



(86) Date de dépôt PCT/PCT Filing Date: 2011/03/25

(87) Date publication PCT/PCT Publication Date: 2011/09/29

(45) Date de délivrance/Issue Date: 2021/12/28

(85) Entrée phase nationale/National Entry: 2013/09/24

(86) N° demande PCT/PCT Application No.: US 2011/029966

(87) N° publication PCT/PCT Publication No.: 2011/119937

(30) Priorités/Priorities: 2010/03/26 (US61/318,273);
2010/05/19 (US61/346,410)

(51) Cl.Int./Int.Cl. *C12N 15/53* (2006.01),
A01H 1/00 (2006.01), *A01H 5/00* (2018.01),
A01H 6/20 (2018.01), *C12N 15/113* (2010.01),
C12N 15/52 (2006.01), *C12N 15/82* (2006.01),
C12N 5/04 (2006.01), *C12N 5/10* (2006.01),
C12N 9/00 (2006.01), *C12N 9/02* (2006.01),
C12P 7/64 (2006.01)

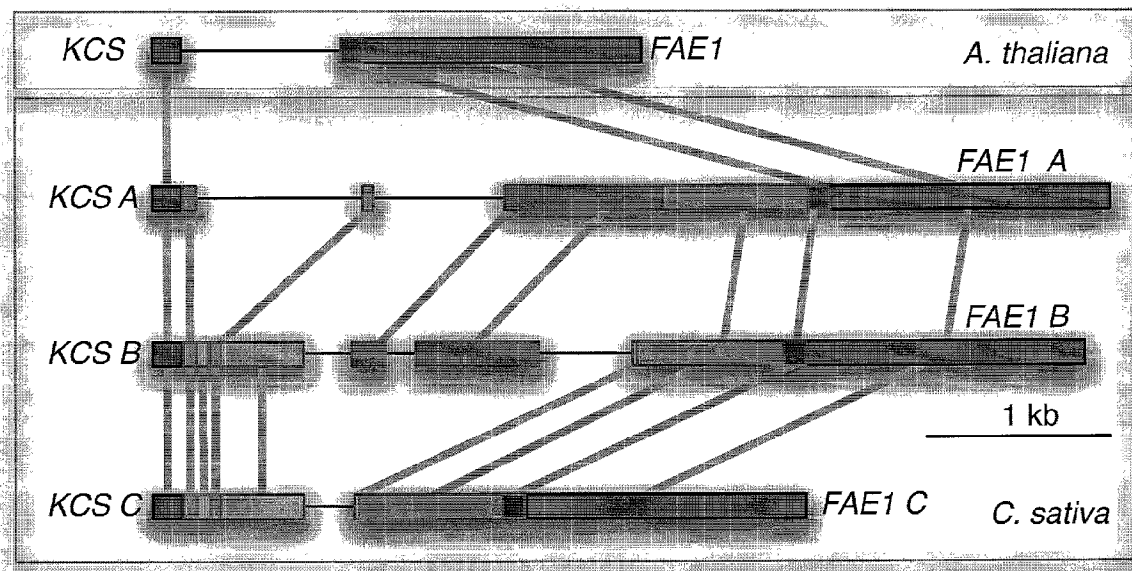
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(54) Titre : ISOLEMENT ET UTILISATION DE GENES FAD2 ET FAE1 DE CAMELINE

(54) Title: THE ISOLATION AND USE OF FAD2 AND FAE1 FROM CAMELINA



(57) Abrégé/Abstract:

The present invention provides isolated FAD2 and FAE1 genes and FAD2 and FAE1 protein sequences of Camelina species, e.g., Camelina sativa, mutations in Camelina FAD2 and FAE1 genes, and methods of using the same. In addition, methods of altering Camelina seed composition and/or improving Camelina seed oil quality are disclosed. Furthermore, methods of breeding Camelina cultivars and/or other closely related species to produce plants having altered or improved seed oil and/or meal quality are provided.

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

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
29 September 2011 (29.09.2011)(10) International Publication Number
WO 2011/119937 A1(51) International Patent Classification:
C07H 21/04 (2006.01) *C07H 21/02* (2006.01)(21) International Application Number:
PCT/US2011/029966(22) International Filing Date:
25 March 2011 (25.03.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/318,273 26 March 2010 (26.03.2010) US
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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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

Declarations under Rule 4.17:

— as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))

Published:

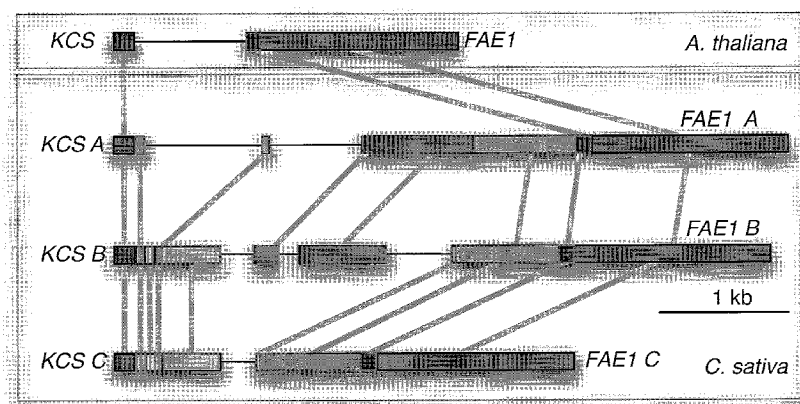
— with international search report (Art. 21(3))

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

— with sequence listing part of description (Rule 5.2(a))

(54) Title: THE ISOLATION AND USE OF FAD2 AND FAE1 FROM CAMELINA

FIGURE 4



(57) Abstract: The present invention provides isolated FAD2 and FAE1 genes and FAD2 and FAE1 protein sequences of Camelina species, e.g., Camelina sativa, mutations in Camelina FAD2 and FAE1 genes, and methods of using the same. In addition, methods of altering Camelina seed composition and/or improving Camelina seed oil quality are disclosed. Furthermore, methods of breeding Camelina cultivars and/or other closely related species to produce plants having altered or improved seed oil and/or meal quality are provided.

5 THE ISOLATION AND USE OF FAD2 AND FAE1 FROM CAMELINA

10

TECHNICAL FIELD

The invention relates to the identification, isolation and use of nucleic acid sequences,
15 including genes, and nucleic acid fragments encoding fatty acid desaturase enzymes and/or
fatty acid elongases, mutants thereof, and methods of altering lipid composition in *Camelina*
species, e.g., *Camelina sativa*.

BACKGROUND

20 The current concern about our global dependence on fossil fuels and the consequent
impact on climate change have brought biofuels to the forefront. This interest in biofuels has
prompted researchers to critically evaluate alternative feedstocks for biofuel production. One
important, emerging biofuel crop is *Camelina sativa* L. Cranz (Brassicaceae), commonly
referred to as “false flax” or “gold-of-pleasure”. Renewed interest in *C. sativa* as a biofuel
25 feedstock is due in part to its drought tolerance and minimal requirements for supplemental
nitrogen and other agricultural inputs (Putnam, Budin et al. 1993; Zubr 1997; Gehringer,
Friedt et al. 2006; Gugel and Falk 2006). Similar to other non-traditional, renewable oilseed
feedstocks such as *Jatropha curcas* L. (“jatropha”), *C. sativa* grows on marginal land. Unlike
jatropha, which is a tropical and subtropical shrub, *C. sativa* is native to Europe and is
30 naturalized in North America, where it grows well in the northern United States and southern
Canada.

In addition to its drought tolerance and broad distribution, several other aspects of *C.*
sativa biology make it well suited for development as an oilseed crop. First, *C. sativa* is a
member of the family Brassicaceae, and thus is a relative of both the genetic model organism
35 *Arabidopsis thaliana* and the oilseed crop *Brassica napus*. The close relationship between *C.*
sativa and *A. thaliana* (Al-Shehbaz, Beilstein et al. 2006; Beilstein, Al-Shehbaz et al. 2006;

5 Beilstein, Al-Shehbaz et al. 2008) makes the *A. thaliana* genome an ideal reference point for the development of genetic and genomic tools in *C. sativa*. Second, the oil content of *C. sativa* seeds is comparable to that of *B. napus*, ranging from 30 to 40% (w/w) (Budin, Breene et al. 1995; Gugel and Falk 2006), suggesting that agronomic lessons from the cultivation of *B. napus* are applicable to *C. sativa* cultivation. Finally, the properties of *C. sativa* biodiesel
10 are already well described (Rice, Frohlich et al. 1997; Frohlich and Rice 2005; Worgetter, Prankl et al. 2006), and both seed oil and biodiesel from *C. sativa* were used as fuel in engine trials with promising results (Bernardo, Howard-Hildige et al. 2003; Frohlich and Rice 2005).

The quality of a biodiesel, regardless of its source, is dependent upon the fatty acid methyl ester (FAME) composition, which affects cold flow and oxidative stability (Knothe
15 2005; Durrett, Benning et al. 2008). For example, saturated FAMES have poor cold flow properties since they can form crystals at lower temperatures, while the FAMES from polyunsaturated fatty acids remain in solution at colder temperatures, and thus have good cold flow properties (Stournas 1995; Serdari, Lois et al. 1999). In contrast, the relationship between saturation and oxidative stability is exactly opposite that of cold flow. Fatty acid
20 saturation is positively correlated with oxidative stability; saturated fatty acids have the best oxidative stability and fatty acids with 2 or greater double bonds have increasing oxidative instability (Knothe and Dunn 2003; Knothe 2005; Durrett, Benning et al. 2008). Additionally, polyunsaturated FAMES can result in increased NO_x emissions, e.g., NO, NO₂ et al (McCormick, Graboski et al. 2001), and thus affect the production of a monitored
25 pollutant. Very long chain fatty acids (VLCFA; as used herein, refers to those fatty acids containing greater than 18 carbons) result in a biodiesel with a high distillation temperature that does not meet existing standards (American Society for Testing and Materials, ASTM), reducing marketability. Given these trade-offs, an ideal biodiesel blend is high in oleic acid (18:1; carbons : double bonds), low in polyunsaturated FAMES, and with few long chain
30 FAMES. This blend is oxidatively stable, has a low cloud point, and meets biodiesel standards (ASTM ; Knothe 2005; Durrett, Benning et al. 2008).

The naturally occurring oil composition of *C. sativa* negatively affects its biodiesel properties. Polyunsaturated fatty acids such as linoleic (18:2) and alpha-linolenic (18:3) acids account for 52.1 – 54.7% of *C. sativa* seed oil (Ní Eidhin, Burke et al. 2003;
35 Abramovic and Abram 2005). This likely accounts for the low oxidative stability of *C. sativa* FAMES (Bernardo, Howard-Hildige et al. 2003). *C. sativa* seeds also contain 21.4 – 22.4% VLCFA, of which 11-eicosenoic acid (20:1) at 14.9-16.2% are especially abundant (Zubr 2002; Ní Eidhin, Burke et al. 2003; Abramovic and Abram 2005), likely resulting in the high

5 distillation temperature of the FAMES. Most desirable for biodiesel is oleic acid (18:1), which accounts for 14.0-17.4% of *C. sativa* seed oil (Budin, Breene et al. 1995; Zubr 2002; Ní Eidhin, Burke et al. 2003; Abramovic and Abram 2005). There is therefore the potential to optimize *Camelina* oil for biodiesel production by decreasing both the amount of polyunsaturated fatty acids being produced from oleic acid and decreasing the production of
10 fatty acids with chain length of 18 carbons or greater.

Genes affecting oil composition are well characterized in *Arabidopsis thaliana*, a close relative of *Camelina sativa*, as well as in some other plants. For example, oleic acid (18:1) is converted to linoleic acid (18:2) in the endoplasmic reticulum by the membrane bound delta-12-desaturase FATTY ACID DESATURASE 2 (FAD2). In *Arabidopsis fad2*
15 mutants, levels of 18:1 oleic acid in the seeds increase by a factor of 2- 3.4 while levels of 18:2 linoleic acids are decreased by a factor of 4-10 (Okuley, Lightner et al. 1994). Thus, mutations affecting FAD2 have been shown to lead to higher levels of oleic acid in *A. thaliana* and other studies have shown FAD2 has a similar role in crops such as canola (Hu, Sullivan-Gilbert et al. 2006), sunflower (Hongtrakul, Slabaugh et al. 1998) and peanut (Patel,
20 Jung et al. 2004).

Very long chain fatty acids are formed in the cytosol of *A. thaliana* by sequential addition of 2 carbon units to 18 carbon fatty acid CoA conjugates. The rate limiting step is thought to be initial condensation, catalyzed in the seed by FATTY ACID ELONGASE 1 (FAE1) (James Jr, Lim et al. 1995) (Kunst, Taylor et al. 1992). In wild type *Arabidopsis*,
25 approximately 25% of fatty acids in seeds are long chain fatty acids, while *fae1* mutants contain less than 1% long chain fatty acids. Interestingly, 18:1 content in seeds increases by a factor of 2 in *A. thaliana fae1* (Kunst, Taylor et al. 1992). In *Brassica napus*, reductions in long chain fatty acids, particularly erucic acid (22:1), are linked to changes in FAE1 activity (Han, Lühs et al. 2001; Katavic, Mietkiewska et al. 2002; Wang, Wang et al. 2008; Wu, Wu
30 et al. 2008).

The close relationship between *A. thaliana* and *C. sativa* suggests that FAD2 and FAE1 may play similar roles in both species, making these genes good targets for manipulation of oil composition in *C. sativa*. To our knowledge, *FAD2* and *FAE1* gene sequences have not been previously reported for *C. sativa*. Indeed, published studies
35 detailing the biology of *C. sativa* and its closest relatives in the genus *Camelina* are few. However, several important findings can be drawn from the literature. Taxonomic treatments describe 11 species in the genus with a center of diversity in Eurasia (Akeroyd J: *Camelina in Flora Europaea*. 2nd edn. Cambridge, UK: Cambridge University Press; 1993.) although *C.*

5 *sativa*, *C. rumelica*, *C. microcarpa*, and *C. alyssum* are naturalized weeds with broad distributions. *Camelina* species can be annual or biennial, with some species requiring vernalization to induce flowering (Mirek Z: Genus *Camelina* in Poland - Taxonomy, Distribution and Habitats. *Fragmenta Floristica et Geobotanica* 1981, 27:445-503.). Chromosome counts range from $n=6$ in *C. rumelica* (Brooks RE: Chromosome number reports LXXXVII *Taxon* 1985, 34:346-351; Baksay L: The chromosome numbers and cytotaxonomical relations of some European plant species. *Ann Hist-Nat Mus Natl Hung* 1957:169-174.) or $n=7$ in *C. hispida* (Maassoumi A: Cruciferes de la flore d'Iran: etude caryosystematique. Thesis. Strasbourg, France, 1980.), upwards to $n=20$ in *C. sativa*, *C. microcarpa*, and *C. alyssum* (Gehringer A, Friedt W, Luhs W, Snowdon RJ: Genetic mapping of agronomic traits in false flax (*Camelina sativa* subsp. *sativa*). *Genome* 2006, 49:1555-1563; Francis A, Warwick S: The Biology of Canadian Weeds. 142. *Camelina alyssum* (Mill.) Thell.; *C. microcarpa* Andr. ex DC.; *C. sativa* (L.) Crantz. *Canadian Journal of Plant Science* 2009, 89:791-810.). Some *Camelina* species are interfertile; crosses of *C. sativa* with *C. alyssum*, and *C. sativa* with *C. microcarpa*, produce viable seed (Tedin O: Vererbung, Variation und Systematik in der Gattung *Camelina*. *Hereditas* 1925, 6:19-386.). More recently, plastid simple sequence repeat (SSR) markers (Flannery ML, Mitchell FJ, Coyne S, Kavanagh TA, Burke JI, Salamin N, Dowding P, Hodgkinson TR: Plastid genome characterisation in Brassica and Brassicaceae using a new set of nine SSRs. *Theor Appl Genet* 2006, 113:1221-1231.) and randomly amplified polymorphic DNA (RAPD) markers have been reported and a mapping study using amplified fragment length polymorphisms (AFLP) has been published (Gehringer A, Friedt W, Luhs W, Snowdon RJ: Genetic mapping of agronomic traits in false flax (*Camelina sativa* subsp. *sativa*). *Genome* 2006, 49:1555-1563). Additionally, the sequences of a few *C. sativa* transcription factors are available from the literature (Martynov VV, Tsvetkov IL, Khavkin EE: Orthologs of arabidopsis CLAVATA 1 gene in cultivated Brassicaceae plants. *Ontogenez* 2004, 35:41-46.) and in GenBank.

As an oilseed crop in the Brassicaceae family, *Camelina sativa* has inspired renewed interest due to its potential for biofuels applications. Little is understood of the nature of the *C. sativa* genome, however. A study was undertaken by the present inventors to characterize two genes in the fatty acid biosynthesis pathway, *fatty acid desaturase (FAD) 2* and *fatty acid elongase (FAE) 1*.

5

SUMMARY OF THE INVENTION

Camelina sativa is a re-emerging oilseed with tremendous potential as an alternative biofuel crop and for which genomic information is becoming increasingly available. The inventors have characterized two genes encoding fatty acid biosynthesis enzymes and, in the process, have discovered unexpected complexity in the *C. sativa* genome.

10

The present inventors disclose herewith the sequences of three copies of both *FAE1* and *FAD2* recovered from *C. sativa*. Southern blots were used to determine whether the recovered copies are allelic or if they represent multiple loci. Moreover, the inventors performed phylogenetic analyses to infer the evolutionary history of the copies, and quantitative PCR (qPCR) to explore whether there is evidence of functional divergence among them. To better understand the *C. sativa* genome and to determine whether the multiple copies recovered are the result of polyploidization, the inventors also analyzed the genome sizes of *C. sativa* and its closest relatives in the genus *Camelina* by flow cytometry. Collectively the inventors' results indicate that *C. sativa* is an allohexaploid whose oil composition is likely influenced by more than one functional copy of *FAE1* and *FAD2*. This should allow highly specialized blends of oil to be produced from *C. sativa* with mutations in *FAE1* and *FAD2*, greatly increasing the utility of this emerging biofuel crop.

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The present inventors unexpectedly discovered by Southern analysis that in *C. sativa*, there are three copies of both *FAD2* and *FAE1* as well as *LFY*, a known single copy gene in other species. All three copies of both *FAD2* and *FAE1* are expressed in developing seeds, and sequence alignments show that previously described conserved sites are present, suggesting that all three copies of both genes could be functional. The regions downstream of *FAD2* and upstream of *FAE1* demonstrate co-linearity with the Arabidopsis genome. In addition, results from flow cytometry indicate that the DNA content of *C. sativa* is approximately three-fold that of diploid *Camelina* relatives. Phylogenetic analyses further support a history of duplication and indicate that *C. sativa* and *C. microcarpa* might share a parental genome. *FAD2* and *FAE1* sequences from species in the tribe of Camelinaeae have been deposited in Genbank at the NCBI [Genbank: GU929417 – GU929441, SEQ ID NOs: 1 to 6, and SEQ ID NOs 45-63, as listed below].

35

GenBank access #	Sequence Name	SEQ ID NO
GU929417	Camelina sativa FAD2 A (upstream, coding and downstream genomic sequence)	1
GU929418	Camelina sativa FAD2 B (upstream, coding and downstream genomic sequence)	2
GU929419	Camelina sativa FAD2 C (upstream, coding and downstream genomic sequence)	3
GU929420	Camelina sativa FAE1 A [upstream gene (KCS17), intergenic region and coding)	4
GU929421	Camelina sativa FAE1 B (upstream gene (KCS17), intergenic region and coding)	5
GU929422	Camelina sativa FAE1 C [upstream gene (KCS17), intergenic region and coding)	6
GU929423	Capsella rubella FAD2	45
GU929424	Arabidopsis lyrata FAD2	46
GU929425	Arabidopsis lyrata FAE1	47
GU929426	Camelina hispida FAD2	48
GU929427	Camelina hispida FAE1-1	49
GU929428	Camelina hispida FAE1-2	50
GU929429	Camelina laxa FAD2	51
GU929430	Camelina laxa FAE1-1	52
GU929431	Camelina laxa FAE1-2	53
GU929432	Camelina microcarpa FAD2 A	54
GU929433	Camelina microcarpa FAD2 B	55
GU929434	Camelina microcarpa FAD2 C	56
GU929435	Camelina microcarpa FAE1 A	57
GU929436	Camelina microcarpa FAE1 B	58
GU929437	Camelina microcarpa FAE1 C	59
GU929438	Camelina rumelica FAD2-1	60
GU929439	Camelina rumelica FAD2-2	61
GU929440	Camelina rumelica FAE1-1	62

GU929441	Camelina rumelica FAE1-2	63
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The *C. sativa* genome appears to be organized in three copies, and can be considered to be an allohexaploid. The discovery of triplication and divergence of genes that in known diploids are present in single copy, the cytometrically determined genome size of *Camelina* species, the pattern of relationship and inferred duplication history in the gene trees, together with the previously known chromosome counts for this taxon, indicate a likely allohexaploid genomic constitution. The characterization of genes encoding key functions of fatty acid biosynthesis lays the foundation for future manipulations of this pathway in *Camelina sativa*, which allows for the future manipulation of oil composition of this emerging biofuel crop.

10

The present invention provides an isolated nucleic acid sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 1 to 6 and 45 to 63, and fragments and variations derived from thereof, which encode a plant fatty acid synthesis gene.

15

In one embodiment, the present invention provides an isolated polynucleotide encoding plant fatty acid desaturase, comprising a nucleic acid sequence that shares at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% identity to SEQ ID NO: 1, 2, 3, 45, 46, 48, 51, 54, 55, 56, 60, and/or 61.

20

In another embodiment, the present invention provides an isolated polynucleotide encoding fatty acid elongase, comprising a nucleic acid sequence that shares at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% identity to SEQ ID NO: 4, 5, 6, 47, 49, 50, 52, 53, 57, 58, 59, 62, and/or 63.

25

The present invention further provides an isolated amino acid sequence (e.g., a peptide, polypeptide and the like) comprising a sequence selected from the group consisting of SEQ ID NOs: 7 to 12, and fragments and variations derived from thereof, which form a plant fatty acid synthesis protein.

30

In some embodiments, the present invention provides an isolated amino acid sequence which forms a protein that shares an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%,

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5 at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% identity to SEQ ID NO: 7, 8, 9, 64, 65, 67, 70, 73, 74, 75, 79, and/or 80.

In one embodiment, the present invention provides an isolated amino acid sequence which forms a protein that shares an amino acid having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% identity to SEQ ID NO: 10, 11, 12, 66, 68, 69, 71, 72, 76, 77, 78, 81, and/or 82.

15 The present invention also provides a chimeric gene comprising the isolated nucleic acid sequence of any one of the polynucleotides described above operably linked to suitable regulatory sequences.

The present invention also provides a recombinant construct comprising the chimeric gene as described above.

20 The present invention further comprises interfering RNA (RNAi) based on the expression of the nucleic acid sequences of the present invention, wherein such RNAi includes but is not limited to microRNA (miRNA) and small interfering RNA (siRNA) which can be used in gene silencing constructs.

The present invention also provides a transformed host cell comprising the chimeric gene as described above. In one embodiment, said host cell is selected from the group consisting of bacteria, yeasts, filamentous fungi, algae, animals, and plants.

The present invention in another aspect, provides a plant comprising in its genome one or more genes as described herein, one or more genes with mutations as described herein, or the chimeric genes as described herein.

30 The present invention in another aspect, provides a plant seed obtained from the plants described herein, wherein the plants comprise in their genomes one or more genes as described herein, one or more genes with mutations as described herein, or the chimeric genes as described herein.

The present invention in another aspect, provides *Camelina* oil obtained from the seeds of a *Camelina* plant comprising the one or more genes described herein, one or more genes with mutations as described herein, or one or more chimeric genes as described herein.

The present invention in another aspect, provides meals made from *Camelina* plants comprising the one or more genes described herein, one or more genes with mutations as

5 described herein, or one or more chimeric genes as described herein. In some embodiments, the meal is a byproduct of the extraction of the oil from said *Camelina* seeds. In some embodiments, said *Camelina* plant has reduced level of erucic acid (22:1) compared to a wild type *Camelina* plant. In some embodiments, said *Camelina* plant has less than 4%, less than 3%, less than 2%, less than 1%, or less than 0.1% erucic acid (22:1) compared to the wild
10 type. In further embodiments, the *Camelina* meal is included in the diets of an animal for about 0.1%, about 0.5%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, or about 10% of their feed on a weight or volume basis.

Thus, the present invention provides methods of altering and/or improving *Camelina* fatty acids composition by disrupting and/or altering one, two, or all three copies of one or
15 more fatty acid synthesis genes in *Camelina*. Methods of disrupting and/or altering gene function include but are not limited to mutagenesis (e.g., chemical mutagenesis, radiation mutagenesis, transposon mutagenesis, insertional mutagenesis, signature tagged mutagenesis, site-directed mutagenesis, and natural mutagenesis), antisense, knock-outs, and/or RNA interference.

20 In some embodiments, the methods comprise introducing mutations in one or more *FAD2* genes and/or one or more *FAE1* genes of *Camelina*. In some embodiments, the methods disclosed herein comprise utilizing *Camelina* mutants with mutations in all three *FAD2* genes (e.g., *FAD2 A*, *FAD2 B*, and *FAD2 C*), and/or *Camelina* mutants with mutations in all three *FAE1* genes (e.g., *FAE1 A*, *FAE1 B*, and *FAE1 C*).

25 The present invention provides mutants in *FAD2 A*, *FAD2 B*, *FAD2 C*, *FAE1 A*, *FAE1 B*, and *FAE1 C*, including but not limited to those as listed in Tables 7-12. In some embodiments, the methods of altering and/or improving *Camelina* fatty acids composition comprise utilizing one or more *Camelina* mutants for any one or more of the mutations listed in Tables 7 to 12 and as described in Example 11. In some embodiments, mutations in one
30 or more copies of *FAD2* genes and/or mutations in one or more copies of *FAE1* genes as described in the Tables 7 to 12 are integrated together to create mutant plants with double, triple, quadruple et al. mutations in one, two, or all three copies of *FAD2* and/or *FAE1* genes. In some embodiments, the mutations described in the Tables 7-12 can be integrated into *Camelina sativa* cultivars other than Cs32 (commercial name as SO30) or other *Camelina*
35 species by classic breeding methods, with or without the help of marker-facilitated inter-cultivar gene transfer methods. In some embodiments, mutations described in the Tables 7-12 can be integrated into species closely related to *Camelina sativa*. In still other embodiments, amino acids in conserved domains or sites compared to *FAD2* or *FAE1*

5 orthologs in other species can be substituted or deleted to make mutants with reduced or abolished activity, mutants that lead to loss-of-function (e.g., protein instability), and/or mutants that lead to gain-of-function (e.g., more stable or more active protein).

In some embodiments, one, two, or all three copies of *Camelina FAD2* and/or *FAE1* genes, and one, two, or all three copies of other non-FAD, non-FAE fatty acid synthesis
10 genes are disrupted. In still some embodiments, one, two, or all three copies of *Camelina FAD2* and/or *FAE1* genes are disrupted, while one or more non-FAD, non-FAE fatty acid synthesis genes are overexpressed. In still more embodiments, one, two, or all three copies of *Camelina FAD2* and/or *FAE1* genes are disrupted, while one or more non-fatty-acid-synthesis genes are disrupted and/or overexpressed.

15 In another aspect, the present invention provides methods of producing *Camelina* seed oil containing altered and/or increased levels of oleic acid (18:1), and/or altered or reduced levels of polyunsaturated fatty acids, and/or decreased very long chain fatty acids. Such methods comprising utilizing the *Camelina* plants comprising the chimeric genes as described herein, or *Camelina* plants with disrupted *FAD2* and/or *FAE1* genes as described
20 herein. As used herein, the phrase “very long chain fatty acid” refers to a fatty acid with more than 18 carbons.

The present invention also provides methods of increasing the activity of a *FAD2* and/or *FAE1* protein in a *Camelina* plant cell, plant part, tissue culture or whole plant comprising transforming the plant cell, plant part, tissue culture or whole plant with a
25 chimeric gene comprising one *FAD2* and/or *FAE1* gene encoding the polypeptide of the present invention, or functional variants thereof. In one embodiment, the chimeric gene is overexpressed. As used herein, a functional variant of a protein refers to a polypeptide comprising one or more amino acid modifications (e.g., substitution, deletion, modification, et al) compared to the original protein, but still maintains the activity of the original protein.

30 In the present invention, “overexpression promoter” means a promoter capable of causing strong expression (large amount expression) of a gene that has been ligated thereto in host plant cells. The overexpression promoter of the present invention may be either an inducible promoter or a constitutive promoter. A promoter is a DNA comprising an expression control region generally located on the 5' upstream of a structural gene or a modified sequence
35 thereof. In the present invention, any promoters appropriate for foreign gene expression in plant cells can be used as overexpression promoters. Non-limiting examples of such overexpression promoters to be used in the present invention include, but are not limited to, a cauliflower mosaic virus (CaMV) 35S promoter, a rice actin promoter, a modified 35S

5 promoter, or an embryo-specific promoter. As used herein an "embryo-specific promoter" refers to a promoter of an embryo-specific gene. An embryo-specific gene is preferentially expressed during embryo development in a plant. For purposes of the present disclosure, embryo development begins with the first cell divisions in the zygote and continues through the late phase of embryo development (characterized by maturation, desiccation, dormancy),
 10 and ends with the production of a mature and desiccated seed. Embryo-specific genes can be further classified as "early phase-specific" and "late phase-specific". Early phase-specific genes are those expressed in embryos up to the end of embryo morphogenesis. Late phase-specific genes are those expressed from maturation through to production of a mature and desiccated seed. An early phase-specific promoter is a promoter that initiates expression of a
 15 protein prior to day 7 after pollination in *Arabidopsis* or an equivalent stage in another plant species. Non-limiting examples of promoter sequences that can be used in the present invention include a promoter for the amino acid permease gene (AAP1) (e.g., the AAP1 promoter from *Arabidopsis thaliana*, Hirner et al, Plant J. 14:535-544, 1998), a promoter for the oleate 12-hydroxylase:desaturase gene (e.g., the promoter designated LFAH 12 from
 20 *Lesquerella fendleri*, Broun et al, Plant J. 13:201-210, 1998), a promoter for the 2S2 albumin gene (e.g., the 2S2 promoter from *Arabidopsis thaliana*, Guerche et al, Plant cell 2:469-478, 1990), a fatty acid elongase gene promoter (FAEl) (e.g., the FAEl promoter from *Arabidopsis thaliana*, Rossak et al, Plant Mol Biol. 46:717-715, 2001), and the leafy cotyledon gene promoter (LEC2) (e.g., the LEC2 promoter from *Arabidopsis thaliana*, Kroj et al
 25 Development 130:6065-6073, 2003). Other early embryo-specific promoters of interest include, but are not limited to, seedstick (Pinyopich et al, Nature 424:85-88, 2003), Fbp7 and Fbpl 1 (*Petunia* Seedstick) (Colombo et al, Plant Cell. 9:703-715, 1997), Banyuls (Devic et al, Plant J. 19:387-398, 1999), agl-15 and agl-18 (Lehti-Shiu et al, Plant Mol Biol. 58:89-107, 2005), Phel (Kohler et al, Genes Develop. 17:1540-1553, 2003), Perl (Haslekas et al, Plant
 30 Mol Biol. 36:833-845, 1998; Haslekas et al, Plant Mol Biol. 53:313-326, 2003), emb175 (Cushing et al, Planta 221 :424-436, 2005), LIL (Kwong et al, Plant Cell 15:5-18, 2003), Lecl (Lotan et al, Cell 93:1195-1205, 1998), Fusca3 (Kroj et al, Development 130:6065-6073, 2003), ttl2 (Debeaujon et al, Plant Cell 13:853-871, 2001), ttl6 (Nesi et al, Plant Cell 14:2463-2479, 2002), A-RZf (Zou and Taylor, Gene 196:291-295, 1997), TtGl (Walker et al,
 35 Plant Cell 11 :1337-1350, 1999; Tsuchiya et al, Plant J. 37:73-81, 2004), TtI (Sagasser et al, Genes Dev. 16:138-149, 2002), TT8 (Nesi et al, Plant Cell 12:1863-1878, 2000), Gea-8 (carrot) (Lin and Zimmerman, J. Exp. Botany 50:1139-1147, 1999), Knox (rice) (Postma-Haarsma et al, Plant Mol Biol. 39:257-271, 1999), Oleosin (Plant et al, Plant Mol Biol.

5 25:193-205, 1994; Keddie et al, Plant Mol Biol. 24:121-145, 1994), ABI3 (Ng et al, Plant Mol Biol. 54:25-38, 2004; Parcy et al, Plant Cell 6:1567-1582, 1994), and the like.

The present invention also provides methods of decreasing the activity of a FAD2 and/or FAE1 protein in a *Camelina* plant cell, plant part, tissue culture or whole plant comprising contacting the plant cell, plant part, tissue culture or whole plant with an
10 inhibitory nucleic acid having complementarity to a gene encoding the FAD2 and/or FAE1 protein.

In one aspect, the present invention provides methods of breeding *Camelina* species producing altered levels of fatty acids in the seed oil and/or meal. In one embodiment, such methods comprise making a cross between a *Camelina* mutant with one or more mutations
15 listed in Tables 7- 12 with a second *Camelina* cultivar to produce an F1 plant; backcrossing the F1 plant to the second *Camelina* cultivar; and repeating the backcrossing step to generate an near isogenic line, wherein the one or more mutations are integrated into the genome of the second *Camelina* cultivar; wherein the near isogenic line derived from the second *Camelina* cultivar with the integrated mutations has altered seed oil composition. Optionally,
20 such methods can be facilitated by molecular markers.

In another aspect, the present invention provides methods of breeding species close to *Camelina sativa*, wherein said species produces altered levels of fatty acids in the seed oil and/or meal. For example, intertribal somatic hybridizations are possible between *C. sativa* and *B. oleracea* (see, e.g., Lise N. Hansen, 1998, Euphytica, Volume 104, No. 3, pages 173-
25 179). In one embodiment, such methods comprise making a cross between the *Camelina* mutants with one or more mutations listed in Tables 7-12 with a species that is closely related to the *Camelina* species containing the mutations to make an F1 plant; backcrossing the F1 plants to the species that is closely related to the *Camelina* species containing the mutations; and, repeating backcrossing step to generate an near isogenic line, wherein the one or more
30 mutations are integrated into the genome of the species that is closely related to the *Camelina* species containing the mutations; wherein the near isogenic line derived from the species that is closely related to the *Camelina* species containing the mutations has integrated these mutations and has altered seed oil composition. Optionally, such method can be facilitated by molecular markers.

35

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts Southern blot analysis of *Camelina sativa* and *Arabidopsis thaliana*. A blot containing genomic DNA from *C. sativa* and *A. thaliana* digested with EcoRI or a

5 combination of EcoRI and BamHI was hybridized with an α -32P dCTP - labeled (A) *FAD2* probe, (B) *FAE1* probe or (C) *LFY* probe obtained from PCR amplification of *C. sativa* DNA.

Figure 2 depicts *FAD2* and *FAE1* protein alignment. Figure 2A shows amino acid sequence comparison of the three *Camelina sativa* *FAD2* sequences, *Arabidopsis thaliana* *FAD2* sequence [Genbank: NM_112047], *Brassica rapa* *FAD2* sequence [Genbank: AJ459107], *Glycine max* *FAD2-3* sequence [Genbank: DQ532371], *Zea mays* *FAD2* sequence [Genbank: AB257309]. Blue underlines below the sequences indicate amino acids conserved in all 50 *FAD2* sequences (Belo, Zheng et al. 2008) while the green underline indicates the ER localization signal (McCartney, Dyer et al. 2004). The three His boxes described by Tocher DR (1998) are indicated with red boxes. Figure 2B shows amino acid sequence comparison of the three *Camelina sativa* *FAE1* sequences, *Arabidopsis thaliana* *FAE1* [Genbank: NM_119617], *Crambe abyssinica* [Genbank: AAX22298], *Brassica rapa* HEAC *FAE1* [Genbank: Y14975], *Brassica rapa* LEAC *FAE1* [Genbank: Y14974], *Limnanthes alba* (meadow foam) [Genbank: AF247134], *Tropaeolum majus* (nasturtium) [Genbank: ABD77097]. Blue underlines below the sequence indicate the asparagine at position 424 and the highly conserved histidine and cysteine residues described by Ghanevati and Jaworski (Ghanevati and Jaworski 2001; Ghanevati and Jaworski 2002). The red box indicates the region highly conserved among condensing enzymes in very long chain fatty acid biosynthesis (Moon, Smith et al. 2001) Abbreviations: Heac= High erucic acid, Leac = Low erucic acid.

Figure 3 depicts *FAD2* and *FAE1* Expression in Developing Seeds. Relative combined expression of all three copies of (A) *FAD2* and (B) *FAE1* measured by real time quantitative PCR at 15, 20, 25, and 30 days post anthesis (DPA) and in 2 week old seedlings. The 20 DPA sample, which expressed *FAD2* and *FAE1* at the highest amount, was used as the calibrator. Error bars represent the standard deviation of 3 replicate experiments. Sequenom SNP analysis demonstrating the expression of each version of (C) *FAD2* or (D) *FAE1* relative to the other versions. Error bars represent the standard deviation of three (for *FAD2*) or four (for *FAE1*) SNP analyses. Because *FAE1* is not expressed in *C. sativa* seedlings (B), the relative expression of the 3 copies of *FAE1* in seedling tissue is not shown (D).

Figure 4 depicts structure and conservation of the *KCS17-FAE1* intergenic region in *Camelina sativa*. The three putative homologous regions in allohexaploid *C. sativa* are aligned to the orthologous region of *Arabidopsis* to display blocks of homology identified on a dot matrix by perfect conservation of a sliding window of 9 bases. The *KCS17* and *FAE1*

gene, respectively blue and red, flank a variable region in which conserved blocks common to two or more genomes are marked by different shades of brown. Lined regions display reduced or no conservation. The large variation in the intergenic region of the triplicated *KCS17-FAEI* DNA of *C. sativa* is consistent with independent evolution before reunion of diverged genomes by allohexaploidization.

Figure 5 depicts genome content of *Camelina* species. 1C nuclei were stained with propidium iodide and analyzed by flow cytometry. Error bars represent the standard deviation of 2-4 replicate samples.

Figure 6 depicts phylogenetic analyses of Camelinae *FAD2* and *FAEI*. Maximum-likelihood trees showing branch length and bootstrap support (100 bootstrap replicates) for (A) 15 *FAD2* sequences from species from the tribe Camelinae calculated using the TVM+I+ Γ model in PAUP* and rooted with *Brassica rapa FAD2* (-LnL 3665.277); and for (B) 15 *FAEI* sequences from species from the tribe Camelinae calculated using the HKY+I+ Γ model in PAUP* and rooted with *Crambe abyssinica FAEI* (-LnL 5051.552). Sequences obtained from Genbank are *Capsella bursa-pastoris FAD2* [Genbank: DQ518293], *Arabidopsis thaliana FAD2* [Genbank: NM_112047], *Brassica rapa FAD2* [Genbank: AJ459107], *Arabidopsis thaliana FAEI* [Genbank: NM_119617], and *Crambe abyssinica FAEI* [Genbank: AY793549].

Figure 7 depicts a simplified version of fatty acid synthesis pathways in plant.

Figure 8 depicts an exemplary field growth of EMS mutagenized *Camelina* M2 population (upper-panel), and exemplary mutant M2 plants with morphological changes (lower-panel).

Figure 9 depicts an exemplary LI-COR® gel identifying mutants in *Camelina FAD2* genes.

Figure 10 depicts proximate locations of mutations in *FAD2 A* and *B*, which were used in the preliminary GC analysis. “H” identifies a His box.

Figure 11 depicts a representative composition of *Camelina sativa* seed oil.

Figure 12 depicts fatty acid compositions in *FAD2* mutants (12A) and in *FAEI* mutants (12B) measured by gas chromatography.

Figure 13 depicts lipid synthesis in the plastid and cytoplasm of oilseeds. Key enzymes are in red text and boxed. ACCase=acetyl co-A carboxylase, KAS=β-ketoacyl-acyl

5 carrier protein (ACP) synthase, GPAT=glycerol phosphate acyltransferase, LPAAT=lysophosphatidic acid acyltransferase, PAP=phosphatidate phosphatase, DAGAT=diacylglycerol acyltransferase, R=fatty acyl group, P=phosphate group, CPT=chloroplast

10

DETAILED DESCRIPTION

Definition

As used herein, the verb “comprise” as is used in this description and in the claims and its conjugations are used in its non-limiting sense to mean that items following the word
15 are included, but items not specifically mentioned are not excluded.

As used herein, the term “plant” refers to any living organism belonging to the kingdom Plantae (i.e., any genus/species in the Plant Kingdom). This includes familiar organisms such as but not limited to trees, herbs, bushes, grasses, vines, ferns, mosses and green algae. The term refers to both monocotyledonous plants, also called monocots, and
20 dicotyledonous plants, also called dicots. Examples of particular plants include but are not limited to corn, potatoes, roses, apple trees, sunflowers, wheat, rice, bananas, tomatoes, opo, pumpkins, squash, lettuce, cabbage, oak trees, guzmania, geraniums, hibiscus, clematis, poinsettias, sugarcane, taro, duck weed, pine trees, Kentucky blue grass, zoysia, coconut trees, brassica leafy vegetables (e.g. broccoli, broccoli raab, Brussels sprouts, cabbage,
25 Chinese cabbage (Bok Choy and Napa), cauliflower, cavalo, collards, kale, kohlrabi, mustard greens, rape greens, and other brassica leafy vegetable crops), bulb vegetables (e.g. garlic, leek, onion (dry bulb, green, and Welch), shallot, and other bulb vegetable crops), citrus fruits (e.g. grapefruit, lemon, lime, orange, tangerine, citrus hybrids, pummelo, and other citrus fruit crops), cucurbit vegetables (e.g. cucumber, citron melon, edible gourds, gherkin,
30 muskmelons (including hybrids and/or cultivars of cucumis melons), water-melon, cantaloupe, and other cucurbit vegetable crops), fruiting vegetables (including eggplant, ground cherry, pepino, pepper, tomato, tomatillo, and other fruiting vegetable crops), grape, leafy vegetables (e.g. romaine), root/tuber and corm vegetables (e.g. potato), and tree nuts (almond, pecan, pistachio, and walnut), berries (e.g., tomatoes, barberries, currants,
35 elderberries, gooseberries, honeysuckles, mayapples, nannyberries, Oregon-grapes, see-buckthorns, hackberries, bearberries, lingonberries, strawberries, sea grapes, lackberries, cloudberry, loganberries, raspberries, salmonberries, thimbleberries, and wineberries), cereal crops (e.g., corn, rice, wheat, barley, sorghum, millets, oats, ryes, triticales,

5 buckwheats, fonio, and quinoa), pome fruit (e.g., apples, pears), stone fruits (e.g., coffees, jujubes, mangos, olives, coconuts, oil palms, pistachios, almonds, apricots, cherries, damsons, nectarines, peaches and plums), vine (e.g., table grapes, wine grapes), fiber crops (e.g. hemp, cotton), ornamentals, and the like. For example, the plant is a species in the tribe of Camelinae, such as *C. alyssum*, *C. anomala*, *C. grandiflora*, *C. hispida*, *C. laxa*, *C.*
10 *microcarpa*, *C. microphylla*, *C. persistens*, *C. rumelica*, *C. sativa*, *C. Stiefelhagenii*, or any hybrid thereof.

