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(54) Expression of fatty acid desaturases in corn

Expression von Fettsäuredesaturasen in Mais

Expression de désaturases d'acides gras dans le maïs

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Description

1. Field of the Invention

[0001] The invention relates generally to expression of desaturase enzymes that modulate the number and location of double bonds in long chain poly-unsaturated fatty acids (LC-PUFA's) in corn and compositions derived therefrom.

2. Description of the Related Art

[0002] The primary products of fatty acid biosynthesis in most organisms are 16- and 18-carbon compounds. The relative ratio of chain lengths and degree of unsaturation of these fatty acids vary widely among species. Mammals, for example, produce primarily saturated and monounsaturated fatty acids, while most higher plants produce fatty acids with one, two, or three double bonds, the latter two comprising polyunsaturated fatty acids (PUFA's).

[0003] Two main families of PUFAs are the omega-3 fatty acids (also represented as "n-3" fatty acids), exemplified by eicosapentaenoic acid (EPA, 20:4, n-3), and the omega-6 fatty acids (also represented as "n-6" fatty acids), exemplified by arachidonic acid (ARA, 20:4, n-6). PUFAs are important components of the plasma membrane of the cell and adipose tissue, where they may be found in such forms as phospholipids and as triglycerides, respectively. PUFAs are necessary for proper development in mammals, particularly in the developing infant brain, and for tissue formation and repair.

[0004] Several disorders respond to treatment with fatty acids. Supplementation with PUFAs has been shown to reduce the rate of restenosis after angioplasty. The health benefits of certain dietary omega-3 fatty acids for cardiovascular disease and rheumatoid arthritis also have been well documented (Simopoulos, 1997; James *et al*., 2000). Further, PUFAs have been suggested for use in treatments for asthma and psoriasis. Evidence indicates that PUFAs may be involved in calcium metabolism, suggesting that PUFAs may be useful in the treatment or prevention of osteoporosis and of kidney or urinary tract stones. The majority of evidence for health benefits applies to the long chain omega-3 fats, EPA and docosahexanenoic acid (DHA, 22:6), which are in fish and fish oil. With this base of evidence, health authorities and nutritionists in Canada (Scientific Review Committee, 1990, Nutrition Recommendations, Minister of National Health and Welfare, Canada, Ottowa), Europe (de Deckerer *et al*., 1998), the United Kingdom (The British Nutrition Foundation, 1992, Unsaturated fatty-acids - nutritional and physiological significance: The report of the British Nutrition Foundation's Task Force, Chapman and Hall, London), and the United States (Simopoulos *et al*., 1999) have recommended increased dietary consumption of these PUFAs.

[0005] PUFAs also can be used to treat diabetes (U.S. Pat. No. 4,826,877; Horrobin et al., 1993). Altered fatty acid metabolism and composition have been demonstrated in diabetic animals. These alterations have been suggested to be involved in some of the long-term complications resulting from diabetes, including retinopathy, neuropathy, nephropathy and reproductive system damage. Primrose oil, which contains γ-linolenic acid (GLA, 18:3, Δ6, 9, 12), has been shown to prevent and reverse diabetic nerve damage.

[0006] PUFAs, such as linoleic acid (LA, 18:2, Δ9, 12) and α-linolenic acid (ALA, 18:3, Δ9, 12, 15), are regarded as essential fatty acids in the diet because mammals lack the ability to synthesize these acids. However, when ingested, mammals have the ability to metabolize LA and ALA to form the n-6 and n-3 families of long-chain polyunsaturated fatty acids (LC-PUFA). These LC-PUFAs are important cellular components conferring fluidity to membranes and functioning as precursors of biologically active eicosanoids such as prostaglandins, prostacyclins, and leukotrienes, which regulate normal physiological functions. Arachidonic acid is the principal precursor for the synthesis of eicosanoids, which include leukotrienes, prostaglandins, and thromboxanes, and which also play a role in the inflammation process. Administration of an omega-3 fatty acid, such as SDA, has been shown to inhibit biosynthesis of leukotrienes (U.S. Patent 5,158,975). The consumption of SDA has been shown to lead to a decrease in blood levels of proinflammatory cytokines TNF-α and IL-1β (U.S. Patent Publication 20040039058).

[0007] In mammals, the formation of LC-PUFA is rate-limited by the step of Δ6 desaturation, which converts LA to γ-linolenic acid (GLA, 18:3, Δ6, 9, 12) and ALA to SDA (18:4, Δ6, 9, 12, 15). Many physiological and pathological conditions have been shown to depress this metabolic step even further, and consequently, the production of LC-PUFA. To overcome the rate-limiting step and increase tissue levels of EPA, one could consume large amounts of ALA. However, consumption of just moderate amounts of SDA provides an efficient source of EPA, as SDA is about four times more efficient than ALA at elevating tissue EPA levels in humans (U.S. Patent Publication 20040039058). In the same studies, SDA administration was also able to increase the tissue levels of docosapentaenoic acid (DPA), which is an elongation product of EPA. Alternatively, bypassing the Δ6-desaturation via dietary supplementation with EPA or DHA can effectively alleviate many pathological diseases associated with low levels of PUFA. However, as set forth in more detail below, currently available sources of PUFA are not desirable for a multitude of reasons. The need for a reliable and economical source of PUFAs has spurred interest in alternative sources of PUFAs.

[0008] Major long chain PUFAs of importance include DHA and EPA, which are primarily found in different types of fish oil, and ARA, found in filamentous fungi such as *Mortierella.* For DHA, a number of sources exist for commercial

production including a variety of marine organisms, oils obtained from coldwater marine fish and egg yolk fractions. Commercial sources of SDA include the plant genera *Trichodesma, Borago* (borage) and *Echium.* However, there are several disadvantages associated with commercial production of PUFAs from natural sources. Natural sources of PUFAs, such as animals and plants, tend to have highly heterogeneous oil compositions. The oils obtained from these sources therefore can require extensive purification to separate out one or more desired PUFAs or to produce an oil which is enriched in one or more PUFAs.

