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- (54) **POLYUNSATURATED FATTY ACIDS IN PLANTS**  
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**Related U.S. Patent Documents**

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- (51) **Int. Cl.**  
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*C12N 9/02* (2006.01)  
*C12P 7/64* (2006.01)

- (52) **U.S. Cl.** ..... **800/281**; 800/298; 435/419;  
 435/468; 435/69.1

- (58) **Field of Classification Search** ..... None  
 See application file for complete search history.

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(57) **ABSTRACT**

The present invention relates to compositions and methods for preparing polyunsaturated long chain fatty acids in plants, plant parts and plant cells, such as leaves, roots, fruits and seeds. Nucleic acid sequences and constructs encoding fatty acid desaturases, including  $\Delta^5$ -desaturases,  $\Delta^6$ -desaturases and  $\Delta^{12}$ -desaturases, are used to generate transgenic plants, plant parts and cells which contain and express one or more transgenes encoding one or more desaturases. Expression of the desaturases with different substrate specificities in the plant system permit the large scale production of polyunsaturated long chain fatty acids such as docosahexaenoic acid, eicosapentaenoic acid,  $\alpha$ -linolenic acid, gamma-linolenic acid, arachidonic acid and the like for modification of the fatty acid profile of plants, plant parts and tissues. Manipulation of the fatty acid profiles allows for the production of commercial quantities of novel plant oils and products.

**18 Claims, 2 Drawing Sheets**

# Pathways of PUFA Metabolism

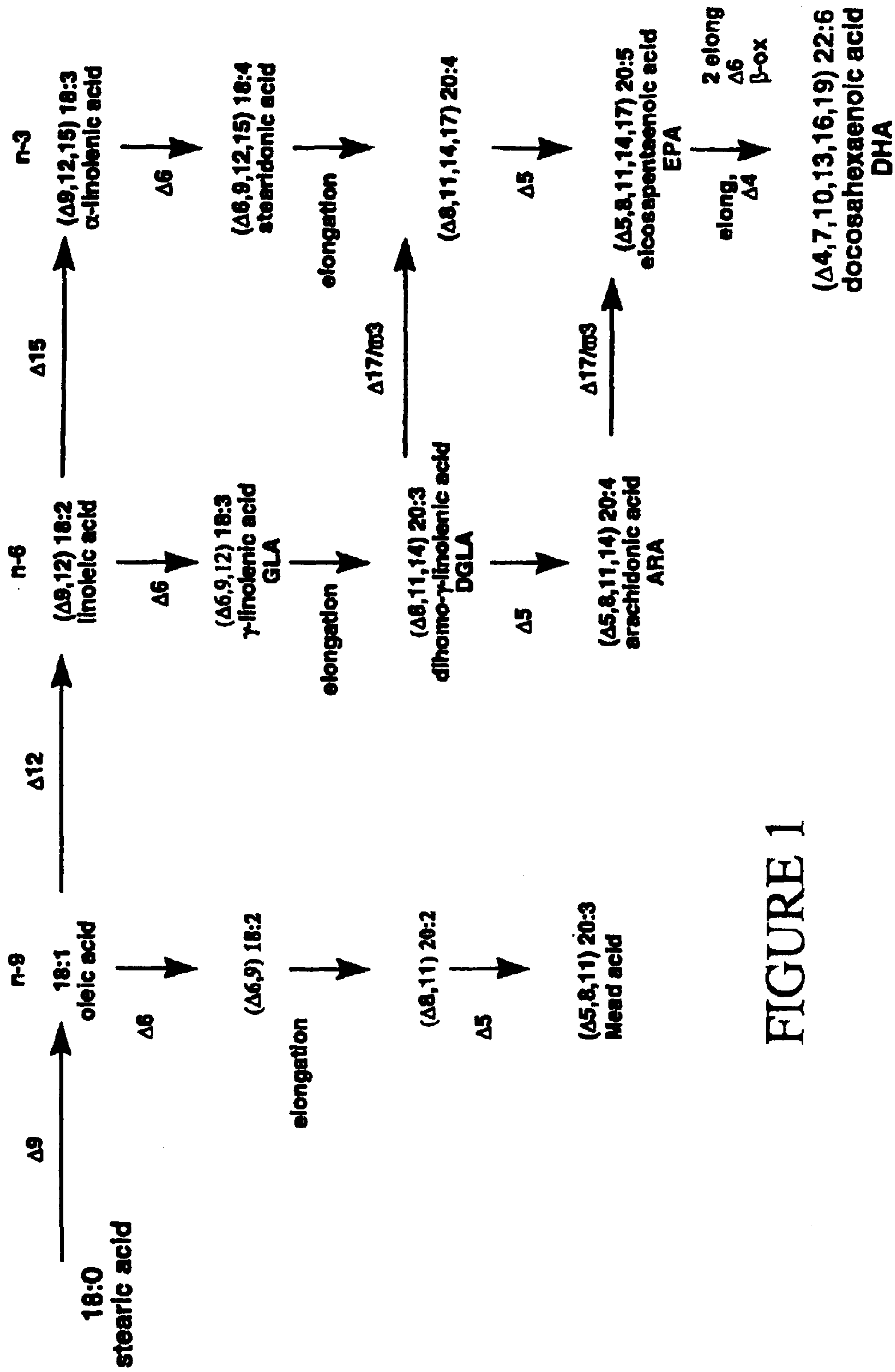


FIGURE 1

# Pathways of PUFA Metabolism

## $\Delta 5$ Desaturation

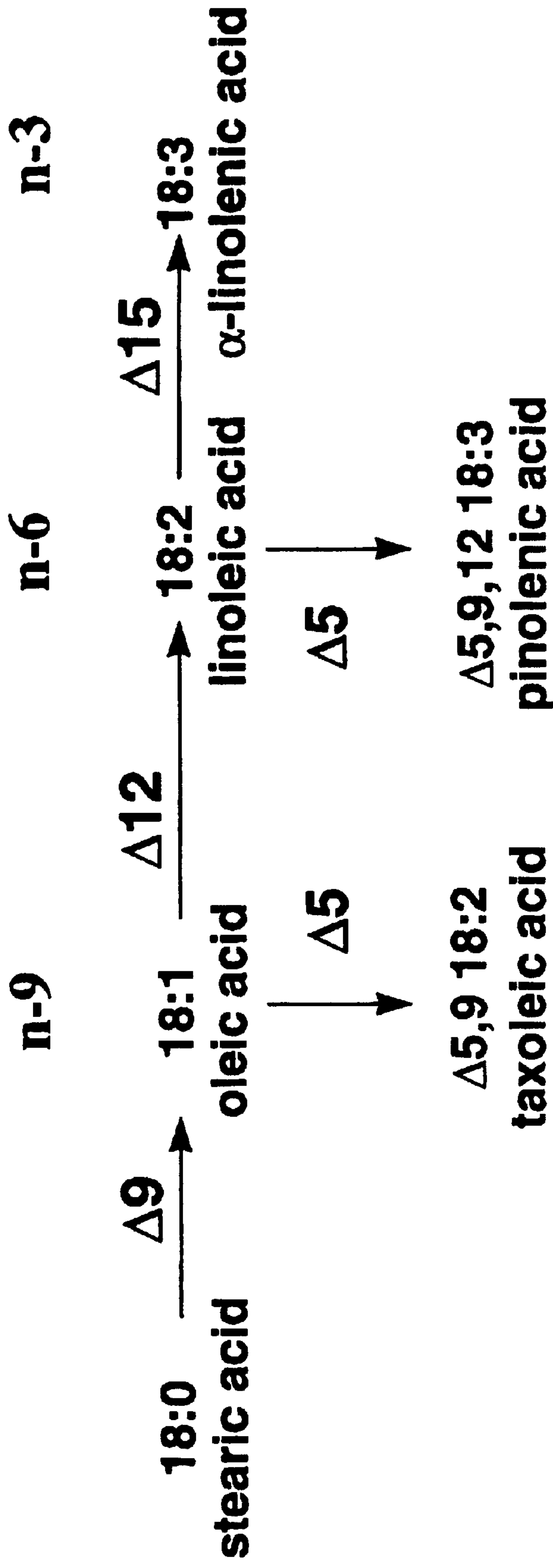


FIGURE 2



## POLYUNSATURATED FATTY ACIDS IN PLANTS

**Matter enclosed in heavy brackets [ ] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue.**

This application claims the benefit of U.S. Provisional Application No. 60/089,043 filed Jun. 12, 1998.

### TECHNICAL FIELD

This invention relates to modulating levels of enzymes and/or enzyme components capable of altering the production of long chain polyunsaturated fatty acids (PUFAs) in a host plant. The invention is exemplified by the production of PUFAs in plants.

### BACKGROUND

Three main families of polyunsaturated fatty acids (PUFAs) are the  $\omega$ 6 fatty acids, exemplified by arachidonic acid, the  $\omega$ 9 fatty acids exemplified by Mead acid, and the  $\omega$ 3 fatty acids, exemplified by eicosapentaenoic acid. PUFAs are important components of the plasma membrane of the cell, where they may be found in such forms as phospholipids. PUFAs also serve as precursors to other molecules of importance in human beings and animals, including the prostacyclins, leukotrienes and prostaglandins. PUFAs are necessary for proper development, particularly in the developing infant brain, and for tissue formation and repair.

Four major long chain PUFAs of importance include docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are primarily found in different types of fish oil, gamma-linolenic acid (GLA), which is found in the seeds of a number of plants, including evening primrose (*Oenothera biennis*), borage (*Borago officinalis*) and black currants (*Ribes nigrum*), and stearidonic acid (SDA), which is found in marine oils and plant seeds. Both GLA and another important long chain PUFA, arachidonic acid (ARA), are found in filamentous fungi. ARA can be purified from animal tissues including liver and adrenal gland. Mead acid accumulates in essential fatty acid deficient animals.

For DHA, a number of sources exist for commercial production including a variety of marine organisms, oils obtained from cold water marine fish, and egg yolk fractions. For ARA, microorganisms, including the genera *Mortierella*, *Entomophthora*, *Phytium* and *Porphyridium* can be used for commercial production. Commercial sources of SDA include the genera *Trichodesma* and *Echium*. Commercial sources of GLA include evening primrose, black currants and borage. However, there are several disadvantages associated with commercial production of PUFAs from natural sources. Natural sources of PUFAs, such as animals and plants, tend to have highly heterogeneous oil compositions. The oils obtained from these sources therefore can require extensive purification to separate out one or more desired PUFAs or to produce an oil which is enriched in one or more PUFA. Natural sources also are subject to uncontrollable fluctuations in availability. Fish stocks may undergo natural variation or may be depleted by overfishing. Fish oils have unpleasant tastes and odors, which may be impossible to economically separate from the desired product, and can render such products unacceptable as food supplements. Animal oils, and particularly fish oils, can accumulate environmental pollutants. Weather and disease can cause fluctuation in yields from both fish and plant sources. Cropland

available for production of alternate oil-producing crops is subject to competition from the steady expansion of human populations and the associated increased need for food production on the remaining arable land. Crops which do produce PUFAs, such as borage, have not been adapted to commercial growth and may not perform well in monoculture. Growth of such crops is thus not economically competitive where more profitable and better established crops can be grown. Large scale fermentation of organisms such as *Mortierella* is also expensive. Natural animal tissues contain low amounts of ARA and are difficult to process. Microorganisms such as *Porphyridium* and *Mortierella* are difficult to cultivate on a commercial scale.

Dietary supplements and pharmaceutical formulations containing PUFAs can retain the disadvantages of the PUFA source. Supplements such as fish oil capsules can contain low levels of the particular desired component and thus require large dosages. High dosages result in ingestion of high levels of undesired components, including contaminants. Care must be taken in providing fatty acid supplements, as overaddition may result in suppression of endogenous biosynthetic pathways and lead to competition with other necessary fatty acids in various lipid fractions in vivo, leading to undesirable results. For example, Eskimos having a diet high in  $\omega$ 3 fatty acids have an increased tendency to bleed (U.S. Pat. No. 4,874,603). Unpleasant tastes and odors of the supplements can make such regimens undesirable, and may inhibit compliance by the patient.

A number of enzymes are involved in PUFA biosynthesis. Linoleic acid (LA, 18:2  $\Delta$ 9, 12) is produced from oleic acid (18:1  $\Delta$ 9) by a  $\Delta$ 12-desaturase. GLA (18:3  $\Delta$ 6, 9, 12) is produced from linoleic acid (LA, 18:2  $\Delta$ 9, 12) by a  $\Delta$ 6-desaturase. ARA (20:4  $\Delta$ 5, 8, 11, 14) production from DGLA (20:3  $\Delta$ 8, 11, 14) is catalyzed by a  $\Delta$ 5-desaturase. However, animals cannot desaturate beyond the  $\Delta$ 9 position and therefore cannot convert oleic acid (18:1  $\Delta$ 9) into linoleic acid (18:2  $\Delta$ 9, 12). Likewise,  $\alpha$ -linolenic acid (ALA, 18:3  $\Delta$ 9, 12, 15) cannot be synthesized by mammals. Other eukaryotes, including fungi and plants, have enzymes which desaturate at positions  $\Delta$ 12 and  $\Delta$ 15. The major polyunsaturated fatty acids of animals therefore are either derived from diet and/or from desaturation and elongation of linoleic acid (18:2  $\Delta$ 9, 12) or -linolenic acid (18:3  $\Delta$ 9, 12, 15).

Poly-unsaturated fatty acids are considered to be useful for nutritional, pharmaceutical, industrial, and other purposes. An expansive supply of poly-unsaturated fatty acids from natural sources and from chemical synthesis are not sufficient for commercial needs. Therefore it is of interest to obtain genetic material involved in PUFA biosynthesis from species that naturally produce these fatty acids and to express the isolated material alone or in combination in a heterologous system which can be manipulated to allow production of commercial quantities of PUFAS.

### SUMMARY OF THE INVENTION

Novel compositions and methods are provided for preparation of poly-unsaturated long chain fatty acids and desaturases in plants and plant cells. The methods involve growing a host plant cell of interest transformed with an expression cassette functional in a host plant cell, the expression cassette comprising a transcriptional and translational initiation regulatory region, joined in reading frame 5' to a DNA sequence encoding a desaturase polypeptide capable of modulating the production of PUFAs. Expression of the desaturase polypeptide provides for an alteration in the PUFA profile of host plant cells as a result of altered concen-



trations of enzymes involved in PUFA biosynthesis. Of particular interest is the selective control of PUFA production in plant tissues and/or plant parts such as leaves, roots, fruits and seeds. The invention finds use for example in the large scale production of DHA, Mead Acid, EPA, ARA, DGLA, stearidonic acid GLA and other fatty acids and for modification of the fatty acid profile of edible plant tissues and/or plant parts.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows possible pathways for the synthesis of Mead acid (20:3  $\Delta$ 5, 8, 11), arachidonic acid (20:4  $\Delta$ 5, 8, 11, 14) and stearidonic acid (18:4  $\Delta$ 6, 9, 12, 15) from palmitic acid ( $C_{16}$ ) from a variety of organisms, including algae, Mortierella and humans. These PUFAs can serve as precursors to other molecules important for humans and other animals, including prostacyclins, leukotrienes, and prostaglandins, some of which are shown.

FIG. 2 shows possible pathways for production of PUFAs in addition to ARA, including taxoleic acid and pinolenic, again compiled from a variety of organisms.

#### DESCRIPTION OF THE PREFERRED EMBODIMENTS

In order to ensure a complete understanding of the invention, the following definitions are provided:

$\Delta$ 5-Desaturase:  $\Delta$ 5 desaturase is an enzyme which introduces a double bond between carbons 5 and 6 from the carboxyl end of a fatty acid molecule.

$\Delta$ 6-Desaturase:  $\Delta$ 6-desaturase is an enzyme which introduces a double bond between carbons 6 and 7 from the carboxyl end of a fatty acid molecule.

$\Delta$ 9-Desaturase:  $\Delta$ 9-desaturase is an enzyme which introduces a double bond between carbons 9 and 10 from the carboxyl end of a fatty acid molecule.

$\Delta$ 12-Desaturase:  $\Delta$ 12-desaturase is an enzyme which introduces a double bond between carbons 12 and 13 from the carboxyl end of a fatty acid molecule.