As used herein, the term “plant part” refers to any part of a plant including but not limited to the shoot, root, stem, seeds, stipules, leaves, petals, flowers, ovules, bracts, branches, petioles, internodes, bark, pubescence, tillers, rhizomes, fronds, blades, pollen,
15 stamen, and the like. The two main parts of plants grown in some sort of media, such as soil, are often referred to as the “above-ground” part, also often referred to as the “shoots”, and the “below-ground” part, also often referred to as the “roots”.

The term “a” or “an” refers to one or more of that entity; for example, “a gene” refers to one or more genes or at least one gene. As such, the terms “a” (or “an”), “one or more” and
20 “at least one” are used interchangeably herein. In addition, reference to “an element” by the indefinite article “a” or “an” does not exclude the possibility that more than one of the elements are present, unless the context clearly requires that there is one and only one of the elements.

As used herein, the term “chimeric protein” refers to a construct that links at least two
25 heterologous proteins into a single macromolecule (fusion protein).

As used herein, the term “nucleic acid” refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides, or analogs thereof. This term refers to the primary structure of the molecule, and thus includes double- and single-stranded DNA, as well as double- and single-stranded RNA. It also includes modified nucleic acids
30 such as methylated and/or capped nucleic acids, nucleic acids containing modified bases, backbone modifications, and the like. The terms “nucleic acid” and “nucleotide sequence” are used interchangeably.

As used herein, the terms “polypeptide,” “peptide,” and “protein” are used interchangeably herein to refer to polymers of amino acids of any length. These terms also
35 include proteins that are post-translationally modified through reactions that include glycosylation, acetylation and phosphorylation.

As used herein, the term “homologous” or “homolog” or “ortholog” is known in the art and refers to related sequences that share a common ancestor or family member and are

5 determined based on the degree of sequence identity. The terms “homology”, “homologous”, “substantially similar” and “corresponding substantially” are used interchangeably herein. They refer to nucleic acid fragments wherein changes in one or more nucleotide bases do not affect the ability of the nucleic acid fragment to mediate gene expression or produce a certain phenotype. These terms also refer to modifications of the nucleic acid fragments of the
10 instant invention such as deletion or insertion of one or more nucleotides that do not substantially alter the functional properties of the resulting nucleic acid fragment relative to the initial, unmodified fragment. It is therefore understood, as those skilled in the art will appreciate, that the invention encompasses more than the specific exemplary sequences. These terms describe the relationship between a gene found in one species, subspecies,
15 variety, cultivar or strain and the corresponding or equivalent gene in another species, subspecies, variety, cultivar or strain. For purposes of this invention homologous sequences are compared. "Homologous sequences" or "homologs" or "orthologs" are thought, believed, or known to be functionally related. A functional relationship may be indicated in any one of a number of ways, including, but not limited to: (a) degree of sequence identity and/or (b) the
20 same or similar biological function. Preferably, both (a) and (b) are indicated. The degree of sequence identity may vary, but in one embodiment, is at least 50% (when using standard sequence alignment programs known in the art), at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about
25 97%, at least about 98%, or at least 98.5%, or at least about 99%, or at least 99.5%, or at least 99.8%, or at least 99.9%. Homology can be determined using software programs readily available in the art, such as those discussed in *Current Protocols in Molecular Biology* (F.M. Ausubel *et al.*, eds., 1987) Supplement 30, section 7.718, Table 7.71. Some alignment programs are MacVector (Oxford Molecular Ltd, Oxford, U.K.) and ALIGN Plus (Scientific and Educational Software, Pennsylvania). Other non-limiting alignment programs include
30 Sequencher (Gene Codes, Ann Arbor, Michigan), AlignX, and Vector NTI (Invitrogen, Carlsbad, CA).

As used herein, the term "nucleotide change" or “nucleotide modification” refers to, e.g., nucleotide substitution, deletion, and/or insertion, as is well understood in the art. For
35 example, mutations containing alterations that produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded protein or how the proteins are made.

5 As used herein, the term "protein modification" refers to, e.g., amino acid substitution, amino acid modification, deletion, and/or insertion, as is well understood in the art.

As used herein, the term "derived from" refers to the origin or source, and may include naturally occurring, recombinant, unpurified, or purified molecules. A nucleic acid
10 or an amino acid derived from an origin or source may have all kinds of nucleotide changes or protein modification as defined elsewhere herein.

As used herein, the term "at least a portion" of a nucleic acid or polypeptide means a portion having the minimal size characteristics of such sequences, or any larger fragment of the full length molecule, up to and including the full length molecule. For example, a portion
15 of a nucleic acid may be 12 nucleotides, 13 nucleotides, 14 nucleotides, 15 nucleotides, 16 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 22 nucleotides, 24 nucleotides, 26 nucleotides, 28 nucleotides, 30 nucleotides, 32 nucleotides, 34 nucleotides, 36 nucleotides, 38 nucleotides, 40 nucleotides, 45 nucleotides, 50 nucleotides, 55 nucleotides, and so on, going up to the full length nucleic acid. Similarly, a portion of a polypeptide may
20 be 4 amino acids, 5 amino acids, 6 amino acids, 7 amino acids, and so on, going up to the full length polypeptide. The length of the portion to be used will depend on the particular application. A portion of a nucleic acid useful as hybridization probe may be as short as 12 nucleotides; in one embodiment, it is 20 nucleotides. A portion of a polypeptide useful as an epitope may be as short as 4 amino acids. A portion of a polypeptide that performs the
25 function of the full-length polypeptide would generally be longer than 4 amino acids.

As used herein, "sequence identity" or "identity" in the context of two nucleic acid or polypeptide sequences includes reference to the residues in the two sequences which are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that
30 residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. Where sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature
35 of the substitution. Sequences which differ by such conservative substitutions are said to have "sequence similarity" or "similarity." Means for making this adjustment are well-known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for

5 example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., according to the algorithm of Meyers and Miller, *Computer Applic. Biol. Sci.*, 4:11-17 (1988).

10 As used herein, the term “suppression” or “disruption” of regulation refers to reduced activity of regulatory proteins, and such reduced activity can be achieved by a variety of mechanisms including antisense, mutation knockout or RNAi. Antisense RNA will reduce the level of expressed protein resulting in reduced protein activity as compared to wild type activity levels. A mutation in the gene encoding a protein may reduce the level of expressed protein and/or interfere with the function of expressed protein to cause reduced protein
15 activity.

As used herein, the terms “polynucleotide”, “polynucleotide sequence”, “nucleic acid sequence”, “nucleic acid fragment”, and “isolated nucleic acid fragment” are used interchangeably herein. These terms encompass nucleotide sequences and the like. A polynucleotide may be a polymer of RNA or DNA that is single- or double-stranded, that
20 optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA, synthetic DNA, or mixtures thereof. Nucleotides (usually found in their 5'-monophosphate form) are referred to by a single letter designation as follows: “A” for adenylate or deoxyadenylate (for RNA or DNA, respectively), “C” for cytidylate or deoxycytidylate, “G” for guanylate or deoxyguanylate, “U” for uridylate, “T” for
25 deoxythymidylate, “R” for purines (A or G), “Y” for pyrimidines (C or T), “K” for G or T, “H” for A or C or T, “I” for inosine, and “N” for any nucleotide.

The term “primer” as used herein refers to an oligonucleotide which is capable of annealing to the amplification target allowing a DNA polymerase to attach, thereby serving
30 as a point of initiation of DNA synthesis when placed under conditions in which synthesis of primer extension product is induced, i.e., in the presence of nucleotides and an agent for polymerization such as DNA polymerase and at a suitable temperature and pH. The (amplification) primer is preferably single stranded for maximum efficiency in amplification. Preferably, the primer is an oligodeoxyribonucleotide. The primer must be sufficiently long
35 to prime the synthesis of extension products in the presence of the agent for polymerization. The exact lengths of the primers will depend on many factors, including temperature and composition (A/T and G/C content) of primer. A pair of bi-directional primers consists of one

5 forward and one reverse primer as commonly used in the art of DNA amplification such as in PCR amplification.

As used herein, “coding sequence” refers to a DNA sequence that codes for a specific amino acid sequence. “Regulatory sequences” refer to nucleotide sequences located upstream (5′ non-coding sequences), within, or downstream (3′ non-coding sequences) of a coding
10 sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence.

As used herein, “regulatory sequences” may include, but are not limited to, promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

As used herein, “promoter” refers to a DNA sequence capable of controlling the
15 expression of a coding sequence or functional RNA. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an “enhancer” is a DNA sequence that can stimulate promoter activity, and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their
20 entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. It is further recognized that since in most cases the exact
25 boundaries of regulatory sequences have not been completely defined, DNA fragments of some variation may have identical promoter activity. Promoters that cause a gene to be expressed in most cell types at most times are commonly referred to as “constitutive promoters”.

As used herein, the “3′ non-coding sequences” or “3′ UTR (untranslated region)
30 sequence” refer to DNA sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3′ end of the mRNA precursor. The use of different 3′ non-coding sequences is exemplified by
35 Ingelbrecht, I. L., et al. (1989) Plant Cell 1:671-680.

As used herein, the term “operably linked” refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is regulated by the other. For example, a promoter is operably linked with a coding sequence when it is capable

5 of regulating the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in a sense or antisense orientation. In another example, the complementary RNA regions of the invention can be operably linked, either directly or indirectly, 5' to the target mRNA, or 3' to the target mRNA, or within the target mRNA, or a
 10 first complementary region is 5' and its complement is 3' to the target mRNA.

As used herein, the term “cross”, “crossing”, “cross pollination” or “cross-breeding” refer to the process by which the pollen of one flower on one plant is applied (artificially or naturally) to the ovule (stigma) of a flower on another plant.

As used herein, the term “gene” refers to any segment of DNA associated with a
 15 biological function. Thus, genes include, but are not limited to, coding sequences and/or the regulatory sequences required for their expression. Genes can also include nonexpressed DNA segments that, for example, form recognition sequences for other proteins. Genes can be obtained from a variety of sources, including cloning from a source of interest or synthesizing from known or predicted sequence information, and may include sequences
 20 designed to have desired parameters.

As used herein, the term “vector”, “plasmid”, or “construct” refers broadly to any plasmid or virus encoding an exogenous nucleic acid. The term should also be construed to include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into virions or cells, such as, for example, polylysine compounds and the like. The vector may be
 25 a viral vector that is suitable as a delivery vehicle for delivery of the nucleic acid, or mutant thereof, to a cell, or the vector may be a non-viral vector which is suitable for the same purpose. Examples of viral and non-viral vectors for delivery of DNA to cells and tissues are well known in the art and are described, for example, in Ma et al. (1997, Proc. Natl. Acad. Sci. U.S.A. 94:12744-12746). Examples of viral vectors include, but are not limited to,
 30 recombinant plant viruses. Non-limiting examples of plant viruses include, TMV-mediated (transient) transfection into tobacco (Tuipe, T-H et al (1993), J. Virology Meth, 42: 227-239), ssDNA genomes viruses (e.g., family *Geminiviridae*), reverse transcribing viruses (e.g., families *Caulimoviridae*, *Pseudoviridae*, and *Metaviridae*), dsNRA viruses (e.g., families *Reoviridae* and *Partitiviridae*), (-) ssRNA viruses (e.g., families *Rhabdoviridae* and
 35 *Bunyaviridae*), (+) ssRNA viruses (e.g., families *Bromoviridae*, *Closteroviridae*, *Comoviridae*, *Luteoviridae*, *Potyviridae*, *Sequiviridae* and *Tombusviridae*) and viroids (e.g., families *Pospiviroidae* and *Avsunviroidae*). Detailed classification information of plant viruses can be found in Fauquet et al (2008, "Geminivirus strain demarcation and

5 nomenclature". *Archives of Virology* 153:783–821,
 and Khan et al. (Plant viruses as molecular pathogens; Publisher Routledge, 2002,
 ISBN 1560228954, 9781560228950). . Examples of non-viral vectors include, but are not
 limited to, liposomes, polyamine derivatives of DNA, and the like.

10 *Camelina sativa*

Camelina is a genus of flowering plants belonging to the Brassicaceae family. *Camelina sativa* is a particular species of the genus *Camelina* that is important historically and is a source of oil that can be used in, for example, biofuels and lubricants. *C. sativa* is being investigated for both biofuel and human utility. It is a crop that has not benefited much
 15 from molecular investigation in the past and as such, there is relatively little sequence information available. The utility of a plant oil either for biodiesel or food depends on its fatty acid composition. *Camelina* has a fatty acid composition with high levels of both polyunsaturated fatty acids such as 18:2 and 18:3 (52-54%) as well as long chain fatty acids such as 20:1 (11-15%) and 22:1 (2-5%). For biodiesel, the optimum fatty acid is 18:1
 20 (oleic). Oleic has the best balance of characteristics for cloud point vs. oxidative stability. Polyunsaturated fatty acids such as 18:2 and 18:3 have poor oxidative stability. The long chain fatty acids such as 20:1 and 22:1 contribute to out of range distillation temperatures in biodiesel. For biodiesel utility it is therefore desirable to lower the level of polyunsaturated fatty acids and to lower the level of long chain fatty acids. The ultimate goal is to increase
 25 the percentage of 18:1 fatty acid. 18:1 is also considered a good fatty acid for food utility.

Camelina has not been intensively bred and the germplasm is somewhat limited genetically. An in-house field study of a significant number of cultivars showed little variation in the fatty acid composition. This agrees with published literature (e.g., Putnam et al., 1993. *Camelina: A promising low-input oilseed*. p. 314-322. In: J. Janick and J.E. Simon
 30 (eds.), *New crops*. Wiley, New York.).

Fatty Acids Synthesis in Plants

35 Fatty acid biosynthesis in plants takes place within the endoplasmic reticulum and plastids, the latter of which is an organelle widely thought to have originated from a photosynthetic bacterial symbiont. Fatty acid metabolism in plants closely resembles that of bacteria.

5 During fatty acid biosynthesis, a repeated series of reactions incorporates acetyl moieties of acetyl-CoA into an acyl group 16 or 18 carbons long. The enzymes involved in this synthesis are acetyl-CoA carboxylase (ACCase), malonyl-CoA:ACP transacylase, 3-ketoacyl-ACP synthase I and III (KAS I and KAS III), 3-ketoacyl-ACP reductase, 2,3-trans-Enoyl-ACP reductase, 3-hydroxyacyl-ACP dehydratase (all referred as fatty acid synthase
10 (FAS), except for ACCase). The name fatty acid synthase refers to a complex of several individual enzymes that catalyze the conversion of acetyl-CoA and malonyl-CoA to 16:0 and 18:0 fatty acids. Acyl-carrier protein (ACP), an essential protein cofactor, is generally considered a component of FAS.

The last three steps of the fatty acids synthesis cycle reduce a 3-ketoacyl substrate to
15 form a fully saturated acyl chain. Each cycle of fatty acid synthesis adds two carbons to the acyl chain. Typically, fatty acid synthesis ends at 16:0 or 18:0, when one of several reactions stops the process. The most common reactions are hydrolysis of acyl moiety from ACP by a thioesterase, transfer of the acyl moiety from ACP directly onto a glycerolipid by an acyl transferase, or double-bond formation on the acyl moiety by an acyl-ACP desaturase. The
20 thioesterase reaction yields a sulfhydryl ACP.

Two principal types of acyl-ACP thioesterases occur in plants. For making storage lipids (triglycerides) in the ER, the FAT enzymes convert the fatty acid-ACP to a fatty acid-Co-A. The substrate for FAE1 is an R-CoA and it is an R-CoA that is added to various positions in the glycerol backbone during the Kennedy pathway portion of the synthesis of
25 Triglycerides in the ER (Figure 7). The major class, designated FatA, is most active with 18:1 delta9-ACP. A second class designated FatB, typified by 16:0-ACP thioesterase, is most active with shorter-chain, saturated acyl-ACPs. Thioesterases play important role in plants that have unusually short fatty acids, such as coconut, many species of *Cuphea*, and California bay. These plants have thioesterases that are especially active with C10 to C12
30 acyl-ACPs, by prematurely terminating fatty acid biosynthesis,.

Unsaturated fatty acids are produced by desaturation of saturated lipids with the help of desaturases (FAD enzymes). Most fatty acid desaturases (FADs) in plants are integral membrane proteins, with the exception that plant contains a soluble, plastid-localized stearoyl-ACP desaturase. The number and properties of different FADs in plants are known
35 from the isolation of a comprehensive collection of *Arabidopsis* mutants with defects in each of eight desaturase genes. The enzymes encoded by these genes differ in substrate specificity, subcellular location, mode of regulation, or some combination of these. A summary of the *Arabidopsis* FADs is shown below:

Name	subcellular location	Fatty acid substrates	Site of double-bond insertion	Notes
FAD2	ER	18:1 Δ 9	Δ 12	preferred substrate is phosphatidylcholine, oleate desaturase
FAD3	ER	18:2 Δ 9,12	ω 3	preferred substrate is phosphatidylcholine, linoleate desaturase
FAD4	Chloroplast	16:0	Δ 3	produces 16:1-trans at sn-2 of phosphatidylglycerol
FAD5	Chloroplast	16:0	Δ 7	desaturates 16:0 at sn-2 of monogalactosyldiacylglycerol
FAD6	Chloroplast	16:1 Δ 7 and 18:1 Δ 9	ω 6	acts on all chloroplast glycerolipids, oleate desaturase
FAD7	Chloroplast	16:2 Δ 7,11 and 18:2 Δ 9,12	ω 3	acts on all chloroplast glycerolipids, linoleate desaturase
FAD8	Chloroplast	16:2 Δ 7,11 and 18:2 Δ 9,12	ω 3	isozyme of FAD7, induced by low temperature, linoleate desaturase
FAB2	Chloroplast	18:0	Δ 9	stromal stearyl-ACP desaturase

- 5 The biochemical defect of each class of mutants is shown by breaks in the pathway on page 480 of Buchanan et al., *Biochemistry and Molecular Biology of Plants*, American Society of Plant Physiologists, 2000, ISBN 0943088372, 9780943088372.

Extensive surveys of the fatty acid composition of seed oils from different plant
 10 species have resulted in the identification of more than 200 naturally occurring fatty acids, which can broadly be classified into 18 structural classes, such as labellenic acid, stearolic acid, sterculynic acid, chaulmoogric acid, ricinoleic acid, vernolic acid, furan-containing fatty acid, et al.. Less is known about the mechanisms responsible for the synthesis and
 15 accumulation of unusual fatty acids, or of their significance to the fitness of the plants that accumulate them. However, recent studies indicate that enzymes involved in the synthesis of unusual fatty acids are structurally similar to the desaturases and hydroxylases. Unusual fatty acids occur almost exclusively in seed oils and may serve a defense function.

Synthesis of structural lipids (e.g. cutin, suberin, epicuticular wax) has also been
 studied in *Arabidopsis*. Proposed pathways related to this is shown on page 512 of Buchanan
 20 et al., *Biochemistry and Molecular Biology of Plants*, American Society of Plant Physiologists, 2000, ISBN 0943088372, 9780943088372.

Thus, as used herein, the phrase “fatty acid synthesis genes” or “FAS gene” refers to any genes that are involved in synthesis of fatty acids, cuticle, and wax as described above. For example, such genes include, but are not limited to, malonyl-CoA:ACP transacylase, 3-ketoacyl-ACP synthase I and III (KAS I and KAS III), 3-ketoacyl-ACP reductase, 2,3-trans-Enoyl-ACP reductase, 3-hydroxyacyl-ACP dehydratase, acyl-ACP thioesterases, fatty acid desaturases (e.g., *FAD2*, *FAD3*), fatty acid elongases (e.g., *FAE1*), hydroxylases, and enzymes displayed in Figures 7 and 13.

Seed oil of *Camelina sativa* contains high levels (up to 45%) of omega-3 fatty acids, which is uncommon in vegetable sources. Over 50% of the fatty acids in cold pressed Camelina oil are polyunsaturated. The major components are alpha-linolenic acid - C18:3 (omega-3-fatty acid, approx 35-45%) and linoleic acid - C18:2 (omega-6 fatty acid, approx 15-20%). Figure 11 shows a representative composition of *Camelina* seed oil. The oil is also very rich in natural antioxidants, such as tocopherols, making this highly stable oil very resistant to oxidation and rancidity. It has 1 - 3% erucic acid. The vitamin E content of *Camelina* oil is approximately 110 mg/100g. The present invention relates to increasing oleic acid (18:1) level, decreasing the level of long chain fatty acids, and/or improving the seed oil quality of *Camelina*. As used herein, the term “level” refers to the relative percentage of a component in a mixture.

In the endoplasmic reticulum, oleic acid (18:1) is converted to linoleic acid (18:2) by a delta-12-desaturase, fatty acid desaturase 2 (*FAD2*). Mutations in *Arabidopsis thaliana* *FAD2* have been shown to increase the levels of 18:1 in the seeds 2 – 3.4 fold while decreasing the levels of 18:2 fatty acids 4-10 fold. (Levels of 20:1 also increased approximately 1.5 fold - Okuley 1994.)

Very long chain fatty acids are synthesized in the cytosol by extension of an 18 carbon fatty acid. The rate limiting step is thought to be the initial condensation step, catalyzed in the seed by fatty acid elongase 1 (*FAE1*, Kunst 1992). In *Arabidopsis*, where approximately 25% of seed fatty acids can be long chain fatty acids, mutants in *FAE1* have less than 1%. Interestingly, *Arabidopsis fae1* mutants show a greater than 2-fold increase in 18:1 content in the seeds. (Katavic et al. (2002). "Restoring enzyme activity in nonfunctional low erucic acid *Brassica napus* fatty acid elongase 1 by a single amino acid substitution." Eur J Biochem 269(22): 5625-31.)

FAD2 and FAE1 Genes of Camelina sativa

5 The invention discloses the full genomic sequence of three *FAD2* genes and three *FAE1* genes from *Camelina sativa* with both upstream and downstream regions for *FAD2* and upstream regions for *FAE1* (deposited in Genbank at the NCBI, Genbank IDs: GU929417 – GU929422, SEQ ID NOs. 1-6). These sequences include both the coding region as well as several hundred base pairs upstream and downstream of the genes. The coding sequences for the *Camelina sativa FAD2* were obtained using primers from *Arabidopsis thaliana FAD2* while the coding regions for the *Camelina sativa FAE1* were obtained using primers from *Crambe abyssinica FAE1*. Also obtained are coding sequences for *FAD2* and *FAE1* genes from *Capsella rubella*, *A. Lyrata*, *Camelina hispida*, *Camelina laxa*, *Camelina microcarpa*, and *Camelina rumelica* (GU929423-GU929441, SEQ ID NOs 45-63), which were amplified using *C. Sativa* primers. The upstream regions for all the genes were obtained using a combination of RACE PCR and PCR with primers from upstream Arabidopsis sequences in conjunction with primers to *Camelina* sequences. The downstream regions of *FAD2* were obtained using PCR with primers designed from a combination of downstream Arabidopsis sequence in conjunction with primers to *Camelina* sequences. The *Camelina sativa FAD2* and *FAE1* genes are highly homologous to both *Arabidopsis* and *Brassica napus* (e.g., canola, oilseed rape) *FAD2* and *FAE1*. However, the disclosed sequences are specific to *Camelina sativa*.

 The present invention provides an isolated nucleic acid sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 1 to 6 and SEQ ID NOs: 45-63, and fragments and variations derived from thereof. In one embodiment, the present invention provides an isolated polynucleotide encoding plant fatty acid desaturase, comprising a nucleic acid sequence that shares at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% identity to SEQ ID NO: 1, 2, 3, 45, 46, 48, 51, 54, 55, 56, 60, and/or 61. In another embodiment, the present invention provides an isolated polynucleotide encoding fatty acid elongase, comprising a nucleic acid sequence that shares at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% identity to SEQ ID NO: 4, 5, 6, 47, 49, 50, 52, 53, 57, 58, 59, 62, and/or 63.

 Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith and Waterman (*Adv.*

5 *Appl. Math.*, 2:482, 1981); Needleman and Wunsch (*J. Mol. Biol.*, 48:443, 1970); Pearson and Lipman (*Proc. Natl. Acad. Sci.*, 85:2444, 1988); Higgins and Sharp (*Gene*, 73:237-44, 1988); Higgins and Sharp (*CABIOS*, 5:151-53, 1989); Corpet *et al.* (*Nuc. Acids Res.*, 16:10881-90, 1988); Huang *et al.* (*Comp. Appls Biosci.*, 8:155-65, 1992); and Pearson *et al.* (*Meth. Mol. Biol.*, 24:307-31, 1994). Altschul *et al.* (*Nature Genet.*, 6:119-29, 1994) presents
10 a detailed consideration of sequence alignment methods and homology calculations.

The present invention also provides a chimeric gene comprising the isolated nucleic acid sequence of any one of the polynucleotides described above operably linked to suitable regulatory sequences.

The present invention also provides a recombinant construct comprising the chimeric
15 gene as described above. In one embodiment, said recombinant construct is a gene silencing construct, such as used in RNAi gene silencing.

The expression vectors of the present invention will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance
20 genes for culturing in *E. coli* and other bacteria. Vectors that can be used with the invention comprise vectors for use in bacteria, which comprise pQE70, pQE60 and pQE-9, pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5. Among preferred eukaryotic vectors are pFastBac1 pWINEO, pSV2CAT, pOG44, pXT1 and pSG, pSVK3, pBPV, pMSG, and pSVL. Other suitable
25 vectors will be readily apparent to the skilled artisan.

The present invention also provides a transformed host cell comprising the chimeric gene as described above. In one embodiment, said host cell is selected from the group consisting of bacteria, yeasts, filamentous fungi, algae, animals, and plants.

These sequences allow the design of gene-specific primers and probes for both *FAD2*
30 and *FAE1*. Additional data demonstrates that all three copies of each gene are expressed in the seed, i.e. no one copy is silent in the seed.

Primers are short nucleic acid molecules, for instance DNA oligonucleotides, usually 7 nucleotides or more in length, for example that hybridize to contiguous complementary nucleotides or a sequence to be amplified. Longer DNA oligonucleotides may be about 15,
35 20, 25, 30 or 50 nucleotides or more in length. Primers can be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, and then the primer extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence,

5 for example, by the PCR or other nucleic-acid amplification methods known in the art, as described above.

A probe comprises an identifiable, isolated nucleic acid that recognizes a target nucleic acid sequence. A probe includes a nucleic acid that is attached to an addressable location, a detectable label or other reporter molecule and that hybridizes to a target
10 sequence. Typical labels include radioactive isotopes, enzyme substrates, co-factors, ligands, chemiluminescent or fluorescent agents, haptens, and enzymes. Methods for labelling and guidance in the choice of labels appropriate for various purposes are discussed, for example, in Sambrook *et al.* (ed.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989 and Ausubel *et al.* *Short*
15 *Protocols in Molecular Biology*, 4th ed., John Wiley & Sons, Inc., 1999.

Methods for preparing and using nucleic acid probes and primers are described, for example, in Sambrook *et al.* (ed.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; Ausubel *et al.* *Short*
20 *Protocols in Molecular Biology*, 4th ed., John Wiley & Sons, Inc., 1999; and Innis *et al.* *PCR Protocols, A Guide to Methods and Applications*, Academic Press, Inc., San Diego, CA, 1990. Amplification primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as PRIMER (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge, MA). One of ordinary skill in the art will appreciate that the specificity of a particular probe or primer increases with its length.
25 Thus, in order to obtain greater specificity, probes and primers can be selected that comprise at least 20, 25, 30, 35, 40, 45, 50 or more consecutive nucleotides of a target nucleotide sequences.

The inventor also obtained Real Time qPCR expression data that shows that *FAD2* and *FAE1* genes are expressed in the seed. In addition, SNP expression data demonstrated
30 that all three copies of *FAD2* and of *FAE1* are expressed. Data that supports these SNP results was also obtained from sequencing a cDNA library from developing *Camelina* seed.

The invention also provides an EMS mutant library that has been created in *Camelina sativa* variety CS32 (commercial name as SO30). Initial TILLING® using primers designed to the three *FAD2* genes yielded mutants in all three *FAD2* genes (Hutcheon *et al.*,
35 TILLING® for Altered Fatty Acid Profiles in *Camelina sativa*, July 2009, American Society of Plant Biologists Annual Meeting.

Preliminary analysis on lipid composition of these mutants using Gas Chromatography-Flame Ionization Detector (GC-FID) has also been conducted. In addition,

5 Tilling mutants have been identified in *FAE1* and preliminary analysis of lipid composition using GC-FID has been conducted on these mutants (Tables 19-20).

The close relationship between *C. species* and the model plant *Arabidopsis thaliana* (Al-Shehbaz, Beilstein et al. 2006; Beilstein, Al-Shehbaz et al. 2006; Beilstein, Al-Shehbaz et al. 2008) facilitates the manipulation of known pathways, such as the one regulating fatty acid biosynthesis. *C. sativa* seed oil is high in both polyunsaturated and long chain fatty acids
 10 (Budin, Breene et al. 1995; Zubr 1997; Gugel and Falk 2006), suggesting that both *FAD2* and *FAE1* are present and active. Three copies each of the *FAD2* and *FAE1* genes were isolated from an agronomic accession of *Camelina sativa* using primers designed from *Arabidopsis thaliana* or *Crambe abyssinica* sequence. Previously identified conserved sites in *FAD2*
 15 (Tocher DR 1998; McCartney, Dyer et al. 2004; Belo, Zheng et al. 2008) and *FAE1* (Ghanevati and Jaworski 2001; Moon, Smith et al. 2001; Ghanevati and Jaworski 2002) are present in all three copies of each gene and a 5' intron shown to be important in regulating *FAD2* expression in sesame (Kim, Kim et al. 2006) was identified in all three *CsFAD2* copies. Real Time qPCR data and Sequenom MassARRAY SNP analysis of the *FAD2* and
 20 *FAE1* cDNA showed that all three copies of each gene are expressed in developing seeds. Thus, it seems likely that all three copies of *FAD2* and *FAE1* in *C. sativa* are functional.

The cloning of three copies of *FAD2* and *FAE1* from the *C. sativa* genome, as well as the observation of three LFY hybridization signals by Southern analysis could be explained by at least two possible scenarios: segmental duplications of selected regions within a
 25 diploid genome either through tandem duplications or through transpositions, or whole genome duplications resulting from polyploidization. The possibility that ancient segmental duplications or transpositions affected all three examined loci seems less probable than polyploidy. Furthermore, no evidence of recent segmental duplication involving multiple genes has been observed in sequenced plant genomes (*Arabidopsis* genome (TAIR 2009,
 30 2010); rice genome (TIGR Rice Database); maize genome (Maize Genome Browser 2010); and Soybean Genome (Phytozome, 2010)).

35 *FAD2 and FAE1 Proteins of Camelina sativa*

The present invention also provides polypeptides and amino acid sequences comprising at least a portion of the isolated protein selected from the group consisting of SEQ ID NOs: 7-12, and all variants thereof.

5 The present invention also provides an isolated amino acid sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 7 to 12, and fragments and variations derived from thereof. In some embodiments, the present invention provides an isolated polypeptide comprising an amino acid sequence that shares at least about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%,
10 about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, about 99.5%, about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to SEQ ID NO: 7, 8, 9, 64, 65, 67, 70, 73, 74, 75, 79, and/or 80. In one embodiment, the present invention provides an isolated polypeptide comprising an amino acid sequence which encodes an amino acid sequence that shares at least about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about
15 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, about 99.5%, about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to SEQ ID NO: 10, 11, 12, 66, 68, 69, 71, 72, 76, 77, 78, 81, and/or 82.

 The invention also encompasses variants and fragments of proteins of FAD2 and
20 FAE1 isolated in the present invention. The variants may contain alterations in the amino acid sequences of the constituent proteins. The term “variant” with respect to a polypeptide refers to an amino acid sequence that is altered by one or more amino acids with respect to a reference sequence. The variant can have “conservative” changes, or “nonconservative” changes, *e.g.*, analogous minor variations can also include amino acid deletions or insertions,
25 or both.

 Functional fragments and variants of a polypeptide include those fragments and variants that maintain one or more functions of the parent polypeptide. It is recognized that the gene or cDNA encoding a polypeptide can be considerably mutated without materially altering one or more of the polypeptide’s functions. First, the genetic code is well-known to
30 be degenerate, and thus different codons encode the same amino acids. Second, even where an amino acid substitution is introduced, the mutation can be conservative and have no material impact on the essential function(s) of a protein. See, *e.g.*, *Stryer Biochemistry* 3rd Ed., 1988. Third, part of a polypeptide chain can be deleted without impairing or eliminating all of its functions. Fourth, insertions or additions can be made in the polypeptide chain for
35 example, adding epitope tags, without impairing or eliminating its functions (Ausubel *et al. J. Immunol.* 159(5): 2502-12, 1997). Other modifications that can be made without materially impairing one or more functions of a polypeptide can include, for example, *in vivo* or *in vitro* chemical and biochemical modifications or the incorporation of unusual amino acids. Such

5 modifications include, but are not limited to, for example, acetylation, carboxylation, phosphorylation, glycosylation, ubiquination, labelling, *e.g.*, with radionucleotides, and various enzymatic modifications, as will be readily appreciated by those well skilled in the art. A variety of methods for labelling polypeptides, and labels useful for such purposes, are well known in the art, and include radioactive isotopes such as ^{32}P , ligands which bind to or
 10 are bound by labelled specific binding partners (*e.g.*, antibodies), fluorophores, chemiluminescent agents, enzymes, and anti-ligands. Functional fragments and variants can be of varying length. For example, some fragments have at least 10, 25, 50, 75, 100, 200, or even more amino acid residues. These mutations can be natural or purposely changed. In some embodiments, mutations containing alterations that produce silent substitutions,
 15 additions, or deletions, but do not alter the properties or activities of the proteins or how the proteins are made are an embodiment of the invention.

Conservative amino acid substitutions are those substitutions that, when made, least interfere with the properties of the original protein, that is, the structure and especially the function of the protein is conserved and not significantly changed by such substitutions.
 20 Conservative substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Further information about conservative substitutions can be found, for instance, in Ben Bassat *et al.* (*J. Bacteriol.*, 169:751-757, 1987), O'Regan *et al.* (*Gene*, 77:237-251, 1989), Sahin-Toth *et al.* (*Protein Sci.*, 3:240-247, 1994), Hochuli *et al.* (*Bio/Technology*, 6:1321-1325, 1988) and
 25 in widely used textbooks of genetics and molecular biology. The Blosum matrices are commonly used for determining the relatedness of polypeptide sequences. The Blosum matrices were created using a large database of trusted alignments (the BLOCKS database), in which pairwise sequence alignments related by less than some threshold percentage
 30 identity were counted (Henikoff *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:10915-10919, 1992). A threshold of 90% identity was used for the highly conserved target frequencies of the BLOSUM90 matrix. A threshold of 65% identity was used for the BLOSUM65 matrix. Scores of zero and above in the Blosum matrices are considered "conservative substitutions" at the percentage identity selected. The following table shows exemplary conservative amino
 35 acid substitutions.

Original Residue	Very Highly - Conserved Substitutions	Highly Conserved Substitutions (from the Blosum90 Matrix)	Conserved Substitutions (from the Blosum65 Matrix)
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Ala	Ser	Gly, Ser, Thr	Cys, Gly, Ser, Thr, Val
Arg	Lys	Gln, His, Lys	Asn, Gln, Glu, His, Lys
Asn	Gln; His	Asp, Gln, His, Lys, Ser, Thr	Arg, Asp, Gln, Glu, His, Lys, Ser, Thr
Asp	Glu	Asn, Glu	Asn, Gln, Glu, Ser
Cys	Ser	None	Ala
Gln	Asn	Arg, Asn, Glu, His, Lys, Met	Arg, Asn, Asp, Glu, His, Lys, Met, Ser
Glu	Asp	Asp, Gln, Lys	Arg, Asn, Asp, Gln, His, Lys, Ser
Gly	Pro	Ala	Ala, Ser
His	Asn; Gln	Arg, Asn, Gln, Tyr	Arg, Asn, Gln, Glu, Tyr
Ile	Leu; Val	Leu, Met, Val	Leu, Met, Phe, Val
Leu	Ile; Val	Ile, Met, Phe, Val	Ile, Met, Phe, Val
Lys	Arg; Gln; Glu	Arg, Asn, Gln, Glu	Arg, Asn, Gln, Glu, Ser,
Met	Leu; Ile	Gln, Ile, Leu, Val	Gln, Ile, Leu, Phe, Val
Phe	Met; Leu; Tyr	Leu, Trp, Tyr	Ile, Leu, Met, Trp, Tyr
Ser	Thr	Ala, Asn, Thr	Ala, Asn, Asp, Gln, Glu, Gly, Lys, Thr
Thr	Ser	Ala, Asn, Ser	Ala, Asn, Ser, Val
Trp	Tyr	Phe, Tyr	Phe, Tyr
Tyr	Trp; Phe	His, Phe, Trp	His, Phe, Trp
Val	Ile; Leu	Ile, Leu, Met	Ala, Ile, Leu, Met, Thr

5

In some examples, variants can have no more than 3, 5, 10, 15, 20, 25, 30, 40, 50, or 100 conservative amino acid changes (such as very highly conserved or highly conserved amino acid substitutions). In other examples, one or several hydrophobic residues (such as Leu, Ile, Val, Met, Phe, or Trp) in a variant sequence can be replaced with a different hydrophobic residue (such as Leu, Ile, Val, Met, Phe, or Trp) to create a variant functionally similar to the disclosed FAD2 and FAE1 proteins.

In one embodiment, variants may differ from the disclosed sequences by alteration of the coding region to fit the codon usage bias of the particular organism into which the molecule is to be introduced. In other embodiments, the coding region may be altered by taking advantage of the degeneracy of the genetic code to alter the coding sequence such that, while the nucleotide sequence is substantially altered, it nevertheless encodes a protein having an amino acid sequence substantially similar to the disclosed FAD2 and FAE1 proteins.

20 *Camelina sativa* as an allohexaploid plant

The present inventors for the first time in the art demonstrates that *Camelina sativa* is an allohexaploid plant.

While not wishing to be bound to any particular theory, triplication of the *C. sativa* genome likely occurred through whole genome duplication, either through autopolyploidization or through allopolyploidization. An autopolyploidy event might

5 have triplicated a single diploid genome resulting in an autohexaploid with a haploid genome of 18, 21, or 24 chromosomes. Given that *C. sativa* has a chromosome count of $n=20$, chromosome splitting or fusion could then have increased the chromosomes from 18 to 20, or decreased the chromosomes from 21 or 24 to 20.

10 Alternatively, triplication of the *C. sativa* genome might have resulted from two allopolyploidy events, resulting in first a tetraploid then a hexaploid, similar to the origin of cultivated wheat. According to this hypothesis, the three copies of each gene diverged in different diploid genomes before converging through polyploidy events. Taking into consideration the reported chromosome counts of various *Camelina* species, the basal chromosome number of the diploid parental species contributing to the *C.*
 15 *sativa* haploid genome of 20 chromosomes could be 7+7+6 or 8+6+6. The allopolyploid hypothesis is supported by the observation that *C. sativa* demonstrates diploid inheritance (Gehringer, Friedt et al. 2006; Lu 2008), as would be expected for an allopolyploid (Sybenga 1996). A hexaploid *C. sativa* could also be derived from the combination of an autotetraploid and a diploid species if, in an autopolyploidized genome, homologous
 20 chromosomes differentiated so that the subsequent chromosome-specific pairing mimicked an allopolyploid genome in its diploid inheritance patterns. Regardless of its evolutionary path, the *C. sativa* genome appears organized in three redundant and differentiated copies and can be formally considered to be an allohexaploid.

Results from the inventors' phylogenetic analyses support a history of duplication for
 25 both *FAD2* and *FAE1* in *Camelina*. For *FAD2*, duplications were only recovered for *C. sativa*, *C. microcarpa*, and *C. rumelica*. These data are consistent with genome size data, which indicate that all three genomes are larger than *C. laxa* and *C. hispida*, from which only a single *FAD2* copy was recovered. Taken together, the results suggest that *C. sativa*, *C. microcarpa*, and *C. rumelica* are likely polyploids. Given the slightly smaller genome size of
 30 *C. rumelica*, and the fact that only two *FAD2* copies were recovered from it, the *C. rumelica* sampled may be tetraploid while *C. sativa* and *C. microcarpa* are hexaploid. Interestingly, in both the *FAD2* and *FAE1* trees, one copy each of *C. rumelica* and *C. microcarpa* are strongly supported as sister. Thus, trees from these genes indicate that *C. rumelica* and *C. microcarpa* are closely related. The various placement of *C. microcarpa* *FAD2* and *FAE1* copies can be
 35 explained if *C. microcarpa* is the result of a hybridization event between *C. rumelica* and a currently unsampled, and thus unidentified species of *Camelina*. Two of the three copies of both *FAD2* and *FAE1* are identical, or nearly identical, in *C. sativa* and *C. microcarpa*, suggesting that *C. sativa* and *C. microcarpa* share a parental genome. Thus, the inventors

5 suggest that an unsampled *Camelina* species contributed its genome to the hybrid formation of both *C. sativa* and *C. microcarpa*. In the case of *C. microcarpa*, the hybridization event likely involved *C. rumelica*. Given the chromosome count of $n=6$ for *C. rumelica*, the other putative parent would be expected to have an $x=7$ genome, and furthermore to be tetraploid at $n=14$. Such a cross would result in the observed *C. microcarpa* genome, with chromosome
10 count $n=20$. Interestingly, *C. hispida* is the only species we sampled with a chromosome count of $n=7$; however no strong relationship between *C. hispida* and *C. microcarpa* is inferred in either gene tree. However, a weak relationship between *C. sativa* and *C. hispida* is inferred from the *FAEI* tree, and thus the possibility that *C. hispida* is involved in the polyploid formation of *C. sativa* should be explored further.

15 The likely allohexaploid nature of the *Camelina sativa* genome has multiple implications. Its vigor and adaptability to marginal growth conditions may result at least in part from polyploidy. Polyploids are thought to be more adaptable to new or harsh environments, with the ability to expand into broader niches than either progenitor (Brochmann, Brysting et al. 2004; Salmon 2005). Indeed, *C. hispida* and *C. laxa*, both of
20 which are likely diploids, are found only in Turkey, Iran, Armenia, and Azerbaijan, while *C. microcarpa* and *C. sativa* are distributed throughout Asia, Europe, and North Africa and are naturalized in North America (GRIN ; Akeroyd 1993). The mechanisms behind this increased adaptability are not completely understood, but have been attributed to heterosis, genetic and regulatory network redundancies, and epigenetic factors (Comai 2005; Hegarty and Hiscock
25 2008).

Allohexaploidy might also affect any potential manipulations of the *C. sativa* genome, such as introgression of germplasm or induced mutations. Introgression of an exotic germplasm could be facilitated by the type of polyploidy-dependent manipulations that are possible in wheat, a potentially comparable allohexaploid (Gill and Friebe 1998; Dubcovsky
30 and Dvorak 2007). In addition, polyploids have displayed excellent response to reverse genomics approaches such as Targeting Induced Local Lesions in Genomes (TILLING®) (Slade, Fuerstenberg et al. 2005; Cooper, Till et al. 2008). As in wheat, any recessive induced mutations could be masked by redundant homologous loci that have maintained function (Stadler 1929; Swaminathan and Rao 1960). This implies that multiple knockout
35 alleles at different homologous sites can be combined to achieve partial or complete suppression of a targeted function (Muramatsu 1963; Li, Huang et al. 2008). We also expect that single locus traits, whether transgenic or not, will display diploid inheritance due to preferential intragenomic pairing.

