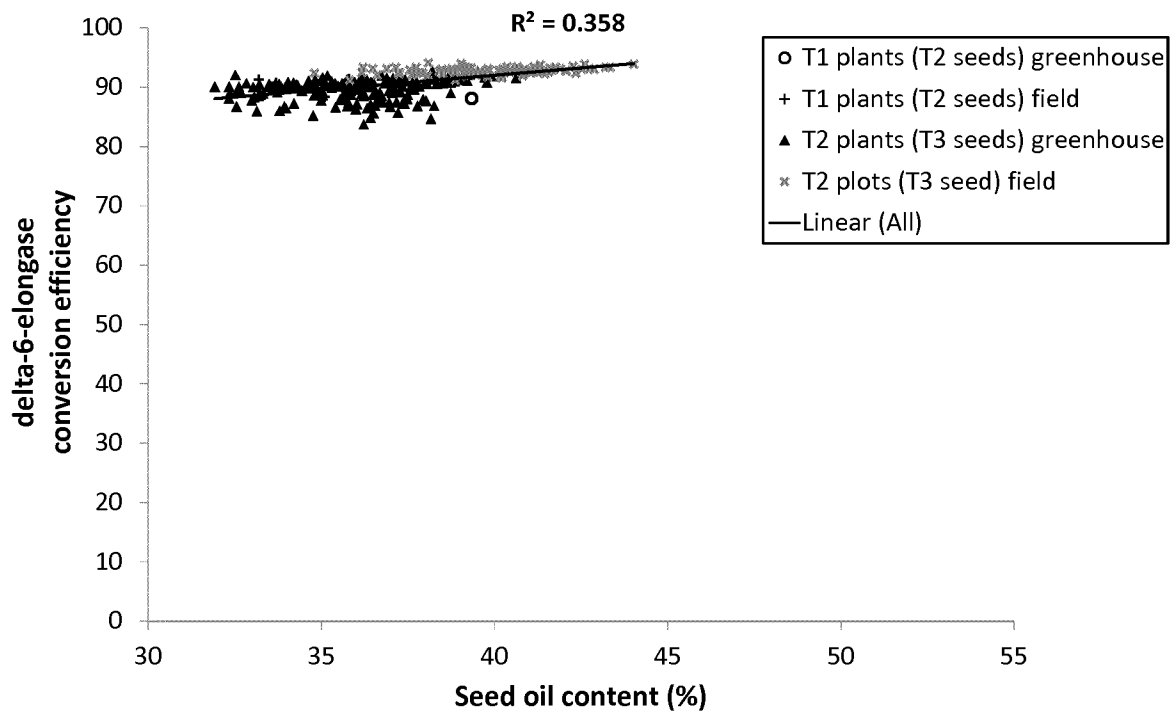


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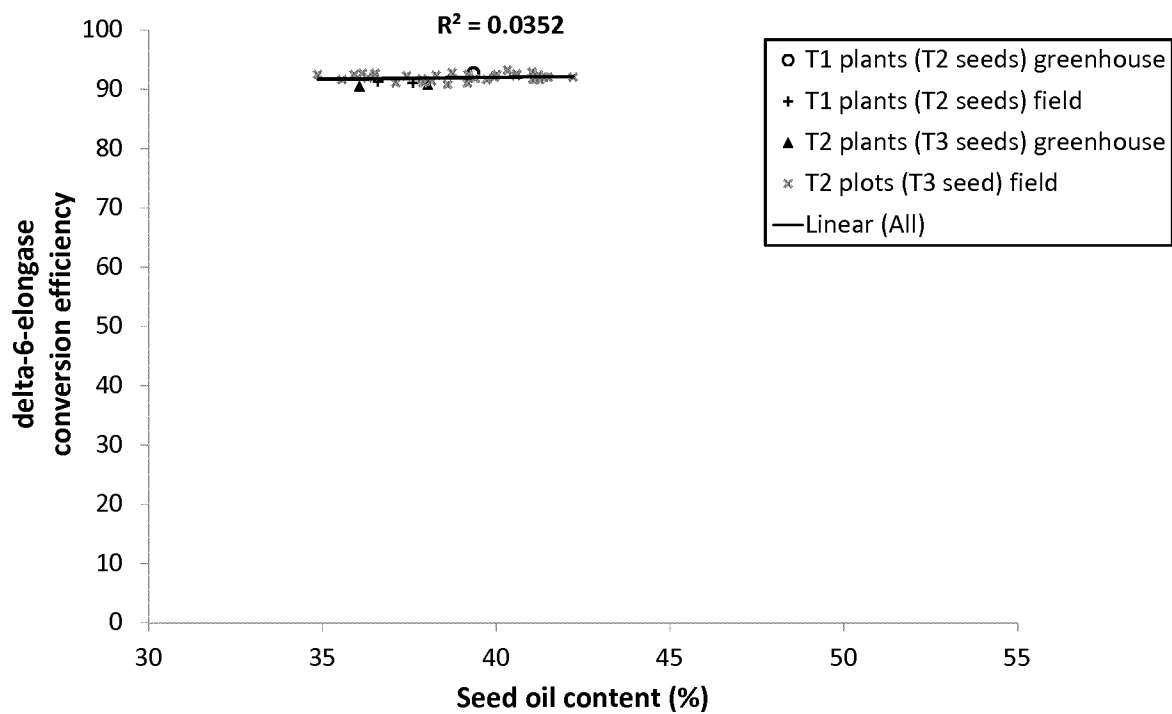


Fig. 56

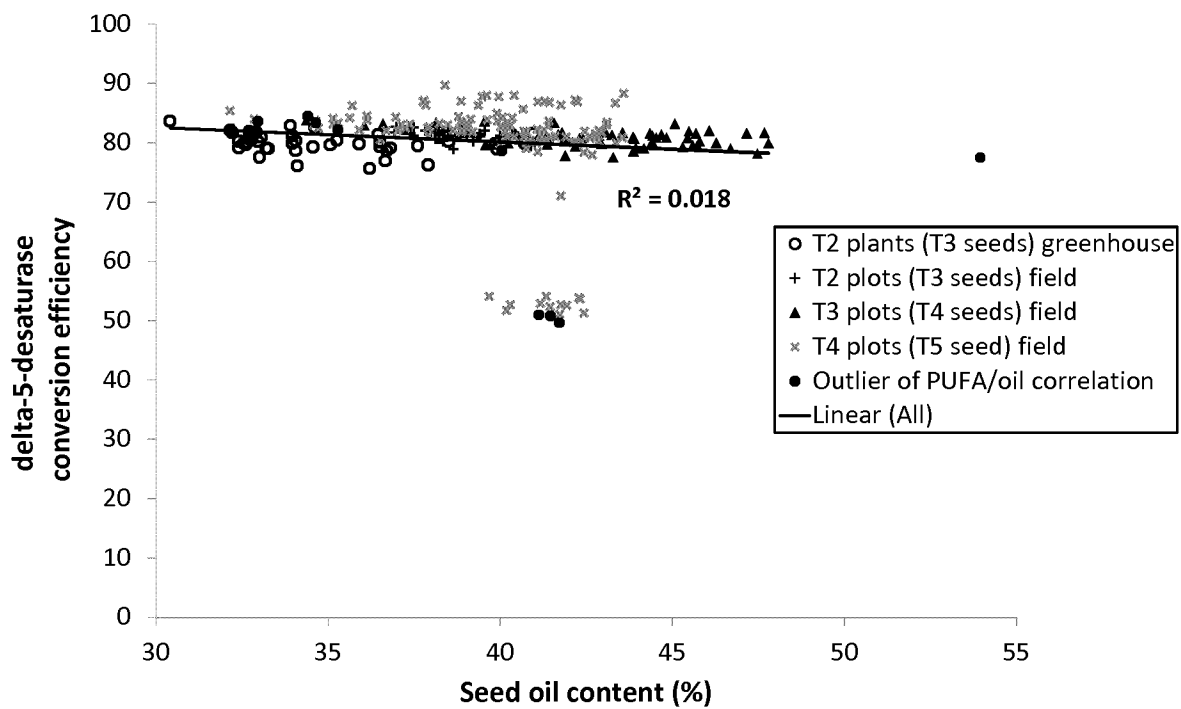


Fig. 57

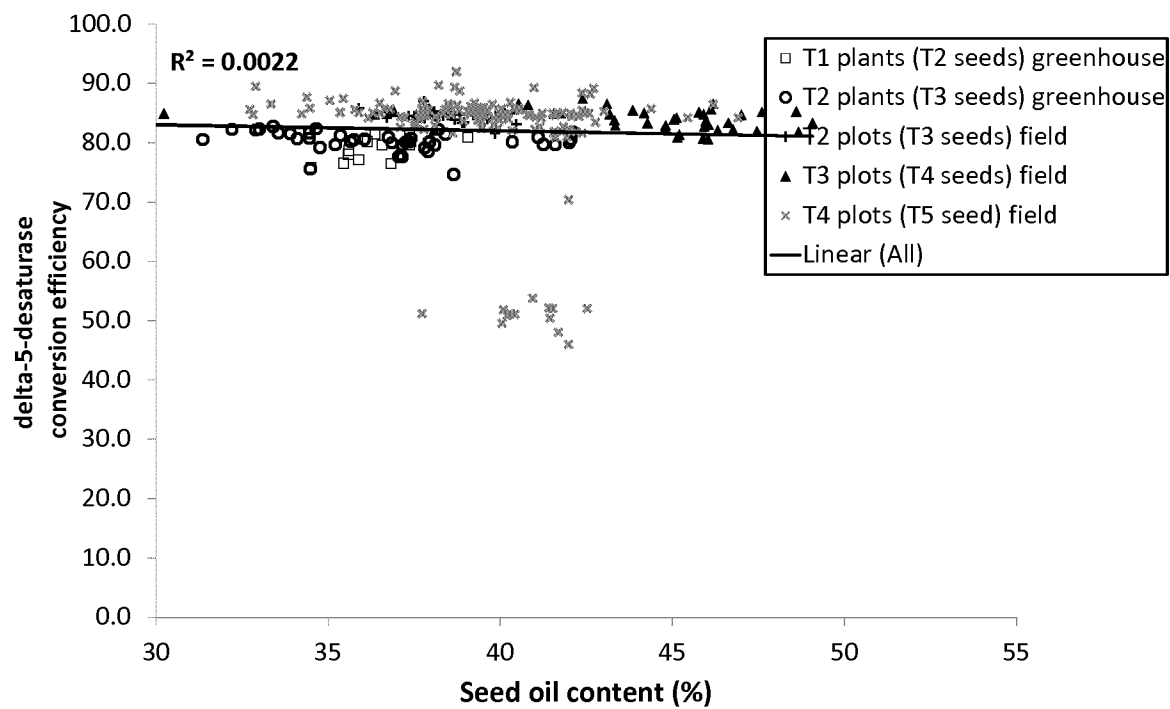


Fig. 58

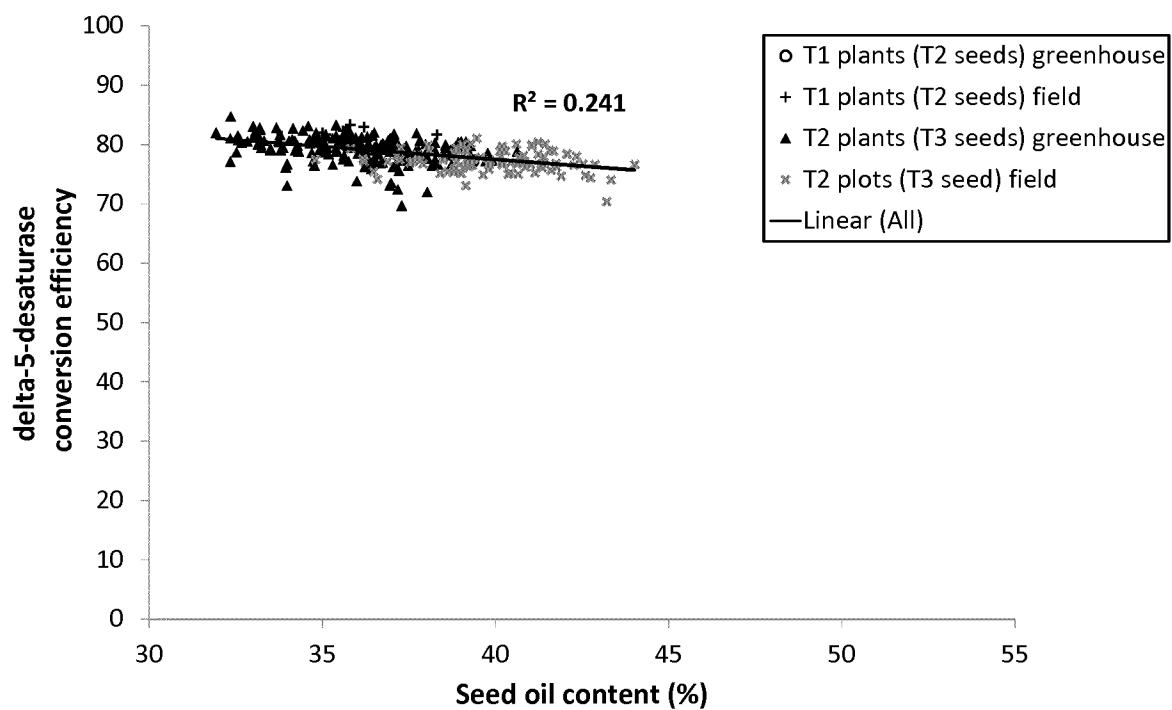


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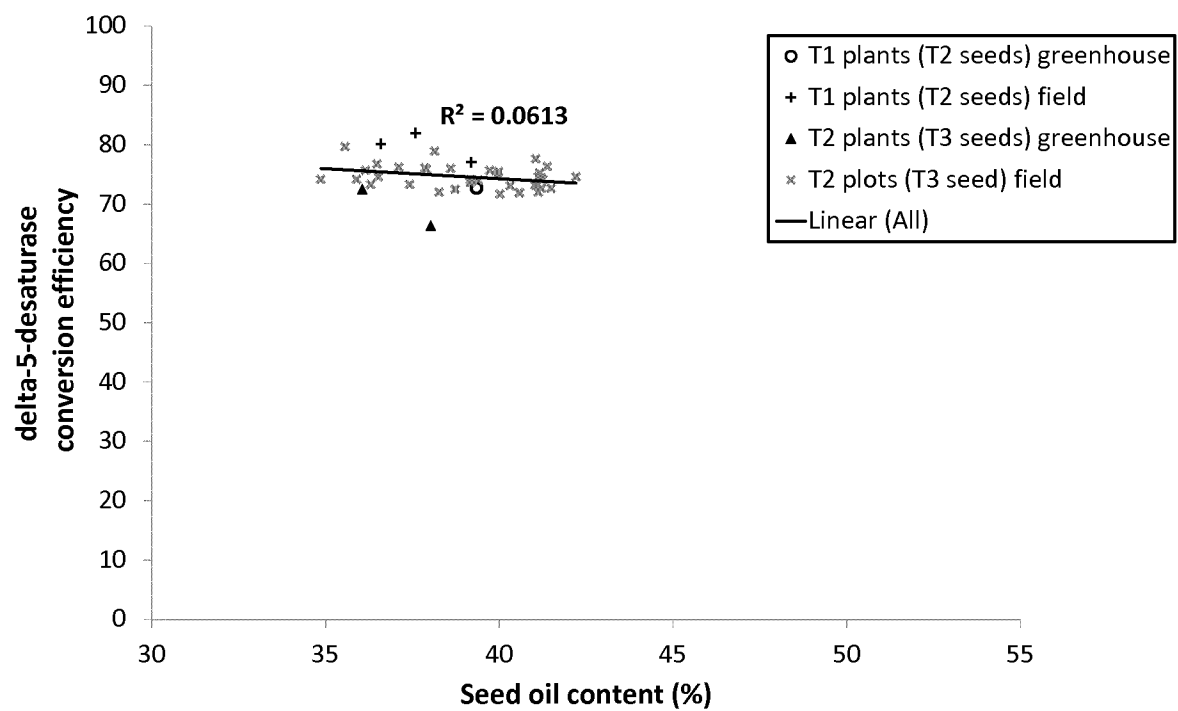


Fig. 60

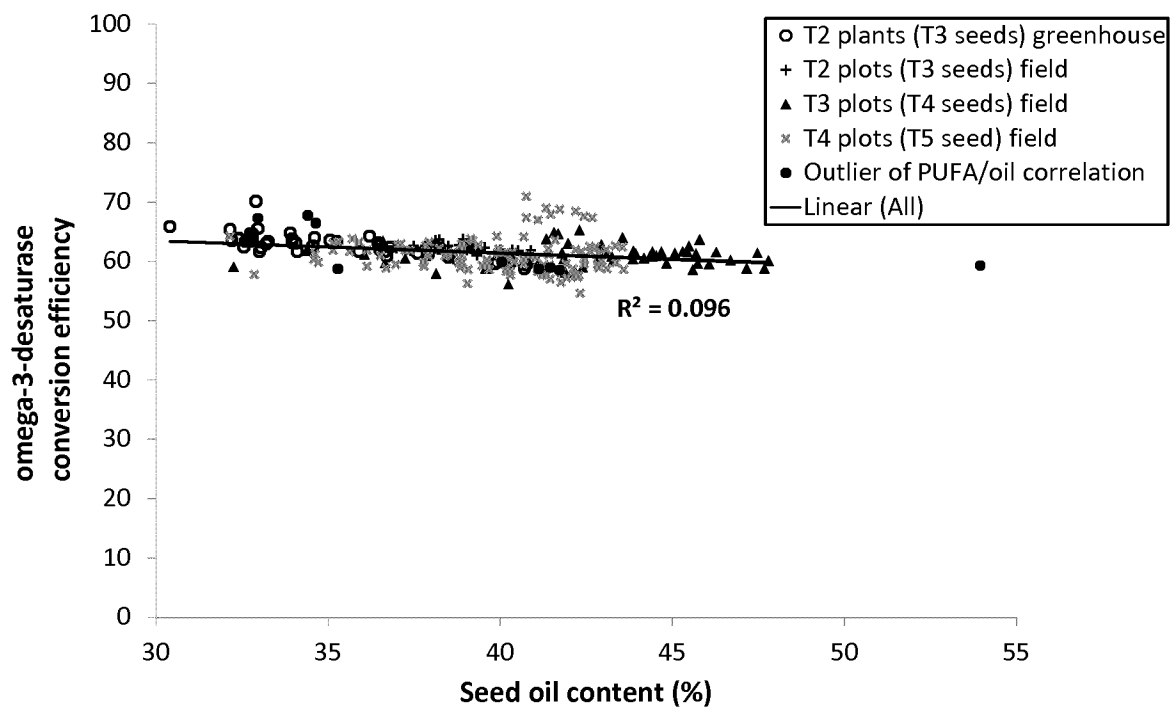


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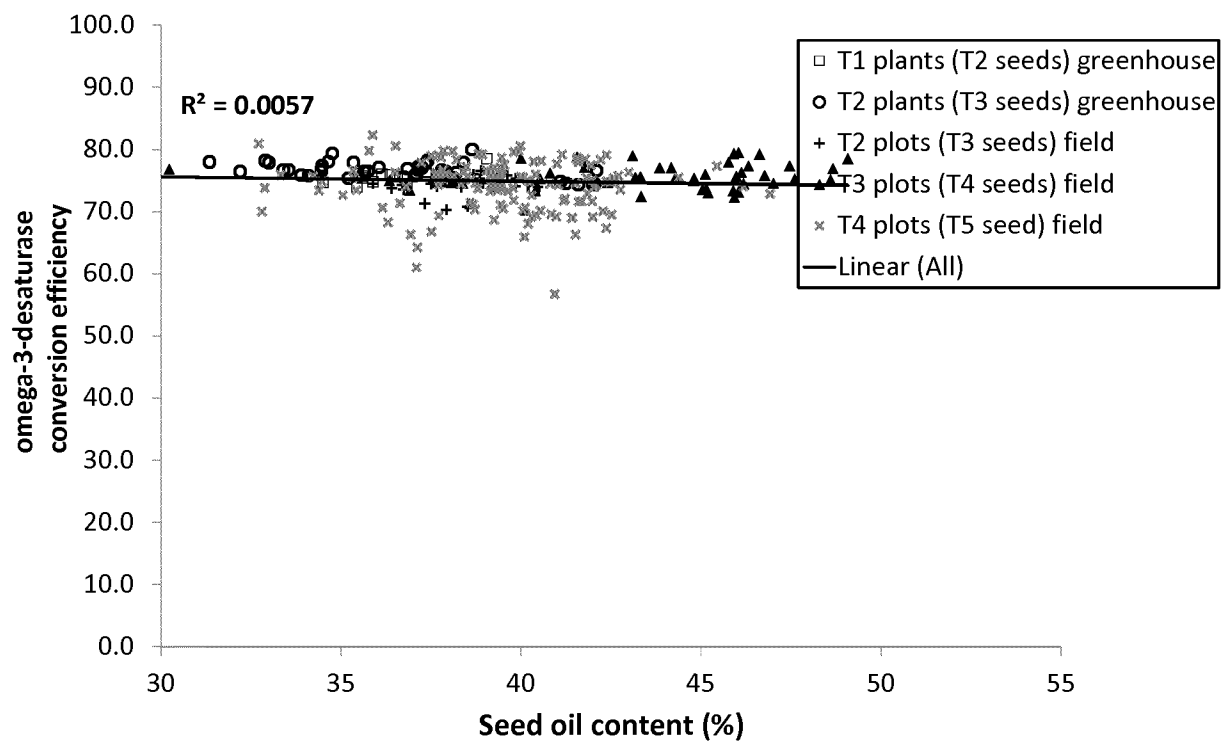


Fig. 62

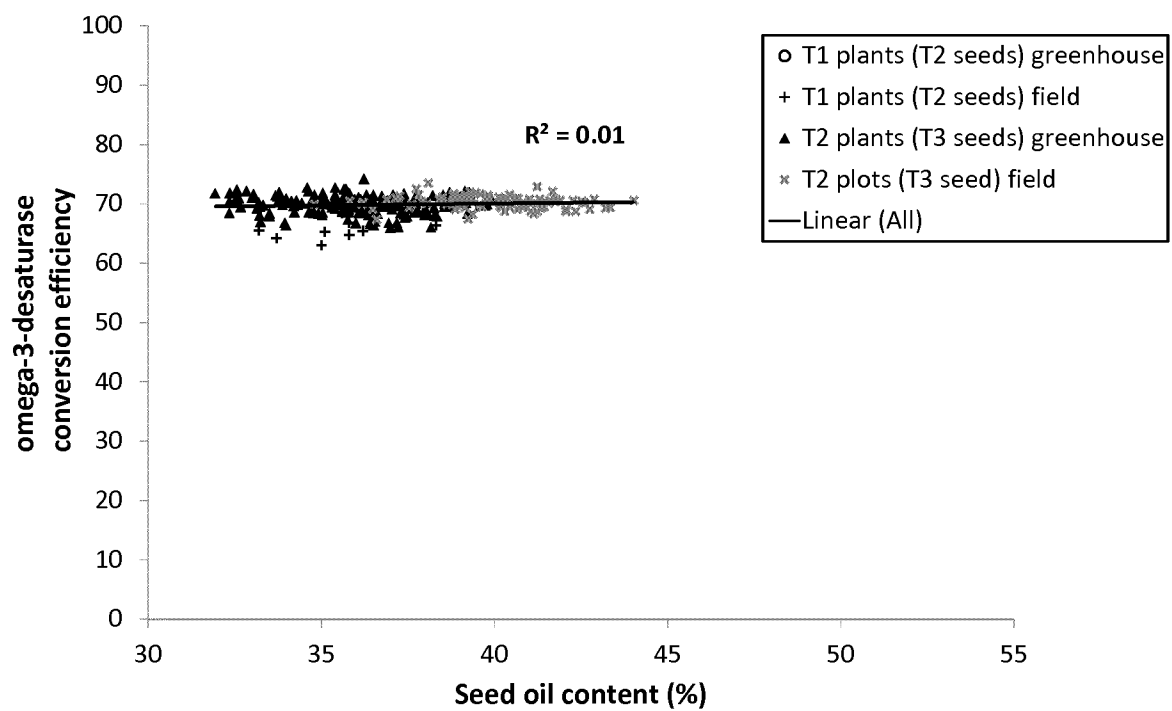


Fig. 63

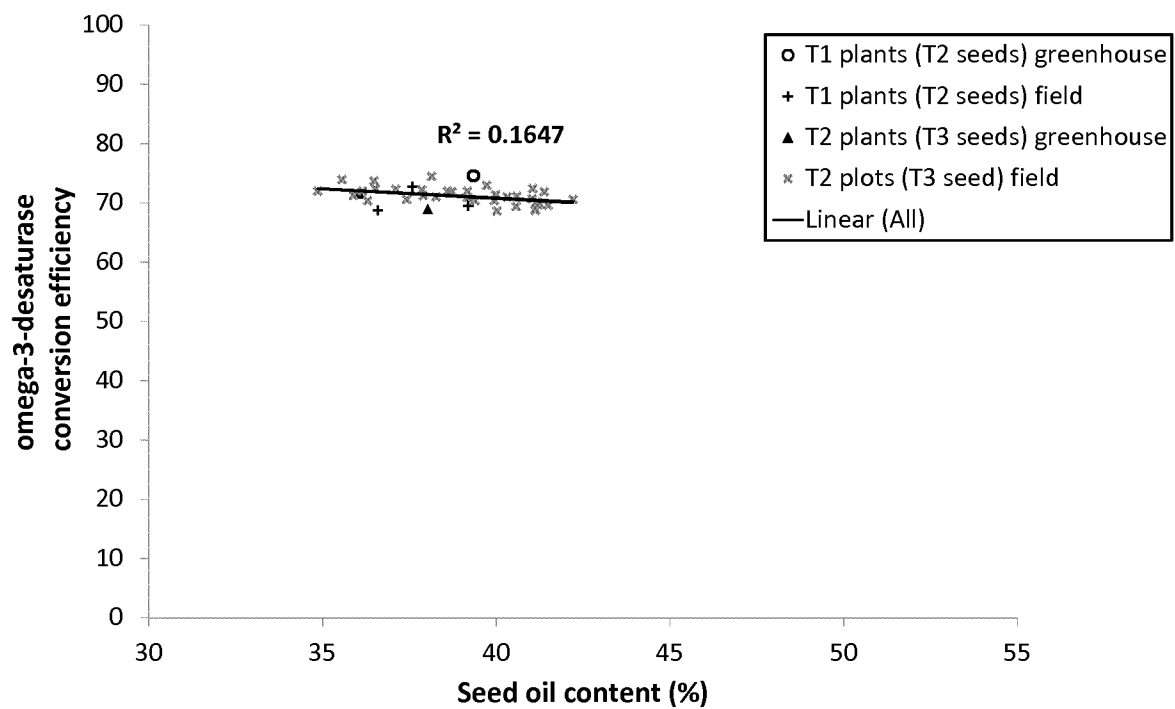


Fig. 64

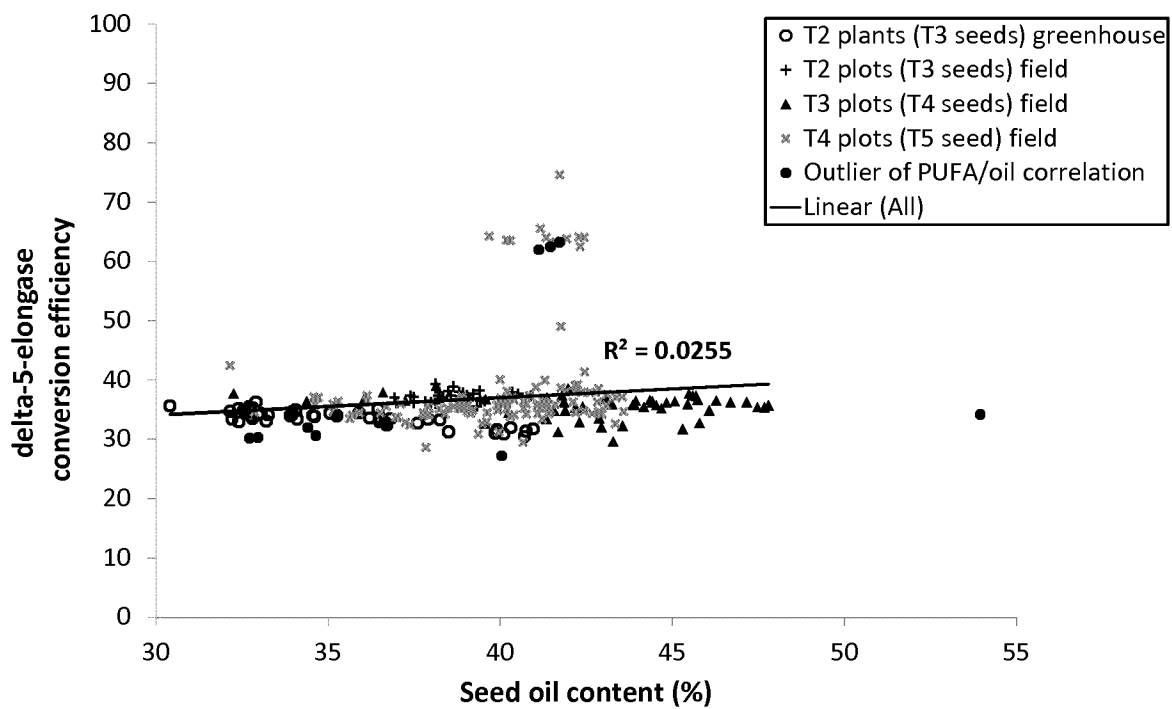


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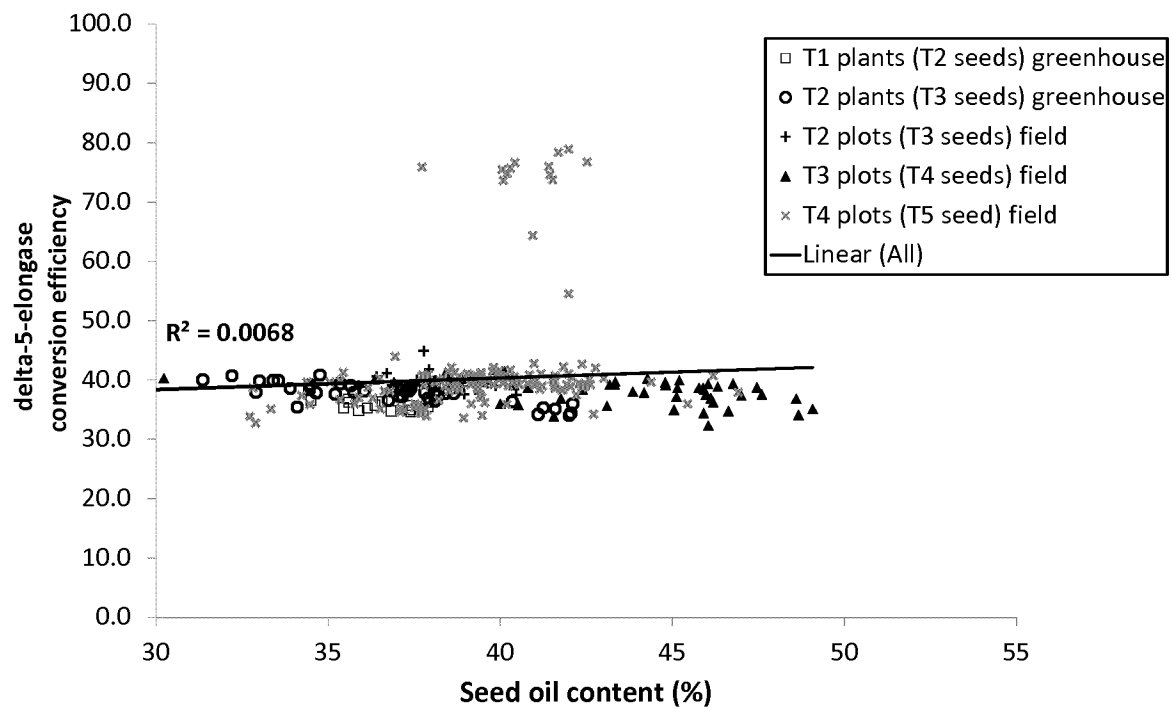


