Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2609367 A1 2006/11/30

(21) 2 609 367

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2006/05/22
(87) Date publication PCT/PCT Publication Date: 2006/11/30
(85) Entrée phase nationale/National Entry: 2007/11/20
(86) N° demande PCT/PCT Application No.: US 2006/020047
(87) N° publication PCT/PCT Publication No.: 2006/127789
(30) Priorités/Priorities: 2005/05/23 (US60/684,134);
2005/11/10 (US60/735,984)

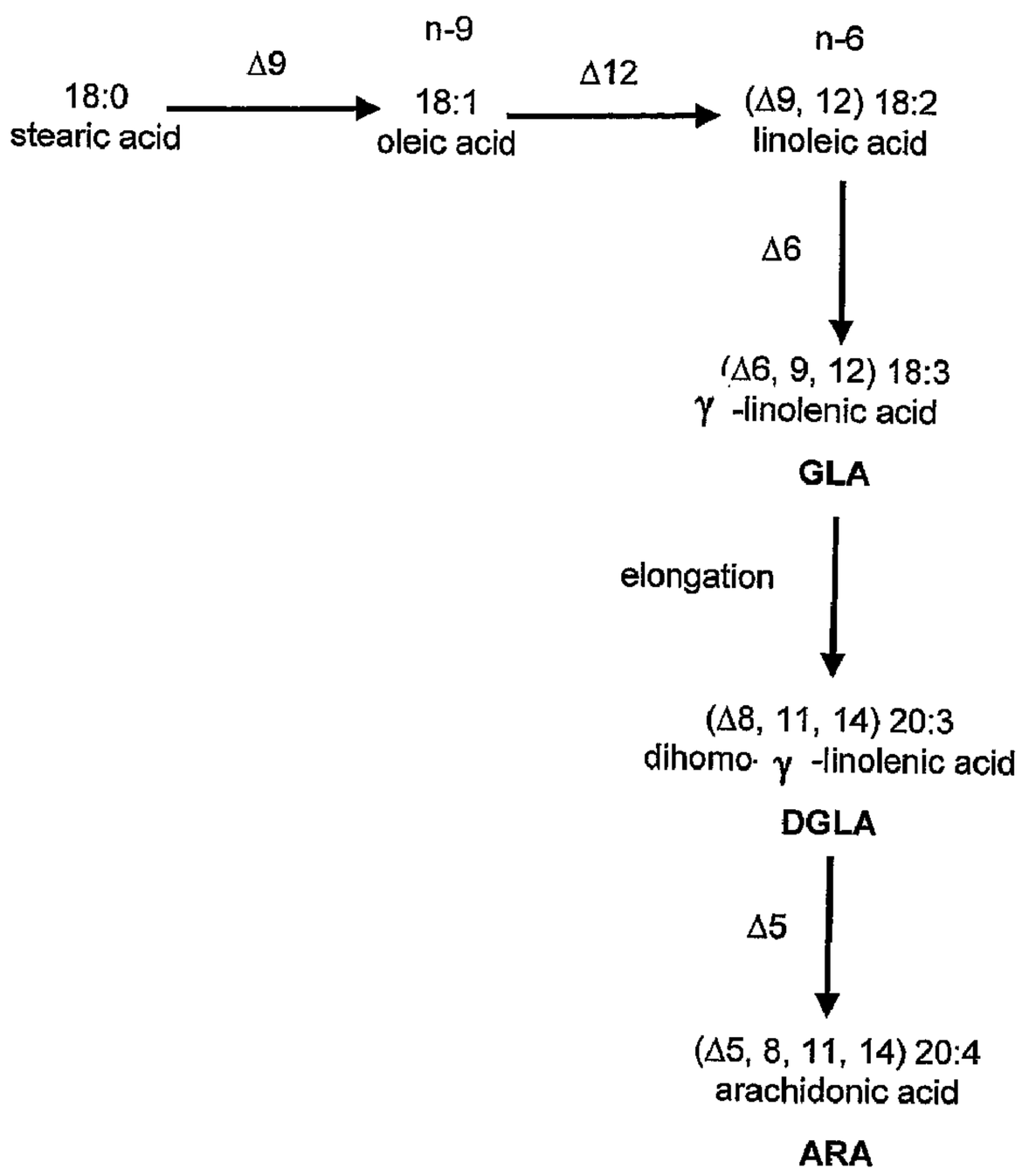
(51) Cl.Int./Int.Cl. *A01H 5/00* (2006.01)

(71) Demandeur/Applicant:
ARCADIA BIOSCIENCES, INC., US

(72) Inventeurs/Inventors:
KNAUF, VIC C., US;
SHEWMAKER, CHRISTINE, US;
FLIDER, FRANK J., US;
EMLAY, DONALD, US;
REY, ERIC, US

(74) Agent: GOWLING LAFLEUR HENDERSON LLP

(54) Titre : SAFRAN A TENEUR ELEVEE EN ACIDE GAMMA-LINOLENIQUE
(54) Title: SAFFLOWER WITH ELEVATED GAMMA-LINOLENIC ACID



(57) Abrégé/Abstract:
The present invention relates to compositions and methods for preparing gamma-linoleic acid (GLA) in safflower plants, particularly from seeds of safflower. Nucleic acid sequences and constructs encoding one or more fatty acid desaturase sequences are used

(21) 2 609 367

(13) A1

(57) Abrégé(suite)/Abstract(continued):
to generate transgenic safflower plants that contain and express one or more of these sequences and produce high levels of GLA in safflower seeds. Provided are transgenic safflower plants and seeds that produce high levels of GLA. Additionally provided are oils produced from seeds of this invention. The invention also relates to methods of treating a variety of diseases including nervous system disorders, inflammatory conditions, cancer and cardiovascular disorders using the oils of this invention.

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 November 2006 (30.11.2006)

PCT

(10) International Publication Number
WO 2006/127789 A2

(51) International Patent Classification:
A01H 5/00 (2006.01)

(21) International Application Number:
PCT/US2006/020047

(22) International Filing Date: 22 May 2006 (22.05.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/684,134 23 May 2005 (23.05.2005) US
60/735,984 10 November 2005 (10.11.2005) US

(71) Applicant *(for all designated States except US)*: **ARCADIA BIOSCIENCES, INC.** [US/US]; 202 Cousteau Place, Suite 200, Davis, CA 95616 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **KNAUF, Vic, C.** [US/US]; 11835 NE Yeomalt Point Drive, Bainbridge Island, WA 98110 (US). **SHEWMAKER, Christine** [US/US]; 1409 Springcreek Drive, Woodland, CA 95776 (US). **FLIDER, Frank, J.** [US/US]; 6922 E. Joan De Arc Avenue, Scottsdale, AZ 85254 (US). **EMLAY, Donald** [US/US]; 3406 Mono Place, Davis, CA 95616 (US). **REY, Eric** [US/US]; 1004 1/2 Cragmont Avenue, Berkeley, CA 94708 (US).

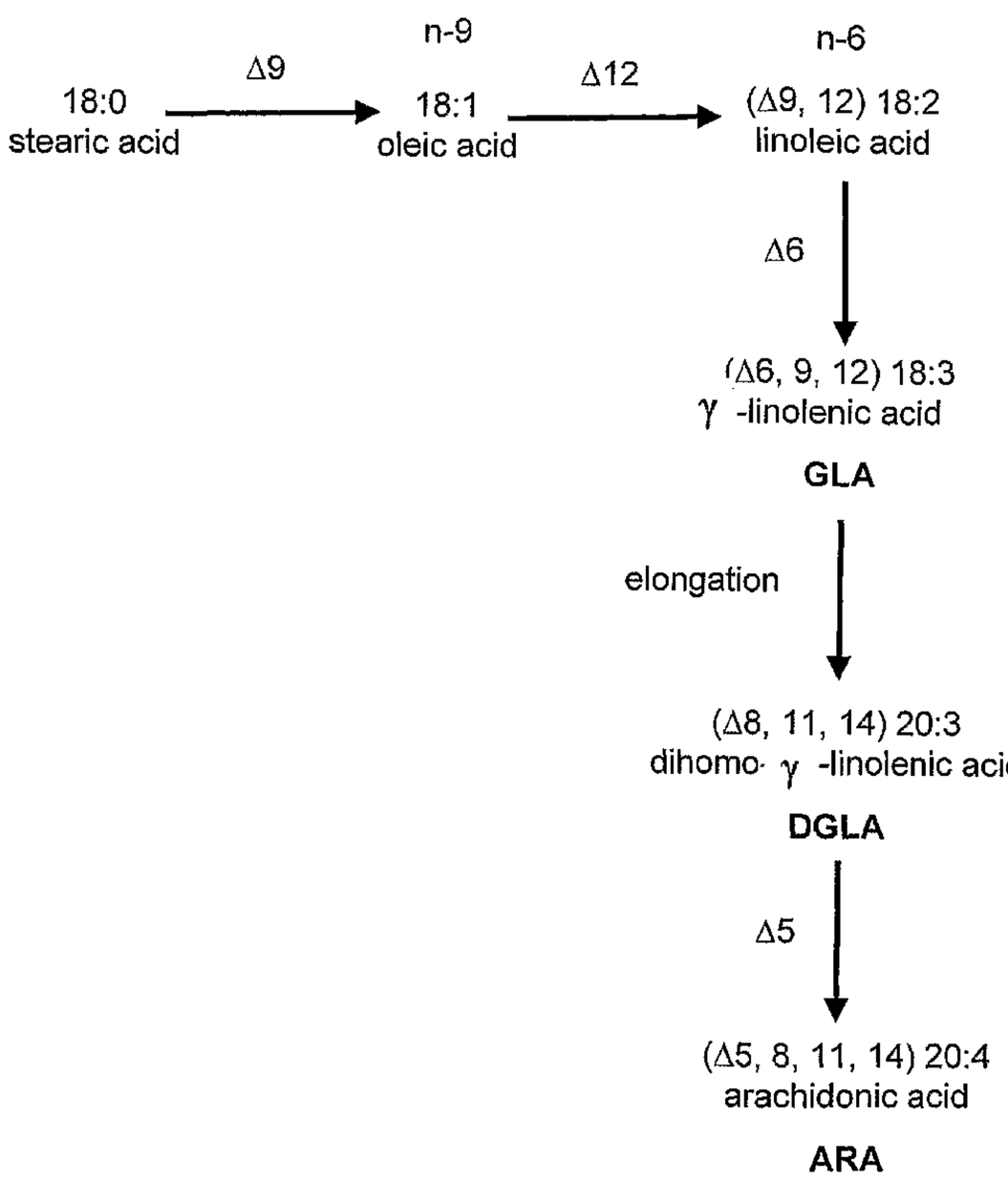
(74) Agents: **WARD, Michael, R.** et al.; MORRISON & FOERSTER LLP, 425 Market Street, San Francisco, CA 94105-2482 (US).

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH,

[Continued on next page]

(54) Title: SAFFLOWER WITH ELEVATED GAMMA-LINOLENIC ACID



(57) Abstract: The present invention relates to compositions and methods for preparing gamma-linoleic acid (GLA) in safflower plants, particularly from seeds of safflower. Nucleic acid sequences and constructs encoding one or more fatty acid desaturase sequences are used to generate transgenic safflower plants that contain and express one or more of these sequences and produce high levels of GLA in safflower seeds. Provided are transgenic safflower plants and seeds that produce high levels of GLA. Additionally provided are oils produced from seeds of this invention. The invention also relates to methods of treating a variety of diseases including nervous system disorders, inflammatory conditions, cancer and cardiovascular disorders using the oils of this invention.

WO 2006/127789 A2

WO 2006/127789 A2

GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 40

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 40

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

SAFFLOWER WITH ELEVATED GAMMA-LINOLENIC ACID

RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 60/684,134, filed May 23, 2005, and U.S. Provisional Application No. 60/735,984, filed November 10, 2005, which are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

[0002] Gamma-linolenic acid (GLA) is an essential fatty acid in the omega-6 family that is found primarily in plant-based oils. GLA is synthesized from linoleic acid (LA) via the action of the enzyme delta-six desaturase (Δ6-desaturase). The beneficial effects of GLA derive from the fact that GLA serves as the precursor to a number of other essential fatty acids such as arachidonic acid, which is a precursor of prostaglandins and other physiologically important molecules.

[0003] Unsaturated fatty acids such as linoleic (C_{18} Δ 9, 12) and α-linolenic (C_{18} Δ 9, 12, 15) acids are essential dietary constituents that cannot be synthesized by vertebrates because while vertebrate cells can introduce double bonds at the Δ 9 position of fatty acids, they cannot introduce additional double bonds between the Δ 9 double bond and the methyl-terminus of the fatty acid chain. Because they are required to synthesize other products, linoleic and α-linolenic acids are essential fatty acids, which are usually obtained from plant sources. LA can be converted by mammals into GLA (C_{18} Δ 6, 9, 12) which can in turn be converted to arachidonic acid (20:4), a critically important fatty acid since it is an essential precursor of most prostaglandins.

[0004] The dietary provision of LA, by virtue of its enzymatic conversion to GLA and then into arachidonic acid, could satisfy the dietary need for GLA and arachidonic acid. However, the consumption of fats that are less highly unsaturated, such as LA, has been correlated with health risks such as hypercholesterolemia, atherosclerosis and other clinical disorders which increase susceptibility to coronary disease. In contrast, the consumption of fats that are more highly unsaturated has been associated with decreased blood cholesterol concentration and reduced risk of atherosclerosis. Consumption of the unsaturated fatty acid GLA has been shown to be

particularly beneficial. Thus, the consumption of the more unsaturated GLA would be preferred over the consumption of LA. It would thus be desirable to generate additional sources rich in GLA for human consumption.

[0005] GLA acts as a precursor for the formation of eicosanoids including prostaglandins. Prostaglandins are vital hormone-like compounds that strengthen cell membranes and serve as cellular signaling molecules. Beneficial effects of GLA have been observed in humans and animals. GLA may help to regulate blood pressure, reduce inflammation and improve immune function. GLA supplementation may benefit a wide range of diseases and conditions including lupus, cancer, allergies, arthritis and ulcerative colitis. GLA may improve the efficacy of drugs used to treat cancer. GLA may help to reduce the symptoms of premenstrual syndrome and menopause; to improve skin health and to treat eczema, acne, rosacea, psoriasis and dandruff; to improve psychiatric and neurological disorders including Alzheimer's disease, Huntington's chorea, multiple sclerosis, attention deficit hyperactivity disorder, depression and Raynaud's phenomenon; to block diabetic neuropathy; to treat cirrhosis of the liver; to improve dry-eye conditions such as Sjögren's syndrome; and to treat cardiovascular disease, osteoporosis, hyperlipidemia and other symptoms associated with aging. Furthermore, GLA has been implicated as a stimulator for the body to burn brown fat. Brown fat is the inner body fat that surrounds vital organs and acts as a fat-burning factory, using calories for heat rather than storing them as white fat. The burning of brown fat is important for the maintenance of ideal body weight. Increased GLA consumption may thus help to stimulate the process of brown fat metabolism.

[0006] Existing GLA supplements are typically derived from plant sources that are naturally higher in GLA such as evening primrose oil, black currant oil and borage oil. However, GLA represents a relatively small fraction of the total fatty acids in these natural sources. Only approximately 7-10% (evening primrose), 14-19% (black currant oil) and 20-26% (borage oil) of the fatty acids from these sources are available as GLA. Despite GLA's broad health benefits, its use is currently limited by the high cost and low concentrations of existing GLA supplements. An average adult would need to consume 10 or more capsules of existing GLA supplements to receive its optimal health benefits. It would be useful to have a less expensive, readily available source of oil that was higher in GLA than the naturally occurring specialty oils currently used for

GLA supplements. Such a source would allow consumers to receive the optimal health benefits of GLA, while spending less money on supplements and ingesting significantly less total oil and fewer calories.

[0007] Safflower is a commercially important agricultural crop. Safflower was first cultivated in the Near East thousands of years ago as a source of dye and other products that could be derived from the plant. Safflower in this century has been utilized as a source of edible oils. Safflower was first introduced to agriculture in the United States in the 1930s as a source of edible oils. Since then, varieties with improved oil content have been developed. Safflower oil primarily comprises the fatty acids palmitic, stearic, oleic and LA. Palmitic (C16:0) and stearic acids (C18:0) are saturated fatty acids; oleic (C18:1) and linoleic (C18:2) are unsaturated fatty acids. However, safflower plants naturally produce only negligible amounts of GLA.

[0008] As such, transgenic safflower plants with seeds containing higher levels of GLA than occur naturally would have great utility.

BRIEF SUMMARY OF THE PREFERRED EMBODIMENTS OF THE INVENTION

[0009] The present invention is directed to safflower plants that produce GLA. In one aspect, safflower plants that produce seeds including at least 1% by weight GLA, the seeds of such plants, and the oil of such plants are described. In preferred embodiments, the oil will have about 1-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30–35, 35-40, 40-45, 45-50, 50-55 or 55-60% or greater by weight GLA.

[0010] In one aspect, safflower plants that contain genetic constructs including nucleic acid sequences that direct expression of one or more desaturase enzymes are described. In one aspect, the Δ6-desaturase is used alone to generate GLA in plants that produce primarily LA. In another aspect, the Δ6-desaturase is used in combination with delta-twelve desaturase (Δ12-desaturase) to produce GLA in plants that produce primarily oleic acid (OA) rather than LA. The constructs include coding sequences for these enzymes and generally include promoter and termination sequences. In one advantageous embodiment, the promoter is a seed specific promoter.

[0011] In one embodiment, a transgenic safflower plant containing a recombinant promoter functional in a safflower plant, operably linked to a recombinant DNA sequence encoding a Δ6-desaturase, in which the safflower plant produces seeds and the seeds contain at least 1% by weight GLA is described. The Δ6-desaturase encoding sequence can be derived from any plant or fungi. Such plant and fungi include but are not limited to *Mucor, Saprolegnia, Saprolegnia diclina, Mortierella, Mortierella alpina, Conidiobolus, Pythium, Phytophthora, Penicillium, Porphyridium, Coidosporium, Mucor circinelloides, Fusarium, Aspergillus, Candida, , Rhodotorula, Entomophthora, Thraustochytrium, Saprolegnia, Borago, Primula,* sunflower, canola, rice, and moss. The promoter used can be a seed specific promoter such as an oleosin promoter or a linin promoter. Also provided by this embodiment is seed derived from these transgenic plants in which the GLA levels in the seed are at least 1% by weight of the total fatty acid content of the seed. Also provided by this embodiment is oil produced from the seeds of these transgenic plants. Such oil can contain 1-60% or greater by weight GLA.

[0012] In another embodiment, the invention provides a transgenic safflower plant containing a first recombinant DNA sequence encoding a Δ6-desaturase, and second recombinant DNA sequence encoding a Δ12-desaturase, where the sequences are operably linked to at least one promoter, in which the safflower plant produces seeds and the seeds contain at least 1% by weight GLA. In some embodiments, the first and second DNA sequences are linked to a single promoter. In other embodiments, the first and second DNA sequences are linked to different promoters. The Δ6- and Δ12-desaturase encoding sequences can be derived from any plant or fungi. Such plant and fungi include but are not limited to *Mucor, Saprolegnia, Saprolegnia diclina, Mortierella, Mortierella alpina, Conidiobolus, Pythium, Phytophthora, Penicillium, Porphyridium, Coidosporium, Mucor circinelloides, Fusarium, Aspergillus, Candida, Euphorbia, Dimorphoteca, Rhodotorula, Entomophthora, Thraustochytrium, Saprolegnia, Borago, Primula,* sunflower, canola, rice, and moss. The promoter used can be a seed specific promoter such as an oleosin promoter or a linin promoter. Also provided by this embodiment is seed derived from these transgenic plants in which the GLA levels in the seed are at least 1% by weight of the total fatty acid content of the seed. Also provided by this embodiment is oil produced from the seeds of these transgenic plants. Such oil can contain 1-60% or greater by weight GLA.

[0013] In yet another embodiment, a method for producing GLA in a safflower seed is provided. The method includes the steps of growing a safflower plant containing a recombinant promoter functional in a safflower plant, operably linked to a recombinant DNA sequence encoding a Δ6-desaturase, and growing the safflower plant under conditions in which the Δ6-desaturase sequence is expressed. The Δ6-desaturase encoding sequence can be derived from any plant or fungi. Such plant and fungi include but are not limited to *Mucor, Saprolegnia, Saprolegnia diclina, Mortierella, Mortierella alpina, Conidiobolus, Pythium, Phytophthora, Penicillium, Porphyridium, Coidosporium, Mucor circinelloides, Fusarium, Aspergillus, Candida, , Rhodotorula, Entomophthora, Thraustochytrium, Saprolegnia, Borago, Primula,* sunflower, canola, rice, and moss. The promoter used can be a seed specific promoter such as an oleosin promoter or a linin promoter. Also provided by this embodiment is seed derived from these transgenic plants in which the GLA levels in the seed are at least 1% by weight of the total fatty acid content of the seed. Also provided by this embodiment is oil produced from the seeds of these transgenic plants. Such oil can contain 1-60% or greater by weight GLA.

[0014] In a further embodiment, a method for producing GLA in a safflower seed is provided. The method includes the steps of growing a safflower plant containing a first recombinant DNA sequence encoding a Δ6-desaturase, and a second recombinant DNA sequence encoding a Δ12-desaturase, where the sequences are operably linked to at least one promoter, and growing the safflower plant under conditions under which the Δ6-desaturase and Δ12-desaturase sequences are expressed. In this embodiment, the Δ6- and Δ12-desaturase encoding sequences can be derived from any plant or fungi. Such plant and fungi include but are not limited to *Mucor, Saprolegnia, Saprolegnia diclina, Mortierella, Mortierella alpina, Conidiobolus, Pythium, Phytophthora, Penicillium, Porphyridium, Coidosporium, Mucor circinelloides, Fusarium, Aspergillus, Candida, Euphorbia, Dimorphoteca, Rhodotorula, Entomophthora, Thraustochytrium, Saprolegnia, Borago, Primula,* sunflower, canola, rice, and moss. The promoter used can be a seed specific promoter such as an oleosin promoter or a linin promoter. Also provided by this embodiment is seed derived from these transgenic plants in which the GLA levels in the seed are at least 1% by weight of the total fatty acid content of the seed. Also provided by this embodiment is oil produced from the seeds of these transgenic plants. Such oil can contain 1-60% or greater by weight GLA.