Fatty acids: Fatty acids are a class of compounds containing a long-hydrocarbon chain and a terminal carboxylate group. Fatty acids include the following:

Fatty Acid	
12:0	Isuric acid
16:0	Palmitic acid
16:1	Palmitoleic acid
18:0	stearic acid
18:1	oleic acid $\Delta$ 9-18:1
18:2 $\Delta$ 5,9	Taxoleic acid $\Delta$ 5,9-18:2
18:2 $\Delta$ 6,9	6,9-octadecadienoic acid $\Delta$ 6,9-18:2
18:2	Linoleic acid $\Delta$ 9,12-18:2 (LA)
18:3 $\Delta$ 6,9,12	Gasima-linoleic acid $\Delta$ 6,9,12-18:3 (GLA)
18:3 $\Delta$ 5,9,12	Pinolenic acid $\Delta$ 5,9,12-18:3
18:3	alpha-linolenic acid $\Delta$ 9,12,15-18:3 (ALA)
18:4	Stearidonic acid $\Delta$ 6,9,12,15-18:4 (SDA)
20:0	Arachidic acid
20:1	Eicosenic Acid
20:2 $\Delta$ 8,11	$\Delta$ 8,11
20:3 $\Delta$ 5,8,11	Mead Acid $\Delta$ 5,8,11
22:0	Behenic acid
22:1	erucic acid
22:2	Docosadienoic acid
20:4 $\gamma$ 6	Arachidonic acid $\Delta$ 5,8,11,14-20:4 (ARA)
20:3 $\gamma$ 6	$\gamma$ 6-eicosatrienoic dihomogamma linolenic $\Delta$ 8,11,14-20:3 (DGLA)

-continued

Fatty Acid		
5	20:5 $\gamma$ 6	Eicosapentaenoic (Timnodonic acid) $\Delta$ 5,8,11,14,17-20:5 (EPA)
	20:3 $\gamma$ 6	$\gamma$ 3-eicosatenoic $\Delta$ 11,16,17-20:3
	20:4 $\gamma$ 6	$\gamma$ 3-eicosatetraenoic $\Delta$ 8,11,14,17-20:4
	22:5 $\gamma$ 6	Docosapentaenoic $\Delta$ 7,10,13,16,19-22:5 ( $\gamma$ 3DPA)
	22:6 $\gamma$ 6	Docosahexaenoic (cervonic acid) $\Delta$ 7,10,13,16,19-22:6 (DHA)
10	24:0	Lignoceric acid

Taking into account these definitions, the present invention is directed to novel DNA sequences, and DNA constructs related to the production of fatty acids in plants. Methods and compositions are provided which permit modification of the poly-unsaturated long chain fatty acid content of plant cells. Plant cells are transformed with an expression cassette comprising a DNA encoding a polypeptide capable of increasing the amount of one or more PUFA in a plant cell. Desirably, integration constructs may be prepared which provide for integration of the expression cassette into the genome of a host cell. Host cells are manipulated to express a sense or antisense DNA encoding a polypeptide(s) that has desaturase activity. By "desaturase" is intended a polypeptide which can desaturate one or more fatty acids to produce a mono- or poly-unsaturated fatty acid or precursor thereof of interest. By "polypeptide" is meant any chain of amino acids, regardless of length or post-translational modification, for example, glycosylation or phosphorylation. The substrate(s) for the expressed enzyme may be produced by the host cell or may be exogenously supplied.

To achieve expression in a host cell, the transformed DNA is operably associated with transcriptional and translational initiation and termination regulatory regions that are functional in the host cell. Constructs comprising the gene to be expressed can provide for integration into the genome of the host cell or can autonomously replicate in the host cell. For production of taxoleic acid, the expression cassettes generally used include a cassette which provides for  $\Delta$ 5 desaturase activity, particularly in a host cell which produces or can take up oleic acid. For production of  $\Delta$ 6,9 18:2 or other  $\omega$ 9 unsaturated fatty acids, the expression cassettes generally used include a cassette which provides for  $\Delta$ 6 desaturase activity, particularly in a host cell which produces or can take up oleic acid. Production of a oleic acid, taxoleic acid, or  $\omega$ 9 unsaturated fatty acids in a plant having  $\Delta$ 12 desaturase activity is favored by providing an expression cassette for an antisense  $\Delta$ 12 transcript, or by disrupting a  $\Delta$ 12 desaturase gene. For production of linoleic acid (LA), the expression cassettes generally used include a cassette which provides for  $\Delta$ 12 desaturase activity, particularly in a host cell which produces or can take up oleic acid. For production of ALA, the expression cassettes generally used include a cassette which provides for  $\Delta$ 15 to  $\omega$ 3 desaturase activity, particularly in a host cell which produces or can take up LA. For production of GLA or SDA, the expression cassettes generally used include a cassette which provides for  $\Delta$ 6 desaturase activity, particularly in a host cell which produces or can take up LA or ALA, respectively. Production of  $\omega$ 6-type unsaturated fatty acids, such as LA or GLA, in a plant capable of producing ALA is favored by inhibiting the activity of  $\Delta$ 15 or  $\omega$ 3 type desaturase; this is accomplished by providing and expression cassette for an antisense  $\Delta$ 15 or  $\omega$ 3 transcript, or by disrupting a  $\Delta$ 15 or  $\omega$ 3 desaturase gene. Similarly, production of LA or ALA in a plant having  $\Delta$ 6 desaturase activity is favored by providing an expression



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cassette for an antisense A 6 transcript, or by disrupting a  $\Delta 6$  desaturase gene. For production of ARA in a host cell which produces or can take up DGLA, the expression cassette generally used provides for  $\Delta 5$  desaturase activity. Production of  $\omega 6$ -type unsaturated fatty acids, such as ARA, in a plant capable of producing ALA is favored by inhibiting the activity of a  $\Delta 15$  or  $\omega 3$  desaturase; this is accomplished by providing an expression cassette for an antisense  $\Delta 15$  or  $\omega 3$  transcript, or by disrupting a  $\Delta 15$  or  $\omega 3$  desaturase gene.

TRANSGENIC PLANT PRODUCTION OF FATTY ACIDS

Transgenic plant production of PUFAs offers several advantages over purification from natural sources such as fish or plants. Production of fatty acids from recombinant plants provides the ability to alter the naturally occurring plant fatty acid profile by providing new synthetic pathways in the host or by suppressing undesired pathways, thereby increasing levels of desired PUFAs, or conjugated forms thereof, and decreasing levels of undesired PUFAs. Production of fatty acids in transgenic plants also offers the advantage that expression of desaturase genes in particular tissues and/or plant parts means that greatly increased levels of desired PUFAs in those tissues and/or parts can be achieved, making recovery from those tissues more economical. For example, the desired PUFAs can be expressed in seed; methods of isolating seed oils are well established. In addition to providing a source for purification of desired PUFAs, seed oil components can be manipulated through expression of desaturase genes, either alone or in combination with other genes such as elongases, to provide seed oils having a particular PUFA profile in concentrated form. The concentrated seed oils then can be added to animal milks and/or synthetic or semi-synthetic milks to serve as infant formulas where human nursing is impossible or undesired, or in cases of malnourishment or disease in both adults and infants.

For production of PUFAs, depending upon the host cell, the availability of substrate, and the desired end product(s), several polypeptides, particularly desaturases, are of interest including those polypeptides which catalyze the conversion of stearic acid to oleic acid, LA to GLA, of ALA to SDA, of oleic acid to LA, or of LA to ALA, oleic acid to taxolic acid, LA to pinolenic acid, oleic acid to 6,9-actadeca-dienoic acid which includes enzymes which desaturate at the  $\Delta 6$ ,  $\Delta 9$ ,  $\Delta 5$ ,  $\Delta 12$ ,  $\Delta 15$ ,  $\Delta 5$ , or  $\omega 3$  positions. Considerations for choosing a specific polypeptide having desaturase activity include the pH optimum of the polypeptide, whether the polypeptide is a rate limiting enzyme or a component thereof, whether the desaturase used is essential for synthesis of a desired polyunsaturated fatty acid, and/or co-factors required by the polypeptide. The expressed polypeptide preferably has parameters compatible with the biochemical environment of its location in the host cell. For example, the polypeptide may have to compete for substrate with other enzymes in the host cell. Analyses of the  $K_m$  and specific activity of the polypeptide in question therefore are considered in determining the suitability of a given polypeptide for modifying PUFA production in a given host cell. The polypeptide used in a particular situation therefore is one which can function under the conditions present in the intended host cell but otherwise can be any polypeptide having desaturase activity which has the desired characteristic of being capable of modifying the relative production of a desired PUFA. A scheme for the synthesis of arachidonic acid (20:4  $\Delta 5$ , 8, 11, 14) from palmitic acid ( $C_{16}$ ) is shown in FIG. 1. A key enzyme in this pathway is a  $\Delta 5$ -desaturase which converts DH- $\gamma$ -linolenic acid (DGLA, eicosatrienoic acid) to ARA.

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Conversion of  $\alpha$ -linolenic acid (ALA) to stearidonic acid by a  $\Delta 6$ -desaturase is also shown. Production of PUFAs in addition to ARA, including EPA and DHA is shown in FIG. 2. A key enzyme in the synthesis of arachidonic acid (20:4  $\Delta 5$ , 8, 11, 14) from stearic acid ( $C_{18}$ ) is a  $\Delta 6$ -desaturase which converts the linoleic acid into  $\gamma$ -linolenic acid. Conversion of  $\alpha$ -linolenic acid (ALA) to stearidonic acid by a  $\Delta 6$ -desaturase also is shown. For production of ARA, the DNA sequence used encodes a polypeptide having  $\Delta 5$  desaturase activity. In particular instances, this can be coupled with an expression cassette which provides for production of a polypeptide having  $\Delta 6$  desaturase activity and, optionally, a transcription cassette providing for production of antisense sequences to a  $\Delta 15$  transcription product. The choice of combination of cassettes used depends in part on the PUFA profile of the host cell. Where the host cell  $\Delta 5$ -desaturase activity is limiting, overexpression of  $\Delta 5$  desaturase alone generally will be sufficient to provide for enhanced ARA production.

SOURCES OF POLYPEPTIDES HAVING DESATURASE ACTIVITY

As sources of polypeptides having desaturase activity and oligonucleotides encoding such polypeptides are organisms which produce a desired poly-unsaturated fatty acid. As an example, microorganisms having an ability to produce ARA can be used as a source of  $\Delta 5$ -desaturase genes; microorganisms which GLA or SDA can be used as a source of  $\Delta 6$ -desaturase and/or  $\Delta 12$ -desaturase genes. Such microorganisms include, for example, those belonging to the genera *Mortierella*, *Conidiobolus*, *Pythium*, *Phytophthora*, *Penicillium*, *Porphyridium*, *Coidosporium*, *Mucor*, *Fusarium*, *Aspergillus*, *Rhodotorula*, and *Entomophthora*. Within the genus *Porphyridium*, of particular interest is *Porphyridium cruentum*. Within the genus *Mortierella*, of particular interest are *Mortierella elongata*, *Mortierella exigua*, *Mortierella hygrophila*, *Mortierella ramanniana*, var. *angulispora*, and *Mortierella alpina*. Within the genus *Mucor*, of particular interest are *Mucor circinelloides* and *Mucor javanicus*.

DNAs encoding desired desaturases can be identified in a variety of ways. As an example, a source of the desired desaturase, for example genomic or cDNA libraries from *Mortierella*, is screened with detectable enzymatically- or chemically-synthesized probes, which can be made from DNA, RNA, or non-naturally occurring nucleotides, or mixtures thereof. Probes may be enzymatically synthesized from DNAs of known desaturases for normal or reduced-stringency hybridization methods. Oligonucleotide probes also can be used to screen sources and can be based on sequences of known desaturases, including sequences conserved among known desaturases, or on peptide sequences obtained from the desired purified protein. Oligonucleotide probes based on amino acid sequences can be degenerate to encompass the degeneracy of the genetic code, or can be biased in favor of the preferred codons of the source organism. Oligonucleotides also can be used as primers for PCR from reverse transcribed mRNA from a known or suspected source; the PCR product can be the full length cDNA or can be used to generate a probe to obtain the desired full length cDNA. Alternatively, a desired protein can be entirely sequenced and total synthesis of a DNA encoding that polypeptide performed.

Once the desired genomic or cDNA has been isolated, it can be sequenced by known methods. It is recognized in the art that such methods are subject to errors, such that multiple sequencing of the same region is routine and is still expected



to lead to measurable rates of mistakes in the resulting deduced sequence, particularly in regions having repeated domains, extensive secondary structure, or unusual base compositions, such as regions with high GC base content. When discrepancies arise, resequencing can be done and can employ special methods. Special methods can include altering sequencing conditions by using: different temperatures; different enzymes; proteins which alter the ability of oligonucleotides to form higher order structures; altered nucleotides such as ITP or methylated dGTP; different gel compositions, for example adding formamide; different primers or primers located at different distances from the problem region; or different templates such as single stranded DNAs. Sequencing of mRNA can also be employed.

For the most part, some or all of the coding sequence for the polypeptide having desaturase activity is from a natural source. In some situations, however, it is desirable to modify all or a portion of the codons, for example, to enhance expression, by employing host preferred codons. Host preferred codons can be determined from the codons of highest frequency in the proteins expressed in the largest amount in a particular host species of interest. Thus, the coding sequence for a polypeptide having desaturase activity can be synthesized in whole or in part. All or portions of the DNA also can be synthesized to remove any destabilizing sequences or regions of a secondary structure which would be present in the transcribed mRNA. All or portions of the DNA also can be synthesized to alter the base composition to one more preferable in the desired host cell. Methods for synthesizing sequences and bringing sequences together are well established in the literature. In vitro mutagenesis and selection, site-directed mutagenesis, or other means can be employed to obtain mutations of naturally occurring desaturase genes to produce a polypeptide having desaturase activity in vivo with more desirable physical and kinetic parameters for function in the host cell, such as a longer half-life or a higher rate of production of a desired polyunsaturated fatty acid.

Desirable cDNAs have less than 60% A+T composition, preferably less than 50% A+T composition. On a localized scale of a sliding window of 20 base pairs, it is preferable that there are no localized regions of the cDNA with greater than 75% A+T composition; with a window of 60 base pairs, it is preferable that there are no localized regions of the cDNA with greater than 60%, more preferably no localized regions with greater than 55% A+T composition.

#### Mortierella Alpina Desaturases

Of particular interest are the *Mortierella alpina*  $\Delta 5$ -desaturase,  $\Delta 6$ -desaturase,  $\Delta 12$ -desaturase and  $\Delta 15$  desaturase. The gene encoding the *Mortierella alpina*  $\Delta 5$ -desaturase can be expressed in transgenic plants to effect greater synthesis of ARA from DGLA, or pinolenic acid from LA, taxoleic acid from oleic acid or Mead and from  $\Delta 8$ , 11-20:2. Other DNAs which are substantially identical in sequence to the *Mortierella alpina*  $\Delta 5$ -desaturase DNA, or which encode polypeptides which are substantially identical in sequence to the *Mortierella alpina*  $\Delta 5$ -desaturase polypeptide, also can be used. The gene encoding the *Mortierella alpina*  $\Delta 6$ -desaturase can be expressed in transgenic plants or animals to effect greater synthesis of GLA from linoleic acid or stearidonic acid (SDA) from ALA or of 6,9-octadecadienoic acid from oleic acid. Other DNAs which are substantially identical in sequence to the *Mortierella alpina*  $\Delta 6$ -desaturase DNA, or which encode polypeptides which are substantially identical in sequence to the *Mortierella alpina*  $\Delta 6$ -desaturase polypeptide, also can be used.

The gene encoding the *Mortierella alpina*  $\Delta 12$ -desaturase can be expressed in transgenic plants to effect greater synthesis of LA from oleic acid. Other DNAs which are substantially identical to the *Mortierella alpina*  $\Delta 12$ -desaturase DNA, or which encode polypeptides which are substantially identical to the *Mortierella alpina*  $\Delta 12$ -desaturase polypeptide, also can be used.

By substantially identical in sequence is intended an amino acid sequence or nucleic acid sequence exhibiting in order of increasing preference at least 60%, 80%, 90% or 95% homology to the *Mortierella alpina*  $\Delta 5$ -desaturase amino acid sequence or nucleic acid sequence encoding the amino acid sequence. For polypeptides, the length of comparison sequences generally is at least 16 amino acids, preferably at least 20 amino acids, or most preferably 35 amino acids. For nucleic acids, the length of comparison sequences generally is at least 50 nucleotides, preferably at least 60 nucleotides, and more preferably at least 75 nucleotides, and most preferably, 110 nucleotides. Homology typically is measured using sequence analysis software, for example, the Sequence Analysis software package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, MEGAlign (DNASar, Inc., 1228 S. Park St., Madison, Wis. 53715), and MacVector (Oxford Molecular Group, 2105 S. Bascom Avenue, Suite 200, Campbell, Calif. 95008). Such software matches similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine and leucine; aspartic acid, glutamic acid, asparagine, and glutamine; serine and threonine; lysine and arginine; and phenylalanine and tyrosine. Substitutions may also be made on the basis of conserved hydrophobicity or hydrophilicity (Kyte and Doolittle, *J. Mol. Biol.* 157: 105-132, 1982), or on the basis of the ability to assume similar polypeptide secondary structure (Chou and Fasman, *Adv. Enzymol.* 47: 45-148, 1978).