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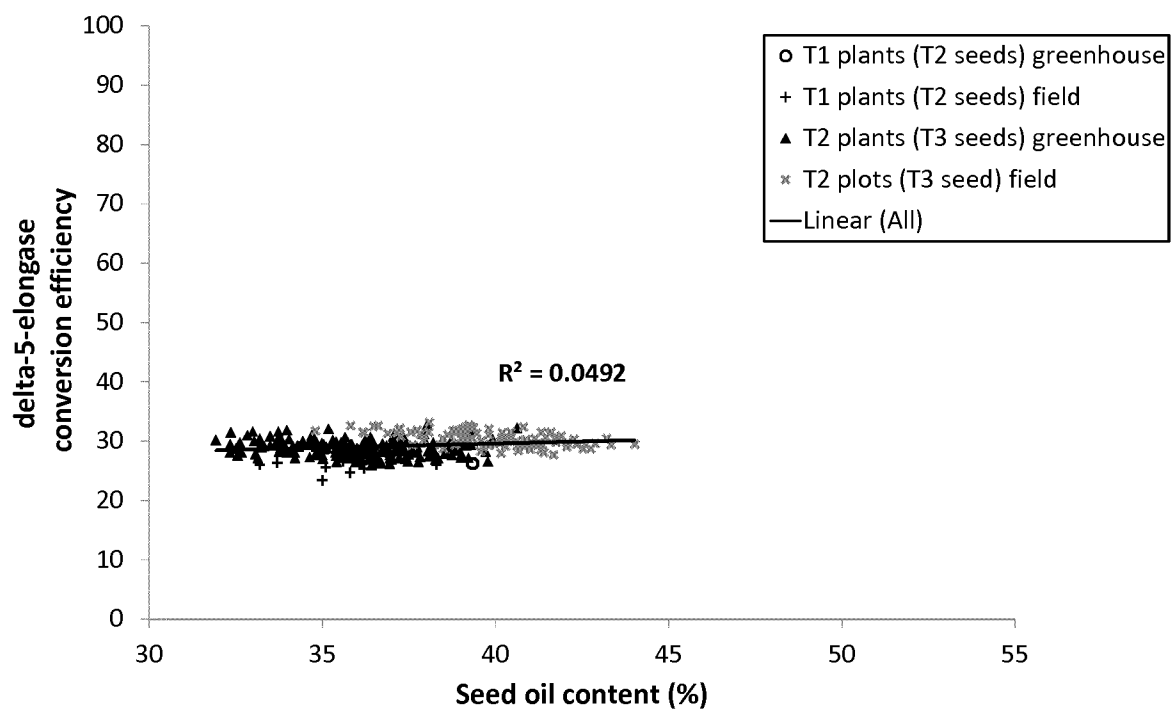


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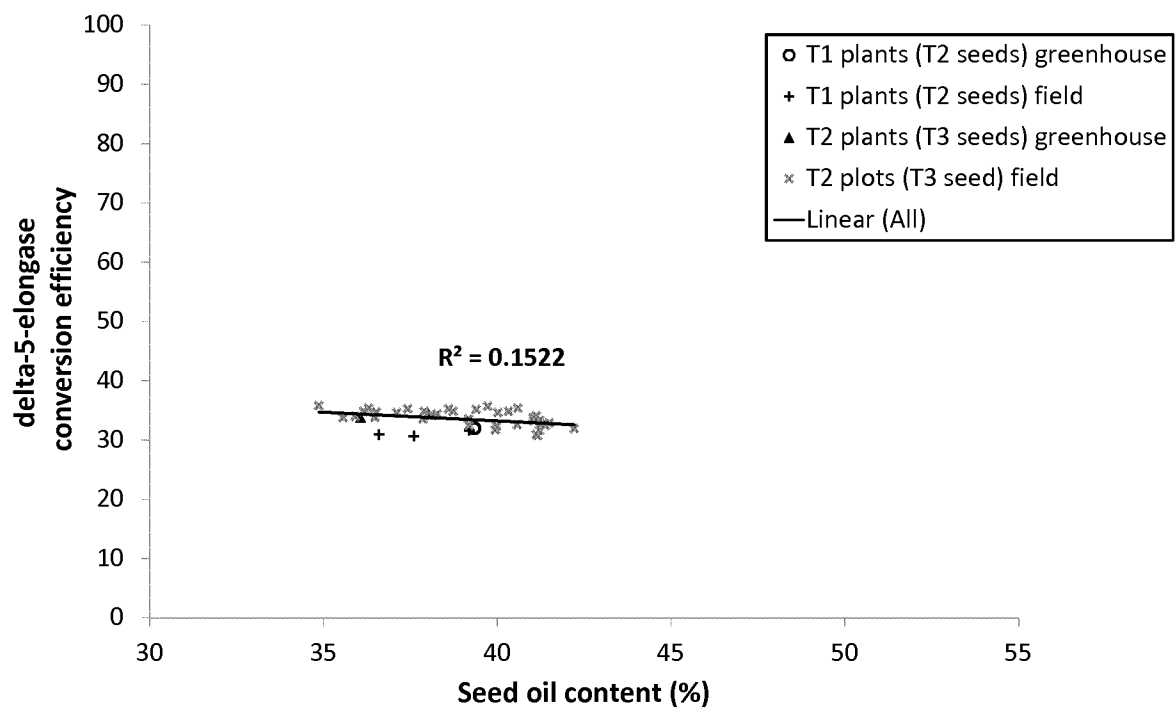


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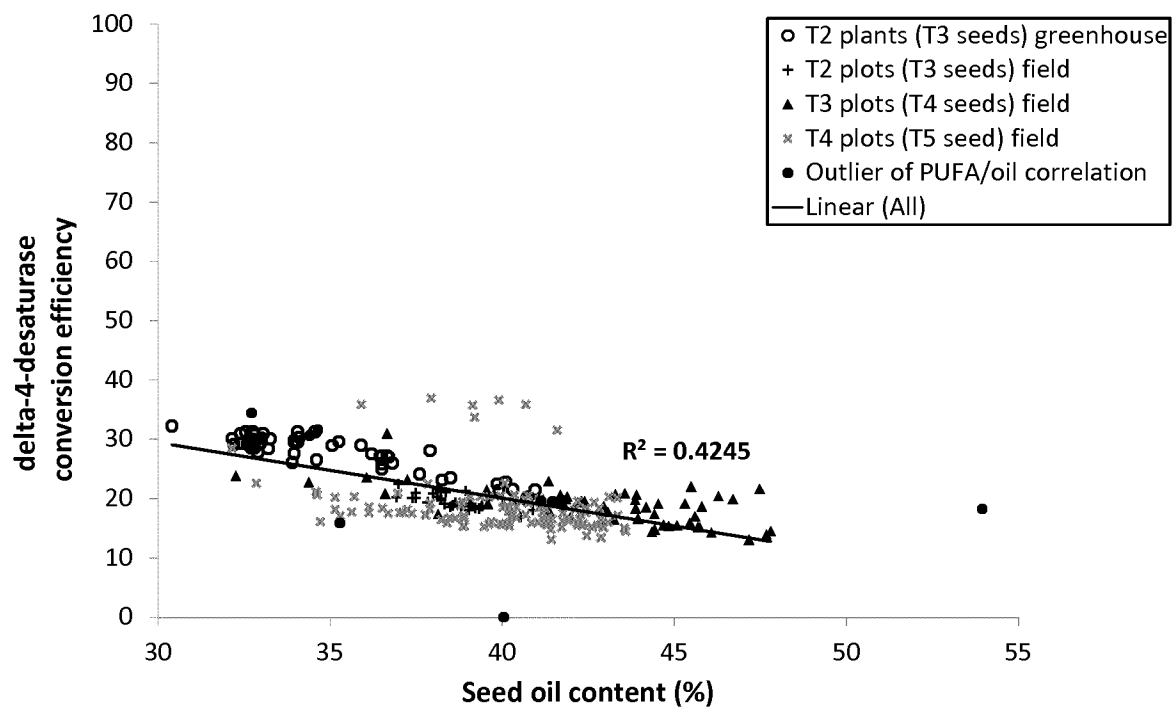


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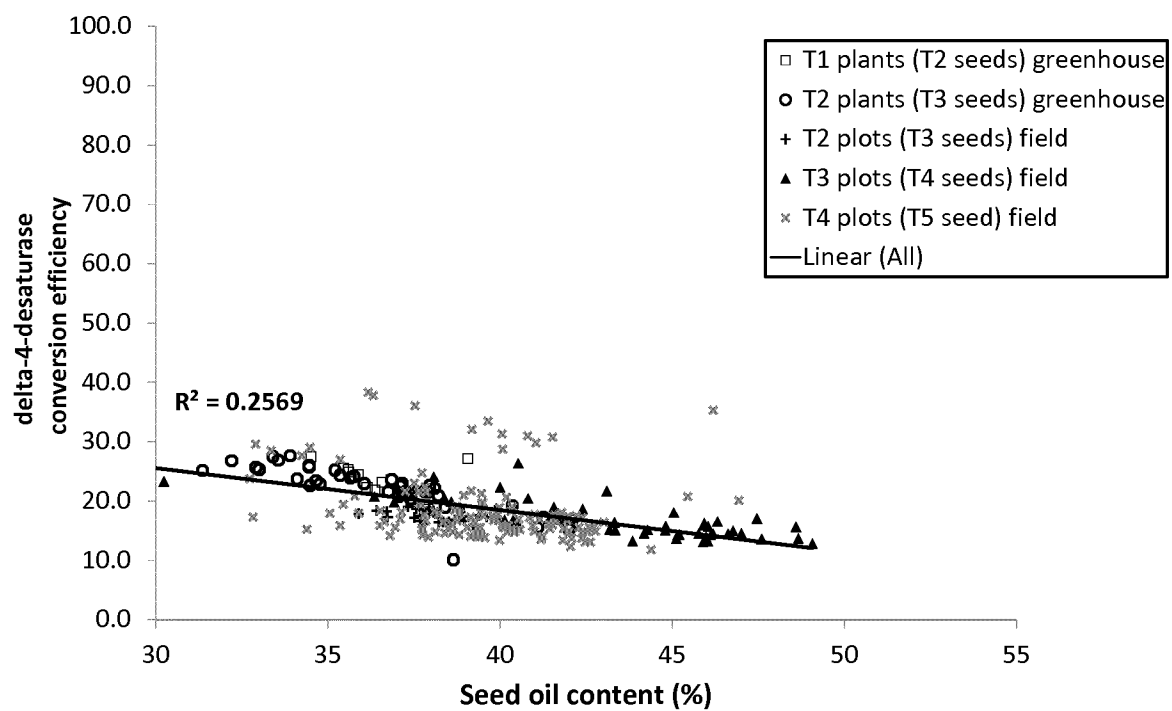


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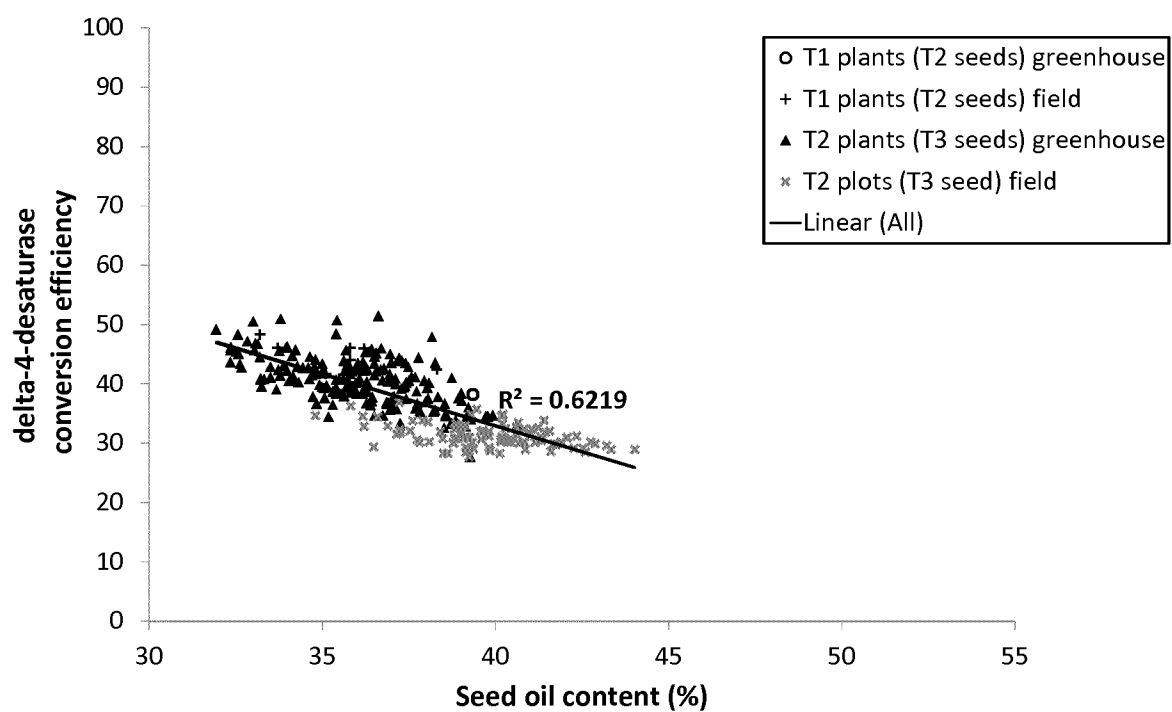


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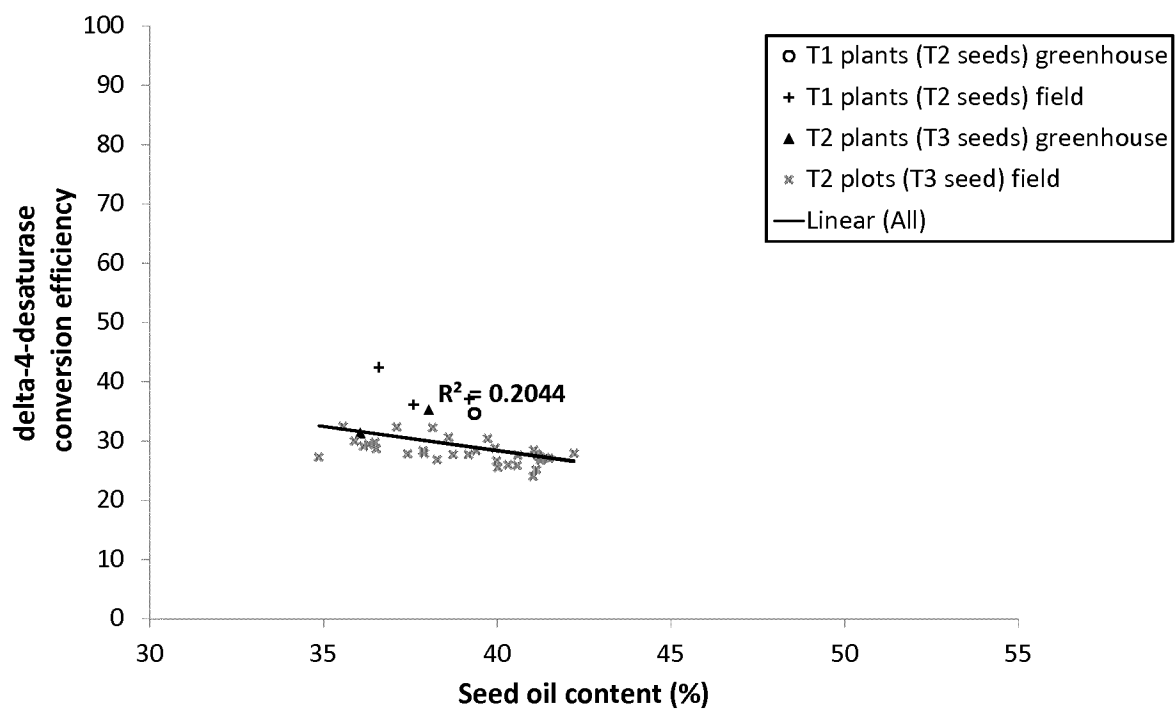


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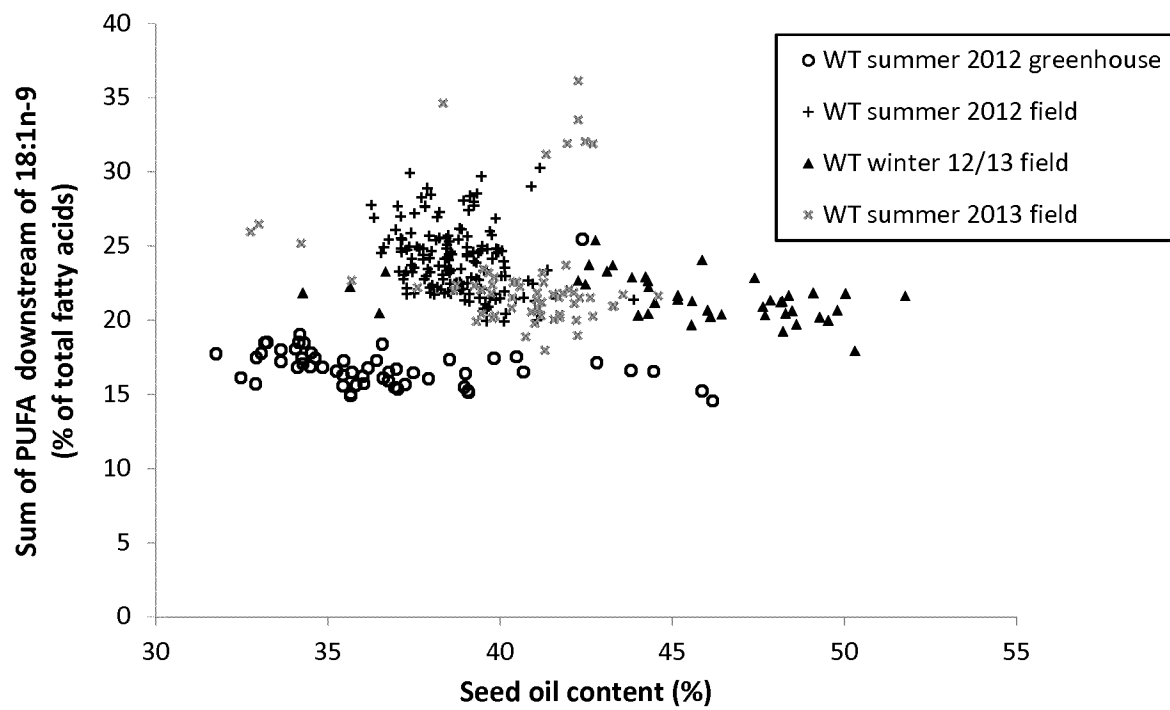


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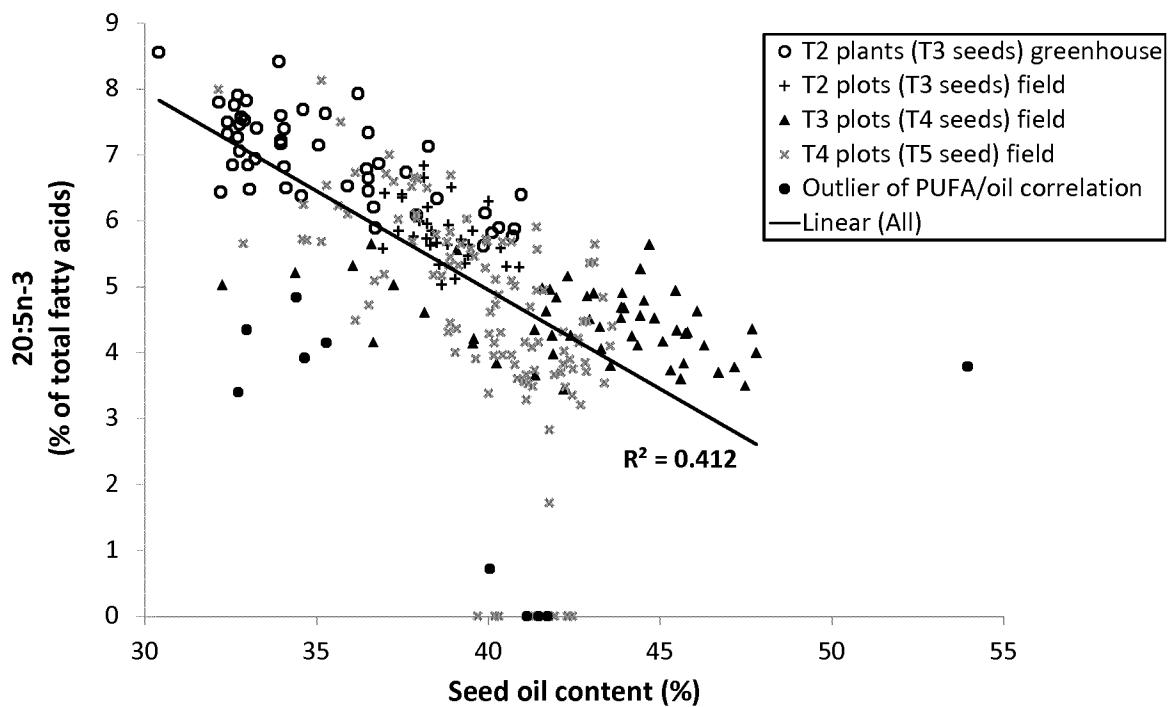


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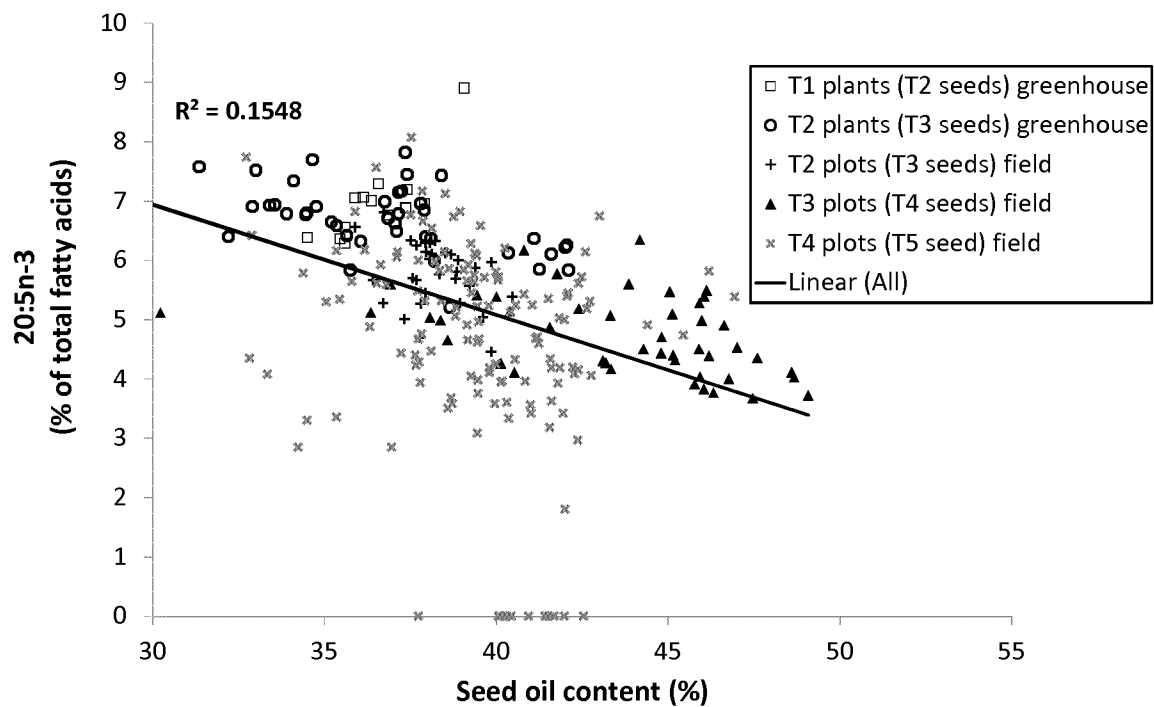


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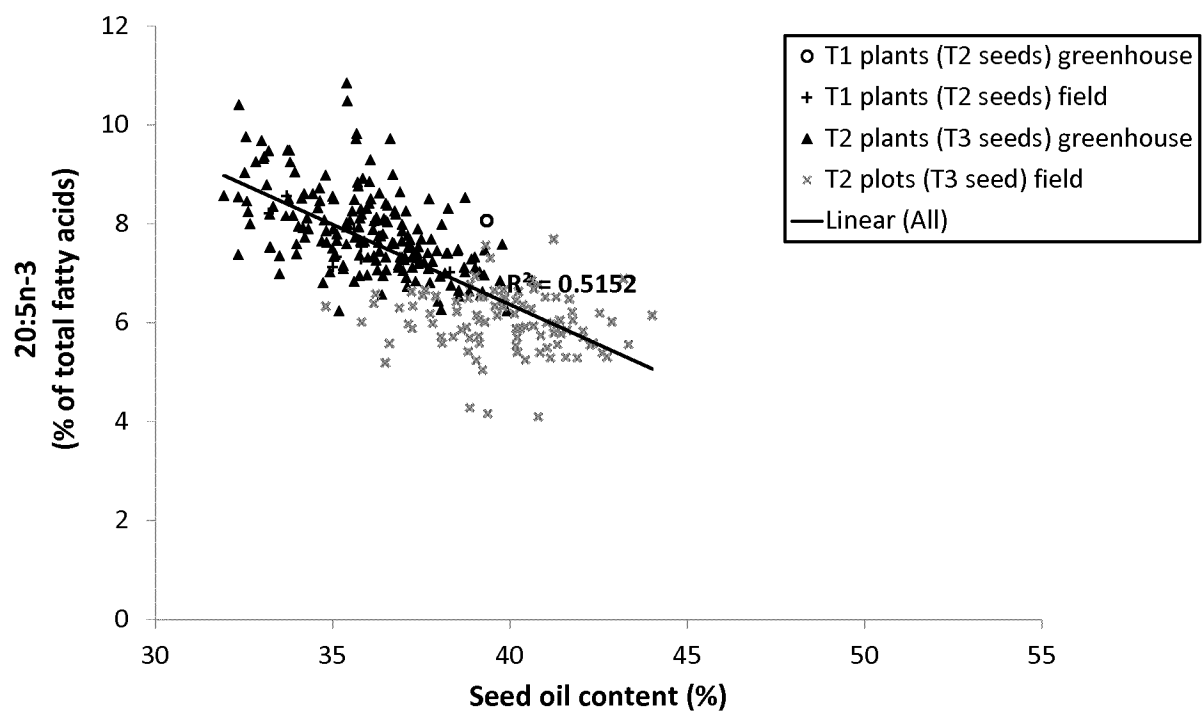


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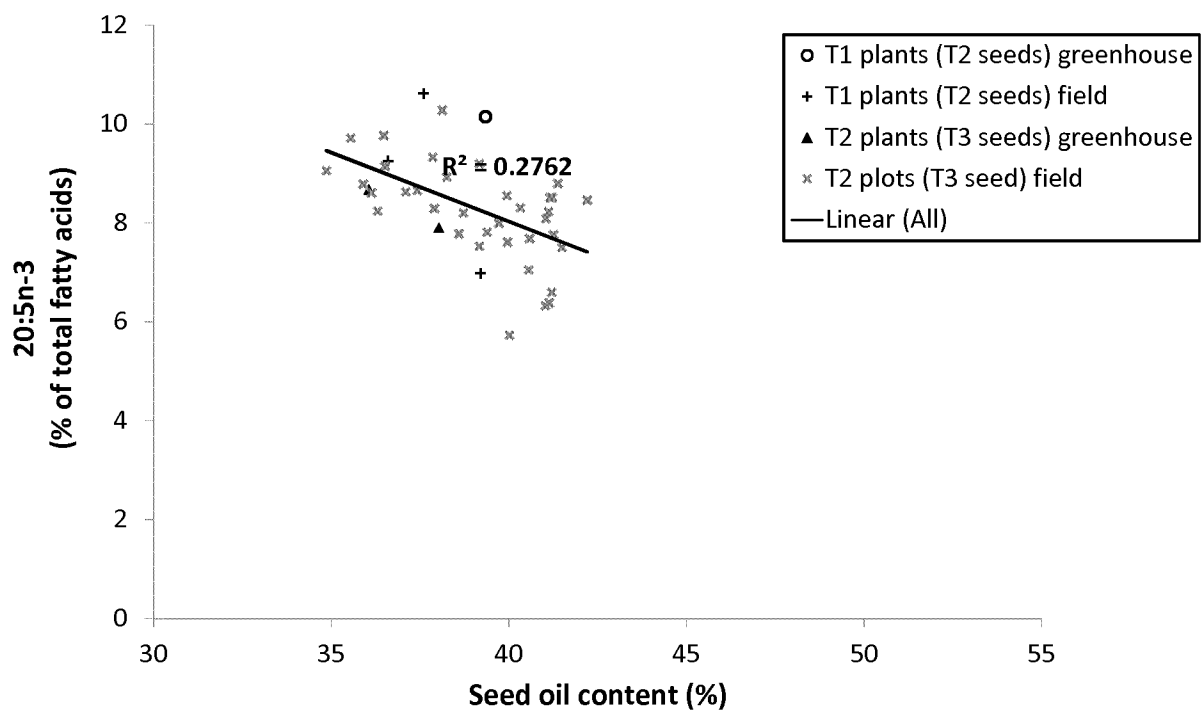


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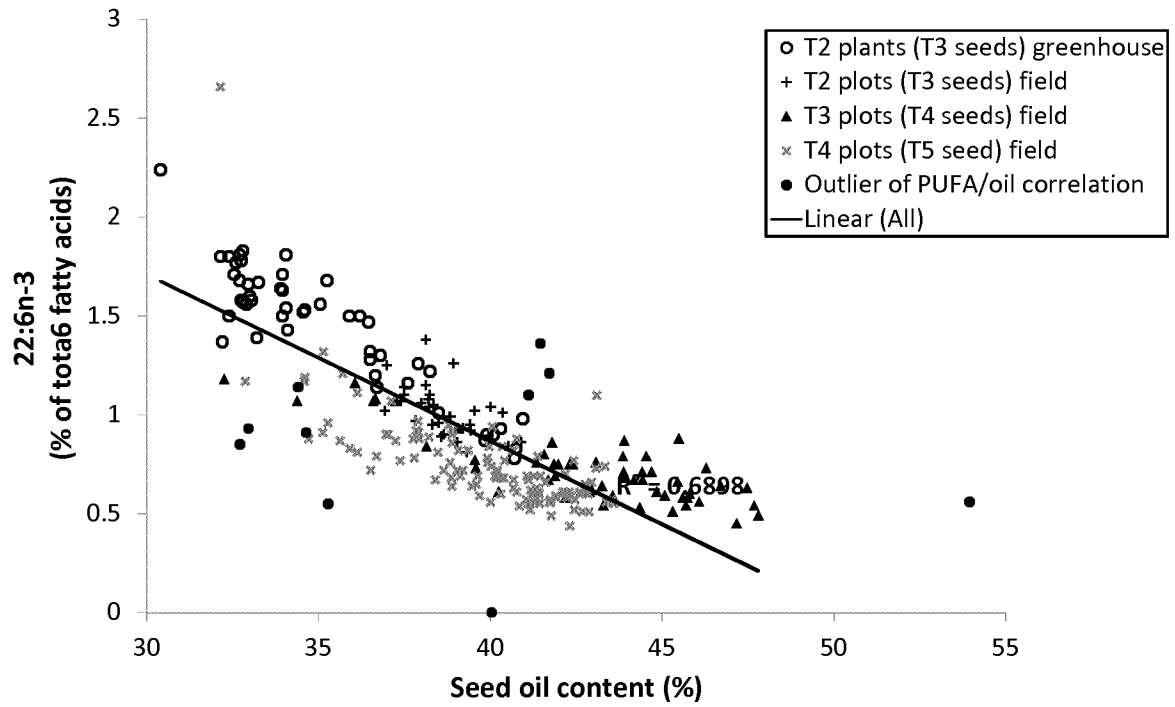


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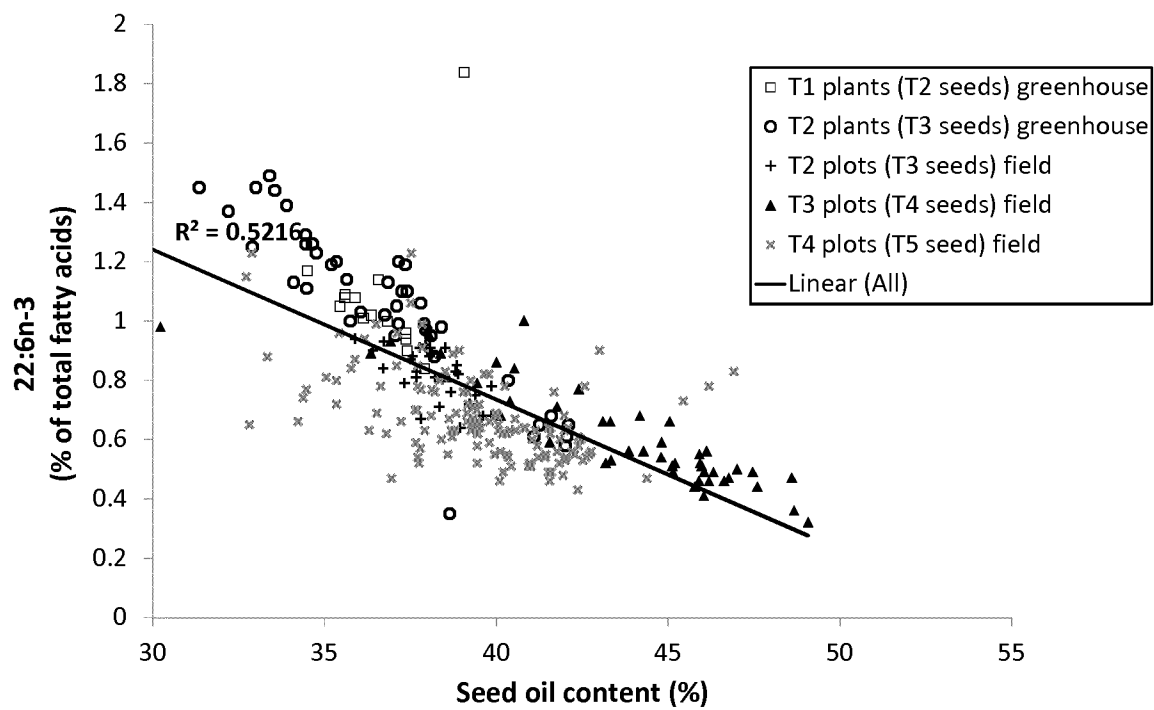