[0015] In yet a further embodiment, safflower oil derived from a transgenic safflower plant in which the safflower oil has a content of GLA 1-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30–35, 35-40, 40-45, 45-50, 50-55 or 55-60% or greater by weight is provided.

[0016] In yet a further embodiment, nutritional and personal care products including safflower oil with a content of GLA 1-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30–35, 35-40, 40-45, 45-50, 50-55 or 55-60% or greater by weight is provided.

[0017] In an additional embodiment, a method of treating or preventing a psychiatric, neurological or other central or peripheral nervous system condition or disease by administering to a subject prone to or afflicted with such condition or diseases an effective amount of the oils described herein is provided.

[0018] In another additional embodiment, a method of treating or preventing an immunological condition or disease by administering to a subject prone to or afflicted with such condition or diseases an effective amount of the oils described herein is provided.

[0019] In a further additional embodiment, a method of treating or preventing an inflammatory condition or disease by administering to a subject prone to or afflicted with such condition or diseases an effective amount of the oils described herein is provided.

[0020] In a yet further additional embodiment, a method of treating or preventing cancer by administering to a subject prone to or afflicted with such diseases an effective amount of the oils described herein is provided.

[0021] In other embodiments, a method of treating or preventing a skin condition or disease by administering to a subject prone to or afflicted with such condition or diseases an effective amount of the oils described herein is provided.

[0022] In further other embodiments, a method of treating or preventing a cardiovascular condition or disease by administering to a subject prone to or afflicted with such diseases an effective amount of the oils described herein is provided.

[0023] In yet further other embodiments, a method of providing nutrition to an infant by administering to an infant an effective amount of the oils of this invention is provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] FIG. 1 shows the pathway for biosynthesis of GLA from the conversion of OA into LA, which is in turn converted into GLA through the consecutive action of the enzymes Δ6- and Δ12-desaturase as shown in the figure. GLA can be converted into arachidonic acid, which is a precursor for a number of prostaglandins, leukotrienes and other physiologically active molecules.

[0025] FIG. 2 shows the sequence alignments of various plant Δ6-desaturases (SEQ ID NO: 4-6) including a consensus sequence.

[0026] FIG. 3 shows the sequence alignments of various fungal Δ6-desaturases (SEQ ID NO: 7-11) including a consensus sequence.

[0027] FIG. 4 shows a linear representation of conserved regions in Δ6-desaturases.

[0028] FIG. 5 shows the sequence alignments of various plant Δ12-desaturases (SEQ ID NO: 12-15) including a consensus sequence.

[0029] FIG. 6 shows the sequence alignments of various fungal Δ12-desaturases (SEQ ID NO: 16-19) including a consensus sequence.

[0030] FIG. 7 shows a linear representation of conserved regions in Δ12-desaturases.

[0031] FIG. 8 shows plasmid pSBS4766 for the expression of Δ6- and Δ12-desaturase from the organism *M. alpina*. Shown are various features of the expression construct including promoters, termination sequences and resistance and marker genes. The plant selectable marker on this plasmid is *pat*, the phosphinothricin acetyl transferase from *Streptomyces viridochromogenes*. The bacterial marker is *SpecR*.

[0032] FIG. 9 shows plasmid pSBS4119 for the expression of Δ6-desaturase from the organism *S. diclina*. Shown are various features of the expression construct including promoters, termination sequences and resistance and marker genes. The plant selectable marker on this plasmid is *pat*, the phosphinothricin acetyl transferase from *Streptomyces viridochromogenes*. The bacterial marker is *SpecR*.

[0033] FIG. 10 shows plasmid pSBS4763 for the expression of Δ6-desaturase from the organism *M. alpina*. Shown are various features of the expression construct including promoters, termination sequences and resistance and marker genes. The plant selectable marker on this plasmid is *pat*, the phosphinothricin acetyl transferase from *Streptomyces viridochromogenes*. The bacterial marker is *SpecR*.

DETAILED DESCRIPTION OF THE INVENTION

[0034] In order to ensure a complete understanding of the invention, the following non-limiting definitions are provided.

[0035] Δ6-desaturase is an enzyme that introduces a double bond between carbons 6 and 7 from the carboxyl end of a fatty acid molecule.

[0036] Δ12-desaturase is an enzyme that introduces a double bond between carbons 12 and 13 from the carboxyl end of a fatty acid molecule.

[0037] As used herein, the abbreviation "GLA" is used to refer to gamma-linolenic acid.

[0038] Percentage by weight is meant to indicate the content of a particular fatty acid in a seed and/or oil from the seed based on weight. Thus, the percentage by weight of GLA or "by weight GLA" is calculated based on the weight of GLA divided by weight of total fatty acids multiplied by 100%. For example, "GLA levels at 5% by weight" or "5% by weight GLA" refers to seeds or oil from seeds that contains 5 grams of GLA and 100 grams of total fatty acid.

Introduction

[0039] As shown in FIG. 1, GLA is produced in a biochemical pathway wherein OA is converted to LA. LA in turn is converted into GLA through the action of fatty acid desaturases, enzymes that introduce double bonds at specific locations in the fatty acid carbon chain. When these enzymes are transferred into cells that produce OA or LA, GLA is produced.

[0040] Safflower is a commercially important crop plant and is a valuable source of vegetable oil. Because safflower plants do not naturally produce GLA in any significant quantity, it would not be an obvious candidate for the production of this fatty acid. For example, because safflower

plants do not normally produce GLA, one might expect that the expression of high levels of this non-endogenous fatty acid might be detrimental to the plant because the exogenously introduced GLA would interfere with the function of endogenous fatty acids. It has been surprisingly found that GLA can be expressed in safflower seeds and that this expression occurs at unexpectedly high levels, even when compared with other plants that express transgenes that are free of the concerns discussed above.

Characteristics of desaturase enzymes

[0041] The reaction catalyzed by desaturases is:

$R_1\text{-}CH_2\text{-}CH_2\text{-}R_2 + O_2 + 2e^- + 2H^+ \rightarrow R_1\text{-}CH{=}CH\text{-}R_2 + 2\ H_2O.$

[0042] Many fatty acid desaturases are membrane bound metalloenzymes. Most are believed to contain two iron atoms at their active site. As shown in FIG. 2, 3, 5 and 6, the Δ6- and Δ12-desaturases share a degree of sequence identity and similarity within each respective class of enzymes. As shown in FIG. 4 and 7 among the regions of conservation within the desaturase family are three strongly conserved histidine-rich sequences (His-boxes) with the general motifs HXXXH, HXXHH and HXXHH or QXXHH. These boxes are required for enzyme activity and are separated by membrane-spanning domains that are required for their correct orientation in the active site. Many enzymes including the Δ5- and Δ6-desaturases contain a cytochrome b5-like N-terminal extension. This is often accompanied by a change in the sequence of the third His box to QXXHH. Electrons acquired from NADH cytochrome b5 reductase are transferred to cytochrome b5 or the cytochrome b5 domain of the desaturase and then to the active site of the desaturase. The mixed oxidation/reduction reaction proceeds through two iron atoms that are stabilized by interaction with the conserved histidine boxes. As discussed below, these structural features and, in particular, the conserved residues that make up the metal binding site, are conserved across species and are responsible for the enzymatic function of this class of enzymes.

Sources of desaturase enzymes

[0043] For the production of GLA, one or more desaturase enzymes will be required depending upon the host cell and the availability of substrates. For instance, in a plant that naturally has abundant amounts of LA, Δ6-desaturase is required to catalyze the conversion of LA into GLA.

In plants that naturally have abundant amounts of OA, but not LA, a combination of Δ12- and Δ6-desaturase enzymes are required to generate GLA.

[0044] Considerations for choosing a specific desaturase polypeptide to use include correct localization and functioning of the polypeptide in the microsomal/endoplasmic reticulum compartment of the cell (these enzymes are membrane bound and must function in conjunction with the existing triglyceride biosynthetic machinery of the cell), whether the polypeptide is a rate limiting enzyme or a component thereof, whether the desaturase used is essential for synthesis of a desired poly-unsaturated 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 GLA 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 that has the desired characteristic of being capable of modifying the relative production of GLA.

[0045] A number of Δ6- and Δ12-desaturases are known including those described in U.S. Patent No. 6,635,451, WO02/081668, U.S. Patent No. 6,635,451, U.S. Patent App. No. 2003/0167525, U.S. Patent No. 6,459,018, U.S. Patent No. 5,972,644, U.S. Patent No. 6,432,684, U.S. Patent No. 5,968,809, U.S. Patent No. 5,972,664, U.S. Patent No. 6,051,754, U.S. Patent No. 6,075,183, U.S. Patent No. 6,136,574, U.S. Patent No. 5,552,306, U.S. Patent No. 5,614,393, 5,663,068, U.S. Patent No. 5,689,050, U.S. Patent No. 5,789,220, U.S. Patent No. 6,355,861 and U.S. Patent No. 6,492,108, which are hereby incorporated by reference in their entirety and for the specific sequences disclosed therein. Among the sources of Δ6- and Δ12-desaturases useful for the practice of this invention are those from plants and fungi. For example, Δ6- and Δ12-desaturases from the genera *Mucor, Saprolegnia diclina, Mortierella, Mortierella alpina, Conidiobolus, Pythium, Phytophthora, Penicillium, Porphyridium, Coidosporium, Mucor circinelloides, Fusarium, Aspergillus, Candida, Euphorbia, Dimorphoteca, Rhodotorula, Entomophthora, Thraustochytrium, Saprolegnia, Borago and Primula* are useful in practice of this invention. Desaturases from sunflower, canola, rice, moss,

and *C. elegans* can also be used in the practice of this invention. Such sequences will include histidine-rich boxes. These sequences can be used as well as sequences that have at least 80%, 85%, 90% or 95% identity based on various alignment methods well known in the art. Also useful are sequences that hybridize to the above sequences under high to moderate stringency. Hybridization and washing conditions that allow identification of additional sequences that correspond to desaturase sequences are also well known in the art, some of which are described below.

[0046] Among the methods for sequence alignment that are well known in the art are the programs and alignment algorithms described in: Smith and Waterman, J Mol Biol 147:195, 1981; Needleman and Wunsch, J Mol Biol 48:443, 1970; Pearson and Lipman, PNAS 85:2444, 1988; Higgins and Sharp, Gene 73:237, 1988; Higgins and Sharp, Comput Appl Biosci 5:151, 1989; Corpet, Nucl Acids Res 16:10881, 1988; Huang, Genomics 14:18, 1992; and Pearson, Methods Mol Biol 24:307, 1994. Altschul *et al.*, (Nature Genetics 6:119, 1994) present a detailed consideration of sequence alignment methods and homology calculations.

[0047] The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, J Mol Biol 215:403, 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx. It can be accessed at the NCBI Website. A description of how to determine sequence identity using this program is available at the NCBI website.

[0048] The AlignX program from Vector NTI was used to generate FIG. 2, 3, 5 and 6. FIG. 2 shows an alignment of Δ6-desaturases from a number of different plant species. FIG. 3 shows an alignment of desaturases from a number of fungal species. FIG. 5 and 6 show alignments of Δ12-desaturases from a number of plant and fungal species, respectively. These figures show the structural and functional relatedness of different Δ6- and Δ12-desaturases within their respective classes of enzymes. Any of the Δ6- or Δ12-desaturases shown in these figures can be used to practice the current invention as well as others that can be identified using the methods of this invention or otherwise available in the art as corresponding to Δ6- or Δ12-desaturases. Also encompassed by this invention are modifications of desaturases that still retain activity or

possessed enhanced enzymatic activity that can be obtained through random or site directed mutagenesis.

[0049] It is well known to the skilled artisan that any of the sequences disclosed herein, as well as others known in the art, and previously unknown desaturases can be isolated using conventional cloning methods such as nucleic acid hybridization or PCR for use in the present invention.

[0050] Examples of hybridization conditions that can be used to isolate desaturase sequences include the following. Stringent conditions are sequence dependent and vary according to the experimental parameters used. Generally, stringent conditions are selected to be about 5° C to 20° C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Conditions for nucleic acid hybridization and calculation of stringencies can be found in Sambrook *et al.* (Molecular Cloning—A Laboratory Manual 2nd Edition, Cold Spring Harbor Laboratory Press, New York, 1989) and Tijssen (Hybridization with Nucleic Acid Probes, Elsevier Science Ltd., Amsterdam, 1993). Examples of factors that affect nucleic acid hybridization include: temperature, salt conditions, the presence of organic solvents in the hybridization mixtures, the lengths and base compositions of the sequences to be hybridized and the extent of base mismatching. An example of high stringency conditions for hybridizing a probe to a filter-bound DNA is 5 X SSC, 2% sodium dodecyl sulfate (SDS), 100 µg/ml single stranded DNA at 55-65° C for 20 minutes and washing in 0.1 X SSC with 0.1% SDS at 60-65° C for 20 minutes.

[0051] Alternatively, PCR primers can be designed to amplify particular desaturases of interest if the sequence of the desaturase cDNA is known. Further, PCR primers can be designed to conserved regions of the desaturases to isolate additional family members. Protocols for performing PCR reactions are well known in the art and are described in manuals such as PCR Protocols: A Guide to Methods and Applications by M. Innes *et al.*, Academic Press, 1989.

[0052] Once sequences have been identified via sequence identity, hybridization, identification of conserved histidine boxes, or other suitable methods, desaturase activity can be tested using several different assays. By way of example is the use of yeast as described in U.S. Pat. No.

5,968,809 in Examples 5 to 7 and Knutzon, et al. J. Biol. Chem. 273 (45): 29360-29366 (1998), both which are hereby incorporated by reference. The yeast may be *Sacharomyces cerevisiae* or an oleaginous species. The sequence of interest is cloned into a yeast expression vector and transformed into yeast. The recombinant yeast strains are grown in media containing various substrates and the fatty acid content of the lipid fraction is analyzed to evaluate desaturase activity. Δ6- desaturase activity can be monitored by using linoleic acid as a substrate and detecting gamma-linolenic acid. Δ12-desaturase activity can be monitored by detecting conversion of endogenous oleic acid to linolenic acid.

[0053] Desaturase activity can also be tested using *Arabidopsis*. Sequences of interest are cloned into appropriate vectors, transformed into *Arabidopsis,* and activity detected by evaluating the phenotype of the transgenic plants. Alternatively, the vectors containing putative desaturase sequences can be expressed in leaves or used to generate transgenic crown galls.

[0054] The resulting desaturase sequences identified and isolated using methods such as those disclosed above are then cloned into plant expression and transformation vectors such as those disclosed below using well known methods in molecular biology such as those disclosed in Sambrook, Fritsch and Maniatis, Molecular Cloning: A Laboratory Manual, 2nd edition (1989) or Current Protocols in Molecular Biology, F.M. Ausubel *et al.*, eds. (1987).

Expression of Desaturase genes

[0055] 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 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 provides certain advantages, particularly where the tissue or part is one that 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. Patent No. 5,463,174, U.S. Patent No. 4,943,674, U.S. Patent No. 5,106,739, U.S. Patent No. 5,175,095, U.S. Patent No. 5,420,034, U.S. Patent No. 5,188,958 and U.S. Patent No. 5,589,379, which are hereby incorporated by

reference in their entirety and for the specific sequences disclosed therein. One particularly useful localization of GLA produced by this invention is in the seed tissue of host plant cells. To direct expression in the seed, seed specific promoters may be used to direct expression of the appropriate desaturases. Examples of such seed specific promoters include those disclosed in U.S. Patent No. 5,623,067, U.S. Patent No. 6,342,657 and U.S. Patent No. 6,642,437, which are hereby incorporated by reference in their entirety and for the specific sequences disclosed therein.

[0056] Expression in a host cell can be accomplished in a transient or stable fashion. Transient expression can occur from introduced constructs that contain expression signals functional in the host cell, but where the 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. Suitable selection markers include resistance to the herbicide Basta provided by the *pat* (phosphothricin acetyl transferase) gene and resistance to kanamycin provided by the *nptII* (neomycin phosphotransferase) gene, among other genes known in the art. 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 translational regulatory regions can be provided by the endogenous locus.

[0057] For expression of the desaturase polypeptide in seeds a seed-specific promoter can be employed. Examples of such promoters include the oleosin or linin promoters. The oleosin promoter is disclosed in U.S. Patent No. 5,792,922 and the linin promoter is disclosed in U.S. Patent No. 6,777,591.

[0058] When it is desirable to express more than one distinct gene, the genes can be contained within a single construct or the genes can be on separate vectors. In either case, one of skill in the art would exercise judicious choice in choosing regulatory regions, selection means and methods of propagation of the introduced construct(s) to provide for optimal expression levels of all enzymes required for the synthesis of the desired products.

[0059] Constructs comprising the gene of interest may be introduced into a host cell by standard techniques. These techniques include transfection, infection, biolistic impact, electroporation, microinjection, scraping or any other method that introduces the gene of interest into the host cell (see U.S. Patent No. 4,743,548, U.S. Patent No. 4,795,855, U.S. Patent No. 5,068,193, U.S. Patent No. 5,188,958, U.S. Patent No. 5,463,174, U.S. Patent No. 5,565,346 and U.S. Patent No. 5,565,347). For convenience, a host cell that 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 more than one site in the genome, with multiple copies at one loci, is amplified and/or is present on an extrachromosomal element having multiple copy numbers.

[0060] A variety of plant transformation methods are known. The Δ6- and Δ12-desaturase genes can be introduced into plants through *Agrobacterium* co-cultivation by a leaf disk transformation-regeneration procedure as described by Horsch *et al.*, Science 227: 1229, 1985. Other methods of *Agrobacterium*-mediated transformation, such as co-cultivation of protoplast (Horsch *et al.,* Science 223:496, 1984; DeBlock *et al.*, EMBO J. 2:2143, 1984), suspension culture of transformed cells (Barton *et al.*, Cell 32:1033, 1983) or vacuum infiltration of flowers (Bechtold *et al.*, CR Acad Scie III, Sci Vie 316:1194, 1993; Wang *et al.*, Plant Cell Rep 22:274, 2003), can also be used and are within the scope of this invention. In a preferred aspect, plants are transformed with *Agrobacterium*-derived or *Agrobacterium*-immobilized vectors such as those described in Klee *et al.*, Annu Rev Plant Physiol 38: 467, 1987. However, other methods are available to insert the Δ6- and Δ12-desaturase genes of the present invention into plant cells. Such alternative methods include, but not limited to, biolistic approaches (Klein *et al.*, Nature 327:70, 1987), protoplast approaches (Shillito and Potrykus, Recombinant DNA Methodology 687, 1989; Davey *et al.*, Plant Mol Biol 13:273, 1989) chemically-induced DNA uptake (Töpfer

et al., Plant Cell 1:133, 1989) and use of viruses or pollen (Ohta, PNAS 83:715, 1986) as vectors.

[0061] When necessary for the transformation method, the Δ6- and Δ12-desaturase genes of the present invention can be inserted into a plant transformation vector, e.g., the binary vector described by Bevan (1984) Nucleic Acids Res. 12, 8111. Plant transformation vectors can be derived by modifying the natural gene transfer system of *Agrobacterium tumefacians*. The natural system comprises large Ti (tumor-inducing)-plasmids containing a large segment, known as T-DNA, which is transferred to transformed plants. Another segment of the Ti plasmid, the vir region, is responsible for T-DNA transfer. The T-DNA region is bordered by terminal repeats. In the modified binary vectors the tumor-inducing genes have been deleted and the functions of the vir region are utilized to transfer foreign DNA bordered by the T-DNA border sequences. The T-region also contains a selectable marker for antibiotic resistance and a multiple cloning site for inserting sequences for transfer. Such engineered strains are known as "disarmed" *A. tumefaciens* strains and allow the efficient transformation of sequences bordered by the T-region into the nuclear genomes of plants.

[0062] Surface-sterilized leaf disks are inoculated with the "disarmed" foreign DNA-containing *A. tumefaciens*, cultured for two days and then transferred to antibiotic-containing medium. Transformed shoots are elected after rooting in medium containing the appropriate antibiotic, transferred to soil and regenerated.

[0063] A 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 therefore 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. Patent 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, β-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.

Transformation of safflower

[0064] At least two basic distinct methods exist for the transformation of safflower plants: (1) shoot regeneration from a callus, which is induced from co-cultivated cotyledons and (2) multiple shoot regeneration directly from co-cultivated excised meristems.

[0065] Method 1 involves induction of a callus from cotyledonary explants subsequent to co-cultivation with *Agrobacterium* (Ying *et al.*, Plant Cell Rep 11:581, 1992); Orlikowska *et al.*, PCTOC 40:85, 1995). The method consists of co-cultivating excised cotyledons during 3 days on callus induction medium (MS salts with B5 vitamins). Explants are transferred to shoot formation medium (MS salts, B5 vitamins and carbenicillin) and cultured for 2 days and then transferred to the same medium containing kanamycin. After 2 to 3 weeks, regenerating leafy structures are transferred together with underlying explant tissue to shoot elongation medium (½MS salts and MS vitamins) containing Geneticin®. After an additional 2 to 3 weeks, elongating shoots are detached from the original explant tissue and transferred to the same medium, at which point the cut ends of non-transformed or chimeric shoots rapidly turn brown while transgenic shoots remain healthy. Healthy shoots are transferred to rooting medium (½MS salts and MS vitamins) when at least 10 mm in length. An average of 2-3 shoots regenerate from one explant.