#### EXPRESSION OF DESATURASE GENES

Once the DNA encoding a desaturase polypeptide has been obtained, it is placed in a vector capable of replication in a host cell, or is propagated in vitro by means of techniques such as PCR or long PCR. Replicating vectors can include plasmids, phage, viruses, cosmids and the like. Desirable vectors include those useful for mutagenesis of the gene of interest or for expression of the gene of interest in host cells. The technique of long PCR has made in vitro propagation of large constructs possible, so that modifications to the gene of interest, such as mutagenesis or addition of expression signals, and propagation of the resulting constructs can occur entirely in vitro without the use of a replicating vector or a host cell.

For expression of a desaturase polypeptide, functional transcriptional and translational initiation and termination regions are operably linked to the DNA encoding the desaturase polypeptide. Transcriptional and translational initiation and termination regions are derived from a variety of nonexclusive sources, including the DNA to be expressed, genes known or suspected to be capable of expression in the desired system, expression vectors, chemical synthesis, or from an endogenous locus in a host cell. Expression in a plant tissue and/or plant part presents certain efficiencies, particularly where the tissue or part is one which is easily harvested, such as seed, leaves, fruits, flowers, roots, etc. Expression can be targeted to that location within the plant by using specific regulatory sequences, such as those of U.S.



Pat. No. 5,463,174, U.S. Pat. No. 4,943,674, U.S. Pat. No. 5,106,739, U.S. Pat. No. 5,175,095, U.S. Pat. No. 5,420,034, U.S. Pat. No. 5,188,958, and U.S. Pat. No. 5,589,379.

Alternatively, the expressed protein can be an enzyme which produces a product which may be incorporated, either directly or upon further modifications, into a fluid fraction from the host plant. In the present case, expression of desaturase genes, or antisense desaturase transcripts, can alter the levels of specific PUFAs, or derivatives thereof, found in plant parts and/or plant tissues. The  $\Delta 5$ -desaturase polypeptide coding region is expressed either by itself or with other genes, in order to produce tissues and/or plant parts containing higher proportions of desired PUFAs or in which the PUFA composition more closely resembles that of human breast milk (Prieto et al., PCT publication WO 95/24494). The termination region can be derived from the 3' region of the gene from which the initiation region was obtained or from a different gene. A large number of termination regions are known to and have been found to be satisfactory in a variety of hosts from the same and different genera and species. The termination region usually is selected more as a matter of convenience rather than because of any particular property.

The choice of a host cell is influenced in part by the desired PUFA profile of the transgenic cell, and the native profile of the host cell. As an example, for production of linoleic acid from oleic acid, the DNA sequence used encodes a polypeptide having  $\Delta 12$  desaturase activity, and for production of GLA from linoleic acid, the DNA sequence used encodes a polypeptide having  $\Delta 6$  desaturase activity. Use of a host cell which expresses  $\Delta 12$  desaturase activity and lacks or is depleted in  $\Delta 15$  desaturase activity, can be used with an expression cassette which provides for overexpression of  $\Delta 6$  desaturase alone generally is sufficient to provide for enhanced GLA production in the transgenic cell. Where the host cell expresses  $\Delta 9$  desaturase activity, expression of both a  $\Delta 12$ - and a  $\Delta 6$ -desaturase can provide for enhanced GLA production. In particular instances where expression of  $\Delta 6$  desaturase activity is coupled with expression of  $\Delta 12$  desaturase activity, it is desirable that the host cell naturally have, or be mutated to have, low  $\Delta 15$  desaturase activity. Alternatively, a host cell for  $\Delta 6$  desaturase expression may have, or be mutated to have, high  $\Delta 12$  desaturase activity.

Expression in a host cell can be accomplished in a transient or stable fashion. Transient expression can occur from introduced constructs which contain expression signals functional in the host cell, but which constructs do not replicate and rarely integrate in the host cell, or where the host cell is not proliferating. Transient expression also can be accomplished by inducing the activity of a regulatable promoter operably linked to the gene of interest, although such inducible systems frequently exhibit a low basal level of expression. Stable expression can be achieved by introduction of a construct that can integrate into the host genome or that autonomously replicates in the host cell. Stable expression of the gene of interest can be selected for through the use of a selectable marker located on or transfected with the expression construct, followed by selection for cells expressing the marker. When stable expression results from integration, integration of constructs can occur randomly within the host genome or can be targeted through the use of constructs containing regions of homology with the host genome sufficient to target recombination with the host locus. Where constructs are targeted to an endogenous locus, all or some of the transcriptional and translation regulatory regions can be provided by the endogenous locus.

When increased expression of the desaturase polypeptide in the source plant is desired, several methods can be employed. Additional genes encoding the desaturase polypeptide can be introduced into the host organism. Expression from the native desaturase locus also can be increased through homologous recombination, for example by inserting a stronger promoter into the host genome to cause increased expression, by removing destabilizing sequences from either the mRNA or the encoded protein by deleting that information from the host genome, or by adding stabilizing sequences to the mRNA (see U.S. Pat. No. 4,910,141 and U.S. Pat. No. 5,500,365.)

When it is desirable to express more than one different gene, appropriate regulatory regions and expression methods, introduced genes can be propagated in the host cell through use of replicating vectors or by integration into the host genome. Where two or more genes are expressed from separate replicating vectors, it is desirable that each vector has a different means of replication. Each introduced construct, whether integrated or not, should have a different means of selection and should lack homology to the other constructs to maintain stable expression and prevent reassortment of elements among constructs. Judicious choices of regulatory regions, selection means and method of propagation of the introduced construct can be experimentally determined so that all introduced genes are expressed at the necessary levels to provide for synthesis of the desired products.

Constructs comprising the gene of interest may be introduced into a host cell by standard techniques. These techniques include transfection, infection, bolistic impact, electroporation, microinjection, scraping, or any other method which introduces the gene of interest into the host cell (see U.S. Pat. No. 4,743,548, U.S. Pat. No. 4,795,855, U.S. Pat. No. 5,068,193, U.S. Pat. No. 5,188,958, U.S. Pat. No. 5,463,174, U.S. Pat. No. 5,565,346 and U.S. Pat. No. 5,565,347). For convenience, a host cell which has been manipulated by any method to take up a DNA sequence or construct will be referred to as "transformed" or "recombinant" herein. The subject host will have at least have one copy of the expression construct and may have two or more, depending upon whether the gene is integrated into the genome, amplified, or is present on an extrachromosomal element having multiple copy numbers.

The transformed host cell can be identified by selection for a marker contained on the introduced construct. Alternatively, a separate marker construct may be introduced with the desired construct, as many transformation techniques introduce many DNA molecules into host cells. Typically, transformed hosts are selected for their ability to grow on selective media. Selective media may incorporate an antibiotic or lack a factor necessary for growth of the untransformed host, such as a nutrient or growth factor. An introduced marker gene therefor may confer antibiotic resistance, or encode an essential growth factor or enzyme, and permit growth on selective media when expressed in the transformed host cell. Desirably, resistance to kanamycin and the amino glycoside G418 are of interest (see U.S. Pat. No. 5,034,322). Selection of a transformed host can also occur when the expressed marker protein can be detected, either directly or indirectly. The marker protein may be expressed alone or as a fusion to another protein. The marker protein can be detected by its enzymatic activity; for example  $\beta$  galactosidase can convert the substrate X-gal to a colored product, and luciferase can convert luciferin to a light-emitting product. The marker protein can be detected by its light-producing or modifying characteristics; for example, the green fluorescent protein of *Aequorea victoria*



fluoresces when illuminated with blue light. Antibodies can be used to detect the marker protein or a molecular tag on, for example, a protein of interest. Cells expressing the marker protein or tag can be selected, for example, visually, or by techniques such as FACS or panning using antibodies.

The PUFAs produced using the subject methods and compositions may be found in the host plant tissue and/or plant part as free fatty acids or in conjugated forms such as acylglycerols, phospholipids, sulfolipids or glycolipids, and may be extracted from the host cell through a variety of means well-known in the art. Such means may include extraction with organic solvents, sonication, supercritical fluid extraction using for example carbon dioxide, and physical means such as presses, or combinations thereof. Of particular interest is extraction with hexane or methanol and chloroform. Where desirable, the aqueous layer can be acidified to protonate negatively charged moieties and thereby increase partitioning of desired products into the organic layer. After extraction, the organic solvents can be removed by evaporation under a stream of nitrogen. When isolated in conjugated forms, the products are enzymatically or chemically cleaved to release the free fatty acid or a less complex conjugate of interest, and are then subjected to further manipulations to produce a desired end product. Desirably, conjugated forms of fatty acids are cleaved with potassium hydroxide.

Surprisingly, as demonstrated more fully in the examples below, expression of the *Mortierella*  $\Delta 6$  desaturase leads to the production of stearidonic acid in the oil extracted from seed tissue of host plant cells. Furthermore, expression of the  $\Delta 6$  desaturase with additional desaturases provided for the enhanced production of SDA in the seed oil.

Thus, the present invention provides methods for the production of stearidonic acid (C18:4) in host plant cells. The methods allow for the production of SDA in host plant cells ranging from about 0.3 wt % to at least about 30 wt %, preferably, from about 5 wt % to at least about 25 wt %, more preferably from about 7 wt % to at least about 25 wt %. The SDA is preferably produced in the seed oil of host plants containing one or more expression constructs as described herein.

Furthermore, the present invention provides a novel source of plant oils containing stearidonic acid. The oils are preferably obtained from the plant seed tissue. The seed oils contain amounts of SDA ranging from about 0.3 wt % to at least about 30 wt %, preferably, from about 5 wt % to at least about 25 wt %, more preferably from about 7 wt % to at least about 25 wt %.

#### PURIFICATION OF FATTY ACIDS

If further purification is necessary, standard methods can be employed. Such methods include extraction, treatment with urea, fractional crystallization, HPLC, fractional distillation, silica gel chromatography, high speed centrifugation or distillation, or combinations of these techniques. Protection of reactive groups, such as the acid or alkenyl groups, may be done at any step through known techniques, for example alkylation or iodination. Methods used include methylation of the fatty acids to produce methyl esters. Similarly, protecting groups may be removed at any step. Desirably, purification of fractions containing ARA, DHA and EPA is accomplished by treatment with urea and/or fractional distillation.

#### USES OF FATTY ACIDS

The uses of the fatty acids of subject invention are several. Probes based on the DNAs of the present invention may find use in methods for isolating related molecules or in methods to detect organisms expressing desaturases. When used as probes, the DNAs or oligonucleotides need to be detectable. This is usually accomplished by attaching a label either at an internal site, for example, via incorporation of a modified residue, or at the 5' or 3' terminus. Such labels can be directly detectable, can bind to a secondary molecule that is detectably labeled, or can bind to an unlabelled secondary molecule and a detectably labeled tertiary molecule; this process can be extended as long as is practical to achieve a satisfactorily detectable signal without unacceptable levels of background signal. Secondary, tertiary, or bridging systems can include use of antibodies directed against any other molecule, including labels or other antibodies, or can involve any molecules which bind to each other, for example a biotin-streptavidin/avidin system. Detectable labels typically include radioactive isotopes, molecules which chemically or enzymatically produce or alter light, enzymes which produce detectable reaction products, magnetic molecules, fluorescent molecules or molecules whose fluorescence or light-emitting characteristics change upon binding. Examples of labelling methods can be found in U.S. Pat. No. 5,011,770. Alternatively, the binding of target molecules can be directly detected by measuring the change in heat of solution on binding of probe to target via isothermal titration calorimetry, or by coating the probe or target on a surface and detecting the change in scattering of light from the surface produced by binding of target or probe, respectively, as may be done with the BIAcore system.

The invention will be better understood by reference to the following non-limiting examples.

#### EXAMPLES

##### Example 1

##### Expression of $\omega$ -3 Desaturase from *C. Elegans* in Transgenic Plants

The  $\Delta 15/\omega$ -3 activity of *Brassica napus* can be increased by the expression of an  $\omega$ -3 desaturase from *C. elegans*. The fat-1 cDNA clone (Genbank accession L41807; Spychalla, J. P., Kinney, A. J., and Browse, J. 1997 P.A.A.S. 94, 1142-1147, SEQ ID NO:1 and SEQ ID NO:2) was obtained from John Browse at Washington State University. The fat-1 cDNA was modified by PCR to introduce cloning sites using the following primers:

Fat-1 forward (SEQ ID NO:3):

5'-CUACUACUACUACTGCAGACAATGGTCCGCTCATTCTCAGA-3'

Fat-1 reverse (SEQ ID NO:4):

5'-CAUCAUCAUCAUGCGGCCGCTTACTTGCTTTGCCTT-3'

These primers allowed the amplification of the entire coding region and added PstI and NotI sites to the 5'- and 3'-ends, respectively. The PCR product was subcloned into pAMP1 (GIBCOBRL) using the CloneAmp system (GIBCOBRL) to create pCGN5562. The sequence was verified by sequencing of both strands to be sure no changes were introduced by PCR.

A once base pair difference was observed in the fat-1 coding region from pCGN5562 vs. the fat-1 Genbank sequence. The C at position 705 of the fat-1 sequence was changed to an A in pCGN5562. This creates a change of a



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GAC codon to GAA, changing the Asp residue at position 231 of fat-1 to a Glu residue. This identical change was observed in products of two independent PCR reactions using fat-1 template and most likely is not a result of PCR mis-incorporation of a nucleotide. For seed specific expression, the Fat-1 coding region was cut out of pCGN5562 as a PstI/NotI fragment and inserted between the PstI/NotI sites of the binary vector, pCGN8623, to create pCGN5563. PCGN5563 can be introduced into Brassica napus via Agrobacterium-mediated transformation.

## Construction of pCGN8623

The polylinker region of the napin promoter cassette, pCGN7770, was replaced by ligating the following oligonucleotides:

5'-TCGACCTGCAGGAAGCTTTCGGCCGCGGATCC-3' (SEQ ID NO:5) and  
5'-TCGAGGATCCGCGGCCGCAAGCTTCCTGCAAG-3' (SEQ ID NO:6).

These oligonucleotides were ligated into Sall/XhoI-digested pCGN7770 to produce pCGN8619. These oligos encode BamHII, NotI, HindIII, and PstI restriction sites. pCGN8619 contains the oligos oriented such that the PstI site is closest to the napin 5' regulatory region. A fragment containing the napin 5' regulatory region, polylinker, and napin 3' region was removed from pCGN8619 by digestion with Asp7181. The fragment was blunt-ended by filling in the 5' overhangs with Klenow fragment then ligated into pCGN5139 that had been digested with Asp7181 and HindIII and blunt-ended by filling in the 5' overhangs with Klenow fragment. A plasmid containing the insert oriented so that the napin promoter was closest to the blunted Asp7181 site of pCGN5139 and the napin 3' was closest to the blunted HindIII site was subjected to sequence analysis to confirm both the insert orientation and the integrity of cloning junctions. The resulting plasmid was designated pCGN8623.

To produce high levels of stearidonic acid in Brassica, the *C. elegans*  $\omega$ -3 desaturase can be combined with  $\Delta$ 6- and  $\Delta$ 12-desaturases from *Mortierella alpina*. PCGN5563-transformed plants may be crossed with pCGN5544-transformed plants expressing the  $\Delta$ 6-and  $\Delta$ 12-desaturases, described below.

The resulting F1 seeds can be analyzed for stearidonic acid content and selected F1 plants can be used for self-pollination to produce F2 seed, or as donors for production of dihaploids, or additional crosses.

An alternative method to combine the fat-1 cDNA with *M. alpina*  $\Delta$ 6 and  $\Delta$ 12 desaturases is to combine them on one T-DNA for transformation. The fat-1 coding region from pCGN5562 can be cut out as a PstI/NotI fragment and inserted into PstI/NotI digested pCGN8619. The transcriptional unit consisting of the napin 5' regulatory region, the fat-1 coding region, and the napin 3'-regulatory region can be cut out as a Sse83871 fragment and inserted into

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pCGN5544 cut with Sse83871. The resulting plasmid would contain three napin transcriptional units containing the *C. elegans*  $\omega$ -3 desaturase, *M. alpina*  $\Delta$ 6 desaturase, and *M. alpina*  $\Delta$ 12 desaturase, all oriented in the same direction as the 35S/nptII/tml transcriptional unit used for selection of transformed tissue.