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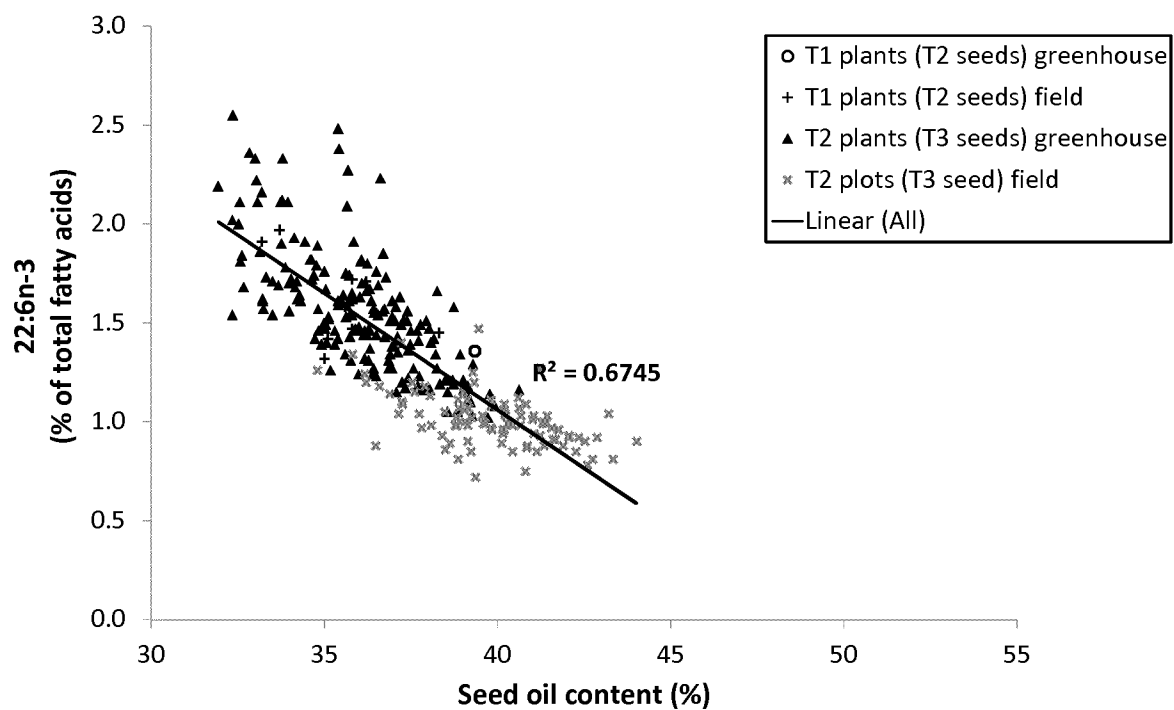
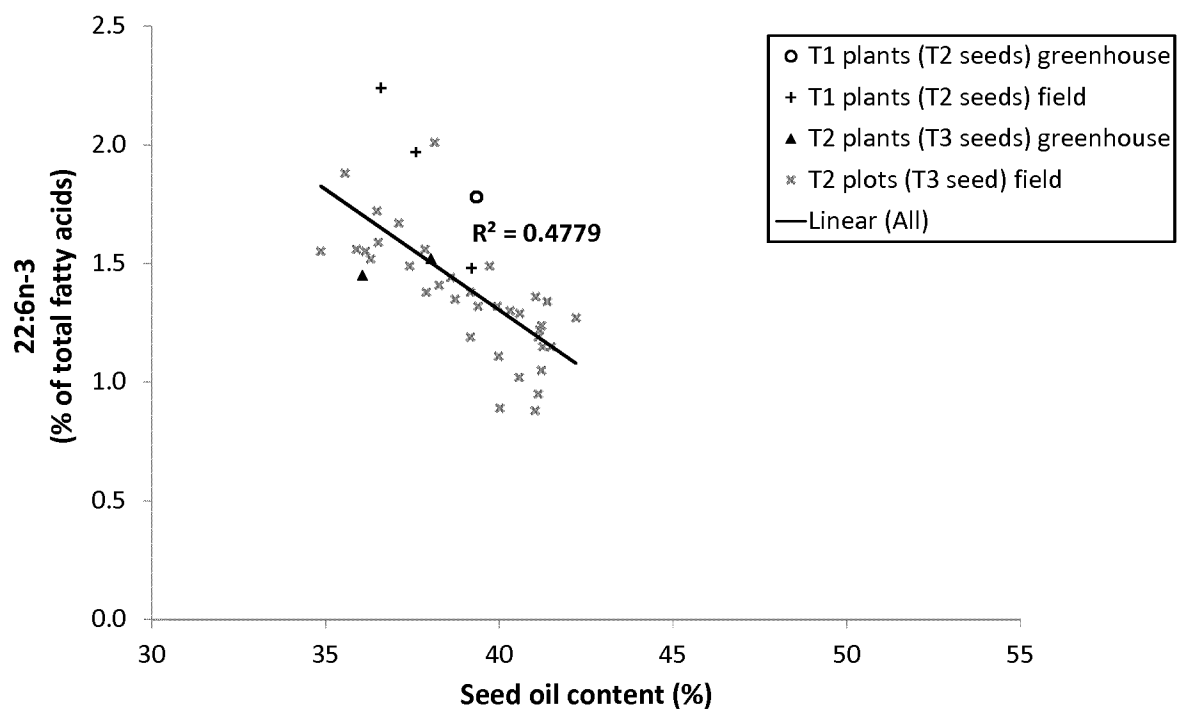


Fig. 80



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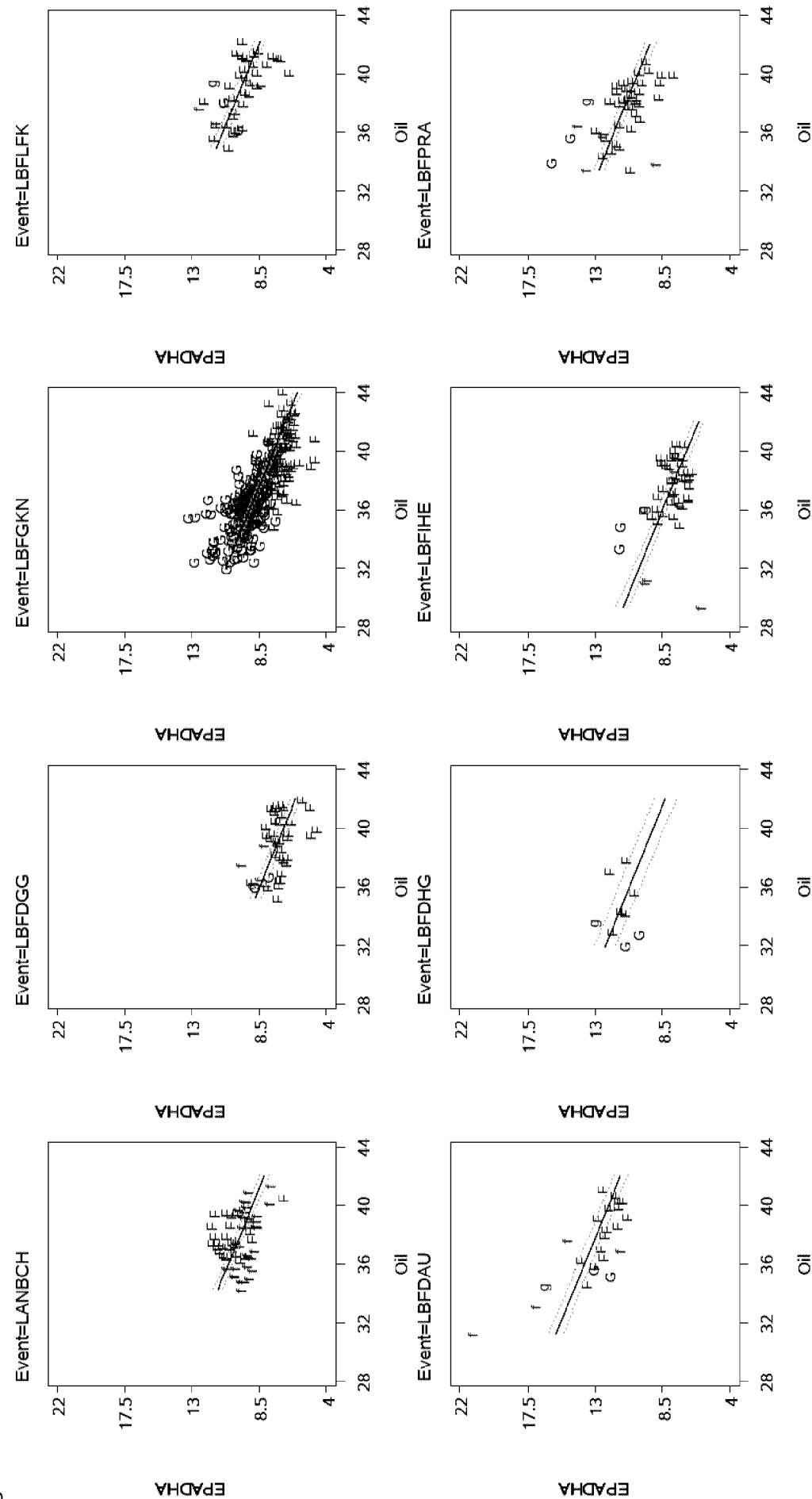
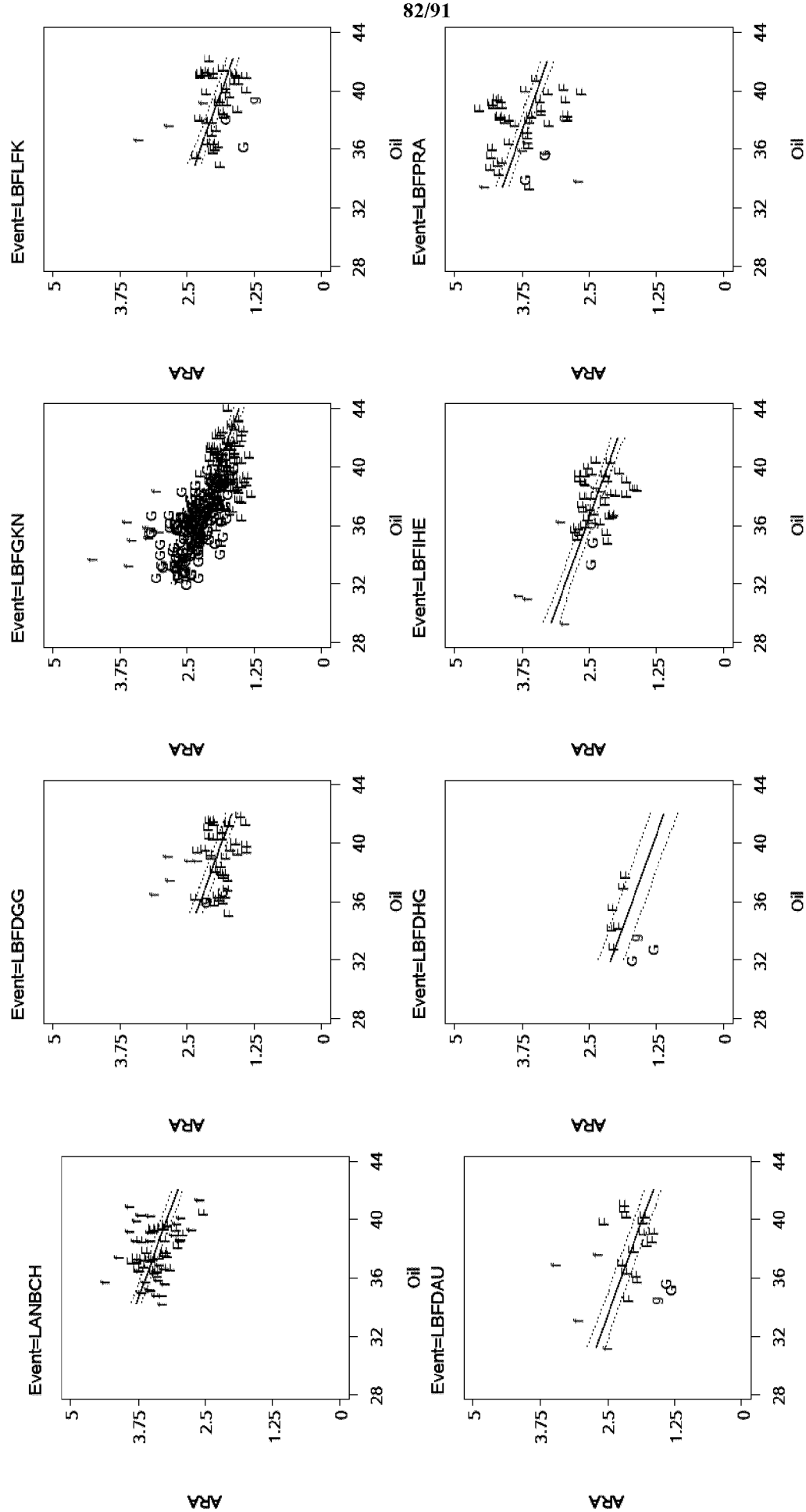


Fig. 81

Fig. 82



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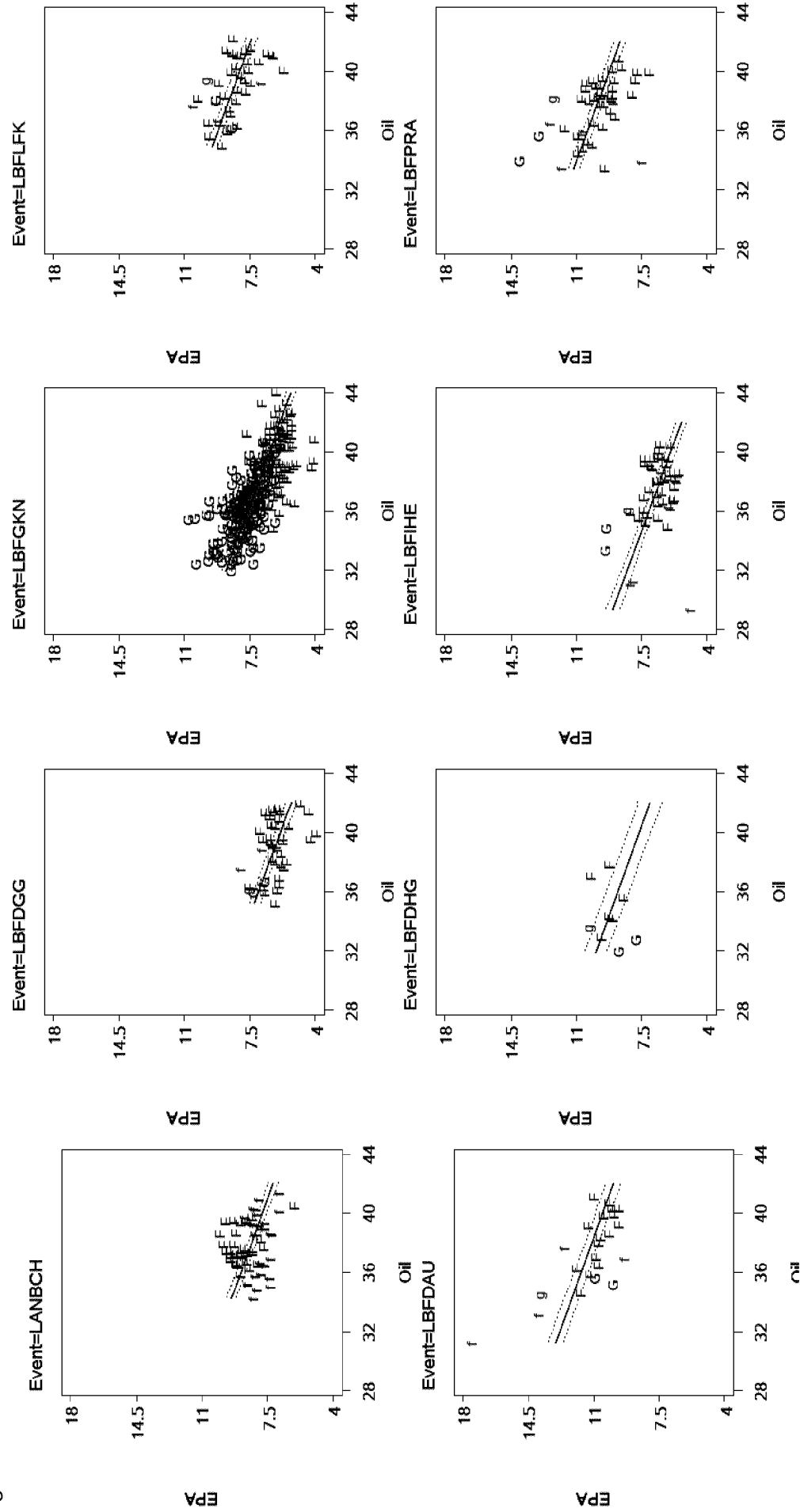


Fig. 83

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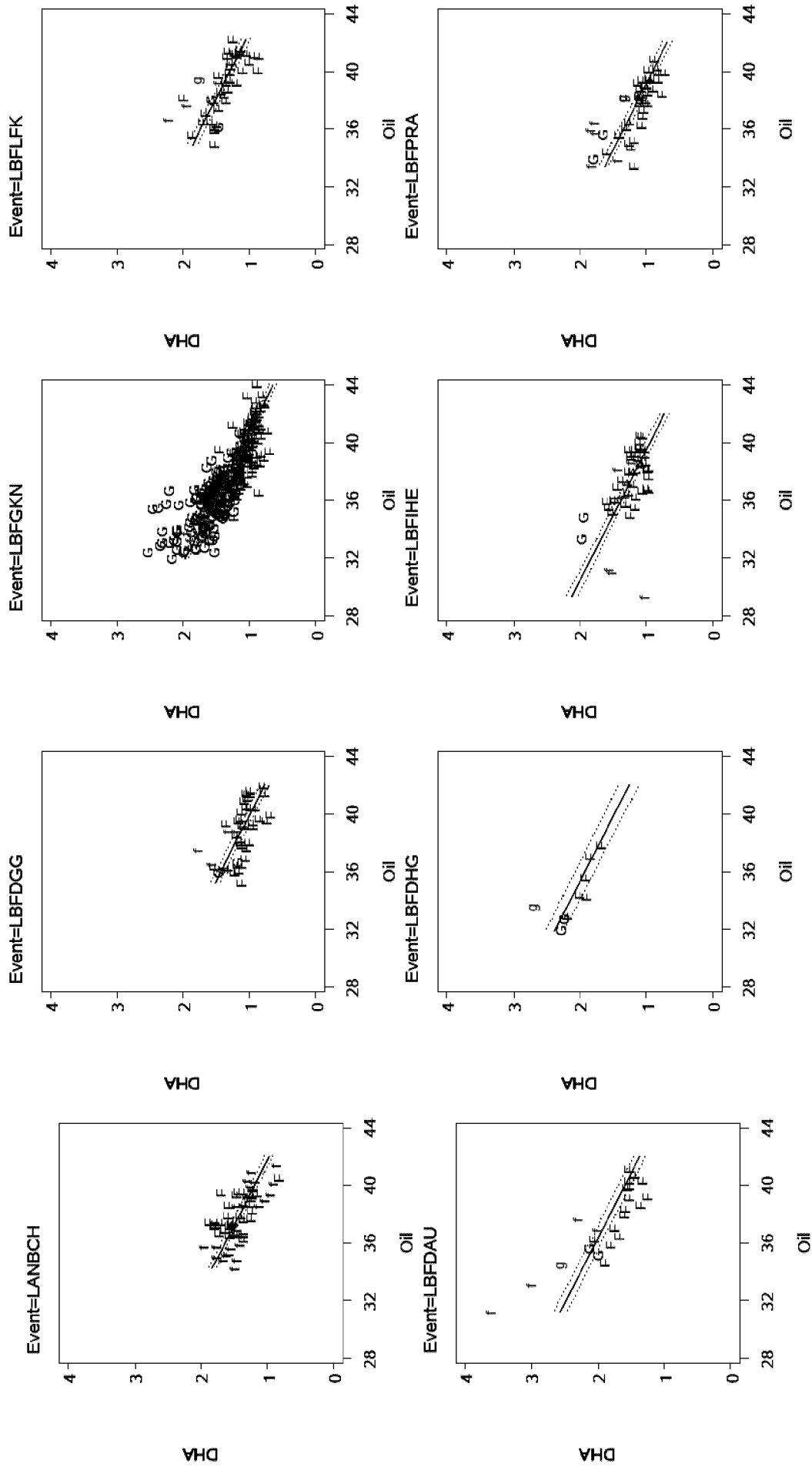


Fig. 84

Fig. 85

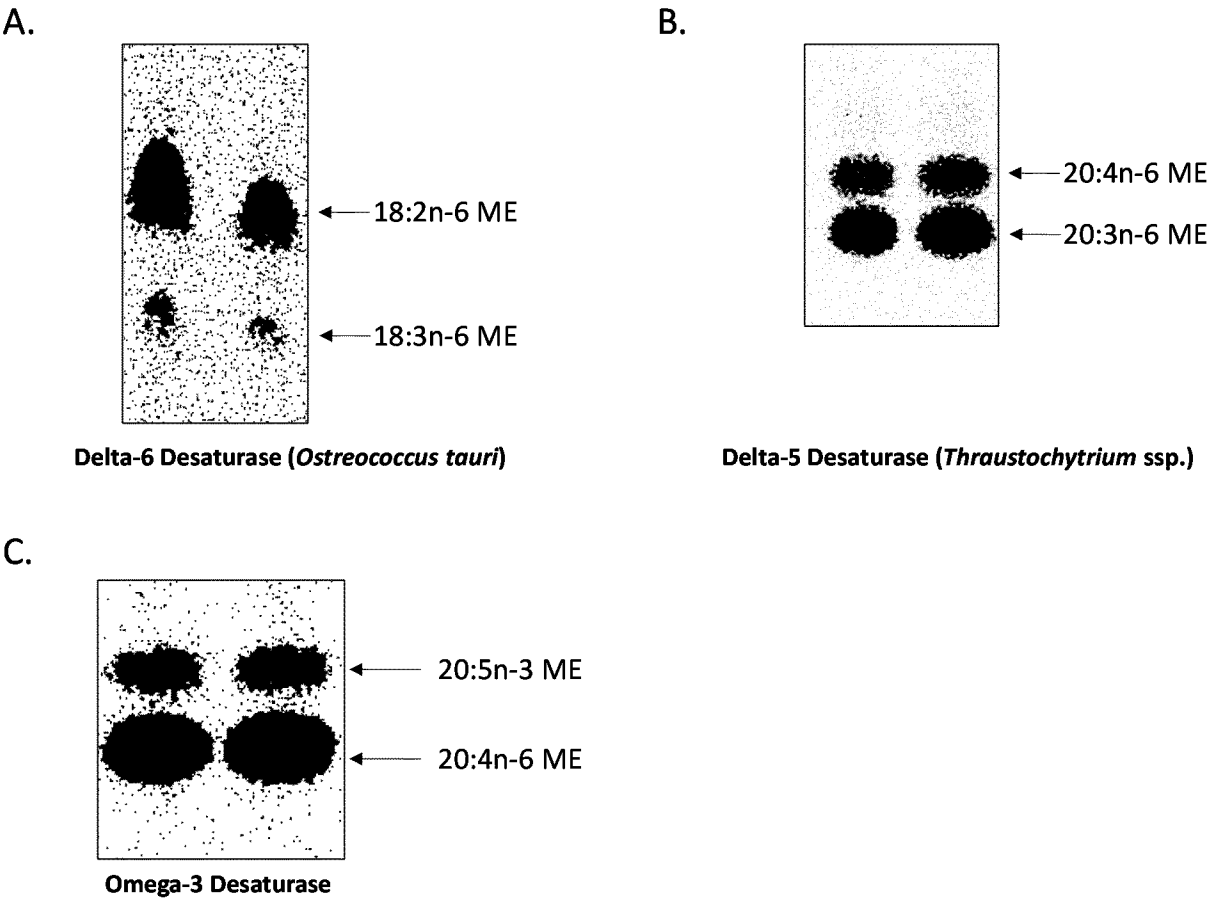
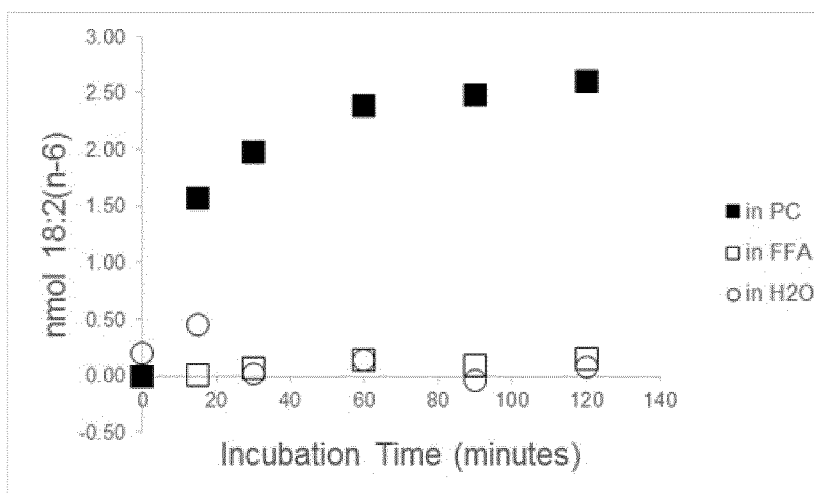


Fig. 86

A.



B.

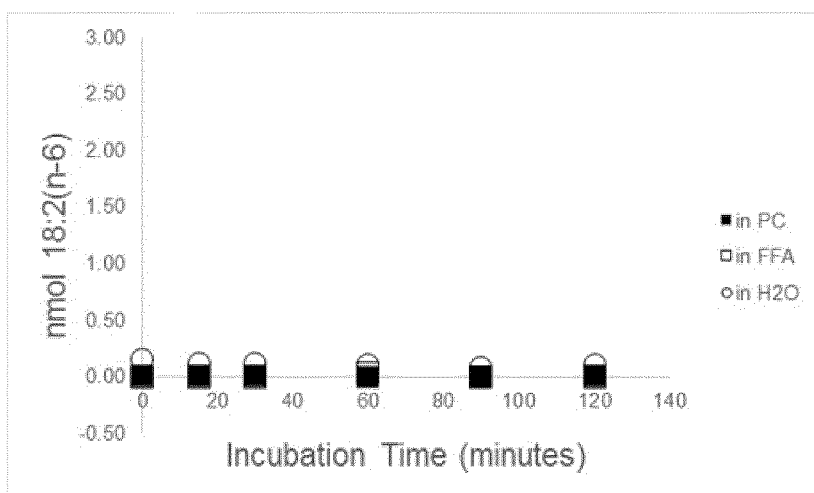
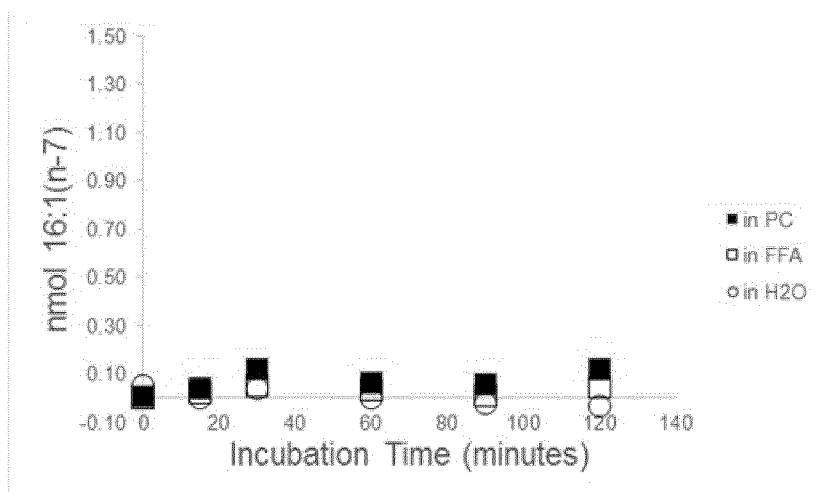


Fig. 87

A.



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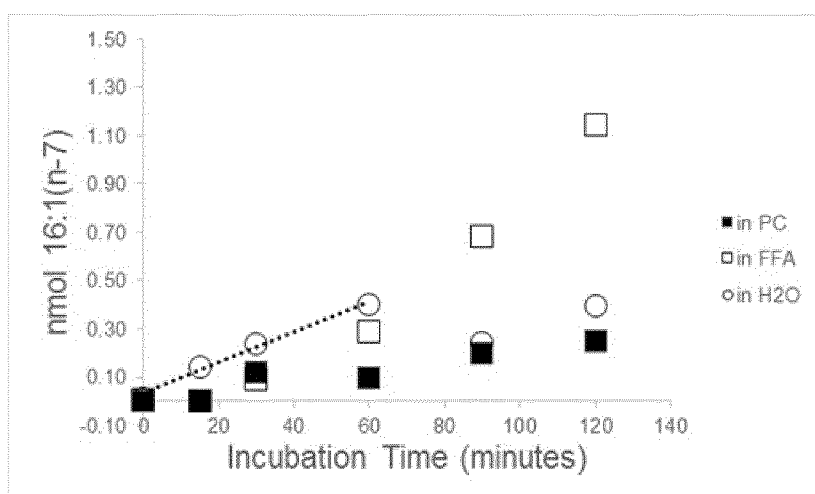


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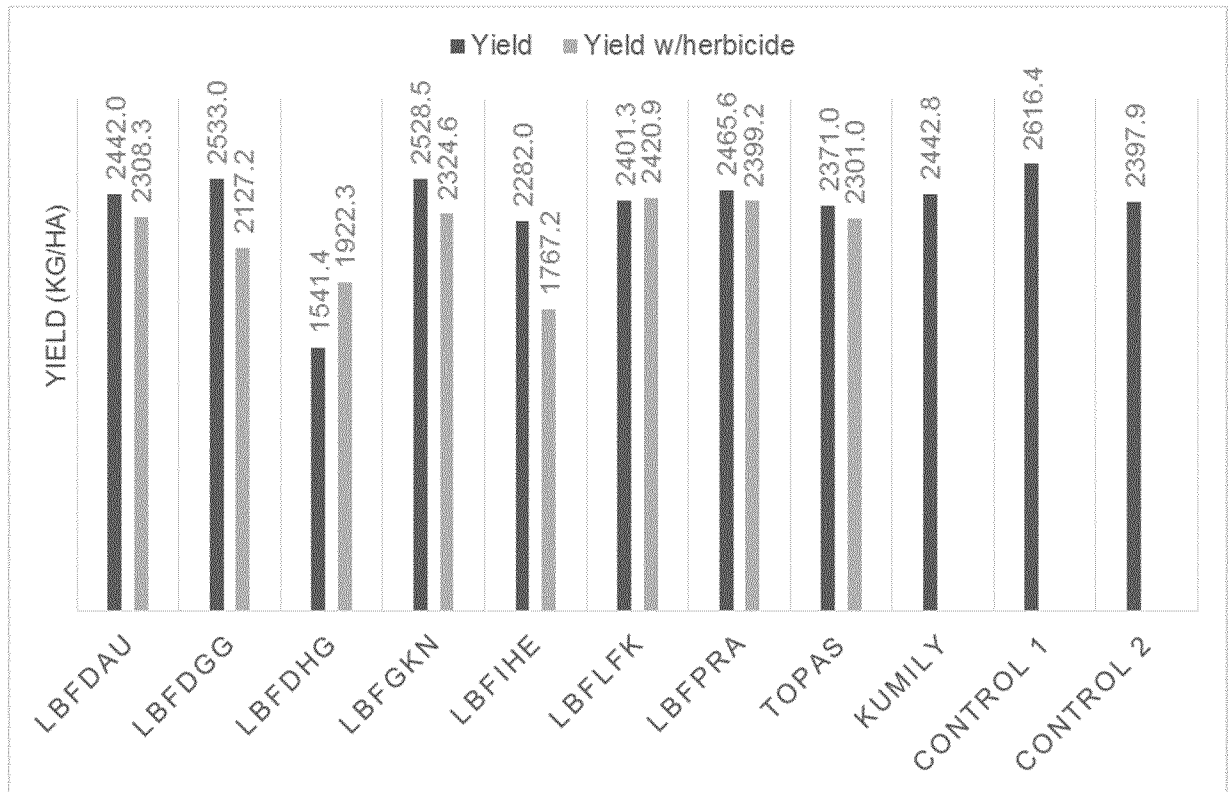