5

Methods of Altering and/or Improving Camelina Seed Oil Composition

In light of the discovery that *Camelina* is an allohexaploid plant, the present invention provides methods of altering and/or improving *Camelina* seed oil composition. As used herein, the term “altering” refers to any change of fatty acid composition in the seed oil, including but not limited to compound structure, distribution, relative ratio, and yield, et al. The term “improving” refers to any change in seed oil composition that makes the seed oil composition better in one or more qualities for industrial or nutritional applications. Such improvement includes, but is not limited to, improved quality as meal, improved quality as raw material to produce biofuel, biodiesel, lubricant, more monounsaturated fatty acids and less polyunsaturated fatty acids, increased stability, lower cloud point, less NOx emissions, reduced trans-fatty acids, et al.

The quality of a biodiesel is greatly dependent upon its composition (Conley SP, Tao B: Biodiesel quality: Is All biodiesel Created Equal? Purdue University Extension; 2006). Polyunsaturated fatty acid methyl esters (FAME) have been shown to disproportionately increase oxidation of biodiesel. The temperatures at which biodiesel forms crystals (the cloud point) and at which it can no longer be poured (the pour point) are also affected by composition: saturated FAMES and long chain FAMES greatly increase cloud point and pour point. Biodiesel higher in unsaturated FAMES are therefore better in colder environments, but have a lower oxidative stability than biodiesel higher in saturated FAMES. Polyunsaturated FAMES have also been shown to result in increased NOx emissions while long chain fatty acids result in a biodiesel with too high of a distillation temperature by ASTM standards. A biodiesel high in 18:1 and low in polyunsaturated FAMES and long chain FAMES is thought to be the best compromise, resulting in higher oxidative stability with a low enough cloud point and a high enough cetane number to meet biodiesel standards (ASTM D6751).

Meal is a significant byproduct of the extraction of the oil from oilseeds for biofuel. To be able to take advantage of this byproduct as a protein supplement for livestock is essential economically for biofuel producers. In order for meal from a particular oilseed to be included in livestock feed in the US, it must be approved by the Association of American Feed Control Officials (AAFCO). The approval takes into account feeding studies in livestock and published studies on the quality of the meal and formulates a definition for the meal that is included in the annually updated AAFCO manual. Currently soybean meal is the best source for animal feed because of its favorable amino acid content and high digestibility.

5 Another widely used meal comes from Canola, an oilseed rape that has been bred to contain
< 2% erucic acid (22:1) and < 30 $\mu\text{mol/g}$ of glucosinolates. High amounts of erucic acid
have been linked to fatty deposits in the heart muscles of animals and glucosinolates lend an
unpalatable taste and confer adverse effects on growth in animals. Camelina oil has about 1-
4% erucic acid, so the development of lines with consistently <2% erucic acid is still
10 desirable. Thus the identification of *FAE1* mutants with reduced very long chain fatty acids
(VLCFA) such as 22:1 is valuable for the potential to create Camelina varieties having oil,
and thus meal, with < 2% erucic acid. Camelina meal has been tested at least in poultry, goat,
cattle (Pilgeram et al., *Camelina sativa*, A montana omega-3 and fuel crop, Issues in new
crops and new uses. 2007. J. Janick and A. Whipkey (eds.) and turkeys (Frame et al., Use of
15 *Camelina sativa* in the Diets of Young Turkeys; J. Appl. Poult. Res. 16:381-386). ASHS
Press, Alexandria, VA). Camelina meal can currently be included in the diets of broiler
chickens and feedlot beef cattle at no more than 10% (FDA, November 2009). Future
feeding studies may enable the expansion of Camelina meal to swine, laying hens and dairy
cattle.

20 In one embodiment, the methods relate to increasing monounsaturated fatty acids
(e.g., oleic acids (18:1)) level and/or reducing polyunsaturated fatty acids level in the seed oil,
wherein the method comprises disrupting and/or altering one or more copies of one or more
Camelina fatty acids synthesis genes. In some embodiments, one, two, or all three copies of
Camelina FAD2 and/or *FAE1* genes are disrupted. For example, the methods comprise
25 utilizing one or more *Camelina* mutants in any one of the mutations listed in Tables 7 to 12
described in Example 11.

In some embodiments, the methods related to increasing monounsaturated fatty acids
(e.g., oleic acids (18:1)) level and/or decreasing very long chain fatty acids (>18 carbons),
wherein the methods comprise disrupting and/or altering one or more copies of two or more
30 *Camelina* fatty acids synthesis genes. In some embodiments, one, two, or all three copies of
Camelina FAD2 and one, two, or all three *FAE1* genes are disrupted.

In some embodiments, mutations in one or more copies of *FAD2* genes and/or one or
more copies of *FAE1* genes described in the Tables 7 to 12 are integrated together to create
mutant plants with double, triple, quadruple et al. mutations. Such mutants can be created by
35 classic breeding methods.

In some embodiments, mutations described in the Tables 7-12 can be integrated into
Camelina cultivars other than Cs32 by classic breeding methods, with or without the help of
marker-facilitated gene transfer methods.

5 In some embodiments, mutations described in the Tables 7-12 can be integrated into species closely related to *Camelina sativa*, such as other species in the Brassicaceae family, such as *Brassica oleracea* (cabbage, cauliflower, etc.), *Brassica rapa* (turnip, Chinese cabbage, etc.), *Brassica napus* (rapeseed, etc.), *Raphanus sativus* (common radish), *Armoracia rusticana* (horseradish), *Matthiola* (stock), and many others, with or without the
10 help of marker-facilitated inter-cultivar gene transfer methods.

In one embodiment, mutants in Tables 7 to 12, wherein the mutants are in evolutionarily conserved regions or sites can be used to produce *Camelina* plants with improved or altered seed oil. In one embodiment, mutants in Table 7 to 12, wherein the mutant is due to nonsense mutation (premature stop codon), can be used to produce *Camelina*
15 plants with improved or altered seed oil.

In one embodiment, mutants in Tables 7 to 12, wherein the mutants are not in evolutionarily conserved regions or sites, can also be used to produce *Camelina* plants with improved or altered seed oil. Non-limiting examples of improved seed oil are those having increased oleic acid, increased fatty acids of C18 or less ($C \leq 18$), decreased very long chain
20 fatty acid ($C > 18$), and/or decreased polyunsaturated fatty acids, in ratio and/or in absolute weight. As used herein, the term " $C \leq 18$ " refers to a chemical compound having not more than 18 carbons; as used herein, the term $C > 18$ refers to a chemical compound that has more than 18 carbons.

In other embodiments, amino acids in conserved domains or sites of *Camelina* FAD2 or FAE1 proteins can be compared to FAD2 or FAE1 orthologs in other species, e.g., closely
25 related Brassicaceae species, or plant species with known FAD/FAE sequences, which do not contain mutations listed in Tables 7 to 12. Then, the FAD/FAE genes in these related species can be substituted or deleted to make mutants with reduced or abolished activity.

In one embodiment, the oleic acid level in the seed oil produced from the *Camelina*
30 plants of the present invention is increased as compared to the same plants known in the prior art (e.g., comparable wild type plant). For example, the level of oleic acid in the seed oil is increased by about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 12%, about 14%, about 16%, about 18%, about 19%, about 20%, about 22%, about 24%, about 26%, about 28%, about 30%, about 32% , about
35 34%, about 36%, about 38%, about 40% , about 42% , about 44%, about 46%, about 48%, about 50%, about 52% , about 54%, about 56% , about 58%, about 59%, about 60%, about 62%, about 64%, about 66%, about 68%, about 70% , about 75%, about 80%, about 85%,

5 about 90%, about 95%, about 100%, about 150%, about 200%, about 250%, about 300%, about 350%, about 400%, about 450%, or about 500%.

In another embodiment, the oleic acid yield in the seed oil produced per *Camelina* plant of the present invention is increased as compared to the same plants known in the prior art (e.g., comparable wild type plant). As used herein, the term “yield” refers to amount of
10 one or more types of fatty acids produced per plant, or per acre. For example, the yield of oleic acid in the seed oil is increased by about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 12%, about 14%, about 16%, about 18%, about 19%, about 20%, about 22%, about 24%, about 26%, about 28%, about 30%, about 32% , about 34%, about 36%, about 38%, about 40% , about 42% , about 44%,
15 about 46%, about 48%, about 50%, about 52% , about 54%, about 56% , about 58%, about 59%, about 60%, about 62%, about 64%, about 66%, about 68%, about 70% , about 75%, about 80%, about 85%, about 90%, about 95%, about 100%, about 150%, about 200%, about 250%, about 300%, about 350%, about 400%, about 450%, or about 500%.

In another embodiment, the polyunsaturated fatty acid level and/or yield in the seed
20 oil produced from the *Camelina* plants of the present invention is decreased as compared to the same plants known in the prior art (e.g., comparable wild type plant). For example, the level and/or yield of polyunsaturated fatty acid in the seed oil is decreased by about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 12%, about 14%, about 16%, about 18%, about 19%, about 20%, about 22%,
25 about 24%, about 26%, about 28%, about 30%, about 32% , about 34%, about 36%, about 38%, about 40% , about 42% , about 44%, about 46%, about 48%, about 50%, about 52% , about 54%, about 56% , about 58%, about 59%, about 60%, about 62%, about 64%, about 66%, about 68%, about 70% , about 75%, about 80%, about 85%, about 90%, about 95%, about 100%, about 150%, about 200%, about 250%, about 300%, about 350%, about 400%,
30 about 450%, or about 500%.

In another embodiment, the very long chain fatty acid (C>18) level and/or yield in the seed oil produced from the *Camelina* plants of the present invention is decreased as compared to the same plants known in the prior art (e.g., comparable wild type plant). For example, the level and/or yield of very long chain fatty acid in the seed oil is decreased by about 1%, about
35 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 12%, about 14%, about 16%, about 18%, about 19%, about 20%, about 22%, about 24%, about 26%, about 28%, about 30%, about 32% , about 34%, about 36%, about 38%, about 40% , about 42% , about 44%, about 46%, about 48%, about 50%, about 52% , about

5 54%, about 56% , about 58%, about 59%, about 60%, about 62%, about 64%, about 66%, about 68%, about 70% , about 75%, about 80%, about 85%, about 90%, about 95%, about 100%, about 150%, about 200%, about 250%, about 300%, about 350%, about 400%, about 450%, or about 500%.

10 In another embodiment, the fatty acids of C18 or less level and/or yield in the seed oil produced from the *Camelina* plants of the present invention is increased as compared to the same plants known in the prior art (e.g., comparable wild type plant). For example, the level and/or yield of fatty acids of C18 or less in the seed oil is increased by about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 12%, about 14%, about 16%, about 18%, about 19%, about 20%, about 22%, about 24%,
15 about 26%, about 28%, about 30%, about 32% , about 34%, about 36%, about 38%, about 40% , about 42% , about 44%, about 46%, about 48%, about 50%, about 52% , about 54%, about 56% , about 58%, about 59%, about 60%, about 62%, about 64%, about 66%, about 68%, about 70% , about 75%, about 80%, about 85%, about 90%, about 95%, about 100%, about 150%, about 200%, about 250%, about 300%, about 350%, or about 400%.

20 Molecular markers are used for the visualization of differences in nucleic acid sequences. This visualization is possible due to DNA-DNA hybridization techniques (RFLP) and/or due to techniques using the polymerase chain reaction (e.g. STS, microsatellites, AFLP, SNP, IMP et al.). All differences between two parental genotypes will segregate in a mapping population based on the cross of these parental genotypes. The segregation of the
25 different markers may be compared and recombination frequencies can be calculated. The recombination frequencies of molecular markers on different chromosomes is generally 50%. Between molecular markers located on the same chromosome the recombination frequency depends on the distance between the markers. A low recombination frequency corresponds to a low distance between markers on a chromosome. Comparing all recombination
30 frequencies will result in the most logical order of the molecular markers on the chromosomes. This most logical order can be depicted in a linkage map.

Molecular markers for the present invention, for example, can be generated by analyzing progeny of a cross between e.g., Cs32 cultivar to another *Camelina* species, e.g., *Camelina microcarpa*. The present inventors have generated such progeny and more Inter
35 MITE Polymorphisms (IMP) markers can be generated following the procedures outlined in the present application. IMP markers are developed by and exclusive to DNA LandMarks Inc. IMP markers are based on Miniature Inverted-repeat Transposable Elements (MITEs), which are short interspersed DNA transposons with terminal inverted repeats (TIRs). They

are small in size (< 500 bp), conserved TIRs, high A+T content, and consist of several distinct families such as Tourist-like, Stowaway-like. They present in plants, fungi, vertebrates, fishes, insects. In plants, they are highly associated with genes (flanking regions, introns). They are also abundant in plants (several thousand copies per genome). IMP markers have many unique advantages:

- * Naturally multiplexed – Greatly lowers cost/data point
- * Reliable – PCR based, reproducible results
- * Portable – Markers are cross-applicable in all crops
- * Practical – Useful in a variety of marker-assisted breeding functions

Similarly, Cs32 can be crossed to other species in the Brassicaceae family to generate molecular markers for further applications.

In some other embodiments, one, two, or all three copies of *Camelina FAD2* and/or *FAE1* genes, and one, two, or all three copies of other non-*FAD2*, non-*FAE1* fatty acid synthesis genes are disrupted. As used herein, the phrase “non-*FAD*, non-*FAE* fatty acid synthesis genes” refers to polynucleotides encoding polypeptides that are involved in plant fatty acid synthesis, but share less than 95% identity to *FAD2* or *FAE1* polypeptide disclosed in the present invention. In still some embodiments, one, two, or all three copies of *Camelina FAD2* and/or *FAE1* genes are disrupted, while one or more non-*FAD*, non-*FAE* fatty acid synthesis genes are overexpressed. In still more embodiments, one, two, or all three copies of *Camelina FAD2* and/or *FAE1* genes are disrupted, while one or more non-fatty-acid-synthesis genes are overexpressed and/or disrupted. As used herein, the phrase “non-fatty-acid-synthesis genes” refers to polynucleotides encoding polypeptides that are not directly involved in the synthesis of fatty acids.

According to the present invention, one skilled in the art will be able to pick preferred target genes and decide when disruption or overexpression is needed to achieve certain goals, e.g., an induction or reduction of certain fatty acids composition, based on the plant fatty acid metabolic pathways and metabolic analysis tools known in the art (e.g., MetaCyc and AraCyc database, see Zhang et al., Plant Physiology, 2005, 138:27-37). For example, one skilled in the art would be able to combine *FAD2* and/or *FAE1* loss-of-function mutants (e.g., mutants with reduced, or abolished *FAD2* and/or *FAE1* protein activity), *FAD2* and/or *FAE1* gain-of-function mutants (e.g., mutants with altered or increased *FAD2* and/or *FAE1* protein activity), or *FAD2* and/or *FAE1* overexpression with overexpression or disruption of non-*FAD*, non-*FAE* fatty acid genes to modulate the fatty acid synthesis in a plant. While not wishing to be bound by any particular theory, knock-down of *FAD2* can potentially lower

5 18:2 fatty acid; knock-down of *FAD3* can potentially lower 18:3 fatty acid; overexpressing plastidial enzyme $\Delta 9$ will give higher 18:1; knock-down of both *FAD2* and *FAD3* will contribute to a higher cloud point of the oil; knock-down of thioesterases (e.g., FAT A and/or FAT B) will lower the amount of 16:0 fatty acids; knock-down of fatty acid elongase (FAE) will lower the amount of long-chain fatty acids; a dominant negative KRP protein or a REV
10 protein can increase cell size and thus increase oil production (see US 2008/263727 and US 2007/056058).

In addition, using the compositions and methods of the present invention, one skilled in the art will be able to combine disruption of *FAD2* and/or *FAE1* genes with other mutants and/or transgenes which can generally improve plant health, plant biomass, plant resistance to
15 biotic and abiotic factors, plant yields, wherein the final preferred fatty acid production is increased. Such mutants and/or transgenes include, but are not limited to, cell cycle controlling genes, cell size controlling genes, cell division controlling genes, pathogen resistance genes, and genes controlling plant traits related to seed yield, which are well known to one skilled in the art (e.g., *REV* genes, *KRP* genes).

20 Methods of disrupting and/or altering a target gene have been known to one skilled in the art. These methods include, but are not limited to, mutagenesis (e.g., chemical mutagenesis, radiation mutagenesis, transposon mutagenesis, insertional mutagenesis, signature tagged mutagenesis, site-directed mutagenesis, and natural mutagenesis), knock-outs/knock-ins, antisense and RNA interference. Various types of mutagenesis can be used
25 to produce and/or isolate variant nucleic acids that encode for protein molecules and/or to further modify/mutate the proteins of the present invention. They include but are not limited to site-directed, random point mutagenesis, homologous recombination (DNA shuffling), mutagenesis using uracil containing templates, oligonucleotide-directed mutagenesis, phosphorothioate-modified DNA mutagenesis, mutagenesis using gapped duplex DNA or the
30 like. Additional suitable methods include point mismatch repair, mutagenesis using repair-deficient host strains, restriction-selection and restriction-purification, deletion mutagenesis, mutagenesis by total gene synthesis, double-strand break repair, and the like. Mutagenesis, e.g., involving chimeric constructs, is also included in the present invention. In one embodiment, mutagenesis can be guided by known information of the naturally occurring
35 molecule or altered or mutated naturally occurring molecule, e.g., sequence, sequence comparisons, physical properties, crystal structure or the like. For more information of mutagenesis in plants, such as agents, protocols, see Acquaah et al. (Principles of plant

5 genetics and breeding, Wiley-Blackwell, 2007, ISBN 1405136464, 9781405136464).

Methods of disrupting plant genes using RNA interference is described later in the specification.

The present invention provides methods of producing *Camelina* seed oil containing altered and/or increased levels of oleic acid (18:1), and/or altered or reduced levels of
10 polyunsaturated fatty acids, and/or decreased very long chain fatty acids (C>18). Such methods comprise utilizing the *Camelina* plants comprising the chimeric genes as described above, or *Camelina* plants with disrupted *FAD2* and/or *FAE1* genes as described above.

The present invention also provides methods of breeding *Camelina* species producing altered levels of fatty acids in the seed oil and/or meal. In one embodiment, such methods
15 comprise

- i) making a cross between the *Camelina* mutants with mutations as described above to a second *Camelina* species to make F1 plants;
- ii) backcrossing said F1 plants to said second *Camelina* species;
- iii) repeating backcrossing step until said mutations are integrated into the genome of said
20 second *Camelina* species. Optionally, such method can be facilitated by molecular markers.

The present invention provides methods of breeding species close to *Camelina sativa*, wherein said species produces altered levels of fatty acids in the seed oil and/or meal. In one embodiment, such methods comprise

- i) making a cross between the *Camelina* mutants with mutations as described above to a
25 species close to *Camelina sativa* to make F1 plants;
- ii) backcrossing said F1 plants to said species that is close to *Camelina sativa*;
- iii) repeating backcrossing step until said mutations are integrated into the genome of said species that is close to *Camelina sativa*. Special techniques (e.g., somatic hybridization) may be necessary in order to successfully transfer a gene from *Camelina sativa* to another species
30 and/or genus, such as to *B. oleracea*. Optionally, such method can be facilitated by molecular markers.

Plant Transformation

The present polynucleotides of the present invention can be transformed into a
35 *Camelina* plant, or other plants.

The most common method for the introduction of new genetic material into a plant genome involves the use of living cells of the bacterial pathogen *Agrobacterium tumefaciens* to literally inject a piece of DNA, called transfer or T-DNA, into individual plant cells

5 (usually following wounding of the tissue) where it is targeted to the plant nucleus for chromosomal integration. There are numerous patents governing *Agrobacterium* mediated transformation and particular DNA delivery plasmids designed specifically for use with *Agrobacterium*---for example, US4536475, EP0265556, EP0270822, WO8504899, WO8603516, US5591616, EP0604662, EP0672752, WO8603776, WO9209696,
10 WO9419930, WO9967357, US4399216, WO8303259, US5731179, EP068730, WO9516031, US5693512, US6051757 and EP904362A1. *Agrobacterium*-mediated plant transformation involves as a first step the placement of DNA fragments cloned on plasmids into living *Agrobacterium* cells, which are then subsequently used for transformation into individual plant cells. *Agrobacterium*-mediated plant transformation is thus an indirect plant
15 transformation method. Methods of *Agrobacterium*-mediated plant transformation that involve using vectors with no T-DNA are also well known to those skilled in the art and can have applicability in the present invention. See, for example, U.S. Patent No. 7,250,554, which utilizes P-DNA instead of T-DNA in the transformation vector.

Direct plant transformation methods using DNA have also been reported. The first of
20 these to be reported historically is electroporation, which utilizes an electrical current applied to a solution containing plant cells (M. E. Fromm et al., *Nature*, 319, 791 (1986); H. Jones et al., *Plant Mol. Biol.*, 13, 501 (1989) and H. Yang et al., *Plant Cell Reports*, 7, 421 (1988). Another direct method, called "biolistic bombardment", uses ultrafine particles, usually tungsten or gold, that are coated with DNA and then sprayed onto the surface of a plant tissue
25 with sufficient force to cause the particles to penetrate plant cells, including the thick cell wall, membrane and nuclear envelope, but without killing at least some of them (US 5,204,253, US 5,015,580). A third direct method uses fibrous forms of metal or ceramic consisting of sharp, porous or hollow needle-like projections that literally impale the cells, and also the nuclear envelope of cells. Both silicon carbide and aluminium borate whiskers
30 have been used for plant transformation (Mizuno et al., 2004; Petolino et al., 2000; US5302523 US Application 20040197909) and also for bacterial and animal transformation (Kaepler et al., 1992; Raloff, 1990; Wang, 1995). There are other methods reported, and undoubtedly, additional methods will be developed. However, the efficiencies of each of these indirect or direct methods in introducing foreign DNA into plant cells are invariably
35 extremely low, making it necessary to use some method for selection of only those cells that have been transformed, and further, allowing growth and regeneration into plants of only those cells that have been transformed.

5 For efficient plant transformation, a selection method must be employed such that whole plants are regenerated from a single transformed cell and every cell of the transformed plant carries the DNA of interest. These methods can employ positive selection, whereby a foreign gene is supplied to a plant cell that allows it to utilize a substrate present in the medium that it otherwise could not use, such as mannose or xylose (for example, refer US 10 5767378; US 5994629). More typically, however, negative selection is used because it is more efficient, utilizing selective agents such as herbicides or antibiotics that either kill or inhibit the growth of nontransformed plant cells and reducing the possibility of chimeras. Resistance genes that are effective against negative selective agents are provided on the introduced foreign DNA used for the plant transformation. For example, one of the most 15 popular selective agents used is the antibiotic kanamycin, together with the resistance gene neomycin phosphotransferase (nptII), which confers resistance to kanamycin and related antibiotics (see, for example, Messing & Vierra, *Gene* 19: 259-268 (1982); Bevan et al., *Nature* 304:184-187 (1983)). However, many different antibiotics and antibiotic resistance genes can be used for transformation purposes (refer US 5034322, US 6174724 and US 20 6255560). In addition, several herbicides and herbicide resistance genes have been used for transformation purposes, including the bar gene, which confers resistance to the herbicide phosphinothricin (White et al., *Nucl Acids Res* 18: 1062 (1990), Spencer et al., *Theor Appl Genet* 79: 625-631(1990), US 4795855, US 5378824 and US 6107549). In addition, the dhfr gene, which confers resistance to the anticancer agent methotrexate, has been used for 25 selection (Bourouis et al., *EMBO J.* 2(7): 1099-1104 (1983).

The expression control elements used to regulate the expression of a given protein can either be the expression control element that is normally found associated with the coding sequence (homologous expression element) or can be a heterologous expression control element. A variety of homologous and heterologous expression control elements are known 30 in the art and can readily be used to make expression units for use in the present invention. Transcription initiation regions, for example, can include any of the various opine initiation regions, such as octopine, mannopine, nopaline and the like that are found in the Ti plasmids of *Agrobacterium tumefaciens*. Alternatively, plant viral promoters can also be used, such as the cauliflower mosaic virus 19S and 35S promoters (CaMV 19S and CaMV 35S promoters, 35 respectively) to control gene expression in a plant (U.S. Patent Nos. 5,352,605; 5,530,196 and 5,858,742 for example). Enhancer sequences derived from the CaMV can also be utilized (U.S. Patent Nos. 5,164,316; 5,196,525; 5,322,938; 5,530,196; 5,352,605; 5,359,142; and 5,858,742 for example). Lastly, plant promoters such as *prolifera* promoter, fruit specific

5 promoters, Ap3 promoter, heat shock promoters, seed specific promoters, etc. can also be used.

10 Either a gamete-specific promoter, a constitutive promoter (such as the CaMV or Nos promoter), an organ-specific promoter (such as the E8 promoter from tomato), or an inducible promoter is typically ligated to the protein or antisense encoding region using standard techniques known in the art. The expression unit may be further optimized by employing supplemental elements such as transcription terminators and/or enhancer elements. For example, the 5' introns of *FAD2* gene in sesame have been demonstrated to increase and/or regulate expression of certain genes (Kim et al. 2006. Mol Genet Genomics 276(4): 351-68). Thus, the 5' intron sequences of the *FAD2* genes of the present invention
15 can be used to increase expression of either a *FAD2* or a non-*FAD2* gene. The expression cassette can comprise, for example, a seed-specific promoter (e.g. the phaseolin promoter (U.S. Pat. No. 5,504,200). The term "seed-specific promoter", means that a gene expressed under the control of the promoter is predominantly expressed in plant seeds with no or no substantial expression, typically less than 10% of the overall expression level, in other plant
20 tissues. Seed specific promoters have been well known in the art, for example, US Patent Nos. 5623067, 5717129, 6403371, 6566584, 6642437, 6777591, 7081565, 7157629, 7192774, 7405345, 7554006, 7589252, 7595384, 7619135, 7642346, and US Application Publication Nos. 20030005485, 20030172403, 20040088754, 20040255350, 20050125861, 20050229273, 20060191044, 20070022502, 20070118933, 20070199098, 20080313771, and
25 20090100551.

Thus, for expression in plants, the expression units will typically contain, in addition to the protein sequence, a plant promoter region, a transcription initiation site and a transcription termination sequence. Unique restriction enzyme sites at the 5' and 3' ends of the expression unit are typically included to allow for easy insertion into a pre-existing
30 vector.

In the construction of heterologous promoter/structural gene or antisense combinations, the promoter is preferably positioned about the same distance from the heterologous transcription start site as it is from the transcription start site in its natural setting. As is known in the art, however, some variation in this distance can be
35 accommodated without loss of promoter function.

In addition to a promoter sequence, the expression cassette can also contain a transcription termination region downstream of the structural gene to provide for efficient termination. The termination region may be obtained from the same gene as the promoter

5 sequence or may be obtained from different genes. If the mRNA encoded by the structural gene is to be efficiently processed, DNA sequences which direct polyadenylation of the RNA are also commonly added to the vector construct. Polyadenylation sequences include, but are not limited to the *Agrobacterium* octopine synthase signal (Gielen *et al.*, EMBO J 3:835-846 (1984)) or the nopaline synthase signal (Depicker *et al.*, Mol. and Appl. Genet. 1:561-573 (1982)). The resulting expression unit is ligated into or otherwise constructed to be included
10 in a vector that is appropriate for higher plant transformation. One or more expression units may be included in the same vector. The vector will typically contain a selectable marker gene expression unit by which transformed plant cells can be identified in culture. Usually, the marker gene will encode resistance to an antibiotic, such as G418, hygromycin,
15 bleomycin, kanamycin, or gentamicin or to an herbicide, such as glyphosate (Round-Up) or glufosinate (BASTA) or atrazine. Replication sequences, of bacterial or viral origin, are generally also included to allow the vector to be cloned in a bacterial or phage host; preferably a broad host range for prokaryotic origin of replication is included. A selectable marker for bacteria may also be included to allow selection of bacterial cells bearing the
20 desired construct. Suitable prokaryotic selectable markers include resistance to antibiotics such as ampicillin, kanamycin or tetracycline. Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art. For instance, in the case of *Agrobacterium* transformations, T-DNA sequences will also be included for subsequent transfer to plant chromosomes.

25 To introduce a desired gene or set of genes by conventional methods requires a sexual cross between two lines, and then repeated back-crossing between hybrid offspring and one of the parents until a plant with the desired characteristics is obtained. This process, however, is restricted to plants that can sexually hybridize, and genes in addition to the desired gene will be transferred.

30 Recombinant DNA techniques allow plant researchers to circumvent these limitations by enabling plant geneticists to identify and clone specific genes for desirable traits, such as improved fatty acid composition, and to introduce these genes into already useful varieties of plants. Once the foreign genes have been introduced into a plant, that plant can then be used in conventional plant breeding schemes (e.g., pedigree breeding, single-seed-descent
35 breeding schemes, reciprocal recurrent selection) to produce progeny which also contain the gene of interest.

Genes can be introduced in a site directed fashion using homologous recombination. Homologous recombination permits site-specific modifications in endogenous genes and thus

inherited or acquired mutations may be corrected, and/or novel alterations may be engineered into the genome. Homologous recombination and site-directed integration in plants are discussed in, for example, U.S. Patent Nos. 5,451,513; 5,501,967 and 5,527,695.

Methods of producing transgenic plants are well known to those of ordinary skill in the art. Transgenic plants can now be produced by a variety of different transformation methods including, but not limited to, electroporation; microinjection; microprojectile bombardment, also known as particle acceleration or biolistic bombardment; viral-mediated transformation; and *Agrobacterium*-mediated transformation. See, for example, U.S. Patent Nos. 5,405,765; 5,472,869; 5,538,877; 5,538,880; 5,550,318; 5,641,664; 5,736,369 and 5,736,369; International Patent Application Publication Nos. WO2002/038779 and WO/2009/117555; Lu et al., (Plant Cell Reports, 2008, 27:273-278); Watson et al., Recombinant DNA, Scientific American Books (1992); Hinchey et al., Bio/Tech. 6:915-922 (1988); McCabe et al., Bio/Tech. 6:923-926 (1988); Toriyama et al., Bio/Tech. 6:1072-1074 (1988); Fromm et al., Bio/Tech. 8:833-839 (1990); Mullins et al., Bio/Tech. 8:833-839 (1990); Hiei et al., Plant Molecular Biology 35:205-218 (1997); Ishida et al., Nature Biotechnology 14:745-750 (1996); Zhang et al., Molecular Biotechnology 8:223-231 (1997); Ku et al., Nature Biotechnology 17:76-80 (1999); and, Raineri et al., Bio/Tech. 8:33-38 (1990)).

Agrobacterium tumefaciens is a naturally occurring bacterium that is capable of inserting its DNA (genetic information) into plants, resulting in a type of injury to the plant known as crown gall. Most species of plants can now be transformed using this method, including cucurbitaceous species.

Microprojectile bombardment is also known as particle acceleration, biolistic bombardment, and the gene gun (Biolistic® Gene Gun). The gene gun is used to shoot pellets that are coated with genes (e.g., for desired traits) into plant seeds or plant tissues in order to get the plant cells to then express the new genes. The gene gun uses an actual explosive (.22 caliber blank) to propel the material. Compressed air or steam may also be used as the propellant. The Biolistic® Gene Gun was invented in 1983-1984 at Cornell University by John Sanford, Edward Wolf, and Nelson Allen. It and its registered trademark are now owned by E. I. du Pont de Nemours and Company. Most species of plants have been transformed using this method.

A transgenic plant formed using *Agrobacterium* transformation methods typically contains a single gene on one chromosome, although multiple copies are possible. Such transgenic plants can be referred to as being hemizygous for the added gene. A more

5 accurate name for such a plant is an independent segregant, because each transformed plant represents a unique T-DNA integration event (U.S. Patent No. 6,156,953). A transgene locus is generally characterized by the presence and/or absence of the transgene. A heterozygous genotype in which one allele corresponds to the absence of the transgene is also designated hemizygous (U.S. Patent No. 6,008,437).

10 *Breeding Methods*

Classic breeding methods can be included in the present invention to introduce one or more mutations of the present invention into other *Camelina* varieties, or other close-related species of the Brassicaceae family that are compatible to be crossed with *Camelina*. In one
15 embodiment, the mutations are on the *FAD2 A*, *FAD2 B*, and/or *FAD2 C* genes. In one embodiment, the mutations are on the *FAE1 A*, *FAE1 B*, and/or *FAE1 C* genes. In one embodiment, the mutations are on any *FAD2* gene and/or any *FAE1* gene.

Open-Pollinated Populations. The improvement of open-pollinated populations of such crops as rye, many maizes and sugar beets, herbage grasses, legumes such as alfalfa and
20 clover, and tropical tree crops such as cacao, coconuts, oil palm and some rubber, depends essentially upon changing gene-frequencies towards fixation of favorable alleles while maintaining a high (but far from maximal) degree of heterozygosity. Uniformity in such populations is impossible and trueness-to-type in an open-pollinated variety is a statistical feature of the population as a whole, not a characteristic of individual plants. Thus, the
25 heterogeneity of open-pollinated populations contrasts with the homogeneity (or virtually so) of inbred lines, clones and hybrids.

Population improvement methods fall naturally into two groups, those based on purely phenotypic selection, normally called mass selection, and those based on selection with progeny testing. Interpopulation improvement utilizes the concept of open breeding
30 populations; allowing genes to flow from one population to another. Plants in one population (cultivar, strain, ecotype, or any germplasm source) are crossed either naturally (e.g., by wind) or by hand or by bees (commonly *Apis mellifera* L. or *Megachile rotundata* F.) with plants from other populations. Selection is applied to improve one (or sometimes both) population(s) by isolating plants with desirable traits from both sources.

35 There are several primary methods of open-pollinated population improvement. First, there is the situation in which a population is changed en masse by a chosen selection procedure. The outcome is an improved population that is indefinitely propagable by random-mating within itself in isolation. Second, the synthetic variety attains the same end

5 result as population improvement but is not itself propagable as such; it has to be reconstructed from parental lines or clones. Third, a method used in plant species that are largely self pollinated in nature, such as soybeans, wheat, rice, safflower, camelina and others is pedigree selection. In this situation, crosses are made and individual plants and lines from individual plants are selected for desired traits. These lines are then advanced as genetically
10 homogeneous varieties. Since the individuals are largely self pollinated these lines are analogous to an inbred line with favorable agronomic characteristics. These plant breeding procedures for improving open-pollinated populations are well known to those skilled in the art and comprehensive reviews of breeding procedures routinely used for improving cross-pollinated plants are provided in numerous texts and articles, including: Allard, *Principles of*
15 *Plant Breeding*, John Wiley & Sons, Inc. (1960); Simmonds, *Principles of Crop Improvement*, Longman Group Limited (1979); Hallauer and Miranda, *Quantitative Genetics in Maize Breeding*, Iowa State University Press (1981); and, Jensen, *Plant Breeding Methodology*, John Wiley & Sons, Inc. (1988).

Mass Selection. In mass selection, desirable individual plants are chosen, harvested,
20 and the seed composited without progeny testing to produce the following generation. Since selection is based on the maternal parent only, and there is no control over pollination, mass selection amounts to a form of random mating with selection. As stated above, the purpose of mass selection is to increase the proportion of superior genotypes in the population.

Synthetics. A synthetic variety is produced by crossing inter se a number of
25 genotypes selected for good combining ability in all possible hybrid combinations, with subsequent maintenance of the variety by open pollination. Whether parents are (more or less inbred) seed-propagated lines, as in some sugar beet and beans (*Vicia*) or clones, as in herbage grasses, clovers and alfalfa, makes no difference in principle. Parents are selected on general combining ability, sometimes by test crosses or topcrosses, more generally by
30 polycrosses. Parental seed lines may be deliberately inbred (e.g. by selfing or sib crossing). However, even if the parents are not deliberately inbred, selection within lines during line maintenance will ensure that some inbreeding occurs. Clonal parents will, of course, remain unchanged and highly heterozygous.

Whether a synthetic can go straight from the parental seed production plot to the
35 farmer or must first undergo one or two cycles of multiplication depends on seed production and the scale of demand for seed. In practice, grasses and clovers are generally multiplied once or twice and are thus considerably removed from the original synthetic.

5 While mass selection is sometimes used, progeny testing is generally preferred for polycrosses, because of their operational simplicity and obvious relevance to the objective, namely exploitation of general combining ability in a synthetic.

The number of parental lines or clones that enter a synthetic vary widely. In practice, numbers of parental lines range from 10 to several hundred, with 100-200 being the average.
10 Broad based synthetics formed from 100 or more clones would be expected to be more stable during seed multiplication than narrow based synthetics.

Pedigreed varieties. A pedigreed variety is a superior genotype developed from selection of individual plants out of a segregating population followed by propagation and seed increase of self pollinated offspring and careful testing of the genotype over several
15 generations. This is an open pollinated method that works well with naturally self pollinating species. This method can be used in combination with mass selection in variety development. Variations in pedigree and mass selection in combination are the most common methods for generating varieties in self pollinated crops.

Hybrids. A hybrid is an individual plant resulting from a cross between parents of
20 differing genotypes. Commercial hybrids are now used extensively in many crops, including corn (maize), sorghum, sugarbeet, sunflower and broccoli. Hybrids can be formed in a number of different ways, including by crossing two parents directly (single cross hybrids), by crossing a single cross hybrid with another parent (three-way or triple cross hybrids), or by crossing two different hybrids (four-way or double cross hybrids).

25 Strictly speaking, most individuals in an out breeding (i.e., open-pollinated) population are hybrids, but the term is usually reserved for cases in which the parents are individuals whose genomes are sufficiently distinct for them to be recognized as different species or subspecies. Hybrids may be fertile or sterile depending on qualitative and/or quantitative differences in the genomes of the two parents. Heterosis, or hybrid vigor, is
30 usually associated with increased heterozygosity that results in increased vigor of growth, survival, and fertility of hybrids as compared with the parental lines that were used to form the hybrid. Maximum heterosis is usually achieved by crossing two genetically different, highly inbred lines.

The production of hybrids is a well-developed industry, involving the isolated
35 production of both the parental lines and the hybrids which result from crossing those lines. For a detailed discussion of the hybrid production process, see, e.g., Wright, *Commercial Hybrid Seed Production* 8:161-176, In Hybridization of Crop Plants.

5 *RNA Interference (RNAi)*

RNA interference (RNAi) is the process of sequence-specific, post-transcriptional gene silencing or transcriptional gene silencing in animals and plants, initiated by double-stranded RNA (dsRNA) that is homologous in sequence to the silenced gene. The preferred RNA effector molecules useful in this invention must be sufficiently distinct in sequence
10 from any host polynucleotide sequences for which function is intended to be undisturbed after any of the methods of this invention are performed. Computer algorithms may be used to define the essential lack of homology between the RNA molecule polynucleotide sequence and host, essential, normal sequences.

The term “dsRNA” or “dsRNA molecule” or “double-strand RNA effector molecule”
15 refers to an at least partially double-strand ribonucleic acid molecule containing a region of at least about 19 or more nucleotides that are in a double-strand conformation. The double-stranded RNA effector molecule may be a duplex double-stranded RNA formed from two separate RNA strands or it may be a single RNA strand with regions of self-complementarity capable of assuming an at least partially double-stranded hairpin conformation (i.e., a hairpin
20 dsRNA or stem-loop dsRNA). In various embodiments, the dsRNA consists entirely of ribonucleotides or consists of a mixture of ribonucleotides and deoxynucleotides, such as RNA/DNA hybrids. The dsRNA may be a single molecule with regions of self-complementarity such that nucleotides in one segment of the molecule base pair with nucleotides in another segment of the molecule. In one aspect, the regions of self-
25 complementarity are linked by a region of at least about 3-4 nucleotides, or about 5, 6, 7, 9 to 15 nucleotides or more, which lacks complementarity to another part of the molecule and thus remains single-stranded (i.e., the “loop region”). Such a molecule will assume a partially double-stranded stem-loop structure, optionally, with short single stranded 5' and/or 3' ends. In one aspect the regions of self-complementarity of the hairpin dsRNA or the
30 double-stranded region of a duplex dsRNA will comprise an Effector Sequence and an Effector Complement (e.g., linked by a single-stranded loop region in a hairpin dsRNA). The Effector Sequence or Effector Strand is that strand of the double-stranded region or duplex which is incorporated in or associates with RISC. In one aspect the double-stranded RNA effector molecule will comprise an at least 19 contiguous nucleotide effector sequence,
35 preferably 19 to 29, 19 to 27, or 19 to 21 nucleotides, which is a reverse complement to the RNA of *Camelina* genes (e.g., *FAD2* and *FAEI* genes), or an opposite strand replication intermediate. In one embodiment, said double-stranded RNA effector molecules are provided by providing to a *Camelina* plant, plant tissue, or plant cell an expression construct

5 comprising one or more double-stranded RNA effector molecules. In one embodiment, the expression construct comprises a double-strand RNA derived from any one of SEQ ID NOs 1-6 and SEQ ID NOs 45-63. In other embodiments, the expression construct comprises a double-strand RNA derived from more than one sequences of SEQ ID NOs 1-6 and SEQ ID NOs 45-63. In further embodiments, the expression construct comprises a double-strand
10 RNA derived from more than one sequences of SEQ ID NOs 1-6 and SEQ ID NOs 45-63, and one or more other genes involved in plant fatty acid synthesis. One skilled in the art will be able to design suitable double-strand RNA effector molecule based on the nucleotide sequences of *Camelina FAD2* and *FAE1* in the present invention and other *Camelina* fatty acid synthesis genes known in the art.

15 In some embodiments, the dsRNA effector molecule of the invention is a “hairpin dsRNA”, a “dsRNA hairpin”, “short-hairpin RNA” or “shRNA”, i.e., an RNA molecule of less than approximately 400 to 500 nucleotides (nt), or less than 100 to 200 nt, in which at least one stretch of at least 15 to 100 nucleotides (e.g., 17 to 50 nt, 19 to 29 nt) is base paired with a complementary sequence located on the same RNA molecule (single RNA strand), and
20 where said sequence and complementary sequence are separated by an unpaired region of at least about 4 to 7 nucleotides (or about 9 to about 15 nt, about 15 to about 100 nt, about 100 to about 1000 nt) which forms a single-stranded loop above the stem structure created by the two regions of base complementarity. The shRNA molecules comprise at least one stem-loop structure comprising a double-stranded stem region of about 17 to about 500 bp; about 17 to about 50 bp; about 40 to about 100 bp; about 18 to about 40 bp; or from about 19 to about 29
25 bp; homologous and complementary to a target sequence to be inhibited; and an unpaired loop region of at least about 4 to 7 nucleotides, or about 9 to about 15 nucleotides, about 15 to about 100 nt, about 250-500bp, about 100 to about 1000 nt, which forms a single-stranded loop above the stem structure created by the two regions of base complementarity. It will be
30 recognized, however, that it is not strictly necessary to include a “loop region” or “loop sequence” because an RNA molecule comprising a sequence followed immediately by its reverse complement will tend to assume a stem-loop conformation even when not separated by an irrelevant “stuffer” sequence.

The expression construct of the present invention comprising DNA sequence which
35 can be transcribed into one or more double-stranded RNA effector molecules can be transformed into a *Camelina* plant, wherein the transformed plant produces different fatty acid compositions than the untransformed plant. The target sequence to be inhibited by the dsRNA effector molecule include, but are not limited to, coding region, 5' UTR region, 3'

5' UTR region of fatty acids synthesis genes. In one embodiment, the target sequence is from one or more *Camelina FAD2* and/or *FAE1* genes.