[0009] Natural sources of PUFAs also are subject to uncontrollable fluctuations in availability. Fish stocks may undergo natural variation or may be depleted by overfishing. In addition, even with overwhelming evidence of their therapeutic benefits, dietary recommendations regarding omega-3 fatty acids are not heeded. Fish oils have unpleasant tastes and odors, which may be impossible to economically separate from the desired product, and can render such products unacceptable as food supplements. Animal oils, and particularly fish oils, can accumulate environmental pollutants. Foods may be enriched with fish oils, but again, such enrichment is problematic because of cost and declining fish stocks worldwide. This problem is also an impediment to consumption and intake of whole fish. Nonetheless, if the health messages to increase fish intake were embraced by communities, there would likely be a problem in meeting demand for fish. Furthermore, there are problems with sustainability of this industry, which relies heavily on wild fish stocks for aquaculture feed (Naylor *et al.*, 2000).

[0010] Other natural limitations favor a novel approach for the production of omega-3 fatty acids. Weather and disease can cause fluctuation in yields from both fish and plant sources. Cropland available for production of alternate oil-producing crops is subject to competition from the steady expansion of human populations and the associated increased need for food production on the remaining arable land. Crops that do produce PUFAs, such as borage, have not been adapted to commercial growth and may not perform well in monoculture. Growth of such crops is thus not economically competitive where more profitable and better-established crops can be grown. Large-scale fermentation of organisms such as *Mortierella* is also expensive. Natural animal tissues contain low amounts of ARA and are difficult to process. Microorganisms such as *Porphyridium* and *Mortierella* are difficult to cultivate on a commercial scale.

[0011] A number of enzymes are involved in the biosynthesis of PUFAs. LA (18:2, Δ9, 12) is produced from oleic acid (OA, 18:1, Δ9) by a Δ12 desaturase while ALA (18:3, Δ9, 12, 15) is produced from LA by a Δ15 desaturase. SDA (18:4, Δ6, 9, 12, 15) and GLA (18:3, Δ6, 9, 12) are produced from LA and ALA by a Δ6 desaturase. However, as stated above, mammals cannot desaturate beyond the Δ9 position and therefore cannot convert oleic acid into LA. Likewise, ALA cannot be synthesized by mammals. Other eukaryotes, including fungi and plants, have enzymes which desaturate at the carbon 12 and carbon 15 positions. The major polyunsaturated fatty acids of animals therefore are derived from diet via the subsequent desaturation and elongation of dietary LA and ALA.

[0012] Various genes encoding desaturases have been described. For example, U.S. Patent 5,952,544 describes nucleic acid fragments isolated and cloned from *Brassica napus* that encode fatty acid desaturase enzymes. Expression of the nucleic acid fragments of the '544 patent resulted in accumulation of ALA. However, in transgenic plants expressing the *B. napus* Δ15 desaturase, substantial LA remains unconverted by the desaturase. It has been demonstrated that certain fungal Δ15 desaturases are capable of converting LA to ALA when expressed in plants. In particular, fungal Δ15 desaturases from *Neurospora crassa* and *Aspergillus (Emericella) nidulans* have been effective (WO 03/099216). Increased ALA levels allow a Δ6 desaturase, when co-expressed with a nucleic acid encoding for the Δ15 desaturase, to act upon the ALA, thereby producing greater levels of SDA. Because of the multitude of beneficial uses for SDA, there is a need to create a substantial increase in the yield of SDA.

[0013] Nucleic acids from various sources have been sought for use in increasing SDA yield. Genes encoding Δ6 desaturases have been isolated from the fungus *Mortierella alpina* (U.S. Patent 6,075,183) and the plant *Primula* (WO 05/021761). These have been shown to be able to convert ALA to SDA in yeast and plants.

[0014] Therefore, it would be advantageous to obtain genetic material involved in PUFA biosynthesis and to express the isolated material in a plant system, in particular, a land-based terrestrial crop plant system, which can be manipulated to provide production of commercial quantities of one or more PUFAs. There is also a need to increase omega-3 fat intake in humans and animals. Thus there is a need to provide a wide range of omega-3 enriched foods and food supplements so that subjects can choose feed, feed ingredients, food and food ingredients which suit their usual dietary habits. Particularly advantageous would be seed oils and meal with increased SDA.

[0015] Currently there is only one omega-3 fatty acid, ALA, available in vegetable oils. However, there is poor conversion of ingested ALA to the longer-chain omega-3 fatty acids such as EPA and DHA. It has been demonstrated in copending U.S. Patent Publication 20040039058 for "Treatment And Prevention Of Inflammatory Disorders," that elevating ALA intake from the community average of 1/g day to 14 g/day by use of flaxseed oil only modestly increased plasma phospholipid EPA levels. A 14-fold increase in ALA intake resulted in a 2-fold increase in plasma phospholipid EPA (Manzioris *et al.*, 1994). Thus, to that end, there is a need for efficient and commercially viable production of PUFAs using fatty acid desaturases, genes encoding them, and recombinant methods of producing them. A need also exists for oils containing higher relative proportions of specific PUFAs, and food and feed compositions and supplements containing them. A need also exists for reliable economical methods of producing specific PUFA's.

[0016] Despite inefficiencies and low yields as described above, the production of omega-3 fatty acids via the terrestrial food chain is an enterprise beneficial to public health and, in particular, the production of SDA. SDA is important because, as described above, there is low conversion of ALA to EPA. This is because the initial enzyme in the conversion, Δ6-desaturase, has low activity in humans and is rate-limiting. Evidence that Δ6-desaturase is rate-limiting is provided by studies which demonstrate that the conversion of its substrate, ALA, is less efficient than the conversion of its product, SDA to EPA in mice and rats (Yamazaki *et al.*, 1992; Huang, 1991).