Fig. 89

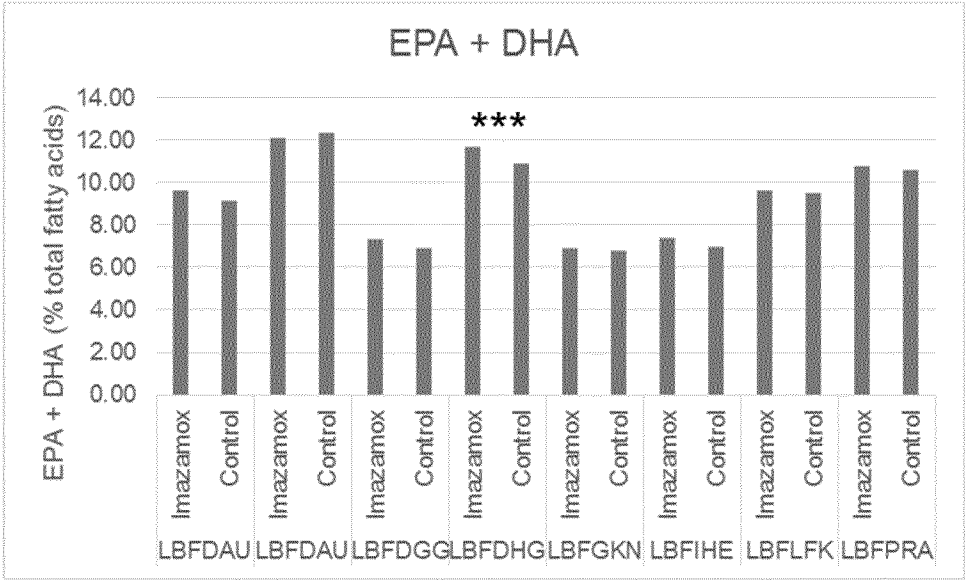


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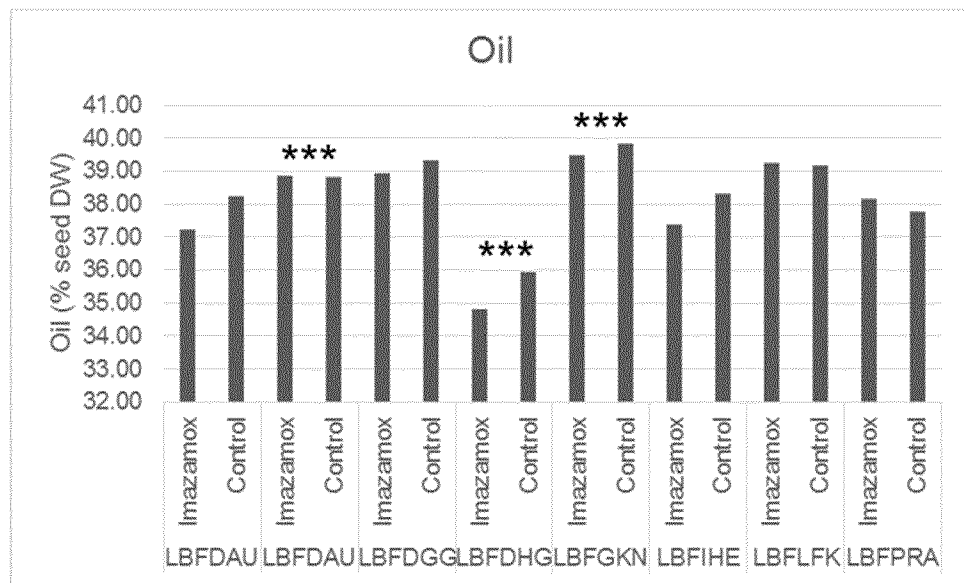
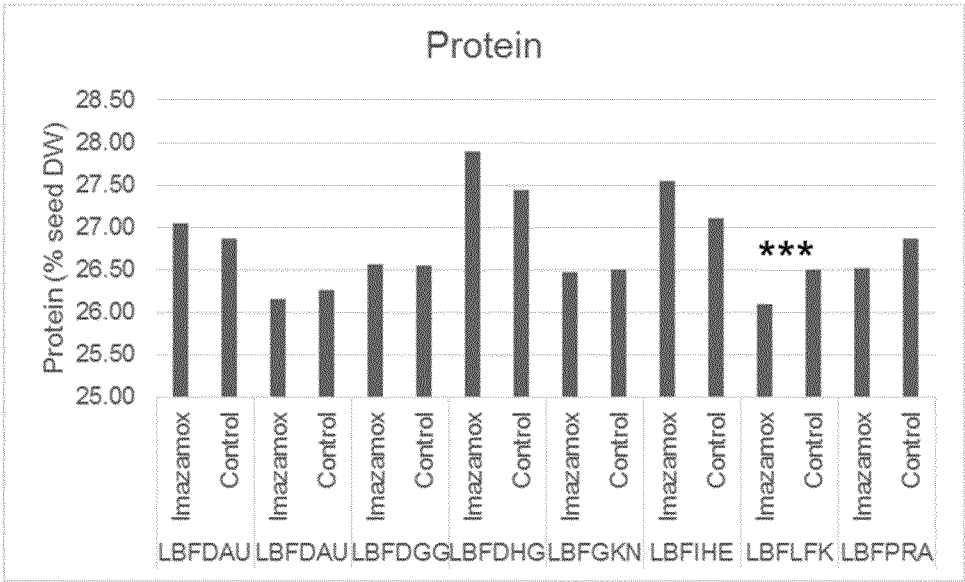


Fig. 91



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eolf-seql

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eolf-seql

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eolf-seql

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eolf-seql

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eolf-seql

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gttaattata	gatatatttt	aggtagtatt	agcaattttac	acttcacaaa	gactatgtaa	60
gttgtaaata	tgatgcgttg	atctcttcat	cattcaatgg	ttagtcaaaa	aaataaaaagc	120
ttactagta	aactaaagta	gtcaaaaatt	gtacttttagt	ttaaaatatt	acatgaataa	180
tccaaaacga	catttatgtg	aaacaaaaac	aatatgtcga	ggcgatcgca	gtacttaatc	240
agtgatcagt	aactaaattc	agtacattaa	agacgtccgc	aatgtgttat	taagttgtct	300
aagcgtcaat	ttgttgctcg	caagatctct	gacactcaac	taaaatgttg	gattttccct	360
ttcaggtgta	tgctcacaag	gtctataaat	atcttgagac	tccactgctg	tctctgagtg	420
agataagaga	caacgaccgt	attgtcgcac	ataggttcaa	ccagatgcac	agaggaccag	480
gaaaagccaa	actagagatt	cttcaactcg	agcagaaaaca	gtaagcattc	gagaattctt	540
taggttctgt	ctatctttca	aatctcacag	gtctgatttt	ggcagagacg	cagagatttc	600
tggaaggtta	tttgaattc	cccttggtac	ttatgtcaac	tcagaacgac	atagtggaag	660
tgacattgat	gcaattattt	ctggattgct	atcaccgcta	caccggactc	attcatcatc	720
tggaagccta	gaggatacta	cagttggtga	aaaagagttt	tctttcagcg	tcttctggtt	780
agatcggtat	tcttccagtc	tcaagccact	tgagtcgcag	tttgttccga	atcctcttag	840
tgctacaagg	ctcgtagtca	agtgggacga	gaaagagcat	gaaaaatatg	attcaagcag	900
ctacttgaat	gatctacctg	aggtttataa	agcaacttcc	tcggtggcgg	aaaagggttc	960
tttgttttca	tgcttggaag	cccttctagc	agaagagcct	ctcggaccag	atgatatgtg	1020
gtgtgttcct	gcttgcaaag	aacacaggca	agcgaacaag	aagctgaact	tgtggaagtt	1080
gccagagatt	cttgtgttcc	acctaaaacg	gttcgcctat	accagatttt	taaagagtaa	1140
gattgatagc	ttcgtggatt	ttccgattca	tgacttagat	ttaagcaagt	acgtgaagaa	1200
taaagagggg	gagtcgtatc	tttacg				1226

<210> 214

<211> 2100

<212> DNA

<213> Artificial Sequence

<220>

<223> flanking sequence

<400> 214

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ggttatcatc	gcagatggct	ccctcttctc	atcgcagatg	gctcaggtct	tccaatcaca	120
catattgggt	cctccattga	tcacaaaaat	tttaaaggaa	tttttaacat	tttttgtaat	180
ttaaagtgtg	tttaaaaatt	tcaaagtata	atatataaga	aaaaatctaa	tttttattat	240
tatatggtta	atgtgattgt	ttattttttt	aataatataa	aattaaacaa	aaatgaggaa	300
agatgcaaaa	gttggtatca	aatcttttatt	attcataatc	attaattgtc	atatatatgt	360
tagttatat	aggtaatttc	gtagctttta	tttaaggaaa	aaatacacac	tgtttttatt	420
ttgggttaat	ataactttcc	ctagtaattg	gatttttacac	caacattttt	tcaattgatt	480
cttaagctgc	catgtaagct	aaatttgcat	actaatttaag	tgacacctaa	gtagggtttc	540
tttttaatta	gtacaaactt	aatgtttacaa	tttttttaaat	gatcttcaat	taatatatag	600
gggataagac	acaacctcct	cttatgattg	tgtaatatata	atagtcctca	tataccttca	660
attattttata	tgcagtagta	gatgcagtat	gaagggaaga	ggtgtgattc	ggagatgcag	720
tagtagtcaa	ttggaaccga	agaatgagag	gtgggttatt	gtgggttatt	gtcgctgcca	780
gatgatgtgg	ctttggattg	cctggttcag	gtgtcgagac	tggacctggg	gggcttagcc	840

eolf-seql

atggtttcca	ggagccaccg	gtttgtggca	gagtcgccga	atatcatggc	catgagatat	900
cggctgggta	actttgaccc	atatttgtgc	gtattttatac	actccaagcc	caaggtgggt	960
cgctctccac	accctgcaac	attggctcaa	accgggccat	tcaagcttgt	atccggctcc	1020
actagccgga	tcctgttttg	tgacgacgga	ttggggatc	tattgtatcg	gtggattcat	1080
gaacggcaag	cccacatcgg	aagtcacgtt	ttttaatgct	atcgaccaca	gcccttaccg	1140
agctgcccc	ataaagatgg	ctcgaagcgg	atcatccgca	agcttgataa	acaagaagaa	1200
ggtatttcta	tttggagggt	gttgggatgt	cgctaattct	tcaaactggg	tagagttata	1260
tgattttaaa	actggaacgt	gagagttttt	gtttgtattt	acgccaaga	tgccccctca	1320
catccaacaa	agtgtggtga	tagaaggcaa	ggttgtttat	ggggtggatg	gggatgggtca	1380
aatctttcac	ttcacaccga	gtactactag	ccttatgttt	actacagacg	gaatagttga	1440
gcctaacc	gaaaatagaa	acgattgggt	tttatttgag	gtaataatgt	gccgtgggtat	1500
cggcgggaga	atactgtggc	gttggccaga	tgagattcct	tagaaagaag	tcaaagggtt	1560
ggaagagctg	cagcaacaac	acatcatcaa	aatctgtctc	ttctctgaga	agacgattgc	1620
catcttctgg	gatgccgggt	ggcctcaaca	aggtctagga	gctttgggtat	gccgagattt	1680
ccatgaagag	acaccagggg	gacgaggggt	ggtggaaggt	ttgggggaac	attgagagtt	1740
ccgctgctgt	tatctcagac	tcacgcata	gttcccttaa	cttggttata	gctgctactg	1800
tccttgcctg	aattactatt	ctcaagcaat	cctttgggtt	tttatataat	ttgtttctat	1860
tttatctttt	gattgcaaca	tgttatcaca	aaccaaacta	atcggtaacg	ttattgctta	1920
aacatatcat	aagctaagca	aatcacacac	ctccccaatc	catggatatg	ttctctcttt	1980
ttcaatcaaa	ttgttttaca	ctcaaacact	gatagtttaa	actgaaggcg	ggaaacgaca	2040
atctgatcac	tgattagtaa	ctaaggcctt	taattaatct	agaggcgcg	cgggccccct	2100

<210> 215

<211> 2065

<212> DNA

<213> Artificial Sequence

<220>

<223> flanking sequence

<400> 215

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gccgtagtc	tgtgcgcact	tgtatcctgc	aggtcaatcg	tttaaacact	gtacggaccg	180
tggcctaata	ggccgggtacc	caactttatt	atacatagtt	gataattcac	tgcccggtatg	240
taccgaattc	gcggccgcaa	gcttgtacac	tagtacgcgt	caattggcga	tcgcagttact	300
taatcagtga	tcagtaacta	aattcagtag	atcaaccgta	tttagtttta	atttttataa	360
gatttttaca	caattataac	tttgtttaaa	ttaaaatttt	attataatat	ttttaggtaa	420
ataaaaagtg	tggttcta	ggtaaatctt	tctgtctgct	ccatctatga	catcttttgc	480
ggttatcacc	tttgctcttt	gtagcatca	tgttttttgt	aactttttta	attctgggtt	540
gtggcagtg	gctccttttc	atctgttttg	gcaatgacac	agctgggttg	ttttgaaatc	600
atgctctgtg	tttatggctg	gtgtgttgtt	tatgatacat	ttgggtgtgt	ccctgaatcc	660
ttgtgttgca	gttgtttatg	atagatttgg	ttgtgtccta	ctattgtttg	cgttccggtat	720
gccgattttg	actggacttg	gtttcttagt	tgtatttcat	ttcgaaaaat	tgtaacctcg	780
tatatctttt	ttttcttttt	gttcttaggg	agaatgatt	attgtctatt	tatgtgatga	840
gctgaaagaa	ctgaaaacaa	ttatgtgcat	ggtaatatct	ccttgcactt	gcaaacgcaa	900
taaccgcaag	attgaatgat	gggtaggtaa	attttttttt	ttttttttgt	ttatgggtcat	960
tgataacat	acttttat	gaaattgtct	tctcttaaga	atgaatgggt	ggtttgagac	1020
tattggagat	aaaatgattc	ttgggagtc	gattctttta	ccaatatgtg	tacacttttt	1080
taagatagat	gtttaatgta	gtatttttat	ttcttatatt	gtgtatatac	ttattatttg	1140
ttttttcctt	aattatata	ttacttattt	tatttttttt	gtttttatat	caaatggtaa	1200
tattacgata	gatgtttaat	gtaatattta	tgtttcttct	attctatatt	tacttattct	1260
ttattttttg	ctgtaataata	ataatggaaa	tgataaaaa	gtataactat	agattttttca	1320
gatagatgtt	taatgtaata	ttccatttct	tatatgttac	atttaattat	tattttatttt	1380
tcttatatta	tataatttatt	tatttttaatt	attttgcctt	tcatcaaatg	gtaatatatt	1440
gatagatgtt	taatgtaata	tttatttttt	ttctattgta	tattttacatc	tttttatgtt	1500
ttcttataat	ttaatataaa	aatgtaaaac	tatacatttt	taaatagatg	tttaattgtag	1560
tatttccatt	tcttatattg	tgtattttact	tatttttaatt	ttcttccttt	tatatcaaat	1620
agtaatatata	caatagatgt	ttaatgtaat	atttttgttt	cctttattgt	atattttactt	1680
tgtttttata	atgattta	gtcgtgtggc	atcttttggg	agaaggattt	tatgttgaga	1740
tgaacattcc	atagagcctc	aaatagcttc	ttttattagt	actagaggtt	ggtcggcctt	1800
acgggagggt	taattacact	aaactatttc	atattagaat	atattttta	tttataaaa	1860
atttgaatat	ttactttata	ttaaaataat	aaatacaatc	tattttttat	tctttttatt	1920
ctaagatcat	atcataactt	ttattaaaa	tttttgggtt	taattataaa	gatcaataac	1980
atttttttct	gagaaaacaa	aattaataaa	ttaaagttta	tgaacattta	atgattttata	2040
tttttctatt	tgattttatat	ttcaa				2065

<210> 216

eolf-seql

<211> 1500
<212> DNA
<213> Artificial Sequence

<220>
<223> flanking sequence

<400> 216
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aagagttata tatgttgttt ttacattagg tgaggactct gttgtaaaaa aaatgtgatt 120
tgttttttta tagatgtggt aattttatat atatctcaag gtctcttgtg gattagatag 180
agagtgaatg attaaatggt tttaaagtgg gctctcctat tgcaataatc taaaaataaa 240
ggtttgtttg ttatacacia taatttttaa gagagaattt taaacaaaaa atattactcc 300
aatcaaaaaa agcattttct aaaaaatatc tcattctcaa ttgatataat ttcttctttt 360
catttgttca ttttatacta cttatttttg gtttaagaaa cttctaaaaa accttcaact 420
gaaaatgctt ttatgcttct aacattttat tcaaagactt ctgatttatg tttggaacaa 480
atgaaataga gaagcaattc catgaattga agtgattaat gtatgttttg caacaattaa 540
aaatgttgct tgtaagtttg caacaattta aaaataaaaaa ggtctaattc gtttttcatt 600
tttcatTTTT tcacatttag gtgatgagta gttgtagtat aacttaaatg gtggtttgtc 660
ttgttatatt tggttgtcat ttcttttatt caagatcttt gatttttttt ttttcaaaaa 720
cacatggaat gaattccatg catttgttaa ttgttttgga attttttttg tactctattt 780
agggttagg actctactgt agtatgacct aacatagttg tgatatgttg atcaaatttt 840
gtgtgacatt tatatgaagg tcttttgatt tatatttagt aaaatgaaat agagagggaa 900
gtttatatga aggtctccag aaattgaagt gactcatgaa tgttttagtta agtaaagatc 960
aatggacttt catcaccaa tatatatatt ttaaactata caaaaaaaaaa tcctttttat 1020
tttaagaag ttttaattata tttaaaaatg attttaaagt acgtaataat tataaacaga 1080
atatcttttt gtagagtggg cctaattgcaa 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 attacgcca gcttggccac 1380
taaggcaaat ttcgcgccct gcagcaaat tacaattgc cactaaacgt ctaaaccctt 1440
gtaatttgtt tttgttttac tatgtgtgtt atgtatttga tttgcgataa atttttatat 1500

<210> 217
<211> 1948
<212> DNA
<213> Artificial Sequence

<220>
<223> flanking sequence

<400> 217
cggccgagct ccctgcaggg ggcccgccgc gcctctagat taattaaagg ccttagttac 60
taatcagtga tcagattgtc gtttcccgcc ttcagtttaa actatcagtg ttgctttagt 120
ggatcaatcaa aagatgaaca gtatcttctc gatcgatgat ttctccgatc ctttttgga 180
atcttctccg cctctcgatt cggactcggc caaggccctc acggcggagg aatggaccgt 240
ggagatgttt ttcgaagaga tcgcttcttc cgtgacctct gcgcctgtcg gatccaacaa 300
caacaacaac aacgcgatcg tcggagtctc gtcgcgcgag tctcttcctt ccgtctccgg 360
acagaacgat ttcgaggaag acagtcggtt tctccgctcg gaatcagatg attaccgtcg 420
cgttcttgag aacaagctcg agactgagtg tgcagctact gttgtagctc ttagggtttg 480
tcttctgttt ctcttttttt tatattgcat gtgtaagaag aagaagaaga tctgaatctt 540
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tcgactgggt ctctgagac tctgtttcaa ccagctctgt ctagtctctt tactcaagg 660
aatagggtct catcatgcaa attagttttt tacactacat agcttgtctc ttagcttcac 720
catgtttgaa attttttatg ccaagaaagt gttctaaaaa tcggtctagg cgcctagtcg 780
gcaaatcggg tctgtacgaa atgatttttt caaatcgatt tgaaaatcgg tctagacgtc 840
cgcctaaca ataattatag cgataaaatt aaggatttgt aatgtagaga tggaaaaaaa 900
aaattttgtt caaatagaga aggaaaatgg ttcttttaga aagcttgaaa ccatttcaag 960
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aaaatctaga aggtaaaaat attccttgtt tcttgtcttt tgatgtttat ctttatagaa 1380
ccttgttgtt tttaggatgc tttcaaatcg agagctcagc aggcgggtcta gaaggagaaa 1440
acaggagcaa acatgtgatc tcgaaacaca ggtttctata aatagatcct ctcaacctcc 1500

eolf-seql

tattaacatc	tccaaggata	ttaatagaaa	cttaattttt	gtgtgtgttt	tgataggtta	1560
atgagctaaa	aggtgaacac	tcctcacttc	tcaagcagct	tagcaacatg	aatcacaaagt	1620
atgatgacgc	tgccggtggc	aatagaatac	taaaagccga	catcgagaca	ctaagagcta	1680
aggtaataac	accttctttt	ttttcataac	cacactaatg	ttttgttact	ataactaatt	1740
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tatgcttctc	ggtagatcaa	atggacataa	caacaacaac	aatagaatgc	cattaactgg	1860
taatagcagg	atgggttggt	cttcatgcat	cccacctttt	caaccgcaat	caaatccaaa	1920
catgggggga	ctaacaacaa	ccattcta				1948

<210> 218

<211> 1600

<212> DNA

<213> Artificial Sequence

<220>

<223> flanking sequence

<400> 218

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aacaaaatcc	attaacaaga	gatgagtaag	tcaccacggt	tggcttacac	ttggtcttac	120
ccatgtctcg	aaaaatcttt	aaggcggtag	tcatatcctg	ttgcttcacg	tacccatcga	180
tgattgtgga	ataagtgaac	tcatcaggta	caagtctttc	ttcgggtcatc	ctattcatac	240
acatcaatgc	ctcattcaac	attccggatc	tgcaaaaccc	ctttatcata	gcgttgtggt	300
gcacgacatc	aaccttcaca	cccttttcaa	ttgagagaga	ggaaaccttc	tttgcttcct	360
caaaatcacc	gcttctgatg	aatccatcta	tgagggtagc	atcacacataa	gcacccgggtg	420
aaattttccg	gtctaacatc	tccaagaaga	ggagcttggc	agatgaaaac	ccacctctct	480
tacacaaccc	actcatcaac	atattgtaga	tggcagcatc	cgggtgaaact	cctctctcta	540
tcattttacc	tttcatctta	attgcatcat	ccatatgacc	tgaagcgaca	agaccatgga	600
taagaatccc	atacgtaact	atatctggtt	tgcaacctct	ctcagccatc	tgcagaagca	660
acttagaagc	aatatcatac	tctttgactc	tgcaataacc	ttgtattaga	ggggcatagg	720
ttatatgttt	ccgaaccaac	cctctctttg	cagcttcac	caaaatccca	acagcatctt	780
ccatccctacc	ttctttacat	aaacgattga	tcaaaatatt	atatgttgct	atatcaggct	840
tgcaaccatt	agctactatc	caccgtatac	tctcagctac	ttcaaccttt	aaaccatgcc	900
tatacttagc	atccataata	ttgttcagaa	accaaact	gacaactaaa	cccctttctt	960
tcacttcctt	caaaagccga	tactcgcga	cgaatcccc	tttcttgag	aaccatttaa	1020
tcactgcgcc	caaaagtctc	caagagtcgg	cataaacc	ttggacttca	actccttgaa	1080
aaccagactc	gcgttctcaa	catcagccat	cttgcagtat	ccactaatga	ttggtgttga	1140
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atttgatctg	tgtttttttg	gccttaatac	taaatcctta	cataagcttt	gttgcttctc	1440
ctcttgtgag	ttgagtgtta	agttgtaata	atggttctact	ttcagcttta	gaagaaacca	1500
tggaaagtgt	tgagagggtc	tacggagagt	tggatggaaa	ggtttcccaa	ggagtgaacg	1560
ctttgttggg	atctttcggg	gttgagttga	ctgatacccc			1600

<210> 219

<211> 955

<212> DNA

<213> Artificial Sequence

<220>

<223> flanking sequence

<400> 219

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ctcttgtaaa	ttgaagtatg	aaaatatact	gaattgggta	ttatatgaat	ttttctgatt	180
taggattcac	atgatccaaa	aaggaaatcc	agaagcacta	atcagacatt	ggaagtagga	240
ttaatcagtg	atcagtaact	attaaattca	attaaccgcg	gacatctaca	tttttgaatt	300
gaaaaaaaat	ttgtaattac	tctttctttt	tctccatatt	gaccatcata	ctcattgctg	360
atccatgtag	atttcccggg	catgaagcca	tttgtcaacg	tcgttgtttg	atttcgttaa	420
ggggagagca	gtccaagtgt	gtttttaatc	tcagataggc	ctaacatcta	gattttattc	480
agactaacc	aaaaccgaaa	cttttgcatt	caagtcattc	ttcgtgttga	ttcgttaattc	540
tttttttcac	attcaacttt	ttggtagcag	caggttcaga	agattcattc	ggtttttcaa	600
tgtttcagag	taacactaga	tttgtttcag	attaaaccga	aaaatcggat	tcgttcagaa	660
gttttatitg	gtatgactat	gttccgggat	gtgatcaata	agaaccactt	gagttcggtc	720
gagttgtttt	ttttttttga	acttttgtat	ctgatcagtt	cggatagact	cgaaaaagga	780

						eolf-seql	
aaagtaaagt	ctaatagaca	tcaatatattt	gacaaaatat	caaaaaaatca	atatacaata		840
ctaaaagggg	atataagcca	tggagagggg	atccacgtaa	gataggaaaa	tcaaccaatc		900
agagaaccca	aaattttcac	atcatctcaa	ttatttttcg	taaaaaatac	aatac		955

<210> 220
 <211> 2199
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> flanking sequence

<400> 220						
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gaagaagaca	aagtaattgg	caaagtaaat	tccattattt	cataccctct	tctttgattc	120
tagtgaagaa	gacaaatcaa	aatattaatc	ttctgctaaa	aaactgaaac	tttcaagttc	180
caaattttcca	aaaaacacat	ttctccttaa	tagacctttt	aacatcaatt	caaactacag	240
tccacaagct	tttcacatga	actggcacaa	taaactgcat	tcatattctt	tggttctagt	300
gaagaagacg	catcaaaaac	tgtagcagct	aaaaaaaaaa	ctgaaacttt	caagttccaa	360
agttgtacca	gtgttttctaa	taatgagcaa	acctgttgaa	agcttttgac	ggttttgcca	420
agactcttgc	caatctcagg	aagcttctta	gggtccgaaca	gcaacgcagc	gactccagcg	480
atcacagcta	gctcagggac	accgagacca	aacagagcat	tacaagttag	tgcattttctc	540
ctctgctccg	gtcggaaacg	cactagagaa	cgggtgtatg	gtcgagtcgt	cacagtgaag	600
cagttgtagt	agaaggaaga	gcgagatgat	gagagaggca	aagaaactgc	tgtgagagaa	660
gaaagagtcg	caacatttgt	cgccattact	gctatcttct	ggttctgatg	ttatgtgtgt	720
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tttgtgaatt	atgaagttaa	ttttttttaa	acaattatta	cataattgct	cagtgaattt	1020
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tcacttaaaa	gaagctgtta	tgtttctaatt	ttaataagat	agatttttaa	taccatgttt	1140
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<210> 221
 <211> 1748
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> flanking sequence

<400> 221						
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taaaaacata	gagaactctt	gtaaattgaa	gtatgaaaat	atactgaatt	gggtattata	180
tgaatttttc	tgatttagga	ttcacatgat	ccaaaaagga	aatccagaag	cactaatcag	240
acattggaag	taggattaat	cagtgatcag	taactattaa	attcaattaa	ccgcggacat	300
ctacattttt	ttactgctat	tcittcataat	tagcaactgg	ttgattactg	aattagtaca	360
gatgtaaacta	acatatatat	tggatatatt	tattatccaa	aagagctatt	caacctttta	420
agcatatgta	tatacatggt	taattgattt	tatttagtatt	tcttgctctt	gttgttttgc	480

eolf-seql

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agatatatTT	tgatcgattg	atatgaatgt	tattataaca	TTTacaatTT	aaaaaataca	660
gTTTTtctt	tctcatTTac	attgTTtcct	ttaggggtga	acacttctgg	ttatTTttatc	720
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acaatcaaac	caaaaataaat	agaaaactaa	aaaccaaca	TTgttaacac	agTTaatagt	1020
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<210> 222

<211> 1500

<212> DNA

<213> Artificial Sequence

<220>

<223> flanking sequence

<400> 222

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tagaagatta	gtaggaaaaa	aaaagatgac	acagagacga	aagatcttaa	cagttgaaca	180
cgTggcggaa	aagcgaaatc	cgatttaaca	gatcggaaaa	taaaaaccgg	cgagattaaa	240
gaagaaaagga	gatagagaga	agaggtacct	tagatccgga	tcctgtagat	gtggagcagc	300
tcgcttgagag	actggTgtcc	tcgaatccat	cagccgaggg	agacgcagat	cgcgcgccgc	360
tccacgagag	cggcgTcttc	ggactgcctc	tagcggaaatc	aacatccttc	ttcaacgaaa	420
cgacaccaa	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
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aagagagaga	gaaaatgacg	aagaaagaca	gagggaaagt	tgatgtatcg	atatagagga	780
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tatttttatt	tttattttta	tttttccttg	tttcattttc	atTTtatattt	tgattttattt	900
tccatttttg	aaatattttta	atcaaatata	atgtgggacc	ttcttggaaa	gtatatctaa	960
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gtaagcctac	gtagcgtcac	gtgacgttag	ctaacgctac	gtagcctcag	ctgacgttac	1260
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gtaacgctag	ctagcgtcac	tcctgcagca	aattttacaca	ttgccactaa	acgtctaaac	1380
ccttgtaatt	tgTTTTgtt	ttactatgtg	tgttatgtat	ttgatttgcg	ataaatTTTT	1440
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<210> 223

<211> 1323

<212> DNA

<213> Artificial Sequence

<220>

<223> flanking sequence

<220>

<221> misc_feature

eolf-seql

<222> (578)..(578)

<223> n is a, c, g, or t

<400> 223

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attaatcagt	gatcagtaac	tattaaattc	aattaaccgc	ggacatctac	atTTTTgaat	180
tgaaaaaaa	ttggtaatta	ctctttcttt	ttctccatat	tgaccatcat	actcattgct	240
gatccatgta	gatttcccg	ttctttttct	cagactactc	actgcccga	gccactacta	300
ctacacacat	tattatctct	ctcacatctc	tactattttac	tcttggattc	cgcaaagtaa	360
gtaaaaaaa	aagaaagcaa	actctgcttc	tcaaattctct	ctctacccat	ttgctctctc	420
acatttttct	ttgacaaaa	ctgaatcttt	acttcaagca	gcgtaagggt	ctcgctcctt	480
tttgttcctc	tgactttggg	tttgatatga	agttttcatg	aaactctttc	attacacttg	540
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gtgattgttt	gctaagtctt	tactttaatc	aatctgatga	ttgtttgctt	cactcaagtg	780
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aaattttttt	ttatgtaaaa	agatatttta	actcaattat	gtgattcatg	atagtatttc	960
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catacaaaat	tacagttaa	ccctcttggt	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 Sequence

<220>

<223> flanking sequence

<400> 224

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aatttaacac	aaaaaaatca	attttttagtt	tttgaattat	gtttccattg	ctgcgacagc	180
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attctttaac	agtttataaa	gtttgtaaat	catttacaaa	gttttttaaat	catttataaa	360
tgtttataaa	ttattttgtaa	gagcatataa	accattttata	aggttttataa	aggtagttaa	420
ataatttatg	agggtttata	aaatgtttat	taagttcata	aaaagggtttt	ataaagttaa	480
gaaacaattt	ataacgactt	attaatcatt	tataaagagt	ctataaacgt	tttataaagg	540
gtatgtaaat	gttttataga	cccttataaa	gtttgtaaac	cctaatagaa	ccccgacatg	600
aagtttataa	aagaacagtt	taggcccga	tagtttgaaa	ttagaaagct	cgcaatttag	660
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tcgtagggtga	aggtggaaat	taatggcgcg	cctgatcact	gattagtaac	tattacgtaa	780
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cgctagctag	cgtcactcct	gcagcaaatt	tacacattgc	cactaaacgt	ctaaaccctt	960
gtaatttggt	tttgttttac	tatgtgtgtt	atgtatttga	tttgcgataa	atttttatat	1020
ttggtactaa	atttataaca	ccttttatgc	taacgtttgc	caacacttag	caatttgcaa	1080
gttgattaat	tgattctaaa					1100

<210> 225

<211> 1413

<212> DNA

<213> Artificial Sequence

<220>

<223> flanking sequence

<400> 225

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eolf-seql

aaattcaatt	aaccgcgga	atctacattt	ttgaattgaa	aaaaaattgg	taattactct	180
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gaagccattt	acaatccatg	tccttataaa	tgggtttatat	actcttataa	ataatttgct	300
tcttcatgcc	tcaagaatat	tactactttc	aagaatagga	aaatctgtgt	actaaaaact	360
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aacaatgtaa	taaaatctca	gacattaaat	gaataagttt	tgactattat	tttataagtc	540
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agtcttgagt	actagaaacg	tcaattcgtg	tatgtatcat	gtttgacata	attaattgtg	660
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gatcagaatc	aaacaaactc	atttcaaggt	ttagttttat	aaatactact	acttacctta	1380
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<210> 226

<211> 2200

<212> DNA

<213> Artificial Sequence

<220>

<223> flanking sequence

<400> 226

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aacgtttgcc	aacacttagc	aatttgcaag	ttgattaatt	gatttctaaat	tattttttgtc	1980
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ggaatttaaa	gtgagtgaat	atggtaccac	aaggttttgg	gatttaattg	ttgcaatgct	2100
gcatggatgg	catatacacc	aaacattcaa	taattcttga	ggataataat	ggtaccacac	2160

eolf-seql

aagattttgag gtgcatgaac gtcacgtgga caaaaggttt 2200

<210> 227
 <211> 3137
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> flanking sequence

<400> 227

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tgaattgaaa	aaaaattggg	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
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atgttttcac	attgcaatat	ggctcagctt	acttaatatg	atgatggtta	agaagaaatc	660
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<210> 228
 <211> 1100
 <212> DNA