The effects of RNAi can be both systemic and heritable in plants. In plants, RNAi is thought to propagate by the transfer of siRNAs between cells through plasmodesmata. The heritability comes from methylation of promoters targeted by RNAi; the new methylation pattern is copied in each new generation of the cell. A broad general distinction between plants and animals lies in the targeting of endogenously produced miRNAs; in plants, miRNAs are usually perfectly or nearly perfectly complementary to their target genes and induce direct mRNA cleavage by RISC, while animals' miRNAs tend to be more divergent in sequence and induce translational repression. Detailed methods for RNAi in plants are described in David Allis et al (Epigenetics, CSHL Press, 2007, ISBN 0879697245, 9780879697242), Sohail et al (Gene silencing by RNA interference: technology and application, CRC Press, 2005, ISBN 0849321417, 9780849321412), Engelke et al. (RNA Interference, Academic Press, 2005, ISBN 0121827976, 9780121827977), and Doran et al. (RNA Interference: Methods for Plants and Animals, CABI, 2009, ISBN 1845934105, 9781845934101).

The present invention is further illustrated by the following examples that should not be construed as limiting. The contents of all references, patents, and published patent applications cited throughout this application, as well as the Figures, are incorporated herein by reference in their entirety for all purposes.

EXAMPLE

30 Example 1.

Methods and materials

Southern blot

Camelina sativa Cs32 and Cs11, and *Arabidopsis thaliana* ecotype Col-0 (Table 2) seeds were germinated on Arabidopsis Growth Media (1x Murashige and Skoog (MS) mineral salts, 0.5 g/L MES, 0.8% PhytaBlend™ all from Caisson Labs, North Logan, UT; pH5.7) and allowed to grow for ~2 weeks under 16/8 hours day/night, 22/18°C, and ~130 $\mu\text{E m}^{-2} \text{ s}^{-1}$ light intensity. Genomic DNA was isolated according to the CTAB method (Saghai-Marooof, Soliman et al. 1984) and 10 μg was digested overnight (~16h) with EcoRI or

5 a combination of EcoRI plus BamHI. DNA electrophoresis and blotting were carried out using standard molecular biology techniques (Tom Maniatis 1982). The probe was labelled with α -32P dCTP according to instructions of the DECAprime II kit (Ambion, Austin, TX). Hybridization was carried out overnight at 42°C. The blot was washed (30 minutes each) at 42°C in 2X SSC, 0.1% SDS, followed by 55°C in 2X SSC, 0.1% SDS, and then 55°C in 0.1X
 10 SSC, 1% SDS, and exposed to a phosphorimager screen. The blot was hybridized with different probes after stripping the membrane in boiling 0.1% SDS for 20 minutes each time.

Cloning of C. sativa FAD2 and FAE1 genes and upstream regions.

FAD2 and *FAE1* genes were amplified from *C. sativa* Cs32 DNA isolated as
 15 described above, using Phusion polymerase (New England Biolabs, Ipswich, MA) and the primers listed in Table 3, according to the manufacturer's directions. The amplified fragments were cloned using the Zero Blunt PCR Cloning kit (Invitrogen, Carlsbad, CA.)

FAD2 and FAE1 Sequence alignments

20 Translated amino acid *FAD2* and *FAE1* sequences were aligned with AlignX (Invitrogen), with a gap opening penalty of 15, a gap extension penalty of 6.66, and a gap separation penalty range of 8. Alignments were imported into Boxshade (EMBNet) to highlight the conserved residues.

25 *RNA isolation and cDNA preparation*

C. sativa Cs32 plants were grown under 24/18°C day/night conditions with a 16/8 hour photoperiod. Flowers were tagged and embryos harvested at the time points indicated. RNA was then isolated using the urea LiCl method described by Tai *et al* (Tai, Pelletier et al. 2004). cDNA were prepared from 0.5 µg of DNAsed RNA that was reverse transcribed with
 30 the High Capacity cDNA RT kit (Applied Biosystems, Foster City, CA) using random primers according to the manufacturer's instructions.

Quantitative Real-time PCR

Relative expression of *FAD2* and *FAE1* cDNA was measured by real-time
 35 quantitative PCR and calculated according to the comparative C_T method ($2^{-\Delta\Delta C_T}$). In brief, separate reactions were prepared in duplicate or triplicate for each of the genes to be measured. Each reaction contained 8 µl of the appropriate primers (200 nM each) and probe

5 (900 nM) for Cs *ACTIN* (reference gene) or Cs *FAD2* or *FAE1* (target gene); 10 µl of Applied Biosystems 2X fast Taqman PCR mix; 2 µl of cDNA. The reactions were run on an Applied Biosystems 7900HT according to the manufacturer's fast PCR method. Real-time primers and probes are listed in Table 4.

10 *Relative Expression Analysis*

Three single nucleotide polymorphisms (SNPs) for each of *FAD2* A, B, and C and *FAE1* A, B, and C were identified. Each identified SNP distinguishes one copy from the other two. An additional SNP, which distinguishes *FAE1* A, B, and C copies from each other, was also identified (Table 5). SNP frequencies were determined in cDNA isolated as
 15 described above by the Sequenom MassARRAY™ allele-specific expression analysis method with no competitor, as described in Park *et al* (Park, Correll et al. 2004).

Genome size estimation

Camelina lines (Table 2) were grown in the greenhouse at temperatures fluctuating
 20 between 16 and 26 C with 16 hour day length supplemented by halogen lights. The nuclei were extracted from leaves according to Henry *et al* [74]. Nuclei were also extracted from approximately 50 seeds of all species, except *C. laxa* and *C. hispida*, which are late flowering. The seeds were crushed with a pestle in 1.4 mL of the same extraction buffer used for the leaves. The fluid was then drawn through four layers of cheesecloth and strained and
 25 processed as for the leaf nuclei. Nuclei of diploid and tetraploids of *Arabidopsis thaliana* accession Col-0 (1 C genome size 157 Mb, and 314 Mb, respectively[75]), and tetraploid *Arabidopsis arenosa* accession Care-1 (1C genome size 480 Mb [Dilkes, unpublished results]) were used as standards for DNA content. Data was collected on two
 30 different days and normalized separately to account for daily fluctuations in flow cytometer performance. The 2C, 4C, and 8C nuclear peaks were used in a regression analysis of measured fluorescence intensity versus nuclear DNA content, producing equations of genome size versus fluorescence that were used to estimate the 2C content of *Camelina* nuclei.

Phylogenetic Inference

35 *FAD2* and *FAE1* were PCR amplified from several *Camelina* species and other species from the tribe Camelinae (Table 2) using primers designed from *C. sativa* *FAD2* and *FAE1* sequences (Table 3). Amplified fragments for *FAD2* and *FAE1* were cloned as described for *C. sativa* above, then aligned by translated amino acids sequences using

5 MacClade 4.05 (Maddison 2004.), ModelTest 3.7 (Posada and Crandall 1998) in PAUP* 4.0b (Swofford 2001) was used to determine the model of sequence evolution favored by the data for each gene. Subsequent maximum likelihood (ML) analyses were performed in PAUP* 4.0b using a heuristic search with tree bisection reconnection (TBR) branch swapping. ML clade support using 100 bootstrap data sets were assessed and this support is presented on the
10 most likely tree recovered from the ML heuristic search.

Camelina alkaline transesterification for FAMES composition and gas chromatography (GC/FID) analysis of Camelina seeds

Approximately 50 mg of seeds were ground up in liquid nitrogen with mortar and
15 pestle. 5mL of 0.2M KOH in methanol was added to each vial containing the ground seeds. Samples were capped, heated at 37C for 1hr and vortexed every 10 minutes. Reaction was stopped with addition of 1mL 1M acetic acid and 2mL heptanes. Samples were vortexed, and then centrifuged for 10 min at room temp at 2990 rpm and the upper organic phase was collected. Before GC analysis, samples were diluted 1/10 in heptanes.

20 The supernatant was transferred to a GC vial, in which 1μL was used for GC analysis. Analysis was carried out on GC/FID 7890A series with a SP_2330 column. Injector and detector temperature were 250°C and 300°C respectively; oven temperature was held at 50°C for 2 min, then programmed to 180°C at a heating rate of 10°C /min, then programmed to hold for 5 min followed by an increase of 5°C/min to 240°C. Total run time
25 was 32.5 min. Flow rates for hydrogen and air to the FID were 30 and 450 mL/min respectively. Helium as the carrier gas flowed at a rate of 1.69mL/min and nitrogen as the make-up gas at 30mL/min.

Example 2.

30 **Southern blot hybridizations show multiple copies of genes in *Camelina sativa***

As a first step to characterize genes involved in fatty acid biosynthesis, the inventors determined the copy number of *FAD2* and *FAE1* by Southern blot analysis. Since *C. sativa* is closely related to *Arabidopsis thaliana* (Al-Shehbaz, Beilstein et al. 2006; Beilstein, Al-Shehbaz et al. 2006; Beilstein, Al-Shehbaz et al. 2008), the inventors designed primers based
35 on Arabidopsis that amplified conserved regions of *FAD2* and *FAE1*. Using these primers, the inventors PCR amplified products of 225 base pairs (bp) (*FAD2*) and 403 bp (*FAE1*) from Arabidopsis and from *C. sativa*. The *C. sativa* products were cloned, sequenced, and

5 compared with Arabidopsis *FAD2* and *FAE1* sequences (TAIR 2009) to confirm their identities. The inventors used the *C. sativa* fragments as probes in Southern blot experiments (Figure 1). Results of the Southern blots revealed three bands in *C. sativa* for both *FAD2* (Fig. 1A) and *FAE1* (Fig. 1B), whereas hybridization revealed only a single band in Arabidopsis for both genes (Fig. 1A & B). These results suggest that *FAD2* and *FAE1* occur
 10 in at least three copies in *C. sativa*, while they are single copy in Arabidopsis (TAIR 2009). Fatty acid genes can be multi-copy in many species, including soybean (Schlueter, Lin et al. 2007), *Brassica napus* (Scheffler, Sharpe et al. 1997), olive (*Olea europaea*) (Hernandez, Mancha et al. 2005), maize (Mikkilineni and Rocheford 2003), and sunflower (Martínez-Rivas, Sperling et al. 2001). Therefore, the inventors designed a probe for Southern blot
 15 hybridization of the gene *LEAFY* (*LFY*), which is known to be single copy in a wide variety of species from several plant families (Frohlich and Estabrook 2000). Three bands were observed following hybridization using the *LFY* probe, suggesting *LFY* also exists as three copies in *C. sativa* (Fig. 1C).

20 Example 3

Copies of *C. sativa* *FAD2* and *FAE1* are highly similar to each other and to their putative orthologs from Arabidopsis

The inventors cloned and sequenced the full length genomic and cDNA sequences of *C. sativa* *FAD2* and *FAE1* (SEQ ID NOs: 1 to 6). Using primers designed from Arabidopsis
 25 *FAD2* and *Crambe abyssinica* *FAE1*, the inventors PCR amplified a band of approximately 1.2kb for *FAD2* and 1.5kb for *FAE1* from *C. sativa*. For each gene, the inventors sequenced more than 60 clones. Three different versions of both *FAD2* and *FAE1* were recovered and designated A, B, and C. It should be noted that the A, B, and C copies were named independently for *FAD2* and *FAE1*, and thus are not associated with a particular genome.

30 The three copies of *C. sativa* *FAD2* are 1155 bp long, lack introns in the coding regions, are 97% identical at the nucleotide level, and encode proteins that are 99% identical in sequence (Table 1). One of the *FAD2* copies contains a BamHI site, and thus this copy likely produced the ~1.3 kb fragment in the Southern blot hybridization of *FAD2* (Figure 1A; BamHI + EcoRI digest). The *C. sativa* nucleotide sequences of *FAD2* are greater than 93%
 35 identical to Arabidopsis *FAD2*, and the putative encoded proteins from the two species share greater than 96% identity (Table 1).

An approximately 1.4 kb intron found within the 5' untranslated region was also recovered from all three copies of *C. sativa* *FAD2*. A similarly sized intron is present in

5 Arabidopsis (TAIR 2009) and in *Sesamum indicum* (sesame) where it has been shown to be involved in regulating *FAD2* expression (Kim et al. 2006).

All three copies of *FAE1* in *C. sativa* are 1518 bp long and lack introns. When the nucleotide sequences and the putative encoded proteins of the three copies are compared they are more than 96% identical (Table 1). In comparison to Arabidopsis, the nucleotide
10 sequences are more than 90% identical, while the encoded proteins are more than 91% identical (Table 1). Thus, the three copies of *C. sativa FAD2* and the three copies of *FAE1* are highly similar to each other and to their putative orthologs from Arabidopsis.

Example 4

15 **Alignments of FAD2 and FAE1 protein sequences from several species reveals conserved and non-conserved domains**

The inventors aligned translated amino acid sequences from the three copies of *C. sativa FAD2* with the *FAD2* protein sequences from Arabidopsis; *Brassica rapa*, an agronomically important member of the Brassicaceae family; *Glycine max*, an agronomically
20 important dicot; and *Zea mays*, an agronomically important monocot (Figure 2A). All three copies of *C. sativa FAD2* have the three conserved HIS boxes found in all membrane-bound desaturases (Tocher DR 1998) as well as the ER localization signal described by McCartney *et al* (Belo, Zheng et al. 2008)(McCartney, Dyer et al. 2004). Furthermore, the conserved amino acids identified in an alignment of the *FAD2* sequences from 34 different species [49]
25 are also present in *C. sativa* with the exception of a positively-charged histidine at position number 44, which is substituted by a polar, uncharged glutamine in *C. sativa*. When the inventors amplified the *FAD2* gene from several species in the tribe Camelinae (Table 2) and aligned the translated amino acid sequences, the inventors found that the *FAD2* proteins from *Capsella rubella*, *Camelina microcarpa*, *Camelina laxa*, and one copy from *Camelina*
30 *rumelica* contain a glutamine at amino acid position 44, while the *FAD2* proteins from *Arabidopsis lyrata*, *Camelina hispida*, and a second copy from *Camelina rumelica* contained a histidine (data not shown).

Table 1 - Nucleotide and Amino Acid identity of *Camelina sativa* and *Arabidopsis thaliana* FAD2 and FAE1 genes.

Gene	% Nucleotide Identity *				% Amino Acid Identity			
	AtFAD2	CsFAD2A	CsFAD2B	CsFAD2C	AtFAD2	CsFAD2A	CsFAD2B	CsFAD2C
FAD2								
	AtFAD2	100	93.6	93.8	93.4	100	96.6	96.4
	CsFAD2A		100	97.3	98.3	100	99.0	99.5
	CsFAD2B			100	97.7		100	99.5
	CsFAD2C				100			100
	AtFAE1	CsFAE1A	CsFAE1B	CsFAE1C	AtFAE1	CsFAE1A	CsFAE1B	CsFAE1C
FAE1	AtFAE1	100	90.7	91.2	91.0	91.9	91.7	91.7
	CsFAE1A		100	97.8	96.8	100	97.6	96.4
	CsFAE1B			100	97.2		100	96.8
	CsFAE1C				100			100

*Nucleotide identity is in coding region only.

Table 2 – Plant species and sources

Species	Source	Catalogue number
<i>Camelina sativa</i> Cs32	USDA	PI 311732
<i>Camelina sativa</i> Cs11	Ames	26668
<i>Arabidopsis thaliana</i> , ecotype Col-0	ABRC	CS28166
<i>Arabidopsis lyrata</i>	ABRC	CS22696
<i>Camelina laxa</i>	USDA	PI 650132
<i>Camelina microcarpa</i>	wild collection; Harvard Herbarium	number “01-22”
<i>Camelina microcarpa</i>	USDA	PI 633188
<i>Capsella bursa-pastoris</i>	Wild collection; Harvard Herbarium collection	number “08-188”
<i>Capsella rubella</i>	ABRC	CS22561
<i>Camelina hispida</i> var <i>grandiflora</i>	Ames	21324
<i>Camelina alyssum</i>	Ames	26658
<i>Camelina rumelica</i>	Ames	21327

Table 3 - Primers used for amplification of genomic regions of *C. sativa*

	Primer Name	Primer sequence (5' – 3')
Southern analysis of FAD2	FAD2_631F	TCAACAACCCTCTTGGACGCATCA (SEQ ID NO: 13)
	FAD2_832R	CTTGTGCAGCAGCGTAACGGTAAA (SEQ ID NO: 14)
Southern analysis of FAE1	AtFAE1 probe F	AGACGGTCCAAGTACAAGCTAGTTC (SEQ ID NO: 15)
	AtFAE1 probe R	CCAAATCTATGTAACGTTGATCT (SEQ ID NO: 16)

Southern analysis of LFY	AtLFY probe F	GATGCGGCGGGGAATAACGGCGGAG (SEQ ID NO: 17)
	AtLFY probe R	CCTGAAGAAGGAACTCACGGCATT (SEQ ID NO: 18)
Cloning of FAD2 coding region	AtFAD2_start	AACATGGGTGCAGGTGGAAGAATG (SEQ ID NO: 19)
	AtFAD2_stop2	TCATAACTTATTGTTGTACCAGTAC (SEQ ID NO: 20)
Cloning of FAE1 coding region	CaFAE1 start	ATGACGTCCATTAACGTAAAGCTC (SEQ ID NO: 21)
	CaFAE1 stop	TTAGGACCGACCGTTTTGGGC (SEQ ID NO: 22)
KCS17-FAE1 intergenic region "A" and "C" (initial clones)	AtKCS F	GGGTGGCTCTTCGCAATGTCGAGCCC (SEQ ID NO: 23)
	CsFAE1 5' RACE	GAGGCTTTTCCGGCAAGTAACGCCG (SEQ ID NO: 24)
KCS17-FAE1 intergenic region "A"	AtKCS cons F	GGTATGAATTGGCTTACACGGAAG (SEQ ID NO: 25)
	CsKCSA_F	TATGAATTGGCTTACACGGAAGCC (SEQ ID NO: 26)
	CsFAE1A_R2	TATATTGCCAATATAAGTATTAAAGGTCC (SEQ ID NO: 27)
KCS17-FAE intergenic region "B"	AtKCS cons F	GGTATGAATTGGCTTACACGGAAG (SEQ ID NO: 28)
	CsFAE1B_R	TATATTGCCAATATAAGTATTAAAGGTCC (SEQ ID NO: 29)
KCS17-FAE intergenic region "C"	AtKCS cons F	GGTATGAATTGGCTTACACGGAAG (SEQ ID NO: 30)
	CsFAE1C_R	GGTAGAGATCGTTTGTGGTAAGCG (SEQ ID NO: 31)

Camelinae FAD2	CsFAD2 start	ATGGGTGCAGGTGGAAGAATGC (SEQ ID NO: 32)
	CsFAD2 stop	TCATAACTTATTGTTGTACCAGTACACACC (SEQ ID NO: 33)
Camelinae FAE1	CsFAE1 start	ATGACGTCCGTTAACGCAAAGCTC (SEQ ID NO: 34)
	CsFAE1 stop	TTAGGACCGACCGTTTTTGACATG (SEQ ID NO: 35)

Table 4 - Primers used for qPCR analyses

	Primer or Probe Name	Sequence (5' – 3')
qPCR of CsACTIN	CsACT For	ACA ATT TCC CGC TCT GCT GTT GTG (SEQ ID NO: 36)
	CsACT Rev	AGG GTT TCT CTC TTC CAC ATG CCA (SEQ ID NO: 37)
	CsACT probe	FAM - TGT TTC AAA CGC TCT ATC CCT CGC TC – IABLFQ (SEQ ID NO: 38)
qPCR of CsFAD2	CsFAD2 A For1	CTG CGA GAA ACC ACC GTT CAC CC (SEQ ID NO: 39)
	CsFAD2 all Rev	CAC GAG TAG TCA ACG AGG TAA ACC GG (SEQ ID NO: 40)
	CsFAD2 all probe	FAM - CCA CTT CTA TTC CCA TCT CCA ACA CAA CC – IABLFQ (SEQ ID NO: 41)
qPCR of CsFAE1	CsFAE1 all For	AAC CTT TGC TTG TTT CCG TTA ACG GC (SEQ ID NO: 42)
	CsFAE1 all Rev	CAC GAG TAG TCA ACG AGG TAA ACC GG (SEQ ID NO: 43)
	CsFAE1 all probe	FAM - CCA CTT CTA TTC CCA TCT CCA ACA CAA CC – IABLFQ (SEQ ID NO: 44)

Table 5 – SNPs distinguishing each copy of FAD2 and FAE1

SNP_ID	Nucleotide position from beginning of coding region
FAD2_A4	51
FAD2_A2	453
FAD2_A6	549
FAD2_B4	288
FAD2_B5	687
FAD2_B8	1109
FAD2_C1	78
FAD2_C5	615
FAD2_C3	966
FAE1_A4	624
FAE1_A3	1368
FAE1_A7	1475
FAE1_B4	414
FAE1_B5	783
FAE1_B8	1438
FAE1_C1	336
FAE1_C2	721
FAE1_C7	1419
FAE1_ABC1	104

The inventors aligned the translated amino acid sequences from the three copies of *C. sativa* FAE1 with the seed-specific FAE1 proteins from *A. thaliana*, *Crambe abyssinica*, a high and low erucic acid *Brassica rapa*, *Limnanthes alba*, and *Tropaeolum majus* (Figure 2B). *L. alba* and *T. majus* are both in the order Brassicales and their seeds accumulate high levels of very long chain fatty acids (Cahoon, Marillia et al. 2000; Mietkiewska, Giblin et al. 2004). Four conserved histidine residues and six conserved cysteine residues, including the active site at cysteine 223, as well as an asparagine residue at 424 required for FAE1 activity were previously identified by Ghanevati and Jaworski (Ghanevati and Jaworski 2001;

Ghanevati and Jaworski 2002). All conserved residues were found to be present in all three copies of *C. sativa* FAE1. More differences were apparent between the three *C. sativa* FAE1 sequences and the other FAE1 sequences than observed in the FAD2 comparison (Figure 2A and B), an observation consistent with the level of amino acid identity seen between Arabidopsis and *C. sativa* FAD2 versus FAE1 (Table 1).

Example 5

All three copies of *FAD2* and *FAE1* are expressed in developing seeds of *C. sativa*

The conservation of amino acids as well as the presence of the 5' regulatory intron in *FAD2* suggests that all three copies of FAD2 and of FAE1 could be functional. To determine whether these genes are also expressed, the inventors first evaluated total *FAD2* and *FAE1* gene expression in developing seeds and in seedling tissue using quantitative real time PCR (qPCR) with primer/probe combinations designed to detect all three copies of each gene. *FAD2* expression in seedling tissue is present but minimal (0.4% of that seen in seeds at 20 days post-anthesis (DPA)), while *FAE1* expression could not be detected in seedlings (Figure 3A and B). In developing seeds, both *FAD2* and *FAE1* expression peaks at 20 DPA and is reduced by 30 DPA (Figure 3A and B). In Arabidopsis, *FAD2* peaks earlier and decreases sooner than *FAE1* (Ruuska, Girke et al. 2002).

The inventors wondered whether the expression of each of the *FAD2* and *FAE1* copies present in *C. sativa* are equally or differentially expressed in the seed. Duplicated genes are frequently silenced either throughout the plant or in a tissue-specific manner (Comai, Tyagi et al. 2000; Kashkush, Feldman et al. 2002; He, Friebe et al. 2003; Adams, Percifield et al. 2004); hence the inventors hypothesized that one or more of the copies of each gene could be significantly down-regulated. The inventors used the Sequenom MassARRAYTM method for determining allele-specific expression of a gene (Park, Correll et al. 2004) to evaluate the relative expression of each of the copies of *FAD2* and *FAE1*. The inventors identified at least three single nucleotide polymorphisms (SNPs) specific to each of the *FAD2* A, B, and C and the *FAE1* A, B, and C copies and then calculated the frequency of each SNP in seed cDNA. Controls consisting of the cloned *FAE1* A, B, and C copies combined to known frequencies showed that the method is greater than 80% accurate (Table 6). No evidence of silencing of any particular copy of either *FAE1* or *FAD2* was discovered. The inventors did observe differential expression, especially of *FAE1* A, which accounts for approx 40-50% of *FAE1* expression in seeds at 20-30 DPA (Figure 3 C and D).

Six cloned DNA positive controls were also included in the analysis and the relative amount of “B” version in each measured with the *FAE1_B5* SNP and all 3 versions with the *FAE1_ABC* SNP:

Table 6. Expression level of *FAE1* genes relative to *FAE1 B*

		FAE1_B5	FAE1 ABC		
		relative B	relative A	relative B	relative C
C1	100% Version A	0.00	1	0	0
C2	100% Version B	1.00	0	1	0
C3	100% Version C	0.00	0	0	1
C4	60%A, 20%B, 20%C	0.20	0.59	0.20	0.22
C5	20%A, 60%B, 20%C	0.54	0.29	0.48	0.23
C6	20%A, 20%B, 60%C	0.20	0.24	0.28	0.48

5

As the results indicate, all three *FAE1* genes are expressed in the seed. A dosage effect may still be expected, however, since *FAE1 B* appears to account for only approximately 25-30% of *FAE1* expression in the seeds. A mutation in *FAE1 A* would be expected to have a greater effect on fatty acids composition in the seeds since it accounts for ~41-48% of *FAE1* expression.

10

Example 6

15 **Characterization of sequences upstream of *C. sativa FAE1* and downstream of *C. sativa FAD2* suggests colinearity with *A. thaliana***

To investigate whether the different copies of *C. sativa FAD2* and *FAE1* are the result of allelic variation or are in fact independent loci, the inventors obtained sequence from the region upstream of *FAE1* and downstream of *FAD2*. Assuming colinearity between *C. sativa* and Arabidopsis for the region around *FAE1*, the inventors PCR amplified the region 5' to *FAE1* using a forward primer for the upstream gene *KCSI7* with reverse primers for *C. sativa FAE1*. The resulting sequences obtained for the putative *C. sativa KCSI7* were highly similar to the last 189 bp of Arabidopsis *KCSI7*, suggesting that the inventors had in fact amplified the orthologous *C. sativa* region upstream of *FAE1*, confirming colinearity between the two

20

species. The inventors then used a dot plot (see details for Nucleic Acid Dot Plots in Maizel et al., 1981; Pustell et al. 1982; Quigley et al., 1984) to compare the three *C. sativa* upstream sequences to each other and to Arabidopsis with parameters set for perfect match on a sliding window of 9 bases. The coordinates from the dot plot were used to define blocks of
 5 homology between Arabidopsis and the three *C. sativa* copies (Figure 4). The results show a variable intergenic region containing conserved blocks common to two or more genomes.

Co-linearity with Arabidopsis was also found for a region downstream of *FAD2* containing the *ACTIN11* (*ACT11*) gene for two out of the three *C. sativa* copies (data not shown). For the third copy, the region downstream of *FAD2* A could have been missed if the
 10 length of the amplified product was too large. Alternatively, the region downstream of *FAD2* A might not exhibit colinearity with Arabidopsis.

Example 7

**The genomes of *C. sativa*, *C. alyssum*, and *C. microcarpa* are larger than the genomes of
 15 other *Camelina* species**

The inventors calculated DNA content in several accessions of *C. sativa* and related species from flow cytometry analyses using propidium iodide-stained nuclei. The inventors used Arabidopsis accession Col-0 (2X) and its tetraploid (4X) derivative as genome size standards. *C. sativa*, *C. alyssum*, and *C. microcarpa* diploid (2C) genomes had a haploid
 20 content between 650 and 800 Mb (Figure 5). *C. sativa* accessions uniformly displayed a genome size close to 750 Mb. North American isolates of *C. sativa*, *C. alyssum*, and *C. microcarpa* have reported chromosome counts of $n=20$ (Francis and Warwick 2009). The genomes of *C. rumelica* (600 Mb), *C. hispida* (300Mb) and *C. laxa* (210Mb) are smaller than those of *C. sativa*, *C. alyssum*, and *C. microcarpa*. Chromosome counts of both $n=6$ (Baksay
 25 1957; Brooks 1985) and $n=12$ (Maassoumi 1980) have been recorded for *C. rumelica*, while only a single count of $n=7$ exists for *C. hispida* (Maassoumi 1980). To our knowledge, no published counts exist for *C. laxa*.

Example 8

**Phylogenetic analysis of *FAD2* and *FAE1* indicate that *C. sativa* and *C. microcarpa* are
 30 closely related**

To understand the duplication history of the multiple *FAD2* and *FAE1* copies recovered from *C. sativa*, the inventors amplified the *FAD2* and *FAE1* genes from several

species in the tribe Camelinae (Table 2), and inferred phylogeny for each gene. The sampling of taxa chosen allowed the inventors to test whether *FAD2* and *FAE1* duplication events occurred after *Camelina* diverged from its closest relatives or within the genus. Results from the evaluation of 55 different models of sequence evolution using Modeltest 3.7 (Posada and Crandall 1998) indicated that the *FAD2* sequence data are best described by the TVM+I+ Γ model, while the *FAE1* data are best described by the HKY+I+ Γ model. Likelihood phylogenetic analyses in PAUP* 4.0 (Swofford 2001) produced a single *FAD2* tree (–LnL 3665.277; Fig. 6A), and a single *FAE1* tree (–LnL 5051.552; Fig. 6B).

Phylogenies inferred from *FAD2* and *FAE1* data indicate a history of duplication for both markers. Both *C. microcarpa* and *C. sativa* have three distinct copies of *FAD2* and *FAE1*. Moreover, for *FAD2*, the A and C copies from these two species are monophyletic with strong (100%) bootstrap support (bs); for *FAE1* the A and B copies from these species are strongly monophyletic (100% bs). In contrast, neither the *FAD2* B copies of *C. sativa* and *C. microcarpa*, nor the *FAE1* C copies of these species form a monophyletic group with each other. Instead, results indicate that *C. rumelica* has two distinct copies of *FAD2* and that one of these copies (*FAD2*-2) is strongly monophyletic with *C. microcarpa* *FAD2* B. The inventors recovered only a single *FAD2* copy for *C. laxa* and *C. hispida*. In contrast, at least two distinct copies of *FAE1* were recovered from all sampled *Camelina* species. The *FAE1*-1 copy of *C. laxa*, *C. hispida*, and *C. rumelica* form a monophyletic group (91% bs), with the former two species sister to one another with strong support (100% bs). Similar to the results from *FAD2*, *C. rumelica* *FAE1*-2 is sister to one of the *C. microcarpa* copies (*FAE1* C; 99% bs). Neither the *C. sativa* *FAD2* B copy, nor the *C. sativa* *FAE1* C copy, shows a well supported sister relationship to other *FAD2* or *FAE1* sequences. However, in the *FAE1* tree, *C. sativa* *FAE1* C is very weakly supported as sister to *C. hispida* *FAE1*-2 (53%). Finally, all recovered *FAD2* and *FAE1* copies from species of the genus *Camelina* are monophyletic and sister to other sampled members of the tribe Camelinae, consistent with phylogenies based on other markers (Beilstein, Al-Shehbaz et al. 2006; Beilstein, Al-Shehbaz et al. 2008).

Example 9

Camelina Breeding Program

Since *Camelina* has not been intensively bred and the germplasm is somewhat limited genetically, the inventors established three strategies for long term development of *Camelina* germplasm. These three, non-mutually exclusive strategies for *Camelina* germplasm

enhancement include: transgenic approach, classical and molecular breeding, and mutation breeding. The long term goals are to achieve increased yield, increased seed oil content and improved fatty acid composition (e.g., higher percentage oleic acid (18:1), which is an optimal fatty acid for biodiesel and/or lower percentage of very long chain fatty acids (VLCFA, such as 20:1, 20:2, 22:1, etc)).

In the transgenic approach, REV and KRP yield technology (US 2008/263727 and US 2007/056058) can be introduced into *Camelina* to obtain events with increased seed yield or seed size, agronomic properties beneficial to obtaining *Camelina* germplasm with increased oil yield per unit land for biofuel purposes. Efficient transformation of *Camelina* has been established before (WO 2009/117555).

In the classical and molecular breeding approach, broad field evaluations of more than 100 accessions of *Camelina* in Northern United States and Canada was initiated across multiple field locations and over multiple years. Different accessions were evaluated for seed yield, oil yield, fatty acid composition, and agronomic performance under different environmental conditions. Superior lines with higher yield identified in the evaluations are used in the breeding program. In addition, molecular breeding studies are also in progress. Preliminary results show that existing *Camelina* cultivars are closely related, as indicated by AFLP analysis in which 379 markers were scored. Jaccard analysis suggested there is more than 90% genetic similarity across existing cultivars. Therefore, there is much room for improvement of *Camelina* germplasm, which will be realized by classical and molecular breeding programs. In the mutation breeding approach, an EMS mutagenized population was created in a selected *Camelina* cultivar, and Targeting Induced Local Lesions In Genomes (TILLING®) method was used to find mutations in known gene sequences. Especially, mutations with altered fatty acid compositions and improved yield as expressed in amount of oil produced per acre are of the most interest. M2 plants/M3 seed were harvested, and gene sequences for select targets were isolated and characterized. Preferred fatty acids include 16:1 and 18:1 monounsaturated fatty acids, since they have the best combination of proper cetane number, cloud point, oxidative stability, and less NOx emissions, as compared to saturated fatty acids (e.g., 12:0, 14:0, 16:0, 18:0, 20:0, and 22:0), or poly unsaturated fatty acids (e.g., 18:2, 18:3).

Example 10**TILLING® Method to Isolate *Camelina* Mutants in *FAD2* and *FAE1* Genes**

As described above, the goal is to improve *Camelina sativa* fatty acid composition for biodiesel. For example, since oleic acid (18:1) is optimal for fatty acid biodiesel, one specific goal is to increase 18:1 and decrease polyunsaturated fatty acids and long chain fatty acids. One way is to lower the activity of *FAD2* and of *FAE1*, as indicated by the fatty acids synthesis pathway shown in Figure 7.

An EMS mutant library has been created in *Camelina sativa* line CS32. This library has a population of about 8000 mutants and was used to screen for mutants of *FAD2* genes (Figure 8). Initial TILLING® using primers designed to the three *FAD2* genes yielded mutants in all three *FAD2* genes. Later, TILLING® using primers designed to the three *FAE1* genes also yielded mutants in all three *FAE1* genes. Lu et al (*Camelina sativa*: A Potential Oilseed Crop for Biofuels and Genetically Engineered Products, Information Systems for Biotechnology New Report, January 2008) describes a preliminary mutant screen where a random screen was carried out for fatty acid composition *Camelina* mutant using gas chromatography (GC). The TILLING® method of the present invention is superior to this because it is not necessary to GC screen thousands of mutants; rather, mutants in known fatty acid genes are identified (Hutcheon et al., TILLING® for Altered Fatty Acid Profiles in *Camelina sativa*, July 2009, American Society of Plant Biologists Annual Meeting).

Also the identification of *Camelina* sequences allows for the design of gene-specific TILLING® primers which can make it much easier to get mutations in all three versions of any given gene, *FAD2* or *FAE1*.

A non-limiting exemplary protocol of TILLING® is described below:

1. Seeds are mutagenized to induce point mutations throughout the genome.
2. A founder population is grown from mutagenized seeds.
3. Founder population is self-fertilized to produce a crossed population.
4. DNA samples from the crossed population are collected in 96-well plates and seeds from the crossed population are stored.
5. Up to eight 96-well plates are pooled into one and the samples (768) subjected to PCR with two gene-specific primers labelled with different IRDye® infrared dyes.
6. Resulting amplicons are heated and cooled, resulting in heteroduplexes between wild type and mutant samples.
7. CEL I nuclease is used to cleave at base mismatches.

8. Samples are denatured and electrophoresed on a LI-COR® DNA Analysis System.
9. In lanes that have a mutation in the pool, a band will be visible below the wild type band on the IRDye® 700 infrared dye image. A counterpart band will be visible in the same lane on the IRDye® 800 infrared dye image. This band is the cleavage product labeled with IRDye® 800 infrared dye from the complementary DNA strand. The sum of the length of the two counterpart bands is equal to the size of the amplicon, which makes it easy to distinguish mutations from amplification artefacts. An exemplary LI-COR gel identifying mutants in *Camelina* FAD2 genes is shown in Figure 9.
10. After detection of a mutation in a pool (lane), the individual DNA samples in the pool are screened again to find out which of the eight pooled samples from the crossed population has the mutation.

More information on TILLING® is described by Colbert et al. (2001. High Throughput Screening for Induced Point Mutations. *Plant Physiology* 126: 480-484.); McCallum et al. (2000. Target Induced Local Lesions In Genomes (TILLING) for Plant Functional Genomics. *Plant Physiology* 123:439-442); Henikoff et al. (Single-Nucleotide Mutations for Plant Functional Genomics. *Annual Review of Plant Biology*. 54:15.1-15.27.); and Till et al. (2003. Large-Scale Discovery of Induced Point Mutations With High-Throughput TILLING. *Genome Research* 13:524-530).

A pilot study determined that the mutation density of the inventors' mutant *Camelina* population was 1/25 kb. TILLING® of an initial 768 M2 individuals for *FAD2* has identified 60 mutants, 60% of which are non-silent mutations. Of the non-silent mutations, about 30% are predicted to be severe missense or truncation mutations. Mutations were identified in all 3 copies of *Camelina FAD2*. The inventors' previous finding that *Camelina sativa* may be polyploid is further supported by the high density of lesions this plant is willing to tolerate in its genome. The mutant M3 plants were grown and a preliminary analysis of their fatty acid profiles by GC was performed.

Example 11**Mutations of *Camelina FAD2* and *FAE1* Genes identified in TILLING®**

Initial screening of the TILLING® population for *FAD2* mutants resulted in plants with silent, STOP (nonsense) and/or severe missense mutations in *FAD2 A*, *B*, and *C*; and

5 *FAE1 A*, *B* and *C* genes.

Positions and effects of mutations in *FAD2 A*, *B*, and *C* genes and *FAE1 A*, *B* and *C* genes are displayed in Tables 7 to 12 below (* indicates the mutation results in a stop codon, = indicates silent mutation).