## Example 2

Over-Expression of  $\Delta$ 15-desaturase Activity in Transgenic Canola

The  $\Delta$ 15-desaturase activity of Brassica napus can be increased by over-expression of the  $\Delta$ 15-desaturase cDNA clone.

*A. B. napus*  $\Delta$ 15-desaturase cDNA clone was obtained by PCR amplification of first-strand cDNA derived from *B. napus* cv. 212/86. The primers were based on published sequence: Genbank #L01418 Arondel et al, 1992 Science 258:1353-1355 (SEQ ID NO:7 and SEQ ID NO:8).

The following primers were used:

Bnd15-FORWARD (SEQ ID NO:9)  
5'-CUACUACUACUAGAGCTCAGCGATGGTTGTTGCTATGGAC-3'  
Bnd15-REVERSE (SEQ ID NO:10)  
5'-CAUCAUCAUGAATTCTTAATTGATTTT AGATTTG-3'

These primers allowed the amplification of the entire coding region and added SacI and EcoRI sites to the 5'- and 3'-ends, respectively. The PCR product was subcloned into pAMP1 (GIBCOBRL) using the CloneAmp system (GIBCOBRL) to create pCGN5520. The sequence was verified by sequencing of both strands to be sure that the open reading frame remained intact. For seed specific expression, the  $\Delta$ 15-desaturase coding region was cut out of pCGN5520 as a BamHI/Sall fragment and inserted between the BgIII and XhoI sites of the pCGN7770, to create pCGN5557. The PstI fragment of pCGN5557 containing the napin 5'-regulatory region, *B. napus*  $\Delta$ 15-desaturase, and napin 3'-regulatory region was inserted into the PstI site of the binary vector, pCGN5138 to produce pCGN5558. pCGN5558 was introduced into Brassica napus via Agrobacterium-mediated transformation.

To produce high levels of stearidonic acid in Brassica, the  $\Delta$ 15-desaturase can be combined with  $\Delta$ 6- and  $\Delta$ 12-desaturases from *Mortierella alpina*. PCGN5558-transformed plants may be crossed with pCGN5544-transformed plants expressing the  $\Delta$ 6 and  $\Delta$ 12-desaturases. The resulting F1 seeds are analyzed for stearidonic acid content. GC-FAME analysis of F1 half-seeds revealed a significant accumulation of SDA in the seed oil of the Brassica lines. SDA levels (18:4) of greater than approximately 25% were obtained in hemizygous lines and are provided in Table 1. Selected F1 plants can be used for self-pollination to produce F2 seed, or as donors for production of dihaploids, or additional crosses.

TABLE 1

Strain ID*	16:0	18:0	18:1	18:2	18:2	18:3	18:3	18:3	18:4	20:0	20:1	20:2	22:0	22:1	22:2
					C912		C91215	C6912							
(5558-SP30021-26 X 5544-LP30108-6-16-1-3)	6	1.34	2.97	9.58	9.58	52.79	34.76	18.03	25.21	0.7	0.56	0.16	0.41	0.03	0
(5558-SP30021-26 X 5544-LP30108-6-16-1-3)	4.45	0.86	10.42	9.06	9.06	49.08	25.68	23.4	23.45	0.5	0.84	0.55	0.49	0	0.06
(5558-SP30021-26 X 5544-LP30108-6-16-1-3)	5.8	2.36	12.5	11.13	11.13	47.47	18.86	28.61	17.55	1.01	0.86	0.3	0.85	0	0.07



TABLE 1-continued

Strain ID*	16:0	18:0	18:1	18:2	18:2	18:3	18:3	18:3	18:4	20:0	20:1	20:2	22:0	22:1	22:2
(5558-SP30021-26 X 5544-LP30108-6-16-1-3)	3.65	0.66	14.26	14.97	14.97	50.94	23.3	27.64	13.22	0.43	0.88	0.23	0.48	0.04	0
(5558-SP30021-26 X 5544-LP30108-6-16-1-3)	4.86	2.42	18.74	14.23	14.23	46.22	23	23.22	10.67	0.89	0.92	0.18	0.7	0.02	0
(5558-SP30021-26 X 5544-LP30108-6-16-1-3)	6.57	1.07	16.79	14	14	48.98	32.88	16.09	10.24	0.52	0.94	0.22	0.39	0.02	0.01
(5558-SP30021-26 X 5544-LP30108-6-16-1-3)	5.85	2.09	8.81	19.12	19.12	50.89	12.03	38.86	9.09	1.39	0.78	0.45	1.23	0	0.04
(5558-SP30021-26 X 5544-LP30108-6-16-1-3)	4.69	2.04	17.46	21.1	21.1	43.38	24.28	19.1	8.5	0.73	0.96	0.37	0.56	0	0
(5558-SP30021-26 X 5544-LP30108-6-16-1-3)	5.43	1.69	16.59	22.2	22.2	44.4	16.57	27.83	6.34	0.9	1.03	0.32	0.81	0.03	0.05
(5558-SP30021-26 X 5544-LP30108-6-16-1-3)	4.28	1.34	18.83	27.24	27.24	40.54	20.91	19.63	5.03	0.73	0.88	0.27	0.7	0	0
(5558-SP30021-26 X 5544-LP30108-6-16-1-3)	4.47	1.38	21.43	26.89	26.89	39.04	18.78	20.26	4.06	0.73	0.91	0.41	0.48	0	0
(5558-SP30021-26 X 5544-LP30108-6-16-1-3)	4.77	1.12	18.4	31.1	31.1	38.51	19.62	18.88	3.52	0.64	0.8	0.21	0.7	0	0
(5558-SP30021-26 X 5544-LP30108-6-16-1-3)	4.34	1.86	24.73	35.49	35.49	28.79	10.79	18	1.91	0.67	1.07	0.45	0.48	0	0.02
					C912		C91515- ALA	C6912- GLA							
(5558-SP30021-26 X 5544-LP30108-6-16-1-3)	4.71	1.75	20.72	34.68	34.68	34.01	4.65	29.36	1.62	0.71	0.89	0.1	0.63	0	0
(5558-SP30021-26 X 5544-LP30108-6-16-1-3)	4.3	0.8	40.79	14.34	14.36	37	36.88	0.12	0	0.43	1.47	0.29	0.29	0	0
(5558-SP30021-19 X 5544-LP30108-6-16-1-1)	6.61	1.5	22.09	11.23	11.23	39.9	25.84	14.06	16.25	0.75	0.75	0.27	0.57	0	0
(5558-SP30021-19 X 5544-LP30108-6-16-1-1)	4.64	1.89	22.73	15.12	15.12	44.48	32.21	12.27	8.78	0.73	0.89	0.23	0.43	0	0
(5558-SP30021-19 X 5544-LP30108-6-16-1-1)	5.51	1.45	24.82	17.79	17.79	41.84	27.46	14.38	6.45	0.59	0.82	0.23	0.39	0	0
(5558-SP30021-19 X 5544-LP30108-6-16-1-1)	4.06	1.67	26.39	16.93	16.93	42.64	32.65	9.99	6	0.64	0.96	0.24	0.41	0	0
(5558-SP30021-19 X 5544-LP30108-6-16-1-1)	5.24	1.44	22.2	20.02	20.02	42.76	28.69	14.07	5.98	0.67	0.79	0.26	0.45	0	0
(5558-SP30021-19 X 5544-LP30108-6-16-1-1)	5.34	2.2	22.68	18.6	18.6	43.14	31.45	11.69	5.5	0.82	0.87	0.25	0.53	0	0
(5558-SP30021-19 X 5544-LP30108-6-16-1-1)	3.98	2.9	25.23	21.21	21.21	38.78	24.6	14.18	4.98	1.02	1.04	0.24	0.57	0	0
(5558-SP30021-19 X 5544-LP30108-6-16-1-1)	3.94	1.77	28.92	20.89	20.89	37.02	21.71	15.32	4.96	0.64	1.09	0.3	0.43	0	0
(5558-SP30021-19 X 5544-LP30108-6-16-1-1)	5.12	1.24	27.7	19.02	19.02	40.2	31.05	9.16	4.76	0.48	0.77	0.23	0.35	0	0
(5558-SP30021-19 X 5544-LP30108-6-16-1-1)	4.16	1.52	28.59	21.99	21.99	36.85	23.33	13.53	4.55	0.6	0.98	0.27	0.41	0	0
(5558-SP30021-19 X 5544-LP30108-6-16-1-1)	4.91	1.32	30.46	18.01	18.01	38.59	30.23	8.36	4.34	0.58	0.93	0.25	0.4	0	0
(5558-SP30021-36 X 5544-LP30108-6-16-1-1)	3.66	1.52	29.52	20.52	20.52	36.61	20.09	16.52	5.63	0.67	1.12	0.14	0.52	0	0
(5558-SP30021-36 X 5544-LP30108-6-16-1-1)	5.09	1.81	25.81	21.54	21.54	38.2	22.52	15.68	4.92	0.75	0.96	0.12	0.57	0.02	0
(5558-SP30021-36 X 5544-LP30108-6-16-1-1)	3.77	1.5	29.79	22.36	22.36	35.46	14.84	20.62	4.39	0.74	1.17	0.18	0.59	0.02	0
(5558-SP30021-36 X 5544-LP30108-6-16-1-1)	3.71	1.45	32.18	23.86	23.86	32.32	17	15.32	3.92	0.63	1.12	0.15	0.5	0.02	0
(5558-SP30021-36 X 5544-LP30108-6-16-1-1)	3.55	1.56	33.27	25.21	25.21	30.69	16.63	14.06	3.08	0.68	1.2	0.16	0.54	0.03	0
(5558-SP30021-36 X 5544-LP30108-6-16-1-1)	4.04	1.52	33.63	24.47	24.47	30.72	18.19	12.53	3.07	0.63	1.17	0.14	0.46	0	0
(5558-SP30021-36 X 5544-LP30108-6-16-1-1)	3.67	1.58	31.98	26.13	26.13	30.89	15.92	14.97	3.05	0.69	1.21	0.16	0.51	0	0
(5558-SP30021-36 X 5544-LP30108-6-16-1-1)	3.58	1.8	30.2	27.22	27.22	31.42	15.48	15.94	2.85	0.79	1.21	0.17	0.61	0.02	0.01
(5558-SP30021-36 X 5544-LP30108-6-16-1-1)	4.68	1.41	28.32	28	28	32.22	14.92	17.3	2.74	0.65	1.1	0.18	0.53	0.01	0
(5558-SP30021-36 X 5544-LP30108-6-16-1-1)	3.5	1.46	34.13	25.92	25.92	29.7	16.77	12.93	2.65	0.67	1.26	0.15	0.51	0.01	0
(5558-SP30021-36 X 5544-LP30108-6-16-1-1)	3.9	1.68	33.44	26.18	26.18	29.43	16.11	13.31	2.6	0.72	1.23	0.18	0.5	0.02	0
(5558-SP30021-36 X 5544-LP30108-6-16-1-1)	3.82	1.71	31.84	27.78	27.78	29.49	15.28	14.2	2.59	0.73	1.19	0.16	0.55	0.02	0
(5558-SP30021-36 X 5544-LP30108-6-16-1-1)	3.6	1.78	29.45	28.14	28.14	31.64	12.83	18.81	2.57	0.76	1.21	0.17	0.58	0	0



An alternative method to combine the *B. napus*  $\Delta 15$ -desaturase with *M. alpina*  $\Delta 6$  and  $\Delta 12$  desaturases is to combine them on one T-DNA for transformation. The transcription cassette consisting of the napin 5'-regulatory region, the  $\Delta 15$ -desaturase coding region, and the napin 3'-regulatory region can be cut out of pCGN5557 as a *Swa*I fragment and inserted into *Swa*I-digested pCGN5544. The resulting plasmid, pCGN5561, contains three napin transcriptional units containing the *M. alpina*  $\Delta 6$  desaturase, the *B. napus*  $\Delta 15$ -desaturase, and the *M. alpina*  $\Delta 12$  desaturase, all oriented in the same direction as the 35S/nptII/tml transcriptional unit used for selection of transformed tissue. In

addition, the *C. elegans*  $\omega$ -3 desaturase coding sequence was also cloned into pCGN5544 to create the construct pCGN5565.

Pooled T2 seeds of plants containing 5561 contain significant amounts of SDA (18:4), shown in Table 2. Levels of greater than about 7% SDA are obtained in pooled 5561 segregating seed. Furthermore, significant levels of SDA were obtained from seeds of 5565 Brassica lines, also shown in Table 2. As shown in Table 2, with constructs 5561 and 5565, levels of SDA ranging from about 0.8 wt % to greater than about 7 wt % can be obtained.

TABLE 2

STRAIN ID	16:0		18:0		18:1	18:2	18:2	18:3	18:3	18:4	20:0	20:1	20:2	22:0	22:1	22:2
	16:0	16:1	18:0	18:1	C6,9	C9,12	C6,9,12	C9,12,15								
5561-6	4.46	0.21	3.5	22.85	0	18.33	18.71	21.61	7.79	1.04	0.76	0.19	0.47	0	0	
5561-4	4.14	0.15	2.62	33.07	0	21.07	17.61	14.56	4.39	0.87	0.92	0.14	0.39	0	0.02	
5561-2	4.26	0.15	2.21	30.42	0	22.02	21.06	12.88	4.25	0.89	0.98	0.2	0.51	0	0.02	
5561-8	4.29	0.18	2	33.05	0	22.44	16.23	15.3	3.95	0.84	0.96	0.19	0.43	0	0.04	
5561-6	3.95	0.12	2.04	32.93	0	24.48	17.42	13.33	3.27	0.79	0.94	0.21	0.4	0.03	0.03	
5561-7	4.26	0.17	2.02	38.4	0	23.3	13.35	13.3	2.73	0.75	1.06	0.16	0.41	0	0	
5561-13	4.38	0.18	1.86	58.94	0.65	13.98	7.1	8.26	1.88	0.77	1.27	0.29	0.35	0.03	0	
5561-15	4.29	0.15	2.3	40.96	0	26.63	8.58	12.98	1.51	0.83	1.07	0.19	0.45	0	0.02	
5561-1	4.25	0.15	1.91	47.41	0	24.46	5.56	12.81	1	0.72	1.14	0.15	0.39	0	0	
5561-5	4.07	0.16	1.96	52.29	0	20.88	5.02	12.17	0.97	0.72	1.16	0.21	0.28	0	0.06	
CONTROL	3.89	0.21	1.65	58.48	0	22.44	0	11.03	0	0.6	1.15	0.16	0.27	0.01	0	

STRAIN ID	16:0	18:0	18:1	18:2-C69	18:2-LA	18:3-GLA	18:3-ALA	18:4	20:0
5565-SP30021-7	4.03	1.93	41.24	0.43	14.46	21.39	6.62	7.38	0.68
5565-SP30021-12	3.95	2.46	40.19	0	30.35	7.3	10.92	2.57	0.7
5565-SP30021-9	4.03	1.82	35.76	0	33.49	8.63	11.58	2.54	0.51
5565-SP30021-3	3.86	1.8	32.3	0	35.57	11.3	10.05	2.37	0.61
5565-SP30021-1	3.98	1.92	59.99	1.84	11.24	8.07	7.46	2.32	0.76
5565-SP30021-8	4.67	1.72	38.95	0	30.38	8.99	10.83	2.25	0.52
5565-SP30021-10	4.03	1.43	47.04	0	26.96	5.97	11.1	1.35	0.52
5565-SP30021-6	3.87	1.77	46.73	0	28.79	5.31	10.4	0.79	0.56
CONTROL	3.89	1.65	58.48	0	22.44	0	11.03	0	0.6



Expression of  $\Delta 5$  Desaturase in Plants Expression in Leaves

Ma29 is a putative *M. alpina*  $\Delta 5$  desaturase as determined by sequence homology (SEQ ID NO:11 and SEQ ID NO:12). This experiment was designed to determine whether leaves expressing Ma29 (as determined by Northern) were able to convert exogenously applied DGLA (20:3) to ARA (20:4).

The Ma29 desaturase cDNA was modified by PCR to introduce convenient restriction sites for cloning. The desaturase coding region has been inserted into a d35 cassette under the control of the double 35S promoter for expression in Brassica leaves (pCGN5525) following stan-

were selected for one of three treatments: water, GLA or DGLA, GLA and DGLA were purchased as sodium salts from NuChek Prep and dissolved in water at 1 mg/ml. Aliquots were capped under N<sub>2</sub> and stored at -70 degrees C.

Leaves were treated by applying a 50  $\mu$ l drop to the upper surface and gently spreading with a gloved finger to cover the entire surface. Applications were made approximately 30 minutes before the end of the light cycle to minimize any photo-oxidation of the applied fatty acids. After 6 days of treatment one leaf from each treatment was harvested and cut in half through the mid rib. One half was washed with water to attempt to remove unincorporated fatty acid. Leaf samples were lyophilized overnight, and fatty acid composition determined by gas chromatography (GC). The results are shown in Table 3.