<213> Artificial Sequence

<220>

<223> flanking sequence

<400> 228

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atg	gtct	caact	tccac	taatt	agtt	180
atatt	aagt	agaaa	aagcc	tggac	catct	240
tggt	atag	gacaa	atcaca	tagtt	atag	300
atga	taata	aatta	cgctt	ctaaa	taata	360
taaat	taaa	taatta	tggtt	tgcat	atag	420
aaaat	agtgt	acgag	agatt	gtgac	catcat	480
gacat	aaatc	taaga	ttgaa	aaagc	attgag	540
accaa	gaaat	agctc	ccagtc	tcacac	aaaagt	600
cccga	ttgaa	aaagc	attgag	acagg	ttcgct	660
gccgt	attact	ggtgc	ccccat	aggtga	ggaaat	720
ggcgc	atcact	agtaac	acgta	acgtag	acgtg	780
agcta	acgtg	agctg	acgtag	acgtg	acgtg	840
agcta	acgtg	agctg	acgtag	acgtg	acgtg	900
caaatt	cattg	aaacg	accct	ttgtt	ttttact	960
tggtg	atttg	cgataa	ttatat	tactaa	ataac	1020
ttatg	gtttg	acttag	ttgca	attaat	tctaa	1080
ttttg	taata					1100

<210> 229

<211> 811

<212> DNA

<213> Artificial Sequence

<220>

<223> flanking sequence

<400> 229

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acatt	tttga	ttgta	tactc	tttcc	attga	180
atact	cattg	ctgat	ccatt	ttact	tgacc	240
ccaca	aatat	atatt	ggcatt	tgat	gcgtat	300
gaata	aaaaat	gtgtc	atatt	gtaa	tggtc	360
taaat	gaaga	tactag	catgt	gtcgc	ctact	420
tatct	caaga	ttctt	ttttc	tacg	taata	480
ttgta	acaaa	attgt	ttgac	attata	aaaaa	540
actga	tgaat	gtcga	gataa	ccacg	gttcc	600
aaaag	gatcc	gagtc	gaat	ccacc	gacac	660
tttcg	cattc	tcgtc	ctga	gaatg	gttct	720
cgtgt	gaat	ctagg	tttat	aatat	gctga	780
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<210> 230

<211> 2900

<212> DNA

<213> Artificial Sequence

<220>

<223> flanking sequence

<400> 230

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attag	ataga	tctac	cttta	tagaa	cacac	aaaaa	atacat	atctaa	aaatt	attaa	atctt	120
ccttt	aaatg	agtga	agat	gaga	accatg	tgatg	aaaaa	cctgca	aaaac	aagata	aaatt	180
agta	agaaaa	acatg	agaca	gaaac	aatata	attgat	ataa	agttt	gatgt	ttata	agttc	240
aaagg	gatta	aagag	aggtt	tgag	agtttt	agaac	gagga	acata	accatt	tttgt	tcag	300
ccatt	tgaga	ggaga	agaga	gaatg	tgtaa	atgtt	ttttt	atata	aggag	acaaa	aatcc	360
caata	aggtt	aaat	attttt	gatc	agaaga	cttact	agac	gactt	acttg	taagt	cgccc	420
agaag	acttc	aata	tttttt	gcggg	aaact	aaaat	atttt	tagcg	ggagt	tagaa	gaccc	480
taaac	ataac	cctta	aaacta	aatta	actaa	ctaa	atactt	cataa	aatca	aatta	aaactt	540

eolf-seql

aaaaagtgtt	tactatacac	agaaataatc	acatgtagat	ataaatttaa	tttttcaaaa	600
aaacatttaa	gctttccaaa	atctaaccct	agaatacat	acaatactac	aacatatgtt	660
gccaaaccct	agaccaaaga	atatcatgat	tcactacttt	cactcatcta	tggtgaaaac	720
aattcaattt	tattatatct	taattttatat	cacttaaaac	tgtttataat	tacatgattt	780
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tatgtaatat	ttttatataa	gtaataatgt	gaatagaatt	tatcaaatac	tatgtagtaa	1260
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ctgacgttac	gtaacgctag	ctagcgtcac	tcctgcagca	aattttacaca	ttgccactaa	2820
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ataaattttt	atatttggtg					2900

<210> 231

<211> 1800

<212> DNA

<213> Artificial Sequence

<220>

<223> flanking sequence

<400> 231

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atttttgaat	tgaaaaaaa	ttggtaatta	ctctttcttt	ttctccatat	tgaccatcat	180
actcattgct	gatccatgta	gatttcccg	acatgaagcc	atttacaatt	gaatatatcc	240
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aagtaaaatg	gagtgtattt	taattttatc	ttcagaccaa	tgttatcaat	gtagcccata	480
tattaatact	aaaacaactt	ctgcacaaac	acacgaatca	aagcctcgtg	tttcatcgta	540
gctttagcta	aaatttccca	aaagcaaatt	caatagtatt	ttactaggtc	aaaccacaaa	600
gagaaaaaga	aagtcacatc	caaggatcaa	gaaatgagaa	gtgagaggag	aatgctttat	660
tggttttgct	aataactaat	aagacatgaa	cgagactgaa	aacatctgg	tttgtccaaa	720
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acattcatgt	ttttactggt	tatacacagt	caataattta	tacacagctc	catgttttaa	840
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tagctgatca	gaacaatctt	actaacgtgt	gtctttgtaa	gaaaatccaa	cttttacttt	1080

eolf-seql

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catcagtata	tggcccaacc	caagaaaagc	tgaacaaaat	tagcaacaat	agcaagggac	1740
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<210> 232

<211> 1600

<212> DNA

<213> Artificial Sequence

<220>

<223> flanking sequence

<400> 232

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ctgaagaaga	tgatgtgaat	gagatggttg	cttggagcaa	tgatgaggat	gaggaccttg	180
gttggcaaac	aaatgaacct	ataaggataa	ataatgagct	caaggtagct	atccttcagc	240
atttcacatt	tcataatcat	agctgttagt	ggttgagttc	tttttattgt	ctctaagcct	300
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cacctgtgga	tgtcactttg	gaaccacatc	atgtcatgct	tcctgtttgga	gttggttctt	420
cggatatcata	aaataaactc	tacctgtgcg	ctattgcttg	ctaaacctaa	aaccattgtg	480
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aaagcatagt	cttttgccg	aaggttctat	cctatggata	agtgaagaa	gaacgccatt	600
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<210> 233

<211> 3284

<212> DNA

<213> Artificial Sequence

<220>

<223> flanking sequence

<400> 233

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ggcctaactt	ttgggtgtgat	gaaagtgtga	gaatatttcc	atatttagaca	attctgtaga	180
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aatataatat	aatttaagat	gaaccaacat	atttctctaa	attctgaatt	ttattttcat	300
ggtgacacgt	ggatacgaaa	caatgtttga	atgtttatcg	attaatatat	aatgtatggt	360
atatgtttgt	tgtactaaca	acaagatttc	atacatttca	aacgaaaaag	agagattgat	420
gtgatcagta	ttattgcttt	tacaaaacta	ttgaaggttc	agcgagttct	tagtaaaagtc	480
gaatccttgt	aataatctct	gttccaacgg	gcaaacatgc	agctgttaaa	ctatcgacgc	540

eolf-seql

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gcttacaaag	tgattcaagc	tatgcctgag	aagccaacgg	ctaagacatg	gggggctctg	3240
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<210> 234

<211> 1700

<212> DNA

<213> Artificial Sequence

<220>

<223> flanking sequence

<400> 234

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cgacgccggt	tgacctattt	tttcttcttc	tttgttttct	tcctcatttg	tattactatt	180
ttataagttt	tgtgtagcgt	tgtaaagctt	tgacgctacg	tagcgttacc	taaggctcacg	240
tgagcttagg	taggcttagc	taatctcagg	tgacgctacg	tagccttacc	taacgtcagg	300
tgaggctagc	tagcgttagc	taacctcacc	tgacgctacg	tagcgttacg	taacgtcagc	360
tgagcctagg	tagccttagc	taacgtcagg	tgacgctacg	tagacaatca	gtaaattgaa	420
cggagaatat	tattcataaa	aatac gatag	taacgggtga	tatattcatt	agaatgaacc	480
gaaaccggcg	gtaaggatct	gagctacaca	tgctcaggtt	ttttacaacg	tgacacaacg	540
aattgaaagc	aaatacatg	cgatcatagg	cgtctcgcct	atctcattaa	agcagcaatc	600
aattattcat	taactactac	tcagccttac	cacccttctc	agcaccaggt	ctagttccca	660
tcttaccat	atgctgaagc	attgagctaa	ggttagatcc	aacggtaggg	aagtgacggt	720

eolf-seql

acttaaaacc	gagcttctct	atgtgagtc	taaccacag	agctatttga	gcgtagtaag	780
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<210> 235

<211> 1532

<212> DNA

<213> Artificial Sequence

<220>

<223> flanking sequence

<400> 235

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gcattgtgtag	ctcagatcct	taccgccggt	ttcggttcat	tctaataaat	atatcaccgg	180
ttactatcgt	atttttatga	ataatattct	ccgttcaatt	tactgattgt	ctacgtagcg	240
tcacctgacg	ttacgtaagc	ctacctaggg	tcacgtgacg	ttacgtaacg	ctacgtagcg	300
tcaggtgagg	ttagctaagc	ctagctagcc	tcacctgacg	ttaggtaagg	ctacgtagcg	360
tcacctgaga	ttagctaagc	ctacctagac	tcacgtgacc	ttaggtaacg	ctacgtagcg	420
tcaaagcttt	acaacgctac	acaaaactgc	ttgctgttct	tctctcatta	cgagaatata	480
cttttttcaa	tattgcttca	aaaactttct	gtcatgtgaa	tttgatttta	gatatatgtt	540
taaaaaatac	ttctccccg	aaaataagat	tttctaaagt	gttcgattat	taagaattca	600
ttaaatatatt	ataatttaatt	ttttctttta	ctttattata	catttttctaa	taacttttta	660
ccaataaaaat	ttaatcaatt	caaataatttt	tatttaattgt	ttcttaaaaag	tataaaaaaat	720
atcttaataca	tatagaaaat	atatttttgt	ggaacaagta	aaaatctaaa	acatcttact	780
ttcaggaacg	gaggtaatat	atagttacat	taaaggtctc	aacattacta	ttttaagtct	840
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<210> 236

<211> 1200

<212> DNA

<213> Artificial Sequence

<220>

<223> flanking sequence

<400> 236

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gtgacgttag	gtaacgctac	gtagcgctcaa	agctttataca	cgctacacaa	aaatgcttgc	180
gtttcttctc	tcattacgag	aatatacttt	tttcaattatt	gcttcaaaaa	ctttctgtca	240
tgtgaattttg	atttttagata	tatgttttaa	aaatacttcc	tccccgaaaa	taagattttc	300

eolf-seql

taaagtgttc	gattattaag	aattcattaa	atatttataa	tttaattttt	cttttacttt	360
attatacatt	ttctaataac	tttttaccac	taaaatttaa	tcaattcaaa	tattttttatt	420
taatgtttct	taaaagtata	aaaaatatct	taatcatata	gaaaatatat	ttttgtggaa	480
caagtaaaaa	tctaaaacat	cttactttca	ggaacggagg	taatagatag	ttacattaaa	540
ggctcaca	ttactatttt	aagtctattc	acttgttttg	cttgcttgct	aaccaaacaa	600
agtttactag	tttaattaat	atattaataa	aaatatccaa	tatatgacct	tgaccaagac	660
aaatgtgcta	gagtagtatt	aacggcgtca	gttgccgtta	atcccattta	ccaaaatata	720
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caacgggcta	gtttatcaat	atctttcaca	aataaagaag	aagaagggtga	tatctttgag	900
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<210> 237

<211> 1600

<212> DNA

<213> Artificial Sequence

<220>

<223> flanking sequence

<400> 237

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tgcaagatat	ttacttttgt	tggtcgatca	tattttaatga	ttatttttgt	tttgaaacaa	180
atattaacat	tatatataat	gtgtctatat	tgaactgttg	taaattataa	acatcaaaaat	240
tttaatgtta	tcttaattat	aattttcta	actagtatat	tcaaaaatca	aaataaacat	300
attttataaa	atagtgccag	tacgtagtat	gggagataat	actagtggct	ttataaaggg	360
aaacattgtc	tctaaaatct	cagataaaaat	gttaaaaacac	acttattcac	aattatgaag	420
atttgaaata	tctgaaatct	caaattgatg	cacttggtag	aaagcaaagg	ttcaacgcta	480
agtctacaag	gtgtaataat	gaagtgaaaa	tgctagttaa	gattaccctt	gatatgtgac	540
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aagttgatta	attgatttcta	aattattttt	gtctttctaaa	tacatatata	aatcaactgg	1560
aaatgtaaat	atttgcta	atttctacta	taggagaatt			1600

<210> 238

<211> 1677

<212> DNA

<213> Artificial Sequence

<220>

<223> flanking sequence

<400> 238

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ccgtgggttc	caaattaaat	aaaactacga	tgatcatcag	aagtaaaatc	aactgtgtcc	180
acattatcag	ttttgtgtat	acgatgaaat	agggtaattc	aaaatctagc	ttgatatgcc	240
ttttggttca	ttttaacctt	ctgtaaacat	tttttcagat	tttgaacaag	taaatccaaa	300
aaaaaaaaaa	aaaatctcaa	ctcaacacta	aattatttta	atgtataaaa	gatgcttaaa	360

eolf-seql

acatttggct	taaaagaaa	aagctaaaaa	catagagaac	tcttgtaaat	tgaagtatga	420
aaatatactg	aattgggtat	tatatgaatt	tttctgattt	aggattcaca	tgatccaaaa	480
aggaaatcca	gaagcactaa	tcagacattg	gaagtaggat	taatcagtga	tcagtaacta	540
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ttattttgcc	caagggccta	tagattcatt	gggccgacc	tgggggttggg	gttgtgtgtg	1620
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<210> 239

<211> 5600

<212> DNA

<213> Artificial Sequence

<220>

<223> flanking sequence

<400> 239

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tagttaaatt	tatatgatat	ttacttcagt	aatattaaaa	tattatatac	atttttatta	180
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attaatttta	aaatcatcat	tatctaaaaa	gatttatatat	tatgttatcc	aaaaatattt	480
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eolf-seql

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accagcactc	taagcaccac					5600

<210> 240

<211> 1321

<212> DNA

<213> Artificial Sequence

<220>

<223> flanking sequence

<400> 240

eolf-seql

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attgaaaagc	attattttta	tcgaaatgaa	tctagtatac	aatcaatatt	tatgtttttt	180
catcagatac	tttcctat	tttggcacct	ttcatcgga	tactgattta	tttcaatgtg	240
tatgcatgca	tgagcatgag	tatacacatg	tcttttaaaa	tgcatgtaaa	gcgtaacgga	300
ccacaaaaga	ggatccatac	aaatacatct	catcgcttcc	tctactattc	tccgacacac	360
acactgagca	tggtgcttaa	acactctggg	gagttctagt	acttctgcta	taatgttaaa	420
ttttatatta	tatacctact	tcctctctct	cgctctgtta	tgttcgattt	cgaaggatt	480
tcaagatcaa	agatgatgag	aaaagggtacc	ttttcgatat	ttaagacaag	gaaagaaagg	540
acgaggttga	aatttttcggg	acttgagggg	ctaaagtggg	agagactgaa	tctgaagatg	600
tcgtttctcg	aaactttgag	atacagaatc	atgtctatca	ttgaagggaat	ggtttttggtt	660
tctaagcttg	ctttcttctt	tctctgttgc	ggttgcagat	tttaacacgt	tagttttttt	720
tttttcgttt	ttttgaacgt	caacaatgtc	ttttttgtac	tcttttagctc	atgtgtaaaa	780
ttctaaattc	ttccaataac	ataccaaca	aattattcgt	atctgatttt	tatagttttt	840
aacctgttaa	tgaattaat	ctaagtgtaa	tttttaggct	aaatgttaaa	ttttatatta	900
aagttttgta	acttgaaatt	acattcttct	tatagcggat	aaacagaaaa	tgctcttaaa	960
caaactcctga	aacaagtaaa	aaatacaaca	gaaaaatcta	acgtttaatt	cttaaaacct	1020
caaaatcctt	atttttacag	ctttcaaagt	ttaacagctg	gaaacctgta	gaaaatcaga	1080
cacagcctct	caagttttct	ggacaataaa	tactggtaac	gtaagaaaac	caattaatga	1140
taccgtcgtt	cagtagatag	aactgacgat	gtgaagatta	attgtttctg	taatatactg	1200
aatttgaaaa	tttatcatca	tcatgttaac	ggaagtgtgc	tgtaaaagta	gttgattacc	1260
tgttatcgtg	taaagtagtt	agtaatttct	tgcttatttg	aaaaatagag	aacatttaac	1320
atgtattttt	aaataggcac	gacctgcta	ctgaacttta	tgaaatgctt	tggaatctta	1321

<210> 241
 <211> 100
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> amplicon

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aagacgtccg	caatgtgtta	ttaagttgtc	taagcgtcaa	tttgtttgtcg	gcaagatctc	100
tgacactcaa	ctaaaatggt	ggattttccc	tttcagggtgt			

<210> 242
 <211> 148
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> amplicon

t	t	t	t	t	t	60
cacacacctc	cccaatccat	ggatatgttc	tctcttttct	caatcaattt	gtttacactc	120
aaacactgat	agtttaaact	gaaggcggga	aacgacaatc	tgatcactga	ttagtaacta	148
aggcctttaa	ttaatctaga	ggcgcgcc				

<210> 243
 <211> 147
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> amplicon

t	t	t	t	t	t	60
ggcgcgcctc	tagattaatt	aaaggcctta	gttactaatc	agtgatcaga	ttgtcgtttc	120
ccgccttcag	tttaaactat	cagtgtttgt	ttagtgggtca	atcaaaaagat	gaacagtatc	147
ttctcgaatc	atgatttctc	cgatccc				

<210> 244
 <211> 118
 <212> DNA
 <213> Artificial Sequence

<220>

eolf-seql

<223> amplicon

<400> 244
 caccaaaagc aaccaagaga gacgtcttta atgtactgaa tttagtaact gaaggcggga 60
 aacgacaatc tgatcactga ttagtaacta aggcctttta ttaatctaga ggcgcgcc 118

<210> 245
 <211> 110
 <212> DNA
 <213> Artificial Sequence

<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 Sequence

<220>
 <223> amplicon

<400> 246
 cactgagcat ggtgcttaaa cactctgggtg agttctagta cttctgctat aatgttaaatt 60
 tttataattat atacctactt cctctctctc gctct 95

<210> 247
 <211> 101
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> amplicon

<400> 247
 ctctttcttt ttctccatat tgaccatcat actcattgct gatccatgta gatttcccgg 60
 acatgaagcc atttgtaac gtcgttggtt gatttcgtta a 101

<210> 248
 <211> 197
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> amplicon

<400> 248
 caattaaccg cggacatcta cattttttta ctgctattct ttcattattag caactgggtg 60
 attactgaat tagtacagat gtaactaaca tatatattgg atattattat tatccaaaag 120
 agctattcaa ccttttaagc atatgtatat acatgtttta ttgattttat tagtatttct 180
 tgctcttggt gttttgc 197

<210> 249
 <211> 138
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> amplicon

<400> 249
 aattaaccgc ggacatctac atttttgaat tgaaaaaaa ttggttaatta ctctttcttt 60
 ttctccatat tgaccatcat actcattgct gatccatgta gatttcccgg ttctttttct 120
 cagactactc actgcccg 138

eolf-seql

<210> 250
 <211> 137
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> amplicon

<400> 250
 ctcttttcttt ttctccatat tgaccatcat actcattgct gatccatgta gattttcccgg 60
 acatgaagcc atttacaatc catgtcctta taaatggttt atatactctt ataaataatt 120
 tgcttcttca tgcctca 137

<210> 251
 <211> 113
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> amplicon

<400> 251
 ctcttttcttt ttctccatat tgaccatcat actcattgct gatccatgta gattttcccgg 60
 acatgaagcc atttacaatg agtcaaaaaa aagttgtttt gatgagtcac tgc 113

<210> 252
 <211> 160
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> amplicon

<400> 252
 ctcttttcttt ttctccatat tgaccatcat actcattgct gatccatgta gattttcccgg 60
 acatgaagcc atttactctg accctactcc acaaataatat ttttatttat aaaaaggtgg 120
 ccattgtata ctatgtgtgc gtatacagga ataaaaatgt 160

<210> 253
 <211> 169
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> amplicon

<400> 253
 ccatattgac catcatactc attgctgac catgtagatt tcccggacat gaagccattt 60
 acaattgaat atatcctcac atatgaaata tatttttttt ttacaaatta cacctattaa 120
 attatacttg atcgggtcatc tgatatattt gaaagaaccc tatcagcca 169

<210> 254
 <211> 576
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> amplicon

<400> 254
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 atattttttt gtacactatt tatcaattta ataaaaaaat ataataaat 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 aatctctggt 420

eolf-seql

Thr	Pro	Thr	Thr	Lys	Gly	Leu	Pro	Leu	Val	Asp	Ser	Pro	Thr	Pro	Ile
		35					40					45			
Val	Leu	Gly	Val	Ser	Val	Tyr	Leu	Thr	Ile	Val	Ile	Gly	Gly	Leu	Leu
	50					55					60				
Trp	Ile	Lys	Ala	Arg	Asp	Leu	Lys	Pro	Arg	Ala	Ser	Glu	Pro	Phe	Leu
65					70					75					80
Leu	Gln	Ala	Leu	Val	Leu	Val	His	Asn	Leu	Phe	Cys	Phe	Ala	Leu	Ser
				85					90					95	
Leu	Tyr	Met	Cys	Val	Gly	Ile	Ala	Tyr	Gln	Ala	Ile	Thr	Trp	Arg	Tyr
			100					105					110		
Ser	Leu	Trp	Gly	Asn	Ala	Tyr	Asn	Pro	Lys	His	Lys	Glu	Met	Ala	Ile
		115					120					125			
Leu	Val	Tyr	Leu	Phe	Tyr	Met	Ser	Lys	Tyr	Val	Glu	Phe	Met	Asp	Thr
	130					135					140				
Val	Ile	Met	Ile	Leu	Lys	Arg	Ser	Thr	Arg	Gln	Ile	Ser	Phe	Leu	His
145					150					155					160
Val	Tyr	His	His	Ser	Ser	Ile	Ser	Leu	Ile	Trp	Trp	Ala	Ile	Ala	His
				165					170					175	
His	Ala	Pro	Gly	Glu	Ala	Tyr	Trp	Ser	Ala	Ala	Leu	Asn	Ser	Gly	
			180				185					190			
Val	His	Val	Leu	Met	Tyr	Ala	Tyr	Tyr	Phe	Leu	Ala	Ala	Cys	Leu	Arg
		195					200				205				
Ser	Ser	Pro	Lys	Leu	Lys	Asn	Lys	Tyr	Leu	Phe	Trp	Gly	Arg	Tyr	Leu
		210				215					220				
Thr	Gln	Phe	Gln	Met	Phe	Gln	Phe	Met	Leu	Asn	Leu	Val	Gln	Ala	Tyr
225					230					235					240
Tyr	Asp	Met	Lys	Thr	Asn	Ala	Pro	Tyr	Pro	Gln	Trp	Leu	Ile	Lys	Ile
				245					250					255	
Leu	Phe	Tyr	Tyr	Met	Ile	Ser	Leu	Leu	Phe	Leu	Phe	Gly	Asn	Phe	Tyr
			260					265					270		
Val	Gln	Lys	Tyr	Ile	Lys	Pro	Ser	Asp	Gly	Lys	Gln	Lys	Gly	Ala	Lys
		275					280					285			
Thr	Glu														
	290														

<210> 259

<211> 1320

<212> DNA

<213> Artificial Sequence

<220>

<223> d5Des(Tc) as by plasmid VC-LTM593-1qcz

<400> 259

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gataagagaa	agaccatcct	cattgagggg	gtgttgtagc	atgctaccaa	cttcaaacac	120
ccaggagggt	ccattattaa	cttcctcacc	gagggagaag	ctggagttga	tgctacccaa	180
gcttacagag	agttccatca	gagatccgga	aaggctgata	agtacctcaa	gtccctccca	240
aagttggatg	cttctaaggt	ggagtctagg	ttctctgcta	aggagcaggc	tagaagggac	300
gctatgacca	gggattacgc	tgctttcaga	gaggagttgg	ttgctgaggg	atacttcgat	360
ccatctatcc	cacacatgat	ctacagagtg	gtggagattg	tggctttgtt	cgctttgtct	420
ttctggttga	tgtctaaggc	ttctccaacc	tctttggttt	tgggagtggt	gatgaacgga	480
atcgctcaag	gaagatgcgg	atgggttatg	cacgagatgg	gacacggatc	tttctactgga	540
gttatctggc	tcgatgatag	gatgtgcgag	ttcttctacg	gagttggatg	tggaatgtct	600
ggacactact	ggaagaacca	gcactctaag	caccacgctg	ctccaaacag	attggagcac	660
gatgtggatt	tgaacacctt	gccactcggt	gctttcaacg	agagagttgt	gaggaagggt	720
aagccaggat	ctttgttggc	tttgtggctc	agagttcagg	cttattttgt	cgctccagtg	780
tcttgcttgt	tgatcggatt	gggatggacc	ttgtacttgc	acccaagata	tatgctcagg	840
accaagagac	acatggagtt	tgtgtggatc	ttcgctagat	atatcggatg	gttctccttg	900
atgggagctt	tgggatattc	tcctggaact	tctgtgggaa	tgtacctctg	ctcttttcgga	960
cttggtgcca	tctacatctt	cctccaattc	gctgtgtctc	acaccactt	gccagttacc	1020
aaccagagg	atcaattgca	ctggcttgag	tacgctgctg	atcacaccgt	gaacatctct	1080
accaagtctt	ggttggttac	ctggtggatg	tctaacctca	acttccaaat	cgagcaccac	1140
ttgttcccaa	ccgctccaca	attcaggttc	aaggagatct	ctccaagagt	tgaggctctc	1200
ttcaagagac	acaacctccc	ttactacgat	ttgccataca	cctctgctgt	ttctactacc	1260
ttcgctaacc	tctactctgt	tggacactct	gttggagctg	ataccaagaa	gcaggattga	1320

<210> 260

eolf-seql

<211> 439

<212> PRT

<213> Thraustochytrium sp.

<220>

<223> d5Des(Tc) as by plasmid VC-LTM593-1qcz

<400> 260

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Met Gly Lys Gly Ser Glu Gly Arg Ser Ala Ala Arg Glu Met Thr Ala
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Glu Ala Asn Gly Asp Lys Arg Lys Thr Ile Leu Ile Glu Gly Val Leu
      20      25      30
Tyr Asp Ala Thr Asn Phe Lys His Pro Gly Gly Ser Ile Asn Phe
      35      40      45
Leu Thr Glu Gly Glu Ala Gly Val Asp Ala Thr Gln Ala Tyr Arg Glu
      50      55      60
Phe His Gln Arg Ser Gly Lys Ala Asp Lys Tyr Leu Lys Ser Leu Pro
65      70      75      80
Lys Leu Asp Ala Ser Lys Val Glu Ser Arg Phe Ser Ala Lys Glu Gln
      85      90      95
Ala Arg Arg Asp Ala Met Thr Arg Asp Tyr Ala Ala Phe Arg Glu Glu
      100      105      110
Leu Val Ala Glu Gly Tyr Phe Asp Pro Ser Ile Pro His Met Ile Tyr
      115      120      125
Arg Val Val Glu Ile Val Ala Leu Phe Ala Leu Ser Phe Trp Leu Met
      130      135      140
Ser Lys Ala Ser Pro Thr Ser Leu Val Leu Gly Val Val Met Asn Gly
145      150      155      160
Ile Ala Gln Gly Arg Cys Gly Trp Val Met His Glu Met Gly His Gly
      165      170      175
Ser Phe Thr Gly Val Ile Trp Leu Asp Arg Met Cys Glu Phe Phe
      180      185      190
Tyr Gly Val Gly Cys Gly Met Ser Gly His Tyr Trp Lys Asn Gln His
      195      200      205
Ser Lys His His Ala Ala Pro Asn Arg Leu Glu His Asp Val Asp Leu
210      215      220
Asn Thr Leu Pro Leu Val Ala Phe Asn Glu Arg Val Val Arg Lys Val
225      230      235      240
Lys Pro Gly Ser Leu Leu Ala Leu Trp Leu Arg Val Gln Ala Tyr Leu
      245      250      255
Phe Ala Pro Val Ser Cys Leu Leu Ile Gly Leu Gly Trp Thr Leu Tyr
      260      265      270
Leu His Pro Arg Tyr Met Leu Arg Thr Lys Arg His Met Glu Phe Val
      275      280      285
Trp Ile Phe Ala Arg Tyr Ile Gly Trp Phe Ser Leu Met Gly Ala Leu
      290      295      300
Gly Tyr Ser Pro Gly Thr Ser Val Gly Met Tyr Leu Cys Ser Phe Gly
305      310      315      320
Leu Gly Cys Ile Tyr Ile Phe Leu Gln Phe Ala Val Ser His Thr His
      325      330      335
Leu Pro Val Thr Asn Pro Glu Asp Gln Leu His Trp Leu Glu Tyr Ala
      340      345      350
Ala Asp His Thr Val Asn Ile Ser Thr Lys Ser Trp Leu Val Thr Trp
      355      360      365
Trp Met Ser Asn Leu Asn Phe Gln Ile Glu His His Leu Phe Pro Thr
      370      375      380
Ala Pro Gln Phe Arg Phe Lys Glu Ile Ser Pro Arg Val Glu Ala Leu
385      390      395      400
Phe Lys Arg His Asn Leu Pro Tyr Tyr Asp Leu Pro Tyr Thr Ser Ala
      405      410      415
Val Ser Thr Thr Phe Ala Asn Leu Tyr Ser Val Gly His Ser Val Gly
420      425      430
Ala Asp Thr Lys Lys Gln Asp
435

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<210> 261

<211> 1371

<212> DNA

<213> Artificial Sequence

<220>

<223> d6Des(0t) as by plasmid VC-LTM593-1qcz

<400> 261

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ttggctaaga	ccctcgctag	aagatacgtg	gttatcgagg	gagttgagta	cgatgtgacc	180
gatttcaaac	atcctggagg	aaccgtgatt	ttctacgctc	tctctaacac	tggagctgat	240
gctactgagg	ctttcaagga	gttccaccac	agatctagaa	aggctaggaa	ggctttggct	300
gctttgcctt	ctagacctgc	taagaccgct	aaagtggatg	atgctgagat	gctccaggat	360
ttcgctaagt	ggagaaagga	gttggagagg	gacggattct	tcaagccttc	tcctgctcat	420
gttgcttaca	gattcgctga	gttggctgct	atgtacgctt	tgggaacctt	cttcatgtac	480
gctagatacg	ttgtgtcctc	tgtgttggtt	tacgcttgct	tcttcggagc	tagatgtgga	540
tgggttcaac	acgagggagg	acactcttct	ttgaccggaa	acatctggtg	ggataagaga	600
atccaagctt	tcactgctgg	attcggattg	gctggatctg	gagatatgtg	gaactccatg	660
cacaacaagc	accacgctac	tcctcaaaaa	gtgaggcacg	atatggattt	ggataccact	720
cctgctgttg	ctttcttcaa	caccgctgtg	gaggataata	gacctagggg	attctctaag	780
tactggctca	gattgcaagc	ttggaccctt	attcctgtga	cttctggatt	ggtgttgctc	840
ttctggatgt	tcttcttcca	cccttctaag	gctttgaagg	gaggaaagta	cgaggagctt	900
gtgtggatgt	tggctgctca	cgtgattaga	acctggacca	ttaaggctgt	tactggattc	960
accgctatgc	aatcctacgg	actcttcttg	gctacttctt	gggtttccgg	atgctacttg	1020
ttcgctcact	tctctacttc	tcacaccac	ttggatgttg	ttcctgctga	tgagcacttg	1080
tcttgggtta	ggtacgctgt	ggatcacacc	attgatatcg	atccttctca	gggatgggtt	1140
aactggttga	tgggatactt	gaactgccaa	gtgattcacc	acctcttccc	ttctatgcct	1200
caattcagac	aacctgaggt	gtccagaaga	ttcgttgctt	tcgctaagaa	gtggaacctc	1260
aactacaagg	tgatgactta	tgctggagct	tggaaggcta	ctttgggaaa	cctcgataat	1320
gtgggaaagc	actactacgt	gcacggacaa	cactctggaa	agaccgcttg	a	1371