[0017] Certain seed oils such as corn lack SDA or other important omega-3 fatty acids altogether and thus there is a great need in the art for plants comprising seed oil with improved PUFA profiles. Such oils can be utilized to produce foods and food supplements enriched in omega-3 fatty acids and consumption of such foods effectively increases tissue levels of EPA. Foods and food stuffs, such as milk, margarine and sausages, all made or prepared with omega-3 enriched oils, will result in health benefits. Animal feedstocks containing the extracted oil or meal or full-fat grain enriched in omega-3 fatty acids can also be used to effectively increase tissue levels of EPA and provide health benefits for the livestock as well as productivity. Thus, there exists a strong need for novel plants expression desaturases for the creation of oils enriched in PUFAs.

SUMMARY OF THE INVENTION

[0018] In one aspect, the invention provides a transgenic corn seed comprising a seed oil having a stearidonic acid (18:4 n-3) content of from 20 % to 33 %, comprising gamma-linolenic acid in a content of less than 5 %, and comprising a polynucleotide sequence encoding a *Neurospora crassa* Δ15 desaturase mutagenized to increase expression in a monocot such as corn and a polynucleotide sequence encoding a Δ6 desaturase. In certain embodiments of the invention, the gamma-linolenic acid content of the oil is less than 3%.

[0019] In further embodiments of the invention, the seed oil of the transgenic corn seed of the invention comprises a ratio of stearidonic acid to gamma-linolenic acid of from 1:1 to 10:1, from 2:1 to 10:1, from 3:1 to 5:1 or at least 3:1. A corn seed oil provided by the invention may further comprise a ratio of omega-3 to omega-6 fatty acids of from 0.5%:1 to 10:1, from 5:1 to 10:1, and at least 5:1.

[0020] Another aspect of the invention provides an endogenous corn seed oil having a stearidonic acid (18:4 n-3) content of from 20 % to 33 %, comprising gamma-linolenic acid in a content of less than 5 %, and being obtainable from the transgenic corn seed described above.

[0021] In another aspect the invention provides a transgenic corn plant that produces a seed oil having a stearidonic acid (18:4 n-3) content of from 20 % to 33 %, comprising gamma-linolenic acid in a content of less than 5 %, and comprising a polynucleotide sequence encoding a *Neurospora crassa* Δ15 desaturase mutagenized to increase expression in a monocot such as corn and a polynucleotide sequence encoding a Δ6 desaturase.

[0022] Another aspect of the invention provides a method of producing seed oil, comprising growing the transgenic plant according to the invention under plant growth conditions until the transgenic plant produces said seed oil.

[0023] In still another aspect, the invention provides a transgenic plant transformed with a recombinant construct comprising a polynucleotide sequence encoding a *Neurospora crassa* Δ15 desaturase mutagenized to increase expression in a monocot such as corn and a polynucleotide sequence encoding a Δ6 desaturase, wherein the plant produces the corn seed oil of the invention.

[0024] In still yet another aspect, the invention provides a method of increasing the nutritional value of an edible product for human or non-human animal consumption, comprising adding a corn seed oil provided by the invention to the edible product. In certain embodiments, the product is human food, animal feed or a food supplement. In the method, the oil may increase the SDA content of the edible product or may increase the ratio of omega-3 to omega-6 fatty acids of the edible product. The edible product may lack SDA prior to adding the oil.

[0025] In still yet another aspect, the invention provides a method of manufacturing food and/or feed, comprising adding the corn seed oil provided by the invention to starting ingredients to produce the food and/or feed. The invention also provides food or feed made by the method.

[0026] In yet another aspect, the invention provides composition for providing stearidonic acid to a human or non-human animal, said composition comprising the corn seed oil of the invention.

BRIEF DESCRIPTION OF THE FIGURES

[0027] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein. The invention can be more fully understood from the following description of the figures:

FIG. 1 shows a map of vector pMON82812.

FIG. 2 shows a map of vector pMON78175.
FIG. 3 shows a map of vector pMON78171.

DETAILED DESCRIPTION OF THE INVENTION

[0028] The invention overcomes the limitations of the prior art by providing methods and compositions for creation of plants with improved PUFA content and the seed oils produced thereby. In one embodiment of the invention, Applicants have provided transgenic corn (*Zea mays*) plants that produce an endogenous corn seed oil containing stearidonic acid (SDA) and also γ-linolenic acid (GLA). This is significant because corn seed oil normally lacks these components, each of which have been shown to have important health benefits. The corn seed oil is endogenous in that it may be produced by a corn seed without the need for external addition of, for example, SDA. Such an endogenous oil may be an extracted oil composition that can be used as a food and feed ingredient and thereby benefit human or animal health. The modification of fatty acid content of an organism such as a plant thus presents many benefits such as improved nutrition and health benefits. Modification of fatty acid content can be used to achieve beneficial levels or profiles of desired PUFAs in plants such as corn, plant parts, and plant products, including plant seed oils. For example, when the desired PUFAs are produced in the seed tissue of a plant, the oil may be isolated from the seeds typically resulting in an oil high in desired PUFAs or an oil having a desired fatty acid content or profile, which may in turn be used to provide beneficial characteristics in food stuffs and other products. The invention in particular provides endogenous corn seed oil having a SDA content of from 20 % to 33 % and comprising GLA in a content of less than 5 %.