TABLE 3

Fatty Acid Analysis of Leaves from Ma29 Transgenic <i>Brassica</i> Plants											
Treatment	SPL #	16:00 %	16:01 %	18:00 %	18:01 %	18:02 %	18:3g %	18:03 %	18:04 %	20:00 %	20:01 %
Water	33	12.95	0.08	2.63	2.51	16.76	0	45.52	0	0.09	
	34	13.00	0.09	2.67	2.56	16.86	0	44.59	0	0.15	
	35	14.13	0.09	2.37	2.15	16.71	0	49.91	0	0.05	
	36	13.92	0.08	2.32	2.07	16.16	0	50.25	0	0.05	
	37	13.79	0.11	2.10	2.12	15.90	0.08	46.29	0	0.54	
	38	12.80	0.09	1.94	2.08	14.54	0.11	45.61	0	0.49	
GLA	39	12.10	0.09	2.37	2.10	14.85	1.65	43.66	0	0	
	40	12.78	0.10	2.34	2.22	15.29	1.72	47.22	0	0.02	
	41	13.71	0.07	2.68	2.16	15.92	2.12	46.55	0	0	
	42	14.10	0.07	1.75	2.35	16.66	1.56	46.41	0	0.01	
	43	13.62	0.09	2.22	1.94	14.68	2.42	46.69	0	0.01	
	44	14.92	0.09	2.20	2.17	15.22	2.30	46.05	0	0.02	
DGLA	45	12.45	0.14	2.30	2.28	15.65	0.07	44.62	0	0.01	
	46	12.67	0.15	2.69	2.50	15.96	0.09	42.77	0	0.01	
	47	12.56	0.23	3.40	1.98	13.57	0.03	45.52	0	0.01	
	48	13.07	0.24	3.60	2.51	13.54	0.04	45.13	0	0.01	
	49	13.26	0.07	2.81	2.34	16.05	0.04	43.89	0	0	
	50	13.53	0.07	2.84	2.41	16.07	0.02	44.90	0	0.01	

Treatment	SPL #	20:02 %	20:03 %	20:04 %	20:05 %	22:00 %	22:01 %	22:02 %	22:03 %	22:06 %	24:0 %	24:1 %
Water	33	0	0	0.29	0	0.01	0.09	16.26	0	0	0.38	0.18
	34	0.01	0	0	0	0.14	0.10	16.82	0.02	0.05	0.36	0.27
	35	0.01	0	0.25	0	0.12	0.06	11.29	0.04	0.05	0.29	0.25
	36	0	0.01	0.26	0	0.07	0.04	11.82	0.03	0.36	0.28	0.21
	37	0.02	0	0.21	0	0.18	0.08	15.87	0.06	0.20	0.30	0.17
	38	0.01	0	0.24	0	0.15	0.07	13.64	0.09	0.08	5.89	0.23
GLA	39	0.02	0.01	0.27	0	0.10	0.08	16.25	3.42	0.19	0.37	0.17
	40	0.01	0	0.27	0	0.10	0.10	14.74	0.05	0.10	0.36	0.14
	41	0	0	0.27	0	0.20	0.10	13.15	0.13	0.29	0.33	0.20
	42	0	0	0.28	0	0.11	0.11	12.60	0.02	0.24	0.38	0.13
	43	0.01	0	0.28	0	0.10	0.03	14.73	0.01	0.24	0.34	0.14
	44	0.02	0	0.26	0	0.13	0.07	14.43	0.05	0.16	0.33	0.17
DGLA	45	0.06	1.21	0.26	0	0.07	0.07	18.67	0.02	0.21	0.36	0.13
	46	0	1.94	0.27	0	0.11	0.09	17.97	0.09	0.39	0.41	0.11
	47	0.01	0.69	0.96	0	0.11	0.07	17.96	0	0.22	0.49	0.20
	48	0.01	0.70	0.74	0	0.14	0.09	17.14	0.05	0.32	0.52	0.10
	49	0	0.35	1.11	0	0.10	0.07	17.26	0.07	0.23	0.39	0.18
	50	0	0.20	0.87	0	0.21	0.07	15.73	0.04	0.15	0.37	0.18

ard protocols (see U.S. Pat. No. 5,424,200 and U.S. Pat. No. 5,106,739). Transgenic Brassica plants containing pCGN5525 were generated following standard protocols (see U.S. Pat. No. 5,188,958 and U.S. Pat. No. 5,463,174).

In the first experiment, three plants were used: a control, LPO04-1, and two transgenics, 5525-23 and 5525-29. LP004 is a low-linolenic Brassica variety. Leaves of each

Leaves treated with GLA contained from 1.56 to 2.4 wt % GLA. The fatty acid analysis showed that the lipid composition of control and transgenic leaves was essentially the same. Leaves of control plants treated with DGLA contained 1.2–1.9 w % DGLA and background amounts of ARA (0.26–0.27 wt %). Transgenic leaves contained only 0.2–0.7 wt % DGLA, but levels of ARA were increased (0.74–1.1 wt %) indicating that the DGLA was converted to ARA in these leaves.



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## Expression in Seed

The purpose of this experiment was to determine whether a construct with the seed specific napin promoter would enable expression in seed.

The Ma29 cDNA was modified by PCR to introduce XhoI cloning sites upstream and downstream of the start and stop codons, respectively, using the following primers:

Madxho-forward (SEQ ID NO:13):

5'-CUACUACUACUACTCGAGCAAGATGGGA  
ACGGACCAAGG

Madxho-reverse (SEQ ID NO:14):

5'-CAUCAUCAUCAUCTCGAGCTACTCTTCCT  
TGGGACGGAG

The PCR product was subcloned into pAMP1 (GIBCOBRL) using the CloneAmp system (GIBCOBRL) to create pCGN5522 and the  $\Delta 5$  desaturase sequence was verified by sequencing of both strands.

For seed-specific expression, the Ma29 coding region was cut out of pCGN5522 as an XhoI fragment and inserted into the Sall site of the napin expression cassette, pCGN3223, to create pCGN5528. The HindIII fragment of pCGN5528 containing the napin 5' regulatory region, the Ma29 coding region, and the napin 3' regulatory region was inserted into the HindIII site of pCGN1557 to create pCGN5531. Two copies of the napin transcriptional unit were inserted in tandem. This tandem construct can permit higher expression of the desaturases per genetic loci. pCGN5531 was introduced into Brassica napus cv.LP004 via Agrobacterium mediated transformation.

The fatty acid composition of twenty-seed pools of mature T2 seeds was analyzed by GC. Table 2 shows the

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expected products of  $\Delta 5$  desaturation of oleic and linoleic acids. No other differences in fatty acid composition were observed in the transgenic seeds.

## Example 4

## Production of D5-desaturated Fatty Acids in Transgenic Plants

The construction of pCGN5531 ( $\Delta 5$ -desaturase) and fatty acid composition of T2 seed pools is described in Example 3. This example takes the seeds through one more generation and discusses ways to maximize the  $\Delta 5$ -desaturated fatty acids.

Example 3 describes the fatty acid composition of T2 seed pools of pCGN5531-transformed B. napus cv. LP004 plants. To investigate the segregation of  $\Delta 5$ -desaturated fatty acids in the T2 seeds and to identify individual plants to be taken on to subsequent generations, half-seed analysis was done. Seeds were germinated overnight in the dark at 30 degrees on water-soaked filter paper. The outer cotyledon was excised for GC analysis and the rest of the seedling was planted in soil. Results of some of these analyses are shown in the accompanying Table 4.  $\Delta 5,9$ -18:2 accumulated to as high as 12% of the total fatty acids and A 5,9,12-18:3 accumulated to up to 0.77% of the fatty acids. These and other individually selected T2 plants were grown in the greenhouse to produce T3 seed.

TABLE 4

Composition of T2 Pooled Seed														
	16:0 %	16:1 %	18:0 %	18:1 % $\Delta$	$\Delta 5,9$ 18:2 %	18:2 % $\Delta$	$\Delta 5,9,12$ 18:3 %	18:3 %	20:0 %	20:1 %	20:2 %	22:0 %	22:1 %	24:0 %
LP004 control	3.86	0.15	3.05	69.1	0	18.51	0.01	1.65	1.09	1.40	0.03	0.63	0.05	0.42
5531-1	4.26	0.15	3.23	62.33	4.07	21.44	0.33	1.38	0.91	1.04	0.05	0.41	0.03	0.27
5531-2	3.78	0.14	3.37	66.18	4.57	17.31	0.27	1.30	1.03	1.18	0	0.47	0.01	0.30
5531-6	3.78	0.13	3.47	63.61	6.21	17.97	0.38	1.34	1.04	1.14	0.05	0.49	0.02	0.26
5531-10	3.96	0.17	3.28	63.82	5.41	18.58	0.32	1.43	0.98	1.11	0.02	0.50	0	0.31
5531-16	3.91	0.17	3.33	64.31	5.03	18.98	0.33	1.39	0.96	1.11	0	0.44	0	0
5531-28	3.81	0.13	2.58	62.64	5.36	20.95	0.45	1.39	0.83	1.15	0.01	0.36	0.05	0.21

Fatty acid analysis of selected T2 half-seeds from pCGN5531-LP004 events														
CYCLE ID	SPL NO	STRAIN ID	12:0	14:0	16:0	16:1	18:0	18:1	18:2 $\Delta 5,9$	18:2 $\Delta 9,12$	18:3 $\Delta 5,9,12$	18:3 $\Delta 9,12,15$		
97XX1539	93	5531-LP004-6	0.03	0.07	3.92	0.17	3.5	61.32	12.22	15.36	0.77	1.36		
97XX1539	29	5531-LP004-6	0.01	0.04	3.6	0.09	3.23	63.77	10.63	14.47	0	1.22		
97XX1539	38	5531-LP004-6	0.01	0.05	3.71	0.09	3.02	65.13	10.57	13.98	0	1.06		
97XX1539	41	5531-LP004-6	0.01	0.05	3.64	0.07	3.22	62.51	9.7	16.63	0	1.28		
97XX1539	18	5531-LP004-6	0.02	0.06	3.69	0.09	3.36	63.79	9.63	15.29	0.63	1.15		
97XX1539	85	5531-LP004-6	0.01	0.06	3.6	0.09	3.54	64.81	9.54	13.69	0.6	1.26		
98GC0023	98	5531-LP004-23	0.01	0.05	3.5	0.09	3.12	64.97	9.92	13.62	0.55	1.25		
98GC0023	32	5531-LP004-23	0.01	0.05	3.43	0.08	2.62	65.21	9.83	14.28	0.59	1.15		
98GC0023	78	5531-LP004-23	0.01	0.05	3.45	0.07	2.78	64.97	9.34	14.69	0.58	1.17		
98GC0023	86	5531-LP004-23	0.01	0.05	3.32	0.08	2.7	64.18	9.08	15.99	0.68	1.18		
98GC0023	67	5531-LP004-23	0.01	0.04	3.49	0.08	3.03	64.14	8.78	15.95	0.62	1.08		
98GC0023	52	5531-LP004-23	0.01	0.03	3.38	0.07	2.56	67.44	8.65	13.55	0.5	1.02		

results obtained with independent transformed lines as compared to non-transformed LP004 seed. The transgenic seeds containing pCGN5531 contain two fatty acids that are not present in the control seeds, identified as taxoleic acid (5,9-18:2) and pinolenic acid (5,9,12-18:3), based on their elution relative to oleic and linoleic acid. These would be the

To maximize the accumulation of  $\Delta 5,9$  18:2 in seed oil, the pCGN5531 construct could be introduced into a high oleic acid variety of canola. A high-oleic variety could be obtained by mutation, so-suppression, or antisense suppression of the  $\Delta 12$  and  $\Delta 15$  desaturases or other necessary co-factors.



To maximize accumulation of  $\Delta 5,9,12$  18:3 in canola, the pCGN5531 construct could be introduced into a high linoleic strain of canola. This could be achieved by crossing pCGN5531-transformed plants with pCGN5542-(*M. alpina*  $\Delta 12$ -desaturase) transformed plants. Alternatively, the  $\Delta 5$  and  $\Delta 12$  desaturases could be combined on one T-DNA for transformation. The transcriptional unit consisting of the napin 5' regulatory region, the *M. alpina*  $\Delta 12$ -desaturase coding region, and the napin 3'-regulatory region can be cut out of pCGN5541 as a NotI fragment. NotI/XbaI linkers could be ligated and the resulting fragment inserted into the XbaI site of pCGN5531. The resulting plasmid would contain three napin transcriptional units containing the *M. alpina*  $\Delta 12$  desaturase, and two copies of the napin/*M. alpina*  $\Delta 5$  desaturase/napin unit, all oriented in the same direction as the 35S/nptII/tml transcriptional unit used for selection of transformed tissue.

#### Example 5

##### Expression of *M. Alpina* $\Delta 6$ Desaturase in Brassica Napus

A nucleic acid sequence from a partial cDNA clone, Ma524, encoding a  $\Delta 6$  fatty acid desaturase from *Mortierella alpina* was obtained by random sequencing of clones from the *M. alpina* cDNA library. The Ma524 cDNA was modified by PCR to introduce cloning sites using the following primers:

Ma524PCR-1 (SEQ ID NO:15)

5'-CUACUACUACUATCTAGACTCGAGACCA  
TGGCTGCTGCT CCAGTGTG

Ma524PCR-2 (SEQ ID NO:16)

5'-CAUCAUCAUCAUAGGCCTCGAGTTACTG  
CGCCTTACCCAT

These primers allowed the amplification of the entire coding region and added XbaI and XhoI sites to the 5'-end and XhoI and StuI sites to the 3' end. The PCR product was subcloned into pAMP1 (GIBCOBRL) using the CloneAmp system (GIBCOBRL) to create pCGN5535 and the  $\Delta 6$  desaturase sequence was verified by sequencing of both strands.

##### Construction of pCGN5544

Plant expression constructs were prepared to express the *Mortierella alpina*  $\Delta 6$  desaturase and the *Mortierella alpina*  $\Delta 12$  desaturase in a plant host cell. The constructs prepared utilized transcriptional initiation regions derived from genes preferentially expressed in a plant seed. Isolation of the cDNA sequences encoding the *M. alpina*  $\Delta 6$  desaturase (SEQ ID NO:17 and SEQ ID NO:18) and *M. alpina*  $\Delta 12$  desaturase (SEQ ID NO:19 and SEQ ID NO:20) are described in PCT Publications WO 98/46763 and WO 98/46764, the entireties of which are incorporated herein by reference.

For seed-specific expression, the Ma524 coding region was cut out of pCGN5535 as an XhoI fragment and inserted

into the Sall site of the napin expression cassette, pCGN3223, to create pCGN5536. The NotI fragment of pCGN5536 containing the napin 5' regulatory region, the Ma524 coding region, and the napin 3' regulatory region was inserted into the NotI site of pCGN1557 to create pCGN5538.

The 5542 cDNA, encoding the *M. alpina*  $\Delta 12$  desaturase, was modified by PCR to introduce cloning sites using the following primers:

Ma648PCR-for (SEQ ID NO:21)

5'-CUACUACUACUAGGATCCATGGCACCT  
CCCAACACT

Ma648PCR-for (SEQ ID NO:22)

5'-CAUCAUCAUCAUGGTACCTCGAGTTA  
CTTCTTGAAAAAGAC

These primers allowed the amplification of the entire coding region and added a BamHI site to the 5' end and KpnI and XhoI sites to the 3' end. The PCR product was subcloned into pAMP1 (Gibco-BRL, Gaithersburg, Md.) using the CloneAmp system (Gibco-BRL) to create pCGN5540, and the  $\Delta 12$  desaturase sequence was verified by sequencing of both strands.

A seed preferential expression construct was prepared for the  $\Delta 12$  desaturase cDNA sequence. The Ma648 coding region was cut out of pCGN5540 as a BamHI/XhoI fragment and inserted between the BglIII and XhoI sites of the napin expression cassette, pCGN3223 (described in U.S. Pat. No. 5,639,790), to create pCGN5542.

In order to express the *M. alpina*  $\Delta 6$  and  $\Delta 12$  desaturase sequences from the same T-DNA, the following construct for seed-preferential expression was prepared.

The NotI fragment of pCGN5536 containing the napin 5' transcriptional initiation region, the Ma524 coding region, and the napin 3' transcriptional termination region was inserted into the NotI site of pCGN5542 to create pCGN5544. The expression cassettes were oriented in such a way that the direction of transcription from Ma524 and Ma648 and the nptII marker is the same.