[0066] With method 2, multiple shoots are briefly induced from excised meristems prior to cocultivation with *Agrobacterium* (Rao and Rohini, Plant Biotechnol 16:201, 1999); Rohini and Rao, Annals Bot 86:1043, 2000). It involves using a needle to prick the embryo axis of

germinating seeds that have had one of the cotyledons removed at the cotyledonary node. The embyro is then immersed and gently agitated at 28-30° C in a suspension of *Agrobacterium* in Winans' AB medium for 10 minutes. Following co-cultivation on semi-solid MS basal medium for 24 hours, embryo axes are washed thoroughly with 500 µg/ml of cefotaxime in liquid MS basal medium with gentle agitation (80 rpm) for 1 hour and placed on autoclaved Soilrite (vermiculite equivalent) (Chowgule Industries Ltd. Bangalore, India) moistened with water for germination under aseptic conditions in a growth room. After 5 to 6 days, the germlings are transferred to Soilrite in pots and allowed to grow under growth room conditions for at least 10 days before they are transferred to the greenhouse. The pots are initially covered with polythene bags to maintain humidity. The growth chamber is maintained at 26-28° C under a 14-hour photoperiod with a fluorescent light. In contrast to method 1, the majority of shoots produced with this method generally do not show vitrification. The developing plantlets might be chimeric and, in that case, successful transformation depends on whether T-DNA is integrated in the meristematic cell layer that generates the future reproductive organs. This method requires substantially more starting material (mature seed) and growth chamber space than method 1.

[0067] A preferred aspect of the present disclosure provides transgenic safflower plants or progeny of these plants expressing DNA encoding desaturases that overproduce GLA. Safflower is an advantageous host plant because it is widely used as a source of vegetable oils. Safflower plant cells are transformed with the isolated DNA encoding Δ6-desaturase or Δ6-desaturase and Δ12-desaturases by any of the plant transformation methods described above. The transformed safflower plant cell, usually in a callus culture or leaf disk, is regenerated into a complete transgenic plant by methods well known to one of ordinary skill in the art (e.g., Horsch *et al.*, Science 227:1129, 1985). Since progeny of transformed safflower plants inherit the DNA encoding desaturase genes, seeds or cuttings from transformed plants are used to maintain the transgenic plant line.

[0068] In one specific aspect, the method comprises introducing DNA encoding Δ6-desaturase into safflower plants that lack GLA or have low levels of GLA but produce LA. In another aspect, the method comprises introducing one or more expression vectors that comprise DNA encoding Δ12-desaturase and Δ6-desaturase safflower plants that are deficient in both GLA and LA. Accordingly, safflower plants deficient in both LA and GLA are induced to produce LA by

the expression of Δ12-desaturase and GLA is then generated due to the expression of Δ6-desaturase. Expression vectors comprising DNA encoding Δ12-desaturase or Δ12-desaturase and Δ6-desaturase can be constructed by methods of recombinant technology known to one of ordinary skill in the art (Sambrook *et al.*, 1989) and the published sequence of Δ12-desaturase (Wada *et al.*, Nature (London) 347:200, 1990). Examples of such vectors are disclosed herein.

Oil Containing GLA

[0069] The resulting GLA in safflower plants can be extracted from various safflower plant parts, particularly seeds, utilizing methods well known in the art as described above. In particular, seeds are harvested and the oil from the safflower seed can be extracted, typically by crushing the seed, and then refined using any conventional method. Methods for extracting oil from safflower seeds are well known in the art and are presented in sources such as Smith, J.R., Safflower, AOCS Press, pp. 185-212 (1996).

[0070] The GLA 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 esterified 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, propane, acetone or ethanol.

[0071] The GLA described herein can be included in nutritional and personal care compositions. Examples of nutritional compositions invention include but are not limited to infant formulas, dietary supplements, dietary substitutes and rehydration compositions. For example, the composition may be added to food of any type including but not limited to margarines, modified butters, cheeses, milk, yogurt, chocolate, candy, snacks, salad oils, cooking oils, cooking fats, meats, fish and beverages. Examples of personal care compositions include skin creams, balms and lotions, moisturizers, tanning and after tanning products, shampoos, hair conditioners and lipsticks. Examples of uses to which the GLA of this invention can be applied are described, for example, in U.S. Patent Nos. 6,635,451 and 5,709,888, which are hereby incorporated by reference in their entirety and for the specific uses disclosed therein.

[0072] The patents cited herein are incorporated by reference in their entirety. The following Examples are provided by way of illustration and are not intended to limit the scope of the invention.

Examples:

Example 1: Plasmid pSBS4766 and transgenic plants expressing this plasmid.

[0073] FIG. 8 shows the map of a construct used to co-express the Δ6-desaturase and Δ12-desaturase from *Mortierella alpina*. The plant selectable marker used in this construct was *pat* which corresponds to the phosphinothricin acetyl transferase gene from *Streptomyces viridochromogenes*. The bacterial marker used in this construct was *SpecR*. The base binary vector used to construct this vector is a derivative of pPZP200. See Hajdukiewicz *et al*. Plant Mol Biol 25: 989, 1994. The sequence of the insert contained within the borders of the pPZP200 plasmid is shown below.

[0074] pSBS4766 (*M. alpina* Δ6- and Δ12-desaturase double expression cassette with PAT selection) (SEQ ID NO: 1)

[0075] ctgcaggaattcgatctctattgattcaaattacgatctgatactgataacgtctagatttttagggttaaagcaatcaatcacctgac
gattcaaggtggttggatcatgacgattccagaaaacatcaagcaagctctcaaagctacactctttgggatcatactgaactctaacaacctc
gttatgtcccgtagtgccagtacagacatcctcgtaactcggattgtgcacgatgccatgactatacccaacctcggtcttggtcacaccagg
aactctctggtaagctagctccactccccagaaacaaccggcgccaaattgcgcgaattgctgacctgaagacggaacatcatcgtcgggt
ccttgggcgattgcggcggaagatgggtcagcttgggcttgaggacgagacccgaatccgagtctgttgaaaaggttgttcattggggattt
gtatacggagattggtcgtcgagaggtttgagggaaaggacaaatgggtttggctctggagaaagagagtgcggctttagagagaagaattg
agaggtttagagagagatgcggcggcgatgagcggaggagagacgacgaggacctgcattatcaaagcagtgacgtggtgaaatttgga
acttttaagaggcagatagatttattatttgtatccattttcttcattgttctagaatgtcgcggaacaaattttaaaactaaatcctaaatttttctaatt
ttgttgccaatagtggatatgtgggccgtatagaaggaatctattgaaggcccaaacccatactgacgagcccaaaggttcgttttgcgttttat
gtttcggttcgatgccaacgccacattctgagctaggcaaaaaacaaacgtgtctttgaatagactcctctcgttaacacatgcagcggctgc
atggtgacgccattaacacgtggcctacaattgcatgatgtctccattgacacgtgacttctcgtctcctttcttaatatatctaacaaacactcct
acctcttccaaaatatatacacatcttttgatcaatctctcattcaaaatctcattctctctagtaaacaagaacaaaaaaccatggctgctgctcc
cagtgtgaggacgtttactcgggccgaggttttgaatgccgaggctctgaatgagggcaagaaggatgccgaggcacccttcttgatgatc
atcgacaacaaggtgtacgatgtccgcgagttcgtccctgatcatcccggtggaagtgtgattctcacgcacgttggcaaggacggcactg

acgtctttgacacttttcaccccgaggctgcttgggagactcttgccaacttttacgttggtgatattgacgagagcgaccgcgatatcaagaat
gatgactttgcggccgaggtccgcaagctgcgtaccttgttccagtctcttggttactacgattcttccaaggcatactacgccttcaaggtctc
gttcaacctctgcatctggggtttgtcgacggtcattgtggccaagtggggccagacctcgaccctcgccaacgtgctctcggctgcgctttt
gggtctgttctggcagcagtgcggatggttggctcacgacttttgcatcaccaggtcttccaggaccgtttctggggtgatcttttcggcgcct
tcttgggaggtgtctgccagggcttctcgtcctcgtggtggaaggacaagcacaacactcaccacgccgcccccaacgtccacggcgagg
atcccgacattgacacccacccctctgttgacctggagtgagcatgcgttggagatgttctcggatgtcccagatgaggagctgacccgcatg
tggtcgcgtttcatggtcctgaaccagacctggttttacttccccattctctcgtttgcccgtctctcctggtgcctccagtccattctctttgtgctg
cctaacggtcaggcccacaagccctcgggcgcgcgtgtgcccatctcgttggtcgagcagctgtcgcttgcgatgcactggacctggtacc
tcgccaccatgttcctgttcatcaaggatcccgtcaacatgctggtgtacttttggtgtcgcaggcggtgtgcggaaacttgttggcgatcgtg
ttctcgctcaaccacaacggtatgcctgtgatctcgaaggaggaggcggtcgatatggatttcttcacgaagcagatcatcacgggtcgtgat
gtccacccgggtctatttgccaactggttcacgggtggattgaactatcagatcgagcaccacttgttcccttcgatgcctcgccacaacttttc
aaagatccagcctgctgtcgagaccctgtgcaaaaagtacaatgtccgataccacaccaccggtatgatcgagggaactgcagaggtcttt
agccgtctgaacgaggtctccaaggctgcctccaagatgggtaaggcgcagtaagcttgttaccccactgatgtcatcgtcatagtccaata
actccaatgtcggggagttagtttatgaggaataaagtgtttagaatttgatcaggggggagataataaaagccgagtttgaatcttttgttataa
gtaatgtttatgtgtgtttctatatgttgtcaaatggtcccatgtttttcttcctctcttttgtaacttgcaagtgttgtgttgtactttatttggcttctttgt
aagttggtaacggtggtctatatatggaaaaggtcttgttttgttaaacttatgttagttaactggattcgtctttaaccacaaaaagttttcaataag
ctacaaatttagacacgcaagccgatgcagtcattagtacatatatttattgcaagtgattacatggcaacccaaacttcaaaaacagtaggttg
ctccatttagtaacctgaattgcctcctgattctagttgatcccggtaccgaattccaggaattcgatctctattgattcaaattacgatctgatact
gataacgtctagatttttagggttaaagcaatcaatcacctgacgattcaaggtggttggatcatgacgattccagaaaacatcaagcaagctc
tcaaagctacactctttgggatcatactgaactctaacaacctcgttatgtcccgtagtgccagtacagacatcctcgtaactcggattgtgcac
gatgccatgactatacccaacctcggtcttggtcacaccaggaactctctggtaagctagctccactccccagaaacaaccggcgccaaatt
gcgcgaattgctgacctgaagacggaacatcatcgtcgggtccttgggcgattgcggcggaagatgggtcagcttgggcttgaggacgag
acccgaatccgagtctgttgaaaaggttgttcattggggatttgtatacggagattggtcgtcgagaggtttgagggaaaggacaaatgggtt
tggctctggagaaagagagtgcggctttagagagagaattgagaggtttagagagagatgcggcggcgatgagcggaggagagacgac
gaggacctgcattatcaaagcagtgacgtggtgaaatttggaacttttaagaggcagatagatttattatttgtatccattttcttcattgttctaga
atgtcgcggaacaaattttaaaactaaatcctaaatttttctaattttgttgccaatagtggatatgtgggccgtatagaaggaatctattgaaggc
ccaaacccatactgacgagcccaaaggttcgttttgcgttttatgtttcggttcgatgccaacgccacattctgagctaggcaaaaaacaaacg
tgtctttgaatagactcctctcgttaacacatgcagcggctgcatggtgacgccattaacacgtggcctacaattgcatgatgtctccattgaca
cgtgacttctcgtctcctttcttaatatatctaacaaacactcctacctcttccaaaatatatacacatcttttgatcaatctctcattcaaaatctcat
tctctctagtaaacaagaacaaaaaaccatggcacctcccaacactatcgatgccggtttgacccagcgtcatatcagcacctcggccccaa
actcggccaagcctgccttcgagcgcaactaccagctccccgagttcaccatcaaggagatccgagagtgcatccctgcccactgctttga

gcgctccggtctccgtggtctctgccacgttgccatcgatctgacttgggcgtcgctcttgttcctggctgcgacccagatcgacaagtttgag
aatcccttgatccgctatttggcctggcctgtttactggatcatgcagggtattgtctgcaccggtgtctgggtgctggctcacgagtgtggtca
tcagtccttctcgacctccaagaccctcaacaacacagttggttggatcttgcactcgatgctcttggtcccctaccactcctggagaatctcgc
actcgaagcaccacaaggccactggccatatgaccaaggaccaggtctttgtgcccaagacccgctcccaggttggcttgcctcccaagg
agaacgctgctgctgccgttcaggaggaggacatgtccgtgcacctggatgaggaggctcccattgtgactttgttctggatggtgatccag
ttcttgttcggatggcccgcgtacctgattatgaacgcctctggccaagactacggccgctggacctcgcacttccacacgtactcgcccatc
tttgagccccgcaacttttcgacattattatctcggacctcggtgtgttggctgccctcggtgccctgatctatgcctccatgcagttgtcgctct
tgaccgtcaccaagtactatattgtcccctacctctttgtcaacttttggttggtcctgatcaccttcttgcagcacaccgatcccaagctgcccc
attaccgcgagggtgcctggaatttccagcgtggagctctttgcaccgttgaccgctcgtttggcaagttcttggaccatatgttccacggcatt
gtccacacccatgtggcccatcacttgttctcgcaaatgccgttctaccatgctgaggaagctacctatcatctcaagaaactgctgggagag
tactatgtgtacgacccatccccgatcgtcgttgcggtctggaggtcgttccgtgagtgccgattcgtggaggatcagggagacgtggtctttt
tcaagaagtaagcttgttaccccactgatgtcatcgtcatagtccaataactccaatgtcggggagttagtttatgaggaataaagtgtttagaat
ttgatcaggggagataataaaagccgagtttgaatcttttgttataagtaatgtttatgtgtgtttctatatgttgtcaaatggtcccatgttttctt
cctctctttttgtaacttgcaagtgttgtgttgtactttatttggcttctttgtaagttggtaacggtggtctatatatggaaaaggtcttgttttgttaaa
cttatgttagttaactggattcgtctttaaccacaaaaagttttcaataagctacaaatttagacacgcaagccgatgcagtcattagtacatatatt
tattgcaagtgattacatggcaacccaaacttcaaaaacagtaggttgctccatttagtaacctgaattgcctcctgattctagttgatcccggtg
aatccaaaaattacggatatgaatataggcatatccgtatccgaattatccgtttgacagctagcaacgattgtacaattgcttctttaaaaaagg
aagaaagaaagaaagaaaagaatcaacatcagcgttaacaaacggccccgttacggcccaaacggtcatatagagtaacggcgttaagc
gttgaaagactcctatcgaaatacgtaaccgcaaacgtgtcatagtcagatcccctcttccttcaccgcctcaaacacaaaaataatcttctaca
gcctatatatacaacccccccttctatctctcctttctcacaattcatcatctttctttctctaccccccaattttaagaaatcctctcttctcctcttcattt
tcaaggtaaatctctctctctctctctctctgttattccttgttttaattaggtatgtattattgctagtttgttaatctgcttatcttatgtatgccttatg
tgaatatctttatcttgttcatctcatccgtttagaagctataaatttgttgatttgactgtgtatctacacgtggttatgtttatatctaatcagatatga
atttcttcatattgttgcgtttgtgtgtaccaatccgaaatcgttgatttttttcatttaatcgtgtagctaattgtacgtatacatatggatctacgtatc
aattgttcatctgtttgtgtttgtatgtatacagatctgaaaacatcacttctctcatctgattgtgttgttacatacatagatatagatctgttatatcat
ttttttattaattgtgtatatatatatgtgcatagatctggattacatgattgtgattatttacatgattttgttatttacgtatgtatatatgtagatctgga
ctttttggagttgttgacttgattgtatttgtgtgtgtatatgtgtgttctgatcttgatatgttatgtatgtgcagccaaggctacgggcgatccacc
atgtctccggagaggagaccagttgagattaggccagctacagcagctgatatggccgcggtttgtgatatcgttaaccattacattgagacg
tctacagtgaactttaggacagagccacaaacaccacaagagtggattgatgatctagagaggttgcaagatagataccttggttggttgct
gaggttgagggtgttgtggctggtattgcttacgctgggccctggaaggctaggaacgcttacgattggacagttgagagtactgtttacgtg
tcacataggcatcaaaggttgggcctaggttccacattgtacacacatttgcttaagtctatggaggcgcaaggttttaagtctgtggttgctgtt
ataggccttccaaacgatccatctgttaggttgcatgaggctttgggatacacagcccggggtacattgcgcgcagctggatacaagcatgg

tggatggcatgatgttggtttttggcaaagggattttgagttgccagctcctccaaggccagttaggccagttacccagatctgagtcgaccga
atgagttccaagatggtttgtgacgaagttagttggttgtttttatggaactttgtttaagctagcttgtaatgtggaaagaacgtgtggctttgtgg
tttttaaatgttggtgaataaagatgtttcctttggattaactagtatttttcctattggtttcatggttttagcacacaacattttaaatatgctgttagat
gatatgctgcctgctttattatttacttacccctcaccttcagtttcaaagttgttgcaatgactctgtgtagtttaagatcgagtgaaagtagattttg
tctatatttattaggggtatttgatatgctaatggtaaacatggtttatgacagcgtacttttttggttatggtgttgacgtttccttttaaacattatagt
agcgtccttggtctgtgttcattggttgaacaaaggcacactcacttggagatgccgtctccactgatatttgaacaaa

[0076] Transformation of safflower with this construct was performed by SemBioSys Genetics Inc. (Calgary, Canada). Techniques utilized by SemBioSys Genetics Inc. include those described in WO 2004/111244, which is hereby incorporated by reference in its entirety. Transgenic plants were grown and seed were harvested.

[0077] Measurement of fatty acid levels was performed in seeds derived from transgenic plants. Seeds were collected from transgenic plants and fatty acid composition was determined by gas chromatography using a modification of a method described in "Official Methods and Recommended Practices of the AOCS", 5th Ed., Method Ce 1-62, American Oil Chemists Society: Champaign, Illinois (1997). In this method, oil is hexane extracted from the seed, hydrolyzed with hydrochloric acid and reacted with methanol to form methyl esters. The methyl esters are then quantified against an internal standard by gas chromatography.

[0078] The fatty acid composition in 10 seed pools of T1 seed of transgenic plants expressing the pSBS4766 construct are shown in Table 1 below. The activity of the Δ6-desaturase gene is clearly evidenced by the presence of GLA in the transgenic lines. While GLA ranges from 0.03% to 0.04% in the S317 controls in Table 1, it ranges form 0.5% to 30.8% in the T1 pooled seeds. This is over a fifty-fold increase in the concentration of GLA. Small but significant increases in the 18:4 are seen in the lines with the highest GLA. This is expected, as the Δ6-desaturase gene can act both on 18:2 to produce GLA and 18:3 (ALA) to produce 18:3. The activity of the Δ12-desaturase is evidenced by the decrease in 18:1 fatty acids. In the S317 controls in Table 1, the OA ranges from 73.76% to 75.8% while in the transgenic lines in ranges from 3.68% to 73.51%. Overall, the data show a wide range of GLA concentrations that can be achieved in safflower via this invention.

[0079] Table 1: Examples of fatty acid composition (expressed as percentages) in 10 seed pools of T1 seed of pSBS4766 construct expressed in S317

Table 1 Line number	C16:0 (Palmitic)	C18:0 (Stearic)	C18:1n9 (Oleic)	C18:2 other	C18:2n6 (Linoleic)	C18:3n6 (gamma-Linolenic)	C18:3n3 (alpha-Linolenic)	C18:4n3 (Octadecate-traenoic)
4766-24	7.40	1.87	3.68		53.00	30.80	0.66	0.17
4766-12	6.77	1.78	3.69		54.22	30.48	0.68	0.16
4766-27	6.71	1.78	18.73		46.82	23.18	0.60	0.13
4766-1	6.52	1.64	20.06		45.88	22.35	0.99	0.13
4766-30	6.44	1.63	17.51		56.99	15.16	0.34	0.02
4766-21	5.91	1.64	17.04		58.00	15.11	0.52	0.03
4766-11	6.06	1.56	14.38		60.75	14.99	0.44	0.04
4766-26	6.34	1.66	15.64		61.66	12.48	0.39	
4766-13	5.83	1.67	27.48		49.92	12.30	0.72	0.04
4766-19	5.94	1.74	23.34		55.54	11.08	0.44	0.02
4766-10	5.70	1.53	24.56		57.28	8.68	0.40	0.01
4766-5	5.31	1.73	33.82		48.63	8.24	0.38	0.01
4766-31	5.27	1.51	46.85		36.17	7.77	0.30	0.01
4766-4	4.50	1.34	73.51	1.89	11.14	5.08	0.32	0.01
4766-14	5.40	1.66	11.74		74.16	4.93	0.37	0.01
4766-41	4.74	1.58	54.76	0.66	33.36	2.66	0.16	
4766-22	5.13	1.5	58.60		31.92	0.50	0.21	
Centennial	6.94	1.88	11.31		76.74	0.07	0.38	
S317	4.92	2.25	73.76		16.34	0.04	0.28	
S317	4.72	2.31	74.73		15.76	0.04	0.07	
S317	4.57	2.25	75.80		14.96	0.03	0.07	

[0080] The fatty acid composition in single seed samples from the S317 control line is shown in Table 2 below. Four replicates (S0-1, S0-2, S0-3, S0-4) were run. The single seed data parallel the 10 seed pool data.

[0081] Table 2: The fatty acid composition in four single seed samples from the control line (S317, denoted S0).