[0029] Various aspects of the invention include methods and compositions for modification of PUFA content of a cell, for example, modification of the PUFA content of a corn plant cell. Compositions related to the invention include isolated polynucleotide sequences and polynucleotide constructs introduced into plants and/or plant parts. An example of such an isolated polynucleotide is a *Primula* fatty acid desaturase such as a *Primula* Δ6-desaturase. Corn cells prepared in accordance with the invention may comprise other fatty acid desaturases, including known Δ6 desaturases such as that from *Mortierella alpina.* The inventors have shown in particular that expression of different Δ6 and Δ15 fatty acid desaturases yields corn seed oil containing SDA. Certain embodiments of the invention therefore provide corn plants and cells transformed with coding sequences of Δ6 and Δ15 fatty acid desaturases. In accordance with the invention, the Δ15-desaturase is from a fungal source, namely *Neurospora crassa.* Various embodiments of the invention may use combinations of desaturase polynucleotides and the encoded polypeptides that typically depend upon the host cell, the availability of substrate(s), and the desired end product(s). "Desaturase" refers to a polypeptide that can desaturate or catalyze formation of a double bond between consecutive carbons of one or more fatty acids to produce a mono- or poly-unsaturated fatty acid or precursor thereof. Of particular interest are polypeptides that can catalyze the conversion of oleic acid to LA, LA to ALA, or ALA to SDA, which includes enzymes which desaturate at the 12, 15, or 6 positions. The term "polypeptide" refers to any chain of amino acids, regardless of length or post-translational modification (*e.g.*, glycosylation or phosphorylation). Considerations for choosing a specific polypeptide having desaturase activity include the pH optimum of the polypeptide, whether the polypeptide is a rate limiting enzyme or a component thereof, whether the desaturase used is essential for synthesis of a desired PUFA, and/or whether a co-factor is required by the polypeptide. The expressed polypeptide preferably has characteristics that are compatible with the biochemical environment of its location in the host cell. For example, the polypeptide may have to compete for substrate(s).

[0030] Analyses of the K_m and specific activity of a polypeptide in question may be considered in determining the suitability of a given polypeptide for modifying PUFA(s) production, level, or profile in a given host cell. The polypeptide used in a particular situation is one which typically can function under the conditions present in the intended host cell, but otherwise may be any desaturase polypeptide having a desired characteristic or being capable of modifying the relative production, level or profile of a desired PUFA(s) or any other desired characteristics as discussed herein. The substrate(s) for the expressed enzyme may be produced by the host cell or may be exogenously supplied. To achieve expression, the polypeptide(s) of the instant invention are encoded by polynucleotides as described below.

[0031] In another aspect of the invention, vectors containing a nucleic acid, or fragment thereof, may be used containing a promoter, a desaturase coding sequence and a termination region for transfer into an organism in which the promoter and termination regions are functional. Accordingly, corn plants producing recombinant Δ6-desaturase are provided by this invention. An example of such a Δ6-desaturase coding sequence disclosed herein that has been optimized for expression in corn is given by SEQ ID NO:8 and SEQ ID NO:9. Therefore nucleic acids comprising this sequence, as well as sequences having at least 90 % sequence identity with these sequences, including at least 93 %, 95 %, 98 % and 99 % identity are contemplated herein. Polypeptide or polynucleotide comparisons may be carried out and identity determined using sequence analysis software, for example, the Sequence Analysis software package of the GCG Wisconsin Package (Accelrys, San Diego, CA), MEGAlign (DNAStar, Inc., 1228 S. Park St., Madison, Wis. 53715), and MacVector (Oxford Molecular Group, 2105 S. Bascom Avenue, Suite 200, Campbell, Calif. 95008). Such software matches similar sequences by assigning degrees of similarity or identity.

[0032] Nucleic acid constructs may be provided that integrate into the genome of a host cell or are autonomously

replicated (*e.g.*, episomally replicated) in the host cell. For production of ALA and/or SDA, the expression cassettes (*i.e.*, a polynucleotide encoding a protein that is operatively linked to nucleic acid sequence(s) that directs the expression of the polynucleotide) generally used include an expression cassette which provides for expression of a polynucleotide encoding a Δ6- and /or Δ15-desaturase. A host cell may have wild type oleic acid content.

[0033] Methods and compositions for the construction of expression vectors, when taken in light of the teachings provided herein, for expression of desaturase enzymes will be apparent to one of ordinary skill in the art. Expression vectors, as described herein, are DNA or RNA molecules engineered for controlled expression of a desired polynucleotide, *e.g.*, the desaturase-encoding polynucleotide. Examples of vectors include plasmids, bacteriophages, cosmids or viruses. Shuttle vectors, *e.g.* (Wolk *et al.* 1984; Bustos *et al.,* 1991) are also contemplated. Reviews of vectors and methods of preparing and using them can be found in Sambrook *et al.* (2001); Goeddel (1990); and Perbal (1988). Sequence elements capable of effecting expression of a polynucleotide include promoters, enhancer elements, upstream activating sequences, transcription termination signals and polyadenylation sites.

[0034] Polynucleotides encoding desaturases may be placed under transcriptional control of a strong promoter. In some cases this leads to an increase in the amount of desaturase enzyme expressed and concomitantly an increase in the fatty acid produced as a result of the reaction catalyzed by the enzyme. Examples of such promoters include the 35S CaMV (cauliflower mosaic virus), 34S FMV (figwort mosaic virus) (see, *e.g.*, U.S. Patentent 5,378,619) and Lec (from corn). There are a wide variety of plant promoter sequences which may be used to drive tissue-specific expression of polynucleotides encoding desaturases in transgenic plants. Indeed, the promoter used may be a seed specific promoter. Examples of promoters that may be used in this regard include the 5' regulatory regions from such genes as napin, which are regulated during plant seed maturation (Kridl et al., Seed Sci. Res. 1:209:219, 1991), phaseolin (Bustos, et al., Plant Cell, 1(9):839-853, 1989), soybean trypsin inhibitor (Riggs, et al., Plant Cell 1(6):609-621, 1989), ACP (Baerson et al., Plant Mol. Biol., 22(2):255-267, 1993), stearoyl-ACP desaturase (Slocombe et al., Plant Physiol. 104(4):167-176, 1994), soybean α' subunit of β-conglycinin (P-Gm7S, see for example, Chen et al., Proc. Natl. Acad. Sci. 83:8560-8564, 1986), *Vicia faba* USP (P-Vf.Usp, see for example, SEQ ID NO: 1, 2, and 3, U.S. Patent 7,078,588), the globulin promoter (see for example Belanger and Kriz, Genet. 129: 863-872 (1991), soybean alpha subunit of β-conglycinin (7S alpha) (U.S. Patent 6,825,398) barley seed peroxidin PERI promoter (see for example, Stacey et al., Plant Mol. Biol., 31:1205-1216, 1996), and *Zea mays* L3 oleosin promoter (P-Zm.L3, see, for example, Hong et al., Plant Mol. Biol., 34(3):549-555, 1997; see also U.S. Patent 6,433,252).