For seed-specific expression, the Ma524 coding region was cut out of pCGN5535 as an XhoI fragment and inserted into the Sall site of the napin expression cassette, pCGN3223, to create pCGN5536. The NotI fragment of pCGN5536 containing the napin 5 regulatory region, the Ma524 coding region, and the napin 3' regulatory region was inserted into the NotI site of pCGN1557 to create pCGN5538. pCGN5538 was introduced into Brassica napus cv.LP004 via Agrobacterium mediated transformation.

Maturing T2 seeds were collected from 6 independent transformation events in the greenhouse. The fatty acid compositions of single seeds was analyzed by GC. Table 5 shows the results of control LP004 seeds and six 5538 lines. All of the 5538 lines except #8 produced seeds containing GLA. Presence of GLA segregated in these seeds as is expected for the T2 selfed seed population. In addition to GLA, the *M. alpina*  $\Delta 6$  desaturase is capable of producing 18:4 (stearidonic) and another fatty acid:  $\Delta 6,9$ -18:2.

TABLE 5

Fatty Acid Analysis of Seeds from Ma524 Transgenic <i>Brassica</i> Plants															
SPL #	16:00 %	16:01 %	18:0 %	18:1 %	6,9 18:2 %	18:2 %	18:3gs %	18:3 %	18:4 %	20:1 %	22:0 %	22:1 %	24:0 %	24:1 %	
LPOO4-1	4.33	0.21	3.78	72.49	0	13.97	0	1.7	0	1.34	0.71	0.02	0.58	0.27	
-2	4.01	0.16	3.09	73.59	0	14.36	0.01	1.4	0	1.43	0.66	0.02	0.5	0.2	



TABLE 5-continued

Fatty Acid Analysis of Seeds from Ma524 Transgenic <i>Brassica</i> Plants															
SPL #	16:00 %	16:01 %	18:0 %	18:1 %	6,9 18:2 %	18:2 %	18:3gs %	18:3 %	18:4 %	20:1 %	22:0 %	22:1 %	24:0 %	24:1 %	
-3	4.12	0.19	3.56	70.25	0	17.28	0	1.57	0	1.28	0.5	0.02	0.39	0.2	
-4	4.22	0.2	2.7	70.25	0	17.86	0	1.61	0	1.31	0.53	0.02	0.4	0.24	
-5	4.02	0.16	3.41	72.91	0	14.45	0.01	1.45	0	1.37	0.7	0.02	0.51	0.26	
-6	4.22	0.18	3.23	71.47	0	15.92	0.01	1.52	0	1.32	0.69	0.02	0.51	0.27	
-7	4.1	0.16	3.47	72.06	0	15.23	0	1.52	0	1.32	0.63	0.03	0.49	0.23	
-9	4.01	0.17	3.71	72.98	0	13.97	0.01	1.41	0	1.45	0.74	0.03	0.58	0.23	
-10	4.04	0.16	3.57	70.03	0	17.46	0	1.5	0	1.33	0.61	0.03	0.36	0.24	
5538-1-1	4.61	0.2	3.48	68.12	1.37	10.68	7.48	1.04	0.33	1.19	0.49	0.02	0.33	0.13	
-2	4.61	0.22	3.46	68.84	1.36	10.28	7.04	1.01	0.31	1.15	0.48	0.02	0.39	0	
-3	4.78	0.24	3.24	65.86	0	21.36	0	1.49	0	1.08	0.46	0.02	0.38	0.22	
-4	4.84	0.3	3.89	67.64	1.67	9.9	6.97	1.02	0.36	1.14	0.53	0.02	0.5	0.18	
-5	4.64	0.2	3.58	64.5	3.61	8.85	10.14	0.95	0.48	1.19	0.47	0.01	0.33	0.12	
-6	4.91	0.27	3.44	66.51	1.48	11.14	7.74	1.15	0.33	1.08	0.49	0.02	0.34	0.13	
-7	4.87	0.22	3.24	65.78	1.27	11.92	8.38	1.2	0	1.12	0.47	0.02	0.37	0.16	
-8	4.59	0.22	3.4	70.77	0	16.71	0	1.35	0	1.14	0.48	0.02	0.39	0.15	
-9	4.63	0.23	3.51	69.66	2.01	8.77	7.24	0.97	0	1.18	0.52	0.02	0.3	0.11	
-10	4.56	0.19	3.55	70.68	0	16.89	0	1.37	0	1.22	0.54	0.02	0.22	0.03	
5538-3-1	4.74	0.21	3.43	67.52	1.29	10.91	7.77	1.03	0.28	1.11	0.5	0.02	0.35	0.14	
-2	4.72	0.21	3.24	67.42	1.63	10.37	8.4	0.99	0	1.12	0.49	0.02	0.36	0.15	
-3	4.24	0.21	3.52	71.31	0	16.53	0	1.33	0	1.12	0.45	0.02	0.4	0.14	
-4	4.64	0.21	3.45	67.92	1.65	9.91	7.97	0.91	0.33	1.14	0.47	0.02	0.37	0.14	
-5	4.91	0.25	3.31	67.19	0	19.92	0.01	1.39	0	1.05	0.48	0.02	0.37	0.14	
-6	4.67	0.21	3.25	67.07	1.23	11.32	8.35	0.99	0	1.16	0.47	0.02	0.33	0.16	
-7	4.53	0.19	2.94	64.8	4.94	8.45	9.95	0.93	0.44	1.13	0.37	0.01	0.27	0.12	
-8	4.66	0.22	3.68	67.33	0.71	12	6.99	1.1	0.24	1.18	0.48	0.03	0.36	0.17	
-9	4.65	0.24	3.11	67.42	0.64	12.71	6.93	1.16	0.25	1.08	0.45	0.02	0.32	0.17	
-10	4.88	0.27	3.33	65.75	0.86	12.89	7.7	1.1	0.24	1.08	0.46	0.01	0.34	0.16	
5538-4-1	4.65	0.24	3.8	62.41	0	24.68	0	1.6	0.01	0.99	0.45	0.02	0.33	0.13	
-2	5.37	0.31	3	57.98	0.38	18.04	10.5	1.41	0	0.99	0.48	0.02	0.3	0.19	
-3	4.61	0.22	3.07	63.62	0.3	16.46	7.67	1.2	0	1.18	0.45	0.02	0.29	0.14	
-4	4.39	0.19	2.93	65.97	0	22.36	0	1.45	0	1.17	0.41	0.03	0.32	0.15	
-5	5.22	0.29	3.85	62.1	2.35	10.25	11.39	0.93	0.41	1.04	0.6	0.02	0.47	0.17	
-6	4.66	0.18	2.85	66.79	0.5	13.03	7.66	0.97	0.22	1.28	0.42	0.02	0.31	0.14	
-7	4.85	0.26	3.03	57.43	0.26	28.04	0.01	2.59	0.01	1.13	0.56	0.02	0.4	0.23	
-8	5.43	0.28	2.94	54.8	1.84	13.79	15.67	1.36	0.53	1.1	0.55	0.02	0.35	0.19	
-9	4.88	0.24	3.32	62.3	0.58	14.86	9.04	1.34	0.29	1.13	0.52	0.02	0.37	0.19	
-10	4.53	0.2	2.73	64.2	0.07	24.15	0	1.52	0	1.09	0.39	0.02	0.27	0.17	
5538-5-1	4.5	0.15	3.35	66.71	0.88	11.7	8.38	1.04	0.3	1.24	0.49	0.02	0.29	0.17	
-2	4.77	0.23	3.06	62.67	0.68	15.2	8.8	1.31	0.28	1.15	0.46	0.02	0.3	0.19	
-3	4.59	0.22	3.61	64.33	2.29	9.95	10.57	1.01	0.45	1.21	0.48	0.02	0.26	0.16	
-4	4.86	0.26	3.4	67.69	0.65	12.24	6.61	1.09	0.23	1.07	0.45	0.02	0.32	0.15	
-5	4.49	0.21	3.3	69.25	0.04	16.51	2.18	1.2	0	1.11	0.44	0.02	0.33	0.16	
-6	4.5	0.21	3.47	70.48	0.08	14.9	2.19	1.22	0	1.13	0.49	0.02	0.33	0.16	
-7	4.39	0.21	3.44	67.59	2.38	9.24	8.98	0.89	0	1.18	0.44	0.02	0.28	0.14	
-8	4.52	0.22	3.17	68.33	0.01	18.91	0.73	1.32	0.01	1.08	0.45	0.02	0.29	0.17	
-9	4.68	0.2	3.05	64.03	1.93	11.03	11.41	1.02	0.01	1.15	0.39	0.02	0.21	0.15	
-10	4.57	0.2	3.1	67.21	0.61	12.62	7.68	1.07	0.25	1.14	0.43	0.02	0.25	0.15	
5538-8-1	4.95	0.26	3.14	64.04	0	23.38	0	1.54	0	0.99	0.42	0.02	0.38	0.17	
-2	4.91	0.26	3.71	62.33	0	23.97	0	1.77	0	0.95	0.53	0.02	0.42	0.19	
-3	4.73	0.25	4.04	63.83	0	22.36	0.01	1.73	0	1.05	0.55	0.02	0.45	0.16	
-4	5.1	0.35	3.8	60.45	0	24.45	0.01	2.13	0	1.07	0.65	0.03	0.53	0.24	
-5	4.98	0.3	3.91	62.48	0	23.44	0	1.77	0	1.01	0.51	0.01	0.43	0.21	
-6	4.62	0.21	3.99	66.14	0	20.38	0	1.48	0	1.15	0.53	0.02	0.48	0.19	
-7	4.64	0.22	3.55	64.6	0	22.65	0	1.38	0	1.09	0.45	0.02	0.41	0.19	
-8	5.65	0.38	3.18	56.6	0	30.83	0.02	0.02	0	0.98	0.55	0.03	0.39	0.26	
-9	8.53	0.63	6.9	51.76	0	26.01	0	0.01	0	1.41	1.21	0.07	0.96	0.33	
-10	5.52	0.4	3.97	57.92	0	28.95	0	0.02	0	0.95	0.52	0.02	0.41	0.16	
5538-10-1	4.44	0.19	3.5	68.42	0	19.51	0	1.32	0	1.14	0.45	0.02	0.31	0.16	
-2	4.57	0.21	3.07	66.08	0	21.99	0.01	1.36	0	1.12	0.41	0.02	0.31	0.16	
-3	4.63	0.21	3.48	67.43	0	20.27	0.01	1.32	0	1.12	0.46	0.02	0.21	0.08	
4	4.69	0.19	3.22	64.62	0	23.16	0	1.35	0	1.08	0.46	0.02	0.33	0.2	
-5	4.58	0.2	3.4	68.75	0	20.17	0.01	0.02	0	1.1	0.45	0.02	0.34	0.17	
-8	4.55	0.21	0	73.55	0.05	14.91	2.76	1.21	0.07	1.24	0.51	0.02	0.19	0	
-9	4.58	0.21	3.28	66.19	0	21.55	0	1.36	0	1.12	0.43	0.02	0.33	0.16	
-10	4.52	0.2	3.4	68.37	0	19.33	0.01	1.3	0	1.13	0.46	0.02	0.35	0.18	

Cross were made between transgenic Brassica 5544 lines producing GLA and standard non-transformed canola varieties. Crosses between 5544 lines with Quantum, Eagle, and Ebony were conducted.

F1 half seeds were analyzed for SDA content and selected plants were grown and allowed to self-pollinate to produce F2 seeds. GC-FAME analysis of both single seed and half-

seed samples from such crosses revealed accumulation of significant levels of SDA (Table 6). Half-seed analysis of 5544-LP108-6-16 with canola variety Eagle yielded a level of approximately 6.3% SDA. Analysis of F2 seed from a cross of 5544-LP108-12-1 with the canola variety Ebony produced levels of SDA as high as about 7.4% SDA.



TABLE 6

STRAIN ID	16:0	16:1	18:0	18:1	18:2	18:2	18:3	18:3	18:4	20:0	20:1	20:2	22:0
					C69	C912	C6912	C91215					
(SP30035-46 x 5544-LP30108-6-16)	6.34	0.84	1.9	4.7	0	14.81	56.73	3.78	6.29	2.12	0.66	0.59	1.04
(SP30035-46 x 5544-LP30108-6-16)	10.18	1.43	4.23	4.34	0	15.96	48.78	3.79	5.51	2.65	0.72	0.77	1.32
(SP30035-46 x 5544-LP30108-6-16)	4.81	0.45	2.53	12.2	0	21.61	46.74	4.83	4.11	0.98	0.79	0.4	0.43
(SP30035-46 x 5544-LP30108-6-16)	4.74	0.48	3.33	16.06	0	20.68	43.02	4.82	3.73	1.25	0.7	0.33	0.75
(SP30035-46 x 5544-LP30108-6-16)	6.02	0.53	1.25	17.29	0	27.34	33.97	7.52	3.41	0.85	0.77	0.27	0.59
(SP30035-46 x 5544-LP30108-6-16)	3.68	0.13	1.99	19.75	0.09	22.75	39.98	5.76	3.41	0.8	0.87	0.27	0.44
(SP30052-7 x 5544-LP30108-12-1)	8.92	0.96	1.64	14.61	0	18.69	36.98	7.44	7.43	1.01	0.49	0.49	0.95
(SP30052-7 x 5544-LP30108-12-1)	9.02	0.89	1.88	10.69	0	16.73	43.39	6.8	6.76	1.05	0.57	0.75	1.07
(SP30052-7 x 5544-LP30108-12-1)	7.76	0.59	1.86	8.15	0	16.04	52.24	4.65	5.3	1.04	0.59	0.69	0.83
(SP30052-7 x 5544-LP30108-12-1)	9.21	0.87	2.23	17.44	0	18.77	36.87	6.79	5.05	0.84	0.64	0.31	0.71
(SP30052-7 x 5544-LP30108-12-1)	5.76	0.31	1.6	20.38	0	24.36	29.94	10.9	4.41	0.69	0.78	0.23	0.48
(SP30052-7 x 5544-LP30108-12-1)	4.03	0.22	1.3	16.87	0	19.3	46.67	5.33	4.17	0.53	0.75	0.35	0.37
(SP30052-7 x 5544-LP30108-12-1)	4.66	0.29	4.47	18.09	0.05	19.07	41.92	5.06	3.47	1.13	0.73	0.35	0.57
(SP30052-7 x 5544-LP30108-12-1)	4.91	0.26	3.13	18.16	0	18.53	43.99	4.64	3.43	1.01	0.79	0.37	0.66

## Example 6

Production of  $\Delta$ 6,9 18:2 in Canola Oil

Example 5 described construction of pCGN5538 designed to express the *M. alpina*  $\Delta$ 6 desaturase in seeds of transgenic canola. Table 4 in that example showed examples of single seed analyses from 6 independent transgenic events. Significant amounts of GLA were produced, in addition to the  $\Delta$ -6,9 18:2 fatty acid.

A total of 29 independent pCGN5538-transformed transgenic plants of the low-linolenic LP004 cultivar were regenerated and grown in the greenhouse. Table 7 shows the fatty acid composition of 20-seed pools of T2 seed from each event. Seven of the lines contained more than 2% of the  $\Delta$ -6,9 18:2 in the seed pools. To identify and select plants with high amounts of  $\Delta$ -6,9 18:2 to be taken on to subsequent generations, half-seed analysis was done. Seeds were germinated overnight in the dark at 30 degrees on water-

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soaked filter paper. The outer cotyledon was excised for GC analysis and the rest of the seedling was planted in soil. Based on results of fatty acid analysis, selected T2 plants were grown in the greenhouse to produce T3 seed. The selection cycle was repeated; pools of T3 seed were analyzed for  $\Delta$ -6,9 18:2, T3 half-seeds were dissected and analyzed, and selected T3 plants were grown in the greenhouse to produce T4 seed. Pools of T4 seed were analyzed for fatty acid composition. Table 6 summarizes the results of this process for lines derived from one of the original transgenic events, 5538-LP004-25. Levels of  $\Delta$ -6,9 18:2 have thus been maintained through 3 generations.

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To maximize the amount of  $\Delta$ -6,9 18:2 that could be produced, the pCGN5538 construct could be introduced into a high oleic acid variety of canola either by transformation or crossing. A high-oleic variety could be obtained by mutation, co-suppression, or antisense suppression of the  $\Delta$ 12 and  $\Delta$ 15 desaturases or other necessary co-factors.