Table 2—Fatty Acids	S0-1	S0-2	S0-3	S0-4
C10:0 Capric	0.7%	0.3%	0.5%	0.5%
C11:0	0.3%	0.1%	0.3%	0.2%
C12:0 Lauric	0.2%	0.1%	0.2%	0.1%
C13:0 Tridecanoic	0.0%	0.0%	0.0%	0.0%
C14:0 Myristic	0.3%	0.2%	0.2%	0.2%
C14:1w5, Myristoleic	0.2%	0.0%	0.1%	0.1%
C15:0 Pentadecanoic	0.0%	0.0%	0.0%	0.0%
C15:1w5cis 10-Pentadecenoid	0.0%	0.0%	0.1%	0.0%
C16:0 Palmitic	6.0%	6.2%	5.7%	5.7%

Table 2—Fatty Acids	S0-1	S0-2	S0-3	S0-4
C16:1w7c Palmitoleic	0.2%	0.1%	0.1%	0.2%
C17:0 Heptadecanoic	0.1%	0.1%	0.1%	0.1%
c17:1w7	0.0%	0.0%	0.0%	0.1%
C18:0 Stearic	3.2%	1.8%	3.4%	1.7%
C18:1w9t	0.1%	0.1%	0.1%	0.1%
C18:1w9c	73.0%	74.2%	74.3%	75.6%
INTERNAL STANDARD				
C18:2w6t	0.1%	0.0%	0.1%	0.0%
C18:2w6c Linoleic (LA)	13.6%	14.8%	12.9%	13.5%
C20:0 Arachidic	0.4%	0.5%	0.5%	0.5%
C18:3w6 γ-linolenic (GLA)	0.0%	0.0%	0.0%	0.0%
C20:1w9	0.3%	0.3%	0.3%	0.3%
C18:3w3, α-linolenic (ALA)	0.1%	0.1%	0.1%	0.1%
C21:0 Heneicosanoic	0.1%	0.1%	0.1%	0.1%
C20:2w6 Eicosadienoic	0.0%	0.0%	0.0%	0.0%
C22:0 Behenic	0.3%	0.3%	0.3%	0.3%
C20:3w6 Dihomo-γ-linolenic (DGLA)	0.0%	0.0%	0.0%	0.0%
C22:1w9 Erucic	0.0%	0.0%	0.0%	0.0%
C20:3w3	0.0%	0.0%	0.0%	0.0%
C20:4w6 Arachidonic (AA)	0.0%	0.0%	0.0%	0.0%
C23:0 Tricosanoic	0.0%	0.0%	0.0%	0.0%
C22:2w6	0.1%	0.0%	0.1%	0.1%
C24:0 Lignoceric	0.2%	0.2%	0.2%	0.2%
C20:5w3 Eicosapentaenoic (EPA)	0.1%	0.0%	0.0%	0.0%
C24:1w9c	0.1%	0.2%	0.1%	0.1%
C22:6w3 Docosahexaenoic (DHA)	0.3%	0.1%	0.1%	0.1%
Total fatty acids	100.0%	100.0%	100.0%	100.0%
Saturated fatty acids	11.8%	9.8%	11.5%	9.6%
Total W7's & W5's	0.4%	0.3%	0.4%	0.4%
Total W9's	73.4%	74.6%	74.6%	76.0%
Total W6's	13.8%	14.9%	13.1%	13.7%
Total W3's	0.5%	0.2%	0.2%	0.2%
Total monounsaturated fatty acids	73.7%	74.9%	75.0%	76.4%
Total trans fatty acids	0.2%	0.1%	0.2%	0.1%
Polyunsaturated fatty acids	14.2%	15.1%	13.3%	13.9%
Ratios:				
Polyunsaturated/saturated	1.2	1.5	1.2	1.5
Omega 6/Omega 3	30.1	72.5	61.6	60.8
AA / EPA	0.2	0.1	1.0	0.8
AA / DHA	0.0	0.1	0.4	0.4

[0082] The fatty acid composition in single seeds from 5 lines (S1, S4, S5, S24, S27) of transgenic plants expressing the pSBS4766 construct are shown in Tables 3-7 below. Data from

8 to 9 replicate seeds are provided. When available, values for single seeds of a NULL control line for each transgenic line are provided for comparison.

Table 3

Fatty Acids	Individual Seed Samples of Transgenic Line S1								
	NULL	S1-1	S1-2	S1-3	S1-4	S1-5	S1-6	S1-7	S1-8
C10:0 Capric	0.6%	0.6%	0.4%	0.6%	0.5%	0.4%	0.4%	0.1%	0.6%
C11:0	0.2%	0.2%	0.2%	0.3%	0.1%	0.1%	0.1%	0.1%	0.2%
C12:0 Lauric	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
C13:0 Tridecanoic	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C14:0 Myristic	0.1%	0.3%	0.3%	0.3%	0.2%	0.3%	0.2%	0.2%	0.2%
C14:1w5, Myristoleic	0.1%	0.1%	0.1%	0.2%	0.1%	0.1%	0.1%	0.1%	0.1%
C15:0 Pentadecanoic	0.0%	0.0%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
C15:1w5cis 10-Pentadecenoid	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C16:0 Palmitic	5.4%	6.1%	8.7%	8.9%	8.5%	8.0%	8.6%	8.9%	8.2%
C16:1w7c Palmitoleic	0.2%	0.3%	0.1%	0.1%	0.1%	0.2%	0.2%	0.1%	0.2%
C17:0 Heptadecanoic	0.1%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.1%	0.1%
c17:1w7	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C18:0 Stearic	2.2%	1.6%	3.2%	2.5%	1.4%	3.6%	2.9%	1.4%	2.1%
C18:1w9t	0.1%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
C18:1w9c	74.9%	59.8%	0.7%	0.8%	0.8%	0.7%	0.7%	0.7%	0.7%
INTERNAL STANDARD									
C18:2w6t	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
C18:2w6c Linoleic (LA)	14.0%	27.3%	37.8%	33.7%	48.9%	47.7%	41.4%	39.3%	41.0%
C20:0 Arachidic	0.3%	0.3%	0.3%	0.4%	0.3%	0.3%	0.2%	0.3%	0.3%
C18:3w6 γ-linolenic (GLA)	0.0%	1.4%	46.1%	49.7%	37.0%	36.7%	43.4%	46.8%	44.5%
C20:1w9	0.3%	0.2%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
C18:3w3, α-linolenic (ALA)	0.1%	0.1%	0.5%	0.6%	0.5%	0.6%	0.5%	0.8%	0.6%
C21:0 Heneicosanoic	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.0%	0.0%	0.1%
C20:2w6 Eicosadienoic	0.0%	0.0%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
C22:0 Behenic	0.2%	0.2%	0.2%	0.3%	0.2%	0.1%	0.2%	0.2%	0.1%
C20:3w6 Dihomo-γ-linolenic (DGLA)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C22:1w9 Erucic	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C20:3w3	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C20:4w6 Arachidonic (AA)	0.0%	0.0%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
C23:0 Tricosanoic	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C22:2w6	0.0%	0.1%	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
C24:0 Lignoceric	0.2%	0.2%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
C20:5w3 Eicosapentaenoic (EPA)	0.0%	0.0%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
C24:1w9c	0.2%	0.2%	0.1%	0.2%	0.1%	0.1%	0.1%	0.1%	0.1%
C22:6w3 Docosahexaenoic (DHA)	0.2%	0.2%	0.2%	0.1%	0.1%	0.2%	0.2%	0.2%	0.0%
Total fatty acids	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Saturated fatty acids	9.6%	9.9%	13.9%	13.9%	11.8%	13.3%	13.1%	11.6%	12.3%
Total W7's & W5's	0.3%	0.5%	0.3%	0.3%	0.3%	0.3%	0.3%	0.2%	0.4%
Total W9's	75.4%	60.3%	0.9%	1.1%	1.0%	0.9%	0.9%	0.9%	0.9%
Total W6's	14.1%	28.8%	84.1%	83.7%	86.0%	84.6%	84.9%	86.3%	85.7%
Total W3's	0.4%	0.4%	0.8%	0.8%	0.8%	0.8%	0.8%	1.0%	0.7%
Total monounsaturated fatty acids	75.7%	60.7%	1.2%	1.4%	1.3%	1.1%	1.2%	1.1%	1.3%

Table 3

Fatty Acids	Individual Seed Samples of Transgenic Line S1								
	NULL	S1-1	S1-2	S1-3	S1-4	S1-5	S1-6	S1-7	S1-8
Total trans fatty acids	0.1%	0.2%	0.0%	0.2%	0.0%	0.1%	0.0%	0.0%	0.0%
Polyunsaturated fatty acids	14.5%	29.2%	84.9%	84.5%	86.8%	85.5%	85.7%	87.3%	86.4%
Ratios:									
Polyunsaturated/saturated	1.5	3.0	6.1	6.1	7.3	6.4	6.6	7.5	7.0
Omega 6/Omega 3	35.3	75.7	109.0	99.3	108.2	100.1	105.3	83.6	127.4
AA / EPA	0.4	0.4	0.6	0.5	0.4	0.7	0.7	1.2	0.6
AA / DHA	0.1	0.1	0.3	0.5	0.3	0.3	0.3	0.4	4.2

Table 4

Fatty Acids	Individual Seed Samples of Transgenic Line S4									
	NULL	S4-1	S4-2	S4-3	S4-4	S4-5	S4-6	S4-7	S4-8	S4-9
C10:0 Capric	0.6%	0.7%	0.7%	0.6%	0.5%	0.8%	0.4%	0.5%	1.0%	0.6%
C11:0	0.1%	0.2%	0.2%	0.2%	0.2%	0.2%	0.1%	0.2%	0.3%	0.2%
C12:0 Lauric	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.2%	0.1%
C13:0 Tridecanoic	0.0%	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%
C14:0 Myristic	0.3%	0.2%	0.2%	0.2%	0.4%	0.3%	0.2%	0.2%	0.2%	0.3%
C14:1w5, Myristoleic	0.1%	0.2%	0.1%	0.0%	0.0%	0.1%	0.1%	0.1%	0.1%	0.0%
C15:0 Pentadecanoic	0.1%	0.0%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
C15:1w5cis 10-Pentadecenoid	0.0%	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C16:0 Palmitic	5.8%	5.4%	5.3%	6.0%	6.4%	6.5%	5.2%	5.5%	5.7%	5.9%
C16:1w7c Palmitoleic	0.3%	0.3%	0.3%	0.2%	0.2%	0.2%	0.3%	0.2%	0.3%	0.3%
C17:0 Heptadecanoic	0.2%	0.1%	0.1%	0.2%	0.2%	0.2%	0.2%	0.2%	0.1%	0.2%
c17:1w7	0.1%	0.1%	0.0%	0.1%	0.0%	0.1%	0.0%	0.0%	0.1%	0.1%
C18:0 Stearic	2.6%	1.2%	1.5%	1.7%	4.9%	2.4%	1.5%	1.5%	1.1%	2.5%
C18:1w9t	0.0%	0.2%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.1%
C18:1w9c	75.0%	76.8%	63.0%	75.4%	72.2%	71.0%	74.5%	74.7%	73.7%	73.6%
INTERNAL STANDARD										
C18:2w6t	0.0%	0.1%	0.1%	0.1%	0.1%	0.1%	0.0%	0.1%	0.0%	0.1%
C18:2w6c Linoleic (LA)	12.8%	6.0%	12.5%	4.2%	3.6%	7.7%	5.4%	4.7%	7.4%	4.3%
C20:0 Arachidic	0.3%	0.3%	0.3%	0.4%	0.4%	0.4%	0.3%	0.4%	0.3%	0.4%
C18:3w6 γ-linolenic (GLA)	0.0%	6.9%	13.7%	9.5%	8.9%	8.2%	10.4%	10.3%	8.0%	9.9%
C20:1w9	0.3%	0.3%	0.4%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%
C18:3w3, α-linolenic (ALA)	0.1%	0.1%	0.2%	0.1%	0.0%	0.1%	0.1%	0.1%	0.1%	0.1%
C21:0 Heneicosanoic	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.0%	0.1%	0.1%	0.0%
C20:2w6 Eicosadienoic	0.0%	0.0%	0.0%	0.0%	0.1%	0.1%	0.0%	0.0%	0.1%	0.1%
C22:0 Behenic	0.2%	0.2%	0.3%	0.2%	0.2%	0.3%	0.2%	0.3%	0.3%	0.3%
C20:3w6 Dihomo-γ-linolenic (DGLA)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C22:1w9 Erucic	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C20:3w3	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C20:4w6 Arachidonic (AA)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C23:0 Tricosanoic	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C22:2w6	0.0%	0.1%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%	0.1%	0.0%
C24:0 Lignoceric	0.2%	0.1%	0.2%	0.2%	0.2%	0.2%	0.1%	0.2%	0.2%	0.2%
C20:5w3 Eicosapentaenoic (EPA)	0.0%	0.1%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.1%	0.0%
C24:1w9c	0.2%	0.2%	0.3%	0.2%	0.1%	0.2%	0.2%	0.2%	0.2%	0.2%
C22:6w3 Docosahexaenoic (DHA)	0.2%	0.0%	0.1%	0.2%	0.4%	0.2%	0.1%	0.1%	0.0%	0.2%

Table 4

Fatty Acids	Individual Seed Samples of Transgenic Line S4									
	NULL	S4-1	S4-2	S4-3	S4-4	S4-5	S4-6	S4-7	S4-8	S4-9
Total fatty acids	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Saturated fatty acids	10.7%	8.8%	9.1%	9.7%	13.8%	11.6%	8.5%	9.1%	9.6%	10.8%
Total W7's & W5's	0.5%	0.6%	0.5%	0.3%	0.3%	0.4%	0.4%	0.3%	0.5%	0.4%
Total W9's	75.5%	77.2%	63.7%	75.9%	72.6%	71.5%	74.9%	75.2%	74.2%	74.1%
Total W6's	12.9%	13.0%	26.2%	13.8%	12.7%	16.0%	15.9%	15.1%	15.5%	14.3%
Total W3's	0.4%	0.2%	0.4%	0.3%	0.5%	0.4%	0.2%	0.1%	0.2%	0.3%
Total monounsaturated fatty acids	75.9%	77.8%	64.2%	76.1%	72.9%	71.9%	75.3%	75.6%	74.7%	74.5%
Total trans fatty acids	0.1%	0.2%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.0%	0.1%
Polyunsaturated fatty acids	13.3%	13.1%	26.6%	14.0%	13.1%	16.4%	16.1%	15.2%	15.7%	14.5%
Ratios:										
Polyunsaturated/saturated	1.2	1.5	2.9	1.4	1.0	1.4	1.9	1.7	1.6	1.3
Omega 6/Omega 3	35.3	76.9	69.1	50.9	27.1	42.6	85.3	104.7	79.0	55.2
AA / EPA	0.3	0.3	0.3	0.5	0.6	0.3	0.5	0.1	0.1	0.1
AA / DHA	0.1	0.4	0.1	0.1	0.0	0.1	0.1	0.0	0.2	0.0

Table 5

Fatty Acids	Individual Seed Samples of Transgenic Line S5									
	NULL	S5-1	S5-2	S5-3	S5-4	S5-5	S5-6	S5-7	S5-8	S5-9
C10:0 Capric	0.5%	0.3%	2.6%	0.6%	0.6%	0.4%	0.4%	0.5%	0.2%	0.6%
C11:0	0.1%	0.1%	0.1%	0.2%	0.2%	0.1%	0.1%	0.2%	0.6%	0.2%
C12:0 Lauric	0.2%	0.1%	0.6%	0.2%	0.2%	0.1%	0.1%	0.2%	0.3%	0.2%
C13:0 Tridecanoic	0.0%	0.0%	0.1%	0.1%	0.1%	0.0%	0.0%	0.0%	0.1%	0.1%
C14:0 Myristic	0.3%	0.2%	0.2%	0.2%	0.2%	0.3%	0.2%	0.3%	1.0%	0.2%
C14:1w5, Myristoleic	0.1%	0.0%	0.2%	0.1%	0.1%	0.0%	0.0%	0.0%	0.2%	0.1%
C15:0 Pentadecanoic	0.1%	0.1%	0.3%	0.1%	0.1%	0.1%	0.1%	0.1%	0.3%	0.1%
C15:1w5cis 10-Pentadecenoid	0.0%	0.0%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%
C16:0 Palmitic	5.5%	7.4%	8.3%	6.8%	7.4%	7.9%	7.2%	7.7%	12.9%	8.0%
C16:1w7c Palmitoleic	0.2%	0.2%	0.1%	0.1%	0.2%	0.2%	0.1%	0.2%	0.4%	0.3%
C17:0 Heptadecanoic	0.1%	0.1%	0.3%	0.1%	0.2%	0.2%	0.1%	0.2%	0.7%	0.2%
c17:1w7	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%
C18:0 Stearic	1.6%	1.7%	2.8%	1.6%	1.6%	4.4%	1.5%	2.2%	10.5%	1.5%
C18:1w9t	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C18:1w9c	75.9%	0.7%	1.0%	0.7%	0.7%	0.8%	0.7%	0.7%	0.8%	0.9%
INTERNAL STANDARD										
C18:2w6t	0.1%	0.0%	0.6%	0.1%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%
C18:2w6c Linoleic (LA)	13.5%	67.2%	69.9%	76.5%	67.2%	70.9%	67.1%	64.7%	52.0%	74.2%
C20:0 Arachidic	0.4%	0.3%	0.4%	0.2%	0.3%	0.3%	0.2%	0.2%	0.5%	0.3%
C18:3w6 γ-linolenic (GLA)	0.0%	20.4%	10.6%	11.2%	19.9%	12.9%	21.1%	21.4%	16.3%	11.7%
C20:1w9	0.2%	0.1%	0.1%	0.1%	0.1%	0.1%	0.0%	0.1%	0.0%	0.1%
C18:3w3, α-linolenic (ALA)	0.1%	0.2%	0.2%	0.2%	0.2%	0.1%	0.2%	0.2%	0.7%	0.3%
C21:0 Heneicosanoic	0.0%	0.0%	0.0%	0.1%	0.0%	0.1%	0.0%	0.1%	0.1%	0.0%
C20:2w6 Eicosadienoic	0.1%	0.1%	0.1%	0.1%	0.1%	0.0%	0.1%	0.1%	0.1%	0.1%
C22:0 Behenic	0.3%	0.2%	0.2%	0.2%	0.1%	0.2%	0.1%	0.2%	0.2%	0.2%
C20:3w6 Dihomo-γ-linolenic (DGLA)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Table 5

Fatty Acids	Individual Seed Samples of Transgenic Line S5									
	NULL	S5-1	S5-2	S5-3	S5-4	S5-5	S5-6	S5-7	S5-8	S5-9
C22:1w9 Erucic	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C20:3w3	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C20:4w6 Arachidonic (AA)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C23:0 Tricosanoic	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C22:2w6	0.0%	0.0%	0.2%	0.1%	0.0%	0.0%	0.0%	0.1%	0.1%	0.0%
C24:0 Lignoceric	0.2%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.2%	0.2%
C20:5w3 Eicosapentaenoic (EPA)	0.0%	0.1%	0.2%	0.1%	0.1%	0.0%	0.0%	0.0%	0.1%	0.1%
C24:1w9c	0.2%	0.2%	0.1%	0.2%	0.1%	0.1%	0.2%	0.2%	0.3%	0.2%
C22:6w3 Docosahexaenoic (DHA)	0.2%	0.1%	0.1%	0.0%	0.0%	0.3%	0.1%	0.1%	0.7%	0.1%
Total fatty acids	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Saturated fatty acids	9.3%	10.6%	16.0%	10.4%	11.1%	14.1%	10.2%	12.0%	27.8%	11.8%
Total W7's & W5's	0.3%	0.3%	0.7%	0.2%	0.3%	0.3%	0.2%	0.3%	0.8%	0.5%
Total W9's	76.3%	1.0%	1.3%	1.1%	1.0%	1.1%	0.9%	1.0%	1.2%	1.3%
Total W6's	13.6%	87.7%	81.0%	87.9%	87.2%	84.0%	88.3%	86.3%	68.6%	86.0%
Total W3's	0.3%	0.3%	0.4%	0.3%	0.3%	0.5%	0.3%	0.3%	1.5%	0.4%
Total monounsaturated fatty acids	76.7%	1.3%	1.9%	1.3%	1.3%	1.4%	1.1%	1.3%	1.9%	1.7%
Total trans fatty acids	0.1%	0.0%	0.6%	0.1%	0.1%	0.0%	0.0%	0.1%	0.1%	0.1%
Polyunsaturated fatty acids	13.9%	88.1%	81.4%	88.2%	87.5%	84.4%	88.6%	86.6%	70.2%	86.4%
Ratios:										
Polyunsaturated/saturated	1.5	8.3	5.1	8.5	7.9	6.0	8.7	7.2	2.5	7.3
Omega 6/Omega 3	44.5	260.6	180.6	293.7	299.0	183.9	258.7	276.3	44.4	207.7
AA / EPA	0.1	0.1	0.2	0.3	0.3	0.1	0.1	0.2	0.1	0.4
AA / DHA	0.0	0.1	0.3	0.6	0.4	0.0	0.1	0.1	0.0	0.4

Table 6

Fatty Acids	Individual Seed Samples of Transgenic Line S24									
	S24-1	S24-2	S24-3	S24-4	S24-5	S24-6	S24-7	S24-8	S24-9	S24-10
C10:0 Capric	0.3%	0.5%	0.5%	0.7%	0.4%	0.5%	0.3%	0.4%	0.3%	0.5%
C11:0	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C12:0 Lauric	0.1%	0.1%	0.1%	0.1%	0.1%	0.2%	0.1%	0.1%	0.1%	0.1%
C13:0 Tridecanoic	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C14:0 Myristic	0.3%	0.3%	0.2%	0.5%	0.3%	0.5%	0.3%	0.2%	0.3%	0.2%
C14:1w5, Myristoleic	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%
C15:0 Pentadecanoic	0.1%	0.1%	0.0%	0.1%	0.1%	0.1%	0.1%	0.1%	0.0%	0.1%
C15:1w5cis 10-Pentadecenoid	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C16:0 Palmitic	7.3%	6.7%	7.1%	8.4%	8.2%	9.8%	7.3%	6.5%	7.9%	5.4%
C16:1w7c Palmitoleic	0.1%	0.1%	0.2%	0.3%	0.1%	0.2%	0.1%	0.1%	0.1%	0.2%
C17:0 Heptadecanoic	0.2%	0.2%	0.1%	0.3%	0.2%	0.3%	0.1%	0.2%	0.2%	0.2%
c17:1w7	0.0%	0.0%	0.1%	0.0%	0.1%	0.0%	0.1%	0.1%	0.5%	0.5%
C18:0 Stearic	3.0%	2.7%	2.9%	4.9%	3.9%	5.3%	3.4%	1.7%	3.6%	2.1%
C18:1w9t	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C18:1w9c	3.7%	4.2%	4.7%	2.5%	3.7%	2.0%	5.3%	3.3%	3.4%	73.9%
INTERNAL STANDARD										
C18:2w6t	0.0%	0.0%	0.0%	0.1%	0.0%	0.1%	0.1%	0.1%	0.1%	0.1%