<210> 262

<211> 456

<212> PRT

<213> *Ostreococcus tauri*

<220>

<223> d6Des(0t) as by plasmid VC-LTM593-1qcz

<400> 262

Met	Cys	Val	Glu	Thr	Glu	Asn	Asn	Asp	Gly	Ile	Pro	Thr	Val	Glu	Ile
1				5					10					15	
Ala	Phe	Asp	Gly	Glu	Arg	Glu	Arg	Ala	Glu	Ala	Asn	Val	Lys	Leu	Ser
			20					25					30		
Ala	Glu	Lys	Met	Glu	Pro	Ala	Ala	Leu	Ala	Lys	Thr	Phe	Ala	Arg	Arg
		35					40					45			
Tyr	Val	Val	Ile	Glu	Gly	Val	Glu	Tyr	Asp	Val	Thr	Asp	Phe	Lys	His
	50					55					60				
Pro	Gly	Gly	Thr	Val	Ile	Phe	Tyr	Ala	Leu	Ser	Asn	Thr	Gly	Ala	Asp
65				70					75					80	
Ala	Thr	Glu	Ala	Phe	Lys	Glu	Phe	His	His	Arg	Ser	Arg	Lys	Ala	Arg
			85					90						95	
Lys	Ala	Leu	Ala	Ala	Leu	Pro	Ser	Arg	Pro	Ala	Lys	Thr	Ala	Lys	Val
		100						105					110		
Asp	Asp	Ala	Glu	Met	Leu	Gln	Asp	Phe	Ala	Lys	Trp	Arg	Lys	Glu	Leu
		115					120					125			
Glu	Arg	Asp	Gly	Phe	Phe	Lys	Pro	Ser	Pro	Ala	His	Val	Ala	Tyr	Arg
	130					135					140				
Phe	Ala	Glu	Leu	Ala	Ala	Met	Tyr	Ala	Leu	Gly	Thr	Tyr	Leu	Met	Tyr
145				150					155					160	
Ala	Arg	Tyr	Val	Val	Ser	Ser	Val	Leu	Val	Tyr	Ala	Cys	Phe	Phe	Gly
			165					170					175		
Ala	Arg	Cys	Gly	Trp	Val	Gln	His	Glu	Gly	Gly	His	Ser	Ser	Leu	Thr
		180						185					190		
Gly	Asn	Ile	Trp	Trp	Asp	Lys	Arg	Ile	Gln	Ala	Phe	Thr	Ala	Gly	Phe
	195					200						205			
Gly	Leu	Ala	Gly	Ser	Gly	Asp	Met	Trp	Asn	Ser	Met	His	Asn	Lys	His
	210					215					220				
His	Ala	Thr	Pro	Gln	Lys	Val	Arg	His	Asp	Met	Asp	Leu	Asp	Thr	Thr

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225	Pro	Ala	Val	Ala	Phe	230	Asn	Thr	Ala	Val	235	Glu	Asp	Asn	Arg	Pro	240	Arg
				245	Tyr	Trp	Leu	Arg	Leu	250	Gln	Ala	Trp	Thr	Phe	255	Ile	Pro
	Gly	Phe	Ser	Lys	260	Leu	Val	Leu	265	Phe	Trp	Met	Phe	Phe	270	Leu	His	Pro
	Val	Thr	Ser	Gly	275	Leu	Val	Leu	280	Thr	Glu	Glu	Leu	285	Val	Trp	Met	Leu
	Ser	Lys	Ala	Leu	Lys	Gly	Gly	Lys	Tyr	Thr	Ile	Lys	Ala	Val	Thr	Gly	Phe	
	Ala	Ala	His	Val	Ile	Arg	Thr	Trp	Thr	310	Leu	Ala	Thr	Ser	Trp	Val	Ser	
305	Thr	Ala	Met	Gln	Ser	235	Tyr	Gly	Leu	330	Leu	Ala	Thr	Ser	Trp	Val	Ser	
	Gly	Cys	Tyr	Leu	340	Phe	Ala	His	Phe	345	Thr	Ser	His	Thr	His	Leu	Asp	
	Val	Val	Pro	Ala	Asp	Glu	His	Leu	Ser	360	Trp	Val	Arg	Tyr	Ala	Val	Asp	
	His	Thr	Ile	Asp	Ile	Asp	Pro	Ser	Gln	375	Gly	Trp	Val	Asn	Trp	Leu	Met	
	Gly	Tyr	Leu	Asn	Cys	Gln	Val	Ile	His	380	Leu	Phe	Pro	Ser	Met	Pro		
385	Gln	Phe	Arg	Gln	Pro	390	Glu	Val	Ser	Arg	Arg	Phe	Val	Ala	Phe	Ala	Lys	
	Lys	Trp	Asn	Leu	Asn	Tyr	Lys	Val	Met	410	Thr	Tyr	Ala	Gly	Ala	Trp	Lys	
	Ala	Thr	Leu	Gly	Asn	Leu	Asp	Asn	425	Val	Gly	Lys	His	Tyr	Tyr	Val	His	
	Gly	Gln	His	Ser	Gly	Lys	Thr	Ala	440									
									450									

<210> 263

<211> 819

<212> DNA

<213> Artificial Sequence

<220>

<223> d6Elo(Tp) as by plasmid VC-LTM593-1qcz

<400> 263

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ccagatggaa	agttcagagc	tgatagggag	gattgggtgt	tgtgcgattt	cagatccgct	120
atcaccattg	ctctcatcta	catcgctttc	gtgatcttgg	gatctgctgt	gatgcaatct	180
ctcccagcta	tggaaccata	ccctatcaag	ttcctctaca	acgtgtctca	aatcttcctc	240
tgcgcttaca	tgactgttga	ggctggattc	ctcgcttata	ggaacggata	caccgttatg	300
ccatgcaacc	acttcaacgt	gaacgatcca	ccagttgcta	acttgctctg	gctcttctac	360
atctccaaag	tgtgggattt	ctgggatacc	atcttcattg	tgctcggaaa	gaagtggaga	420
caactctctt	tcttgcacgt	gtaccaccac	accaccatct	tcctcttcta	ctggttgaac	480
gctaacgtgc	tctacgatgg	agatatcttc	ttgaccatcc	tcctcaacgg	attcattcac	540
accgtgatgt	acacctacta	cttcactctc	atgcacacca	aggattctaa	gaccggaag	600
tctttgccaa	tctgttgtaa	gtcatctttg	accgctttcc	aactcttgca	attcaccatc	660
atgatgtccc	aagctaccta	cttggttttc	cacggatgcg	ataaggtttc	cctcagaatc	720
accatcgtgt	acttcgtgta	cattctctcc	cttttcttcc	tcttcgctca	gttcttcgtg	780
caatcctaca	tggtctccaa	gaagaagaag	tccgcttga			819

<210> 264

<211> 272

<212> PRT

<213> Thalassiosira pseudonana

<220>

<223> d6Elo(Tp) as by plasmid VC-LTM593-1qcz

<400> 264

Met	Asp	Ala	Tyr	Asn	Ala	Ala	Met	Asp	Lys	Ile	Gly	Ala	Ala	Ile	Ile
1				5					10					15	
Asp	Trp	Ser	Asp	Pro	Asp	Gly	Lys	Phe	Arg	Ala	Asp	Arg	Glu	Asp	Trp
			20					25					30		
Trp	Leu	Cys	Asp	Phe	Arg	Ser	Ala	Ile	Thr	Ile	Ala	Leu	Ile	Tyr	Ile

eolf-seql

Ala	Phe	35	Val	Ile	Leu	Gly	Ser	40	Ala	Val	Met	Gln	Ser	45	Leu	Pro	Ala	Met
50	50						55	55					60	60				
Asp	Pro	Tyr	Pro	Ile	Lys	Phe	Leu	Tyr	Asn	Val	Ser	Gln	Ile	Phe	Leu			
65	65				70	70			75	75				80	80			
Cys	Ala	Tyr	Met	Thr	Val	Glu	Ala	Gly	Phe	Leu	Ala	Tyr	Arg	Asn	Gly			
				85					90					95				
Tyr	Thr	Val	Met	Pro	Cys	Asn	His	Phe	Asn	Val	Asn	Asp	Pro	Pro	Val			
			100					105					110					
Ala	Asn	Leu	Leu	Trp	Leu	Phe	Tyr	Ile	Ser	Lys	Val	Trp	Asp	Phe	Trp			
		115				120						125						
Asp	Thr	Ile	Phe	Ile	Val	Leu	Gly	Lys	Lys	Trp	Arg	Gln	Leu	Ser	Phe			
130	130					135					140							
Leu	His	Val	Tyr	His	His	Thr	Thr	Ile	Phe	Leu	Phe	Tyr	Trp	Leu	Asn			
145	145				150					155					160			
Ala	Asn	Val	Leu	Tyr	Asp	Gly	Asp	Ile	Phe	Leu	Thr	Ile	Leu	Leu	Asn			
			165						170					175				
Gly	Phe	Ile	His	Thr	Val	Met	Tyr	Thr	Tyr	Tyr	Phe	Ile	Cys	Met	His			
			180					185					190					
Thr	Lys	Asp	Ser	Lys	Thr	Gly	Lys	Ser	Leu	Pro	Ile	Trp	Trp	Lys	Ser			
		195					200					205						
Ser	Leu	Thr	Ala	Phe	Gln	Leu	Leu	Gln	Phe	Thr	Ile	Met	Met	Ser	Gln			
	210				215						220							
Ala	Thr	Tyr	Leu	Val	Phe	His	Gly	Cys	Asp	Lys	Val	Ser	Leu	Arg	Ile			
225	225				230				235					240				
Thr	Ile	Val	Tyr	Phe	Val	Tyr	Ile	Leu	Ser	Leu	Phe	Phe	Leu	Phe	Ala			
			245					250					255					
Gln	Phe	Phe	Val	Gln	Ser	Tyr	Met	Ala	Pro	Lys	Lys	Lys	Lys	Ser	Ala			
			260					265					270					

<210> 265
 <211> 1197
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> d12Des(Ps) as by plasmid VC-LTM593-1qcz

<400> 265																		
atggctattt	tgaaccctga	ggctgattct	gctgctaacc	tcgctactga	ttctgaggct													60
aagcaaagac	aattggctga	ggctggatac	actcacgttg	aggggtgctcc	tgctcctttg													120
cctttggagt	tgccctcactt	ctctctcaga	gatctcagag	ctgctattcc	taagcactgc													180
ttcagagagt	ctttcgtgac	ctccacctac	tacatgatca	agaacgtgtt	gacttgcgct													240
gctttgttct	acgctgctac	cttcattgat	agagctggag	ctgctgctta	tgttttgtgg													300
cctgtgtact	ggttcttcca	gggatcttac	ttgactggag	tgtgggttat	cgctcacgag													360
tgtggacacc	aggcttattg	ctcttctgag	gtgggtgaaca	acttgattgg	actcgtgttg													420
cactctgctt	tgttgggtgcc	ttaccactct	tggagaatct	ctcacagaaa	gcaccactcc													480
aacactggat	cttgcgagaa	cgatgaggtt	ttcgttccctg	tgaccagatc	tgtgttggct													540
tcttcttgga	acgagacctt	ggaggattct	cctctctacc	aactctaccg	tatcgtgtac													600
atgttggttg	ttggatggat	gcctggatac	ctcttcttca	acgctactgg	acactactaag													660
tactggggaa	agtctaggtc	tcacttcaac	ccttactccg	ctatctatgc	tgatagggag													720
aggtggatga	tcgtgctctc	cgatattttc	ttgggtggcta	tgttggctgt	tttggctgct													780
ttgggtgcaca	ctttctcctt	caacacgatg	gtgaagtctt	acgtgggtgcc	ttacttcatt													840
gtgaacgctt	acttgggtgtt	gattacctac	ctccaacaca	ccgataccta	catccctcac													900
ttcagagagg	gagagtggaa	ttggttgaga	ggagctttgt	gcactgtgga	tagatcattt													960
ggtccattcc	tcgattctgt	ggtgcataga	atcgtggata	cccacgtttg	ccaccatatt													1020
ttctccaaga	tgcccttcta	tcactgagag	gaggctacca	acgctattaa	gcctctcctc													1080
ggaaagtctt	acttgaagga	tactactcct	gttctgtgtg	ctctctggag	atcttacacc													1140
cactgcaagt	tcgttgagga	tgatggaaag	gtgggtgttct	acaagaacaa	gttatag													1197

<210> 266
 <211> 398
 <212> PRT
 <213> Phytophthora sojae

<220>
 <223> d12Des(Ps) as by plasmid VC-LTM593-1qcz

eolf-seql

<400> 266

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Met Ala Ile Leu Asn Pro Glu Ala Asp Ser Ala Ala Asn Leu Ala Thr
1      5      10      15
Asp Ser Glu Ala Lys Gln Arg Gln Leu Ala Glu Ala Gly Tyr Thr His
20     25     30
Val Glu Gly Ala Pro Ala Pro Leu Pro Leu Glu Leu Pro His Phe Ser
35     40     45
Leu Arg Asp Leu Arg Ala Ala Ile Pro Lys His Cys Phe Glu Arg Ser
50     55     60
Phe Val Thr Ser Thr Tyr Tyr Met Ile Lys Asn Val Leu Thr Cys Ala
65     70     75     80
Ala Leu Phe Tyr Ala Ala Thr Phe Ile Asp Arg Ala Gly Ala Ala Ala
85     90     95
Tyr Val Leu Trp Pro Val Tyr Trp Phe Gln Gly Ser Tyr Leu Thr
100    105    110
Gly Val Trp Val Ile Ala His Glu Cys Gly His Gln Ala Tyr Cys Ser
115    120    125
Ser Glu Val Val Asn Asn Leu Ile Gly Leu Val Leu His Ser Ala Leu
130    135    140
Leu Val Pro Tyr His Ser Trp Arg Ile Ser His Arg Lys His His Ser
145    150    155    160
Asn Thr Gly Ser Cys Glu Asn Asp Glu Val Phe Val Pro Val Thr Arg
165    170    175
Ser Val Leu Ala Ser Ser Trp Asn Glu Thr Leu Glu Asp Ser Pro Leu
180    185    190
Tyr Gln Leu Tyr Arg Ile Val Tyr Met Leu Val Val Gly Trp Met Pro
195    200    205
Gly Tyr Leu Phe Phe Asn Ala Thr Gly Pro Thr Lys Tyr Trp Gly Lys
210    215    220
Ser Arg Ser His Phe Asn Pro Tyr Ser Ala Ile Tyr Ala Asp Arg Glu
225    230    235    240
Arg Trp Met Ile Val Leu Ser Asp Ile Phe Leu Val Ala Met Leu Ala
245    250    255
Val Leu Ala Ala Leu Val His Thr Phe Ser Phe Asn Thr Met Val Lys
260    265    270
Phe Tyr Val Val Pro Tyr Phe Ile Val Asn Ala Tyr Leu Val Leu Ile
275    280    285
Thr Tyr Leu Gln His Thr Asp Thr Tyr Ile Pro His Phe Arg Glu Gly
290    295    300
Glu Trp Asn Trp Leu Arg Gly Ala Leu Cys Thr Val Asp Arg Ser Phe
305    310    315    320
Gly Pro Phe Leu Asp Ser Val Val His Arg Ile Val Asp Thr His Val
325    330    335
Cys His His Ile Phe Ser Lys Met Pro Phe Tyr His Cys Glu Glu Ala
340    345    350
Thr Asn Ala Ile Lys Pro Leu Leu Gly Lys Phe Tyr Leu Lys Asp Thr
355    360    365
Thr Pro Val Pro Val Ala Leu Trp Arg Ser Tyr Thr His Cys Lys Phe
370    375    380
Val Glu Asp Asp Gly Lys Val Val Phe Tyr Lys Asn Lys Leu
385    390    395

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<210> 267

<211> 1092

<212> DNA

<213> Artificial Sequence

<220>

<223> o3Des(Pir) as by plasmid VC-LTM593-1qcz

<400> 267

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atggccttcta catctgctgc tcaagacgct gctccttacg agttcccttc tctcactgag      60
atcaagaggg ctcttccttc tgagtgtttc gaggcttctg ttctcttttc tctctactac      120
accgctagat ctcttgctct tgctggatct ctcgctgttg ctctctctta cgctagagct      180
ttgcctcttg ttcaggctaa cgctcttctt gatgctactc tctgcactgg atacgttctt      240
ctccagggaa tcgttttctg gggattcttc accgttggtc acgattgtgg acacggagct      300
ttctctagat ctcacgtgct caacttctct gttggaacc tcatgcactc tatcatcctt      360
acccctttcg agtcttgga gctctctcac agacaccacc acaagaacac cggaacatc      420

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eolf-seql

gataaggacg	agatcttcta	ccctcaaaga	gaggctgatt	ctcaccctgt	ttctagacac	480
cttgtgatgt	cicctggatc	tgcttggttc	gcttaccttt	tcgctggatt	ccctcctaga	540
accatgaacc	acttcaacc	ttgggaggct	atgtatgtta	gaagagtggc	tgctgtgatc	600
atctctctcg	gagttctttt	cgctttcgct	ggactctact	cttacctcac	cttcgttctt	660
ggattcacca	ctatggctat	ctactacttc	ggacctctct	tcactcttcg	taccatgctt	720
gttgttacca	ctttcctcca	ccacaacgat	gaggagacac	cttggtacgc	tgattctgag	780
tggaattacg	tgaagggaaa	cctctcttct	gtggacagat	cttacgggtg	tctcatcgac	840
aaccttagcc	acaacatcgg	aactcaccag	atccaccacc	tcttccttat	catccctcac	900
tacaagctca	acgatgctac	tgctgctttc	gctaaggctt	tccctgagct	tgttaggaaa	960
aacgctgctc	ctatcatccc	aactttcttc	aggatggctg	ctatgtacgc	taagtacgga	1020
gttgttgaca	ctgatgctaa	gaccttcact	ctcaaggagg	ctaaggctgc	tgctaagact	1080
aagtcattct	ga					1092

<210> 268

<211> 363

<212> PRT

<213> Pythium irregulare

<220>

<223> o3Des(Pir) as by plasmid VC-LTM593-1qcz

<400> 268

Met	Ala	Ser	Thr	Ser	Ala	Ala	Gln	Asp	Ala	Ala	Pro	Tyr	Glu	Phe	Pro
1				5				10						15	
Ser	Leu	Thr	Glu	Ile	Lys	Arg	Ala	Leu	Pro	Ser	Glu	Cys	Phe	Glu	Ala
			20					25					30		
Ser	Val	Pro	Leu	Ser	Leu	Tyr	Tyr	Thr	Ala	Arg	Ser	Leu	Ala	Leu	Ala
		35					40					45			
Gly	Ser	Leu	Ala	Val	Ala	Leu	Ser	Tyr	Ala	Arg	Ala	Leu	Pro	Leu	Val
	50					55					60				
Gln	Ala	Asn	Ala	Leu	Leu	Asp	Ala	Thr	Leu	Cys	Thr	Gly	Tyr	Val	Leu
65				70						75				80	
Leu	Gln	Gly	Ile	Val	Phe	Trp	Gly	Phe	Phe	Thr	Val	Gly	His	Asp	Cys
			85					90						95	
Gly	His	Gly	Ala	Phe	Ser	Arg	Ser	His	Val	Leu	Asn	Phe	Ser	Val	Gly
			100					105					110		
Thr	Leu	Met	His	Ser	Ile	Ile	Leu	Thr	Pro	Phe	Glu	Ser	Trp	Lys	Leu
		115					120					125			
Ser	His	Arg	His	His	His	Lys	Asn	Thr	Gly	Asn	Ile	Asp	Lys	Asp	Glu
	130					135					140				
Ile	Phe	Tyr	Pro	Gln	Arg	Glu	Ala	Asp	Ser	His	Pro	Val	Ser	Arg	His
145				150						155				160	
Leu	Val	Met	Ser	Leu	Gly	Ser	Ala	Trp	Phe	Ala	Tyr	Leu	Phe	Ala	Gly
			165					170						175	
Phe	Pro	Pro	Arg	Thr	Met	Asn	His	Phe	Asn	Pro	Trp	Glu	Ala	Met	Tyr
			180					185					190		
Val	Arg	Arg	Val	Ala	Ala	Val	Ile	Ile	Ser	Leu	Gly	Val	Leu	Phe	Ala
		195					200					205			
Phe	Ala	Gly	Leu	Tyr	Ser	Tyr	Leu	Thr	Phe	Val	Leu	Gly	Phe	Thr	Thr
	210					215					220				
Met	Ala	Ile	Tyr	Tyr	Phe	Gly	Pro	Leu	Phe	Ile	Phe	Ala	Thr	Met	Leu
225					230					235				240	
Val	Val	Thr	Thr	Phe	Leu	His	His	Asn	Asp	Glu	Glu	Thr	Pro	Trp	Tyr
			245					250						255	
Ala	Asp	Ser	Glu	Trp	Thr	Tyr	Val	Lys	Gly	Asn	Leu	Ser	Ser	Val	Asp
			260					265					270		
Arg	Ser	Tyr	Gly	Ala	Leu	Ile	Asp	Asn	Leu	Ser	His	Asn	Ile	Gly	Thr
		275					280					285			
His	Gln	Ile	His	His	Leu	Phe	Pro	Ile	Ile	Pro	His	Tyr	Lys	Leu	Asn
	290				295						300				
Asp	Ala	Thr	Ala	Ala	Phe	Ala	Lys	Ala	Phe	Pro	Glu	Leu	Val	Arg	Lys
305				310						315				320	
Asn	Ala	Ala	Pro	Ile	Ile	Pro	Thr	Phe	Phe	Arg	Met	Ala	Ala	Met	Tyr
			325					330						335	
Ala	Lys	Tyr	Gly	Val	Val	Asp	Thr	Asp	Ala	Lys	Thr	Phe	Thr	Leu	Lys
			340					345					350		
Glu	Ala	Lys	Ala	Ala	Ala	Lys	Thr	Lys	Ser	Ser					
	355					360									

eolf-seql

<210> 269
 <211> 1086
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> o3Des(Pi) as by plasmid VC-LTM593-1qcz

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<400> 269
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aaggattgct tcgaggcttc tgtgcctttg tctctctact acactgtgag atgcttggtt    120
attgctgtgg ctttgacctt cggattgaac tacgctagag ctttgccaga gggtgagtct    180
ttctgggctt tggatgctgc tttgtgcaact ggatataatc tcctccaggg aattgtgttc    240
tggggattct tcactgttgg acacgatgct ggacacggag ctttctctag ataccacctc    300
ttgaacttcg ttgtgggaac cttcatgcac tctctcatct tgacccatt cgagtcttgg    360
aagttgaccc acagacacca ccacaagaac accggaacaa tcgatagaga tgaggtgttc    420
taccacacaga gaaaggctga tgatcaccca ttgtccagga acttgatctt ggctttggga    480
gctgcttggc ttgcttattt ggtggaggga ttcccaccaa gaaagggtgaa ccacttcaac    540
ccattcgagc cactttttgt gagacaagtg tccgctgtgg ttatctcttt gctcgctcac    600
ttcttcgttg ctggactctc tatctacttg tctctccagt tgggacttaa gaccatggct    660
atctactact acggaccagt ttctgtgttc ggatctatgt tgggtgattac caccttcttg    720
caccacaacg atgaggagac tccatggtat gctgattctg agtggactta cgtgaaggga    780
aacttgtcct ctgtggatag atcttacggg gctctcatcg ataacctctc ccacaacatc    840
ggaactcacc agatccacca cctcttccca attatccac actacaagct caagaaggct    900
actgctgctt tccaccaagc tttcccagag cttgtgagaa agtccgatga gccaatcatc    960
aaggctttct tcagagtggg aaggttgatg gctaactacg gagtggttga tcaagaggct   1020
aagctcttca ctttgaagga ggctaaggct gctactgaag ctgctgctaa gaccaagtct   1080
acctga                                           1086
    
```

<210> 270
 <211> 361
 <212> PRT
 <213> Phytophthora infestans

<220>
 <223> o3Des(Pi) as by plasmid VC-LTM593-1qcz

```

<400> 270
Met Ala Thr Lys Glu Ala Tyr Val Phe Pro Thr Leu Thr Glu Ile Lys
1      5      10      15
Arg Ser Leu Pro Lys Asp Cys Phe Glu Ala Ser Val Pro Leu Ser Leu
20     25     30
Tyr Tyr Thr Val Arg Cys Leu Val Ile Ala Val Ala Leu Thr Phe Gly
35     40     45
Leu Asn Tyr Ala Arg Ala Leu Pro Glu Val Glu Ser Phe Trp Ala Leu
50     55     60
Asp Ala Ala Leu Cys Thr Gly Tyr Ile Leu Leu Gln Gly Ile Val Phe
65     70     75     80
Trp Gly Phe Phe Thr Val Gly His Asp Ala Gly His Gly Ala Phe Ser
85     90     95
Arg Tyr His Leu Leu Asn Phe Val Val Gly Thr Phe Met His Ser Leu
100    105    110
Ile Leu Thr Pro Phe Glu Ser Trp Lys Leu Thr His Arg His His His
115    120    125
Lys Asn Thr Gly Asn Ile Asp Arg Asp Glu Val Phe Tyr Pro Gln Arg
130    135    140
Lys Ala Asp Asp His Pro Leu Ser Arg Asn Leu Ile Leu Ala Leu Gly
145    150    155    160
Ala Ala Trp Leu Ala Tyr Leu Val Glu Gly Phe Pro Pro Arg Lys Val
165    170    175
Asn His Phe Asn Pro Phe Glu Pro Leu Phe Val Arg Gln Val Ser Ala
180    185    190
Val Val Ile Ser Leu Leu Ala His Phe Phe Val Ala Gly Leu Ser Ile
195    200    205
Tyr Leu Ser Leu Gln Leu Gly Leu Lys Thr Met Ala Ile Tyr Tyr Tyr
210    215    220
Gly Pro Val Phe Val Phe Gly Ser Met Leu Val Ile Thr Thr Phe Leu
    