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Table 7. Summary of *Camelina FAD2 A* mutants

Nucleotide Change	Effect	Primer set	Plant ID	Mutation Score
G1516A	G35R	FAD2A	2480	severe missense
C1645T	L78F	FAD2A	2487	severe missense
C1746T	H111=	FAD2A	2782	silent
C1813T	P134S	FAD2A	2085	severe missense
G1844A	R144H	FAD2A	2764	severe missense
C1977T	V188=	FAD2A	2484	silent
G2015A	G201D	FAD2A	2993	severe missense
C2099T	S229F	FAD2A	2579	severe missense
G2155A	G248R	FAD2A	2200	severe missense
G1495A, G2272A	E28K, E287K	FAD2A	2983	missense, severe missense
G2138A	R242H	FAD2A	2986	missense

Table 8. Summary of *Camelina FAD2 B* mutants

Nucleotide Change	Effect	Primer set	Plant ID	Mutation Score
C207T	S53F	FAD2B	2474 or 2199	severe missense
C213T	S55F	FAD2B	3142	Severe Missense
G785A	A246T	FAD2B	3363	Missense
C476T	R143C	FAD2B	3314	Severe Missense
C176T	P43S	FAD2B	3325	Severe Missense
G462A	W138*	FAD2B	3489	Nonsense
G498A	G150E	FAD2B	3702	Severe Missense
G779A	A244T	FAD2B	3732	Missense
G737A	D230N	FAD2B	3814	Missense
C812T	L255F	FAD2B	4245	Missense
C882T	P278L	FAD2B	4408	Missense
G410A	D121N	FAD2B	4875	Missense
G675A	C209Y	FAD2B	4916	Missense
C459T	S137F	FAD2B	5155	Severe Missense
C528T	P160L	FAD2B	5746	Severe Missense
C987T	T313M	FAD2B	6023	Severe Missense
C284T	P79S	FAD2B	6107	Severe Missense

G416A	V123I	FAD2B	6122	Severe Missense
G650A	G201S	FAD2B	6105	Severe Missense
C656T	P203S	FAD2B	6277	Missense
C203T	R52C	FAD2B	6493	Severe Missense
G582A	G178E	FAD2B	6486	Severe Missense
G372A	C108Y	FAD2B	6479	Severe Missense
G322A	W91*	FAD2B	6490	Nonsense
G374A	G109S	FAD2B	6752	Severe Missense
G926A	G293R	FAD2B	6778	Severe Missense
C490T	S147=	FAD2B	3207	silent
C940T	T297=	FAD2B	3423	silent
G148A	T33=	FAD2B	3521	silent

Table 9. Summary of *Camelina FAD2 C* mutants

Nucleotide Change	Effect	Primer set	plant ID	Mutation Score
G1429A	E28K	FAD2C	6431	Missense
C1501T	R52C	FAD2C	3168	Severe Missense
C1542T	S65=	FAD2C	5756	silent
C1576T	L77F	FAD2C	5550	Missense
C1582T	P79S	FAD2C	5655	Severe Missense
G1607A	W87*	FAD2C	4506	Nonsense
C1609T	P88S	FAD2C	3210	Severe Missense
G1619A	W91*	FAD2C	3284	Nonsense
G1672A	G109S	FAD2C	3690	Severe Missense
G1717A	G124S	FAD2C	5644	Severe Missense
C1720T	L125F	FAD2C	4933	Missense
C1741T	L132F	FAD2C	4995	Missense
G1795A	G150R	FAD2C	3147	Severe Missense
G1796A	G150E	FAD2C	4608	Severe Missense
C1799T	S151F	FAD2C	3275	Severe Missense
G1808A	R154K	FAD2C	3490	Missense
G1810A	D155N	FAD2C	2578, 2586	Severe Missense
C1857T	G170=	FAD2C	4716	silent
C1873T	P176S	FAD2C	3267	Severe Missense
G1880A	G178E	FAD2C	5903	Severe Missense
G1883A	R179H	FAD2C	4846	Severe Missense
G1890A	M181I	FAD2C	4400	Missense
G1915A	G190R	FAD2C	5524	Severe Missense
G1948A	G201S	FAD2C	6120	Severe Missense
G1963A	G206R	FAD2C	4556	Missense
C2029T	L228F	FAD2C	4802	Missense
G2072A	R242H	FAD2C	5122	Missense
G2080A	A245T	FAD2C	3152	Missense
C2081T	A245V	FAD2C	5318	Missense
C2084T	A246V	FAD2C	4884	Missense
C2096T	A250V	FAD2C	3318	Missense
C2110T	L255F	FAD2C	5734	Missense
C2112T	L255=	FAD2C	4677	silent

G2117A	G257E	FAD2C	5491	Severe Missense
G2117A	G257E	FAD2C	6470	Severe Missense
G2140A	A265T	FAD2C	3924	Missense
G2149A	V268I	FAD2C	6068	Severe Missense
C2188T	P281S	FAD2C	4864	Severe Missense
C2204T	S286F	FAD2C	5183	Severe Missense
G2255A	G303E	FAD2C	4467	Severe Missense
G2268A	K307=	FAD2C	6509	silent
C2285T	T313M	FAD2C	5426	Severe Missense
C2293T	H316Y	FAD2C	2785, 2487, 2488, or 2786	Severe Missense
C2315T	S323L	FAD2C	6060	Severe Missense
G2422A	E359K	FAD2C	4997	Severe Missense
G2443A	V366I	FAD2C	6579	Missense
C1595T	S83F	FAD2C	4138	Severe Missense
C2383T	Q346*	FAD2C	6077	Nonsense

Table 10. Summary of *Camelina FAE1 A* mutants

Nucleotide Change	Effect	Primer set	Plant ID	Mutation Score
G621A	V55I	FAE1-A	4696	Missense
C695T	L79=	FAE1-A	3920	silent
C714T	L86F	FAE1-A	4489	Severe Missense
G798A	V114M	FAE1-A	5495	Missense
G801A	A115T	FAE1-A	3436	Missense
G805A	C116Y	FAE1-A	3533	Missense
G810A	D118N	FAE1-A	3424	Missense
G810A	D118N	FAE1-A	5977	Missense
C817T	S120F	FAE1-A	3821	Severe Missense
C820T	S121L	FAE1-A	4703	Missense
G821A	S121=	FAE1-A	6126	silent
G867A	E137K	FAE1-A	6361	Missense
G877A	S140N	FAE1-A	3284	Severe Missense
G997A	R180K	FAE1-A	3390	Missense
G997A	R180K	FAE1-A	5346	Missense
G1005A	G183S	FAE1-A	6655	Severe Missense
C1042T	T195I	FAE1-A	5557	Severe Missense
G1061A	M201I	FAE1-A	4088	Severe Missense
G1065A	V203I	FAE1-A	4469	Missense
C1072T	T205I	FAE1-A	4500	Severe Missense
C1083T	R209*	FAE1-A	3395	Nonsense
C1091T	N211=	FAE1-A	5486	silent
G1120A	G221D	FAE1-A	6386	Severe Missense
C1141T	A228V	FAE1-A	4467	Severe Missense
C1167T	H237Y	FAE1-A	4164	Severe Missense
C1167T	H237Y	FAE1-A	4318	Severe Missense
G1254A	V266I	FAE1-A	3365	Missense
G1258A	S267N	FAE1-A	3783	Severe Missense
C1272T	R272C	FAE1-A	5401	Severe Missense

G1311A	G285R	FAE1-A	3799	Missense
G1354A	R299Q	FAE1-A	5095	Severe Missense
G1366A	G303E	FAE1-A	3820	Severe Missense
G1387A	R310Q	FAE1-A	6528	Missense
G1390A	C311Y	FAE1-A	3631	Severe Missense
G1401A	G315R	FAE1-A	4257	Missense
G1402A	G315E	FAE1-A	6186	Missense
G1402A	G315E	FAE1-A	6446	Missense
G1407A	D317N	FAE1-A	3897	Severe Missense
G1416A	G320S	FAE1-A	5197	Severe Missense
G1426A	G323E	FAE1-A	5680	Severe Missense
G1426A	G323E	FAE1-A	6284	Severe Missense
C1450T	T331I	FAE1-A	4412	Missense
G1463A	G335=	FAE1-A	5117	silent
G1518A	E354K	FAE1-A	3597	Severe Missense

Table 11. Summary of *Camelina* FAE1 B mutants

Nucleotide Change	Effect	Primer set	PLANT ID	Mutation Score
C710T	P76L	FAE1B	5778	Severe Missense
C718T	L79F	FAE1B	5840	Severe Missense
G724A	D81N	FAE1B	6324	Severe Missense
C731T	S83L	FAE1B	4318	Severe Missense
C817T	R112W	FAE1B	4140	Severe Missense
G823A	V114M	FAE1B	3768	Missense
G823A	V114M	FAE1B	5966	Missense
C845T	S121L	FAE1B	3758	Missense
G858A	L125=	FAE1B	3709	silent
G887A	G135D	FAE1B	4015	Severe Missense
C907T	Q142*	FAE1B	5951	Nonsense
C928T	P149S	FAE1B	5107	Severe Missense
C952T	R157C	FAE1B	4840	Severe Missense
G953A	R157H	FAE1B	4239	Severe Missense
G958A	E159K	FAE1B	6322	Severe Missense
G969A	Q162=	FAE1B	3529	silent
G988A	E169K	FAE1B	3734	Severe Missense
C1019T	P179L	FAE1B	3873	Severe Missense
G1031A	G183D	FAE1B	4135	Missense
G1042A	V187M	FAE1B	6517	Severe Missense
C1063T	P194S	FAE1B	6478	Severe Missense
C1082T	A200V	FAE1B	3986	Severe Missense
G1086A	M201I	FAE1B	3895	Missense
G1109A	R209Q	FAE1B	4139	Severe Missense
C1154T	A224V	FAE1B	3352	Severe Missense
C1229T	T249I	FAE1B	4169	Severe Missense
G1231A	E250K	FAE1B	6678	Severe Missense
C1271T	S263F	FAE1B	3829	Severe Missense
C1271T	S263F	FAE1B	6700	Severe Missense
G1275A	M264I	FAE1B	6308	Severe Missense
G1306A	G275R	FAE1B	5333	Severe Missense
C1310T	A276V	FAE1B	3241	Severe Missense

G1314A	A277=	FAE1B	4884	silent
C1310T	A276V	FAE1B	3284	Severe Missense
C1325T	S281F	FAE1B	5343	Severe Missense
G1337A	G285E	FAE1B	3358	Missense
G1337A	G285E	FAE1B	3821	Missense
G1343A	R287Q	FAE1B	5930	Silent
C1352T	S290F	FAE1B	4882	Severe Missense
C1384T	H301Y	FAE1B	4687	Severe Missense
C1389T	T302=	FAE1B	5840	silent
G1412A	R310Q	FAE1B	3936	Missense
G1417A	V312M	FAE1B	3173	Severe Missense
G1427A	G315E	FAE1B	3926	Missense
G1435A	E318K	FAE1B	6479	Missense
G1441A	G320S	FAE1B	3842	Severe Missense
C1493T	A337V	FAE1B	4630	Severe Missense
C1522T	P347S	FAE1B	3912	Severe Missense

Table 12. Summary of *Camelina FAE1 C* mutants

Nucleotide Change	Effect	Primer set	Plant ID	Mutation Score
A506T	T15S	FAE1-C	3688	Missense
A506T	T15S	FAE1-C	4325	Missense
A506T	T15S	FAE1-C	4907	Missense
A506T	T15S	FAE1-C	6025	Missense
A506T	T15S	FAE1-C	6695	Missense
C564T	S34F	FAE1-C	4965	Missense
C605T	L48F	FAE1-C	6835	Missense
G704A	D81N	FAE1-C	4510	Severe Missense
C719T	L86F	FAE1-C	5015	Severe Missense
G798A	R112Q	FAE1-C	4184	Missense
C802T	N113=	FAE1-C	6130	silent
C822T	S120F	FAE1-C	3886	Severe Missense
C825T	S121L	FAE1-C	4255	Missense
G840A	R126K	FAE1-C	5936	Missense
G855A	R131H	FAE1-C	3725	Severe Missense
G855A	R131H	FAE1-C	4813	Severe Missense
C858T	S132L	FAE1-C	5951	Severe Missense
C887T	P142S	FAE1-C	3918	Missense
C887T	P142S	FAE1-C	4198	Missense
C906T	P148L	FAE1-C	4068	Severe Missense
C911T	Q150*	FAE1-C	5566	Nonsense
C911T	Q150*	FAE1-C	6139	Nonsense
G926A	A155T	FAE1-C	3923	Missense
G933A	R157H	FAE1-C	5576	Severe Missense
G982A	E173=	FAE1-C	3367	silent
C987T	T175I	FAE1-C	3247	Severe Missense
G1010A	G183S	FAE1-C	3365	Severe Missense
C1047T	T195I	FAE1-C	5891	Severe Missense
G1067A	V202I	FAE1-C	5975	Missense
C1088T	R209*	FAE1-C	6476	Nonsense
G1115A	G218R	FAE1-C	3970	Severe Missense

G1137A	G225D	FAE1-C	3911	Severe Missense
C1154T	L231F	FAE1-C	6643	Severe Missense
G1175A	V238I	FAE1-C	3380	Missense
C1251T	S263F	FAE1-C	5793	Severe Missense
C1252T	S263=	FAE1-C	3885	silent
G1255A	M264I	FAE1-C	5422	Severe Missense
G1283A	G274S	FAE1-C	4945	Severe Missense
G1287A	G275E	FAE1-C	3749	Severe Missense
C1305T	S281F	FAE1-C	3401	Severe Missense
C1305T	S281F	FAE1-C	4608	Severe Missense
G1316A	G285R	FAE1-C	4123	Missense
C1353T	T297M	FAE1-C	3427	Severe Missense
G1359A	R299Q	FAE1-C	3166	Severe Missense
C1400T	Q313*	FAE1-C	5114	Nonsense
C1403T	Q314*	FAE1-C	4162	Nonsense
G1406A	G315R	FAE1-C	3776	Missense
G1472A	A337T	FAE1-C	4852	Missense
C1486T	N341=	FAE1-C	4399	silent
C1494T	T344M	FAE1-C	5013	Severe Missense
C1502T	P347S	FAE1-C	6553	Severe Missense

As tables 7-12 indicate, multiple mutants were isolated in each *FAD2* or *FAE1* gene copy. The types of mutants include missense, severe missense, nonsense and silent mutations.

5

Example 12

Fatty Acids Composition in *FAD2* and *FAE1* mutants

Fatty acid methyl ester (FAME) composition in *Camelina FAD2* mutants was analyzed in a preliminary test by gas chromatography (GC) following the protocol described in Example 1. The results were shown in Table 13.

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Table 13 % FAME content in *Camelina FAD2* mutants

Mutation	Cs 32 wild type	Combined Null Population	FAD2A Q44*	FAD2A missense G150E		FAD2A missense S229F		FAD2B W91*	
			HOMO	HOMO	NULL	HOMO	NULL	HOMO	NULL
sample size	10	14	8	7	4	5	4	6	6
C18:1	14.4 ± 0.4	14.3 ± 2.0	22.6 ± 1.2	24.0 ± 1.2	17.1 ± 0.9	19.2 ± 1.3	13.9 ± 0.9	18.4 ± 0.6	12.8 ± 0.5
C18:2	21.4 ± 0.8	28.8 ± 2.8	20.3 ± 1.1	19.0 ± 0.5	26.9 ± 1.2	22.2 ± 1.1	26.8 ± 1.4	28.7 ± 0.4	31.5 ± 2.0
C18:3	33.7 ± 0.6	25.4 ± 1.9	26.2 ± 1.8	26.1 ± 1.6	26.0 ± 1.1	25.7 ± 1.2	26.5 ± 1.3	23.8 ± 1.5	24.2 ±

									2.2
C20:1	15.5 ± 1.0	10.7 ± 1.4	12.2 ± 1.0	13.1 ± 1.4	10.5 ± 1.3	13.4 ± 1.0	10.8 ± 1.3	10.2 ± 1.6	10.7 ± 1.2
% increase in 18:1 relative to wild type seeds			56.9%	66.7%		33.3%		27.8%	
Note: HOMO means the plants are all homozygous mutants at the specified locus. NULL means there is no mutation at the specified locus. % means % of FAME composition									

As the results indicate, an obvious increase of oleic acid (18:1) was observed in certain *FAD2* mutants tested compared to NULL control plants. Thus, the data supports the inventors' prediction very well that disruption in one, two or more *FAD2* gene in *Camelina* is sufficient to alter its fatty acid composition, and more specifically, to increase the oleic acid (18:1) concentration.

More mutants in *FAD2* genes and *FAE1* genes were subjected to GC analysis. To select mutants with potentially the most profound phenotype, *FAD2 A*, *B*, and *C*, or *FAE1 A*, *B*, and *C* protein sequences were analyzed against orthologs in *Arabidopsis*, *Crambe*, *B. rapa* *HEAC*, *B. rapa* *LEAC*, meadow foam, and nasturtium. It is preferred that a mutation happens at the position which is conserved through reference species, and/or a position described before as conserved in orthologs or close-related genes in other species (e.g., see reference 52, Ghanevati and Jaworski, 2002, and Jet et al., Dissection of malonyl-coenzyme A decarboxylation from polyketide formation in the reaction mechanism of a plant polyketide synthase, *Biochemistry*, 39:890-902). For example, the G150E, Q44* (nonsense), S229F and W91* (nonsense) mutations in *FAD2* genes are potentially very promising as are the following mutants in *FAE1*: Q142* (nonsense), R209* (nonsense), G221D and H301Y.

Two independent GC analyses of fatty acid compositions in *FAD2 A* and *FAD2 B* mutants were conducted, and the results are shown in Tables 14 and 15. Mutants with clear increases in oleic acid were selected, and their results from both GC runs were averaged together to produce Table 16. Some of these tested mutations have obvious increased oleic acid (18:1), such as *FAD2 A* mutants G150E, Q44*, S229F, and *FAD2 B* mutants W91* compared to NULL population or wild-type Cs32 control plants, while no significant difference was found between NULL population and wild type Cs32 plants. Table 17 shows the fatty acid compositions of selected *FAD2* mutants for one of the independent GC

analyses. The result of Table 16 is further summarized in Figure 12A. As the results indicate, these mutants have evident increased oleic acid (18:1) and reduced polyunsaturated fatty acids (e.g., 18:2 and 18:3) in seed oil, just as the inventors predicted.

A third independent GC analysis was conducted in which *FAD2 C* mutants were included. This was a preliminary analysis where seeds from heterozygous plants were used, resulting in a mixed population containing null, heterozygous and homozygous seeds. The results

showed that all tested *FAD2 C* mutants do not have significant induction of 18:1 fatty acid, as compared to Cs32 control plants. While not wishing to be bound by any particular theory, the results suggest that any potential increase in 18:1 in a *FAD2 C* mutant plant is not detectable in progeny from heterozygous plants, where the mixture of wild type, heterozygous and homozygous seeds may dilute the effects of the homozygous seed.

The same preliminary third GC run analyzed mutants at the *FAE1* loci. Though results showed that some of these tested mutants, for example *FAE1 A* mutant R272C, *FAE1 B* mutants S281F and R209Q, and *FAE1 C* mutants Q313* and Q150* had obvious decreased 20:1 and/or 22:1 in seed oil relative to wild type Cs32 plants, the inclusion of a significant number of heterozygous lines may have confounded the results as was the case with the *FAD2 C* results above.

A fourth independent GC analysis (results shown in Tables 18a and 19a of the present specification) was conducted on M4 or M5 generation *FAD2 A*, *FAD2 B*, *FAD2 C*, *FAE1 A*, *FAE1 B* and *FAE1 C* mutants. This analysis included multiple homozygous lines for a given *FAD2* or *FAE1* mutation, which conferred more confidence in the results due to multiple samples for a given mutation. In addition, the inventors limited the number of heterozygous lines analyzed where the seeds were a mixture of homozygous (designated 'hom'), heterozygous (designated 'het') and null because of ambiguous results in the third GC run. In test 4, Arabidopsis *FAD2* and *FAE1* mutants, wild type *Camelina sativa* CS32, and null sibling lines not carrying a *FAD2* or *FAE1* mutation were included as controls. From this analysis, some *FAD2 A* Q44* and G150E, *FAD2 B* W91* and G150E, and *FAD2 C* W87* homozygous or heterozygous lines clearly had greater 18:1 fatty acid levels compared to their null sibling lines or the CS32 control.

For *FAEI*, some *FAEI A* G221D, *FAEI B* Q142* and H301Y, and *FAEI C* R209* homozygous or heterozygous lines clearly had lower 20:1 fatty acid levels and/or lower 22:1 fatty acid levels compared to their null sibling lines or the CS32 control. The *FAEI* data from the fourth GC run is summarized in Figure 12B.

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This data supports the inventors' prediction that disruption in one, two or more *FAEI* genes in *Camelina* is sufficient to alter its fatty acid composition, and more specifically, to decrease the very long chain (for example 20:1 and 22:1) fatty acid content.

The fourth GC analysis did not include some *FAD2 C*, *FAEI A*, *FAEI B* and *FAEI C* mutants included in the third GC analysis due to pursuance of a select number of mutant lines in the breeding program for *FAD2* (A, B and C) and *FAEI* (A, B and C) mutants. In particular, *FAD2 C* mutants Q346*, G150R, R242H, G190R were included in the third but not the fourth GC analysis. Similarly, *FAEI A* mutants G183S, R272C, C311Y, *FAEI B* mutants P76L, L79F, R157H, R209Q, E250K, W91*, and *FAEI C* mutants R157H, G225D, L231F, G274S, Q313*, Q314* were included in the third but not the fourth GC analysis. The *FAEI C* Q150* mutant, which was analyzed in test 3 but not test 4, will be tested for fatty acid composition in future GC runs. In test 3, homozygous *FAEI C* Q150* mutant plants were used for analysis. According to the GC data in test 3, the fatty acids composition in homozygous *FAEI C* Q150* mutant is as following: C16:0, 6.19%; C18:0, 2.74%; C18:1, 13.81%; C18:2, 24.05%; C20:0, 0.97%; C18:3, 33.28%; C20:1, 14.25%; C20:2, 1.79%; C20:3, 0.70%; and C22:1, 2.23%.

Table 14. Fatty Acids Composition in *FAD2* mutants, sorted by mutation, Test No. 1

Line	Gene	SNP	mutation	Plant #	genotype	C16:0	C18:0	C18:1	C18:2	C20:0	C18:3	C20:1	C20:2	C20:3	C22:1
2362	FAD2A	5	G150E	4	HOMO	7.9%	4.3%	22.9%	19.1%	1.9%	28.1%	12.3%	0.8%	0.7%	2.1%
2362	FAD2A	5	G150E	5	HOMO	7.8%	3.8%	23.0%	19.1%	1.8%	28.1%	13.1%	0.7%	0.6%	2.1%
2362	FAD2A	5	G150E	1	HET	7.3%	3.8%	22.3%	20.7%	2.1%	25.7%	14.3%	0.9%	0.6%	2.3%
2362	FAD2A	5	G150E	2	HET	11.0%	5.4%	13.6%	15.0%	2.8%	34.6%	12.9%	1.4%	0.8%	2.4%
2362	FAD2A	5	G150E	11	Null	8.3%	5.2%	17.9%	25.3%	2.7%	27.4%	10.0%	1.0%	0.5%	1.7%
2362	FAD2A	5	G150E	18	Null	9.0%	5.1%	17.8%	28.2%	3.2%	24.9%	8.9%	1.2%	0.6%	1.1%
2510	FAD2A	4	S147F	3	HOMO	7.2%	5.1%	15.9%	24.4%	3.8%	28.4%	10.3%	1.3%	0.8%	2.7%
2510	FAD2A	4	S147F	10	HOMO	9.0%	6.4%	15.9%	29.2%	3.5%	25.0%	7.1%	1.2%	0.6%	2.1%
2510	FAD2A	4	S147F	1	HET	8.2%	5.5%	15.1%	27.2%	3.3%	27.9%	8.0%	1.3%	0.8%	2.8%
2510	FAD2A	4	S147F	4	HET	8.4%	5.7%	15.8%	30.1%	3.7%	23.7%	8.5%	1.3%	0.3%	2.4%
2510	FAD2A	4	S147F	2	Null	8.5%	4.7%	12.6%	28.2%	3.5%	27.7%	9.2%	1.7%	0.9%	2.9%
2510	FAD2A	4	S147F	5	Null	7.7%	3.5%	14.0%	26.9%	2.5%	29.3%	10.7%	1.5%	0.9%	3.0%
2579	FAD2A	7	S229F	1	HOMO	7.3%	3.4%	19.3%	23.6%	1.7%	26.5%	13.0%	1.2%	0.7%	3.2%
2579	FAD2A	7	S229F	5	HOMO	8.0%	4.4%	20.9%	22.3%	2.2%	27.4%	10.6%	1.1%	0.4%	2.8%
2579	FAD2A	7	S229F	2	HET	7.5%	3.6%	17.0%	25.8%	0.1%	31.7%	10.1%	1.6%	0.4%	2.3%
2579	FAD2A	7	S229F	3	HET	7.7%	4.6%	16.5%	25.0%	0.1%	30.5%	11.2%	1.3%	0.8%	2.4%
2579	FAD2A	7	S229F	10	Null	8.4%	3.6%	12.0%	26.8%	2.6%	28.9%	11.9%	1.9%	0.9%	3.1%
2579	FAD2A	7	S229F	12	Null	8.0%	5.2%	14.4%	26.1%	3.5%	28.9%	8.9%	1.5%	0.8%	2.8%
2764	FAD2A	3	R144H	5	HOMO	7.5%	3.8%	22.1%	26.3%	0.1%	26.0%	10.5%	1.1%	0.5%	2.1%
2764	FAD2A	3	R144H	8	HOMO	7.5%	3.3%	17.0%	21.6%	0.1%	34.4%	11.3%	1.2%	0.9%	2.9%
2764	FAD2A	3	R144H	10	HOMO	6.9%	4.3%	17.6%	22.5%	2.9%	28.4%	12.3%	1.2%	0.8%	3.2%
2764	FAD2A	3	R144H	16	HOMO	6.5%	3.0%	18.7%	24.5%	1.7%	26.9%	13.3%	1.3%	0.7%	3.4%
2764	FAD2A	3	R144H	1	HET	6.9%	3.4%	18.4%	24.5%	1.5%	27.7%	12.7%	1.3%	0.5%	3.0%
2764	FAD2A	3	R144H	2	HET	7.7%	3.9%	15.7%	25.5%	0.1%	32.5%	10.1%	1.4%	0.8%	2.3%
2764	FAD2A	3	R144H	3	HET	7.3%	3.5%	17.6%	26.5%	0.1%	29.1%	11.4%	1.4%	0.7%	2.4%
2764	FAD2A	3	R144H	6	HET	7.7%	3.3%	16.8%	22.3%	0.1%	34.5%	10.8%	1.1%	0.9%	2.6%

Line	Gene	SNP	mutation	Plant #	genotype	C16:0	C18:0	C18:1	C18:2	C20:0	C18:3	C20:1	C20:2	C20:3	C22:1
2764	FAD2A	3	R144H	4	Null	6.8%	3.5%	17.1%	27.8%	1.7%	24.9%	12.8%	1.5%	0.7%	3.1%
2764	FAD2A	3	R144H	7	Null	7.1%	3.2%	17.1%	25.2%	1.5%	28.2%	12.5%	1.5%	0.5%	3.3%
2785	FAD2C	11	H316Y	3	HOMO	8.1%	3.7%	16.6%	20.5%	2.6%	32.1%	11.2%	1.3%	0.8%	3.2%
2785	FAD2C	11	H316Y	10	HOMO	8.3%	3.7%	15.2%	16.7%	2.3%	34.2%	14.3%	1.2%	1.1%	3.1%
2785	FAD2C	11	H316Y	12	HOMO	8.5%	3.5%	14.3%	18.0%	2.7%	32.8%	14.1%	1.2%	1.2%	3.7%
2785	FAD2C	11	H316Y	18	HOMO	8.1%	3.9%	14.8%	19.1%	2.1%	35.0%	11.0%	1.3%	1.3%	3.4%
2785	FAD2C	11	H316Y	1	HET	8.8%	4.0%	14.3%	22.0%	2.0%	33.3%	9.9%	1.6%	0.8%	3.4%
2785	FAD2C	11	H316Y	6	HET	8.9%	3.9%	14.4%	20.5%	0.1%	36.0%	11.1%	1.3%	1.1%	2.8%
2785	FAD2C	11	H316Y	7	HET	8.2%	3.9%	14.4%	20.5%	0.1%	36.1%	11.1%	1.3%	1.1%	3.1%
2785	FAD2C	11	H316Y	8	HET	9.0%	3.9%	12.6%	21.0%	0.1%	38.2%	10.2%	1.5%	1.0%	2.5%
2785	FAD2C	11	H316Y	2	Null	8.6%	3.8%	11.3%	20.6%	2.8%	35.1%	11.1%	1.6%	1.4%	3.7%
2785	FAD2C	11	H316Y	5	Null	8.8%	4.7%	12.8%	20.7%	3.0%	34.2%	10.3%	1.4%	1.1%	3.0%
2812	FAD2B	9	H145Y	1	HOMO	8.3%	4.1%	15.1%	27.4%	2.9%	24.6%	12.4%	1.5%	0.7%	3.1%
2812	FAD2B	9	H145Y	2	HOMO	9.9%	3.6%	13.3%	28.7%	2.4%	27.0%	9.3%	1.5%	0.8%	3.5%
2812	FAD2B	9	H145Y	12	HET	7.8%	3.9%	16.6%	29.1%	2.6%	24.6%	10.7%	1.5%	0.7%	2.6%
2812	FAD2B	9	H145Y	25	HET	7.8%	4.1%	15.9%	30.5%	2.2%	25.8%	9.0%	1.6%	0.7%	2.4%
2826	FAD2A	2	Q44*	1	HOMO	7.7%	4.9%	22.2%	20.1%	2.1%	28.4%	11.0%	0.8%	0.6%	2.1%
2826	FAD2A	2	Q44*	2	HOMO	7.8%	4.9%	19.9%	19.2%	2.6%	29.5%	11.7%	1.0%	0.8%	2.6%
2826	FAD2A	2	Q44*	3	HOMO	7.5%	4.6%	22.9%	19.8%	2.1%	27.7%	11.7%	0.8%	0.6%	2.3%
2826	FAD2A	2	Q44*	4	HOMO	7.7%	5.4%	24.7%	20.3%	2.5%	25.7%	10.6%	0.7%	0.5%	1.9%
2826	FAD2A	2	Q44*	5	HOMO	7.6%	5.2%	22.9%	19.1%	2.2%	28.1%	11.5%	0.8%	0.6%	2.0%
2826	FAD2A	2	Q44*	37	HET	7.3%	4.8%	22.9%	19.1%	2.0%	28.5%	12.2%	0.7%	0.6%	1.9%
3006	FAD2B	8	W91*	1	HOMO	7.9%	4.6%	18.9%	28.5%	1.7%	26.8%	8.9%	1.0%	0.5%	1.1%
3006	FAD2B	8	W91*	2	HOMO	8.2%	5.3%	18.8%	29.2%	1.7%	26.3%	7.8%	0.9%	0.4%	1.3%
3006	FAD2B	8	W91*	3	HOMO	8.0%	5.5%	18.4%	28.9%	2.7%	24.4%	8.6%	1.1%	0.5%	1.8%
3006	FAD2B	8	W91*	4	HOMO	7.5%	5.1%	18.0%	28.9%	2.9%	22.9%	10.6%	1.3%	0.5%	2.3%
3006	FAD2B	8	W91*	5	Null	7.8%	4.2%	12.3%	33.5%	2.9%	23.8%	9.3%	2.1%	0.7%	3.5%

Line	Gene	SNP	mutation	Plant #	genotype	C16:0	C18:0	C18:1	C18:2	C20:0	C18:3	C20:1	C20:2	C20:3	C22:1
3006	FAD2B	8	W91*	7	Null	7.9%	4.4%	17.6%	30.0%	2.1%	24.9%	9.7%	1.2%	0.5%	1.6%
3006	FAD2B	8	W91*	8	HET	8.0%	3.9%	13.4%	30.6%	2.2%	26.9%	9.3%	2.0%	0.5%	3.1%

Note: * stands for nonsense mutation;

HOMO means the plants are all homozygous mutants at the specified locus.

HET means the plants are heterozygous mutants at the specified locus.

NULL means there is no mutation at the specified locus.

% means % of FAME composition

Table 15. Fatty Acids Composition in *FAD2* mutants, sorted by mutation, Test No. 2

Gene	SNP	mutation	Plant #	genotype	# of samples	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C20:3	C22:1
none	none		1	CS32 controls	10	5.8%	2.3%	14.9%	20.3%	34.0%	1.2%	16.2%	1.8%	0.9%	2.7%
none	none		2	CS32 controls		6.1%	2.3%	14.6%	21.3%	33.3%	1.2%	15.6%	1.8%	1.2%	2.6%
none	none		3	CS32 controls		6.2%	2.3%	14.1%	21.7%	34.2%	1.1%	15.5%	1.8%	1.0%	2.1%
none	none		4	CS32 controls		6.0%	2.4%	14.2%	20.8%	34.5%	1.2%	15.8%	1.6%	1.0%	2.4%
none	none		5	CS32 controls		6.7%	2.6%	14.6%	23.1%	33.5%	0.7%	12.9%	1.8%	1.3%	2.7%
none	none		6	CS32 controls		6.2%	2.5%	14.6%	21.6%	33.4%	1.0%	15.4%	1.8%	1.1%	2.5%
none	none		7	CS32 controls		5.9%	2.4%	14.6%	22.2%	32.7%	1.3%	15.7%	1.8%	1.0%	2.5%
none	none		8	CS32 controls		6.0%	2.3%	13.6%	21.0%	34.3%	1.4%	16.0%	1.9%	1.0%	2.6%
none	none		9	CS32 controls		5.9%	2.4%	14.3%	20.6%	33.6%	1.3%	16.2%	1.9%	1.1%	2.7%
none	none		10	CS32 controls		6.2%	2.4%	13.9%	21.4%	33.2%	1.3%	15.9%	1.9%	1.2%	2.6%
FAD2B/C	51	D60N	Y1	605	1	8.7%	2.5%	10.4%	29.0%	28.4%	1.7%	13.3%	2.1%	0.9%	3.0%
FAD2A	5	G150E	4	HOMO	1	8.0%	4.4%	22.5%	18.8%	27.5%	2.2%	12.7%	0.5%	1.7%	1.7%
FAD2A	5	G150E	5	HOMO	1	7.8%	4.2%	23.2%	18.4%	26.6%	2.2%	14.2%	0.7%	0.6%	2.2%
FAD2A		G150E	20	Null	2 new	8.7%	4.2%	16.2%	26.7%	25.7%	2.2%	12.0%	1.3%	0.4%	2.4%

Gene	SNP	mutation	Plant #	genotype	# of samples	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C20:3	C22:1
FAD2A		G150E	24	Null		8.7%	5.1%	16.4%	27.4%	26.0%	2.2%	10.9%	1.1%	0.6%	1.6%
FAD2A		G150E	6	HOMO	5 new	7.7%	4.2%	26.1%	18.8%	25.2%	1.9%	13.3%	0.6%	0.5%	1.7%
FAD2A		G150E	8	HOMO		8.1%	4.6%	24.2%	18.7%	28.1%	2.2%	10.2%	0.7%	0.5%	2.7%
FAD2A		G150E	14	HOMO		8.2%	4.5%	24.6%	19.4%	24.7%	2.0%	13.7%	0.6%	0.3%	1.9%
FAD2A		G150E	23	HOMO		8.6%	4.7%	23.0%	19.0%	26.7%	2.1%	13.1%	0.6%	0.5%	1.8%
FAD2A		G150E	25	HOMO		8.0%	4.0%	24.2%	20.0%	23.7%	1.8%	14.6%	0.7%	0.5%	2.5%
FAD2A		G150E	3	Het	3 new at least	7.9%	4.4%	21.1%	22.2%	27.2%	2.0%	12.4%	0.8%	0.3%	1.7%
FAD2A		G150E	7	Het		8.5%	4.5%	19.1%	24.3%	24.8%	2.1%	12.8%	1.0%	0.6%	2.2%
FAD2A		G150E	9	Het		8.4%	4.7%	17.9%	23.3%	27.5%	2.5%	12.2%	1.0%	0.6%	1.8%
FAD2A	2	Q44*	2	HOMO	1	8.3%	5.1%	22.8%	20.8%	26.7%	2.0%	11.1%	0.8%	0.4%	2.0%
FAD2A	2	Q44*	1	HOMO	1	7.8%	4.9%	19.7%	18.9%	28.3%	2.6%	13.9%	0.9%	0.7%	2.4%
FAD2A	2	Q44*	4	HOMO	1	7.6%	5.1%	23.7%	20.2%	25.7%	2.4%	12.8%	0.6%	0.4%	1.5%
FAD2A		Q44*	40	Null	1	8.2%	6.1%	25.6%	20.4%	24.3%	2.4%	10.8%	0.6%	0.3%	1.4%
FAD2A		Q44*	36	HOMO	3	7.8%	5.5%	23.2%	21.4%	23.8%	2.5%	13.0%	0.7%	0.2%	1.8%
FAD2A		Q44*	38	HOMO		8.0%	6.2%	22.3%	20.4%	25.4%	2.9%	12.4%	0.7%	0.3%	1.5%
FAD2A		Q44*	39	HOMO		9.2%	5.6%	22.9%	22.0%	23.7%	2.3%	11.0%	0.7%	0.4%	2.0%
FAD2A	3	R144H	16	HOMO	1	6.9%	2.9%	18.5%	26.2%	26.5%	1.6%	13.1%	1.2%	0.3%	2.9%
FAD2A	3	R144H	5	HOMO	1	7.1%	4.0%	21.7%	26.0%	23.2%	2.4%	12.1%	0.9%	0.3%	2.3%
FAD2A		R144H	19	Null	2 (34)	7.1%	3.0%	17.8%	27.1%	26.0%	1.7%	12.8%	1.3%	0.4%	2.7%
FAD2A		R144H	25	Null		7.4%	4.3%	15.2%	25.6%	29.6%	2.4%	11.5%	1.2%	0.4%	2.4%
FAD2A	7	S229F	5	HOMO	1	7.6%	3.8%	19.6%	21.4%	27.4%	2.0%	13.9%	1.0%	0.6%	2.6%
FAD2A	7	S229F	3	HET	1	7.9%	5.1%	18.2%	26.0%	25.7%	2.0%	10.4%	1.2%	0.7%	2.6%
FAD2A	7	S229F	12	Null	1	8.6%	5.6%	15.0%	27.8%	26.6%	3.4%	8.2%	1.4%	0.7%	2.8%
FAD2A	7	S229F	10	Null	1	9.3%	5.7%	12.8%	28.1%	25.4%	3.7%	10.1%	1.8%	0.4%	2.7%
FAD2A		S229F	7	HOMO	3	8.4%	5.8%	18.4%	22.7%	24.5%	3.5%	11.8%	1.1%	0.6%	3.2%
FAD2A		S229F	9	HOMO		7.3%	5.1%	17.8%	22.4%	25.2%	3.6%	14.0%	1.1%	0.6%	2.9%
FAD2A		S229F	19	HOMO		6.9%	5.0%	21.0%	20.9%	24.8%	2.7%	14.3%	0.9%	0.6%	2.9%

Gene	SNP	mutation	Plant #	genotype	# of samples	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C20:3	C22:1
FAD2A		S229F	13	Null	2	7.8%	5.6%	14.1%	26.4%	25.6%	3.6%	12.2%	1.5%	0.7%	2.5%
FAD2A		S229F	14	Null		7.6%	4.8%	13.8%	25.0%	28.3%	3.2%	12.6%	1.2%	0.8%	2.6%
FAD2A		S229F	4	Het	3 (46)	7.6%	3.5%	15.6%	23.8%	27.3%	2.3%	14.2%	1.6%	0.8%	3.2%
FAD2A		S229F	6	Het		7.7%	2.8%	14.8%	24.5%	29.4%	1.9%	13.5%	1.5%	0.8%	3.1%
FAD2A		S229F	8	Het		7.3%	5.0%	14.2%	22.6%	28.2%	3.7%	13.5%	1.4%	1.0%	3.2%
FAD2A		S229F	Y1	610	7	5.3%	2.3%	16.5%	21.8%	29.8%	1.5%	16.2%	2.0%	1.2%	3.4%
FAD2A		S229F	Y2	610		5.5%	2.3%	16.1%	22.0%	30.6%	1.2%	16.2%	1.9%	1.2%	2.9%
FAD2A		S229F	Y3	610		6.5%	2.2%	17.0%	23.3%	29.5%	1.1%	15.3%	1.7%	0.9%	2.4%
FAD2A		S229F	Y4	610		5.9%	2.0%	14.8%	21.8%	34.2%	0.9%	14.7%	2.0%	1.1%	2.5%
FAD2A		S229F	Y5	610		5.9%	2.0%	14.4%	22.3%	34.7%	1.0%	14.7%	1.8%	1.0%	2.3%
FAD2A		S229F	Y6	610		5.5%	2.2%	16.7%	21.6%	31.9%	1.2%	15.2%	1.8%	1.1%	2.8%
FAD2A		S229F	Y7	610		6.3%	2.5%	17.8%	23.7%	28.4%	1.4%	14.8%	1.6%	0.9%	2.6%
FAD2B		W91*	1	HOMO	1	7.7%	5.1%	19.4%	28.6%	23.9%	1.9%	10.9%	0.9%	0.4%	1.2%
FAD2B		W91*	8	HET	1	7.8%	5.5%	18.5%	27.5%	24.4%	2.5%	10.9%	0.9%	0.5%	1.5%
FAD2B		W91*	7	Null	1	7.6%	3.9%	12.4%	32.4%	23.9%	2.6%	11.5%	1.9%	0.7%	3.1%
FAD2B		W91*	5	Null	1	7.9%	4.2%	12.8%	29.4%	26.1%	2.6%	11.8%	1.7%	0.7%	2.9%
FAD2B		W91*	10	Null	4	8.4%	5.0%	12.1%	28.8%	26.9%	2.8%	11.4%	1.7%	0.8%	2.0%
FAD2B		W91*	13	Null		7.6%	4.8%	13.0%	33.7%	21.9%	2.8%	10.8%	1.8%	0.6%	2.9%
FAD2B		W91*	23	Null		8.3%	5.8%	13.4%	31.9%	21.4%	4.0%	10.6%	1.7%	0.4%	2.6%
FAD2B		W91*	24	Null		9.1%	5.1%	13.1%	32.8%	24.8%	2.2%	8.4%	1.8%	0.3%	2.5%
FAD2B		W91*	21	HOMO	2	7.9%	5.0%	17.9%	28.5%	23.5%	2.2%	11.5%	1.1%	0.5%	1.8%
FAD2B		W91*	22	HOMO		7.7%	6.0%	18.1%	28.1%	21.8%	3.3%	11.5%	1.1%	0.4%	2.0%
FAD2B		W91*	Y1	1105	9	5.9%	2.5%	19.7%	22.9%	33.6%	0.6%	12.3%	1.0%	0.7%	0.8%
FAD2B		W91*	Y2	1105		6.4%	2.5%	19.0%	24.5%	30.4%	1.0%	13.0%	1.2%	0.7%	1.3%
FAD2B		W91*	Y3	1105		6.8%	2.7%	18.8%	25.4%	31.6%	0.6%	11.5%	1.2%	0.5%	1.0%
FAD2B		W91*	Y4	1105		7.1%	3.0%	19.8%	26.8%	28.0%	1.0%	11.9%	1.0%	0.5%	0.9%
FAD2B		W91*	Y5	1105		6.4%	2.4%	19.8%	25.1%	29.7%	0.8%	12.7%	1.1%	0.6%	1.5%

Gene	SNP	mutation	Plant #	genotype	# of samples	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C20:3	C22:1
FAD2B		W91*	Y6	1105		6.8%	2.6%	18.1%	26.1%	29.6%	1.1%	12.7%	1.3%	0.5%	1.3%
FAD2B		W91*	Y7	1105		6.0%	2.7%	14.6%	24.5%	30.3%	1.0%	14.9%	2.0%	1.1%	2.9%
FAD2B		W91*	Y8	1105		5.9%	2.5%	19.0%	23.0%	32.0%	0.9%	13.7%	1.2%	0.5%	1.2%
FAD2B		W91*	Y9	1105		6.5%	2.5%	17.2%	24.2%	31.7%	1.1%	13.4%	1.1%	0.7%	1.5%

Note: * stands for nonsense mutation;
HOMO means the plants are all homozygous mutants at the specified locus.
HET means the plants are heterozygous mutants at the specified locus.
NULL means there is no mutation at the specified locus.
% means % of FAME composition

Table 16. Fatty Acids Composition in selected *FAD2* mutants, sorted by mutation, Average of Test No. 1 and Test No. 2

Gene	SNP	mutation	Plant #	genotype	# of samples	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C20:3	C22:1
none	none	CS32 controls	1	CS32 controls	10	5.79%	2.28%	14.91%	20.26%	34.01%	1.20%	16.16%	1.75%	0.92%	2.71%
none	none	CS32 controls	2	CS32 controls		6.07%	2.30%	14.63%	21.35%	33.27%	1.19%	15.64%	1.80%	1.17%	2.56%
none	none	CS32 controls	3	CS32 controls		6.17%	2.30%	14.13%	21.74%	34.22%	1.08%	15.46%	1.79%	1.02%	2.09%
none	none	CS32 controls	4	CS32 controls		6.03%	2.40%	14.23%	20.78%	34.53%	1.23%	15.81%	1.56%	1.01%	2.41%
none	none	CS32 controls	5	CS32 controls		6.73%	2.57%	14.63%	23.08%	33.52%	0.75%	12.87%	1.83%	1.34%	2.67%
none	none	CS32 controls	6	CS32 controls		6.22%	2.46%	14.57%	21.59%	33.42%	0.98%	15.40%	1.84%	1.05%	2.47%
none	none	CS32 controls	7	CS32 controls		5.88%	2.42%	14.58%	22.23%	32.70%	1.26%	15.70%	1.79%	0.97%	2.46%
none	none	CS32 controls	8	CS32 controls		6.03%	2.26%	13.61%	20.96%	34.27%	1.35%	15.97%	1.89%	1.04%	2.62%
none	none	CS32 controls	9	CS32 controls		5.93%	2.38%	14.34%	20.60%	33.61%	1.26%	16.23%	1.85%	1.11%	2.70%

Gene	SNP	mutation	Plant #	genotype	# of samples	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C20:3	C22:1
none	none	controls		controls								%			
		CS32 controls	10	CS32 controls		6.19%	2.42%	13.93%	21.40%	33.20%	1.35%	15.85%	1.88%	1.20%	2.57%
FAD2A				CS32 AVE				14.36%	21.40%	33.67%		15.51%			
FAD2A				CS32 SD				0.39%	0.83%	0.57%		0.96%			
FAD2A	5	G150E	4	HOMO	1	7.98%	4.39%	22.51%	18.83%	27.52%	2.20%	12.69%	0.47%	1.71%	1.71%
FAD2A	5	G150E	5	HOMO	1	7.80%	4.19%	23.22%	18.43%	26.56%	2.16%	14.17%	0.68%	0.56%	2.24%
FAD2A		G150E	6	HOMO	5 new	7.68%	4.22%	26.13%	18.78%	25.20%	1.94%	13.30%	0.55%	0.46%	1.74%
FAD2A		G150E	8	HOMO		8.09%	4.58%	24.20%	18.71%	28.10%	2.24%	10.23%	0.73%	0.46%	2.66%
FAD2A		G150E	14	HOMO		8.24%	4.46%	24.63%	19.43%	24.68%	2.02%	13.72%	0.64%	0.29%	1.89%
FAD2A		G150E	23	HOMO		8.60%	4.73%	22.96%	19.00%	26.72%	2.07%	13.07%	0.58%	0.52%	1.76%
FAD2A		G150E	25	HOMO		8.05%	4.01%	24.16%	19.98%	23.65%	1.84%	14.60%	0.72%	0.52%	2.46%
FAD2A		G150E		Homo AVE				23.97%	19.02%	26.06%		13.11%			
FAD2A		G150E		Homo SD				1.22%	0.52%	1.60%		1.43%			
FAD2A		G150E	20	Null	2 new	8.73%	4.20%	16.24%	26.75%	25.73%	2.17%	12.03%	1.30%	0.44%	2.41%
FAD2A	5	G150E	11	Null		8.29%	5.23%	17.87%	25.33%	27.41%	2.72%	9.99%	0.98%	0.49%	1.69%
FAD2A	5	G150E	18	Null		8.98%	5.13%	17.82%	28.18%	24.86%	3.19%	8.92%	1.20%	0.57%	1.14%
FAD2A		G150E	24	Null		8.74%	5.08%	16.38%	27.40%	26.04%	2.17%	10.88%	1.09%	0.63%	1.59%
FAD2A		G150E		Null AVE				17.08%	26.91%	26.01%		10.46%			
FAD2A		G150E		null SD				0.89%	1.21%	1.06%		1.32%			
FAD2A	2	Q44*	2	HOMO	1	8.29%	5.10%	22.83%	20.76%	26.73%	2.03%	11.13%	0.78%	0.39%	1.96%
FAD2A	2	Q44*	1	HOMO	1	7.80%	4.89%	19.67%	18.86%	28.30%	2.61%	13.90%	0.87%	0.73%	2.36%