TABLE 7

Fatty Acid Composition of 20-seed Pools of pCON5538 T2 Seeds																	
SPL #	5538-LP004 event	12:0 %	14:0 %	16:0 %	16:1 %	18:0 %	18:1 %	$\Delta$ 6,9	$\Delta$ 6,9,12	$\Delta$ 9,12,15	18:4 %	20:0 %	20:1 %	20:2 %	22:0 %	22:2 %	
								18:2 %	18:3 %	18:3 %							
	31	0.02	0.06	4.07	0.07	0	59.4	5.4	10.07	15.93	1.2	0.6	0.98	1.16	0.0	0.44	0.03
	29	0.01	0.05	3.81	0.14	0	60.7	4.53	10.9	14.77	1.03	0.55	1.09	1.26	0.0	0.46	0.02
	19	0.02	0.07	4.27	0.13	0	62.9	4.17	10.03	13.14	1.02	0.59	1.18	1.25	0.0	0.53	0.02
	14	0	0	5.29	0.24	3.8	49.1	1.02	23.44	11.21	2.26	0.34	1.45	0.93	0.0	0.76	0
	22	0.02	0.05	3.87	0.09	0	64.1	2.59	12.57	11.18	1.27	0.6	1.18	1.08	0.1	0.56	0
	9	0.01	0.06	4.57	0.16	0	62.9	3.4	12.05	11.15	1.27	0.6	1.28	1.18	0.0	0.56	0.03
	25	0.01	0.06	4.17	0.14	0	62.4	2.49	14.42	11.03	1.2	0.46	1.18	1.15	0.0	0.53	0.01
	15	0.01	0.05	3.94	0.11	0	65.2	2.08	12.77	10.9	1.04	0.43	1.1	1.24	0.0	0.48	0.01
	18	0	0.06	5.34	0.29	0	58.4	1.42	18.19	10.53	1.8	0.49	1.2	1	0.0	0.58	0.02
	20	0.01	0.04	3.95	0.1	0	65.6	1.31	13.83	10.22	1.09	0.39	1.06	1.3	0.0	0.46	0.01
	7	0.02	0.07	4.04	0.11	0	62.1	0.92	18.12	8.72	1.77	0.35	1.26	1.19	0.0	0.58	0



TABLE 7-continued

11	0.01	0.06	4.23	0.17	0	62.9	1.6	17.19	8.58	1.48	0.38	1.16	1.03	0.0	0.49	0.01
27	0.02	0	3.99	0.15	0	65.3	0.64	17.85	7.89	1.36	0.31	1.08	1.21	0.0	0	0
2	0.01	0.05	4.02	0.14	0	66.4	1.2	15.74	7.58	1.22	0.32	10.6	1.19	0.0	0.45	0
28	0.01	0.04	3.77	0.11	0	67.5	0.79	15.56	7.58	1.12	0.28	0.97	1.23	0.0	0.44	0
3	0.01	0.05	3.96	0.13	0	68.5	1.81	13.23	7.44	1.1	0.35	1.12	1.21	0.0	0.46	0.01
21	0.01	0.05	3.74	0.1	0	66.9	1.16	15.9	6.99	1.35	0.28	1.15	1.27	0.0	0.52	0
5	0.01	0.04	3.81	0.12	0	69.1	0.74	14.58	6.95	1.14	0.28	1.06	1.18	0.0	0.45	0
6	0	0	2.84	0	3.06	62.5	1.55	18.44	6.94	1.21	0.39	1.04	1.33	0.1	0	0
4	0.01	0.05	3.88	0.11	0	66.9	0.64	16.21	6.89	1.52	0.31	1.09	1.21	0.0	0.5	0
30	0.01	0.04	3.89	0.12	0	68.6	0.72	15.58	6.47	1.17	0.23	1.03	1.07	0.0	0.46	0
16	0.02	0.05	3.75	0.13	0	70.4	0.91	13.56	6.39	1.13	0.28	1.04	1.2	0.0	0.44	0.01
26	0.01	0	3.77	0.12	0	67.6	0	21.08	3.61	1.37	0.13	0.96	1.16	0.0	0	0
23	0	0	4.92	0.22	0	65.2	0	22.23	3	1.79	0.11	1.28	1.11	0.0	0	0
24	0.01	0	3.84	0.13	0	68.4	0.36	21	2.09	1.53	0.08	1.06	1.27	0.0	0	0
10	0.01	0	3.74	0.11	0	70.4	0	20.82	0.65	1.3	0.03	1.05	1.18	0.0	0.46	0
12	0.01	0	3.83	0.12	0	69.9	0	21.61	0.34	1.34	0	1.06	1.12	0.0	0.47	0
17	0.01	0	0	0.13	0	72.6	0	23.03	0.24	1.51	0	1.18	1.21	0.0	0	0
8	0.01	0	4.54	0.2	0	64.9	0	25.65	0.22	1.94	0	1.38	1.01	0.0	0	0
13	0.01	0	3.99	0.16	0	65.8	0	25.9	0	1.58	0	1.17	1.16	0.0	0	0
LP004 control	0.01	0.04	3.46	0.09	0	69.9	0	21.95	0	1.37	0.01	0.9	1.25	0.0	0.42	0

STRAIN IDA	T2 Pool Δ6,9 18:2	GLA	T3 Pool Δ6,9 18:2	GLA	T3 selection Δ6,9 18:2	GLA	T4 Pool Δ6,9 18:2	Δ6,9,12 18:3
5538-LP004-25	2.49	11.03						
5538-LP004-25-3			9.1	11.92				
5538-LP004-25-3-31					13.61	7.82	11.02	9.41
5538-LP004-25-3-30					6.51	7.93	10.27	8.7
5538-LP004-25-3-29					13.35	11.23	9.42	10.5
5538-LP004-25-3-28					9.92	24.1	9.37	10.19
5538-LP004-25-3-25					5.3	30.34	7.95	11.34
5538-LP004-25-2			3.87	11.08				
5538-LP004-25-2-29					13.63	7.41	9.6	11.07
5538-LP004-25-2-	27				5.02	22.04	6.95	9.61
5538-LP004-25-2-26					1.21	26.84	4.31	7.45
5538-LP004-25-2-25					5.83	34.16	8.77	11.58
5538-LP004-25-13			10.53	11.19				
5538-LP004-25-13-27					14.65	11.46	7.86	10.49
5538-LP004-25-13-26					11.18	13.04	9.33	10.01
5538-LP004-25-13-25					4.18	36.78	7.2	12.22
5538-LP004-25-1			3.05	11.16				
5538-LP004-25-1-41					0	0	0.01	0.04
5538-LP004-25-1-28					3.43	19.98	4.63	6.53
5538-LP004-25-1-27					5.52	20.13	8.35	11.21
5538-LP004-25-1-26					0.1	25.16	5.52	8.59
5538-LP004-25-1-25					6.5	31.83	9.85	10.88

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

## SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 22

<210> SEQ ID NO 1

<211> LENGTH: 1391

<212> TYPE: DNA

<213> ORGANISM: *Caenorhabditis elegans*

<400> SEQUENCE: 1

caagtttgag gtatggtcgc tcattcctca gaagggttat ccgccacggc tccggtcacc 60

ggcggagatg ttctggttga tgctcgtgca tctcttgaag aaaaggaggc tccacgtgat 120

gtgaatgcaa aactaaaca ggccaccact gaagagccac gcatccaatt accaactgtg 180



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gatgctttcc gtcgtgcaat tccagcacac tgtttcgaaa gagatctcgt taaatcaatc 240
agatatttgg tgcaagactt tgcggcactc acaattctct actttgctct tccagctttt 300
gagtactttg gattgtttgg ttacttgggt tggaaacattt ttatgggagt ttttggatc 360
gcgttgttcg tcggttgaca cgattgtctt catggatcat tctctgataa tcagaatctc 420
aatgatttca ttggacatat cgccttctca ccaactctct ctccatactt cccatggcag 480
aaaagtcaca agcttcacca tgctttcacc aaccacattg acaaagatca tggacacgtg 540
tggattcagg ataaggattg ggaagcaatg ccatcatgga aaagatggtt caatccaatt 600
ccattctctg gatggcttaa atggttccca gtgtacactt tattcggttt ctgtgatgga 660
tctcacttct ggccatactc ttcacttttt gttcgttaact ctgaccgtgt tcaatgtgta 720
atctctggaa tctgttctg tgtgtgtgca tatattgctc taacaattgc tggatcatat 780
tccaattggt tctggtacta ttgggttcca ctttctttct tcggattgat gctcgtcatt 840
gttacctatt tgcaacatgt cgatgatgct gctgaggtgt acgaggctga tgaatggagc 900
ttcgtccgtg gacaaacca aaccatcgat cgttactatg gactcggatt ggacacaacg 960
atgcaccata tcacagacgg acacgttgc catcacttct tcaacaaaat cccacattac 1020
catctcatcg aagcaaccga aggtgtcaaa aaggcttgg agccgttctc cgacacccaa 1080
tacgggtaca aatctcaagt gaactacgat ttctttgccc gtttctctgt gttcaactac 1140
aagctcgact atctcgttca caagaccgcc ggaatcatgc aattccgaac aactctcgag 1200
gagaaggcaa aggccaagta aaagaatata ccgtgccgtt ctagagtaca acaacaactt 1260
ctcgcgtttc accggttttg ctctaattgc aatttttctt tgttctatat atattttttt 1320
gctttttaat tttattctct ctaaaaaact tctacttttc agtgcgttga atgcataaag 1380
ccataactct t 1391

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<210> SEQ ID NO 2
<211> LENGTH: 402
<212> TYPE: PRT
<213> ORGANISM: Caenorhabditis elegans

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<400> SEQUENCE: 2

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Met Val Ala His Ser Ser Glu Gly Leu Ser Ala Thr Ala Pro Val Thr
1          5          10          15
Gly Gly Asp Val Leu Val Asp Ala Arg Ala Ser Leu Glu Glu Lys Glu
20          25          30
Ala Pro Arg Asp Val Asn Ala Asn Thr Lys Gln Ala Thr Thr Glu Glu
35          40          45
Pro Arg Ile Gln Leu Pro Thr Val Asp Ala Phe Arg Arg Ala Ile Pro
50          55          60
Ala His Cys Phe Glu Arg Asp Leu Val Lys Ser Ile Arg Tyr Leu Val
65          70          75          80
Gln Asp Phe Ala Ala Leu Thr Ile Leu Tyr Phe Ala Leu Pro Ala Phe
85          90          95
Glu Tyr Phe Gly Leu Phe Gly Tyr Leu Val Trp Asn Ile Phe Met Gly
100         105         110
Val Phe Gly Phe Ala Leu Phe Val Val Gly His Asp Cys Leu His Gly
115         120         125
Ser Phe Ser Asp Asn Gln Asn Leu Asn Asp Phe Ile Gly His Ile Ala
130         135         140
Phe Ser Pro Leu Phe Ser Pro Tyr Phe Pro Trp Gln Lys Ser His Lys

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

<210> SEQ ID NO 3  
 <211> LENGTH: 41  
 <212> TYPE: DNA  
 <213> ORGANISM: synthetic primer

<400> SEQUENCE: 3

cuacuacuac uactgcagac aatggtcgct cattcctcag a

41

<210> SEQ ID NO 4  
 <211> LENGTH: 38  
 <212> TYPE: DNA  
 <213> ORGANISM: synthetic primer

<400> SEQUENCE: 4

caucaucauc augeggccgc ttacttggcc ttgcctt

38

<210> SEQ ID NO 5  
 <211> LENGTH: 32  
 <212> TYPE: DNA  
 <213> ORGANISM: synthetic polylinker

<400> SEQUENCE: 5

tcgacctgca ggaagcttgc ggccgcgat cc

32



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<210> SEQ ID NO 6  
 <211> LENGTH: 32  
 <212> TYPE: DNA  
 <213> ORGANISM: synthetic polylinker

<400> SEQUENCE: 6

tcgaggatcc gcggccgcaa gcttcctgca gg 32

<210> SEQ ID NO 7  
 <211> LENGTH: 1353  
 <212> TYPE: DNA  
 <213> ORGANISM: Brassica napus

<400> SEQUENCE: 7

aatccatcaa acctttattc accacatttc actgaaaggc cacacatcta gagagagaaa 60  
 cttcgtccaa atctctctct ccagcgatgg ttgttgctat ggaccagcgc agcaatgtta 120  
 acggagattc cggtgcccgg aaggaagaag ggtttgatcc aagcgcacaa ccaccgttta 180  
 agatcggaga tataagggcg gcgattccta agcattgctg ggtgaagagt cctttgagat 240  
 ctatgagcta cgtcaccaga gacattttcg ccgtcgcggc tctggccatg gccgcccgtg 300  
 attttgatag ctggttcctc tggccactct actgggttgc ccaaggaacc cttttctggg 360  
 ccatcttcgt tcttggccac gactgtggac atgggagttt ctcagacatt cctctgctga 420  
 acagtgtggt tggtcacatt cttcattcat tcactcctgt tccttaccat ggttggagaa 480  
 taagccatcg gacacaccac cagaacctg gccatgttga aaacgacgag tcttgggttc 540  
 cgttgccaga aaagtgttac aagaacttgc cccatagtac tcggatgctc agatacactg 600  
 tcctctgccc catgctcgtc taccgatct atctgtgta cagaagtcct ggaaaagaag 660  
 ggtcacattt taaccatac agtagtttat ttgctccaag cgagaggaag cttattgcaa 720  
 cttcaactac ttgctggtcc ataatggttg ccaactctgt ttatctatcg ttctcgttg 780  
 atccagtcac agttctcaaa gtctatggcg ttccttaccat tatctttgtg atgtggttg 840  
 acgctgtcac gtacttgcac catcatggtc acgatgagaa gttgccttgg tacagaggca 900  
 aggaatggag ttatttacgt ggaggattaa caactattga tagagattac ggaatcttca 960  
 acaacatcca tcacgacatt ggaactcac tgatccatca tcttttccca caaatccctc 1020  
 actatcactt ggtcgatgcc acgagagcag ctaaactatgt gttaggaaga tactacagag 1080  
 agccgaagac gtcaggagca ataccgatc acttggtgga gagtttggtc gcaagtatta 1140  
 aaaaagatca ttacgtcagt gacactggtg atattgtctt ctacgagaca gatccagatc 1200  
 tctacgttta tgcttctgac aaatctaaaa tcaattaact tttcttcta gctctattag 1260  
 gaataaacac tccttctctt ttacttattt gtttctgctt taagtttaaa atgtactcgt 1320  
 gaaacctttt ttttattaat gtatttacgt tac 1353

<210> SEQ ID NO 8  
 <211> LENGTH: 383  
 <212> TYPE: PRT  
 <213> ORGANISM: Brassica napus

<400> SEQUENCE: 8

Met Val Val Ala Met Asp Gln Arg Ser Asn Val Asn Gly Asp Ser Gly  
 1 5 10 15

Ala Arg Lys Glu Glu Gly Phe Asp Pro Ser Ala Gln Pro Pro Phe Lys  
 20 25 30

Ile Gly Asp Ile Arg Ala Ala Ile Pro Lys His Cys Trp Val Lys Ser



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

<210> SEQ ID NO 9  
 <211> LENGTH: 40  
 <212> TYPE: DNA  
 <213> ORGANISM: synthetic primer

<400> SEQUENCE: 9

cuacuacuac uagagctcag cgatgggttg tgctatggac

40

<210> SEQ ID NO 10  
 <211> LENGTH: 37  
 <212> TYPE: DNA



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<213> ORGANISM: synthetic primer