```

eolf-seql

225	His	His	Asn	Asp	Glu	230	Glu	Thr	Pro	Trp	Tyr	235	Ala	Asp	Ser	Glu	Trp	240	Thr
					245	Asn	Leu	Ser	Ser	Val	250	Asp	Arg	Ser	Tyr	Gly	Ala	Leu	
				260	Ser	His	Asn	Ile	265	Gly	Thr	His	Gln	Ile	270	His	His	Leu	
			275	Phe	Pro	Ile	Ile	Pro	His	Tyr	280	Leu	Lys	Lys	Ala	Thr	Ala	Ala	Phe
			290	His	Gln	Ala	Phe	Pro	Glu	Leu	Val	Arg	Lys	Ser	Asp	Glu	Pro	Ile	Ile
			305	Lys	Ala	Phe	Phe	Arg	Val	Gly	Arg	Leu	Tyr	310	Ala	Asn	Tyr	Gly	Val
					325	Leu	Phe	Thr	Leu	330	Lys	Glu	Ala	Lys	Ala	Ala	Thr		
					340	Lys	Thr	Lys	Ser	Thr									
					355														

<210> 271
 <211> 1560
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> d4Des(Tc) as by plasmid VC-LTM593-1qcz

<400> 271

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gacgacgctt	ggtgtgctat	tcacggacac	gtgtacgacg	ttaccaagtt	cgcttcagtt	120
cacccaggag	gagatattat	cttgctcgct	gctggaaagg	aagctactgt	cctctacgag	180
acctaccatg	ttagaggagt	gtctgacgct	gtgctcagaa	agtacagaat	aggaaagtgt	240
ccagacggac	aaggaggagc	taacgagaag	gagaagagaa	ccttgctctg	attgtcctct	300
gcttcttact	acacctggaa	ctccgatttc	tacagagtga	tgaggagag	agttgtggct	360
agattgaagg	agagagggaa	ggctagaaga	ggaggatacg	aactctggat	caaggctttc	420
ttgctccttg	ttggattctg	gtcctctctt	tactggatgt	gcaccctcga	tccatctttc	480
ggagctatct	tggctgctat	gtctttggga	gtgttcgctg	cttttggttg	aacctgcac	540
caacacgatg	gaaaccacgg	agctttcgct	caatctagat	gggttaacaa	ggtggcagga	600
tggactttgg	atatgatcgg	agcttctgga	atgacttggg	agttccaaca	cgtgttgga	660
caccacccat	acactaactt	gatcgaggag	gagaacggat	tgcaaaagg	gtccggaaag	720
aagatggata	ccaagttggc	tgatcaagag	tctgatccag	atgtgttctc	cacctacca	780
atgatgagat	tgcacccttg	gcaccagaag	aggtggtatc	acaggttcca	gcacatctac	840
ggacccttca	tcttcggatt	catgaccatc	aacaagggtg	tgactcaaga	tggtggagt	900
gtgttgagaa	agagactctt	ccaaatcgat	gctgagtga	gatatgcttc	cccaatgtac	960
gttgctagg	tctggattat	gaaggctttg	accgtgttgt	atatggttgc	tttgctttgt	1020
tatatgcaag	gaccttgga	cggattgaaa	ctcttcgcta	tcgctcactt	cacttgcgga	1080
gaggttttgg	ctaccatgtt	catcgtgaac	cacattatcg	agggagtgtc	ttacgcttct	1140
aaggatgctg	ttaagggaac	tatggctcca	ccaaagacta	tgacaggagt	gacccaatg	1200
aacaacacta	gaaaggaggt	tgaggctgag	gcttctaagt	ctggagctgt	ggttaagtct	1260
gtgccattgg	atgattgggc	tgctgttcag	tgccaaacct	ctgtgaactg	gtctgttgg	1320
tcttggtttt	ggaaccactt	ctctggagga	ctcaaccacc	aaatcgagca	ccacctcttc	1380
ccaggattgt	ctcacgagac	ctactaccac	atccaagacg	tggttcaatc	tacctgtgct	1440
gagtacggag	ttccatacca	acacgagcca	tctttgtgga	ctgcttactg	gaagatgctc	1500
gaacaccta	gacaattggg	aaacgaggag	actcacgagt	catggcagag	agctgcttga	1560

<210> 272
 <211> 519
 <212> PRT
 <213> Thraustochytrium sp.

<220>
 <223> d4Des(Tc) as by plasmid VC-LTM593-1qcz

<400> 272

Met	Thr	Val	Gly	Tyr	Asp	Glu	Glu	Ile	Pro	Phe	Glu	Gln	Val	Arg	Ala
1				5					10				15		
His	Asn	Lys	Pro	Asp	Asp	Ala	Trp	Cys	Ala	Ile	His	Gly	His	Val	Tyr
			20					25				30			
Asp	Val	Thr	Lys	Phe	Ala	Ser	Val	His	Pro	Gly	Gly	Asp	Ile	Ile	Leu

		35					40					45			
Leu	Ala	Ala	Gly	Lys	Glu	Ala	Thr	Val	Leu	Tyr	Glu	Thr	Tyr	His	Val
	50					55					60				
Arg	Gly	Val	Ser	Asp	Ala	Val	Leu	Arg	Lys	Tyr	Arg	Ile	Gly	Lys	Leu
65					70					75					80
Pro	Asp	Gly	Gln	Gly	Gly	Ala	Asn	Glu	Lys	Glu	Lys	Arg	Thr	Leu	Ser
				85					90					95	
Gly	Leu	Ser	Ser	Ala	Ser	Tyr	Tyr	Thr	Trp	Asn	Ser	Asp	Phe	Tyr	Arg
			100					105					110		
Val	Met	Arg	Glu	Arg	Val	Val	Ala	Arg	Leu	Lys	Glu	Arg	Gly	Lys	Ala
		115					120					125			
Arg	Arg	Gly	Gly	Tyr	Glu	Leu	Trp	Ile	Lys	Ala	Phe	Leu	Leu	Leu	Val
	130					135					140				
Gly	Phe	Trp	Ser	Ser	Leu	Tyr	Trp	Met	Cys	Thr	Leu	Asp	Pro	Ser	Phe
145					150					155					160
Gly	Ala	Ile	Leu	Ala	Ala	Met	Ser	Leu	Gly	Val	Phe	Ala	Ala	Phe	Val
				165					170					175	
Gly	Thr	Cys	Ile	Gln	His	Asp	Gly	Asn	His	Gly	Ala	Phe	Ala	Gln	Ser
			180					185					190		
Arg	Trp	Val	Asn	Lys	Val	Ala	Gly	Trp	Thr	Leu	Asp	Met	Ile	Gly	Ala
		195					200					205			
Ser	Gly	Met	Thr	Trp	Glu	Phe	Gln	His	Val	Leu	Gly	His	His	Pro	Tyr
	210					215					220				
Thr	Asn	Leu	Ile	Glu	Glu	Glu	Asn	Gly	Leu	Gln	Lys	Val	Ser	Gly	Lys
225					230					235					240
Lys	Met	Asp	Thr	Lys	Leu	Ala	Asp	Gln	Glu	Ser	Asp	Pro	Asp	Val	Phe
				245					250					255	
Ser	Thr	Tyr	Pro	Met	Met	Arg	Leu	His	Pro	Trp	His	Gln	Lys	Arg	Trp
			260					265					270		
Tyr	His	Arg	Phe	Gln	His	Ile	Tyr	Gly	Pro	Phe	Ile	Phe	Gly	Phe	Met
		275					280					285			
Thr	Ile	Asn	Lys	Val	Val	Thr	Gln	Asp	Val	Gly	Val	Val	Leu	Arg	Lys
	290					295					300				
Arg	Leu	Phe	Gln	Ile	Asp	Ala	Glu	Cys	Arg	Tyr	Ala	Ser	Pro	Met	Tyr
305					310					315					320
Val	Ala	Arg	Phe	Trp	Ile	Met	Lys	Ala	Leu	Thr	Val	Leu	Tyr	Met	Val
				325					330					335	
Ala	Leu	Pro	Cys	Tyr	Met	Gln	Gly	Pro	Trp	His	Gly	Leu	Lys	Leu	Phe
			340					345					350		
Ala	Ile	Ala	His	Phe	Thr	Cys	Gly	Glu	Val	Leu	Ala	Thr	Met	Phe	Ile
		355					360					365			
Val	Asn	His	Ile	Ile	Glu	Gly	Val	Ser	Tyr	Ala	Ser	Lys	Asp	Ala	Val
	370					375					380				
Lys	Gly	Thr	Met	Ala	Pro	Pro	Lys	Thr	Met	His	Gly	Val	Thr	Pro	Met
385					390		</								

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<210> 273
<211> 1338
<212> DNA
<213> Artificial Sequence
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 $\langle 220 \rangle$

eolf-seql

<223> d4Des(Pl) as by plasmid VC-LTM593-1qcz

<400> 273

atgccaccta	gtgctgctag	tgaaggtggt	gttgctgaac	ttagagctgc	tgaagttgct	60
agctacacta	gaaaggctgt	tgacgaaaga	cctgacctca	ctatagttgg	tgacgctggt	120
tacgacgcta	aggcttttag	ggacgagcac	cctgggtggtg	ctcacttcgt	tagccttttc	180
ggaggtaggg	acgctactga	ggcttttatg	gaatatcacc	gtagagcttg	gcctaaggct	240
aggatgtcta	agttcttcgt	tggttcactt	gacgctagcg	agaagcctac	tcaagctgat	300
tcagcttacc	ttagactttg	cgctgagggt	aacgctcttt	tgccctaaggg	tagcggagga	360
ttcgctcctc	ctagctactg	gcttaaggct	gctgctcttg	ttgttgctgc	tgttagtata	420
gaggggtata	tgctccttag	gggtaagacc	cttttgctta	gcgttttcct	tggactcgtg	480
ttcgcttgga	taggacttaa	tattcagcac	gacgctaata	acggtgctct	tagtagacac	540
tcagtgatta	actactgcct	cggttacgct	caggattgga	taggtggtaa	tatggtgctt	600
tggcttcaag	agcacgttgt	gatgcaccac	ctccacacta	acgacgttga	cgctgaccc	660
gatcaaaagg	ctcacggtgt	tcttagactt	aagcctactg	acggttggtg	gccttggcac	720
gcacttcaac	aactctatat	ccttcctggg	gaggctatgt	acgcttttaa	gcttcttttc	780
ttggacgccc	ttgagcttct	tgcttgagg	tgaggagggtg	agaagattag	ccctcttgct	840
agagctttgt	tcgctcctgc	tggtgcttgt	aagcttggtg	tctgggctag	attcggtgct	900
ctccctctct	ggcttcaacc	tactgttcac	actgctttgt	gtatctgtgc	tactgtgtgt	960
actggttagt	tctacctcgc	cttcttcttc	tttatctctc	acaacttcga	cggtgttggt	1020
agcgttggtg	ctaagggatc	acttcctaga	tcagctactt	tcgttcaacg	tcagggttgag	1080
actagctcta	acgttggtgg	ttactggctt	ggagttctta	acggtggact	taactttcag	1140
atagagcacc	acttggtccc	taggcttcac	cactcttact	acgctcaa	agctcctgtg	1200
gtaggactc	acatagagaa	gctcggtttt	aagtaccgtc	acttccttac	cggttggtat	1260
aaccttagct	caatgcttca	gcataatggg	aagatgggaa	ctagacctgg	tgctgagaag	1320
ggtggttaagg	ctgagtag					1338

<210> 274

<211> 445

<212> PRT

<213> Pavlova lutheri

<220>

<223> d4Des(Pl) as by plasmid VC-LTM593-1qcz

<400> 274

Met	Pro	Pro	Ser	Ala	Ala	Ser	Glu	Gly	Gly	Val	Ala	Glu	Leu	Arg	Ala
1				5				10					15		
Ala	Glu	Val	Ala	Ser	Tyr	Thr	Arg	Lys	Ala	Val	Asp	Glu	Arg	Pro	Asp
			20					25					30		
Leu	Thr	Ile	Val	Gly	Asp	Ala	Val	Tyr	Asp	Ala	Lys	Ala	Phe	Arg	Asp
		35					40					45			
Glu	His	Pro	Gly	Gly	Ala	His	Phe	Val	Ser	Leu	Phe	Gly	Gly	Arg	Asp
	50					55					60				
Ala	Thr	Glu	Ala	Phe	Met	Glu	Tyr	His	Arg	Arg	Ala	Trp	Pro	Lys	Ala
65					70				75					80	
Arg	Met	Ser	Lys	Phe	Phe	Val	Gly	Ser	Leu	Asp	Ala	Ser	Glu	Lys	Pro
			85					90					95		
Thr	Gln	Ala	Asp	Ser	Ala	Tyr	Leu	Arg	Leu	Cys	Ala	Glu	Val	Asn	Ala
			100					105					110		
Leu	Leu	Pro	Lys	Gly	Ser	Gly	Gly	Phe	Ala	Pro	Pro	Ser	Tyr	Trp	Leu
		115					120					125			
Lys	Ala	Ala	Ala	Leu	Val	Val	Ala	Ala	Val	Ser	Ile	Glu	Gly	Tyr	Met
	130					135					140				
Leu	Leu	Arg	Gly	Lys	Thr	Leu	Leu	Ser	Val	Phe	Leu	Gly	Leu	Val	
145					150				155					160	
Phe	Ala	Trp	Ile	Gly	Leu	Asn	Ile	Gln	His	Asp	Ala	Asn	His	Gly	Ala
			165					170						175	
Leu	Ser	Arg	His	Ser	Val	Ile	Asn	Tyr	Cys	Leu	Gly	Tyr	Ala	Gln	Asp
		180						185					190		
Trp	Ile	Gly	Asn	Met	Val	Leu	Trp	Leu	Gln	Glu	His	Val	Val	Met	
	195					200					205				
His	His	Leu	His	Thr	Asn	Asp	Val	Asp	Ala	Asp	Pro	Asp	Gln	Lys	Ala
	210					215				220					
His	Gly	Val	Leu	Arg	Leu	Lys	Pro	Thr	Asp	Gly	Trp	Met	Pro	Trp	His
225					230				235					240	
Ala	Leu	Gln	Gln	Leu	Tyr	Ile	Leu	Pro	Gly	Glu	Ala	Met	Tyr	Ala	Phe
			245						250					255	

eolf-seql

Lys Leu Leu Phe Leu Asp Ala Leu Glu Leu Leu Ala Trp Arg Trp Glu
 260 270
 Gly Glu Lys Ile Ser Pro Leu Ala Arg Ala Leu Phe Ala Pro Ala Val
 275 280 285
 Ala Cys Lys Leu Gly Phe Trp Ala Arg Phe Val Ala Leu Pro Leu Trp
 290 295 300
 Leu Gln Pro Thr Val His Thr Ala Leu Cys Ile Cys Ala Thr Val Cys
 305 310 315 320
 Thr Gly Ser Phe Tyr Leu Ala Phe Phe Phe Ile Ser His Asn Phe
 325 330 335
 Asp Gly Val Gly Ser Val Gly Pro Lys Gly Ser Leu Pro Arg Ser Ala
 340 345 350
 Thr Phe Val Gln Arg Gln Val Glu Thr Ser Ser Asn Val Gly Gly Tyr
 355 360 365
 Trp Leu Gly Val Leu Asn Gly Gly Leu Asn Phe Gln Ile Glu His His
 370 375 380
 Leu Phe Pro Arg Leu His His Ser Tyr Tyr Ala Gln Ile Ala Pro Val
 385 390 395 400
 Val Arg Thr His Ile Glu Lys Leu Gly Phe Lys Tyr Arg His Phe Pro
 405 410 415
 Thr Val Gly Ser Asn Leu Ser Ser Met Leu Gln His Met Gly Lys Met
 420 425 430
 Gly Thr Arg Pro Gly Ala Glu Lys Gly Gly Lys Ala Glu
 435 440 445

<210> 275

<211> 903

<212> DNA

<213> Artificial Sequence

<220>

<223> d5Elo(0t) as by plasmid VC-LTM593-1qcz

<400> 275

atgtctgcta	gcggagcttt	gttgcctgct	atagctttcg	ctgcttacgc	ttacgctacc	60
tacgcttatg	ctttcgagtg	gagccacgct	aacggaatcg	ataacgtgga	tgctagagag	120
tggattggag	ctttgtcttt	gagactccct	gcaattgcaa	ccacaatgta	cctcttgttc	180
tgccctgtgg	gacctagatt	gatggctaag	agggaggctt	ttgataccta	gggatttatg	240
ctcgcctaca	acgcttacca	aaccgctttc	aacgcttggtg	tgctcggaat	gttcgctaga	300
gagatctctg	gattgggaca	acctgtttgg	ggatctacta	tgccctggag	cgataggaag	360
tccttcaaga	ttttgttggg	agtgtggctc	cactacaaca	ataagtacct	cgagttggtg	420
gatactgtgt	tcattggtggc	taggaaaaag	accaagcagc	tctctttctt	gcacgtgtac	480
caccacgctt	tggtgatttg	ggcttggtgg	cttgcttctc	acctcatggc	taccaacgat	540
tgcatcgtatg	cttatttcgg	agctgcttgc	aactctttca	tccacatcgt	gatgtactcc	600
tactacctca	tgctctgctt	gggaattagg	tgcccttgga	agagatatat	caccagggtc	660
cagatgttgc	aattcgtgat	cgtgttcgct	cacgctgttt	tcgtgctcag	acaaaagcac	720
tgccctgtta	ctttgccttg	ggcacaaatg	ttcgtgatga	caaatatgtt	ggtgctcttc	780
ggaaacttct	acctcaaggc	ttactctaac	aagtctaggg	gagatggagc	ttcttctggt	840
aagcctgctg	agactactag	agcaccttct	gtgagaagaa	ccaggtcaag	gaagatcgat	900
tga						903

<210> 276

<211> 300

<212> PRT

<213> *Ostreococcus tauri*

<220>

<223> d5Elo(0t) as by plasmid VC-LTM593-1qcz

<400> 276

Met Ser Ala Ser Gly Ala Leu Leu Pro Ala Ile Ala Phe Ala Ala Tyr
 1 5 10 15
 Ala Tyr Ala Thr Tyr Ala Tyr Ala Phe Glu Trp Ser His Ala Asn Gly
 20 25 30
 Ile Asp Asn Val Asp Ala Arg Glu Trp Ile Gly Ala Leu Ser Leu Arg
 35 40 45
 Leu Pro Ala Ile Ala Thr Thr Met Tyr Leu Leu Phe Cys Leu Val Gly
 50 55 60

eolf-seql

Pro	Arg	Leu	Met	Ala	Lys	Arg	Glu	Ala	Phe	Asp	Pro	Lys	Gly	Phe	Met
65					70				75						80
Leu	Ala	Tyr	Asn	Ala	Tyr	Gln	Thr	Ala	Phe	Asn	Val	Val	Val	Leu	Gly
				85					90					95	
Met	Phe	Ala	Arg	Glu	Ile	Ser	Gly	Leu	Gly	Gln	Pro	Val	Trp	Gly	Ser
			100					105					110		
Thr	Met	Pro	Trp	Ser	Asp	Arg	Lys	Ser	Phe	Lys	Ile	Leu	Leu	Gly	Val
		115					120					125			
Trp	Leu	His	Tyr	Asn	Asn	Lys	Tyr	Leu	Glu	Leu	Leu	Asp	Thr	Val	Phe
		130				135					140				
Met	Val	Ala	Arg	Lys	Lys	Thr	Lys	Gln	Leu	Ser	Phe	Leu	His	Val	Tyr
145				150						155					160
His	His	Ala	Leu	Leu	Ile	Trp	Ala	Trp	Trp	Leu	Val	Cys	His	Leu	Met
			165						170					175	
Ala	Thr	Asn	Asp	Cys	Ile	Asp	Ala	Tyr	Phe	Gly	Ala	Ala	Cys	Asn	Ser
		180						185					190		
Phe	Ile	His	Ile	Val	Met	Tyr	Ser	Tyr	Tyr	Leu	Met	Ser	Ala	Leu	Gly
		195					200					205			
Ile	Arg	Cys	Pro	Trp	Lys	Arg	Tyr	Ile	Thr	Gln	Ala	Gln	Met	Leu	Gln
	210				215						220				
Phe	Val	Ile	Val	Phe	Ala	His	Ala	Val	Phe	Val	Leu	Arg	Gln	Lys	His
225				230					235						240
Cys	Pro	Val	Thr	Leu	Pro	Trp	Ala	Gln	Met	Phe	Val	Met	Thr	Asn	Met
			245					250						255	
Leu	Val	Leu	Phe	Gly	Asn	Phe	Tyr	Leu	Lys	Ala	Tyr	Ser	Asn	Lys	Ser
		260						265					270		
Arg	Gly	Asp	Gly	Ala	Ser	Ser	Val	Lys	Pro	Ala	Glu	Thr	Thr	Arg	Ala
		275					280					285			
Pro	Ser	Val	Arg	Arg	Thr	Arg	Ser	Arg	Lys	Ile	Asp				
	290					295					300				

<210> 277

<211> 2013

<212> DNA

<213> Artificial Sequence

<220>

<223> AHAS as by plasmid VC-LTM593-1qcz

<400> 277

atggcgcgcg	caacaacaac	aacaacaaca	tcttcttcga	tctccttctc	caccaaacca	60
tctccttcct	cctccaaatc	accattacca	atctccagat	tctccctccc	attctcccta	120
aaccccaaca	aatcatcctc	ctcctcccgc	cgccgcggta	tcaaattccag	ctctccctcc	180
tccatctccg	ccgtgctcaa	cacaaccacc	aatgtcacaa	ccactccctc	tccaacaaaa	240
cctaccaaac	ccgaacatt	catctcccga	ttcgctccag	atcaaccccg	caaaggcgct	300
gatactctcg	tcgaggcttt	agaacgtcaa	ggcgtagaaa	ccgtattcgc	ttaccctgga	360
ggtacatcaa	tggagattca	ccaagcctta	acccgctctt	cctcaatccg	taacgtcctt	420
cctcgtcacg	aacaaggagg	tgtattcgca	gcagaaggat	acgctcgatc	ctcaggtaaa	480
ccaggatatt	gtatagccac	ttcaggctcc	ggagctacaa	atctcgttag	cggattagcc	540
gatgcgttgt	tagatagtgt	tcctcttgta	gcaatcacag	gacaagtccc	tcgtcgtatg	600
attggtacag	atgcgtttca	agagactccg	attggtgagg	taacgcgttc	gattacgaag	660
cataactatc	ttgtgatgga	tgttgaagat	atcccaagga	ttattgaaga	ggctttcttt	720
ttagctactt	ctggtagacc	tggacctgtt	ttggttgatg	ttcctaaaga	tattcaacaa	780
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eolf-seql

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eolf-seql

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eolf-seql

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eolf-seql

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eolf-seql

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eolf-seql

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eolf-seql

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eolf-seql

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eolf-seql

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<213> Artificial sequence

<220>
<223> LBFLFK Locus 2 flanking sequence up to and including the left border of the T-DNA

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eolf-seql

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eolf-seql

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<223> LBFDAU Locus 1 RB junction region

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<223> LBFDAU Locus 1 LB junction region

<400> 301

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<210> 302

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<212> DNA

<213> Artificial sequence

<220>

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eolf-seql

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<223> LBFDAU Locus 1 flanking sequence up to and including the left border of the T-DNA

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<220>

<223> LBFDAU Locus 1_Foward primer

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eolf-seql

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eolf-seq1

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eolf-seql

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eolf-seql

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aggcttacgt	aacgtcagct	gaggctacgt	agcgttagct	aacgtcacgt	gacgctacgt	3960
aggcttacgt	aatagttact	aatcagtgat	caggcgccgc	attaatttcc	accttccact	4020
acgatggggg	gcatcgacc	ggtgagtaat	attgttaggc	taagagcgaa	tttggcctgt	4080
agacctcaat	tgcgagcttt	ctaatttcaa	actattcggg	cctaactttt	ggtgtgatga	4140

eolf-seql

tgctgactgt	ttcgacgtta	attgatccta	cactatgtag	gtcatatcca	tcgttttaat	4200
ttttggccac	cattcaattc	tgtcttgcc	ttagggatgt	gaatatgaac	ggccaaggta	4260
agagaataaa	aataatccaa	attaaagcaa	gagaggccaa	gtaagataat	ccaaatgtac	4320
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aacccaccat	ccccaatctc	accaaacc	ccacacaact	cacaactcac	tctcacacct	4560
taaagaacca	atcaccacca	aaaaatttca	cgatttggaa	tttgattcct	gcgatcacag	4620
gtatgacagg	ttagattttg	ttttgtatag	ttgtatacat	acttctttgt	gatgttttgt	4680
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aggagtttgg	ggaaaaagga	agagtttttt	tggttgggtc	gagtgtattat	gaggttattt	4920
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agggaaagtc	tgctgctaga	gagatgactg	ctgaggctaa	cggagataag	agaaagacca	5040
tcctcattga	gggagtgttg	tacgatgcta	ccaacttcaa	acaccagga	ggttccatta	5100
ttaaacttct	caccgagggg	gaagctggag	ttgatgctac	ccaagcttac	agagagtacc	5160
atcagagatc	cggaaaggct	gataagtacc	tcaagtccct	cccaaagttg	gatgcttcta	5220
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aggcttctcc	aacctctttg	gttttgggag	tggatgataa	cggaaatcgt	caaggaagat	5460
gcggtatggg	tatgcacgag	atgggacacg	gatctttcac	tggagtattc	tggctcgatg	5520
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<210> 312

<211> 1321

<212> DNA

<213> Artificial sequence

<220>

<223> LBFDAU Locus 2 flanking sequence up to and including the left border of the T-DNA

<400> 312

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catcagatac	tttcttattt	tttggcacct	ttcatcggac	tactgattta	tttcaatgtg	180
tatgcatgca	tgagcatgag	tatacacatg	tcttttaaaa	tgcatgtaaa	gcgtaacgga	240
ccacaaaaga	ggatccatac	aaatacatct	catcgcttcc	tctactattc	tccgacacac	300
acactgagca	tgggtgctta	acactctggt	gagttctagt	acttctgcta	taatgttaaa	360
ttttatatta	tatacctact	tcctctctct	cgctctgtta	tgttcgattt	cgaaaggatt	420
tcaagatcaa	agatgatgag	aaaagggtacc	ttttcgatat	ttaagacaag	gaaagaaagg	480
acgaggttga	aattttcggg	acttggaggg	ctaaagtggg	agagactgaa	tctgaagatg	540
tcgtttctcg	aaactttgag	atacagaatc	atgtctatca	ttgaaggaa	ggttttgggt	600
tctaagcttg	ctttcttctt	tctctgttgc	ggttgcagat	tttaacacgt	tagttttttt	660
tttttcggtt	ttttgaacgt	caacaatgtc	ttttttgtac	tcttttagctc	atgtgtaaaa	720
ttctaaattc	ttccaataac	atacccaaca	aatttattcg	atctgatttt	tatagttttt	780
aacctgttaa	tgtaattaat	ctaagtgtaa	tttttaggct	aaatgttaaa	ttttatatta	840
aagttttgta	acttgaaatt	acattcttct	tatagcggat	aaacagaaaa	tgctcttaaa	900
caaactctga	aacaagtaaa	aaatacaaca	gaaaaatcta	acgtttaatt	cttaaaacct	960
caaaatcctt	atttttacag	ctttcaaagt	ttacacagct	gaaacctgta	gaaaatcaga	1020
cacagcctct	caagttttct	ggacaataaa	tactggtaac	gtaagaaaac	caattaatga	1080
taccgtcggt	cagtatatag	aactgacgat	gtgaagatta	attgtttctg	taatatactg	1140
aatttgaaaa	tttatcatca	tcagtgttaac	ggaagtgtgc	tgtaaaagta	gttgattacc	1200
tggtatcgtg	taaagtagtt	agtaatttct	tgcttatttg	aaaaatagag	aacatttaac	1260
atgtattttt	aaataggcac	gacctgcta	ctgaacttta	tgaatgctt	tggaatctta	1320
t						1321

<210> 313

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> LBFDAU Locus 2_Forward primer

eolf-seq1

<400> 313
cactgagcat ggtgcttaaa cac 23

<210> 314
<211> 29
<212> DNA
<213> Artificial sequence

<220>
<223> LBFDAU Locus 2_Reverse primer

<400> 314
agagcgagag agaggaagta ggtatataa 29

<210> 315
<211> 19
<212> DNA
<213> Artificial sequence

<220>
<223> LBFDAU locus 2_Probe

<400> 315
ctggtgagtt ctagtactt 19

<210> 316
<211> 19
<212> DNA
<213> Artificial sequence

<220>
<223> Primer for determining zygosity of LBFLFK Locus 1

<400> 316
agaagtgtac gcgacgaga 19

<210> 317
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Primer for determining zygosity of LBFLFK Locus 1

<400> 317
tcaggagcga gaatgcgaaa g 21

<210> 318
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Primer for determining zygosity of LBFLFK Locus 2

<400> 318
acccatacat acgcataagt g 21

<210> 319
<211> 19
<212> DNA
<213> Artificial sequence

<220>
<223> Primer for determining zygosity of LBFLFK Locus 2

<400> 319
aatatatggg ctacattga 19

eolf-seql

<210> 320
 <211> 18
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer for determining zygosity of LBFDAU Locus 1

<400> 320
 ggcaggcgtg atcttatt 18

<210> 321
 <211> 21
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer for determining zygosity of LBFDAU Locus 1

<400> 321
 cataatttgc agtcgctgat t 21

<210> 322
 <211> 21
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer for determining zygosity of LBFDAU Locus 2

<400> 322
 agataacgat acatccacga a 21

<210> 323
 <211> 21
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer for determining zygosity of LBFDAU Locus 2

<400> 323
 cgaacataac agagcgagag a 21

<210> 324
 <211> 1197
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> d12Des(Ps) on LBFLFK Locus 1

<400> 324
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 aagcaaagac aattggctga ggctggatac actcacgttg aggggtgctcc tgctcctttg 120
 cctttggagt tgccctactt ctctctcaga gatctcagag ctgctattcc taagcactgc 180
 ttcgagagat ctttcgtgac ctccacctac tacatgatca agaacgtgtt gacttgcgct 240
 gctttgttat acgctgctac cttcattgat agagctggag ctgctgctta tgttttgtgg 300
 cctgtgtact ggttcttcca gggatcttac ttgactggag tgtgggttat cgctcacgag 360
 tgtggacacc aggcttattg ctcttctgag gtggtgaaca acttgattgg actcgtgttg 420
 cactctgctt tgttgggtgcc ttaccactct tggagaatct ctcacagaaa gcaccactcc 480
 aacactggat cttgcgagaa cgatgaggtt ttcgttcctg tgaccagatc tgtgttggct 540
 tcttcttgga acgagacctt ggaggattct cctctctacc aactctaccg tatcgtgtac 600
 atgttgggtt ttggatggat gcctggatac ctcttcttca acgctactgg acctactaag 660
 tactggggaa agtctaggtc tcacttcaac ctttactccg ctatctatgc tgatagggag 720
 aggtggatga tcgtgctctc cgatattttc tttgtggcta tgttggctgt tttggctgct 780
 ttggtgcaca ctttctcctt caacacgatg gtgaagtctt acgtggtgcc ttacttcatt 840

eolf-seql

gtgaacgctt	acttgggtgtt	gattacctac	ctccaacaca	ccgataccta	catccctcac	900
ttcagagagg	gagagtggaa	ttggttgaga	ggagctttgt	gcactgtgga	tagatcattt	960
ggtccattcc	tcgattctgt	ggtgcataga	atcgtggata	cccacgtttg	ccaccatata	1020
ttctccaaga	tgcctttcta	tcactgagag	gaggctacca	acgctattaa	gcctctcctc	1080
ggaaagtctt	acttgaagga	tactactcct	gttcctgttg	ctctctggag	atcttacacc	1140
cactgcaagt	tcgttgagga	tgatggaaag	gtgggtgttct	acaagaacaa	gttatag	1197

<210> 325

<211> 398

<212> PRT

<213> Artificial Sequence

<220>

<223> d12Des(Ps) on LBFLFK Locus 1

<400> 325

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Asp	Ser	Glu	Ala	Lys	Gln	Arg	Gln	Leu	Ala	Glu	Ala	Gly	Tyr	Thr	His
			20					25					30		
Val	Glu	Gly	Ala	Pro	Ala	Pro	Leu	Pro	Leu	Glu	Leu	Pro	His	Phe	Ser
			35				40					45			
Leu	Arg	Asp	Leu	Arg	Ala	Ala	Ile	Pro	Lys	His	Cys	Phe	Glu	Arg	Ser
			50			55					60				
Phe	Val	Thr	Ser	Thr	Tyr	Tyr	Met	Ile	Lys	Asn	Val	Leu	Thr	Cys	Ala
65					70					75					80
Ala	Leu	Leu	Tyr	Ala	Ala	Thr	Phe	Ile	Asp	Arg	Ala	Gly	Ala	Ala	Ala
				85					90					95	
Tyr	Val	Leu	Trp	Pro	Val	Tyr	Trp	Phe	Phe	Gln	Gly	Ser	Tyr	Leu	Thr
			100					105					110		
Gly	Val	Trp	Val	Ile	Ala	His	Glu	Cys	Gly	His	Gln	Ala	Tyr	Cys	Ser
			115				120					125			
Ser	Glu	Val	Val	Asn	Asn	Leu	Ile	Gly	Leu	Val	Leu	His	Ser	Ala	Leu
			130			135					140				
Leu	Val	Pro	Tyr	His	Ser	Trp	Arg	Ile	Ser	His	Arg	Lys	His	His	Ser
145					150					155					160
Asn	Thr	Gly	Ser	Cys	Glu	Asn	Asp	Glu	Val	Phe	Val	Pro	Val	Thr	Arg
				165					170					175	
Ser	Val	Leu	Ala	Ser	Ser	Trp	Asn	Glu	Thr	Leu	Glu	Asp	Ser	Pro	Leu
			180					185					190		
Tyr	Gln	Leu	Tyr	Arg	Ile	Val	Tyr	Met	Leu	Val	Val	Gly	Trp	Met	Pro
			195				200					205			
Gly	Tyr	Leu	Phe	Phe	Asn	Ala	Thr	Gly	Pro	Thr	Lys	Tyr	Trp	Gly	Lys
					215						220				
Ser	Arg	Ser	His	Phe	Asn	Pro	Tyr	Ser	Ala	Ile	Tyr	Ala	Asp	Arg	Glu
225					230					235					240
Arg	Trp	Met	Ile	Val	Leu	Ser	Asp	Ile	Phe	Leu	Val	Ala	Met	Leu	Ala
				245					250					255	
Val	Leu	Ala	Ala	Leu	Val	His	Thr	Phe	Ser	Phe	Asn	Thr	Met	Val	Lys
			260					265					270		
Phe	Tyr	Val	Val	Pro	Tyr	Phe	Ile	Val	Asn	Ala	Tyr	Leu	Val	Leu	Ile
			275				280					285			
Thr	Tyr	Leu	Gln	His	Thr	Asp	Thr	Tyr	Ile	Pro	His	Phe	Arg	Glu	Gly
			290			295					300				
Glu	Trp	Asn	Trp	Leu	Arg	Gly	Ala	Leu	Cys	Thr	Val	Asp	Arg	Ser	Phe
305					310					315					320
Gly	Pro	Phe	Leu	Asp	Ser	Val	Val	His	Arg	Ile	Val	Asp	Thr	His	Val
				325					330					335	
Cys	His	His	Ile	Phe	Ser	Lys	Met	Pro	Phe	Tyr	His	Cys	Glu	Glu	Ala
			340					345					350		
Thr	Asn	Ala	Ile	Lys	Pro	Leu	Leu	Gly	Lys	Phe	Tyr	Leu	Lys	Asp	Thr
			355				360					365			
Thr	Pro	Val	Pro	Val	Ala	Leu	Trp	Arg	Ser	Tyr	Thr	His	Cys	Lys	Phe
			370			375					380				
Val	Glu	Asp	Asp	Gly	Lys	Val	Val	Phe	Tyr	Lys	Asn	Lys	Leu		
385					390					395					

<210> 326

eolf-seql

<211> 1338
<212> DNA
<213> Artificial Sequence

<220>
<223> d4Des(Pl) on LBFLFK Locus 2

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<400> 326
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tacgacgcta aggccttttag ggacgagcac cctgggtggtg ctcacttcgt tagccttttc   180
ggaggtaggg acgctactga ggcttttatg gaatatcacc gtagagcttg gcctaaggct   240
aggatgtcta agttcttcgt tggttcactt gacgctagcg agaagcctac tcaagctgat   300
tcatcttacc ttagactttg cgctgaggtt aacgctcttt tgcctaaggg tagcggagga   360
ttcgctcctc ctactactg gcttaaggct gctgctcttg ttgttgctgc tgttagtata   420
gaggggtata tgctccttag gggtaagacc cttttgctta gcgttttcct tggactcgtg   480
ttcgcttgga taggacttaa tattcagcac gacgctaate acggtgctct tagtagacac   540
tcagtgatta actactgcct cggttacgct caggattgga taggtggtaa tatggtgctt   600
tggcttcaag agcacgttgt gatgcaccac ctccacacta acgacgttga cgctgacctt   660
gatcaaaagg ctacaggtgt tcttagactt aagcctactg acggttggat gccttggcac   720
gcacttcaac aactctatat ccttcctggt gaggctatgt acgcttttaa gcttcttttc   780
ttggacgccc ttgagcttct tgcttgaggg tgggaggggtg agaagattag ccctcttgct   840
agagctttgt tcgctcctgc tgttgcttgt aagcttggat tctgggctag attcggtgct   900
ctccctctct ggcttcaacc tactgttcac actgctttgt gtatctgtgc tactgtgtgt   960
actggtagct tctacctgcg cttcttcttc tttatctctc acaacttcga cgggtgttgg   1020
agcgttggac ctaagggatc acttcctaga tcagctactt tcgttcaacg tcaggttgag   1080
actagctcta acgttgggtg ttactggcct ggagttctta acggtggact taactttcag   1140
atagagcacc acttggtccc taggcttcac cactcttact acgctcaaata agctcctgtg   1200
gtaggactc acatagagaa gctcgggttt aagtagcgtc acttccctac cggttgatct   1260
aaccttagct caatgcttca gcatatgggt aagatgggaa ctagacctgg tgctgagaag   1320
ggtggttaagg ctgagtag                                     1338
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<210> 327
<211> 445
<212> PRT
<213> Artificial Sequence

<220>
<223> d4Des(Pl) on LBFLFK Locus 2

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<400> 327
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Ala Glu Val Ala Ser Tyr Thr Arg Lys Ala Val Asp Glu Arg Pro Asp
20     25     30
Leu Thr Ile Val Gly Asp Ala Val Tyr Asp Ala Lys Ala Phe Arg Asp
35     40     45
Glu His Pro Gly Gly Ala His Phe Val Ser Leu Phe Gly Gly Arg Asp
50     55     60
Ala Thr Glu Ala Phe Met Glu Tyr His Arg Arg Ala Trp Pro Lys Ala
65     70     75     80
Arg Met Ser Lys Phe Phe Val Gly Ser Leu Asp Ala Ser Glu Lys Pro
85     90     95
Thr Gln Ala Asp Ser Ser Tyr Leu Arg Leu Cys Ala Glu Val Asn Ala
100    105    110
Leu Leu Pro Lys Gly Ser Gly Gly Phe Ala Pro Pro Ser Tyr Trp Leu
115    120    125
Lys Ala Ala Ala Leu Val Val Ala Ala Val Ser Ile Glu Gly Tyr Met
130    135    140
Leu Leu Arg Gly Lys Thr Leu Leu Leu Ser Val Phe Leu Gly Leu Val
145    150    155    160
Phe Ala Trp Ile Gly Leu Asn Ile Gln His Asp Ala Asn His Gly Ala
165    170    175
Leu Ser Arg His Ser Val Ile Asn Tyr Cys Leu Gly Tyr Ala Gln Asp
180    185    190
Trp Ile Gly Glu Asn Met Val Leu Trp Leu Gln Glu His Val Val Met
195    200    205
His His Leu His Thr Asn Asp Val Asp Ala Asp Pro Asp Gln Lys Ala
```

eolf-seql

210	His	Gly	Val	Leu	Arg	Leu	215	Lys	Pro	Thr	Asp	Gly	220	Trp	Met	Pro	Trp	His		
225	Ala	Leu	Gln	Gln	Leu	230	Tyr	Ile	Leu	Pro	Gly	Glu	235	Ala	Met	Tyr	Ala	240	Phe	
					245	Leu	Asp	Ala	Leu	Glu	250	Leu	Leu	Ala	Trp	Arg	255	Trp	Glu	
				260	Ser	Pro	Leu	Ala	Arg	Ala	Leu	Phe	Ala	270	Pro	Ala	Val			
			275	Ile	Gly	Phe	Trp	Ala	Arg	Phe	Val	Ala	Leu	285	Pro	Leu	Trp			
			290	Cys	Lys	Leu	Gly	Phe	Trp	Ala	Arg	Phe	Val	300	Cys	Ala	Thr	Val	Cys	
			305	Gln	Pro	Thr	Val	His	Thr	Ala	Leu	Cys	Ile	315	Cys	Ala	Thr	Val	Cys	
			320	Gly	Ser	Phe	Tyr	Leu	Ala	Phe	Phe	Phe	Ile	330	Ile	Ser	His	Asn	320	Phe
			325	Gly	Ser	Val	Gly	Pro	Lys	Gly	Ser	Leu	Pro	335	Arg	Ser	Ala			
			340	Val	Gln	Arg	Gln	Val	Glu	Thr	Ser	Ser	Asn	350	Val	Gly	Gly	Tyr		
			355	Val	Leu	Asn	Gly	Gly	Leu	Asn	Phe	Gln	Ile	365	Glu	His	His			
			370	Gly	Val	Leu	Asn	Gly	Gly	Leu	Asn	Phe	Gln	380	Ile	Ala	Pro	Val		
			385	Pro	Arg	Leu	His	His	Ser	Tyr	Tyr	Ala	Gln	395	Ile	Ala	Pro	Val		
			400	Arg	Thr	His	Ile	Glu	Lys	Leu	Gly	Phe	Lys	410	Tyr	Arg	His	Phe	Pro	
			405	Thr	Val	Gly	Ser	Asn	Leu	Ser	Ser	Met	Leu	415	Gln	His	Met	Gly	Lys	Met
			420	Arg	Pro	Gly	Ala	Glu	Lys	Gly	Gly	Lys	Ala	430	Glu					
			435						440					445						