Gene	SNP	mutation	Plant #	genotype	# of samples	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C20:3	C22:1
FAD2A	2	Q44*	4	HOMO	1	7.63%	5.05%	23.75%	20.19%	25.73%	2.39%	12.83%	0.60%	0.38%	1.45%
FAD2A	2	Q44*	5	HOMO		7.61%	5.25%	22.91%	19.10%	28.07%	2.20%	11.49%	0.78%	0.62%	1.95%
FAD2A	2	Q44*	3	HOMO		7.50%	4.63%	22.86%	19.84%	27.72%	2.09%	11.66%	0.80%	0.63%	2.26%
FAD2A		Q44*	36	HOMO	3	7.79%	5.55%	23.24%	21.36%	23.80%	2.46%	13.03%	0.71%	0.22%	1.84%
FAD2A		Q44*	38	HOMO		7.98%	6.21%	22.35%	20.37%	25.41%	2.87%	12.40%	0.66%	0.28%	1.47%
FAD2A		Q44*	39	HOMO		9.19%	5.64%	22.93%	22.02%	23.75%	2.34%	10.96%	0.74%	0.42%	2.00%
FAD2A		Q44*		Homo AVE				22.57%	20.31%	26.19%		12.18%			
FAD2A		Q44*		homo SD				1.24%	1.07%	1.82%		1.04%			
FAD2A	7	S229F	5	HOMO	1	7.60%	3.79%	19.62%	21.37%	27.42%	2.01%	13.94%	0.97%	0.63%	2.64%
FAD2A	7	S229F	1	HOMO		7.31%	3.36%	19.34%	23.61%	26.47%	1.74%	13.03%	1.25%	0.73%	3.17%
FAD2A		S229F	7	HOMO	3	8.44%	5.78%	18.39%	22.68%	24.53%	3.52%	11.78%	1.09%	0.56%	3.22%
FAD2A		S229F	9	HOMO		7.32%	5.15%	17.75%	22.35%	25.15%	3.56%	14.04%	1.11%	0.65%	2.91%
FAD2A		S229F	19	HOMO		6.92%	4.96%	21.02%	20.89%	24.77%	2.73%	14.28%	0.92%	0.63%	2.87%
FAD2A		S229F	20	HOMO AVE				19.23%	22.18%	25.67%		13.42%			
FAD2A		S229F	21	HOMO SD				1.25%	1.08%	1.23%		1.03%			
FAD2A	7	S229F	12	Null	1	8.62%	5.59%	14.98%	27.76%	26.56%	3.41%	8.20%	1.36%	0.69%	2.82%
FAD2A	7	S229F	10	Null	1	9.35%	5.67%	12.84%	28.10%	25.37%	3.68%	10.10%	1.77%	0.41%	2.71%
FAD2A		S229F	13	Null	2	7.77%	5.64%	14.08%	26.36%	25.64%	3.59%	12.18%	1.49%	0.75%	2.51%

Gene	SNP	mutation	Plant #	genotype	# of samples	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C20:3	C22:1
FAD2A		S229F	14	Null		7.57%	4.84%	13.78%	24.96%	28.33%	3.24%	12.64%	1.21%	0.85%	2.59%
FAD2A		S229F	15	Null AVE				13.92%	26.80%	26.47%		10.78%			
FAD2A		S229F	16	Null SD				0.88%	1.44%	1.34%		2.05%			
FAD2B		W91*	1	HOMO	1	7.72%	5.07%	19.39%	28.57%	23.91%	1.93%	10.91%	0.92%	0.44%	1.15%
FAD2B		W91*	21	HOMO	2	7.85%	5.02%	17.95%	28.51%	23.54%	2.22%	11.50%	1.10%	0.53%	1.78%
FAD2B		W91*	22	HOMO		7.73%	5.99%	18.08%	28.11%	21.75%	3.34%	11.49%	1.09%	0.44%	1.97%
FAD2B	8	W91*	2	HOMO		8.23%	5.35%	18.80%	29.20%	26.29%	1.68%	7.84%	0.86%	0.41%	1.34%
FAD2B	8	W91*	3	HOMO		8.05%	5.50%	18.37%	28.87%	24.40%	2.69%	8.63%	1.15%	0.53%	1.82%
FAD2B	8	W91*	4	HOMO		7.54%	5.09%	17.96%	28.89%	22.95%	2.92%	10.62%	1.25%	0.51%	2.27%
FAD2B		W91*	5	HOMO AVE				18.43%	28.69%	23.80%		10.17%			
FAD2B		W91*	6	HOMO SD				0.57%	0.38%	1.52%		1.55%			
FAD2B		W91*	7	Null	1	7.61%	3.94%	12.42%	32.37%	23.94%	2.61%	11.48%	1.87%	0.68%	3.09%
FAD2B		W91*	5	Null	1	7.93%	4.16%	12.77%	29.39%	26.08%	2.58%	11.78%	1.68%	0.74%	2.89%
FAD2B		W91*	10	Null	4	8.35%	5.04%	12.13%	28.77%	26.89%	2.84%	11.44%	1.69%	0.82%	2.04%
FAD2B		W91*	13	Null		7.61%	4.83%	12.99%	33.71%	21.94%	2.81%	10.77%	1.84%	0.62%	2.88%
FAD2B		W91*	23	Null		8.31%	5.84%	13.38%	31.92%	21.35%	4.00%	10.55%	1.66%	0.36%	2.62%
FAD2B		W91*	24	Null		9.08%	5.10%	13.10%	32.82%	24.75%	2.16%	8.39%	1.77%	0.34%	2.49%
FAD2B		W91*	25	Null AVE				12.80%	31.50%	24.16%		10.73%			
FAD2B		W91*	26	Null SD				0.46%	1.97%	2.21%		1.24%			

Note: * stands for nonsense mutation;

Gene	SNP	mutation	Plant #	genotype	# of samples	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C20:3	C22:1
HOMO means the plants are all homozygous mutants at the specified locus.															
HET means the plants are heterozygous mutants at the specified locus.															
NULL means there is no mutation at the specified locus.															
% means % of FAME composition															

Table 17. Fatty Acids Composition in selected *FAD2* mutants, sorted by mutation, Test 2

Gene	SNP	mutation	Plant #	genotype	# of samples	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C20:3	C22:1
none	none	CS32 controls	1	CS32 controls	10	5.8%	2.3%	14.9%	20.3%	34.0%	1.2%	16.2%	1.8%	0.9%	2.7%
none	none	CS32 controls	2	CS32 controls		6.1%	2.3%	14.6%	21.3%	33.3%	1.2%	15.6%	1.8%	1.2%	2.6%
none	none	CS32 controls	3	CS32 controls		6.2%	2.3%	14.1%	21.7%	34.2%	1.1%	15.5%	1.8%	1.0%	2.1%
none	none	CS32 controls	4	CS32 controls		6.0%	2.4%	14.2%	20.8%	34.5%	1.2%	15.8%	1.6%	1.0%	2.4%
none	none	CS32 controls	5	CS32 controls		6.7%	2.6%	14.6%	23.1%	33.5%	0.7%	12.9%	1.8%	1.3%	2.7%
none	none	CS32 controls	6	CS32 controls		6.2%	2.5%	14.6%	21.6%	33.4%	1.0%	15.4%	1.8%	1.1%	2.5%
none	none	CS32 controls	7	CS32 controls		5.9%	2.4%	14.6%	22.2%	32.7%	1.3%	15.7%	1.8%	1.0%	2.5%
none	none	CS32 controls	8	CS32 controls		6.0%	2.3%	13.6%	21.0%	34.3%	1.4%	16.0%	1.9%	1.0%	2.6%
none	none	CS32 controls	9	CS32 controls		5.9%	2.4%	14.3%	20.6%	33.6%	1.3%	16.2%	1.9%	1.1%	2.7%
none	none	CS32 controls	10	CS32 controls		6.2%	2.4%	13.9%	21.4%	33.2%	1.3%	15.9%	1.9%	1.2%	2.6%
FAD2A	5	G150E	4	HOMO	1	8.0%	4.4%	22.5%	18.8%	27.5%	2.2%	12.7%	0.5%	1.7%	1.7%
FAD2A	5	G150E	5	HOMO	1	7.8%	4.2%	23.2%	18.4%	26.6%	2.2%	14.2%	0.7%	0.6%	2.2%
FAD2A		G150E	6	HOMO	5 new	7.7%	4.2%	26.1%	18.8%	25.2%	1.9%	13.3%	0.6%	0.5%	1.7%

FAD2A		G150E	8	HOMO			8.1%	4.6%	24.2%	18.7%	28.1%	2.2%	10.2%	0.7%	0.5%	2.7%
FAD2A		G150E	14	HOMO			8.2%	4.5%	24.6%	19.4%	24.7%	2.0%	13.7%	0.6%	0.3%	1.9%
FAD2A		G150E	23	HOMO			8.6%	4.7%	23.0%	19.0%	26.7%	2.1%	13.1%	0.6%	0.5%	1.8%
FAD2A		G150E	25	HOMO			8.0%	4.0%	24.2%	20.0%	23.7%	1.8%	14.6%	0.7%	0.5%	2.5%
FAD2A	2	Q44*	2	HOMO	1		8.3%	5.1%	22.8%	20.8%	26.7%	2.0%	11.1%	0.8%	0.4%	2.0%
FAD2A	2	Q44*	1	HOMO	1		7.8%	4.9%	19.7%	18.9%	28.3%	2.6%	13.9%	0.9%	0.7%	2.4%
FAD2A	2	Q44*	4	HOMO	1		7.6%	5.1%	23.7%	20.2%	25.7%	2.4%	12.8%	0.6%	0.4%	1.5%
FAD2A	2	Q44*	5	HOMO												
FAD2A	2	Q44*	3	HOMO												
FAD2A		Q44*	36	HOMO	3		7.8%	5.5%	23.2%	21.4%	23.8%	2.5%	13.0%	0.7%	0.2%	1.8%
FAD2A		Q44*	38	HOMO			8.0%	6.2%	22.3%	20.4%	25.4%	2.9%	12.4%	0.7%	0.3%	1.5%
FAD2A		Q44*	39	HOMO			9.2%	5.6%	22.9%	22.0%	23.7%	2.3%	11.0%	0.7%	0.4%	2.0%
FAD2A	7	S229F	5	HOMO	1		7.6%	3.8%	19.6%	21.4%	27.4%	2.0%	13.9%	1.0%	0.6%	2.6%
FAD2A	7	S229F	1	HOMO												
FAD2A		S229F	7	HOMO	3		8.4%	5.8%	18.4%	22.7%	24.5%	3.5%	11.8%	1.1%	0.6%	3.2%
FAD2A		S229F	9	HOMO			7.3%	5.1%	17.8%	22.4%	25.2%	3.6%	14.0%	1.1%	0.6%	2.9%
FAD2A		S229F	19	HOMO			6.9%	5.0%	21.0%	20.9%	24.8%	2.7%	14.3%	0.9%	0.6%	2.9%
FAD2B		W91*	1	HOMO	1		7.7%	5.1%	19.4%	28.6%	23.9%	1.9%	10.9%	0.9%	0.4%	1.2%
FAD2B		W91*	21	HOMO	2		7.9%	5.0%	17.9%	28.5%	23.5%	2.2%	11.5%	1.1%	0.5%	1.8%
FAD2B		W91*	22	HOMO			7.7%	6.0%	18.1%	28.1%	21.8%	3.3%	11.5%	1.1%	0.4%	2.0%
FAD2B	8	W91*	2	HOMO												
FAD2B	8	W91*	3	HOMO												
FAD2B	8	W91*	4	HOMO												
FAD2A		G150E	20	Null	2		8.7%	4.2%	16.2%	26.7%	25.7%	2.2%	12.0%	1.3%	0.4%	2.4%
FAD2A	5	G150E	11	Null			8.3%	5.2%	17.9%	25.3%	27.4%	2.7%	10.0%	1.0%	0.5%	1.7%
FAD2A	5	G150E	18	Null			9.0%	5.1%	17.8%	28.2%	24.9%	3.2%	8.9%	1.2%	0.6%	1.1%
FAD2A		G150E	24	Null			8.7%	5.1%	16.4%	27.4%	26.0%	2.2%	10.9%	1.1%	0.6%	1.6%
FAD2A	7	S229F	12	Null	1		8.6%	5.6%	15.0%	27.8%	26.6%	3.4%	8.2%	1.4%	0.7%	2.8%
FAD2A	7	S229F	10	Null	1		9.3%	5.7%	12.8%	28.1%	25.4%	3.7%	10.1%	1.8%	0.4%	2.7%

FAD2A		S229F	13	Null	2		7.8%	5.6%	14.1%	26.4%	25.6%	3.6%	12.2%	1.5%	0.7%	2.5%
FAD2A		S229F	14	Null			7.6%	4.8%	13.8%	25.0%	28.3%	3.2%	12.6%	1.2%	0.8%	2.6%
FAD2B		W91*	7	Null	1		7.6%	3.9%	12.4%	32.4%	23.9%	2.6%	11.5%	1.9%	0.7%	3.1%
FAD2B		W91*	5	Null	1		7.9%	4.2%	12.8%	29.4%	26.1%	2.6%	11.8%	1.7%	0.7%	2.9%
FAD2B		W91*	10	Null	4		8.4%	5.0%	12.1%	28.8%	26.9%	2.8%	11.4%	1.7%	0.8%	2.0%
FAD2B		W91*	13	Null			7.6%	4.8%	13.0%	33.7%	21.9%	2.8%	10.8%	1.8%	0.6%	2.9%
FAD2B		W91*	23	Null			8.3%	5.8%	13.4%	31.9%	21.4%	4.0%	10.6%	1.7%	0.4%	2.6%
FAD2B		W91*	24	Null			9.1%	5.1%	13.1%	32.8%	24.8%	2.2%	8.4%	1.8%	0.3%	2.5%

Note: * stands for nonsense mutation;

HOMO means the plants are all homozygous mutants at the specified locus.

HET means the plants are heterozygous mutants at the specified locus.

NULL means there is no mutation at the specified locus.

% means % of FAME composition

Table 18a. Fatty Acids Composition in selected *FAD2* mutants, sorted by gene, Test 4

Sample	Genotype	Seed generation	gene	mutation	C16:0	C18:0	C18:1	C18:2	C20:0	C18:3	C20:1	C20:2	C20:3	C22:1
2362-Q10	HOM	M5	FAD2 A	G150E	8.0%	4.3%	21.6%	16.2%	2.3%	30.3%	13.4%	0.8%	0.7%	2.2%
2362-Q11	HOM	M5	FAD2 A	G150E	8.0%	3.6%	20.4%	17.1%	1.8%	32.3%	13.1%	0.8%	0.7%	2.2%
2362-Q12	HOM	M5	FAD2 A	G150E	8.3%	3.6%	19.4%	17.9%	1.5%	32.5%	13.0%	0.9%	0.7%	2.2%
2362-Q13	HOM	M5	FAD2 A	G150E	8.2%	4.2%	20.4%	17.3%	2.3%	31.0%	13.1%	0.9%	0.6%	2.1%
2826-P1	Het	M5	FAD2 A	Q44*	7.1%	3.4%	16.3%	16.3%	2.2%	35.1%	14.6%	1.2%	1.1%	2.8%
2826-P2	Het	M5	FAD2 A	Q44*	7.7%	4.0%	17.2%	16.4%	2.5%	33.7%	14.1%	1.0%	0.9%	2.5%
2826-P3	Het	M5	FAD2 A	Q44*	8.4%	4.0%	17.8%	19.8%	2.7%	28.9%	12.4%	1.4%	1.2%	3.3%
2826-P4	Het	M5	FAD2 A	Q44*	7.7%	3.9%	15.5%	16.6%	2.5%	34.4%	14.5%	1.2%	1.0%	2.6%
3006-R1	HOM	M5	FAD2 B	W91*	7.7%	3.5%	11.9%	21.5%	2.3%	35.6%	12.3%	1.6%	1.1%	2.6%
3006-R2	HOM	M5	FAD2 B	W91*	7.8%	3.6%	12.1%	22.6%	2.3%	33.4%	12.7%	1.6%	1.0%	2.7%
3006-R3	HOM	M5	FAD2 B	W91*	8.1%	3.5%	12.3%	22.5%	2.1%	34.5%	12.0%	1.6%	1.1%	2.4%

Sample	Genotype	Seed generation	gene	mutation	C16:0	C18:0	C18:1	C18:2	C20:0	C18:3	C20:1	C20:2	C20:3	C22:1
3006-R4	HOM	M5	FAD2 B	W91*	7.8%	3.6%	12.7%	22.2%	2.1%	34.4%	12.2%	1.6%	1.0%	2.4%
3489-N2	HOM	M4	FAD2 B	W138*	8.0%	3.4%	11.9%	23.2%	2.5%	31.7%	12.8%	1.9%	1.1%	3.6%
3489-N5	HOM	M4	FAD2 B	W138*	8.2%	3.5%	11.5%	23.7%	2.6%	31.0%	12.8%	2.0%	1.0%	3.6%
3489-N9	HOM	M4	FAD2 B	W138*	7.9%	3.3%	12.3%	22.2%	2.6%	31.0%	13.7%	1.9%	1.2%	3.8%
3489-N12	HOM	M4	FAD2 B	W138*	7.8%	3.4%	11.9%	22.9%	2.6%	30.7%	14.0%	2.0%	1.1%	3.8%
3489-N16	HOM	M4	FAD2 B	W138*	7.8%	3.5%	11.9%	22.7%	2.6%	31.2%	13.6%	2.0%	1.1%	3.8%
3702-O2	HOM	M4	FAD2 B	G150E	7.7%	4.2%	12.8%	23.5%	3.0%	31.1%	12.0%	1.8%	1.0%	2.9%
3702-O3	HOM	M4	FAD2 B	G150E	9.7%	5.6%	17.1%	34.2%	3.7%	18.0%	8.2%	1.0%	0.3%	2.0%
3702-O4	Het	M4	FAD2 B	G150E	7.5%	4.4%	11.6%	24.7%	4.5%	31.1%	10.4%	1.8%	0.9%	3.2%
3702-O6	HOM	M4	FAD2 B	G150E	7.8%	4.4%	12.0%	24.4%	3.0%	31.4%	11.5%	1.8%	0.9%	2.7%
3702-O7	HOM	M4	FAD2 B	G150E	8.0%	5.8%	13.6%	25.2%	4.2%	29.7%	9.1%	1.4%	0.7%	2.2%
3702-O9	Het	M4	FAD2 B	G150E	7.1%	4.0%	12.1%	23.7%	3.2%	31.2%	12.4%	1.9%	1.0%	3.3%
6490-M1	HOM	M4	FAD2 B	W91*	6.3%	3.2%	13.2%	21.8%	2.3%	32.1%	14.4%	1.8%	1.2%	3.7%
6490-M2	HOM	M4	FAD2 B	W91*	6.1%	2.9%	12.0%	20.2%	2.2%	34.2%	14.8%	2.1%	1.3%	4.1%
6490-M3	HOM	M4	FAD2 B	W91*	6.2%	3.0%	12.5%	20.8%	2.3%	33.5%	14.5%	2.0%	1.3%	4.0%
6490-M4	HOM	M4	FAD2 B	W91*	8.3%	3.2%	12.3%	21.3%	2.3%	32.0%	14.0%	1.8%	1.2%	3.6%
6490-M5	HOM	M4	FAD2 B	W91*	8.0%	2.5%	12.0%	20.5%	1.8%	33.8%	14.7%	1.9%	1.3%	3.6%
6490-M10	null	M4	FAD2 B	W91*	9.0%	2.6%	10.9%	18.5%	2.1%	35.5%	14.4%	2.1%	1.4%	3.3%
3284-B11	null	M4	FAD2 C	W91*	8.9%	4.7%	10.1%	22.3%	2.8%	35.8%	10.3%	2.0%	1.2%	2.0%
3284-B12	Het	M4	FAD2 C	W91*	8.6%	5.2%	11.9%	23.2%	3.2%	33.4%	10.2%	1.6%	0.9%	1.7%
3284-B13	Het	M4	FAD2 C	W91*	8.1%	4.4%	11.6%	21.1%	2.7%	36.3%	11.1%	1.7%	1.2%	1.9%
3284-B15	null	M4	FAD2 C	W91*	9.0%	4.2%	9.3%	22.1%	2.9%	36.9%	10.5%	1.9%	1.3%	1.9%
3284-B21	Het	M4	FAD2 C	W91*	8.4%	4.5%	10.6%	20.3%	2.9%	36.5%	11.7%	1.7%	1.2%	2.2%
4506-A2	null	M4	FAD2 C	W87*	7.5%	3.5%	11.9%	23.6%	3.0%	30.8%	12.9%	2.1%	1.1%	3.5%
4506-A10	Hom	M4	FAD2 C	W87*	6.9%	3.6%	14.7%	20.7%	2.6%	31.5%	14.1%	1.6%	1.0%	3.3%
4506-A12	Hom	M4	FAD2 C	W87*	7.3%	4.0%	15.9%	20.4%	2.9%	30.0%	14.1%	1.4%	0.9%	3.2%
4506-A15	Hom	M4	FAD2 C	W87*	8.2%	3.3%	7.1%	24.1%	2.5%	33.8%	14.0%	1.9%	1.1%	4.1%

Sample	Genotype	Seed generation	gene	mutation	C16:0	C18:0	C18:1	C18:2	C20:0	C18:3	C20:1	C20:2	C20:3	C22:1
4506-A16	null	M4	FAD2 C	W87*	8.0%	3.5%	12.8%	23.6%	3.0%	30.9%	12.5%	1.7%	1.0%	3.1%
4608-C4	Hom	M4	FAD2 C	G150E	8.8%	3.5%	9.2%	23.3%	2.9%	35.0%	11.2%	1.9%	1.2%	2.9%
4608-C12	Hom	M4	FAD2 C	G150E	9.3%	4.0%	10.0%	25.9%	3.0%	31.6%	10.8%	1.9%	1.0%	2.7%
4608-C13	Het	M4	FAD2 C	G150E	9.0%	3.9%	9.2%	25.4%	2.8%	32.8%	11.0%	2.1%	1.1%	2.8%
4608-C15	null	M4	FAD2 C	G150E	8.9%	3.9%	8.9%	26.0%	2.9%	32.7%	10.7%	2.2%	1.1%	2.7%
4608-C17	Het	M4	FAD2 C	G150E	9.0%	3.8%	8.7%	23.6%	3.1%	34.1%	11.1%	2.2%	1.3%	3.1%
Cs32-1					7.8%	4.7%	12.4%	27.1%	4.3%	25.5%	12.1%	2.0%	0.8%	3.3%
Cs32-2					8.0%	4.5%	12.0%	26.7%	3.9%	27.1%	11.8%	2.0%	0.8%	3.2%
Cs32-3					8.0%	4.1%	12.1%	26.6%	3.7%	27.7%	11.7%	2.1%	0.9%	3.2%
Cs32-4					7.9%	3.9%	12.2%	26.2%	3.4%	28.7%	11.7%	2.0%	0.9%	3.0%
At FAD2_1					5.4%	3.0%	49.9%	4.2%	1.5%	10.3%	24.2%	0.0%	0.0%	1.5%
At FAD2_2					5.6%	3.6%	50.5%	4.5%	0.0%	10.3%	24.1%	0.1%	0.0%	1.4%
At FAE1_1					10.3%	4.7%	28.8%	34.2%	1.0%	20.9%	0.1%	0.0%	0.0%	0.0%
At FAE1_2					10.2%	5.2%	28.7%	33.9%	1.0%	20.9%	0.1%	0.0%	0.0%	0.0%

Note: * stands for nonsense mutation;
Hom means the plants are all homozygous mutants at the specified locus.
Het means the plants are heterozygous mutants at the specified locus.
Null means there is no mutation at the specified locus.
% means % of FAME composition
Gene indicates in which gene the mutation is located

Table 19a. Fatty Acids Composition in selected *FAE1* mutants, sorted by gene, Test 4

Sample	Genotype	Seed generation	gene	mutation	C16:0	C18:0	C18:1	C18:2	C20:0	C18:3	C20:1	C20:2	C20:3	C22:1
3395-D10	Hom	M4	FAE1 A	R209*	9.7%	4.2%	13.2%	20.8%	2.4%	33.6%	11.4%	1.6%	1.1%	2.0%
3395-D12	Hom	M4	FAE1 A	R209*	8.3%	4.7%	15.8%	21.0%	2.4%	32.7%	11.0%	1.3%	1.0%	1.6%
3395-D13	Hom	M4	FAE1 A	R209*	7.8%	4.2%	14.1%	20.7%	2.2%	35.7%	11.0%	1.5%	1.1%	1.7%

Sample	Genotype	Seed generation	gene	mutation	C16:0	C18:0	C18:1	C18:2	C20:0	C18:3	C20:1	C20:2	C20:3	C22:1
3395-D17	null	M4	FAE1 A	R209*	9.8%	3.4%	11.7%	20.8%	2.1%	34.6%	11.7%	1.8%	1.3%	2.8%
3395-D18	null	M4	FAE1 A	R209*	7.4%	4.3%	14.0%	21.4%	2.5%	33.5%	11.6%	1.6%	1.2%	2.6%
3395-D19	null	M4	FAE1 A	R209*	7.7%	3.5%	14.2%	20.2%	2.4%	33.5%	12.7%	1.6%	1.1%	3.1%
3395-D20	Hom	M4	FAE1 A	R209*	7.8%	4.4%	14.8%	21.4%	2.4%	33.8%	11.3%	1.4%	1.0%	1.7%
6386-F1	Het	M4	FAE1 A	G221D	11.1%	5.0%	10.6%	32.7%	3.6%	23.4%	9.2%	1.7%	0.5%	2.1%
6386-F2	HOM	M4	FAE1 A	G221D	9.2%	4.7%	13.3%	26.8%	2.2%	31.3%	9.1%	1.3%	0.7%	1.2%
6386-F7	Hom	M4	FAE1 A	G221D	8.8%	4.6%	12.7%	26.3%	2.4%	32.7%	9.0%	1.5%	0.8%	1.1%
6386-F9	Hom	M4	FAE1 A	G221D	9.0%	4.4%	11.5%	26.8%	2.6%	30.9%	10.2%	1.8%	0.9%	1.9%
6386-F13	null	M4	FAE1 A	G221D	8.9%	4.6%	12.7%	25.4%	2.3%	33.6%	9.0%	1.4%	0.8%	1.2%
6386-F15	null	M4	FAE1 A	G221D	8.1%	4.2%	11.2%	25.3%	3.4%	30.1%	11.8%	1.9%	1.0%	2.9%
6386-F19	Het	M4	FAE1 A	G221D	8.2%	4.2%	11.4%	24.8%	2.7%	32.8%	10.9%	1.8%	1.0%	2.2%
4687-I4	HOM	M4	FAE1 B	H301Y	7.1%	2.6%	14.6%	20.7%	1.2%	41.0%	9.2%	1.3%	1.1%	1.3%
4687-I10	null	M4	FAE1 B	H301Y	7.3%	3.2%	14.5%	21.0%	1.8%	37.8%	10.5%	1.4%	1.1%	1.5%
4687-I11	HOM	M4	FAE1 B	H301Y	7.7%	3.2%	15.9%	21.8%	1.4%	38.9%	8.2%	1.1%	0.9%	0.9%
4687-I14	null	M4	FAE1 B	H301Y	7.6%	3.6%	16.0%	21.6%	1.8%	34.1%	11.5%	1.4%	1.0%	1.5%
4687-I17	HOM	M4	FAE1 B	H301Y	7.2%	3.0%	14.7%	19.6%	1.3%	43.0%	8.2%	1.1%	1.0%	0.9%
5343-H6	null	M4	FAE1 B	S281F	7.6%	3.5%	11.5%	19.6%	2.5%	36.5%	12.6%	1.8%	1.4%	3.0%
5343-H7	null	M4	FAE1 B	S281F	7.8%	3.6%	11.6%	20.1%	2.6%	35.7%	12.5%	1.8%	1.2%	3.1%
5343-H10	HOM	M4	FAE1 B	S281F	7.9%	4.0%	13.2%	22.9%	2.3%	34.5%	10.6%	1.5%	1.0%	2.1%
5343-H14	HOM	M4	FAE1 B	S281F	8.3%	3.2%	11.2%	20.6%	1.8%	39.3%	10.6%	1.7%	1.3%	2.1%
5343-H15	HOM	M4	FAE1 B	S281F	8.1%	4.0%	12.0%	22.1%	2.2%	36.5%	10.4%	1.6%	1.1%	2.0%
5343-H16	HOM	M4	FAE1 B	S281F	7.9%	3.0%	11.0%	19.4%	1.7%	40.6%	10.9%	1.8%	1.5%	2.3%
5951-G1	HOM	M4	FAE1 B	Q142*	8.7%	3.6%	14.1%	22.4%	1.1%	43.0%	4.9%	0.8%	0.7%	0.7%
5951-G2	HOM	M4	FAE1 B	Q142*	8.1%	3.8%	14.4%	22.3%	1.4%	40.7%	6.6%	0.9%	0.8%	0.9%
5951-G3	HOM	M4	FAE1 B	Q142*	7.9%	3.1%	11.3%	20.0%	2.1%	39.0%	11.0%	1.7%	1.5%	2.4%
5951-G4	HOM	M4	FAE1 B	Q142*	9.4%	3.3%	14.3%	23.8%	0.9%	44.0%	3.0%	0.5%	0.5%	0.3%
5951-G5	HOM	M4	FAE1 B	Q142*	8.0%	2.8%	10.5%	20.4%	2.2%	39.0%	11.4%	1.7%	1.4%	2.7%

Sample	Genotype	Seed generation	gene	mutation	C16:0	C18:0	C18:1	C18:2	C20:0	C18:3	C20:1	C20:2	C20:3	C22:1
6476-K2	Hom	M4	FAE1 C	R209*	7.8%	3.2%	9.2%	23.2%	2.2%	38.7%	9.4%	1.9%	1.3%	2.9%
6476-K4	HOM	M4	FAE1 C	R209*	7.3%	3.8%	10.6%	23.1%	2.4%	38.3%	9.1%	1.7%	1.2%	2.3%
6476-K6	HOM	M4	FAE1 C	R209*	8.3%	4.0%	10.2%	22.9%	2.5%	36.3%	9.8%	1.9%	1.2%	2.9%
6476-K7	null	M4	FAE1 C	R209*	7.1%	3.6%	9.8%	23.6%	3.0%	32.1%	13.0%	2.5%	1.2%	4.0%
6476-K15	HOM	M4	FAE1 C	R209*	7.5%	4.0%	11.2%	21.6%	2.2%	35.6%	11.7%	1.9%	1.2%	3.0%
Cs32-1					7.8%	4.7%	12.4%	27.1%	4.3%	25.5%	12.1%	2.0%	0.8%	3.3%
Cs32-2					8.0%	4.5%	12.0%	26.7%	3.9%	27.1%	11.8%	2.0%	0.8%	3.2%
Cs32-3					8.0%	4.1%	12.1%	26.6%	3.7%	27.7%	11.7%	2.1%	0.9%	3.2%
Cs32-4					7.9%	3.9%	12.2%	26.2%	3.4%	28.7%	11.7%	2.0%	0.9%	3.0%
At FAE1_1					10.3%	4.7%	28.8%	34.2%	1.0%	20.9%	0.1%	0.0%	0.0%	0.0%
At FAE1_2					10.2%	5.2%	28.7%	33.9%	1.0%	20.9%	0.1%	0.0%	0.0%	0.0%
Note: * stands for nonsense mutation; Hom means the plants are all homozygous mutants at the specified locus. Het means the plants are heterozygous mutants at the specified locus. Null means there is no mutation at the specified locus. % means % of FAME composition Gene indicates in which gene the mutation is located														

Example 13**Fatty Acids Composition in plants with multiple mutations in *FAD2* and/or *FAE1* genes**

To further increase the oleic acid (18:1) level and/or yield and improve *Camelina* seed oil quality, mutations in one or more copies of *FAD2* genes and/or one or more copies of *FAE1* genes are integrated together to create mutant plants with double, triple, quadruple et al. mutations. Such mutants can be created by classic breeding methods. Table 20 below shows a list of non-limiting examples of such mutants.

Table 20. Plants with more than one mutation in Fatty Acid Synthesis Genes

Plant ID	Genotype					
	<i>FAD2 A</i>	<i>FAD2 B</i>	<i>FAD2 C</i>	<i>FAE1 A</i>	<i>FAE1 B</i>	<i>FAE1 C</i>
A1	HOMO	HOMO	NULL	NULL	NULL	NULL
A2	HOMO	NULL	HOMO	NULL	NULL	NULL
A3	NULL	HOMO	HOMO	NULL	NULL	NULL
A4	HOMO	HOMO	HOMO	NULL	NULL	NULL
A5	NULL	NULL	NULL	HOMO	HOMO	NULL
A6	NULL	NULL	NULL	HOMO	NULL	HOMO
A7	NULL	NULL	NULL	NULL	HOMO	HOMO
A8	NULL	NULL	NULL	HOMO	HOMO	HOMO
A9	HOMO	NULL	NULL	HOMO	NULL	NULL
A10	HOMO	NULL	NULL	HOMO	HOMO	NULL
A11	HOMO	NULL	NULL	HOMO	HOMO	HOMO
A12	HOMO	HOMO	NULL	HOMO	NULL	NULL
A13	HOMO	HOMO	NULL	HOMO	HOMO	NULL
A14	HOMO	HOMO	NULL	HOMO	HOMO	HOMO
A15	HOMO	HOMO	HOMO	HOMO	NULL	NULL
A16	HOMO	HOMO	HOMO	HOMO	HOMO	NULL
A17	HOMO	HOMO	HOMO	HOMO	HOMO	HOMO
Note: HOMO means the plants are all homozygous mutants at the specified locus. NULL means there is no mutation at the specified locus.						

Fatty acid compositions in these mutants are then analyzed by gas chromatography (GC). The results will show that one or more of these mutants produce seed oil with higher oleic acid (18:1) levels and/or lower VLCFA levels when compared to Cs32 control plants or

to one or more single mutants that have only one mutation in a *FAD2* gene and/or a *FAE1* gene.

Thus, mutations in more than one *FAD2* and/or *FAE1* genes further increase oleic acid (18:1) levels and/or lower VLCFA levels, and improve *Camelina* seed oil quality.

5

Example 14

Fatty Acids Composition in RNAi transgenic *Camelina* plants

As described in the present invention, RNAi technology can be used to disrupt one or more fatty acid synthesis genes (e.g., *FAD2*, *FAE1*, and other genes) in *Camelina* to obtain
10 an increase in oleic acid (18:1) and/or a decrease in VLCFA in the seed oil as measured by relative percent or absolute yield. The advantage of this method is that an RNAi expression vector can contain a double strand RNA that simultaneously suppresses one or more homologous genes. This is extremely helpful in *Camelina* as the inventors proved it is an allohexaploid species.

15 Using RNAi technology to knock down expression of all *FAD2* genes and/or all *FAE1* genes may be more convenient than classic breeding method. Whereas both sense- and antisense-mediated gene silencing have proven fruitful for PTGS in plant cells, RNAi induction can be more efficiently achieved by specialized expression cassettes that produce self-complementary hairpin (hp)-like RNA molecules. Such cassettes typically include plant
20 promoter and terminator sequences that control the expression of two inversely repeated sequences of the target genes that are separated by a specific spacer.

Upon delivery to plant cells, expression of an RNAi cassette will result in a dsRNA molecule composed of two distinct regions: a single-stranded loop, encoded by the spacer region, and a double-stranded stem, encoded by the inverted repeats. It is the stem region
25 that is used as a substrate by the dicer, but, because the spacer itself can potentially be recognized as a substrate as well, intron sequences are often used in the construction of such RNAi vectors (e.g. Meyer et al., 2004, Vectors for RNAi technology in poplar. Plant Biol (Stuttg) 6: 100–103). These vectors include, but are not limited to, pHANNIBAL, pKANNIBAL, pHELLSGATE, pSAT, pCAMBIA, pGREEN, et al.. RNAi (or hpRNA)
30 plant expression can potentially be delivered to plant cells by various means of transformation but are typically used by incorporating into binary plasmids to be delivered to plant cells by Agrobacterium-mediated transformation.

Fatty acid synthesis genes that are potential targets include, any one of *FAD2* genes and/or any one of *FAE1* genes as provided in the present invention, or alternatively along with any other genes involved in *Camelina* fatty acid synthesis as described herein or elsewhere.

5 A non-limiting example of using RNAi technology to suppress *Camelina FAD2* genes is described below. A complete hpRNA expression cassette is composed of four distinct regions: a promoter and terminator sequence, the ChsA intron sequence, and a dual MCS. The dual MCS results from cloning of the ChsA intron sequence into pSAT6-MCS and dividing the original MCS into two new, distinct regions, designated MCS-I and MCS-II,
10 which contain the following unique restriction endonuclease recognition sites: *NcoI*, *BspEI*, *BglII*, *XhoI*, *SacI*, and *EcoRI* in MCS-I and *PstI*, *SalI*, *KpnI*, *SacII*, *ApaI*, *XmaI*, *SmaI*, *BamHI*, and *XbaI* in MCS-II. The two MCS regions allow the successive cloning of the target gene sequence in reverse orientation and assembly of a hpRNA sequence. In pSAT6.35S.RNAi, expression of hpRNA is controlled by tandem CaMV 35S promoter (35SP) and CaMV 35S
15 terminator (35ST), conferring a complete expression cassette. In pSAT6.Napin.RNAi, expression of hpRNA is controlled by Napin plant seed-specific promoter. hpRNA designed according to conserved, specific 19 to 29, 19 to 27, or 19 to 21 polynucleotides of *FAD2 A*, *FAD2 B*, and *FAD2 C* genes, which does not share homology to other genes, are introduced into either pSAT6.35S.RNAi or pSAT6.Napin.RNA vector to make the final RNAi construct.
20 Such conserved, specific 15-21 polynucleotides sequences can be designed by one of ordinary skill in the art based on *FAD2* genes disclosed in the present invention and known *Camelina* non-*FAD2* gene sequences deposited in the GenBank.

Further, pSAT6.35S.RNAi or pSAT6.Napin.RNA vector containing the hpRNA targeting *FAD2* genes is transformed into *Camelina* plant using the method described in
25 WO2009/117555, and positive transformants are selected. Northern blot or qPCR is used to verify if one or more *FAD2* genes are suppressed in the transformants. The transgenic lines with the most efficient suppression in all *FAD2* genes are subjected to fatty acid composition analysis by GC, and the results indicate such transgenic *Camelina* plants have an increased oleic acid (18:1) level and/or reduced polyunsaturated fatty acids level in the seed oil
30 compared to transgenic *Camelina* plants with empty control vector.

In addition, another pSAT6.35S.RNAi or pSAT6.Napin.RNA vector containing the hpRNA targeting *FAE1* genes is transformed into *Camelina* plant using the method described in WO2009/117555, and positive transformants are selected. Northern blot or qPCR is used

to verify if one or more *FAE1* genes are suppressed in the transformants. The transgenic lines with the most efficient suppression in all *FAE1* genes are subjected to fatty acid composition analysis by GC, and the results indicate such transgenic *Camelina* plants have a decreased long chain fatty acid level, and/or reduced long chain polyunsaturated fatty acids level in the seed oil compared to transgenic *Camelina* plants with empty control vector.

Example 15

Fatty Acid Composition in *Camelina* plants having suppressed FAD2 and/or FAE1 gene functions in combination with overexpression or suppression of other non-FAD and non-FAE fatty acid synthesis genes

Other fatty acid synthesis enzymes may be manipulated in the fatty acid synthesis pathways to increase the amount of oleic acid (18:1) or decrease the amount of palmitic acid (16:0) to create Camelina oil with fatty acid profiles optimal for biodiesel production. Lower amounts of 16:0 saturated fatty acid and higher amounts of 18:1 monounsaturated fatty acid is desirable for a good balance of proper cetane number, cloud point, oxidative stability, and reduced NOx emissions, as mentioned in the Background and Example 9.

Three key enzymes regulate the amount of 16:0, 18:0 and 18:1 fatty acids (Figure 13): acyl-acyl carrier protein thioesterase (also known as FATB), β -ketoacyl-acyl carrier protein (ACP) synthase II (KAS II) and Δ -9 desaturase. FATB hydrolyzes the fatty acyl group from acyl carrier protein (ACP) and thus determines the amount and type of fatty acid that is exported from the plastid. Suppression of FATB leads to a reduction in 16:0 and 18:0 (stearic acid) released to the cytoplasm. KAS II converts palmitoyl-ACP (16:0-ACP) to stearoyl-ACP (18:0 ACP), and thus the overexpression of KAS II leads to an increase in the amount of 16:0 being converted to 18:0. Δ -9 desaturase converts 18:0-ACP to oleoyl-ACP (18:1-ACP), and thus the overexpression of Δ -9 desaturase leads to an increase in the amount of 18:0 being converted to 18:1. Since the product of KAS II activity (18:0-ACP) is the substrate for Δ -9 desaturase, the overexpression of both KAS II and Δ -9 desaturase will lead to a further decrease in 16:0 and 18:0 and an increase in 18:1.

Camelina lines having suppressed FAD2 and/or FAE1 gene functions, as described in the present invention, obtained either by TILLING or transgenic means (e.g., antisense, RNAi), may be combined with overexpression or suppression of the non-FAD and non-FAE genes described in this example to create new Camelina lines with even greater percentages

of 18:1 fatty acid and/or lesser percentages of 16:0 and/or 18:0 fatty acids compared to lines with only FAD2/FAE1 modifications or only non-FAD/non-FAE modifications.

For example, Camelina FAD2 and/or FAE1 mutant plants, permutations of which are described in Example 13, may be combined by breeding with a Camelina plant overexpressing KAS II in a seed-specific manner to create a new Camelina line where the amount of 18:1 is higher and the amount of 16:0 is lower compared to either parent plant alone. The seed-specific overexpression of KAS II may also indirectly decrease the amount of 18:2 and/or 18:3 polyunsaturated fatty acids.

Alternatively, Camelina FAD2 and/or FAE1 mutant plants may be combined by breeding with a Camelina plant overexpressing Δ -9 desaturase in a seed-specific manner to create a new Camelina line where the amount of 18:1 is higher and the amount of 16:0 is lower compared to either parent plant alone. This combination may also decrease the amount of very long chain fatty acids.

In addition, Camelina FAD2 and/or FAE1 mutant plants may be combined by breeding with a Camelina plant overexpressing both KAS II and Δ -9 desaturase in a seed-specific manner to create a new Camelina line where the amount of 18:1 is higher and the amount of 16:0 is lower compared to any of the original parent modifications (FAD2/FAE1 suppression, KAS II overexpression or Δ -9 desaturase overexpression) alone.