[0035] Examples of promoters highly expressed in the endosperm include promoters from genes encoding zeins, which are a group of storage proteins found in maize endosperm. Genomic clones for zein genes have been isolated (Pedersen et al., Cell 29:1015-1026 (1982), and Russell et al., Transgenic Res. 6(2):157-168) and the promoters from these clones, including the 15 kD, 16 kD, 19 kD, 22 kD, and 27 kD genes, could also be used to provide expression in the endosperm (see, *e.g.*, U.S. Patent 6,326,527). Other suitable promoters known to function in maize, and in other plants, include the promoters for the following genes: waxy (granule bound starch synthase), Brittle and Shrunken 2 (ADP glucose pryophosphorylase), Shrunken 1(sucrose synthase), branching enzymes I and II, starch synthases, de-branching enzymes, oleosins, glutelins, sucrose synthases (Yang *et al.*, 1990), Betl1 (basal endosperm transfer layer) and globulin1. Other useful promoters that are known by one of skill in the art are also contemplated.

[0036] The ordinarily skilled artisan can determine vectors and regulatory elements (including operably linked promoters and coding regions) suitable for expression in a particular host cell. "Operably linked" in this context means that the promoter and terminator sequences effectively function to regulate transcription. As a further example, a vector appropriate for expression of Δ6 and/or Δ15 desaturase in transgenic corn plants can comprise a seed-specific promoter sequence operably linked to the desaturase coding region and further operably linked to a seed storage protein termination signal or the nopaline synthase termination signal. As a still further example, a vector for use in expression of desaturases in plants can comprise a constitutive promoter or a tissue specific promoter operably linked to the desaturase coding region and further operably linked to a constitutive or tissue specific terminator or the nopaline synthase termination signal.

[0037] Modifications of the nucleotide sequences or regulatory elements disclosed herein which maintain the functions are contemplated herein. Such modifications include insertions, substitutions and deletions, and specifically substitutions which reflect the degeneracy of the genetic code.

[0038] Standard techniques for the construction of such recombinant vectors are well-known to those of ordinary skill in the art and can be found in references such as Sambrook *et al.* (2001), or any of the myriad of laboratory manuals on recombinant DNA technology that are widely available. A variety of strategies are available for ligating fragments of DNA, the choice of which depends on the nature of the termini of the DNA fragments. It is further contemplated to include in a nucleic acid vector other nucleotide sequence elements which facilitate cloning, expression or processing, for example sequences encoding signal peptides, a sequence encoding KDEL, which is required for retention of proteins in the endoplasmic reticulum or sequences encoding transit peptides which direct Δ6-desaturase to the chloroplast. Such sequences are known to one of ordinary skill in the art. An optimized transit peptide is described, for example, by Van den Broeck *et al.* (1985). Prokaryotic and eukaryotic signal sequences are disclosed, for example, by Michaelis *et al.* (1982).

[0039] Once the desired genomic or cDNA has been isolated, it can be sequenced by known methods. It is recognized in the art that such methods are subject to errors, such that multiple sequencing of the same region is routine and is still expected to lead to measurable rates of mistakes in the resulting deduced sequence, particularly in regions having repeated domains, extensive secondary structure, or unusual base compositions, such as regions with high GC base content. When discrepancies arise, resequencing can be done and can employ special methods. Special methods can include altering sequencing conditions by using: different temperatures; different enzymes; proteins which alter the ability of oligonucleotides to form higher order structures; altered nucleotides such as ITP or methylated dGTP; different gel compositions, for example adding formamide; different primers or primers located at different distances from the problem region; or different templates such as single stranded DNAs. Sequencing of mRNA also can be employed.

[0040] Some or all of the coding sequence for a polypeptide having desaturase activity may be from a natural source. In some situations, however, it is desirable to modify all or a portion of the codons, for example, to enhance expression, by employing host preferred codons. Host-preferred codons can be determined from the codons of highest frequency in the proteins expressed in the largest amount in a particular host species and/or tissue of interest. Thus, the coding sequence for a polypeptide having desaturase activity can be synthesized in whole or in part. All or portions of the DNA also can be synthesized to remove any destabilizing sequences or regions of secondary structure which would be present in the transcribed mRNA. All or portions of the DNA also can be synthesized to alter the base composition to one more preferable in the desired host cell. Methods for synthesizing sequences and bringing sequences together are well established in the literature. *In vitro* mutagenesis and selection, site-directed mutagenesis, or other means can be employed to obtain mutations of naturally-occurring desaturase genes to produce a polypeptide having desaturase activity *in vivo* with more desirable physical and kinetic parameters for function in the host cell, such as a longer half-life or a higher rate of production of a desired polyunsaturated fatty acid.

[0041] Once the polynucleotide encoding a desaturase polypeptide has been obtained, it is placed in a vector capable of replication in a host cell, or is propagated *in vitro* by means of techniques such as PCR or long PCR. Replicating vectors can include plasmids, phage, viruses and cosmids. Desirable vectors include those useful for mutagenesis of the gene of interest or for expression of the gene of interest in host cells. The technique of long PCR has made *in vitro* propagation of large constructs possible, so that modifications to the gene of interest, such as mutagenesis or addition of expression signals, and propagation of the resulting constructs can occur entirely *in vitro* without the use of a replicating vector or a host cell.