Table 6

Fatty Acids	Individual Seed Samples of Transgenic Line S24									
	S24-1	S24-2	S24-3	S24-4	S24-5	S24-6	S24-7	S24-8	S24-9	S24-10
C18:2w6c Linoleic (LA)	50.8%	53.1%	57.3%	35.3%	47.1%	35.6%	51.8%	54.9%	50.0%	8.4%
C20:0 Arachidic	0.2%	0.2%	0.2%	0.3%	0.3%	0.3%	0.3%	0.2%	0.2%	0.3%
C18:3w6 γ-linolenic (GLA)	32.1%	30.4%	25.3%	43.7%	34.0%	43.3%	29.3%	30.7%	31.7%	6.4%
C20:1w9	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.3%
C18:3w3, α-linolenic (ALA)	0.3%	0.3%	0.3%	0.5%	0.3%	0.5%	0.3%	0.4%	0.4%	0.1%
C21:0 Heneicosanoic	0.1%	0.1%	0.1%	0.2%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
C20:2w6 Eicosadienoic	0.1%	0.1%	0.1%	0.2%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
C22:0 Behenic	0.2%	0.2%	0.1%	0.3%	0.2%	0.2%	0.2%	0.1%	0.1%	0.3%
C20:3w6 Dihomo-γ-linolenic (DGLA)	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C22:1w9 Erucic	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C20:3w3	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C20:4w6 Arachidonic (AA)	0.1%	0.0%	0.0%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%
C23:0 Tricosanoic	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C22:2w6	0.1%	0.0%	0.0%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%
C24:0 Lignoceric	0.1%	0.1%	0.1%	0.2%	0.1%	0.1%	0.1%	0.1%	0.1%	0.2%
C20:5w3 Eicosapentaenoic (EPA)	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.0%
C24:1w9c	0.1%	0.1%	0.1%	0.3%	0.1%	0.2%	0.2%	0.1%	0.2%	0.2%
C22:6w3 Docosahexaenoic (DHA)	0.3%	0.1%	0.2%	0.4%	0.2%	0.2%	0.2%	0.0%	0.2%	0.3%
Total fatty acids	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Saturated fatty acids	11.9%	11.1%	11.5%	16.0%	13.9%	17.5%	12.1%	9.7%	13.0%	9.5%
Total W7's & W5's	0.1%	0.2%	0.2%	0.4%	0.3%	0.2%	0.3%	0.2%	0.6%	0.8%
Total W9's	4.0%	4.5%	4.9%	3.0%	4.0%	2.2%	5.6%	3.6%	3.6%	74.3%
Total W6's	83.2%	83.7%	82.8%	79.4%	81.2%	79.2%	81.3%	85.8%	81.9%	14.9%
Total W3's	0.7%	0.5%	0.5%	1.1%	0.6%	0.8%	0.5%	0.6%	0.7%	0.4%
Total monounsaturated fatty acids	4.2%	4.6%	5.2%	3.4%	4.2%	2.5%	6.0%	3.8%	4.3%	75.1%
Total trans fatty acids	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Polyunsaturated fatty acids	83.9%	84.2%	83.3%	80.5%	81.9%	79.9%	81.8%	86.4%	82.6%	15.3%
Ratios:										
Polyunsaturated/saturated	7.1	7.6	7.2	5.0	5.9	4.6	6.7	8.9	6.4	1.6
Omega 6/Omega 3	111.6	162.9	162.1	73.1	128.9	102.1	169.0	149.7	112.7	37.8
AA / EPA	0.5	0.4	0.6	0.6	0.7	1.0	0.7	0.4	0.4	0.3
AA / DHA	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.8	0.2	0.0

Table 7

	Individual Seed Samples of Transgenic Line S27								
	NULL	S27-1	S27-2	S27-3	S27-4	S27-5	S27-6	S27-7	S27-8
C10:0 Capric	0.6%	0.6%	0.4%	0.4%	0.6%	0.4%	0.5%	0.3%	0.4%
C11:0	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C12:0 Lauric	0.2%	0.2%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
C13:0 Tridecanoic	0.1%	0.0%	0.0%	0.1%	0.1%	0.0%	0.1%	0.0%	0.0%
C14:0 Myristic	0.4%	0.4%	0.3%	0.3%	0.4%	0.2%	0.3%	0.3%	0.3%
C14:1w5, Myristoleic	0.1%	0.1%	0.1%	0.1%	0.1%	0.0%	0.2%	0.1%	0.1%
C15:0 Pentadecanoic	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
C15:1w5cis 10-Pentadecenoid	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Table 7

	Individual Seed Samples of Transgenic Line S27								
	NULL	S27-1	S27-2	S27-3	S27-4	S27-5	S27-6	S27-7	S27-8
C16:0 Palmitic	7.4%	8.0%	8.7%	10.6%	8.6%	7.6%	9.0%	8.9%	8.1%
C16:1w7c Palmitoleic	0.3%	0.3%	0.2%	0.2%	0.2%	0.1%	0.2%	0.1%	0.2%
C17:0 Heptadecanoic	0.3%	0.2%	0.2%	0.2%	0.2%	0.2%	0.1%	0.1%	0.3%
c17:1w7	0.0%	0.0%	0.0%	0.0%	0.4%	0.4%	0.6%	0.3%	0.1%
C18:0 Stearic	5.0%	3.6%	3.6%	3.7%	5.1%	3.0%	4.1%	2.7%	3.7%
C18:1w9t	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C18:1w9c	66.9%	2.8%	1.8%	3.2%	3.4%	3.7%	3.4%	1.6%	3.4%
INTERNAL STANDARD									
C18:2w6t	0.1%	0.1%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.2%
C18:2w6c Linoleic (LA)	16.2%	46.6%	31.5%	48.8%	45.4%	55.7%	50.8%	35.7%	53.3%
C20:0 Arachidic	0.5%	0.3%	0.4%	0.5%	0.4%	0.2%	0.5%	0.3%	0.3%
C18:3w6 γ-linolenic (GLA)	0.0%	34.4%	50.7%	29.8%	33.1%	26.8%	28.1%	47.7%	27.8%
C20:1w9	0.3%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
C18:3w3, α-linolenic (ALA)	0.2%	0.6%	0.6%	0.5%	0.5%	0.5%	0.7%	0.7%	0.4%
C21:0 Heneicosanoic	0.1%	0.1%	0.1%	0.1%	0.1%	0.0%	0.1%	0.1%	0.1%
C20:2w6 Eicosadienoic	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
C22:0 Behenic	0.2%	0.2%	0.2%	0.3%	0.2%	0.1%	0.2%	0.2%	0.2%
C20:3w6 Dihomo-γ-linolenic (DGLA)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C22:1w9 Erucic	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C20:3w3	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C20:4w6 Arachidonic (AA)	0.0%	0.0%	0.1%	0.0%	0.1%	0.1%	0.0%	0.1%	0.1%
C23:0 Tricosanoic	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C22:2w6	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C24:0 Lignoceric	0.2%	0.2%	0.1%	0.3%	0.1%	0.1%	0.2%	0.1%	0.1%
C20:5w3 Eicosapentaenoic (EPA)	0.1%	0.1%	0.1%	0.1%	0.1%	0.0%	0.1%	0.1%	0.1%
C24:1w9c	0.3%	0.2%	0.1%	0.2%	0.1%	0.1%	0.2%	0.1%	0.2%
C22:6w3 Docosahexaenoic (DHA)	0.3%	0.3%	0.1%	0.3%	0.4%	0.1%	0.0%	0.0%	0.3%
Total Fatty acids	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Saturated Fatty acids	15.1%	14.0%	14.4%	16.6%	15.9%	12.2%	15.4%	13.2%	13.7%
Total W7's & W5's	0.4%	0.5%	0.3%	0.3%	0.7%	0.6%	1.0%	0.5%	0.3%
Total W9's	67.4%	3.1%	2.1%	3.4%	3.6%	3.9%	3.7%	1.8%	3.7%
Total W6's	16.4%	81.2%	82.5%	78.8%	78.7%	82.7%	79.0%	83.6%	81.3%
Total W3's	0.6%	1.0%	0.7%	0.8%	0.9%	0.6%	0.8%	0.8%	0.8%
Total Monounsaturated Fatty acids	67.9%	3.6%	2.4%	3.7%	4.3%	4.5%	4.7%	2.3%	4.0%
Total Trans Fatty Acids	0.1%	0.1%	0.1%	0.0%	0.1%	0.0%	0.1%	0.1%	0.2%
Polyunsaturated Fatty acids	17.0%	82.2%	83.2%	79.6%	79.6%	83.3%	79.8%	84.4%	82.1%
Ratios:									
Polyunsaturated/Saturated	1.1	5.9	5.8	4.8	5.0	6.8	5.2	6.4	6.0
Omega 6/Omega 3	29.3	78.7	113.9	95.8	83.3	139.2	102.0	109.9	107.0
AA / EPA	0.3	0.3	1.1	0.5	0.9	1.2	0.6	1.0	1.0
AA / DHA	0.1	0.1	0.8	0.2	0.1	0.7	2.6	1.3	0.2

[0083] The single seed data follow the trend seen in the pooled seed data. Since T1 lines are still segregating, some variability can be present in single seed samples due to null, heterozygous and homozygous insertions. Observed are GLA concentrations ranging from 1.4% (Table 3: seed 1 in line 1, S1-1) to 50.8% (Table 7: seed 2 line 27, S27-2). Lines with seed oil profiles similar to those from either the single seed data or pooled seed data may be obtained. Certain lines did not set seed. Those that set seed were selected for the study.

[0084] Fatty acid composition of seed from T1 and T2 generations of lines expressing the pSBS4766 construct is shown below in Table 8.

[0085] Table 8: Examples of single seed fatty acid composition (expressed as percentages) in T1 and T2 individual lines of pSBS4766 construct expressed in S317

Table 8 **Line Number**	**Generation:**	**C18:3n6 (gamma Linolenic)**	**C16:0 (Palmitic)**	**C18:0 (Stearic)**	**C18:1n9 (Oleic)**	**C18:2n6 (Linoleic)**
4766-12-4	T1	25.60	6.78	1.90	5.38	59.12
4766-12-4-6	T2	23.38	8.54	3.57	7.66	56.27
4766-21-25	T1	26.10	7.83	1.91	5.36	58.53
4766-21-25-2	T2	24.41	8.45	3.56	9.92	53.67
4766-21-10	T1	15.35	7.15	1.71	9.37	65.53
4766-21-10-7	T2	25.31	7.00	2.73	7.94	55.17
4766-70-43	T1	17.68	4.87	2.05	10.88	64.52
4766-70-43-9	T2	16.75	4.80	2.33	10.58	64.80
4766-110-10	T1	23.37	6.65	2.00	5.77	61.26
4766-110-10-25	T2	29.84	8.27	3.66	6.51	50.59
4766-110-11	T1	19.65	6.66	2.06	7.48	63.85
4766-110-11-32	T2	29.89	8.43	2.25	5.11	52.26
4766-95-4	T1	10.22	6.20	2.00	15.52	65.06
4766-95-4-1	T2	18.05	6.72	2.24	11.13	61.12
S317	VAR	0.00	5.29	2.72	74.81	16.10
S317	VAR	0.00	5.44	1.64	74.61	17.82

[0086] Fatty acid composition of T2 seed is consistent with that measured in T1 seed. These data show that the transgene is stable and heritable, producing consistent elevations in GLA across generations.

Example 2: Plasmid pSBS4119 and transgenic plants expressing this plasmid.

[0087] FIG. 9 shows the map of a construct used to express the Δ6-desaturase from *Saprolegnia diclina*. The plant selectable marker used in this construct was *pat* which corresponds to the phosphinothricin acetyl transferase gene from *Streptomyces viridochromogenes*. The bacterial marker used in this construct was *SpecR*. The base binary vector used to construct this vector is a derivative of pPZP200. See Hajdukiewicz *et al.*, Plant Mol Biol 25: 989, 1994. The sequence of the insert contained within the borders of the pPZP200 plasmid is shown below.

[0088] pSBS4119 (*S. diclina* Δ6-desaturase expression cassette with PAT selection) (SEQ ID NO: 2)

[0089] ctgcaggaattcgatctctattgattcaaattacgatctgatactgataacgtctagatttttagggttaaagcaatcaatcacctgac
gattcaaggtggttggatcatgacgattccagaaaacatcaagcaagctctcaaagctacactctttgggatcatactgaactctaacaacctc
gttatgtcccgtagtgccagtacagacatcctcgtaactcggattgtgcacgatgccatgactatacccaacctcggtcttggtcacaccagg
aactctctggtaagctagctccactccccagaaacaaccggcgccaaattgcgcgaattgctgacctgaagacggaacatcatcgtcgggt
ccttgggcgattgcggcggaagatgggtcagcttgggcttgaggacgagacccgaatccgagtctgttgaaaaggttgttcattggggattt
gtatacggagattggtcgtcgagaggtttgagggaaaggacaaatgggtttggctctggagaaagagagtgcggctttagagagagaattg
agaggtttagagagagatgcggcggcgatgagcggaggagagacgacgaggacctgcattatcaaagcagtgacgtggtgaaatttgga
acttttaagaggcagatagatttattatttgtatccattttcttcattgttctagaatgtcgcggaacaaattttaaaactaaatcctaaatttttctaatt
ttgttgccaatagtggatatgtgggccgtatagaaggaatctattgaaggcccaaacccatactgacgagcccaaaggttcgttttgcgttttat
gtttcggttcgatgccaacgccacattctgagctaggcaaaaaacaaacgtgtctttgaatagactcctctcgttaacacatgcagcggctgc
atggtgacgccattaacacgtggcctacaattgcatgatgtctccattgacacgtgacttctcgtctcctttcttaatatatctaacaaacactcct
acctcttccaaaatatatacacatcttttgatcaatctctcattcaaaatctcattctctctagtaaacaagaacaaaaaaccatggtccaggggc
aaaaggccgagaagatctcgtgggcgaccatccgtgagcacaaccgccaagacaacgcgtggatcgtgatccaccacaaggtgtacga
catctcggcctttgaggaccacccgggcggcgtcgtcatgttcacgcaggccggcgaagacgcgaccgatgcgttcgctgtcttccaccc
gagctcggcgctcaagctcctcgagcagtactacgtcggcgacgtcgaccagtcgacggcggccgtcgacacgtcgatctcggacgag
gtcaagaagagccagtcggacttcattgcgtcgtaccgcaagctgcgccttgaagtcaagcgcctcggcttgtacgactcgagcaagctct
actacctctacaagtgcgcctcgacgctgagcattgcgcttgtgtcggcggccatttgcctccactttgactcgacggccatgtacatggtcg
cggctgtcatccttggcctcttttaccagcagtgcggctggctcgcccatgactttctgcaccaccaagtgtttgagaaccacttgtttggcgac
ctcgtcggcgtcatggtcggcaacctctggcagggcttctcggtgcagtggtggaagaacaagcacaacacgcaccatgcgatccccaac
ctccacgcgacgcccgagatcgccttccacggcgacccggacattgacacgatgccgattctcgcgtggtcgctcaagatggcgcagca
cgcggtcgactcgcccgtcgggctcttcttcatgcgctaccaagcgtacctgtactttcccatcttgctctttgcgcgtatctcgtgggtgatcc
agtcggccatgtacgccttctacaacgttgggcccggcggcacctttgacaaggtccagtacccgctgctcgagcgcgccggcctcctcct

ctactacggctggaacctcggccttgtgtacgcagccaacatgtcgctgctccaagcggctgcgttcctctttgtgagccaggcgtcgtgcg
gcctcttcctcgcgatggtctttagcgtcggccacaacggcatggaggtctttgacaaggacagcaagcccgatttttggaagctgcaagtg
ctctcgacgcgcaacgtgacgtcgtcgctctggatcgactggttcatgggcggcctcaactaccagatcgaccaccacttgttcccgatggt
gccccggcacaacctcccggcgctcaacgtgctcgtcaagtcgctctgcaagcagtacgacatcccataccacgagacgggcttcatcgc
gggcatggccgaggtcgtcgtgcacctcgagcgcatctcgatcgagttcttcaaggagtttcccgccatgtaagcttgttaccccactgatgt
catcgtcatagtccaataactccaatgtcggggagttagtttatgaggaataaagtgtttagaatttgatcaggggggagataataaaagccgag
tttgaatctttttgttataagtaatgtttatgtgtgtttctatatgttgtcaaatggtcccatgtttttcttcctctcttttgtaacttgcaagtgttgtgttgt
actttatttggcttctttgtaagttggtaacggtggtctatatatggaaaaggtcttgttttgttaaacttatgttagttaactggattcgtctttaacca
caaaaagttttcaataagctacaaatttagacacgcaagccgatgcagtcattagtacatatatttattgcaagtgattacatggcaacccaaac
ttcaaaaacagtaggttgctccatttagtaacctgaattgcctcctgattctagttgatcccggtaccgaattcgaatccaaaaattacggatatg
aatataggcatatccgtatccgaattatccgtttgacagctagcaacgattgtacaattgcttctttaaaaaaggaagaaagaaagaaagaaaa
gaatcaacatcagcgttaacaaacggccccgttacggcccaaacggtcatatagagtaacggcgttaagcgttgaaagactcctatcgaaat
acgtaaccgcaaacgtgtcatagtcagatcccctcttccttcaccgcctcaaacacaaaaataatcttctacagcctatatatacaacccccct
tctatctctcctttctcacaattcatcatctttctttctctacccccaattttaagaaatcctctcttctcctcttcattttcaaggtaaatctctctctct
ctctctctctgttattccttgttttaattaggtatgtattattgctagtttgttaatctgcttatcttatgtatgccttatgtgaatatctttatcttgttcatctc
atccgtttagaagctataaatttgttgatttgactgtgtatctacacgtggttatgtttatatctaatcagatatgaatttcttcatattgttgcgtttgtgt
gtaccaatccgaaatcgttgatttttttcatttaatcgtgtagctaattgtacgtatacatatggatctacgtatcaattgttcatctgtttgtgtttgtat
gtatacagatctgaaaacatcacttctctcatctgattgtgttgttacatacatagatatagatctgttatatcatttttttattaattgtgtatatatatat
gtgcatagatctggattacatgattgtgattatttacatgattttgttatttacgtatgtatatatgtagatctggacttttttggagttgttgacttgattg
tatttgtgtgtgtatatgtgtgttctgatcttgatatgttatgtatgtgcagccaaggctacgggcgatccaccatgtctccggagaggagacca
gttgagattaggccagctacagcagctgatatggccgcggtttgtgatatcgttaaccattacattgagacgtctacagtgaactttaggacag
agccacaaacaccacaagagtggattgatgatctagagaggttgcaagatagataccttggttggttgctgaggttgagggtgttgtggct
ggtattgcttacgctgggccctggaaggctaggaacgcttacgattggacagttgagagtactgtttacgtgtcacataggcatcaaaggttg
ggcctaggttccacattgtacacacatttgcttaagtctatggaggcgcaaggttttaagtctgtggttgctgttataggccttccaaacgatcca
tctgttaggttgcatgaggctttgggatacacagcccggggtacattgcgcgcagctggatacaagcatggtggatggcatgatgttggttttt
ggcaaagggattttgagttgccagctcctccaaggccagttaggccagttacccagatctgagtcgaccgaatgagttccaagatggtttgtg
acgaagttagttggttgtttttatggaactttgtttaagctagcttgtaatgtggaaagaacgtgtggctttgtggtttttaaatgttggtgaataaag
atgtttcctttggattaactagtatttttcctattggtttcatggttttagcacacaacattttaaatatgctgttagatgatatgctgcctgctttattattt
acttacccctcaccttcagtttcaaagttgttgcaatgactctgtgtagtttaagatcgagtgaaagtagattttgtctatatttattaggggtatttg
atatgctaatggtaaacatggtttatgacagcgtacttttttggttatggtgttgacgtttccttttaaacattatagtagcgtccttggtctgtgttcat
tggttgaacaaaggcacactcacttggagatgccgtctccactgatatttgaacaaa

[0090] Transformation of safflower with this construct was performed by SemBioSys Genetics Inc. (Calgary, Canada). Techniques utilized by SemBioSys Genetics Inc. include those described in WO 2004/111244, which is hereby incorporated by reference in its entirety. Transgenic plants will be grown and seed will be harvested.

[0091] Seeds were collected from transgenic plants and fatty acid composition was performed using a modification of a gas chromatographic method described in "Official Methods and Recommended Practices of the AOCS", 5th Ed., Method Ce 1-62, American Oil Chemists Society: Champaign, Illinois (1997).

[0092] As shown below in Table 9, GLA levels ranged from 11.41% (line 4119-23-1) to 72.89% (line 4119-21-3) in T1 seed from transgenic lines expressing Δ6-desaturase from *S. diclina* in the pSBS4119 construct. GLA levels over 60% were obtained in several transgenic lines. Since T1 lines are still segregating, measurements of single seed samples can vary due to null, heterozygous or homozygous insertions. GLA levels in Centennial controls and Null control lines were not detectable. The Centennial variety is naturally high in LA and transgenic expression of Δ6-desaturase alone is sufficient to increase GLA levels.