<400> SEQUENCE: 10

caucaucauc augaattctt aattgatttt agatttg 37

<210> SEQ ID NO 11

<211> LENGTH: 1482

<212> TYPE: DNA

<213> ORGANISM: Mortierella alpina

<400> SEQUENCE: 11

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gggaacggac caaggaaaaa ccttcacctg ggaagagctg gcggcccata acaccaagga 120

cgacctactc ttggccatcc gcggcagggt gtacgatgtc acaaagttct tgagccgcca 180

tcttgggtgga gtggacactc tctgtctcgg agctggccga gatgttactc cggcttttga 240

gatgtatcac gcgtttgggg ctgcagatgc cattatgaag aagtactatg tcggtacact 300

ggtctcgaat gagctgcca tcttcccgga gccaacgggtg ttccacaaaa ccatcaagac 360

gagagtogag ggctacttta cggatcggaa cattgatccc aagaatagac cagagatctg 420

gggaacgatac gctcttatct ttggatcctt gatcgcttcc tactacgcgc agctctttgt 480

gcctttcggt gtcgaacgca catggcttca ggtgggtggtt gcaatcatca tgggatttgc 540

gtgcgcacaa gtcggactca accctcttca tgatgcgtct cacttttcag tgaccacaaa 600

ccccactgtc tggaagattc tgggagccac gcacgacttt ttcaacggag catcgtacct 660

ggtgtggatg taccaacata tgctcggcca tcaccctac accaacattg ctggagcaga 720

tcccgaactg tcgacgtctg agcccgatgt tcgtcgtatc aagcccaacc aaaagtgggt 780

tgcaaacac atcaaccagc acatgtttgt tcttttctg tacggactgc tggcgttcaa 840

ggtgcgcatt caggacatca acattttgta ctttgtcaag accaatgacg ctattcgtgt 900

caatcccac tcgacatggc aactgtgat gttctggggc ggcaaggctt tctttgtctg 960

gtatcgctg attgttcccc tgcagtatct gccctgggc aagggtgctgc tcttgttcac 1020

ggtcgcggac atggtgtcgt cttactggct ggcgctgacc ttccaggcga accacgttgt 1080

tgaggaagtt cagtggccgt tgctgacga gaacgggatc atccaaaagg actgggcagc 1140

tatgcaggtc gagactacgc aggattacgc acacgattcg cacctctgga ccagcatcac 1200

tggcagcttg aactaccagg ctgtgcacca tetgttcccc aacgtgtcgc agcaccatta 1260

tccgatatt ctggccatca tcaagaacac ctgcagcgag tacaaggttc cttacattgt 1320

caaggatacg ttttggcaag catttgcttc acatttgag cacttgctg ttcttggact 1380

ccgtcccag gaagagtaga agaaaaaag cccgaatga agtattgcc ctttttctc 1440

caagaatggc aaaaggagat caagtggaca ttctctatga ag 1482

<210> SEQ ID NO 12

<211> LENGTH: 446

<212> TYPE: PRT

<213> ORGANISM: Mortierella alpina

<400> SEQUENCE: 12

Met Gly Thr Asp Gln Gly Lys Thr Phe Thr Trp Glu Glu Leu Ala Ala  
1 5 10 15

His Asn Thr Lys Asp Asp Leu Leu Leu Ala Ile Arg Gly Arg Val Tyr  
20 25 30

Asp Val Thr Lys Phe Leu Ser Arg His Pro Gly Gly Val Asp Thr Leu



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35			40			45									
Leu	Leu	Gly	Ala	Gly	Arg	Asp	Val	Thr	Pro	Val	Phe	Glu	Met	Tyr	His
	50					55					60				
Ala	Phe	Gly	Ala	Ala	Asp	Ala	Ile	Met	Lys	Lys	Tyr	Tyr	Val	Gly	Thr
65					70					75					80
Leu	Val	Ser	Asn	Glu	Leu	Pro	Ile	Phe	Pro	Glu	Pro	Thr	Val	Phe	His
				85					90					95	
Lys	Thr	Ile	Lys	Thr	Arg	Val	Glu	Gly	Tyr	Phe	Thr	Asp	Arg	Asn	Ile
			100					105					110		
Asp	Pro	Lys	Asn	Arg	Pro	Glu	Ile	Trp	Gly	Arg	Tyr	Ala	Leu	Ile	Phe
		115					120					125			
Gly	Ser	Leu	Ile	Ala	Ser	Tyr	Tyr	Ala	Gln	Leu	Phe	Val	Pro	Phe	Val
	130					135					140				
Val	Glu	Arg	Thr	Trp	Leu	Gln	Val	Val	Phe	Ala	Ile	Ile	Met	Gly	Phe
145					150					155					160
Ala	Cys	Ala	Gln	Val	Gly	Leu	Asn	Pro	Leu	His	Asp	Ala	Ser	His	Phe
				165					170					175	
Ser	Val	Thr	His	Asn	Pro	Thr	Val	Trp	Lys	Ile	Leu	Gly	Ala	Thr	His
			180					185					190		
Asp	Phe	Phe	Asn	Gly	Ala	Ser	Tyr	Leu	Val	Trp	Met	Tyr	Gln	His	Met
		195					200					205			
Leu	Gly	His	His	Pro	Tyr	Thr	Asn	Ile	Ala	Gly	Ala	Asp	Pro	Asp	Val
	210					215					220				
Ser	Thr	Ser	Glu	Pro	Asp	Val	Arg	Arg	Ile	Lys	Pro	Asn	Gln	Lys	Trp
225					230					235					240
Phe	Val	Asn	His	Ile	Asn	Gln	His	Met	Phe	Val	Pro	Phe	Leu	Tyr	Gly
			245						250					255	
Leu	Leu	Ala	Phe	Lys	Val	Arg	Ile	Gln	Asp	Ile	Asn	Ile	Leu	Tyr	Phe
		260						265					270		
Val	Lys	Thr	Asn	Asp	Ala	Ile	Arg	Val	Asn	Pro	Ile	Ser	Thr	Trp	His
		275					280					285			
Thr	Val	Met	Phe	Trp	Gly	Gly	Lys	Ala	Phe	Phe	Val	Trp	Tyr	Arg	Leu
	290					295					300				
Ile	Val	Pro	Leu	Gln	Tyr	Leu	Pro	Leu	Gly	Lys	Val	Leu	Leu	Leu	Phe
305					310					315					320
Thr	Val	Ala	Asp	Met	Val	Ser	Ser	Tyr	Trp	Leu	Ala	Leu	Thr	Phe	Gln
			325						330					335	
Ala	Asn	His	Val	Val	Glu	Glu	Val	Gln	Trp	Pro	Leu	Pro	Asp	Glu	Asn
			340					345					350		
Gly	Ile	Ile	Gln	Lys	Asp	Trp	Ala	Ala	Met	Gln	Val	Glu	Thr	Thr	Gln
		355					360					365			
Asp	Tyr	Ala	His	Asp	Ser	His	Leu	Trp	Thr	Ser	Ile	Thr	Gly	Ser	Leu
	370					375					380				
Asn	Tyr	Gln	Ala	Val	His	His	Leu	Phe	Pro	Asn	Val	Ser	Gln	His	His
385					390					395					400
Tyr	Pro	Asp	Ile	Leu	Ala	Ile	Ile	Lys	Asn	Thr	Cys	Ser	Glu	Tyr	Lys
			405						410					415	
Val	Pro	Tyr	Leu	Val	Lys	Asp	Thr	Phe	Trp	Gln	Ala	Phe	Ala	Ser	His
			420					425					430		
Leu	Glu	His	Leu	Arg	Val	Leu	Gly	Leu	Arg	Pro	Lys	Glu	Glu		
		435					440					445			



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<211> LENGTH: 39  
 <212> TYPE: DNA  
 <213> ORGANISM: synthetic primer  
  
 <400> SEQUENCE: 13  
  
 cuacuacuac uactcgagca agatgggaac ggaccaagg 39

<210> SEQ ID NO 14  
 <211> LENGTH: 39  
 <212> TYPE: DNA  
 <213> ORGANISM: synthetic primer  
  
 <400> SEQUENCE: 14  
  
 caucaucauc auctcgagct actcttcctt gggacggag 39

<210> SEQ ID NO 15  
 <211> LENGTH: 47  
 <212> TYPE: DNA  
 <213> ORGANISM: synthetic primer  
  
 <400> SEQUENCE: 15  
  
 cuacuacuac uatctagact cgagaccatg gctgctgctc cagtgtg 47

<210> SEQ ID NO 16  
 <211> LENGTH: 40  
 <212> TYPE: DNA  
 <213> ORGANISM: synthetic primer  
  
 <400> SEQUENCE: 16  
  
 caucaucauc auaggcctcg agttactgcg ccttaccat 40

<210> SEQ ID NO 17  
 <211> LENGTH: 1617  
 <212> TYPE: DNA  
 <213> ORGANISM: Mortierella alpina  
  
 <400> SEQUENCE: 17  
  
 cgacactcct tccttcttct caccgcctct agtccccttc aacccccctc tttgacaaag 60  
 acaacaaacc atggctgctg ctcccagtgt gaggacgttt actcgggccg aggttttgaa 120  
 tgccgaggct ctgaatgagg gcaagaagga tgccgaggca cccttcttga tgatcatcga 180  
 caacaagggtg tacgatgtcc gcgagtctct ccctgatcat cccgggtgaa gtgtgattct 240  
 cacgcacggt ggcaaggacg gcaactgacgt ctttgacact tttcaccctg aggetgcttg 300  
 ggagactcct gccaaactttt acgttggtga tattgacgag agcgaccgcg atatcaagaa 360  
 tgatgacttt gggccgagg tccgcaagct gcgtaccttg ttccagtctc ttggttacta 420  
 cgattcttcc aaggcactact acgccttcaa ggtctcgttc aacctctgca tctggggttt 480  
 gtcgacggtc attgtggcca agtggggcca gacctcgacc ctgccaacg tgctctcggc 540  
 tgcgcttttg ggtctgttct ggcagcagtg cggatggttg gctcacgact ttttgcacat 600  
 ccaggctctc caggaccgtt tctgggggtga tcttttcggc gccttcttgg gaggtgtctg 660  
 ccagggtctc tcgtcctcgt ggtggaagga caagcacaac actcaccacg ccgcccccaa 720  
 cgtccacggc gaggatcccg acattgacac ccacctctg ttgacctgga gtgagcatgc 780  
 gttggagatg ttctcggatg tcccagatga ggagctgacc cgcagtgtgt cgcgtttcat 840  
 ggtcctgaac cagacctggt tttacttccc cattctctcg tttgcccgtc tctcctggtg 900  
 cctccagctc attctctttg tgctgcctaa cggtcaggcc cacaagcct cgggcgcgcg 960  
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caccatgttc ctgttcatca aggatcccgt caacatgctg gtgtactttt tgggtgctgca 1080
ggcgggtgtgc ggaaacttgt tggcgatcgt gttctcgctc aaccacaacg gtatgcctgt 1140
gatctcgaag gaggaggcgg tcgatatgga tttcttcacg aagcagatca tcacgggtcg 1200
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gcaccacttg ttccttcga tgctcgcca caacttttca aagatccagc ctgctgctga 1320
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agaggtcttt agccgtctga acgaggtctc caaggctgcc tccaagatgg gtaaggcgca 1440
gtaaaaaaaaa aaacaaggac gttttttttc gccagtgcct gtgctgtgc ctgcttcct 1500
tgcaagtcg agcgtttctg gaaaggatcg ttcagtgcag tatcatcatt ctcttttac 1560
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&lt;210&gt; SEQ ID NO 18

&lt;211&gt; LENGTH: 457

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Mortierella alpina*

&lt;400&gt; SEQUENCE: 18

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Met Ala Ala Ala Pro Ser Val Arg Thr Phe Thr Arg Ala Glu Val Leu
1           5           10          15

Asn Ala Glu Ala Leu Asn Glu Gly Lys Lys Asp Ala Glu Ala Pro Phe
                20           25           30

Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe Val Pro
          35           40           45

Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys Asp Gly
          50           55           60

Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu Thr Leu
65           70           75           80

Ala Asn Phe Tyr Val Gly Asp Ile Asp Glu Ser Asp Arg Asp Ile Lys
          85           90           95

Asn Asp Asp Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu Phe Gln
          100          105          110

Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val
          115          120          125

Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Thr Val Ile Val Ala Lys
          130          135          140

Trp Gly Gln Thr Ser Thr Leu Ala Asn Val Leu Ser Ala Ala Leu Leu
145          150          155          160

Gly Leu Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe Leu His
          165          170          175

His Gln Val Phe Gln Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe
          180          185          190

Leu Gly Gly Val Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys
          195          200          205

His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp Pro Asp
          210          215          220

Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Glu Met
225          230          235          240

Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Arg Met Trp Ser Arg Phe
          245          250          255

Met Val Leu Asn Gln Thr Trp Phe Tyr Phe Pro Ile Leu Ser Phe Ala
          260          265          270

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ctctttgtca acttttggtt ggtcctgatc accttcttgc agcacaccga tcccaagctg 1080
ccccattacc gcgagggtgc ctggaatttc cagcgtggag ctctttgcac cgttgaccgc 1140
tcgtttgga agttcttga ccatatgttc cacggcattg tccacacca tgtggcccat 1200
cacttggtct cgcaaatgcc gttctaccat gctgaggaag ctacctatca tctcaagaaa 1260
ctgctgggag agtactatgt gtacgacca tccccgatcg tcgttgcggt ctggaggctc 1320
ttccgtgagt gccgattcgt ggaggatcag ggagacgtgg tctttttcaa gaagtaaaaa 1380
aaaagacaat ggaccacaca caacctgtc tctacagacc tacgtatcat gtagccatac 1440
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&lt;210&gt; SEQ ID NO 20

&lt;211&gt; LENGTH: 399

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Mortierella alpina

&lt;400&gt; SEQUENCE: 20

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

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

<210> SEQ ID NO 21  
 <211> LENGTH: 36  
 <212> TYPE: DNA  
 <213> ORGANISM: synthetic primer

<400> SEQUENCE: 21

cuacuacuac uaggatccat ggcacctccc aacct

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<210> SEQ ID NO 22  
 <211> LENGTH: 41  
 <212> TYPE: DNA  
 <213> ORGANISM: synthetic primer

<400> SEQUENCE: 22

caucaucauc auggtacctc gagttacttc ttgaaaaaga c

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What is claimed is:

1. A method for producing stearidonic acid in a plant seed, said method comprising:

growing a plant having integrated into its genome:

a first DNA construct comprising, in the 5' to 3' direction of transcription, a promoter functional in a plant seed cell, a DNA sequence encoding a delta-six desaturase *from the genus Mortierella*, and a transcription termination region functional in a plant cell,

a second DNA construct comprising, in the 5' to 3' direction of transcription, a promoter function in a plant seed cell, and a DNA sequence encoding a delta 15 desaturase, and a transcription termination region functional in a plant cell, and

a third DNA construct comprising, in the 5' to 3' direction of transcription, a promoter functional in a plant seed cell, and a DNA sequence encoding a delta 12 desaturase, and a transcription termination region functional in a plant cell,

and growing said plant under conditions whereby said delta-six desaturase, delta 15 desaturase and delta 12 desaturase [is] are expressed.

[2. The method according to claim 1 wherein said plant has a second construct integrated into its genome, wherein said second construct has in the 5' to 3' direction of transcription, a promoter functional in a plant seed cell, and a DNA sequence encoding a delta 12 desaturase.]

[3. The method according to claim 1 wherein said plant has a second construct integrated into its genome, wherein said second construct has in the 5' to 3' direction of

transcription, a promoter functional in a plant seed cell, and a DNA sequence encoding a delta 15 desaturase.]

[4. The method of claim 1 wherein said desaturase encoding sequence is from the genus *Mortierella*.]

5. The method according to claim 1, wherein said promoter *for the first DNA construct* is a napin promoter.

6. The method according to claim 1, wherein said promoter *for the first DNA construct* is from the soybean beta-conglycinin 7S subunit transcription initiation region.

7. The method according to claim 1, wherein said method further comprises extracting oil from said plant seed.

8. The method of claim 7, wherein said oil comprises about 5 weight percent or greater stearidonic acid.

9. The method of claim 7, wherein said oil comprises about 10 weight percent or greater stearidonic acid.

10. The method of claim 7, wherein said oil comprises about 15 weight percent or greater stearidonic acid.

11. The method of claim 7, wherein said oil comprises about 20 weight percent or greater stearidonic acid.

12. The method of claim 7, wherein said oil comprises about 25 weight percent or greater stearidonic acid.

13. A method for producing stearidonic acid in a plant seed, said method comprising:

growing a plant having integrated into its genome;

a first DNA construct comprising, in the 5' to 3' direction of transcription, a promoter functional in a plant seed cell, a DNA sequence encoding a delta-six desaturase, and a transcription termination region functional in a plant cell,

a second DNA construct comprising, in the 5' to 3' direction of transcription, a promoter functional in a plant

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seed cell, and a DNA sequence encoding a delta 15 desaturase, and a transcription termination region functional in a plant cell, and  
 a third DNA construct comprising, in the 5' to 3' direction of transcription, a promoter functional in a plant seed cell, and a DNA sequence encoding a delta 12 desaturase, and a transcription termination region functional in a plant cell, and  
 growing said plant under conditions whereby said delta-six desaturase, delta 15 desaturase, and delta-12 desaturase are expressed.

14. The method according to claim 13, wherein said promoter for the first DNA construct is a napin promoter.

15. The method according to claim 13, wherein said promoter for the first DNA construct is from the soybean  $\beta$ -conglycinin 7S subunit transcription initiation region.

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16. The method according to claim 13, wherein said method further comprises extracting oil from said plant seed.

17. The method of claim 16, wherein said oil comprises about 5 weight percent or greater stearidonic acid.

18. The method of claim 16, wherein said oil comprises about 10 weight percent or greater stearidonic acid.

19. The method of claim 16, wherein said oil comprises about 15 weight percent or greater stearidonic acid.

20. The method of claim 16, wherein said oil comprises about 20 weight percent or greater stearidonic acid.

21. The method of claim 16, wherein said oil comprises about 25 weight percent or greater stearidonic acid.

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