[0042] For expression of a desaturase polypeptide, functional transcriptional and translational initiation and termination regions are operably linked to the polynucleotide encoding the desaturase polypeptide. Expression of the polypeptide coding region can take place *in vitro* or in a host cell. Transcriptional and translational initiation and termination regions are derived from a variety of nonexclusive sources, including the polynucleotide 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.

[0043] Expression in a host cell can be accomplished in a transient or stable fashion. Transient expression can occur from introduced constructs which contain expression signals functional in the host cell, but which constructs do not replicate and rarely integrate in the host cell, or where the host cell is not proliferating. Transient expression also can be accomplished by inducing the activity of a regulatable promoter operably linked to the gene of interest, although such inducible systems frequently exhibit a low basal level of expression. Stable expression can be achieved by introduction of a construct that can integrate into the host genome or that autonomously replicates in the host cell. Stable expression of the gene of interest can be selected for through the use of a selectable marker located on or transfected with the expression construct, followed by selection for cells expressing the marker. When stable expression results from integration, integration of constructs can occur randomly within the host genome or can be targeted through the use of constructs containing regions of homology with the host genome sufficient to target recombination with the host locus. Where constructs are targeted to an endogenous locus, all or some of the transcriptional and translational regulatory regions can be provided by the endogenous locus.

[0044] When increased expression of the desaturase polypeptide in the source organism is desired, several methods can be employed. Additional genes encoding the desaturase polypeptide can be introduced into the host organism. Expression from the native desaturase locus also can be increased through homologous recombination, for example by inserting a stronger promoter into the host genome to cause increased expression, by removing destabilizing sequences from either the mRNA or the encoded protein by deleting that information from the host genome, or by adding stabilizing sequences to the mRNA (U.S. Patent 4,910,141).

[0045] It is contemplated that more than one polynucleotide encoding a desaturase or a polynucleotide encoding more than one desaturase may be introduced and propagated in a host cell through the use of episomal or integrated expression vectors. Where two or more genes are expressed from separate replicating vectors, it is desirable that each vector has a different means of replication. Each introduced construct, whether integrated or not, should have a different means of selection and should lack homology to the other constructs to maintain stable expression and prevent reassortment of elements among constructs. Judicious choices of regulatory regions, selection means and method of propagation of the

introduced construct can be experimentally determined so that all introduced polynucleotides are expressed at the necessary levels to provide for synthesis of the desired products.

[0046] When necessary for transformation, a desaturase coding sequence can be inserted into a plant transformation vector, *e.g.* the binary vector described by Bevan (1984). Plant transformation vectors can be derived by modifying the natural gene transfer system of *Agrobacterium tumefaciens.* 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.

[0047] Probes based on the polynucleotides disclosed herein may find use in methods for isolating related molecules or in methods to detect organisms expressing desaturases. When used as probes, the polynucleotides or oligonucleotides must be detectable. This is usually accomplished by attaching a label either at an internal site, for example via incorporation of a modified residue, or at the 5' or 3' terminus. Such labels can be directly detectable, can bind to a secondary molecule that is detectably labeled, or can bind to an unlabelled secondary molecule and a detectably labeled tertiary molecule; this process can be extended as long as is practical to achieve a satisfactorily detectable signal without unacceptable levels of background signal. Secondary, tertiary, or bridging systems can include use of antibodies directed against any other molecule, including labels or other antibodies, or can involve any molecules which bind to each other, for example a biotin-streptavidin/avidin system. Detectable labels typically include radioactive isotopes, molecules which chemically or enzymatically produce or alter light, enzymes which produce detectable reaction products, magnetic molecules, fluorescent molecules or molecules whose fluorescence or light-emitting characteristics change upon binding. Examples of labeling methods can be found in U.S. Patent 5,011,770. Alternatively, the binding of target molecules can be directly detected by measuring the change in heat of solution on binding of probe to target via isothermal titration calorimetry, or by coating the probe or target on a surface and detecting the change in scattering of light from the surface produced by binding of target or probe, respectively, as may be done with the BIAcore system.

[0048] Constructs comprising the gene of interest may be introduced into a host cell by standard techniques. For convenience, a host cell which has been manipulated by any method to take up a DNA sequence or construct will be referred to as "transformed" or "recombinant" herein. The subject host will have at least one copy of the expression construct and may have two or more, for example, depending upon whether the gene is integrated into the genome, amplified, or is present on an extrachromosomal element having multiple copy numbers.

[0049] The transformed host cell can be identified by selection for a marker contained on the introduced construct. Alternatively, a separate marker construct may be introduced with the desired construct, as many transformation techniques introduce many DNA molecules into host cells. Typically, transformed hosts are selected for their ability to grow on selective media. Selective media may incorporate an antibiotic or lack a factor necessary for growth of the untransformed host, such as a nutrient or growth factor. An introduced marker gene therefor may confer antibiotic resistance, or encode an essential growth factor or enzyme, and permit growth on selective media when expressed in the transformed host. Selection of a transformed host can also occur when the expressed marker protein can be detected, either directly or indirectly. The marker protein may be expressed alone or as a fusion to another protein. The marker protein can be detected by its enzymatic activity; for example, beta-galactosidase can convert the substrate X-gal to a colored product, and luciferase can convert luciferin to a light-emitting product. The marker protein can be detected by its light-producing or modifying characteristics; for example, the green fluorescent protein of *Aequorea victoria* fluoresces when illuminated with blue light. Antibodies can be used to detect the marker protein or a molecular tag on, for example, a protein of interest. Cells expressing the marker protein or tag can be selected, for example, visually, or by techniques such as FACS or panning using antibodies. Desirably, resistance to kanamycin and the amino glycoside G418 are of interest, as well as ability to grow on media lacking uracil, leucine, lysine or tryptophan.