[0093] Table 9: Examples of single seed fatty acid composition (expressed as percentages) in T1 seed of pSBS4119 construct expressed in Centennial

Table 9 Line Number	Type	C18:3n6 (gamma Linolenic)	C16:0 (Palmitic)	C18:0 (Stearic)	C18:1n9 (Oleic)	C18:2n6 (Linoleic)
4119-13-1	Transgenic	46.47	7.11	1.55	7.98	35.87
4119-13-11	Transgenic	51.73	7.07	1.57	6.66	32.00
4119-15-10	Transgenic	61.93	8.02	1.69	6.38	19.68
4119-15-7	Transgenic	69.59	8.03	1.43	5.70	13.33
4119-17-1	Transgenic	69.13	9.58	1.35	5.37	12.06
4119-17-3	Transgenic	67.13	9.33	1.54	6.76	12.29
4119-19-1	NULL	0.00	6.54	1.35	10.23	80.86
4119-19-10	Transgenic	69.85	8.13	1.35	5.42	13.70
4119-20-10	Transgenic	63.22	7.69	1.53	5.88	20.24
4119-21-1	Transgenic	71.06	8.94	1.43	5.02	11.44
4119-21-3	Transgenic	72.89	9.68	1.21	4.12	8.59
4119-2-29	Transgenic	52.33	7.46	1.59	7.00	30.46
4119-2-31	Transgenic	61.23	8.52	1.48	7.38	19.40
4119-23-1	Transgenic	11.41	6.34	1.41	9.28	71.57

Table 9 Line Number	Type	C18:3n6 (gamma Linolenic)	C16:0 (Palmitic)	C18:0 (Stearic)	C18:1n9 (Oleic)	C18:2n6 (Linoleic)
4119-23-2	Transgenic	11.99	6.51	1.48	9.07	70.95
4119-24-1	NULL	0.00	6.62	1.35	10.12	80.69
4119-24-2	Transgenic	65.39	8.04	1.46	6.47	16.90
4119-29-2	Transgenic	62.91	7.68	1.30	6.82	19.44
4119-29-4	Transgenic	62.72	7.42	1.31	6.95	19.74
4119-30-1	Transgenic	66.46	7.75	1.41	6.53	16.16
4119-30-10	Transgenic	28.28	5.97	1.59	6.46	56.93
4119-33-15	Transgenic	72.85	8.33	1.32	4.92	10.17
4119-33-18	Transgenic	69.73	7.53	1.33	5.90	13.29
4119-35-1	Transgenic	59.55	7.63	1.56	10.82	17.91
4119-35-3	Transgenic	63.11	7.27	1.29	5.93	20.63
4119-36-14	Transgenic	64.90	8.19	1.41	5.85	17.98
4119-36-15	Transgenic	61.10	8.30	1.39	8.22	19.07
4119-39-17	Transgenic	63.54	7.72	1.65	5.79	19.38
4119-39-18	Transgenic	64.79	7.66	1.57	5.11	18.68
Centennial-4	Control	0.00	6.63	2.22	25.36	65.80
Centennial-6	Control	0.00	6.59	2.03	13.53	76.87

Example 3. Plasmid pSBS4763 and transgenic plants expressing this plasmid.

[0094] FIG. 10 shows the map of a construct used to express the Δ6-desaturase from *Mortierella alpina*. The plant selectable marker used in this construct was *pat* which corresponds to the phosphinothricin acetyl transferase gene from *Streptomyces viridochromogenes*. The bacterial marker used in this construct was *SpecR*. The base binary vector used to construct this vector is a derivative of pPZP200. See Hajdukiewicz *et al.*, Plant Mol Biol 25: 989 (1994). The sequence of the insert contained within the borders of the pPZP200 plasmid is shown below.

[0095] pSBS4763 (*M. alpina* Δ6-desaturase expression cassette with PAT selection) (SEQ ID NO: 3)

[0096] ctgcaggaattcgatctctattgattcaaattacgatctgatactgataacgtctagatttttagggttaaagcaatcaatcacctgac
gattcaaggtggttggatcatgacgattccagaaaacatcaagcaagctctcaaagctacactctttgggatcatactgaactctaacaacctc
gttatgtcccgtagtgccagtacagacatcctcgtaactcggattgtgcacgatgccatgactatacccaacctcggtcttggtcacaccagg
aactctctggtaagctagctccactccccagaaacaaccggcgccaaattgcgcgaattgctgacctgaagacggaacatcatcgtcgggt
ccttgggcgattgcggcggaagatgggtcagcttgggcttgaggacgagacccgaatccgagtctgttgaaaaggttgttcattggggattt

gtatacggagattggtcgtcgagaggtttgagggaaaggacaaatgggtttggctctggagaaagagagtgcggctttagagagagaattg
agaggtttagagagagatgcggcggcgatgagcggaggagagacgacgaggacctgcattatcaaagcagtgacgtggtgaaatttgga
acttttaagaggcagatagatttattatttgtatccattttcttcattgttctagaatgtcgcggaacaaattttaaaactaaatcctaaatttttctaatt
ttgttgccaatagtggatatgtgggccgtatagaaggaatctattgaaggcccaaacccatactgacgagcccaaaggttcgttttgcgttttat
gtttcggttcgatgccaacgccacattctgagctaggcaaaaaacaaacgtgtctttgaatagactcctctcgttaacacatgcagcggctgc
atggtgacgccattaacacgtggcctacaattgcatgatgtctccattgacacgtgacttctcgtctcctttcttaatatatctaacaaacactcct
acctcttccaaaatatatacacatctttttgatcaatctctcattcaaaatctcattctctctagtaaacaagaacaaaaaaccatggctgctgctcc
cagtgtgaggacgtttactcgggccgaggttttgaatgccgaggctctgaatgagggcaagaaggatgccgaggcacccttcttgatgatc
atcgacaacaaggtgtacgatgtccgcgagttcgtccctgatcatcccggtggaagtgtgattctcacgcacgttggcaaggacggcactg
acgtctttgacacttttcaccccgaggctgcttgggagactcttgccaacttttacgttggtgatattgacgagagcgaccgcgatatcaagaat
gatgactttgcggccgaggtccgcaagctgcgtaccttgttccagtctcttggttactacgattcttccaaggcatactacgccttcaaggtctc
gttcaacctctgcatctggggtttgtcgacggtcattgtggccaagtggggccagacctcgaccctcgccaacgtgctctcggctgcgctttt
gggtctgttctggcagcagtgcggatggttggctcacgacttttgcatcaccaggtcttccaggaccgtttctggggtgatcttttcggcgcct
tcttgggaggtgtctgccagggcttctcgtcctcgtggtggaaggacaagcacaacactcaccacgccgcccccaacgtccacggcgagg
atcccgacattgacacccaccctctgttgacctggagtgagcatgcgttggagatgttctcggatgtcccagatgaggagctgacccgcatg
tggtcgcgtttcatggtcctgaaccagacctggttttacttccccattctctcgtttgcccgtctctcctggtgcctccagtccattctctttgtgctg
cctaacggtcaggcccacaagccctcgggcgcgcgtgtgcccatctcgttggtcgagcagctgtcgcttgcgatgcactggacctggtacc
tcgccaccatgttcctgttcatcaaggatcccgtcaacatgctggtgtacttttggtgtcgcaggcggtgtgcggaaacttgttggcgatcgtg
ttctcgctcaaccacaacggtatgcctgtgatctcgaaggaggaggcggtcgatatggatttcttcacgaagcagatcatcacgggtcgtgat
gtccacccgggtctatttgccaactggttcacgggtggattgaactatcagatcgagcaccacttgttcccttcgatgcctcgccacaacttttc
aaagatccagcctgctgtcgagaccctgtgcaaaaagtacaatgtccgataccacaccaccggtatgatcgagggaactgcagaggtcttt
agccgtctgaacgaggtctccaaggctgcctccaagatgggtaaggcgcagtaagcttgttaccccactgatgtcatcgtcatagtccaata
actccaatgtcggggagttagtttatgaggaataaagtgtttagaatttgatcaggggggagataataaaagccgagtttgaatctttttgttataa
gtaatgtttatgtgtgtttctatatgttgtcaaatggtcccatgtttttcttcctctcttttgtaacttgcaagtgttgtgttgtactttatttggcttctttgt
aagttggtaacggtggtctatatatggaaaaggtcttgttttgttaaacttatgttagttaactggattcgtctttaaccacaaaaagttttcaataag
ctacaaatttagacacgcaagccgatgcagtcattagtacatatatttattgcaagtgattacatggcaacccaaacttcaaaaacagtaggttg
ctccatttagtaacctgaattgcctcctgattctagttgatcccggtaccgaattcgaatccaaaaattacggatatgaatataggcatatccgtat
ccgaattatccgtttgacagctagcaacgattgtacaattgcttctttaaaaaaggaagaaagaaagaaagaaaagaatcaacatcagcgtta
acaaacggccccgttacggcccaaacggtcatatagagtaacggcgttaagcgttgaaagactcctatcgaaatacgtaaccgcaaacgtg
tcatagtcagatcccctcttccttcaccgcctcaaacacaaaaataatcttctacagcctatatatacaaccccccttctatctctcctttctcaca
attcatcatctttctttctctacccccaattttaagaaatcctctcttctcctcttcattttcaaggtaaatctctctctctctctctctctgttattccttg

ttttaattaggtatgtattattgctagtttgttaatctgcttatcttatgtatgccttatgtgaatatctttatcttgttcatctcatccgtttagaagctataa
atttgttgatttgactgtgtatctacacgtggttatgtttatatctaatcagatatgaatttcttcatattgttgcgtttgtgtgtaccaatccgaaatcgt
tgatttttttcatttaatcgtgtagctaattgtacgtatacatatggatctacgtatcaattgttcatctgtttgtgtttgtatgtatacagatctgaaaac
atcacttctctcatctgattgtgttgttacatacatagatatagatctgttatatcatttttttattaattgtgtatatatatatgtgcatagatctggattac
atgattgtgattatttacatgattttgttatttacgtatgtatatatgtagatctggacttttttggagttgttgacttgattgtatttgtgtgtgtatatgtgt
gttctgatcttgatatgttatgtatgtgcagccaaggctacgggcgatccaccatgtctccggagaggagaccagttgagattaggccagcta
cagcagctgatatggccgcggtttgtgatatcgttaaccattacattgagacgtctacagtgaactttaggacagagccacaaacaccacaag
agtggattgatgatctagagaggttgcaagatagatacccttggttggttgctgaggttgagggtgttgtggctggtattgcttacgctgggcc
ctggaaggctaggaacgcttacgattggacagttgagagtactgtttacgtgtcacataggcatcaaaggttgggcctaggttccacattgta
cacacatttgcttaagtctatggaggcgcaaggttttaagtctgtggttgctgttataggccttccaaacgatccatctgttaggttgcatgaggc
tttgggatacacagcccggggtacattgcgcgcagctggatacaagcatggtggatggcatgatgttggtttttggcaaagggattttgagtt
gccagctcctccaaggccagttaggccagttacccagatctgagtcgaccgaatgagttccaagatggtttgtgacgaagttagttggttgttt
ttatggaactttgtttaagctagcttgtaatgtggaaagaacgtgtggctttgtggtttttaaatgttggtgaataaagatgtttcctttggattaacta
gtatttttcctattggtttcatggttttagcacacaacattttaaatatgctgttagatgatatgctgcctgctttattatttacttacccctcaccttcag
tttcaaagttgttgcaatgactctgtgtagtttaagatcgagtgaaagtagattttgtctatatttattaggggtatttgatatgctaatggtaaacat
ggtttatgacagcgtacttttttggttatggtgttgacgtttccttttaaacattatagtagcgtccttggtctgtgttcattggttgaacaaaggcac
actcacttggagatgccgtctccactgatatttgaaca

[0097] Transformation of safflower with this construct was performed by SemBioSys Genetics Inc. (Calgary, Canada). Techniques utilized by SemBioSys Genetics Inc. include those described in WO 2004/111244, which is hereby incorporated by reference in its entirety. Transgenic plants will be grown and seed will be harvested.

[0098] Seeds were collected from transgenic plants and fatty acid composition was performed using a modification of a gas chromatographic method described in "Official Methods and Recommended Practices of the AOCS", 5th Ed., Method Ce 1-62, American Oil Chemists Society: Champaign, Illinois (1997).

[0099] As shown below in Table 10, GLA levels ranged from 7.8% (line 4763-13-2) to 50.19% (line 4763-28-1) in T1 seed from transgenic lines expressing Δ6-desaturase from *M. alpina* in the pSBS4763 construct. Since T1 lines are still segregating, measurements of single seed samples can vary due to null, heterozygous or homozygous insertions. GLA levels in Centennial controls

and Null control lines were 0.05 or below. LA levels in Centennial are naturally high and GLA levels in Centennial can be increased with the expression of Δ6-desaturase only.

[00100] Table 10: Examples of single seed fatty acid composition of T1 seed of pSBS4763 construct expressed in Centennial

Table 10 Line Number	Type	C18:3n6 (gamma Linolenic)	C16:0 (Palmitic)	C18:0 (Stearic)	C18:1n9 (Oleic)	C18:2n6 (Linoleic)
4763-1-1	Transgenic	8.36	6.41	1.50	7.70	74.82
4763-1-2	Transgenic	14.28	6.26	1.56	9.01	67.69
4763-2-1	Transgenic	16.29	6.56	1.53	8.19	66.38
4763-2-2	Transgenic	11.31	6.46	1.59	9.12	70.23
4763-13-2	Transgenic	7.80	6.54	1.53	8.69	74.06
4763-13-3	NULL	0.05	6.27	1.33	8.16	82.98
4763-15-1	Transgenic	11.22	6.24	1.26	7.91	70.36
4763-15-2	Transgenic	19.40	6.56	2.43	7.65	62.65
4763-16-1	Transgenic	17.94	6.22	1.42	7.29	66.36
4763-16-2	Transgenic	11.79	6.08	1.86	7.97	70.78
4763-17-2	NULL	0.04	6.33	1.37	9.19	81.84
4763-17-3	Transgenic	8.43	6.52	1.53	9.56	72.75
4763-18-2	Transgenic	8.73	6.81	2.00	9.33	70.68
4763-18-3	NULL	0.00	6.68	1.88	9.38	80.91
4763-19-4	Transgenic	12.71	6.72	1.87	7.16	68.74
4763-19-15	Transgenic	14.55	6.46	1.75	7.41	67.84
4763-21-2	Transgenic	20.62	6.89	2.37	5.51	59.73
4763-21-11	Transgenic	20.99	6.93	1.77	6.12	61.40
4763-22-4	Transgenic	10.55	6.45	1.53	7.47	73.23
4763-22-5	Transgenic	16.32	6.71	1.47	8.05	66.28
4763-23-12	Transgenic	34.02	6.92	2.06	5.21	49.27
4763-23-14	Transgenic	36.92	7.58	1.60	7.20	45.69
4763-24-6	Transgenic	17.67	8.80	3.89	7.22	56.08
4763-24-7	Transgenic	14.42	8.78	5.30	9.12	57.06
4763-25-2	Transgenic	18.05	8.68	4.35	7.01	54.70
4763-25-3	Transgenic	26.62	10.06	7.29	6.10	38.93
4763-27-3	Transgenic	40.91	8.92	3.40	5.04	24.89
4763-27-9	Transgenic	19.61	14.67	15.70	3.95	19.56
4763-28-1	Transgenic	50.19	9.71	1.88	6.14	30.45
4763-28-2	Transgenic	37.35	7.78	1.61	6.18	46.12
4763-30-12	Transgenic	8.04	7.22	2.11	7.87	73.03
4763-30-13	Transgenic	9.08	7.55	2.17	9.44	69.75
Centennial-1	Control	0.00	6.33	2.18	15.74	74.86
Centennial-3	Control	0.00	6.97	2.13	13.92	76.18

[00101] These data show that Δ6-desaturases from a variety of sources can be used to increase GLA production in safflower. Transgenic expression of Δ6-desaturase in a plant variety that is naturally high in LA, as is the Centennial variety, is effective at increasing GLA content.

[00102] The following statements of the invention are intended to characterize possible elements of the invention according to the foregoing description given in the specification.

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 40

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 40

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

WE CLAIM:

1. A safflower plant that produces seeds comprising at least 1% by weight GLA.

2. The seed of a plant of claim 1.

3. The oil of a seed of claim 2.

4. The oil of claim 3, wherein said oil comprises about 1-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30–35, 35-40, 40-45, 45-50, 50-55 or 55-60% or greater by weight GLA.

5. A transgenic safflower plant comprising a recombinant promoter functional in said safflower plant wherein said promoter is operably linked to a recombinant DNA sequence encoding a Δ6-desaturase, wherein said safflower plant produces seeds and said seeds comprise at least 1% by weight GLA.

6. The transgenic safflower plant of claim 5, wherein said desaturase encoding sequence is a plant or fungal desaturase.

7. The transgenic safflower plant of claim 6,wherein said desaturase encoding sequence is chosen from the group consisting of *Mucor, Saprolegnia, Saprolegnia diclina, Mortierella*, *Mortierella alpina, Conidiobolus*, *Pythium*, *Phytophthora*, *Penicillium*, *Porphyridium*, *Coidosporium*, *Mucor circinelloides*, *Fusarium*, *Aspergillus*, *Candida, Rhodotorula*, *Entomophthora*, *Thraustochytrium, Saprolegnia, Borago, Primula*, sunflower, canola, rice, and moss Δ6-desaturase.

8. The transgenic safflower plant of claim 5, wherein said promoter is a seed specific promoter.

9. The transgenic safflower plant of claim 8, wherein the seed specific promoter is an oleosin promoter or a linin promoter.

10. Seed derived from the transgenic plant of claims 5, 6, 7, 8, or 9, wherein GLA levels in the seed are at least 1% by weight of the total fatty acid content of said seed.

11. Oil produced from the seed of claim 10.

12. The oil of claim 11, wherein said oil comprises about 1-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30–35, 35-40, 40-45, 45-50, 50-55 or 55-60% or greater by weight GLA.

13. A transgenic safflower plant comprising:

a first recombinant DNA sequence encoding a Δ6-desaturase, and

a second recombinant DNA sequence encoding a Δ12-desaturase,

wherein said recombinant DNA sequences are operably linked to at least one promoter and

wherein said safflower plant produces seeds that comprise at least 1% by weight GLA.

14. The transgenic safflower plant of claim 13, wherein said Δ6-desaturase and Δ12-desaturase encoding sequence is a plant or fungal desaturase.

15. The transgenic safflower plant of claim 14, wherein said desaturase encoding sequence is chosen from the group consisting of *Mucor, Saprolegnia, Saprolegnia diclina, Mortierella*, *Mortierella alpina, Conidiobolus*, *Pythium*, *Phytophthora*, *Penicillium*, *Porphyridium*,

Coidosporium, *Mucor circinelloides*, *Fusarium*, *Aspergillus*, *Candida*, *Euphorbia*, *Dimorphoteca*, *Rhodotorula*, *Entomophthora*, *Thraustochytrium*, *Saprolegnia*, *Borago*, *Primula*, sunflower, canola, rice, and moss Δ6- and Δ12-desaturase.

16. The transgenic safflower plant of claim 13, wherein said promoter is a seed specific promoter.

17. The transgenic safflower plant of claim 16, wherein the seed specific promoter is an oleosin or a linin promoter.

18. Seed derived from the transgenic plant of claims 13, 14, 15, 16, or 17, wherein GLA levels in the seed are at least 1% of total fatty acid content.

19. Oil produced from the seed of claim 18.

20. The oil of claim 19, wherein said oil comprises about 1-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-45, 45-50, 50-55 or 55-60% or greater by weight GLA.

21. A method for producing GLA in a safflower seed, said method comprising growing a safflower plant comprising a recombinant promoter functional in said safflower plant wherein said promoter is operably linked to a recombinant DNA sequence encoding a Δ6-desaturase, wherein said safflower plant is grown under conditions whereby said Δ6-desaturase sequence is expressed.

22. The method of claim 21, wherein said Δ6-desaturase and Δ12-desaturase encoding sequence is a plant or fungal desaturase.

23. The method of claim 22, wherein said desaturase encoding sequence is chosen from the group consisting of *Mucor*, *Saprolegnia*,

Saprolegnia diclina, Mortierella, Mortierella alpina, Conidiobolus, Pythium, Phytophthora, Penicillium, Porphyridium, Coidosporium, Mucor circinelloides, Fusarium, Aspergillus, Candida, Euphorbia, Rhodotorula, Thraustochytrium, Saprolegnia, Borago, Primula, sunflower, canola, rice and moss Δ6-desaturase.

24. The method according to claim 21, wherein said promoter is a seed specific promoter.

25. The method according the claim 24, wherein the seed specific promoter is an oleosin or a linin promoter.

26. The method according to claim 21, 22, 23, 24, or 25 wherein said method further comprises isolating seeds from said safflower plant.

27. The method according to claim 26, wherein said seed has GLA levels that are at least 1% of total fatty acid content.

28. The method according to claim 26, wherein said method further comprises extracting oil from said safflower plant seed.

29. The oil of claim 28, wherein said oil comprises about 1-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-45, 45-50, 50-55 or 55-60% or greater by weight GLA.

30. A method for producing GLA in a safflower seed, said method comprising growing a safflower plant comprising:

a first recombinant DNA sequence encoding a Δ6-desaturase, and

a second recombinant DNA sequence encoding a Δ12-desaturase,

wherein said recombinant DNA sequences are operably linked to at least one promoter,

wherein said safflower plant is grown under conditions whereby said Δ6-desaturase and Δ12-desaturase sequences are expressed.

31. The method of claim 30, wherein said Δ6-desaturase and Δ12-desaturase encoding sequence is a plant or fungal desaturase.