Optionally, Camelina FAD2 and/or FAE1 mutant plants may be combined by breeding with a Camelina plant knocked out for FATB function (either by TILLING or transgenic means with a seed-specific promoter) to create a new Camelina line where the amount of 18:1 is higher and the amount of 16:0 is lower compared to either parent plant alone. Arabidopsis FATB knockout plants are compromised in growth and produce less viable seeds (Bonaventure et al, The Plant Cell, Vol. 15, 1020–1033, April 2003). This detrimental phenotype may be alleviated in a polyploid like Camelina, where the presence of multiple copies for a given gene may allow greater flexibility in manipulating the levels of camelina FATB. Alternatively, the detrimental FATB knockout phenotype may be alleviated by only suppressing or knocking out FATB function using a FATB antisense or RNAi construct driven by a seed-specific promoter.

Other combinations of FAD2/FAE1 suppression, KAS II seed-specific overexpression, Δ -9 desaturase seed-specific overexpression and/or FATB suppression may be envisioned to obtain Camelina plants with increased 18:1 and decreased 16:0 and/or 18:0.

Example 16

Camelina plants having mutations in FAD2 and/or FAE1 genes in combination with overexpression of REV/KRP genes for altered fatty acid composition and increased seed yield

The purpose of suppressing Camelina FAD2 and/or FAE1 functions is to obtain an altered fatty acid profile of Camelina oil more suitable for conversion to biodiesel. Another attribute that would contribute to improvement of the oilseed crop for biofuel purposes would be an increase in seed yield, either by an increase in total seed number or seed size, in order to increase the amount of oil recovered per unit of land. Two yield technologies, REV and KRP dominant negative, have been described (US 2008/263727 and US 2007/056058) that give increased seed yield when overexpressed under early embryo-specific promoters.

Camelina FAD2 and/or FAE1 mutant plants, permutations of which are described in Example 13, may be combined by breeding with a Camelina plant overexpressing REV in an early embryo-specific manner to create a new Camelina line with greater seed yield and high 18:1 and/or low VLCFAs compared to either parent plant alone.

Similarly, Camelina FAD2 and/or FAE1 mutant plants may be combined by breeding with a Camelina plant overexpressing KRP dominant negative in an early embryo-specific or constitutive manner to create a new Camelina line with greater seed yield and high 18:1 and/or low VLCFAs compared to either parent plant alone.

Additionally, Camelina FAD2 and/or FAE1 mutant plants may be combined by breeding with a Camelina plant overexpressing both REV and KRP dominant negative in an early embryo-specific (or constitutive for KRP) manner to create a new Camelina line with greater seed yield and high 18:1 and/or low VLCFAs compared to any of the original parent modifications (FAD2/FAE1 suppression, early embryo-specific REV overexpression or embryo-specific or constitutive KRP dominant negative overexpression) alone.

In view of the many possible embodiments to which the principles of the disclosed invention may be applied, it should be recognized that the illustrated embodiments are only examples and should not be taken as limiting the scope of the invention.

Unless defined otherwise, all technical and scientific terms herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes IX*, published by Oxford University Press, 2007 (ISBN-10 0131439812);
5 Kendrew *et al.* (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: A Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8); *Oxford Dictionary of Biochemistry and Molecular Biology*, Revised Edition, 2000. Although any methods and materials, similar or equivalent to those
10 described herein, can be used in the practice or testing of the present invention, the preferred methods and materials are described herein.

All publications, patents, and patent publications cited are incorporated by reference herein in their entirety for all purposes. Also incorporated by reference herein are nucleic acid sequences and polypeptide sequences deposited into the GenBank, which are cited in
15 this specification.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

While the invention has been described in connection with specific embodiments
20 thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope
25 of the appended claims.

REFERENCES

(2010). "Maize Genome Browser." from <http://maizesequence.org/index.html>.

(2010). "Phytozome." from <http://www.phytozome.net/index.php>.

(2010). "TIGR Rice Database." from <http://rice.tigr.org/>.

30 Abramovic, H. and V. Abram (2005). "Physico-chemical properties, composition and oxidative stability of *Camelina sativa* oil." Food Technology and Biotechnology 43: 63-70.

- Adams, K. L., R. Percifield, et al. (2004). "Organ-specific silencing of duplicated genes in a newly synthesized cotton allotetraploid." Genetics 168(4): 2217-26.
- Akeroyd, J. (1993). Camelina in Flora Europaea. Cambridge, UK, Cambridge University Press.
- 5 Al-Shehbaz, I., M. Beilstein, et al. (2006). "Systematics and phylogeny of the Brassicaceae (Cruciferae): an overview." Plant Systematics and Evolution 259(2): 89-120.
- ASTM D6751-09: Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels. West Conshohocken, PA, ASTM.
- Baksay, L. (1957). "The chromosome numbers and cytotaxonomical relations of some
10 European plant species." Ann. Hist.-Nat. Mus. Natl. Hung(8): 169-174.
- Beilstein, M. A., I. A. Al-Shehbaz, et al. (2006). "Brassicaceae phylogeny and trichome evolution." Am. J. Bot. 93(4): 607-619.
- Beilstein, M. A., I. A. Al-Shehbaz, et al. (2008). "Brassicaceae phylogeny inferred from phytochrome A and ndhF sequence data: tribes and trichomes revisited." Am. J. Bot.
15 95(10): 1307-1327.
- Belo, A., P. Zheng, et al. (2008). "Whole genome scan detects an allelic variant of fad2 associated with increased oleic acid levels in maize." Mol Genet Genomics 279(1): 1-10.
- Bernardo, A., R. Howard-Hildige, et al. (2003). "Camelina oil as a fuel for diesel transport
20 engines." Industrial Crops and Products 17(3): 191-197.
- Brochmann, C., A. K. Brysting, et al. (2004). "Polyploidy in arctic plants." Biological Journal of the Linnean Society 82(4): 521-536.
- Brooks, R. E. (1985). "Chromosome number reports LXXXVII " Taxon 34: 346-351.
- Budin, J., W. Breene, et al. (1995). "Some compositional properties of camelina (*camelina sativa* L. Crantz) seeds and oils " Journal of the American Oil Chemists' Society
25 72(3): 309-315.
- Cahoon, E. B., E. F. Marillia, et al. (2000). "Production of fatty acid components of meadowfoam oil in somatic soybean embryos." Plant Physiol 124(1): 243-51.
- Comai, L. (2005). "The advantages and disadvantages of being polyploid." Nat Rev Genet
30 6(11): 836-46.
- Comai, L., A. P. Tyagi, et al. (2000). "Phenotypic instability and rapid gene silencing in newly formed arabidopsis allotetraploids." Plant Cell 12(9): 1551-68.

- Cooper, J., B. Till, et al. (2008). "TILLING to detect induced mutations in soybean." BMC Plant Biology 8(1): 9.
- Dubcovsky, J. and J. Dvorak (2007). "Genome plasticity a key factor in the success of polyploid wheat under domestication." Science 316(5833): 1862-6.
- 5 Durrett, T. P., C. Benning, et al. (2008). "Plant triacylglycerols as feedstocks for the production of biofuels." Plant J 54(4): 593-607.
- EMBnet, S. "Boxshade." from <http://www.ch.embnet.org/>.
- Flannery, M. L., F. J. Mitchell, et al. (2006). "Plastid genome characterisation in Brassica and Brassicaceae using a new set of nine SSRs." Theor Appl Genet 113(7): 1221-31.
- 10 Francis, A. and S. Warwick (2009). "The Biology of Canadian Weeds. 142. *Camelina alyssum* (Mill.) Thell.; *C. microcarpa* Andr. ex DC.; *C. sativa* (L.) Crantz." Canadian Journal of Plant Science 89: 791-810.
- Frohlich, A. and B. Rice (2005). "Evaluation of *Camelina sativa* oil as a feedstock for biodiesel production." Industrial Crops and Products 21(1): 25-31.
- 15 Frohlich, M. W. and G. F. Estabrook (2000). "Wilkinson support calculated with exact probabilities: an example using *Floricaula*/LEAFY amino acid sequences that compares three hypotheses involving gene gain/loss in seed plants." Mol Biol Evol 17(12): 1914-25.
- Gehringer, A., W. Friedt, et al. (2006). "Genetic mapping of agronomic traits in false flax (*Camelina sativa* subsp. *sativa*)." Genome 49(12): 1555-63.
- 20 Ghanevati, M. and J. G. Jaworski (2001). "Active-site residues of a plant membrane-bound fatty acid elongase beta-ketoacyl-CoA synthase, FAE1 KCS." Biochim Biophys Acta 1530(1): 77-85.
- Ghanevati, M. and J. G. Jaworski (2002). "Engineering and mechanistic studies of the *Arabidopsis* FAE1 beta-ketoacyl-CoA synthase, FAE1 KCS." Eur J Biochem 269(14): 3531-9.
- 25 Gill, B. S. and B. Friebe (1998). "Plant cytogenetics at the dawn of the 21st century " Current Opinion in Plant Biology 1(2): 109-115.
- GRIN, U. "USDA Germplasm Resources Information Network." from <http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl?language=en>.
- 30 Gugel, R. K. and K. C. Falk (2006). "Agronomic and seed quality evaluation of *Camelina sativa* in western Canada." Canadian journal of plant science 86(4): 1047-1058

- Han, J., W. Lühs, et al. (2001). "Functional characterization of β -ketoacyl-CoA synthase genes from *Brassica napus* L." Plant Mol Biol 46(2): 229-239.
- He, P., B. R. Friebe, et al. (2003). "Allopolyploidy alters gene expression in the highly stable hexaploid wheat." Plant Mol Biol 52(2): 401-14.
- 5 Hegarty, M. J. and S. J. Hiscock (2008). "Genomic Clues to the Evolutionary Success of Polyploid Plants." 18(10): R435-R444.
- Hernandez, M. L., M. Mancha, et al. (2005). "Molecular cloning and characterization of genes encoding two microsomal oleate desaturases (FAD2) from olive." Phytochemistry 66(12): 1417-26.
- 10 Hongtrakul, V., M. B. Slabaugh, et al. (1998). "A Seed Specific Δ -12 Oleate Desaturase Gene Is Duplicated, Rearranged, and Weakly Expressed in High Oleic Acid Sunflower Lines." Crop Sci 38(5): 1245-1249.
- Hu, X., M. Sullivan-Gilbert, et al. (2006). "Mapping of the loci controlling oleic and linolenic acid contents and development of fad2 and fad3 allele-specific markers in canola (Brassica napus L.)." Theor Appl Genet 113(3): 497-507.
- 15 James Jr, D. W., E. Lim, et al. (1995). "Directed Tagging of the Arabidopsis FATTY ACID ELONGATION1 (FAE1) Gene with the Maize Transposon Activator." Plant Cell 7(3): 309-319.
- Kashkush, K., M. Feldman, et al. (2002). "Gene loss, silencing and activation in a newly synthesized wheat allotetraploid." Genetics 160(4): 1651-9.
- 20 Katavic, V., E. Mietkiewska, et al. (2002). "Restoring enzyme activity in nonfunctional low erucic acid *Brassica napus* fatty acid elongase 1 by a single amino acid substitution." Eur J Biochem 269(22): 5625-31.
- Kim, M. J., H. Kim, et al. (2006). "Seed-specific expression of sesame microsomal oleic acid desaturase is controlled by combinatorial properties between negative cis-regulatory elements in the SeFAD2 promoter and enhancers in the 5'-UTR intron." Mol Genet Genomics 276(4): 351-68.
- 25 Knothe, G. (2005). "Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters " Fuel Processing Technology 86(10): 1059-1070.
- 30 Knothe, G. and R. Dunn (2003). "Dependence of oil stability index of fatty compounds on their structure and concentration and presence of metals." Journal of the American Oil Chemists' Society 80(10): 1021-1026.

- Kunst, L., D. Taylor, et al. (1992). "Fatty acid elongation in developing seeds of *Arabidopsis thaliana*." Plant Physiol Biochem 30(4): 425-434.
- Li, W., L. Huang, et al. (2008). "Recurrent Deletions of Puroindoline Genes at the Grain Hardness Locus in Four Independent Lineages of Polyploid Wheat1." Plant Physiol. 146(1): 200-212.
- Lu, C., Kang, J (2008). "Generation of transgenic plants of a potential oilseed crop *Camelina sativa* by *Agrobacterium*-mediated transformation." Plant Cell Reports 27: 273-278.
- Maassoumi, A. (1980). *Crucifères de la flore d'Iran: étude caryosystematique*. Strasbourg, France, Strasbourg, France. Thesis.
- 10 Maddison, W., and Maddison, DR (2004.). *MacClade: analysis of phylogeny and character evolution*. Sunderland, MA, Sinauer.
- Maizel JV and Lenk RP: Enhanced graphic matrix analysis of nucleic acid and protein sequences. *Proc Natl Acad Sci USA* 78:7665, 1981.
- Martínez-Rivas, J. M., P. Sperling, et al. (2001). "Spatial and temporal regulation of three different microsomal oleate desaturase genes (FAD2) from normal-type and high-oleic varieties of sunflower (*Helianthus annuus* L.)." Molecular Breeding 8(2): 159-168.
- 15 Martynov, V. V., I. L. Tsvetkov, et al. (2004). "Orthologs of *Arabidopsis* CLAVATA 1 gene in cultivated Brassicaceae plants." Ontogenez 35(1): 41-6.
- 20 McCartney, A. W., J. M. Dyer, et al. (2004). "Membrane-bound fatty acid desaturases are inserted co-translationally into the ER and contain different ER retrieval motifs at their carboxy termini." Plant J 37(2): 156-73.
- McCormick, R. L., M. S. Graboski, et al. (2001). "Impact of Biodiesel Source Material and Chemical Structure on Emissions of Criteria Pollutants from a Heavy-Duty Engine." Environmental Science & Technology 35(9): 1742-1747.
- 25 Mietkiewska, E., E. M. Giblin, et al. (2004). "Seed-specific heterologous expression of a nasturtium FAE gene in *Arabidopsis* results in a dramatic increase in the proportion of erucic acid." Plant Physiol 136(1): 2665-75.
- Mikkilineni, V. and T. R. Rocheford (2003). "Sequence variation and genomic organization of fatty acid desaturase-2 (fad2) and fatty acid desaturase-6 (fad6) cDNAs in maize." Theor Appl Genet 106(7): 1326-32.
- 30 Mirek, Z. (1981). "Genus *Camelina* in Poland - Taxonomy, Distribution and Habitats." Fragmenta Floristica et Geobotanica 27: 445-503.

- Moon, H., M. A. Smith, et al. (2001). "A Condensing Enzyme from the Seeds of *Lesquerella fendleri* That Specifically Elongates Hydroxy Fatty Acids." Plant Physiol. 127(4): 1635-1643.
- Muramatsu, M. (1963). "Dosage Effect of the *Spelta* Gene *q* of Hexaploid Wheat." Genetics 5 48(4): 469-482.
- Ní Eidhin, D., J. Burke, et al. (2003). "Oxidative Stability of C9;3-rich Camelina Oil and Camelina Oil-based Spread Compared with Plant and Fish Oils and Sunflower Spread." Journal of Food Science 68(1): 345-353
- Okuley, J., J. Lightner, et al. (1994). "Arabidopsis *FAD2* gene encodes the enzyme that is 10 essential for polyunsaturated lipid synthesis." Plant Cell 6(1): 147-58.
- Park, C., D. Correll, et al. (2004). Measuring Allele-Specific Expression Using MassARRAY. Sequenom Application Note. Doc No. 8876-005 R01.
- Patel, M., S. Jung, et al. (2004). "High-oleate peanut mutants result from a MITE insertion into the *FAD2* gene." TAG Theoretical and Applied Genetics 108(8): 1492-1502.
- 15 Posada, D. and K. Crandall (1998). "Modeltest: testing the model of DNA substitution." Bioinformatics 14(9): 817-818.
- Pustell J and Kafatos FC: A high speed, high capacity homology matrix: Zooming through SV40 and polyoma. Nucleic Acids Res 10:4765, 1982.
- Putnam, D., J. Budin, et al. (1993). Camelina: a promising low-input oilseed. New crops. J. 20 Janick, and Simon, JE New York, Wiley: 314-322.
- Quigley GJ, Gehrke L, Roth DA and Auron PE: Computer-aided nucleic acid secondary structure modeling incorporating enzymatic digestion data. Nucleic Acids Res 12:347, 1984.
- Rice, B., A. Frohlich, et al. (1997). Biodiesel production based on waste cooking oil: 25 Promotion of the Establishment of an Industry in Ireland. Dublin, Ireland, Teagasc Agriculture and Food Development Authority.
- Ruuska, S. A., T. Girke, et al. (2002). "Contrapuntal networks of gene expression during Arabidopsis seed filling." Plant Cell 14(6): 1191-206.
- Saghai-Maroofof, M. A., K. M. Soliman, et al. (1984). "Ribosomal DNA spacer-length 30 polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics." Proc Natl Acad Sci U S A 81(24): 8014-8.

- Salmon, A., Ainouche, ML, Wendel, JF, (2005). "Genetic and epigenetic consequences of recent hybridization and polyploidy in *Spartina* (Poaceae)." Molecular Ecology 14(4): 1163-1175.
- Scheffler, J. A., A. G. Sharpe, et al. (1997). "Desaturase multigene families of *Brassica napus* arose through genome duplication." Theoretical and Applied Genetics 94(5): 583-591.
- Schlueter, J. A., J. Y. Lin, et al. (2007). "Gene duplication and paleopolyploidy in soybean and the implications for whole genome sequencing." BMC Genomics 8: 330.
- Serdari, A., E. Lois, et al. (1999). "Impact of Esters of Mono- and Dicarboxylic Acids on Diesel Fuel Quality." Industrial & Engineering Chemistry Research 38(9): 3543-3548.
- Slade, A. J., S. I. Fuerstenberg, et al. (2005). "A reverse genetic, nontransgenic approach to wheat crop improvement by TILLING." Nat Biotechnol 23(1): 75-81.
- Stadler, L. J. (1929). "Chromosome Number and the Mutation Rate in *Avena* and *Triticum*." Proc Natl Acad Sci U S A 15(12): 876-81.
- Stournas, S., Lois, E., Serdari, A. (1995). "Effects of fatty acid derivatives on the ignition quality and cold flow of diesel fuel " Journal of the American Oil Chemists' Society 72(4): 433-437.
- Swaminathan, M. S. and M. V. Rao (1960). "Frequency of Mutations Induced by Radiations in Hexaploid Species of *Triticum*." Science 132(3442): 1842.
- Swofford, D. (2001). PAUP* 4.0 beta 5: Phylogenetic Analysis Using Parsimony and Other Methods. Sunderland, MA, Sinauer.
- Sybenga, J. (1996). "Chromosome pairing affinity and quadrivalent formation in polyploids: do segmental allopolyploids exist?" Genome 39: 1176-1184.
- Tai, H. H., C. Pelletier, et al. (2004). "Total RNA isolation from *Picea mariana* dry seed " Plant Molecular Biology Reporter 22(1): 93a-93e.
- TAIR. (2009). "The Arabidopsis Information Resource." from <http://www.arabidopsis.org>.
- Tedin, O. (1925). "Vererbung, Variation und Systematik in der Gattung *Camelina*." Hereditas 6: 19-386.
- Tocher DR, L. M., Hodgson PA (1998). "Recent advances in the biochemistry and molecular biology of fatty acyl desaturases " Progress in Lipid Research 37(2-3): 73-117.
- Tom Maniatis, J. S., E. F. Fritsch (1982). Molecular cloning : a laboratory manual. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory.

- Vollmann, J., H. Grausgruber, et al. (2005). "Genetic diversity in camelina germplasm as revealed by seed quality characteristics and RAPD polymorphism." Plant Breeding 124(5): 446-453.
- Wang, N., Y. Wang, et al. (2008). "A functional genomics resource for Brassica napus: development of an EMS mutagenized population and discovery of FAE1 point mutations by TILLING." New Phytol 180(4): 751-65.
- Worgetter, M., H. Prankl, et al. (2006). Local and Innovative Biodiesel. Wieselburg, Austria, FJ-BLT Wieselburg.
- Wu, G., Y. Wu, et al. (2008). "Zero erucic acid trait of rapeseed (Brassica napus L.) results from a deletion of four base pairs in the fatty acid elongase 1 gene." Theor Appl Genet 116(4): 491-9.
- Zubr, J. (1997). "Oil-seed crop: *Camelina sativa*." Industrial Crops and Products 6(2): 113-119.
- Zubr, J., Matthaus, B. (2002). "Effects of growth conditions on fatty acids and tocopherols in *Camelina sativa* oil." Industrial Crops and Products 15: 155-162.
- Pustell J and Kafatos FC: A high speed, high capacity homology matrix: Zooming through SV40 and polyoma. *Nucleic Acids Res* 10:4765, 1982.
- Quigley GJ, Gehrke L, Roth DA and Auron PE: Computer-aided nucleic acid secondary structure modeling incorporating enzymatic digestion data. *Nucleic Acids Res* 12:347, 1984.

THE EMBODIMENTS OF THE INVENTION FOR WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. An isolated polynucleotide encoding a plant fatty acid desaturase, wherein the isolated polynucleotide comprises a sequence sharing at least 93% identity to SEQ ID NO: 1.
2. The isolated polynucleotide of claim 1, wherein the polynucleotide comprises a sequence selected from the group consisting of SEQ ID NO: 1.
3. The isolated polynucleotide of claim 1, wherein the polynucleotide comprises one or more mutations selected from the group consisting of G1516A, C1645T, C1746T, C1813T, G1844A, C1977T, G2015A, C2099T, G2155A, G1495A, G2272A, and G2138A.
4. The isolated polynucleotide of claim 1, wherein the polynucleotide comprises one or more mutations selected from the group consisting of C207T, C213T, G785A, C476T, C176T, G462A, G498A, G779A, G737A, C812T, C882T, G410A, G675A, C459T, C528T, C987T, C284T, G416A, G650A, C656T, C203T, G582A, G372A, G322A, G374A, G926A, C490T, C940T and G148A.
5. The isolated polynucleotide of claim 1, wherein the polynucleotide comprises one or more mutations selected from the group consisting of G1429A, C1501T, C1542T, C1576T, C1582T, G1607A, C1609T, G1619A, G1672A, G1717A, C1720T, C1741T, G1795A, G1796A, C1799T, G1808A, G1810A, C1857T, C1873T, G1880A, G1883A, G1890A, G1915A, G1948A, G1963A, C2029T, G2072A, G2080A, C2081T, C2084T, C2096T, C2110T, C2112T, G2117A, G2140A, G2149A, C2188T, C2204T, G2255A, G2268A, C2285T, C2293T, C2315T, G2422A, G2443A, C1595T and C2383T.

FIGURE 1

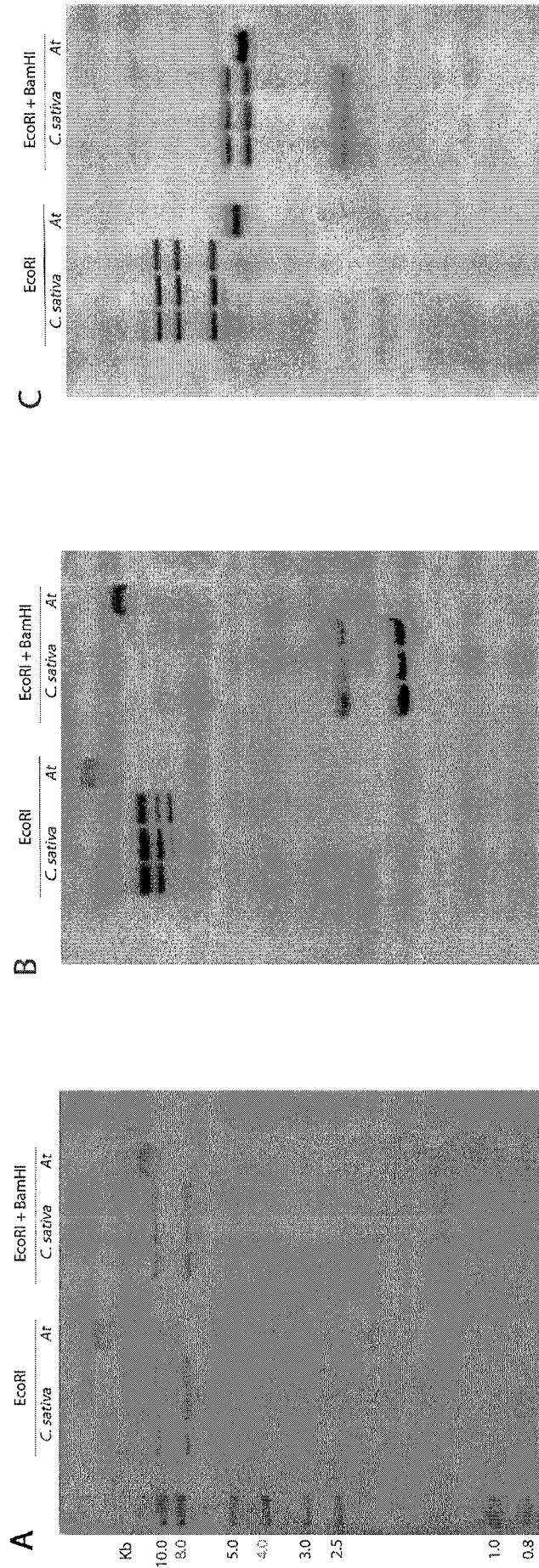


FIGURE 2A

CsFAD2_A	1	MGAGGRMPVPSSSS.....KKSETDAIKRVPCEKPPFTLGGLKKAIPPQCCKRSIPRSFS
CsFAD2_B	1	MGAGGRMPVPSSSS.....KKSETDAIKRVPCEKPPFTLGGLKKAIPPQCCKRSIPRSFS
CsFAD2_C	1	MGAGGRMPVPSSSS.....KKSETDAIKRVPCEKPPFTLGGLKKAIPPQCCKRSIPRSFS
AtFAD2	1	MGAGGRMPVPSSSS.....KKSETDTIKRVPCEKPPFTLGGLKKAIPPCHCFKRSIPRSFS
BrFAD2	1	MGAGGRMPVPSSSS.....KKSETDNKRVPCEKPPFTLGGLKKAIPPCHCFKRSIPRSFS
GmFAD2-3	1	MGAGGRMPVPSSSS.....KKSEVDPIKRVPCEKPPFTLGGLKKAIPPCHCFKRSIPRSFS
ZmFAD2	1	MGAGGRMPTEKEREKQEQQLAAGGAAMCRSPVEKPPFTLGGLKKAIPPCHCFKRSIPRSFS
		H1
CsFAD2_A	56	YLIIDIIIASCFYYVATNYFSLLPQPLSYLAWPLYWACQGCVLTVGVVIAHECGHHAFSD
CsFAD2_B	56	YLIIDIIIASCFYYVATNYFSLLPQPLSYLAWPLYWACQGCVLTVGVVIAHECGHHAFSD
CsFAD2_C	56	YLIIDIIIASCFYYVATNYFSLLPQPLSYLAWPLYWACQGCVLTVGVVIAHECGHHAFSD
AtFAD2	55	YLIIDIIIASCFYYVATNYFSLLPQPLSYLAWPLYWACQGCVLTVGVVIAHECGHHAFSD
BrFAD2	55	YLIIDIIIASCFYYVATNYFSLLPQPLSYLAWPLYWACQGCVLTVGVVIAHECGHHAFSD
GmFAD2-3	55	YLYIDIIIASCFYYVATNYFSLLPQPLSYLAWPLYWACQGCVLTVGVVIAHECGHHAFSD
ZmFAD2	61	YLYIDIIIASCFYYVATNYFSLLPQPLSYLAWPLYWACQGCVLTVGVVIAHECGHHAFSD
		H2
CsFAD2_A	116	YQWLDDTVGLIFHSFLLVPYFSWKYSHRRHHSNTGSLERDEVFVPKQSAIKWYGKYLNN
CsFAD2_B	116	YQWLDDTVGLIFHSFLLVPYFSWKYSHRRHHSNTGSLERDEVFVPKQSAIKWYGKYLNN
CsFAD2_C	116	YQWLDDTVGLIFHSFLLVPYFSWKYSHRRHHSNTGSLERDEVFVPKQSAIKWYGKYLNN
AtFAD2	115	YQWLDDTVGLIFHSFLLVPYFSWKYSHRRHHSNTGSLERDEVFVPKQSAIKWYGKYLNN
BrFAD2	115	YQWLDDTVGLIFHSFLLVPYFSWKYSHRRHHSNTGSLERDEVFVPKQSAIKWYGKYLNN
GmFAD2-3	115	YQWLDDTVGLIFHSFLLVPYFSWKYSHRRHHSNTGSLERDEVFVPKQSAIKWYGKYLNN
ZmFAD2	121	YQWLDDTVGLIFHSFLLVPYFSWKYSHRRHHSNTGSLERDEVFVPKQSAIKWYGKYLNN
		H3
CsFAD2_A	176	PLGRIMMLTVQFVLGWPLYLAFNVSGRPYDG.FACHFFPNAPIYNDRELRQIYLSDAGI
CsFAD2_B	176	PLGRIMMLTVQFVLGWPLYLAFNVSGRPYDG.FACHFFPNAPIYNDRELRQIYLSDAGI
CsFAD2_C	176	PLGRIMMLTVQFVLGWPLYLAFNVSGRPYDG.FACHFFPNAPIYNDRELRQIYLSDAGI
AtFAD2	175	PLGRIMMLTVQFVLGWPLYLAFNVSGRPYDG.FACHFFPNAPIYNDRELRQIYLSDAGI
BrFAD2	175	PLGRIMMLTVQFVLGWPLYLAFNVSGRPYDG.FACHFFPNAPIYNDRELRQIYLSDAGI
GmFAD2-3	175	PLGRIMMLTVQFVLGWPLYLAFNVSGRPYDG.FACHFFPNAPIYNDRELRQIYLSDAGI
ZmFAD2	181	PLGRIMMLTVQFVLGWPLYLAFNVSGRPYDG.FACHFFPNAPIYNDRELRQIYLSDAGI
		H4
CsFAD2_A	234	LAVCFGLYRYAAAQGLASMICLYGVPLLIIVNAFLVLITYLQHTHPALPHYDSSEWDWLRG
CsFAD2_B	234	LAVCFGLYRYAAAQGLASMICLYGVPLLIIVNAFLVLITYLQHTHPALPHYDSSEWDWLRG
CsFAD2_C	234	LAVCFGLYRYAAAQGLASMICLYGVPLLIIVNAFLVLITYLQHTHPALPHYDSSEWDWLRG
AtFAD2	233	LAVCFGLYRYAAAQGLASMICLYGVPLLIIVNAFLVLITYLQHTHPALPHYDSSEWDWLRG
BrFAD2	234	LAVCFGLYRYAAAQGLASMICLYGVPLLIIVNAFLVLITYLQHTHPALPHYDSSEWDWLRG
GmFAD2-3	233	LAVCFGLYRYAAAQGLASMICLYGVPLLIIVNAFLVLITYLQHTHPALPHYDSSEWDWLRG
ZmFAD2	240	LAVCFGLYRYAAAQGLASMICLYGVPLLIIVNAFLVLITYLQHTHPALPHYDSSEWDWLRG
		H5
CsFAD2_A	294	ALATVDRDYGILNKVFHNITDTHVAHHLFSTMPHYNAMEATKAIKPILGDYYQFDGTPWY
CsFAD2_B	294	ALATVDRDYGILNKVFHNITDTHVAHHLFSTMPHYNAMEATKAIKPILGDYYQFDGTPWY
CsFAD2_C	294	ALATVDRDYGILNKVFHNITDTHVAHHLFSTMPHYNAMEATKAIKPILGDYYQFDGTPWY
AtFAD2	293	ALATVDRDYGILNKVFHNITDTHVAHHLFSTMPHYNAMEATKAIKPILGDYYQFDGTPWY
BrFAD2	294	ALATVDRDYGILNKVFHNITDTHVAHHLFSTMPHYNAMEATKAIKPILGDYYQFDGTPWY
GmFAD2-3	293	ALATVDRDYGILNKVFHNITDTHVAHHLFSTMPHYNAMEATKAIKPILGDYYQFDGTPWY
ZmFAD2	300	ALATVDRDYGILNKVFHNITDTHVAHHLFSTMPHYNAMEATKAIKPILGDYYQFDGTPWY
		H6
CsFAD2_A	354	VAMYREAKECIYVEPDREGDKKGVYWYNNKL
CsFAD2_B	354	VAMYREAKECIYVEPDREGDKKGVYWYNNKL
CsFAD2_C	354	VAMYREAKECIYVEPDREGDKKGVYWYNNKL
AtFAD2	353	VAMYREAKECIYVEPDREGDKKGVYWYNNKL
BrFAD2	354	VAMYREAKECIYVEPDREGDKKGVYWYNNKL
GmFAD2-3	353	VAMYREAKECIYVEPDREGDKKGVYWYNNKL
ZmFAD2	360	VAMYREAKECIYVEPDREGDKKGVYWYNNKL

FIGURE 2B

CsFAE1_A	1	MT.....SVN...AKLLYHYVITNFFNLCLFPLTA LAGKASRL
CsFAE1_B	1	MT.....SVN...AKLLYHYVITNFFNLCLFPLTA LAGKASRL
CsFAE1_C	1	MT.....SVN...AKLLYHYVITNFFNLCLFPLTA LAGKASRL
At_FAE1	1	MT.....SVN...VKLLYHYVITNFFNLCLFPLTA LAGKASRL
CaFAE1	1	MT.....SEN...VKLLYHYVITNFFNLCLFPLTA LAGKASRL
BrFAE1_Heac	1	MT.....SEN...VKLLYHYVITNFFNLCLFPLTA LAGKASRL
BrFAE1_Leac	1	MT.....SEN...VKLLYHYVITNFFNLCLFPLTA LAGKASRL
LaFAE1	1	MSGTKPKPLIATVKNTLPDLKLSNNLKHVKLYHYITTHGMYLCLPLALITIFQISTLT
TmFAE1	1	MSGTKATS....VSVLPDFKQSVNLKYVKLYHYITTHAMYLCLPLALLNSQISTLT
CsFAE1_A	37	TSDDLYHYS.HLQHNLTIVLLFAFTAFGLVLYIVTRPKPVYLVDSYCYLFPFPLKVS
CsFAE1_B	37	TANDLYHYS.HLQHNLTIVLLFAFTAFGLVLYIVTRPKPVYLVDSYCYLFPFPLKVS
CsFAE1_C	37	TDDLYHYS.HLQHNLTIVLLFAFTAFGLVLYIVTRPKPVYLVDSYCYLFPFPLKVS
At_FAE1	37	TDDLNLFLS.YLQHNLTIVLLFAFTAFGLVLYIVTRPKPVYLVDSYCYLFPFPLKVS
CaFAE1	37	TDDLHHVYYSYLOHNLTIVLLFAFTAFGLVLYIVTRPKPVYLVDSYCYLFPFPLKVS
BrFAE1_Heac	37	TDDLHHVYYSYLOHNLTIVLLFAFTAFGLVLYIVTRPKPVYLVDSYCYLFPFPLKVS
BrFAE1_Leac	37	TDDLHHVYYSYLOHNLTIVLLFAFTAFGLVLYIVTRPKPVYLVDSYCYLFPFPLKVS
LaFAE1	61	SKPCFNDHEQLQFN.LIYVSSSTLLVSLHLYETPRFPVYLHDAKWKDETFKSR
TmFAE1	56	STLPHHLYNHILMINSSVLCIELLLEVLTYETPRFPVYLHNSCKKDAITHKCDR
CsFAE1_A	96	SRAMDIYQRRKADTSRN.VACDLPSSLDLFRKIQERSGLGDETSRQGLTHVPPRKTFA
CsFAE1_B	96	SRAMDIYQRRKADTSRN.VACDLPSSLDLFRKIQERSGLGDETSRQGLTHVPPRKTFA
CsFAE1_C	96	SKVMDIYQRRKADTSRN.VACDLPSSLDLFRKIQERSGLGDETSRQGLTHVPPRKTFA
At_FAE1	96	SKVMDIYQRRKADTSRN.VACDLPSSLDLFRKIQERSGLGDETSRQGLTHVPPRKTFA
CaFAE1	97	SKVMDIYQRRKADTSRN.GTCDSSWLDLFRKIQERSGLGDETHGPEGLTHVPPRKTFA
BrFAE1_Heac	97	SKVMDIYQRRKADTSRN.GTCDSSWLDLFRKIQERSGLGDETHGPEGLTHVPPRKTFA
BrFAE1_Leac	97	SKVMDIYQRRKADTSRN.GTCDSSWLDLFRKIQERSGLGDETHGPEGLTHVPPRKTFA
LaFAE1	120	EHFMCCKGESGSETEDN.....IDCKRKVARSGLGDAITLPEAGTFAHSMK
TmFAE1	116	RHFMTIRGTYTEEN.....DFCKRLERSGCGESYLEPTVFKFPRVYDK
CsFAE1_A	155	ASRETEQVITGALKLEFENTKVNPRIGILVNVSSMFNPTPSLSAMVVNTFKLRSNIS
CsFAE1_B	155	ASRETEQVITGALKLEFENTKVNPRIGILVNVSSMFNPTPSLSAMVVNTFKLRSNIS
CsFAE1_C	155	ASRETEQVITGALKLEFENTKVNPRIGILVNVSSMFNPTPSLSAMVVNTFKLRSNIS
At_FAE1	156	ASRETEQVITGALKLEFENTKVNPRIGILVNVSSMFNPTPSLSAMVVNTFKLRSNIS
CaFAE1	156	ASRETEQVITGALKLEFENTKVNPRIGILVNVSSMFNPTPSLSAMVVNTFKLRSNIS
BrFAE1_Heac	156	ASRETEQVITGALKLEFENTKVNPRIGILVNVSSMFNPTPSLSAMVVNTFKLRSNIS
BrFAE1_Leac	156	ASRETEQVITGALKLEFENTKVNPRIGILVNVSSMFNPTPSLSAMVVNTFKLRSNIS
LaFAE1	170	ARRRADIIVFGATLLERKYNPRIGILVNVSSMFNPTPSLSAMVVNTFKLRSNIS
TmFAE1	166	ERRRADIIVFGATLLERKYNPRIGILVNVSSMFNPTPSLSAMVVNTFKLRSNIS
CsFAE1_A	215	PSLGGMGCSAGVIAIDLAKDLLHVHKNTYALVVSSENTITQGIYAGENRSMVSNCLFRVG
CsFAE1_B	215	PSLGGMGCSAGVIAIDLAKDLLHVHKNTYALVVSSENTITQGIYAGENRSMVSNCLFRVG
CsFAE1_C	215	PSLGGMGCSAGVIAIDLAKDLLHVHKNTYALVVSSENTITQGIYAGENRSMVSNCLFRVG
At_FAE1	216	FNLGGMGCSAGVIAIDLAKDLLHVHKNTYALVVSSENTITQGIYAGENRSMVSNCLFRVG
CaFAE1	216	FNLGGMGCSAGVIAIDLAKDLLHVHKNTYALVVSSENTITQGIYAGENRSMVSNCLFRVG
BrFAE1_Heac	215	FNLGGMGCSAGVIAIDLAKDLLHVHKNTYALVVSSENTITQGIYAGENRSMVSNCLFRVG
BrFAE1_Leac	216	FNLGGMGCSAGVIAIDLAKDLLHVHKNTYALVVSSENTITQGIYAGENRSMVSNCLFRVG
LaFAE1	230	FNLGGMGCSAGVIAIDLAKDLLHVHKNTYALVVSSENTITQGIYAGENRSMVSNCLFRVG
TmFAE1	226	FNLGGMGCSAGVIAIDLAKDLLHVHKNTYALVVSSENTITQGIYAGENRSMVSNCLFRVG
CsFAE1_A	275	GAAILLSNKPGDRRRSKYLVHTVTRHTGADDSFRCVQGGDDSGKIGVCLSKDITVA
CsFAE1_B	275	GAAILLSNKPGDRRRSKYLVHTVTRHTGADDSFRCVQGGDDSGKIGVCLSKDITVA
CsFAE1_C	275	GAAILLSNKPGDRRRSKYLVHTVTRHTGADDSFRCVQGGDDSGKIGVCLSKDITVA
At_FAE1	276	GAAILLSNKPGDRRRSKYLVHTVTRHTGADDSFRCVQGGDDSGKIGVCLSKDITVA
CaFAE1	276	GAAILLSNKPGDRRRSKYLVHTVTRHTGADDSFRCVQGGDDSGKIGVCLSKDITVA
BrFAE1_Heac	275	GAAILLSNKPGDRRRSKYLVHTVTRHTGADDSFRCVQGGDDSGKIGVCLSKDITVA
BrFAE1_Leac	276	GAAILLSNKPGDRRRSKYLVHTVTRHTGADDSFRCVQGGDDSGKIGVCLSKDITVA
LaFAE1	290	GAAILLSNKPGDRRRSKYLVHTVTRHTGADDSFRCVQGGDDSGKIGVCLSKDITVA
TmFAE1	286	GAAILLSNKPGDRRRSKYLVHTVTRHTGADDSFRCVQGGDDSGKIGVCLSKDITVA
CsFAE1_A	335	GTALKKNIATLGLPLPLSEKLLFFVTFPAKKLLKDKIKHYVVPDFKLAIDHFCIHAGGR
CsFAE1_B	335	GTALKKNIATLGLPLPLSEKLLFFVTFPAKKLLKDKIKHYVVPDFKLAIDHFCIHAGGR
CsFAE1_C	335	GTALKKNIATLGLPLPLSEKLLFFVTFPAKKLLKDKIKHYVVPDFKLAIDHFCIHAGGR
At_FAE1	336	GRTALKKNIATLGLPLPLSEKLLFFVTFPAKKLLKDKIKHYVVPDFKLAIDHFCIHAGGR
CaFAE1	336	GRTALKKNIATLGLPLPLSEKLLFFVTFPAKKLLKDKIKHYVVPDFKLAIDHFCIHAGGR
BrFAE1_Heac	335	GRTALKKNIATLGLPLPLSEKLLFFVTFPAKKLLKDKIKHYVVPDFKLAIDHFCIHAGGR
BrFAE1_Leac	336	GRTALKKNIATLGLPLPLSEKLLFFVTFPAKKLLKDKIKHYVVPDFKLAIDHFCIHAGGR
LaFAE1	350	GDALKTNITTLGLPLPLSEKLLFFVTFPAKKLLKDKIKHYVVPDFKLAIDHFCIHAGGR
TmFAE1	346	GDALKTNITTLGLPLPLSEKLLFFVTFPAKKLLKDKIKHYVVPDFKLAIDHFCIHAGGR
CsFAE1_A	395	AVIDVLEKSLGLSPIDVEASRSTLHFRGNTSSSSIWYELAYIEAKGRMKKGNRAQIALG
CsFAE1_B	395	AVIDVLEKSLGLSPIDVEASRSTLHFRGNTSSSSIWYELAYIEAKGRMKKGNRAQIALG
CsFAE1_C	395	AVIDVLEKSLGLSPIDVEASRSTLHFRGNTSSSSIWYELAYIEAKGRMKKGNRAQIALG
At_FAE1	396	AVIDVLEKSLGLSPIDVEASRSTLHFRGNTSSSSIWYELAYIEAKGRMKKGNRAQIALG
CaFAE1	396	AVIDVLEKSLGLSPIDVEASRSTLHFRGNTSSSSIWYELAYIEAKGRMKKGNRAQIALG
BrFAE1_Heac	395	AVIDVLEKSLGLSPIDVEASRSTLHFRGNTSSSSIWYELAYIEAKGRMKKGNRAQIALG
BrFAE1_Leac	396	AVIDVLEKSLGLSPIDVEASRSTLHFRGNTSSSSIWYELAYIEAKGRMKKGNRAQIALG
LaFAE1	409	AVIDVLEKSLGLSPIDVEASRSTLHFRGNTSSSSIWYELAYIEAKGRMKKGNRAQIALG
TmFAE1	405	AVIDVLEKSLGLSPIDVEASRSTLHFRGNTSSSSIWYELAYIEAKGRMKKGNRAQIALG
CsFAE1_A	455	SGFKCNSAVVVALQNVKASAN.SPWEHCIDRYPVQIDSGSSKSDTVKNGRS
CsFAE1_B	455	SGFKCNSAVVVALQNVKASAN.SPWEHCIDRYPVQIDSGSSKSDTVKNGRS
CsFAE1_C	455	SGFKCNSAVVVALQNVKASAN.SPWEHCIDRYPVQIDSGSSKSDTVKNGRS
At_FAE1	456	SGFKCNSAVVVALQNVKASAN.SPWEHCIDRYPVQIDSGSSKSDTVKNGRS
CaFAE1	456	SGFKCNSAVVVALQNVKASAN.SPWEHCIDRYPVQIDSGSSKSDTVKNGRS
BrFAE1_Heac	455	SGFKCNSAVVVALQNVKASAN.SPWEHCIDRYPVQIDSGSSKSDTVKNGRS
BrFAE1_Leac	456	SGFKCNSAVVVALQNVKASAN.SPWEHCIDRYPVQIDSGSSKSDTVKNGRS
LaFAE1	469	SGFKCNSAVVVALQNVKASAN.SPWEHCIDRYPVQIDSGSSKSDTVKNGRS
TmFAE1	465	SGFKCNSAVVVALQNVKASAN.SPWEHCIDRYPVQIDSGSSKSDTVKNGRS