[0050] Another aspect of the present invention relates to transgenic plants or progeny of plants containing a recombinant construct comprising a polynucleotide sequence encoding a *Neurospora crassa* Δ15 desaturase mutagenized to increase expression in a monocot such as corn and a polynucleotide sequence encoding a Δ6 desaturase. Plant cells may be transformed with one or more isolated DNA(s) encoding Δ6- and Δ15-desaturase by any plant transformation method. The transformed plant cell, often 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.*, 1985). Since progeny of transformed plants inherit the polynucleotide(s) encoding the desaturase, seeds or cuttings from transformed plants may be used to maintain the transgenic plant line.

[0051] Further disclosed herein is a method for providing transgenic plants with an increased content of GLA and/or SDA. A DNA encoding a Δ15- and/or Δ12-desaturase may be introduced into plant cells with a Δ6 desaturase. Such plants may or may not also comprise endogenous Δ12- and/or Δ15-desaturase activity. Further disclosed herein is a

method for providing transgenic corn plants containing elevated levels of PUFAs including GLA and/or SDA, which are lacking in native corn plants. Expression vectors comprising DNA encoding a Δ6-desaturase, and/or a Δ12-desaturase and/or a Δ15-desaturase, can be constructed by methods of recombinant technology known to one of ordinary skill in the art (Sambrook *et al*., 2001).

[0052] For dietary supplementation, the purified PUFAs, transformed plants or plant parts, or derivatives thereof, may be incorporated into cooking oils, fats or margarines formulated so that in normal use the recipient would receive the desired amount. The PUFAs may also be incorporated into infant formulas, nutritional supplements or other food products, and may find use as anti-inflammatory or cholesterol lowering agents.

[0053] As used herein, "edible composition" is defined as compositions which may be ingested by a mammal such as foodstuffs, nutritional substances and pharmaceutical compositions. As used herein "foodstuffs" refer to substances that can be used or prepared for use as food for a mammal and include substances that may be used in the preparation of food (such as frying oils) or food additives. For example, foodstuffs include animals used for human consumption or any product therefrom, such as, for example, eggs. Typical foodstuffs include beverages, (*e.g.*, soft drinks, carbonated beverages, ready to mix beverages), infused foods (*e.g.* fruits and vegetables), sauces, condiments, salad dressings, fruit juices, syrups, desserts (*e.g.*, puddings, gelatin, icings and fillings, baked goods and frozen desserts such as ice creams and sherbets), soft frozen products (*e.g.*, soft frozen creams, soft frozen ice creams and yogurts, soft frozen toppings such as dairy or non-dairy whipped toppings), oils and emulsified products (*e.g.*, shortening, margarine, mayonnaise, butter, cooking oil, and salad dressings) and intermediate moisture foods (*e.g.*, rice and dog foods).

[0054] One example of a foodstuff encompassed by the invention is a food formulated for a companion animal. The term "companion animal" refers to a domesticated animal. The companion animal may be a mammal in particular, and specifically includes dogs, cats, rabbits, rodents, and horses. As described, the companion animal may obtain health benefits by consuming such a foodstuff comprising seed oil according to the invention.

[0055] The formulation of animal food products is well known to those of skill in the art, including food formulated for companion animals. In the area of cat and dog food, for example, wet pet food, semi-moist pet food, dry pet food and pet treats and snacks are well known. Drinks for pets are also available such as milk drinks for cats. An intermediate moisture food, for example, generally has a moisture content above 20 % while a wet food has a moisture of at least 65 %. Semi-moist food typically has a moisture content between 20 to 65 % and can include humectants such as propylene glycol, potassium sorbate, and other ingredients to prevent microbial (i.e., bacteria and mold) growth. Dry pet food (kibble) generally has a moisture content below 20 %, and its production may include extruding, drying and/or baking in heat. Pet treats and snacks are often semi-moist chewable treats or snacks; dry treats or snacks in any number of shapes or forms; chewable bones; baked, extruded or stamped treats; confection treats/snacks; or other kinds of treats, as is well known in the art.

[0056] An intermediate moisture pet food product may include ingredients such as cereal grains, meats, fats, vitamins, minerals, water and functional ingredients that are blended together, cooked and packaged. However, any semi-moist pet food formulation known to one skilled in the art can be used. For example, a pet food can be formed by adding, on a dry matter basis, 5-40 % by weight of protein; 5-45 % by weight of fat; 0.1-12 % by weight of a fiber; 1-90 % by weight carbohydrate, and 0.1-2 % by weight of a functional ingredient. An oil composition of the invention may added in any desired amount, for example, in 1-50 % by weight, including 1-30 % and 3-15 %. Variations may be made based on the desired characteristics of the end product, as is well known to those of skill in the art.

[0057] Furthermore, edible compositions described herein can also be ingested as an additive or supplement contained in foods and drinks. These can be formulated together with a nutritional substance such as various vitamins and minerals and incorporated into substantially liquid compositions such as nutrient drinks, soymilks and soups; substantially solid compositions; and gelatins or used in the form of a powder to be incorporated into various foods. The content of the effective ingredient in such a functional or health food can be similar to the dose contained in a typical pharmaceutical agent.

[0058] The purified PUFAs, transformed plants or plant parts may also be incorporated into animal, particularly livestock, feed. In this way, the animals themselves may benefit from a PUFA rich diet, while human consumers of food products produced from such livestock may benefit as well. It is expected in certain cases that SDA will be converted to EPA in animals and thus such animals may benefit from an increase in EPA by consumption of SDA.