<210> 328
 <211> 398
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Consensus

<220>
 <221> misc_feature
 <222> (94)..(94)
 <223> Xaa can be any proteinogenic amino acid

<220>
 <221> misc_feature
 <222> (186)..(186)
 <223> Xaa can be any proteinogenic amino acid

<400> 328
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 20 25 30
 Val Glu Gly Ala Pro Ala Pro Leu Asp Leu Pro His Phe Ser
 35 40 45
 Leu Arg Asp Leu Arg Ala Ala Ile Pro Lys His Cys Phe Glu Arg Ser
 50 55 60
 Phe Ile Thr Ser Thr Tyr Tyr Met Ile Lys Asn Leu Leu Thr Cys Ala
 65 70 75 80
 Ala Leu Phe Tyr Ala Ala Thr Tyr Ile Asp Gln Thr Gly Xaa Phe Ala
 85 90 95
 Tyr Leu Leu Trp Pro Val Tyr Trp Phe Phe Gln Gly Ser Tyr Leu Thr
 100 105 110
 Gly Ile Trp Val Ile Ala His Glu Cys Gly His Gln Ala Tyr Cys Ser
 115 120 125
 Ser Glu Val Val Asn Asn Leu Ile Gly Leu Val Leu His Ser Ala Leu
 130 135 140
 Leu Val Pro Tyr His Ser Trp Arg Ile Ser His Arg Lys His His Ser

eolf-seql

145	Asn	Thr	Gly	Ser	Cys	150	Glu	Asn	Asp	Glu	Val	155	Phe	Val	Pro	Val	Thr	160	Arg
					165							170					175		
	Ser	Val	Ile	Ala	Ser	Ser	Trp	Asp	Glu	Xaa	Leu	Glu	Asp	Ser	Pro	Leu			
				180						185					190				
	Tyr	Gln	Leu	Tyr	Arg	Ile	Val	Tyr	Met	Leu	Val	Val	Gly	Trp	Met	Pro			
			195					200					205						
	Gly	Tyr	Leu	Phe	Phe	Asn	Ala	Thr	Gly	Pro	Thr	Lys	Tyr	Trp	Gly	Lys			
		210					215					220							
	Ser	Arg	Ser	His	Phe	Asn	Pro	Tyr	Ser	Ala	Ile	Tyr	Ser	Asp	Arg	Glu			
		225				230					235					240			
	Arg	Trp	Met	Ile	Val	Leu	Ser	Asp	Ile	Phe	Leu	Val	Leu	Met	Ile	Gly			
				245						250					255				
	Val	Leu	Ala	Thr	Leu	Val	Tyr	Thr	Phe	Ser	Phe	Tyr	Thr	Met	Leu	Lys			
			260						265					270					
	Phe	Tyr	Val	Val	Pro	Tyr	Phe	Ile	Val	Asn	Ala	Tyr	Leu	Val	Leu	Ile			
		275						280					285						
	Thr	Tyr	Leu	Gln	His	Thr	Asp	Thr	Tyr	Ile	Pro	His	Phe	Arg	Asp	Ser			
		290					295					300							
	Glu	Trp	Asn	Trp	Leu	Arg	Gly	Ala	Leu	Cys	Thr	Val	Asp	Arg	Ser	Phe			
		305				310					315					320			
	Gly	Pro	Tyr	Leu	Asp	Ser	Val	Val	His	Arg	Ile	Val	Asp	Thr	His	Val			
				325						330					335				
	Cys	His	His	Ile	Phe	Ser	Lys	Met	Pro	Phe	Tyr	His	Cys	Glu	Glu	Ala			
			340						345					350					
	Thr	Asn	Ala	Ile	Lys	Pro	Leu	Leu	Gly	Lys	Phe	Tyr	Leu	Lys	Asp	Asp			
		355						360					365						
	Thr	Pro	Val	Pro	Ile	Ala	Leu	Trp	Arg	Ser	Tyr	Thr	His	Cys	Lys	Phe			
		370				375						380							
	Val	Glu	Asp	Asp	Gly	Lys	Ile	Val	Phe	Tyr	Lys	Asn	Lys	Leu					
		385				390					395								

<210> 329

<211> 398

<212> PRT

<213> Phytophthora sojae

<220>

<223> G5A275_PHYSP-Phytophthora_sojae

<400> 329

Met	Ala	Ile	Leu	Asn	Pro	Glu	Ala	Asp	Ser	Ala	Ala	Asn	Leu	Ala	Thr
1				5					10				15		
Asp	Ser	Glu	Ala	Lys	Gln	Arg	Gln	Leu	Ala	Glu	Ala	Gly	Tyr	Thr	His
			20					25				30			
Val	Glu	Gly	Ala	Pro	Ala	Pro	Leu	Pro	Leu	Glu	Leu	Pro	His	Phe	Ser
		35					40					45			
Leu	Arg	Asp	Leu	Arg	Ala	Ala	Ile	Pro	Lys	His	Cys	Phe	Glu	Arg	Ser
	50					55					60				
Phe	Val	Thr	Ser	Thr	Tyr	Tyr	Met	Ile	Lys	Asn	Val	Leu	Thr	Cys	Ala
				70						75					80
Ala	Leu	Phe	Tyr	Ala	Ala	Thr	Phe	Ile	Asp	Arg	Ala	Gly	Ala	Ala	Ala
				85					90					95	
Tyr	Val	Leu	Trp	Pro	Val	Tyr	Trp	Phe	Gln	Gly	Ser	Tyr	Leu	Thr	
		100						105				110			
Gly	Val	Trp	Val	Ile	Ala	His	Glu	Cys	Gly	His	Gln	Ala	Tyr	Cys	Ser
		115					120					125			
Ser	Glu	Val	Val	Asn	Asn	Leu	Ile	Gly	Leu	Val	Leu	His	Ser	Ala	Leu
		130				135					140				
Leu	Val	Pro	Tyr	His	Ser	Trp	Arg	Ile	Ser	His	Arg	Lys	His	His	Ser
				150						155					160
Asn	Thr	Gly	Ser	Cys	Glu	Asn	Asp	Glu	Val	Phe	Val	Pro	Val	Thr	Arg
				165					170					175	
Ser	Val	Leu	Ala	Ser	Ser	Trp	Asn	Glu	Thr	Leu	Glu	Asp	Ser	Pro	Leu
			180					185					190		
Tyr	Gln	Leu	Tyr	Arg	Ile	Val	Tyr	Met	Leu	Val	Val	Gly	Trp	Met	Pro
		195					200					205			
Gly	Tyr	Leu	Phe	Phe	Asn	Ala	Thr	Gly	Pro	Thr	Lys	Tyr	Trp	Gly	Lys

eolf-seql

210	Ser	Arg	Ser	His	Phe	Asn	Pro	Tyr	Ser	Ala	Ile	Tyr	Ala	Asp	Arg	Glu
225	Arg	Trp	Met	Ile	Val	Leu	Ser	Asp	Ile	Phe	Leu	Val	Ala	Met	Leu	Ala
	Val	Leu	Ala	Ala	Leu	Val	His	Thr	Phe	Ser	Phe	Asn	Thr	Met	Val	Lys
	Phe	Tyr	Val	Val	Pro	Tyr	Phe	Ile	Val	Asn	Ala	Tyr	Leu	Val	Leu	Ile
	Thr	Tyr	Leu	Gln	His	Thr	Asp	Thr	Tyr	Ile	Pro	His	Phe	Arg	Glu	Gly
	Glu	Trp	Asn	Trp	Leu	Arg	Gly	Ala	Leu	Cys	Thr	Val	Asp	Arg	Ser	Phe
305	Gly	Pro	Phe	Leu	Asp	Ser	Val	Val	His	Arg	Ile	Val	Asp	Thr	His	Val
	Cys	His	His	Ile	Phe	Ser	Lys	Met	Pro	Phe	Tyr	His	Cys	Glu	Glu	Ala
	Thr	Asn	Ala	Ile	Lys	Pro	Leu	Leu	Gly	Lys	Phe	Tyr	Leu	Lys	Asp	Thr
	Thr	Pro	Val	Pro	Val	Ala	Leu	Trp	Arg	Ser	Tyr	Thr	His	Cys	Lys	Phe
385	Val	Glu	Asp	Asp	Gly	Lys	Val	Val	Phe	Tyr	Lys	Asn	Lys	Leu		

<210> 330

<211> 398

<212> PRT

<213> Phytophthora ramorum

<220>

<223> H3G9L1_PHYRM-Phytophthora ramorum

<400> 330

Met	Ala	Ile	Leu	Asn	Pro	Glu	Ala	Asp	Ser	Ala	Ala	Lys	Val	Ala	Ser
1	Asp	Ser	Glu	Ala	Lys	Gln	Arg	Gln	Leu	Ala	Glu	Ala	Gly	Tyr	Lys
	Val	Glu	Gly	Ala	Pro	Ala	Pro	Leu	Pro	Leu	Glu	Leu	Pro	His	Phe
	Leu	Arg	Glu	Leu	Arg	Ala	Ala	Ile	Pro	Lys	His	Cys	Phe	Glu	Arg
	Phe	Val	Thr	Ser	Thr	Tyr	Tyr	Ala	Ile	Lys	Asn	Met	Leu	Thr	Cys
65	Ala	Leu	Phe	Tyr	Ala	Ala	Ser	Tyr	Ile	Asp	Gln	Thr	Gly	Ala	Ala
	Tyr	Leu	Leu	Trp	Pro	Val	Tyr	Trp	Phe	Phe	Gln	Gly	Ser	Tyr	Leu
	Gly	Ile	Trp	Val	Val	Ala	His	Glu	Cys	Gly	His	Gln	Ala	Tyr	Cys
	Ser	Glu	Val	Val	Asn	Asn	Ile	Ile	Gly	Leu	Val	Leu	His	Ser	Ala
	Leu	Val	Pro	Tyr	His	Ser	Trp	Arg	Ile	Ser	His	Arg	Lys	His	His
145	Asn	Thr	Gly	Ser	Cys	Glu	Asn	Asp	Glu	Val	Phe	Val	Pro	Val	Thr
	Ser	Val	Ala	Ala	Ser	Ser	Trp	Asp	Glu	Thr	Leu	Glu	Asp	Ser	Pro
	Tyr	Gln	Leu	Tyr	Arg	Ile	Val	Tyr	Met	Leu	Val	Val	Gly	Trp	Met
	Gly	Tyr	Leu	Phe	Phe	Asn	Ala	Thr	Gly	Pro	Thr	Lys	Tyr	Trp	Gly
	Pro	Arg	Ser	His	Phe	Asn	Pro	Tyr	Ser	Ala	Ile	Tyr	Ser	Asp	Arg
225	Arg	Trp	Met	Ile	Val	Leu	Ser	Asp	Ile	Phe	Leu	Ile	Val	Ala	Leu
	Thr	Leu	Ala	Val	Leu	Val	His	Thr	Phe	Ser	Leu	Phe	Thr	Met	Val
	Phe	Tyr	Val	Val	Pro	Tyr	Phe	Val	Val	Asn	Ala	Tyr	Leu	Val	Leu

eolf-seql

Thr	Phe	275	Gln	His	Thr	Asp	280	Thr	Tyr	Ile	Pro	His	285	Phe	Arg	Glu	Gly
	290	Leu				295						300					
Glu	Trp	Ser	Trp	Leu	Arg	Gly	Ala	Leu	Cys	Thr	Val	Asp	Arg	Ser	Phe		
305					310					315					320		
Gly	Lys	Tyr	Leu	Asp	Ser	Val	Val	His	Arg	Ile	Val	Asp	Thr	His	Val		
				325					330					335			
Cys	His	His	Ile	Phe	Ser	Lys	Met	Pro	Phe	Tyr	His	Cys	Glu	Glu	Ala		
			340					345					350				
Thr	Asn	Ala	Ile	Lys	Pro	Leu	Leu	Gly	Lys	Phe	Tyr	Leu	Lys	Asp	Thr		
		355					360					365					
Thr	Pro	Val	Pro	Met	Ala	Leu	Trp	Arg	Ser	Tyr	Thr	Tyr	Cys	Lys	Phe		
	370					375					380						
Val	Glu	Asp	Asp	Gly	Asn	Val	Val	Phe	Tyr	Lys	Asn	Lys	Leu				
385					390					395							

<210> 331

<211> 398

<212> PRT

<213> Phytophthora infestans

<220>

<223> G4XUM4_PHYIN-Phytophthora_infestans

<400> 331

Met	Ala	Ile	Leu	Asn	Pro	Glu	Ala	Glu	Ser	Ala	Ala	Lys	Val	Ala	Thr		
1				5				10					15				
Leu	Ser	Glu	Ala	Lys	Gln	Arg	Glu	Leu	Ala	Glu	Ala	Gly	Tyr	Lys	His		
		20					25					30					
Val	Glu	Gly	Ala	Pro	Ala	Pro	Leu	Pro	Leu	Asp	Leu	Pro	His	Phe	Ser		
		35				40					45						
Leu	Arg	Asp	Leu	Arg	Ala	Ala	Ile	Pro	Lys	His	Cys	Phe	Glu	Arg	Ser		
	50				55					60							
Phe	Ile	Thr	Ser	Thr	Tyr	Tyr	Met	Ile	Lys	Asn	Leu	Leu	Thr	Cys	Ala		
65				70				75						80			
Ala	Leu	Phe	Tyr	Ala	Ala	Thr	Tyr	Ile	Asp	Gln	Thr	Gly	Ser	Phe	Ala		
				85				90					95				
Tyr	Leu	Leu	Trp	Pro	Val	Tyr	Trp	Phe	Phe	Gln	Gly	Ser	Tyr	Leu	Thr		
		100					105					110					
Gly	Ile	Trp	Val	Val	Ala	His	Glu	Cys	Gly	His	Gln	Ala	Tyr	Cys	Ser		
		115				120					125						
Ser	Glu	Val	Val	Asn	Asn	Met	Ile	Gly	Leu	Val	Leu	His	Ser	Thr	Leu		
	130				135					140							
Leu	Val	Pro	Tyr	His	Ser	Trp	Arg	Ile	Ser	His	Arg	Lys	His	His	Ser		
145				150				155						160			
Asn	Thr	Gly	Ser	Cys	Glu	Asn	Asp	Glu	Val	Phe	Val	Pro	Val	Thr	Arg		
				165				170					175				
Ser	Val	Ile	Ala	Ser	Ser	Trp	Asp	Glu	Thr	Leu	Glu	Asp	Ser	Pro	Leu		
		180					185					190					
Tyr	Gln	Leu	Tyr	Arg	Ile	Val	Tyr	Met	Leu	Val	Val	Gly	Trp	Met	Pro		
	195					200						205					
Gly	Tyr	Leu	Phe	Phe	Asn	Ala	Thr	Gly	Pro	Ser	Lys	Tyr	Leu	Gly	Lys		
	210				215					220							
Ser	Arg	Ser	His	Phe	Asn	Pro	Tyr	Ser	Ser	Ile	Tyr	Ser	Asp	Arg	Glu		
225					230				235						240		
Arg	Trp	Met	Ile	Val	Leu	Ser	Asp	Ile	Phe	Leu	Val	Leu	Met	Ile	Gly		
				245				250					255				
Ala	Leu	Val	Thr	Leu	Val	Tyr	Thr	Phe	Ser	Phe	Tyr	Thr	Met	Leu	Lys		
		260					265					270					
Phe	Tyr	Val	Val	Pro	Tyr	Phe	Ile	Val	Asn	Ala	Tyr	Leu	Val	Leu	Ile		
	275					280					285						
Thr	Tyr	Leu	Gln	His	Thr	Asp	Thr	Tyr	Ile	Pro	His	Phe	Arg	Asp	Ser		
	290				295					300							
Glu	Trp	Asn	Trp	Leu	Arg	Gly	Ala	Leu	Cys	Thr	Val	Asp	Arg	Ser	Phe		
305					310				315					320			
Gly	Pro	Tyr	Leu	Asp	Ser	Val	Val	His	Arg	Ile	Val	Asp	Thr	His	Ile		
				325				330					335				
Cys	His	His	Ile	Phe	Ser	Lys	Met	Pro	Phe	Tyr	His	Cys	Glu	Glu	Ala		

eofl-seql

Thr Asn Ala 340 Lys Pro Leu Leu 345 Gly Lys Phe Tyr Leu 350 Lys Asp Asp
 Thr Pro Val 355 Pro Ile Ala Leu 360 Trp Arg Ser Tyr Ile 365 His Cys Lys Phe
 Val 370 Glu Asp Asp Gly Lys 375 Ile Val Phe Tyr Lys 380 Asn Lys Leu
 385 390 395

<210> 332

<211> 398

<212> PRT

<213> Hyaloperonospora arabidopsidis

<220>

<223> M4BXW8_HYAAE-Hyaloperonospora_arabidopsidis

<400> 332

Met Ala Ile Leu Asn Pro Asp Ser Glu Ser Pro Thr Lys Gln Val Ala
 1 5 10 15
 Asp Ser Glu Ala Lys Gln Arg Gln Leu 20 25 30 35 40 45
 Val Glu Gly Cys Pro Ala Pro Leu Pro Leu Glu Leu Pro His Phe Ser
 50 55 60
 Leu Arg Asp Leu Arg Ala Ala Thr Pro Glu His Cys Phe Glu Arg Ser
 65 70 75 80
 Phe Val Thr Ser Met Tyr His Leu Val Lys Asn Leu Leu Tyr Cys Gly
 85 90 95
 Ala Leu Leu Tyr Val Ala Thr Arg Leu Asp Asp Leu Gly Ala Ile Ala
 Tyr Leu Leu Trp Pro Val Tyr Trp Phe Phe Gln Gly Ser Tyr Leu Thr
 100 105 110
 Gly Val Trp Val Ile Ala His Glu Cys Gly His Gln Ser Phe Cys Ser
 115 120 125
 Ser Glu Thr Val Asn Asn Leu Ile Gly Leu Val Leu His Ser Ala Leu
 130 135 140
 Leu Val Pro Tyr His Ser Trp Arg Ile Ser His Arg Lys His His Ser
 145 150 155 160
 Asn Thr Gly Ser Cys Glu Asn Asp Glu Val Phe Val Pro Val Thr Arg
 165 170 175
 Ser Ala Ile Lys Ser Ser Trp Asn Glu Val Leu Glu Asp Ser Pro Leu
 180 185 190
 Tyr Gln Leu Tyr Arg Ile Ala Phe Met Leu Val Val Gly Trp Met Pro
 195 200 205
 Gly Tyr Leu Cys Phe Asn Ala Thr Gly Pro Ser Lys Tyr Trp Gly Lys
 210 215 220
 Ala Arg Ser His Phe Asn Pro Phe Ser Thr Leu Tyr Ala Asp Arg Glu
 225 230 235 240
 Arg Trp Leu Ile Val Leu Ser Asp Val Ser Leu Val Leu Val Leu Ala
 245 250 255
 Gly Leu Ala Gly Leu Val His Thr Leu Ser Leu Tyr Thr Met Val Lys
 260 265 270
 Leu Tyr Phe Ala Pro Tyr Leu Ile Val Asn Val Tyr Leu Val Leu Ile
 275 280 285
 Thr Phe Leu Gln His Thr Asp Thr Tyr Ile Pro His Phe Arg Glu Gly
 290 295 300
 Glu Trp Thr Trp Leu Arg Gly Ala Leu Cys Thr Val Asp Arg Ser Phe
 305 310 315 320
 Gly Pro Tyr Leu Asp Ser Val Leu His His Ile Val Asp Thr His Val
 325 330 335
 Val His His Ile Phe Ser Lys Met Pro Phe Tyr His Cys Glu Glu Ala
 340 345 350
 Thr Lys Ala Ile Lys Pro Leu Leu Gly Lys Phe Tyr Leu Lys Asp Thr
 355 360 365
 Thr Pro Phe Pro Leu Ala Met Trp Arg Ser Tyr Lys His Cys Lys Phe
 370 375 380
 Val Glu Asp Asp Gly Lys Ile Val Phe Tyr Lys Asn Lys Leu
 385 390 395

eolf-seql

<210> 333
 <211> 398
 <212> PRT
 <213> Phytophthora parasitica

<220>
 <223> W2PDL4_PHYPN-Phytophthora_parasitica

<400> 333
 Met Ala Ile Leu Asn Pro Glu Ala Glu Ser Ala Ala Lys Val Ala Ser
 1 5 10 15
 Leu Ser Glu Ala Lys Gln Arg Glu Leu Ala Glu Ala Gly Tyr Lys His
 20 25 30
 Val Glu Gly Ala Pro Ala Pro Leu Pro Leu Asp Leu Pro His Phe Ser
 35 40 45
 Leu Arg Asp Leu Arg Ala Ala Ile Pro Lys His Cys Phe Glu Arg Ser
 50 55 60
 Phe Ile Thr Ser Thr Tyr Tyr Met Ile Lys Asn Leu Leu Thr Cys Ala
 65 70 75 80
 Ala Leu Phe Tyr Ala Ala Thr Tyr Ile Asp Gln Thr Gly Asn Phe Ala
 85 90 95
 Tyr Leu Leu Trp Pro Val Tyr Trp Phe Phe Gln Gly Ser Tyr Leu Thr
 100 105 110
 Gly Ile Trp Val Ile Ala His Glu Cys Gly His Gln Ala Tyr Cys Ser
 115 120 125
 Ser Glu Val Val Asn Asn Leu Ile Gly Leu Val Leu His Ser Ala Leu
 130 135 140
 Leu Val Pro Tyr His Ser Trp Arg Ile Ser His Arg Lys His His Ser
 145 150 155 160
 Asn Thr Gly Ser Cys Glu Asn Asp Glu Val Phe Val Pro Val Thr Arg
 165 170 175
 Ser Val Ile Ala Ser Ser Trp Asp Glu Asn Leu Glu Asp Ser Pro Leu
 180 185 190
 Tyr Gln Leu Tyr Arg Ile Val Tyr Met Leu Val Val Gly Trp Met Pro
 195 200 205
 Gly Tyr Leu Phe Phe Asn Ala Thr Gly Pro Thr Lys Tyr Trp Gly Lys
 210 215 220
 Ser Arg Ser His Phe Asn Pro Tyr Ser Ala Ile Tyr Ser Asp Arg Glu
 225 230 235 240
 Arg Trp Met Ile Val Leu Ser Asp Ile Phe Leu Val Leu Val Ile Gly
 245 250 255
 Val Leu Ala Thr Leu Val Tyr Thr Phe Ser Phe Tyr Thr Met Leu Lys
 260 265 270
 Phe Tyr Val Val Pro Tyr Phe Ile Val Asn Ala Tyr Leu Val Leu Ile
 275 280 285
 Thr Tyr Leu Gln His Thr Asp Thr Tyr Ile Pro His Phe Arg Asp Ser
 290 295 300
 Glu Trp Asn Trp Leu Arg Gly Ala Leu Cys Thr Val Asp Arg Ser Phe
 305 310 315 320
 Gly Pro Tyr Leu Asp Ser Val Val His Arg Ile Val Asp Thr His Val
 325 330 335
 Cys His His Ile Phe Ser Lys Met Pro Phe Tyr His Cys Glu Glu Ala
 340 345 350
 Thr Asn Ala Ile Lys Pro Leu Leu Gly Lys Phe Tyr Leu Lys Asp Asp
 355 360 365
 Thr Pro Val Pro Ile Ala Leu Trp Arg Ser Tyr Thr His Cys Lys Phe
 370 375 380
 Val Glu Asp Asp Gly Lys Ile Val Phe Tyr Lys Asn Lys Leu
 385 390 395

<210> 334
 <211> 398
 <212> PRT
 <213> Phytophthora parasitica

<220>
 <223> W2LW72_PHYPR-Phytophthora_parasitica

eolf-seql

<400> 334

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Met Ala Ile Leu Asn Pro Glu Ala Glu Ser Ala Ala Lys Val Ala Ser
1      5      10      15
Leu Ser Glu Ala Lys Gln Arg Glu Leu Ala Glu Ala Gly Tyr Lys His
20      25      30
Val Glu Gly Ala Pro Ala Pro Leu Pro Leu Asp Leu Pro His Phe Ser
35      40      45
Leu Arg Asp Leu Arg Ala Ala Ile Pro Lys His Cys Phe Glu Arg Ser
50      55      60
Phe Ile Thr Ser Thr Tyr Tyr Met Ile Lys Asn Leu Leu Thr Cys Ala
65      70      75      80
Ala Leu Phe Tyr Ala Ala Thr Tyr Ile Asp Gln Thr Gly Asn Phe Ala
85      90      95
Tyr Leu Leu Trp Pro Val Tyr Trp Phe Gln Gly Ser Tyr Leu Thr
100     105     110
Gly Ile Trp Val Ile Ala His Glu Cys Gly His Gln Ala Tyr Cys Ser
115     120     125
Ser Glu Val Val Asn Asn Leu Ile Gly Leu Val Leu His Ser Ala Leu
130     135     140
Leu Val Pro Tyr His Ser Trp Arg Ile Ser His Arg Lys His His Ser
145     150     155     160
Asn Thr Gly Ser Cys Glu Asn Asp Glu Val Phe Val Pro Val Thr Arg
165     170     175
Ser Val Ile Ala Ser Ser Trp Asp Glu Asn Leu Glu Asp Ser Pro Leu
180     185     190
Tyr Gln Leu Tyr Arg Ile Val Tyr Met Leu Val Val Gly Trp Met Pro
195     200     205
Gly Tyr Leu Phe Phe Asn Ala Thr Gly Pro Thr Lys Tyr Trp Gly Lys
210     215     220
Ser Arg Ser His Phe Asn Pro Tyr Ser Ala Ile Tyr Ser Asp Arg Glu
225     230     235     240
Arg Trp Met Ile Val Leu Ser Asp Ile Phe Leu Val Leu Met Ile Gly
245     250     255
Val Leu Ala Thr Leu Val Tyr Thr Phe Ser Phe Tyr Thr Met Leu Lys
260     265     270
Phe Tyr Val Val Pro Tyr Phe Ile Val Asn Ala Tyr Leu Val Leu Ile
275     280     285
Thr Tyr Leu Gln His Thr Asp Thr Tyr Ile Pro His Phe Arg Asp Ser
290     295     300
Glu Trp Asn Trp Leu Arg Gly Ala Leu Cys Thr Val Asp Arg Ser Phe
305     310     315     320
Gly Pro Tyr Leu Asp Leu Val Val His Arg Ile Val Asp Thr His Val
325     330     335
Cys His His Ile Phe Ser Lys Met Pro Phe Tyr His Cys Glu Glu Ala
340     345     350
Thr Asn Ala Ile Lys Pro Leu Leu Gly Lys Phe Tyr Leu Lys Asp Asp
355     360     365
Thr Pro Val Pro Ile Ala Leu Trp Arg Ser Tyr Thr His Cys Lys Phe
370     375     380
Val Glu Asp Asp Gly Lys Ile Val Phe Tyr Lys Asn Lys Leu
385     390     395

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<210> 335

<211> 398

<212> PRT

<213> *Phytophthora parasitica*

<220>

<223> W2ZYI2_PHYPR-*Phytophthora parasitica*

<400> 335

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Met Ala Ile Leu Asn Pro Glu Ala Glu Ser Ala Ala Lys Val Ala Ser
1      5      10      15
Leu Ser Glu Ala Lys Gln Arg Glu Leu Ala Glu Ala Gly Tyr Lys His
20      25      30
Val Glu Gly Ala Pro Ala Pro Leu Pro Leu Asp Leu Pro His Phe Ser
35      40      45
Leu Arg Asp Leu Arg Ala Ala Ile Pro Lys His Cys Phe Glu Arg Ser

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eolf-seql

50	Phe	Ile	Thr	Ser	Thr	Tyr	55	Tyr	Met	Ile	Lys	Asn	60	Leu	Leu	Thr	Cys	Ala
65	Ala	Leu	Phe	Tyr	Ala	Ala	70	Thr	Tyr	Ile	Asp	Gln	75	Thr	Gly	Asn	Phe	Thr
					85	Pro	Val	Tyr	Trp	Phe	90	Phe	Gln	Gly	Ser	Tyr	Leu	Thr
					100					105						110		
					115					120						125		
					130					135						140		
					145					150						155		
					165					170						175		
					180					185						190		
					195					200						205		
					210					215						220		
					225					230						235		
					245					250						255		
					260					265						270		
					275					280						285		
					290					295						300		
					305					310						315		
					325					330						335		
					340					345						350		
					355					360						365		
					370					375						380		
					385					390						395		

<210> 336

<211> 393

<212> PRT

<213> Saprolegnia spp.

<220>

<223> Q6UB74_9STRA-Saprolegnia

<400> 336

Met	Cys	Lys	Gly	Gln	Ala	Pro	Ser	Lys	Ala	Asp	Val	Phe	His	Ala	Ala
1				5				10					15		
Gly	Tyr	Arg	Pro	Val	Ala	Gly	Thr	Pro	Glu	Pro	Leu	Pro	Leu	Glu	Pro
			20					25					30		
Pro	Thr	Ile	Thr	Leu	Lys	Asp	Leu	Arg	Ala	Ala	Ile	Pro	Ala	His	Cys
			35				40					45			
Phe	Glu	Arg	Ser	Ala	Ala	Thr	Ser	Phe	Tyr	His	Leu	Ala	Lys	Asn	Leu
			50			55					60				
Ala	Ile	Cys	Ala	Gly	Val	Phe	Ala	Val	Gly	Leu	Lys	Leu	Ala	Ala	Ala
65				70						75				80	
Asp	Leu	Pro	Leu	Ala	Ala	Lys	Leu	Val	Ala	Trp	Pro	Ile	Tyr	Trp	Phe
				85					90					95	
Val	Gln	Gly	Thr	Tyr	Phe	Thr	Gly	Ile	Trp	Val	Ile	Ala	His	Glu	Cys
			100					105					110		
Gly	His	Gln	Ala	Phe	Ser	Ala	Ser	Glu	Ile	Leu	Asn	Asp	Thr	Val	Gly

eolf-seql

Ile	Ile	115	Leu	His	Ser	Leu	Leu	120	Phe	Val	Pro	Tyr	His	125	Ser	Trp	Lys	Ile
	130						135						140					
Thr	His	Arg	Arg	His	His	Ser	Asn	Thr	Gly	Ser	Cys	Glu	Asn	Asp	Glu			
145					150					155								160
Val	Phe	Thr	Pro	Thr	Pro	Arg	Ser	Val	Val	Glu	Ala	Lys	His	Asp	His			
				165					170									
Ser	Leu	Leu	Glu	Glu	Ser	Pro	Leu	Tyr	Asn	Leu	Tyr	Gly	Ile	Val	Met			
			180					185										
Met	Leu	Leu	Val	Gly	Trp	Met	Pro	Gly	Tyr	Leu	Phe	Phe	Asn	Ala	Thr			
		195					200					205						
Gly	Pro	Thr	Lys	Tyr	Ala	Gly	Leu	Ala	Lys	Ser	His	Phe	Asn	Pro	Tyr			
	210					215					220							
Ala	Ala	Phe	Phe	Leu	Pro	Lys	Glu	Arg	Leu	Ser	Ile	Trp	Trp	Ser	Asp			
225					230					235								
Leu	Cys	Phe	Leu	Ala	Ala	Leu	Tyr	Gly	Phe	Gly	Tyr	Gly	Val	Ser	Val			
				245					250									
Phe	Gly	Leu	Leu	Asp	Val	Ala	Arg	His	Tyr	Ile	Val	Pro	Tyr	Leu	Ile			
			260					265										
Cys	Asn	Ala	Tyr	Leu	Val	Leu	Ile	Thr	Tyr	Leu	Gln	His	Thr	Asp	Thr			
		275					280					285						
Tyr	Val	Pro	His	Phe	Arg	Gly	Asp	Glu	Trp	Asn	Trp	Leu	Arg	Gly	Ala			
	290					295					300							
Leu	Cys	Thr	Val	Asp	Arg	Ser	Phe	Gly	Ala	Trp	Ile	Asp	Ser	Ala	Ile			
305					310					315								
His	His	Ile	Ala	Asp	Thr	His	Val	Thr	His	His	Ile	Phe	Ser	Lys	Thr			
				325					330									
Pro	Phe	Tyr	His	Ala	Ile	Glu	Ala	Thr	Asp	Ala	Ile	Thr	Pro	Leu	Leu			
			340					345										
Gly	Lys	Tyr	Tyr	Leu	Ile	Asp	Pro	Thr	Pro	Ile	Pro	Leu	Ala	Leu	Trp			
		355					360											
Arg	Ser	Phe	Thr	His	Cys	Lys	Tyr	Val	Glu	Asp	Asp	Gly	Asn	Val	Val			
	370					375					380							
Phe	Tyr	Lys	Arg	Lys	Leu	Glu	Glu	Lys										
385					390													