32. The method of claim 31, wherein said desaturase encoding sequence is chosen from the group consisting of *Mucor, Saprolegnia, Saprolegnia diclina, Mortierella*, *Mortierella alpina, Conidiobolus*, *Pythium*, *Phytophathra*, *Penicillium*, *Porphyridium*, *Coidosporium*, *Mucor circinelloides*, *Fusarium*, *Aspergillus*, *Candida, Euphorbia, Dimorphoteca, Rhodotorula*, *Entomophthora*, *Thraustochytrium, Saprolegnia, Borago, Primula,* sunflower, canola, rice, and moss Δ6-and Δ12-desaturase.

33. The method according to claim 30, wherein said promoter is a seed specific promoter.

34. The method according the claim 33, wherein the seed specific promoter is chosen from oleosin promoter or linin promoter.

35. The method according to claim 30, 31, 32, 33, or 34, wherein said method further comprises isolating seeds from said safflower plant.

36. The method according to claim 35, wherein said seeds have GLA levels that are at least 1% of total fatty acid content.

37. The method according to claim 35, wherein said method further comprises extracting oil from said safflower plant seed.

38. The oil of claim 37, wherein said oil comprises about 1-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-45, 45-50, 50-55 or 55-60% or greater by weight GLA.

39. Safflower oil derived from a transgenic safflower plant wherein said safflower oil has a content of gamma-linolenic acid greater than 1% by weight.

40. Safflower oil derived from a transgenic safflower plant wherein said safflower oil has a content of gamma-linolenic acid greater than 5% by weight.

41. Safflower oil derived from a transgenic safflower plant wherein said safflower oil has a content of gamma-linolenic acid greater than 10% by weight.

42. Safflower oil derived from a transgenic safflower plant wherein said safflower oil has a content of gamma-linolenic acid greater than 1-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-45, 45-50, 50-55 or 55-60 per cent by weight.

43. A method of treating or preventing a psychiatric, neurological or other central or peripheral nervous system condition or disease which comprises administering to a subject prone to or afflicted with such disease an effective amount of an oil according to claims 3, 11, 19, 29, 38, 39, 40, 41, or 42.

44. A method of treating or preventing an immunological condition or disease which comprises administering to a subject prone to or afflicted with such disease an effective amount of an oil according to claims 3, 11, 19, 29, 38, 39, 40, 41, or 42.

45. A method of treating or preventing an inflammatory condition or disease which comprises administering to a subject prone to or afflicted with such disease an effective amount of an oil according to claims 3, 11, 19, 29, 38, 39, 40, 41, or 42.

46. A method of treating or preventing cancer which comprises administering to a subject prone to or afflicted with such disease an effective amount of an oil according to claims 3, 11, 19, 29, 38, 39, 40, 41, or 42.

47. A method of treating or preventing a skin condition or disease which comprises administering to a subject prone to or afflicted with such disease an effective amount of an oil according to claims 3, 11, 19, 29, 38, 39, 40, 41, or 42.

48. A method of treating or preventing a cardiovascular condition or disease which comprises administering to a subject prone to or afflicted with such disease an effective amount of an oil according to claims 3, 11, 19, 29, 38, 39, 40, 41, or 42.

49. A method of providing nutrition to an infant comprising administering to an infant an effective amount of an oil according to claims 3, 11, 19, 29, 38, 39, 40, 41, or 42.

Pathway for GLA Biosynthesis

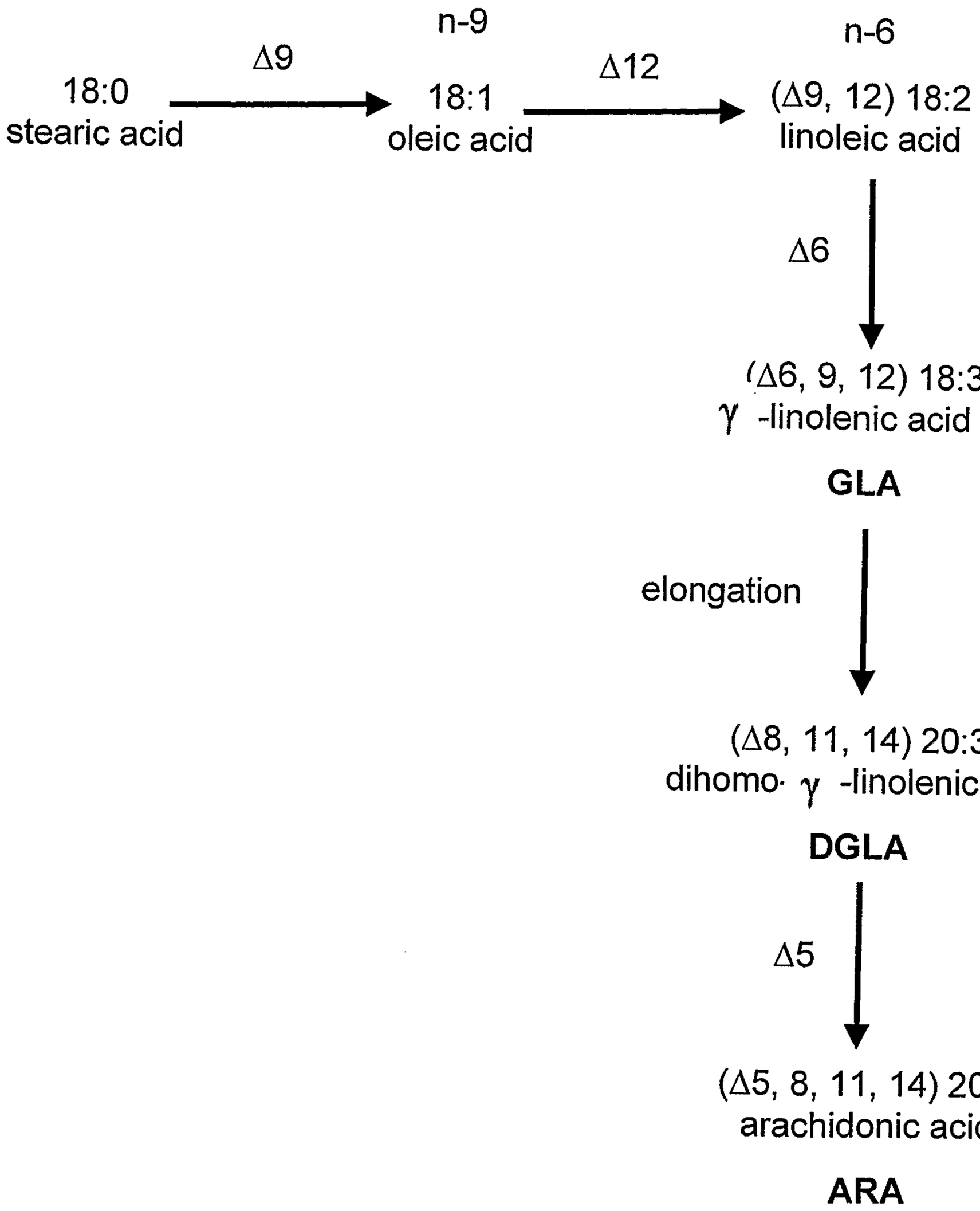


Figure 1

Amino Acid Alignment of Plant Delta-6 Desaturases

```
                                    1
AAC49700 Borago officinalis D6  (1) -----MAAQIKKYITSEELKNHDKPGDLWISIQGKAYDVSDWVKDHPGGSFPLKSLAGQEVTDAFVAFHP   70
  AAP23034 Primula farinosa D6  (1) MANKSPPNPKTGYITSSDLKSHNKAGDLWISIHGQVYDVSSWAALHPGGTAPLMALAGHDVTDAFLAYHP
  AAP23036 Primula vialli D6    (1) MANKSPPNPKTGYITSSDLKGHNKAGDLWISIHGEVYDVSSWAGLHPGGSAPLMALAGHDVTDAFLAYHP
            Consensus           (1) MANKSPPNPKTGYITSSDLK HNKAGDLWISIHG VYDVSSWAALHPGGSAPLMALAGHDVTDAFLAYHP
                                   71
AAC49700 Borago officinalis D6 (66) ASTWKNLDKFFTGYYLKDVSVSEVSKDYRKLVFEFSKMGLYDKKGHIMFATLCFIAMLFAMSVYGVLFCE  140
  AAP23034 Primula farinosa D6 (71) PSTARLLPPLSTNLLLQNHSVSPTSSDYRKLLDNFHKHGLFRARGHTAYATFVFMIAMFLMSVTGVLCSD
  AAP23036 Primula vialli D6   (71) PSTARLLPPLSTNLLLQNHSVSPTSSDYRKLLHNFHKIGMFRARGHTAYATFVIMIVMFLTSVTGVLCSD
            Consensus          (71) PSTARLLPPLSTNLLLQNHSVSPTSSDYRKLL NFHKIGLFRARGHTAYATFVFMIMMFLMSVTGVLCSD
                                  141
AAC49700 Borago officinalis D6 (136) GVLVHLFSGCLMGFLWIQSGWIGHDAGHYMVVSDSRLNKFMGIFAANCLSGISIGWWKWNHNAHHIACNS  210
  AAP23034 Primula farinosa D6 (141) SAWVHLASGGAMGFAWIQCGWIGHDSGHYRIMSDRKWNWFAQILSTNCLQGISIGWWKWNHNAHHIACNS
  AAP23036 Primula vialli D6   (141) SAWVHLASGAAMGFAWIQCGWIGHDSGHYRIMSDRKWNWFAQVLSTNCLQGISIGWWKWNHNAHHIACNS
            Consensus          (141) SAWVHLASGAAMGFAWIQCGWIGHDSGHYRIMSDRKWNWFAQILSTNCLQGISIGWWKWNHNAHHIACNS
                                  211
AAC49700 Borago officinalis D6 (206) LEYDPDLQYIPFLVVSSKFFGSLTSHFYEKRLTFDSLSRFFVSYQHWTFYPIMCAARLNMYVQSLIMLLT  280
  AAP23034 Primula farinosa D6 (211) LDYDPDLQYIPLLVVSPKFFNSLTSRFYDKKLNFDGVSRFLVCYQHWTFYPVMCVARLNMLAQSFITLFS
  AAP23036 Primula vialli D6   (211) LDYDPDLQYIPLLVVSPKFFNSLTSRFYDKKLNFDGVSRFLVCYQHWTFYPVMCVARLNMIAQSFITLFS
            Consensus          (211) LDYDPDLQYIPLLVVSPKFFNSLTSRFYDKKLNFDGVSRFLVCYQHWTFYPVMCVARLNMIAQSFITLFS
                                  281
AAC49700 Borago officinalis D6 (276) KRNVSYRAHELLGCLVFSFWYPLLVSCLPNWGERIMFVIASLSVTGMQQVQFSLNHFSSSVYVGKPKGNN  350
  AAP23034 Primula farinosa D6 (281) SREVCHRAQEVFGLAVFWVWFPLLLSCLPNWGERIMFLLASYSVTGIQHVQFSLNHFSSDVYVGPPVGND
  AAP23036 Primula vialli D6   (281) SREVGHRAQEIFGLAVFWVWFPLLLSCLPNWSERIMFLLASYSVTGIQHVQFSLNHFSSDVYVGPPVAND
            Consensus          (281) SREV HRAQEIFGLAVFWVWFPLLLSCLPNWGERIMFLLASYSVTGIQHVQFSLNHFSSDVYVGPPVGND
                                  351
AAC49700 Borago officinalis D6 (346) WFEKQTDGTLDISCPPWMDWFHGGLQFQIEHHLFPKMPRCNLRKISPYVIELCKKHNLPYNYASFSKANE  420
  AAP23034 Primula farinosa D6 (351) WFKKQTAGTLNISCPAWMDWFHGGLQFQVEHHLFPRMPRGQFRKISPFVRDLCKKHNLPYNIASFTKANV
  AAP23036 Primula vialli D6   (351) WFKKQTAGTLNISCPAWMDWFHGGLQFQVEHHLFPRMPRGQFRKISPFVRDLCKKHNLPYNIASFTKANV
            Consensus          (351) WFKKQTAGTLNISCPAWMDWFHGGLQFQVEHHLFPRMPRGQFRKISPFVRDLCKKHNLPYNIASFTKANV
                                  421                                453
AAC49700 Borago officinalis D6 (416) MTLKTLRNTAIQARDTTKPLPKNLVWEALHTHG (SEQ ID NO: 4)
  AAP23034 Primula farinosa D6 (421) FTLKTLRNTAIEARDLSNPLPKNMVWEALKTLG (SEQ ID NO: 5)
  AAP23036 Primula vialli D6   (421) LTLKTLRNTAIEARDLSNPTPKNMVWEAVHTHG (SEQ ID NO: 6)
            Consensus          (421) LTLKTLRNTAIEARDLSNPLPKNMVWEALHTHG
```

Similarity: 99.1%; Identity: 62.7%
AAC49700—Borago officinalis (448)
AAP23034—Primula farinosa (453)
AAP23036—Primula vialli (453)

Figure 2

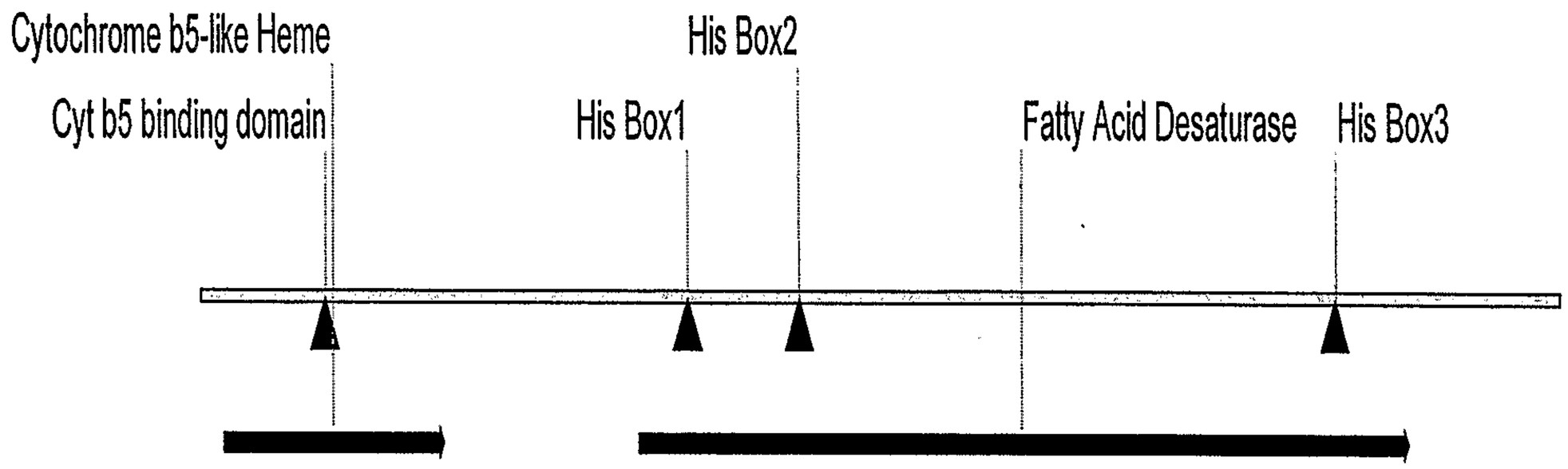
Amino Acid Alignment of Fungal Delta-6 Desaturases

Similarity: 53.7%; Identity: 7.1%

AAF08685 Mortierella alpina D6 (457)
BAB6905 Mucor circinelloides D6 (467)
BAC57562 Mucor circinelloides D6 (523)
Thraustochytrium aureum D6 (456) (SEQ ID NO: 33 from WO02081668)
Saprolegnia diclina D6 (453) (SEQ ID NO: 14 from US 6,635,451)

Figure 3

Conserved Regions in the Delta-6 Desaturases



AAC49700BorageD6

448 aa

Figure 4

Amino Acid Alignment of Plant Delta-12 Desaturases

```
                                         1                                                          60
     AAT02411 Brassica napus D12   (1)   MGAGGRMQVSPPSSSP-------GTNTLKRVPCETPPFTVGELKKAIPPHCFKRSIPRSF
AAC31698 Borago officinalis D12   (1)   MGGGGRMPVPTKGKKS-------KSDVFQRVPSEKPPFTVGDLKKVIPPHCFQRSVLHSF
 AAL68983 Helianthus annuus D12   (1)   MGAGGRMSSPNGKEKD-------GPKPLERALHEKPPFTVGDIKKVIPPHCFKRSVIRSF
     BAD12887 Oriza sativa D12   (1)   MGAGGRMTEKEREEQQKLLGRAGNGAAVQRSPTDKPPFTLGQIKKAIPPHCFQRSVIKSF
                     Consensus   (1)   MGAGGRMSVP K EK        GS  LQRVPSEKPPFTVGDIKKVIPPHCFQRSVIRSF
                                         61                                                        120
     AAT02411 Brassica napus D12  (54)   SYLIWDIIIASCFYYVATTYFPLLPHPLSYFAWPLYWACQGCVLTGVWVIAHECGHHAFS
AAC31698 Borago officinalis D12  (54)   SYVVYDLVIAALFFYTASRYIHLQPHPLSYVAWPLYWFCQGSVLTGVWVIAHECGHHAFS
 AAL68983 Helianthus annuus D12  (54)   SYVVYDLTIASIFYYLANNYIPLLPNSLAYVAWPVYWIFQGCVLTGVWVIAHECGHHAFS
     BAD12887 Oriza sativa D12  (61)   SYVVHDLVIVAALLYFALVMIPVLPSGHEFAAWPLYWIAQGCVLTGVWVIAHECGHHAFS
                     Consensus  (61)   SYVVYDLVIAAIFYYLAS YIPLLPHPLSYVAWPLYWICQGCVLTGVWVIAHECGHHAFS
                                         121                                                       180
     AAT02411 Brassica napus D12 (114)   DYQWLDDTVGLIFHSFPLVPYFSWKYSHRRHHSNTGSLERDEVFVPKKKSDIKWYGKYLN
AAC31698 Borago officinalis D12 (114)   DYQWLDDTVGLLLHSALLVPYFSWKYSHRRHHSNTGSLERDEVFVPKKRSGISWSSEYLN
 AAL68983 Helianthus annuus D12 (114)   DYQWLDDTVGLILHSALLVPYFSWKYSHRRHHSNTGSIEHDEVFVPKLKSSVRSTAKYLN
     BAD12887 Oriza sativa D12 (121)   DYSVLDDIVGLVLHSSLLVPYFSWKYSHRRHHSNTGSLERDEVFVPKQKSAMAWYTPYVY
                     Consensus (121)   DYQWLDDTVGLILHSALLVPYFSWKYSHRRHHSNTGSLERDEVFVPKKKSAIRWYAKYLN
                                         181                                                       240
     AAT02411 Brassica napus D12 (174)   -NPLGRTVMLTVQFTLGWPLYLAFNVSGRPYSDGFACHFHPNAPIYNDRERLQIYISDAG
AAC31698 Borago officinalis D12 (174)   -NPPGRVLVLLVQLTLGWPLYLMFNVSGR-PYDRFACHFDPKSPIYNDRERLQIYISDAG
 AAL68983 Helianthus annuus D12 (174)   -NPPGRILTLLVTLTMGWPLYLMFNVSGR-YYDRFACHFDPNSPIYSNRERAQIFISDAG
     BAD12887 Oriza sativa D12 (181)   HNPIGRLVHIFVQLTLGWPLYLAFNVSGR-PYPRFACHFDPYGPIYNDRERVQIFISDVG
                     Consensus (181)    NPPGRILMLLVQLTLGWPLYLMFNVSGR YYDRFACHFDPNSPIYNDRERLQIFISDAG
                                         241                                                       300
     AAT02411 Brassica napus D12 (233)   VLSVCYGLYRYAGSRGVASMVCVYGVPLMIVNCFLVLITYLQHTHPSLPHYDSSEWDWLR
AAC31698 Borago officinalis D12 (232)   IVAVMYGLYRLVAAKGVAWVVCYYGVPLLVVNGFLVLITYLQHTQPSLPHYDSSEWDWLK
 AAL68983 Helianthus annuus D12 (232)   ILTVFYILFRLASTKGLVWVLTMYGGPLLVVNGFLVLITFLQHTHPSLPHYDSTEWDWLR
     BAD12887 Oriza sativa D12 (240)   VVSAGLALFKLSSAFGFWWVVRVYGVPLLIVNAWLVLITYLQHTHPALPHYDSSEWDWLR
                     Consensus (241)   ILSV YGLFRLASAKGVAWVVCVYGVPLLIVNGFLVLITYLQHTHPSLPHYDSSEWDWLR
                                         301                                                       360
     AAT02411 Brassica napus D12 (293)   GALATVDRDYGILSKVFHNITDTHVAHHLFSTMPHYNAMEATKAIKPILGEYYQFDGTPV
AAC31698 Borago officinalis D12 (292)   GALATVDRDYGFLNKVLHNITDTHVAHHLFSTMPHYHAMEATKAIKPILGDYYQCDRTPV
 AAL68983 Helianthus annuus D12 (292)   GALATVDRDYGILNKVFHNITDTHVTHHLFSTMPHYHAMEATKAIKPILGDYYQFDGTSI
     BAD12887 Oriza sativa D12 (300)   GALATVDRDYGILNKVFHNITDTHVAHHLFSTMPHYHAMEATKAIRPILGEYYQFDPTPV
                     Consensus (301)   GALATVDRDYGILNKVFHNITDTHVAHHLFSTMPHYHAMEATKAIKPILGDYYQFDGTPV
                                         361                             392
     AAT02411 Brassica napus D12 (353)   VKAMWREAKECIYVEPDRQGEKKGVFWYNNKL  (SEQ ID NO: 14)
AAC31698 Borago officinalis D12 (352)   FKAMYREVKECIYVEADEGDNKKGVFWYKNKL  (SEQ ID NO: 12)
 AAL68983 Helianthus annuus D12 (352)   FKAMYRETKECIYVDKDE-DVKDGVYWYRNKI  (SEQ ID NO: 13)
     BAD12887 Oriza sativa D12 (360)   AKATWREAKECIYVEPED---NKGVFWYNNKF  (SEQ ID NO: 15)
                     Consensus (361)   FKAMWREAKECIYVEPDE D KKGVFWYNNKL
```