FIGURE 3

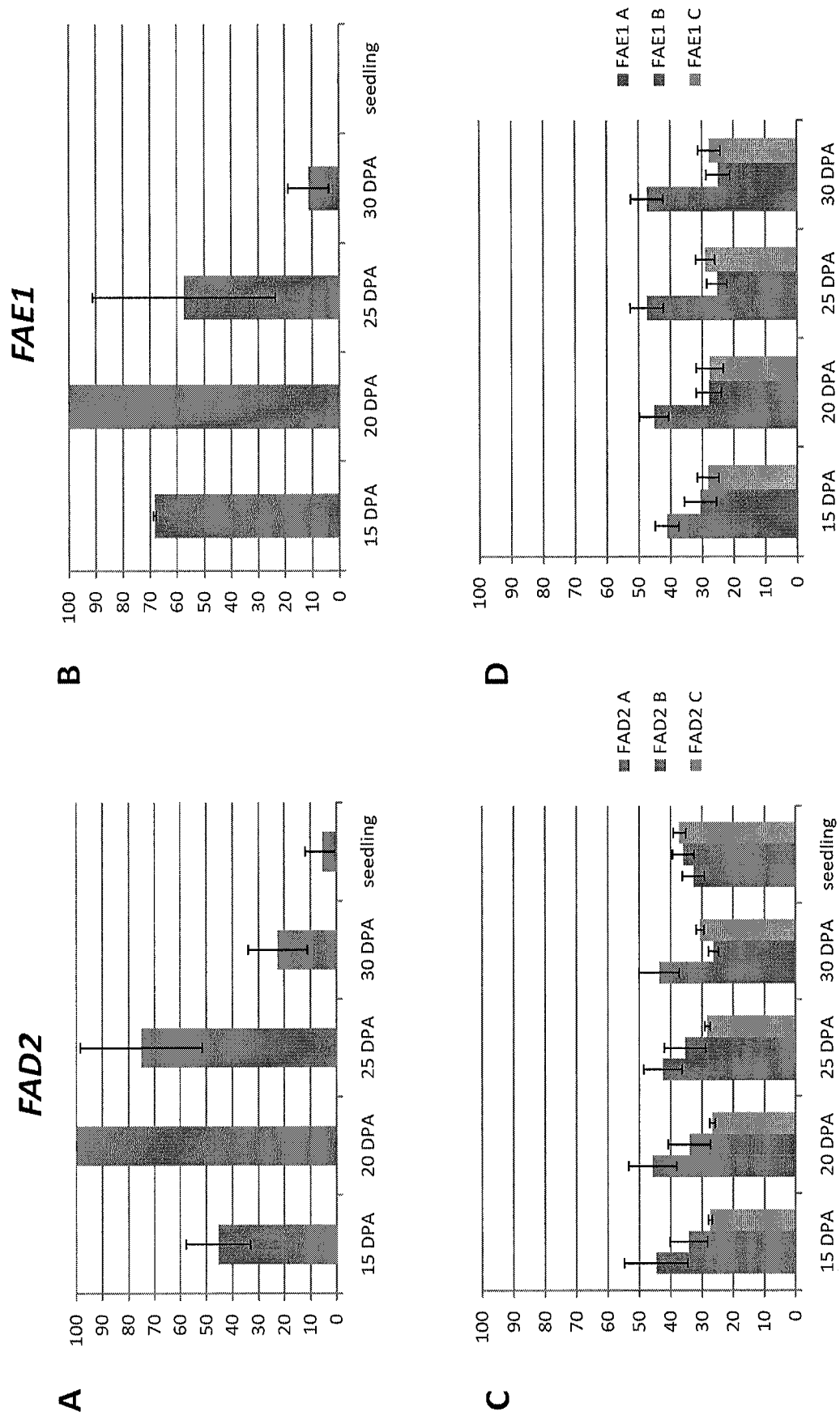


FIGURE 4

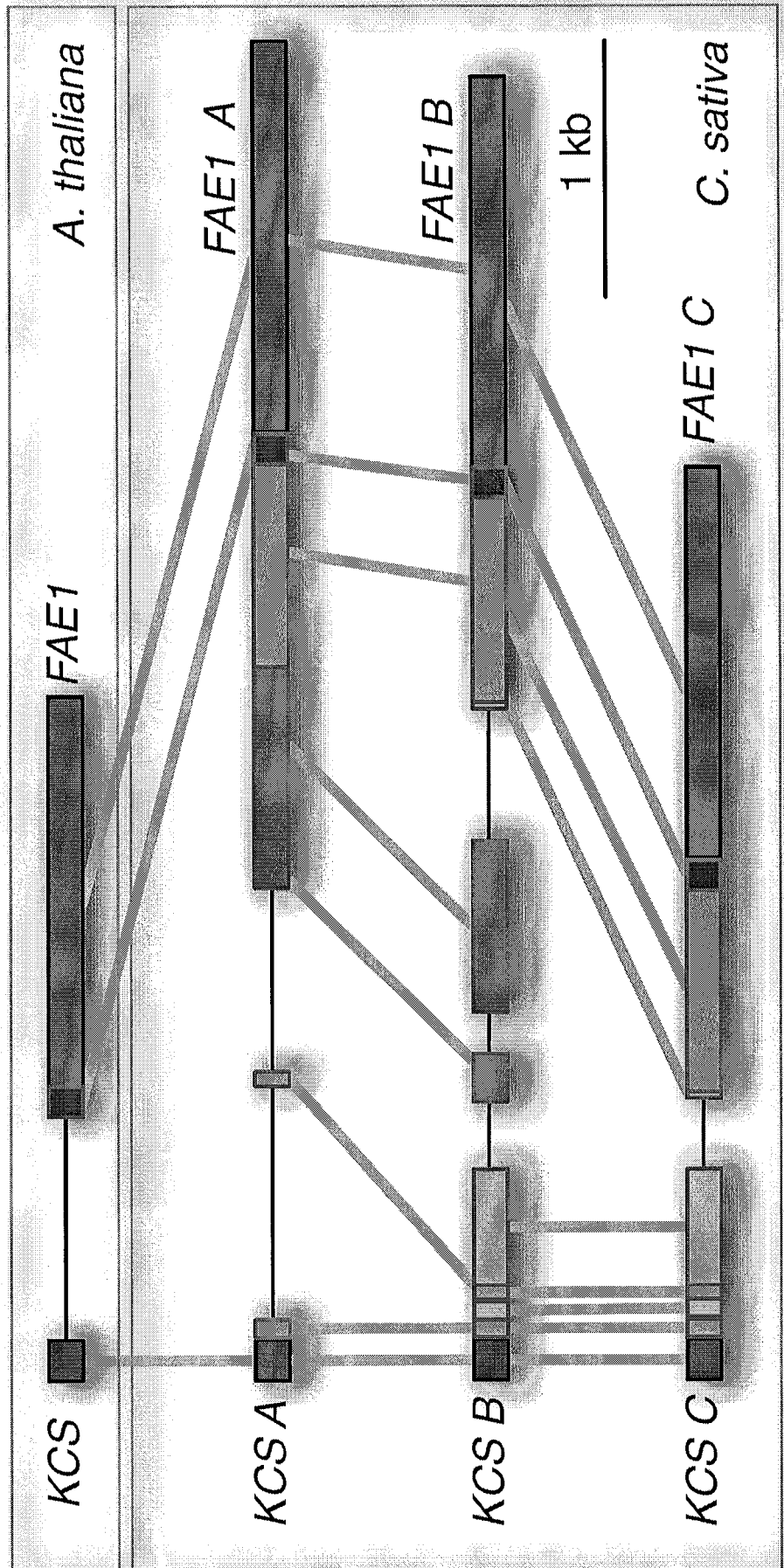


FIGURE 5

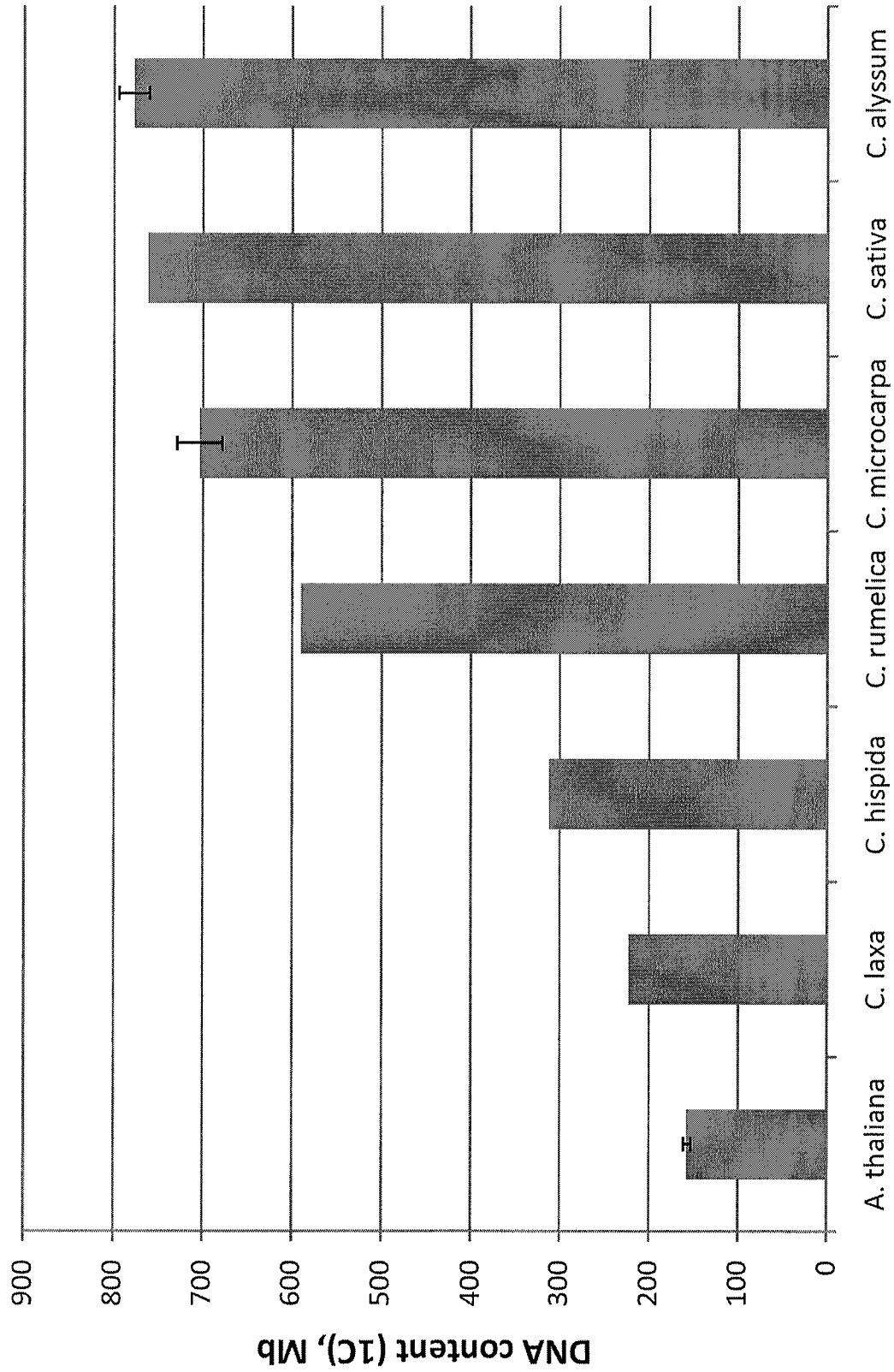


FIGURE 6A

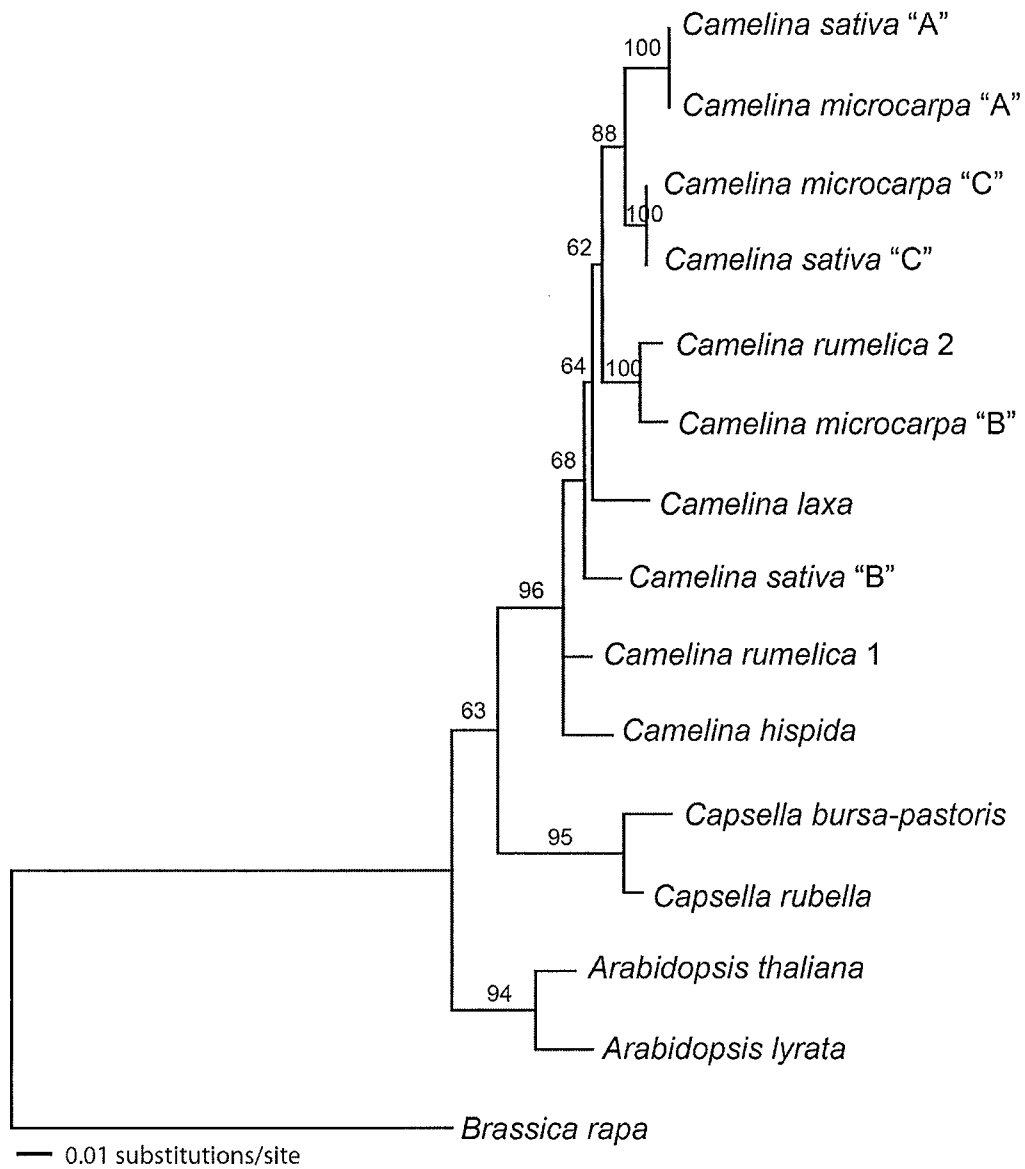


FIGURE 6B

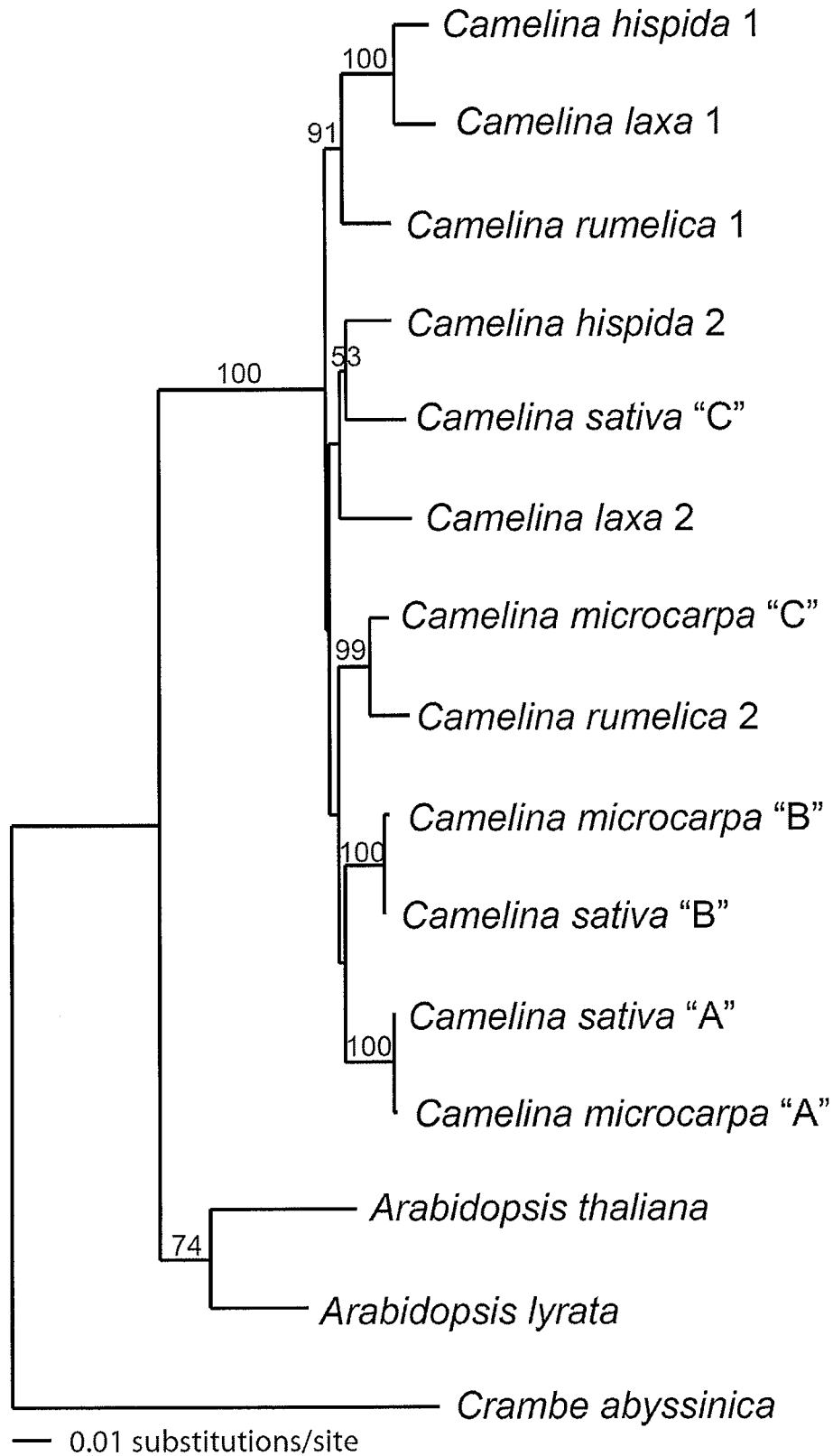


FIGURE 7

Seed Oil: Outline of its Biosynthetic Pathway

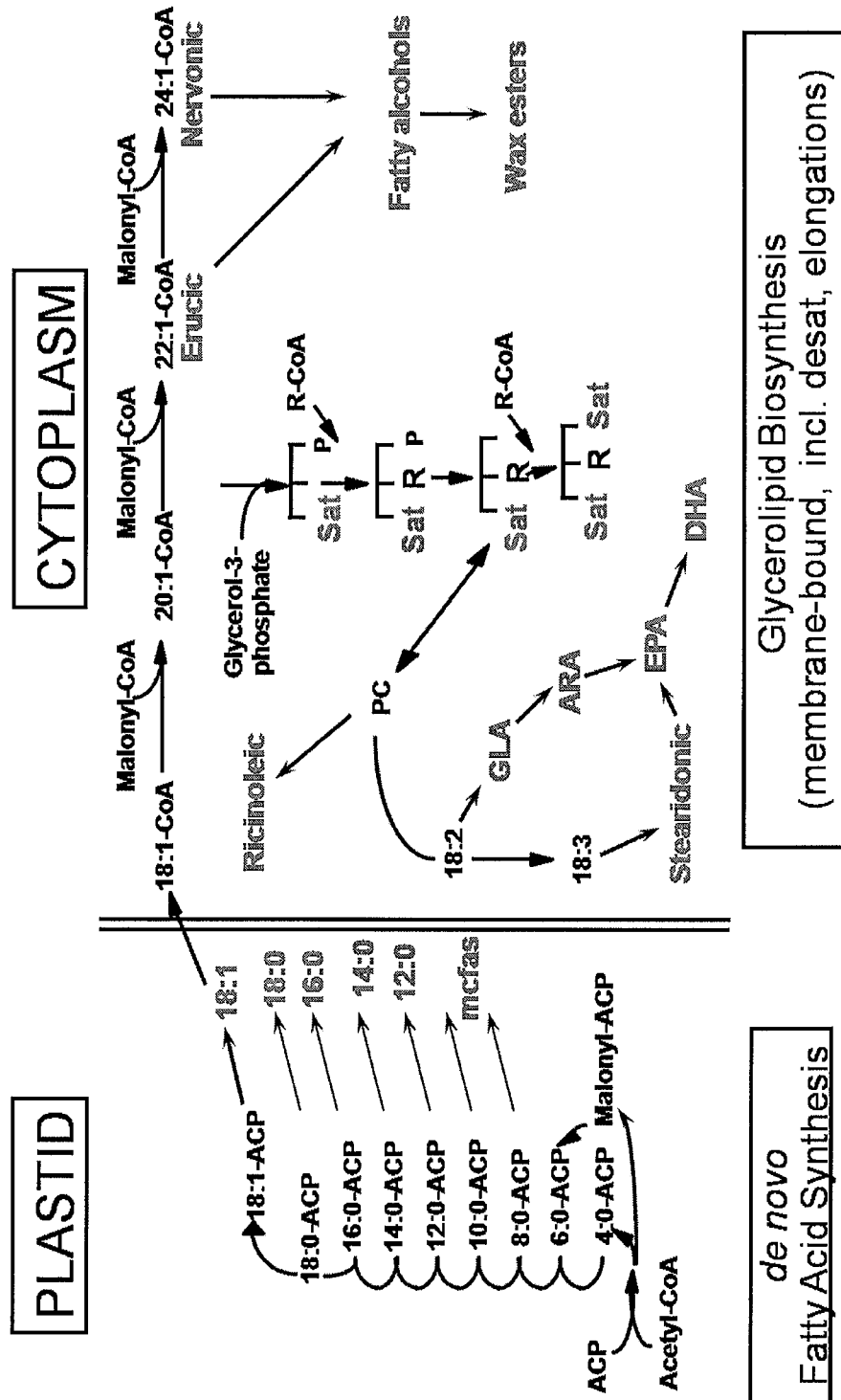
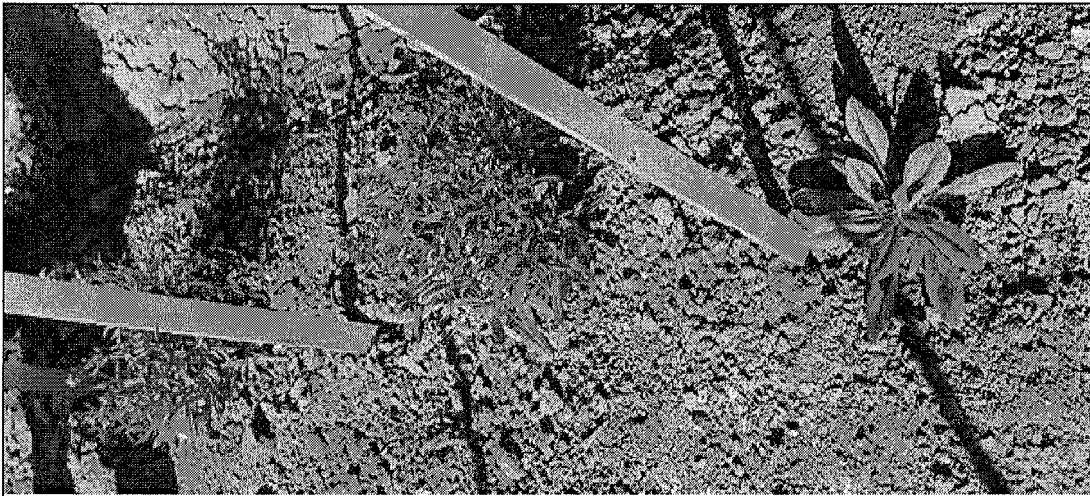


FIGURE 8



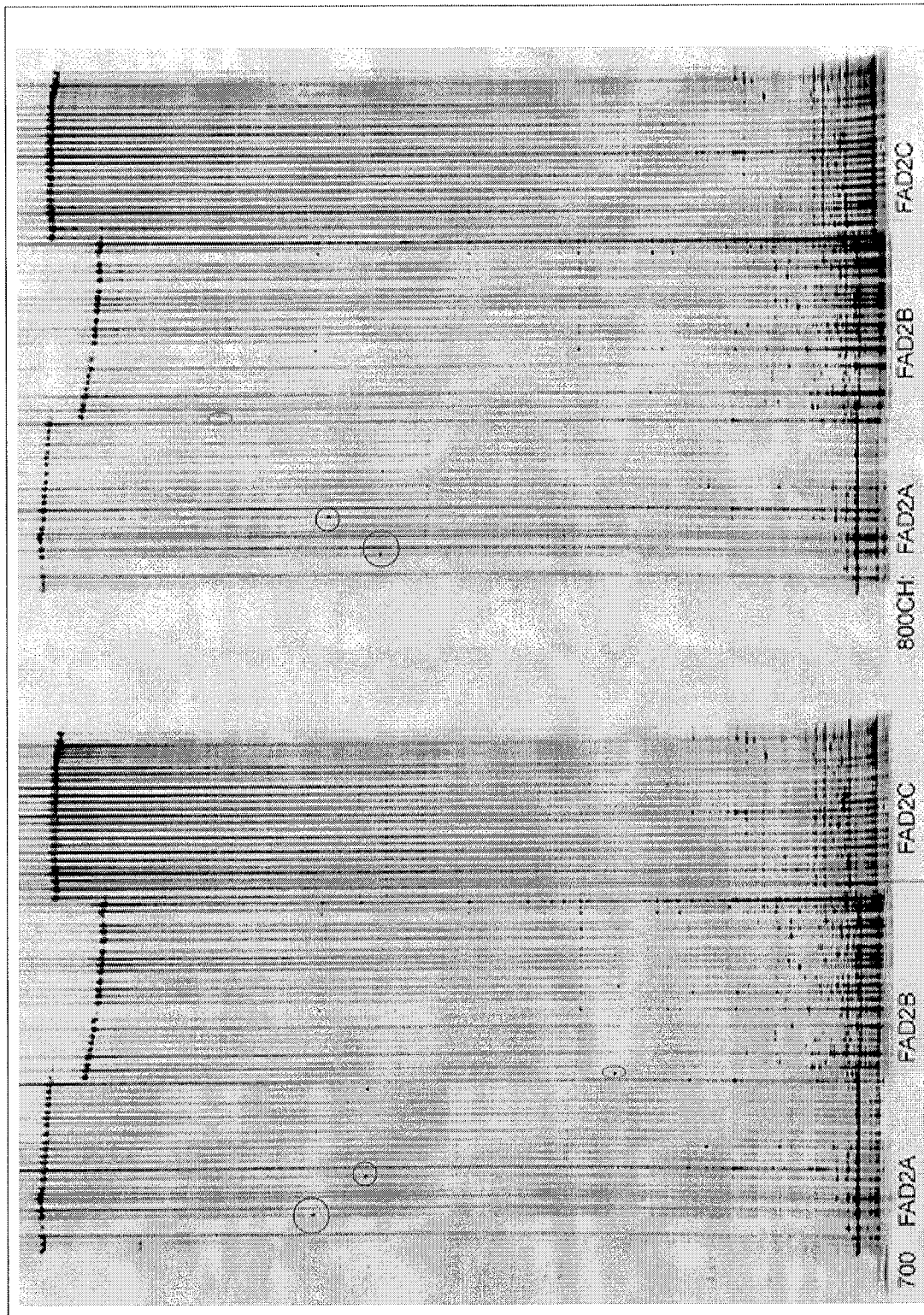
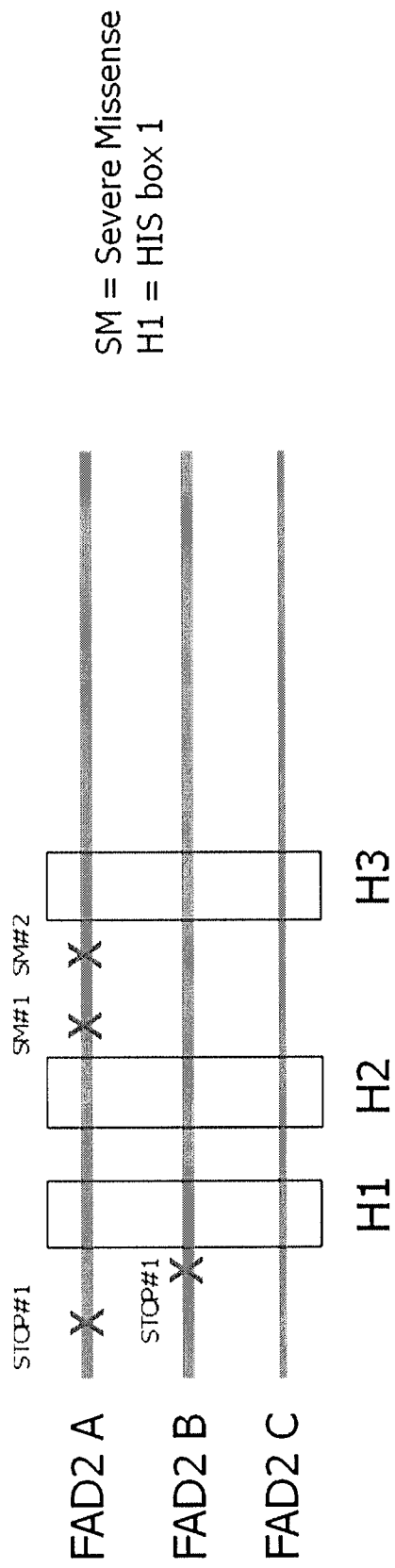


FIGURE 9

FIGURE 10



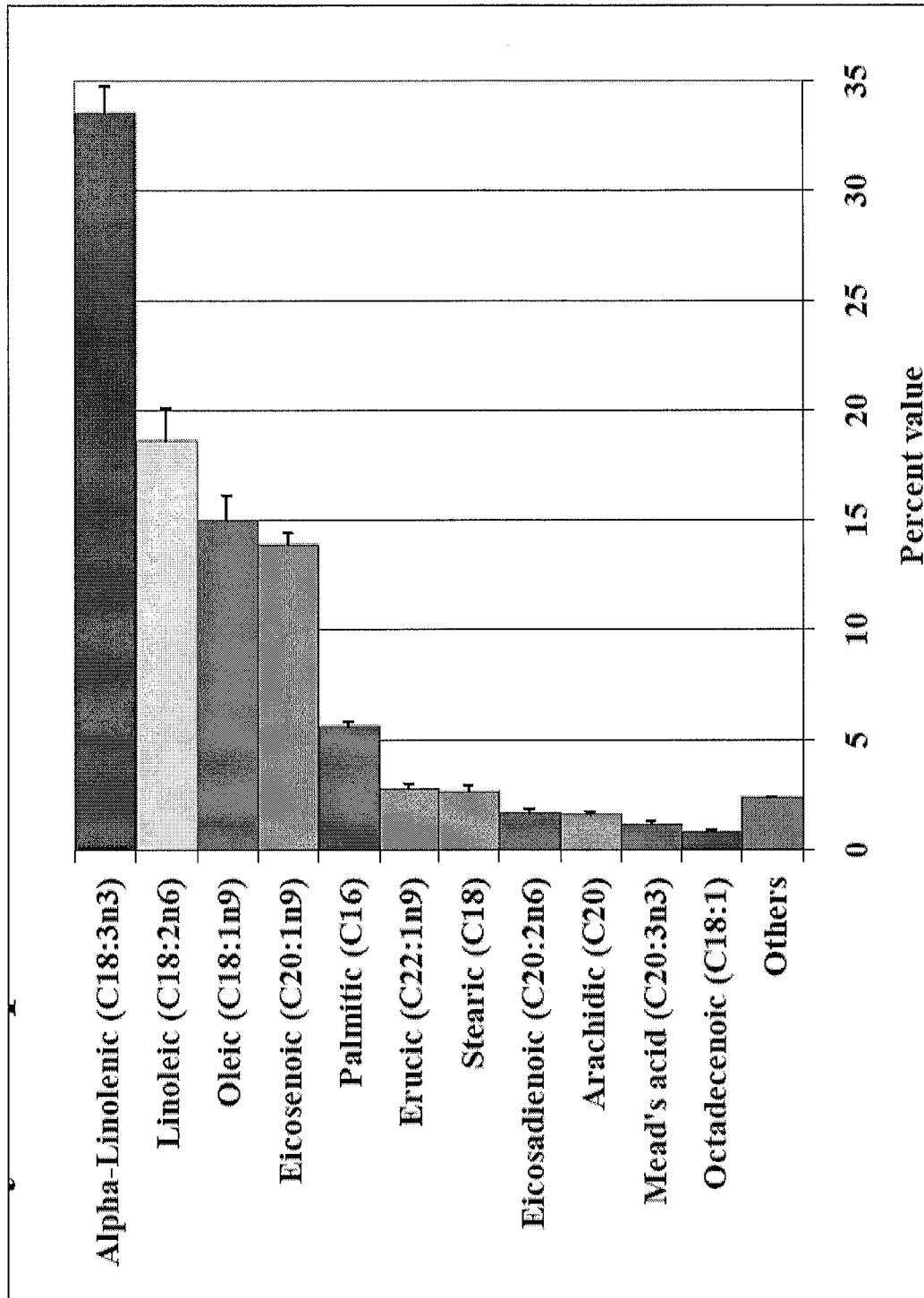


FIGURE 11

FIGURE 12 A

Fatty Acid Compositions in *FAD2* mutants

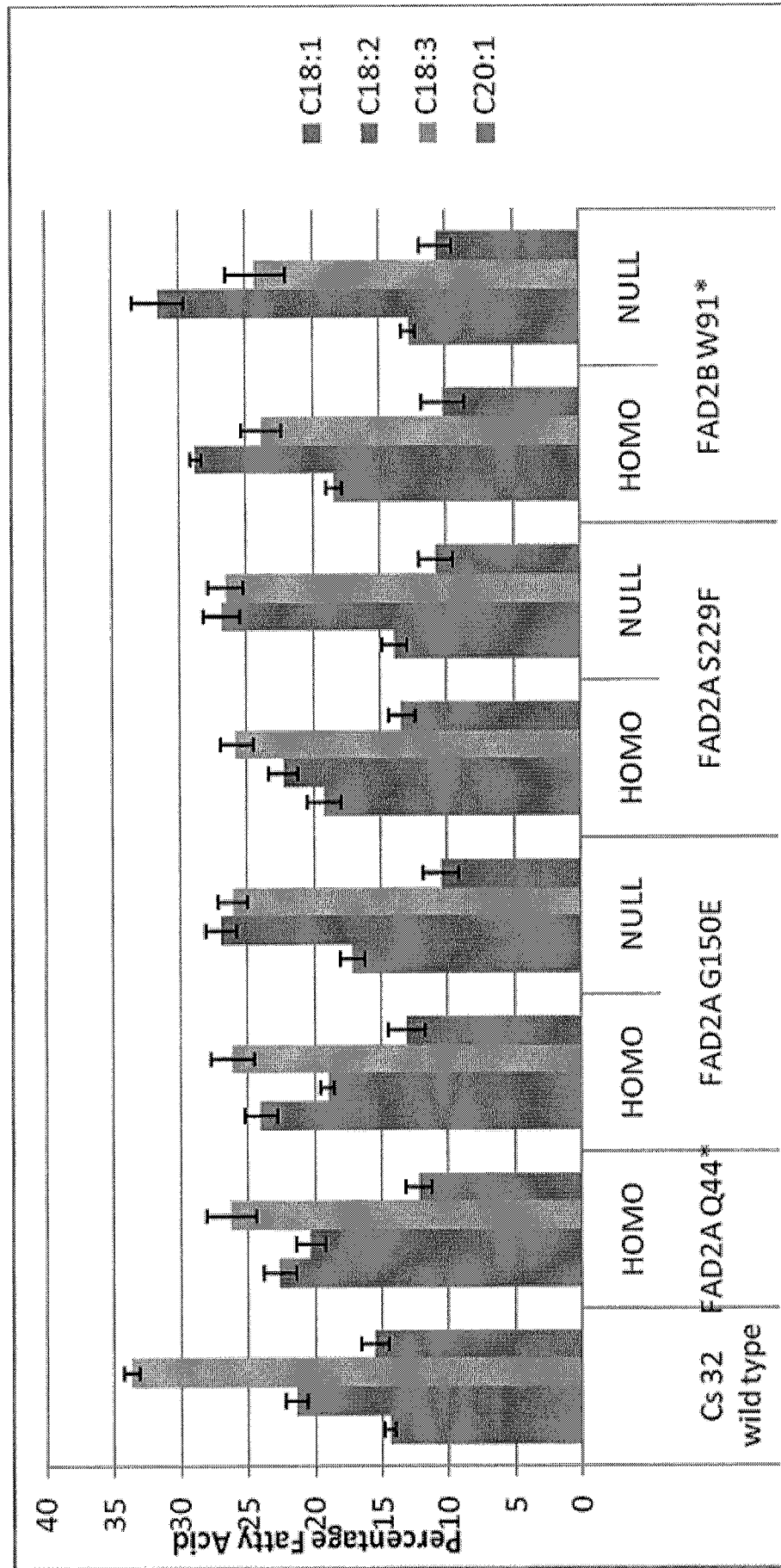


FIGURE 12B

Fatty Acid Compositions in *FAE* mutants, test 4

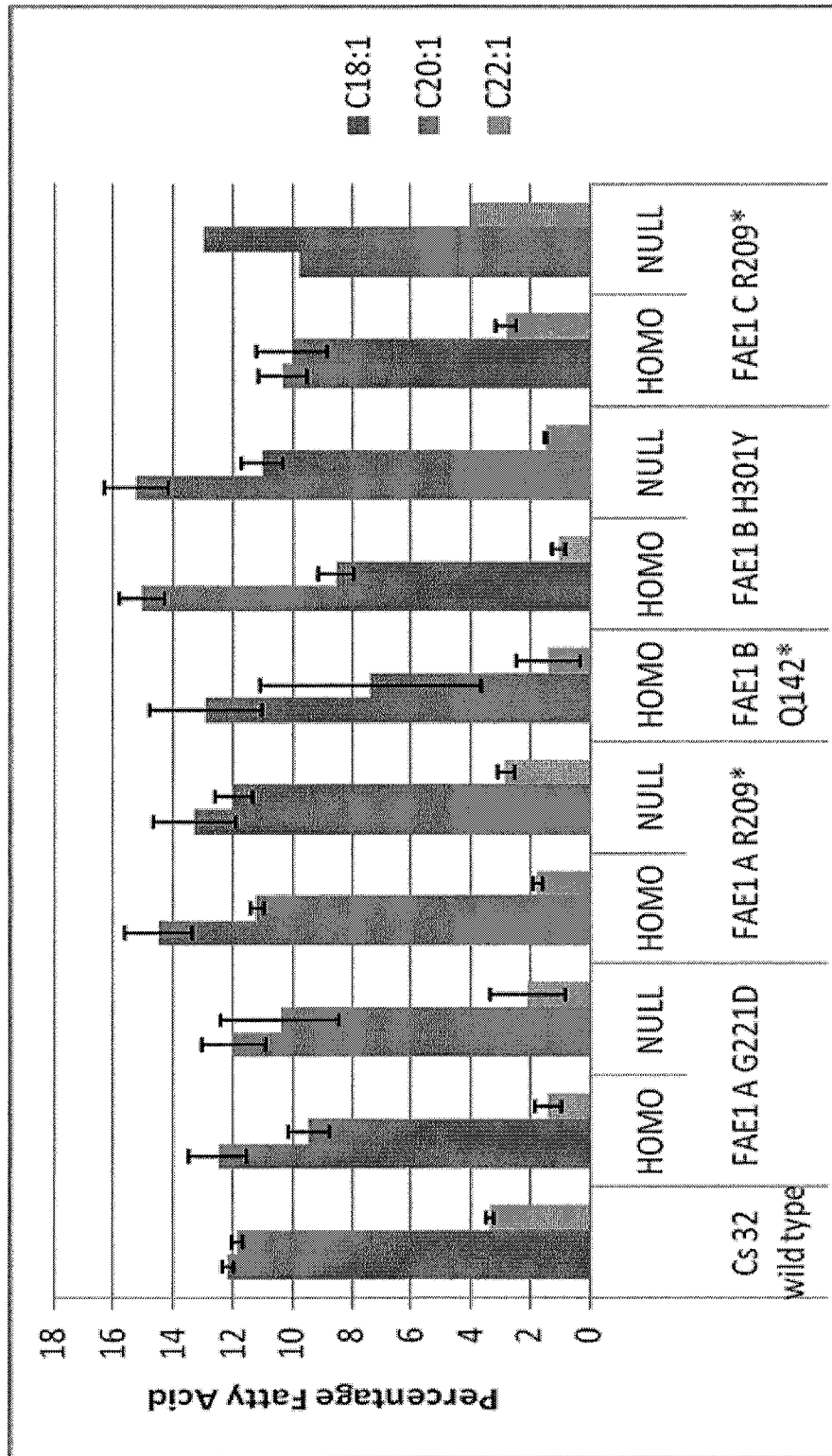


FIGURE 13