Similarity: 95.4%; Identity: 57.7%

AAT02411—Brassica napus (384)

AAC31698—Borago officinalis (383)

AAL68983—Helianthus annuus (382)

BAD12887—Oriza sativa (388)

Figure 5

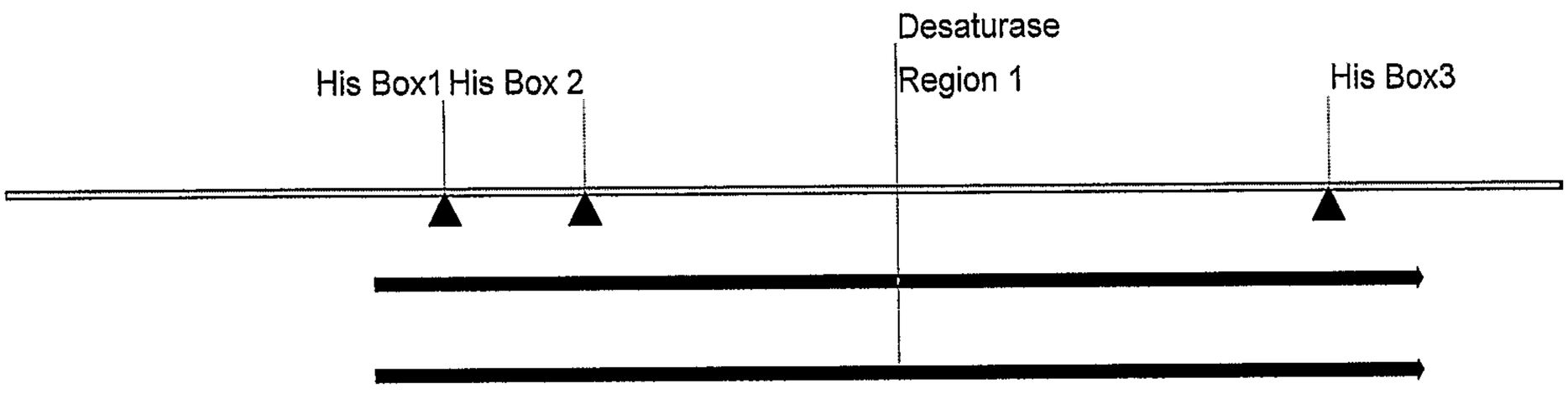
Comparison of Fungal Delta-12 Desaturases

```
                                                1                                                  60
CAE47978 Aspergillus furnigatus D12     (1)  ----------------------------------MASDAEKTSSK-----M--IDTYGNEF
EAK94955 Candida albicans D12           (1)  MSVVEASSSSVVEDS--------TASNVVQRGNISSFASTTASS----NLTTIDTNGKVF
EAL03493 Candida albicans D12           (1)  MAAATTSFSSGFNNNNNADQSTDSSATISKSGNVASFKTTSTTSTYQTNLTAIDTYGNEF
AAF08684 Mortierella alpina D12         (1)  ----------------------MAPPNTIDAGLTQRHISTSAPN------SAKPAFERNY
Consensus                               (1)  MA    S SS              SAANII AGNIASFASTSASS    NLTAIDTYGNEF
                                                61                                                 120
CAE47978 Aspergillus furnigatus D12    (21)  KIPDYTIKQIRDAIPAHCYQRSAATSLYYVFRDMAILASVFYVFHNYVTPETVP-----S
EAK94955 Candida albicans D12          (49)  KVPDYSIKDILQAIPKHCYERSLIRSLGYVVRDITMMVIIGYVGHTFIPMVQIPEYPSLA
EAL03493 Candida albicans D12          (61)  KVPDYTIKDILSAIPTHCYERRLLQSLSYVFRDLFCMVVLGFIANNYIHLIPN-------
AAF08684 Mortierella alpina D12        (33)  QIPEETIKEIRECIPAHCFERSGLRGLCHVAIDLTWASLLFLAATQIDKFENP-------
Consensus                              (61)  KVPDYTIKDIRDAIPAHCYERSLLRSL YVFRDITIMVILGYVAHNYI LENIP     A
                                               121                                                180
CAE47978 Aspergillus furnigatus D12    (76)  MPVRVVLWTIYTVVQGLVGTGVWVLAHECGHQAFSISKVLNDTVGWICHSLLLVPYFSWK
EAK94955 Candida albicans D12         (109)  YGLRGALWMVQSYCIGLFGFGLWILAHECGHGAFSDYQNINDFIGWVLHSYLTVPYFSWK
EAL03493 Candida albicans D12         (114)  QFIRFAAWTGYVWCQGLFGTGIWVLAHECGHQAFSDYGSVNDFVGWVLHSYLLVPYFSWK
AAF08684 Mortierella alpina D12        (86)  -LIRYLAWPVYWIMQGIVCTGVWVLAHECGHQSFSISKTLNNTVGWILHSMLLVPYHSWR
Consensus                             (121)    IRFALWTVYSWCQGLVGTGVWVLAHECGHQAFSTYKSLNDTVGWILHSYLLVPYFSWK
                                               181                                                240
CAE47978 Aspergillus furnigatus D12   (136)  ISHGKHHKATGNIARDMVFVPKTREEYATR-------IGRAAHELSELMEETPILTATNL
EAK94955 Candida albicans D12         (169)  FSHAKHHKATGHLTKDMVFIPYTKEEYLE---------KNKVEKVADLMEESPIYSFLVL
EAL03493 Candida albicans D12         (174)  FSHGKHHKATGHLTRDMVFVPKTKEEFLQ---------NRGVKDLDDLLGDSPMYSLLTL
AAF08684 Mortierella alpina D12       (145)  ISHSKHHKATGHMTKDQVFVPKTRSQVGLPPKENAAAAVQEEDMSVHLDEEAPIVTLFWM
Consensus                             (181)  ISHGKHHKATGHLTKDMVFVPKTKEEYL           RAVDDLADLMEESPIYSLL L
                                               241                                                300
CAE47978 Aspergillus furnigatus D12   (189)  VLQQLFGWPMYLLTNVTGHNNHERQPEGRGKGKRNGYFGGVNHFNPSSPLYEAKDAKLIV
EAK94955 Candida albicans D12         (220)  VFQQLGGLQLYLATNATGQ-----VYPGYSKIAKS-------HYTPTSPVFDKHQYWYIV
EAL03493 Candida albicans D12         (225)  IFQQTFGWISYLVANVSGQ-----KYPGVSFLKLN-------HFNPNSLIFDKKDYWYIL
AAF08684 Mortierella alpina D12       (205)  VIQFLFGWPAYLIMNASGQ----------DYGRWTS------HFHTYSPIFEPRNFFDII
Consensus                             (241)  VFQQLFGWPMYLITNVSGQ      YPG SKGKKN        HFNPSSPIFDKKDYWYIV
                                               301                                                360
CAE47978 Aspergillus furnigatus D12   (249)  LSDLGLFLVGSLLYYIGSTYGWLNLLVWYGIPYLWVNHWLVAITFLQHTDPTLPHYQPEA
EAK94955 Candida albicans D12         (268)  LSDLGIFLAFTTVYQWYKNFGLFNMMINWFVPWLWVNHWLVFVTFLQHTDPTMPHYTSKE
EAL03493 Candida albicans D12         (273)  LSDLGILLQFFNLYVWYQSFGGFNLLVNYVLPYFLVNHWLVFITYLQHSDPQMPHYEASQ
AAF08684 Mortierella alpina D12       (249)  ISDLGVLAALGALIYASMQLSLLTVTKYYIVPYLFVNFWLVLITFLQHTDPKLPHYREGA
Consensus                             (301)  LSDLGILLAFS LYYWY SFGLLNLLVNYIVPYLWVNHWLVFITFLQHTDPTLPHY A A
                                               361                                                420
CAE47978 Aspergillus furnigatus D12   (309)  WDFTRGAAATIDRDFGFVGRHIFHGIIETHVLHHYVSTIPFYHADEASEAIQKVMGPHYR
EAK94955 Candida albicans D12         (328)  WTFARGAAATLDRNFGFVGQHIFHDIIETHVLHHYVSRIPFYNAREATDAIRKVMGEHYR
EAL03493 Candida albicans D12         (333)  WTFARGAAATIDREFGFVGKHIFHDIIETHVLHHYVSRIPFYNAREASEAIKKVMGIHYQ
AAF08684 Mortierella alpina D12       (309)  WNFQRGALCTVDRSFGKFLDHMFHGIVHTHVAHHLFSQMPFYHAEEATYHLKKLLGEYYV
Consensus                             (361)  WTFARGAAATIDRDFGFVGKHIFHGIIETHVLHHYVSRIPFYNAREASEAIKKVMGEHYR
                                               421                                          476
CAE47978 Aspergillus furnigatus D12   (369)  SEAHTGWTGFLKALWTSARTCQWVEPTEGAKGESQYVLFYRNINGIGVPPAKIPAK (SEQ ID NO: 17)
EAK94955 Candida albicans D12         (388)  YEGESMWY-----SLWKCMRMCQFVDDDKED---AKGVMMFRNVNGWGPVKPKD--- (SEQ ID NO: 18)
EAL03493 Candida albicans D12         (393)  HSDENMWV-----SLWKSARWCQFVDGN------N-GVLMYRNTNGFGVDPKKQTH- (SEQ ID NO: 19)
AAF08684 Mortierella alpina D12       (369)  YDPSPIVV-----AVWRSFRECRFVEDQG-------DVVFFKK-------------- (SEQ ID NO: 16)
Consensus                             (421)  YEAESMWV     ALWKSAR CQFVDDN       A GVLMFRNINGFGV P K
```

Figure 6

Similarity: 83.2%; Identity: 23.9%
CAE47978—Aspergillus furnigatus (424)
EAK94955—Candida albicans (436)
EAL03493—Candida albicans (433)
AAF08684—Mortierella alpina (399)

Conserved Regions in the Delta-12 Desaturases



AAF08684MalpinaD12

399 aa

Figure 7

Plasmid pSBS4766 for the Expression of Delta-6 and Delta-12 Desaturases from *M. alpina*

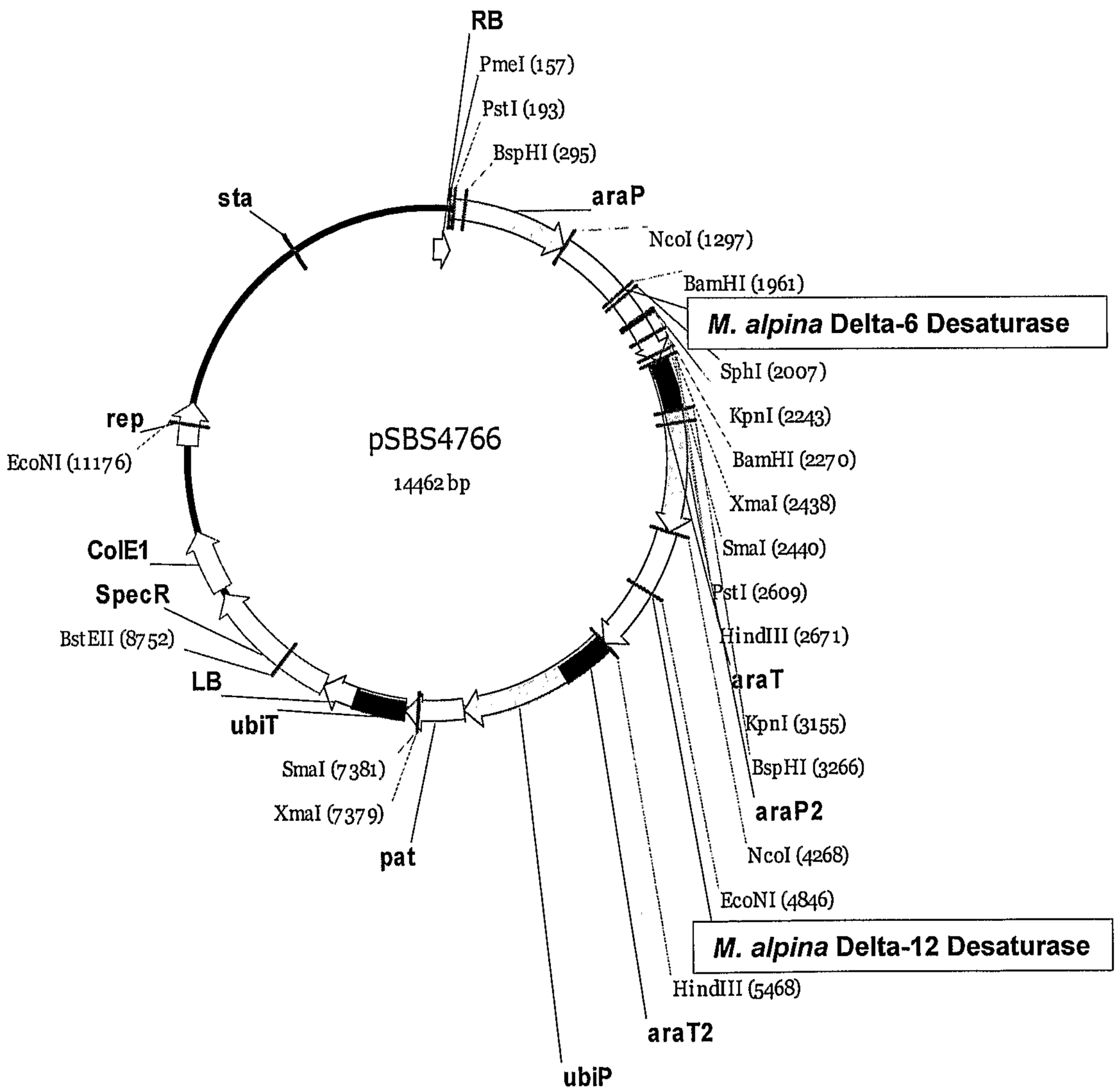


Figure 8

Plasmid pSBS4119 for the Expression of Delta-6 Desaturase from *S. diclina*

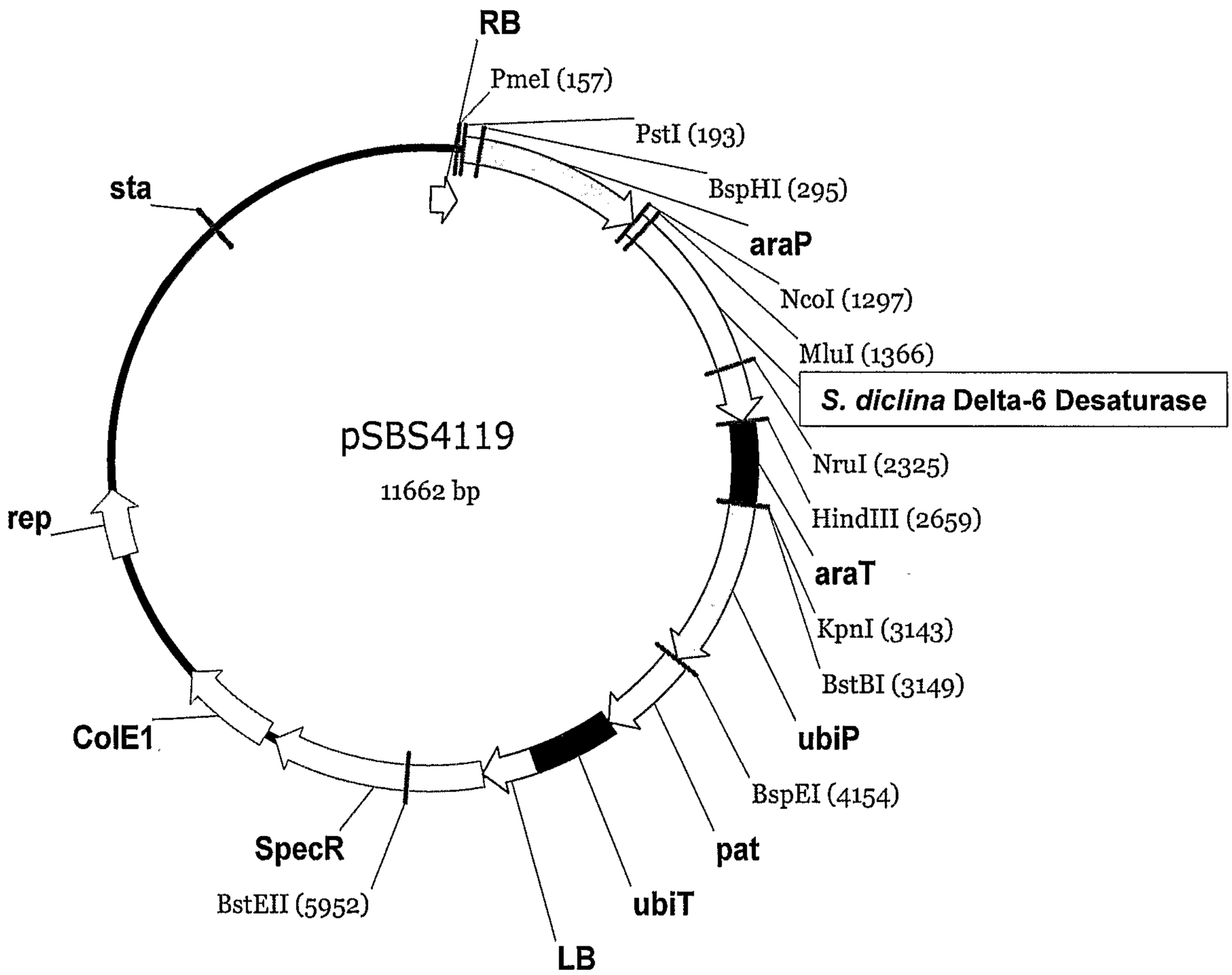


Figure 9

Plasmid pSBS4763 for the Expression of Delta-6 Desaturase from *M. alpina*

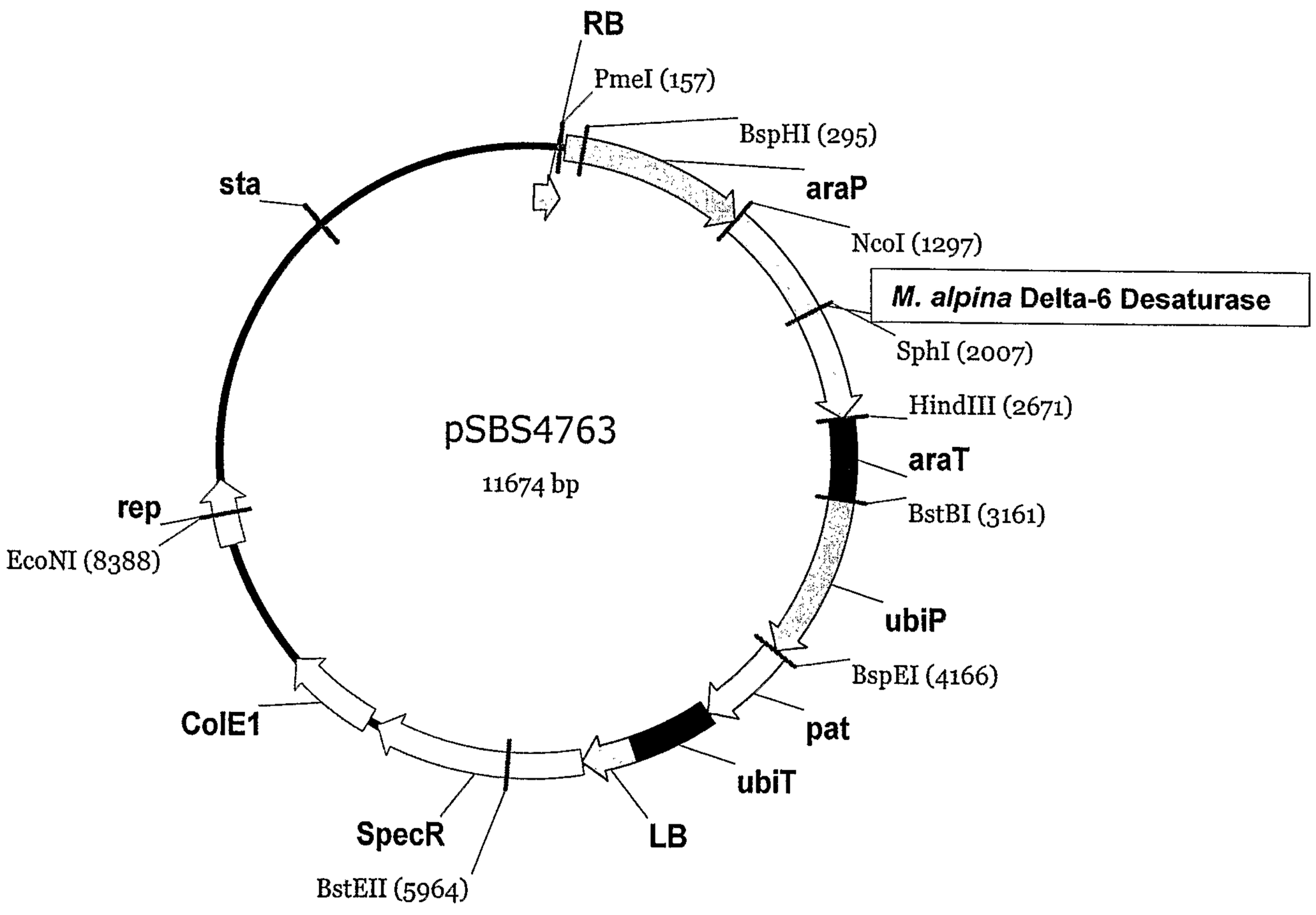


Figure 10

Pathway for GLA Biosynthesis