[0059] For pharmaceutical use (human or veterinary), the compositions may generally be administered orally but can be administered by any route by which they may be successfully absorbed, *e.g.*, parenterally (*i.e.* subcutaneously, intramuscularly or intravenously), rectally, vaginally or topically, for example, as a skin ointment or lotion. The PUFAs, transformed plants or plant parts may be administered alone or in combination with a pharmaceutically acceptable carrier or excipient. Where available, gelatin capsules are the preferred form of oral administration. Dietary supplementation as set forth above can also provide an oral route of administration. The unsaturated acids may be administered in conjugated forms, or as salts, esters, amides or prodrugs of the fatty acids. Any pharmaceutically acceptable salt is contemplated; especially preferred are the sodium, potassium or lithium salts. Also contemplated are the N-alkylpolyhydroxamine salts, such as N-methyl glucamine, found in WO 96/33155. The preferred esters are the ethyl esters. As

solid salts, the PUFAs also can be administered in tablet form. For intravenous administration, the PUFAs or derivatives thereof may be incorporated into commercial formulations such as Intralipids.

[0060] Coding sequences or fragments thereof may be provided operably linked to a heterologous promoter, in either sense or antisense orientation. Expression constructs are also disclosed comprising these sequences, as are plants and plant cells transformed with the sequences. The construction of constructs which may be employed in conjunction with plant transformation techniques using these or other sequences will be known to those of skill of the art in light of the present disclosure (see, for example, Sambrook *et al*., 2001; Gelvin *et al*., 1990). The techniques are thus not limited to any particular nucleic acid sequences.

[0061] One use of the sequences will be in the alteration of oil composition. The desaturase gene may be provided with other sequences. Where an expressible coding region that is not necessarily a marker coding region is employed in combination with a marker coding region, one may employ the separate coding regions on either the same or different DNA segments for transformation. In the latter case, the different vectors are delivered concurrently to recipient cells to maximize cotransformation.

[0062] The choice of any additional elements used in conjunction with the desaturase coding sequences will often depend on the purpose of the transformation. One of the major purposes of transformation of crop plants is to add commercially desirable, agronomically important traits to the plant. As PUFAs are known to confer many beneficial effects on health, concomitant increases in SDA production may also be beneficial and could be achieved by expression of *Primula* Δ6-desaturase. Such increasing of SDA maycomprise expression of Δ12 and/or Δ15 desaturase.

[0063] Vectors used for plant transformation may include, for example, plasmids, cosmids, YACs (yeast artificial chromosomes), BACs (bacterial artificial chromosomes) or any other suitable cloning system, as well as fragments of DNA therefrom. Thus when the term "vector" or "expression vector" is used, all of the foregoing types of vectors, as well as nucleic acid sequences isolated therefrom, are included. It is contemplated that utilization of cloning systems with large insert capacities will allow introduction of large DNA sequences comprising more than one selected gene. This could be used to introduce various desaturase encoding nucleic acids. Introduction of such sequences may be facilitated by use of bacterial or yeast artificial chromosomes (BACs or YACs, respectively), or even plant artificial chromosomes. For example, the use of BACs for Agrobacterium-mediated transformation was disclosed by Hamilton *et al.* (1996).

[0064] Particularly useful for transformation are expression cassettes which have been isolated from such vectors. DNA segments used for transforming plant cells will, of course, generally comprise the cDNA, gene or genes which one desires to introduce into and have expressed in the host cells. These DNA segments can further include structures such as promoters, enhancers, polylinkers, or even regulatory genes as desired. The DNA segment or gene chosen for cellular introduction will often encode a protein which will be expressed in the resultant recombinant cells resulting in a screenable or selectable trait and/or which will impart an improved phenotype to the resulting transgenic plant. However, this may not always be the case, and transgenic plants may harbornon-expressed transgenes. Preferred components likely to be included with vectors are as follows.

[0065] The DNA sequence between the transcription initiation site and the start of the coding sequence, *i.e*., the untranslated leader sequence, can also influence gene expression. One may thus wish to employ a particular leader sequence with a transformation construct. Preferred leader sequences are contemplated to include those which comprise sequences predicted to direct optimum expression of the attached gene, *i.e*., to include a preferred consensus leader sequence which may increase or maintain mRNA stability and prevent inappropriate initiation of translation. The choice of such sequences will be known to those of skill in the art in light of the present disclosure. Sequences that are derived from genes that are highly expressed in plants will typically be preferred.

[0066] Transformation constructs will typically include a 3' end DNA sequence that acts as a signal to terminate transcription and allow for the poly-adenylation of the mRNA produced by coding sequences operably linked to a desaturase gene (*e.g.*, cDNA). The native terminator of a desaturase gene may be used. Alternatively, a heterologous 3' end may enhance the expression of desaturase coding regions. Examples of terminators deemed to be useful include those from the nopaline synthase gene of *Agrobacterium tumefaciens* (nos 3' end) (Bevan *et al*., 1983), the 3' end of the protease inhibitor I or II genes from potato or tomato and the CaMV 35S terminator. Regulatory elements such as an Adh intron (Callis *et al*., 1987), sucrose synthase intron (Vasil *et al*., 1989) or TMV omega element (Gallie *et al*., 1989), may further be included where desired.

[0067] Suitable methods for transformation of plant or other cells for use with the current invention are believed to include virtually any method by which DNA can be introduced into a cell, such as by direct delivery of DNA such as by PEG-mediated transformation of protoplasts (Omirulleh *et al*., 1993), by desiccation/inhibition-mediated DNA uptake (Potrykus *et al*., 1985), by electroporation (U.S. Patent 5,384,253), by agitation with silicon carbide fibers (Kaeppler et al., 1990; U.S. Patents 5,302,523 and 5,464,765), by Agrobacterium-mediated transformation (U.S. Patents 5,591,616 and 5,563,055) and by acceleration of DNA coated particles (U.S. Patents 5,550,318; 5,538,877; and 5,538,880). Through the application of techniques such as these, the cells of virtually any plant species may be stably transformed, and these cells developed into transgenic plants.

[0068] After effecting delivery of exogenous DNA to recipient cells, the next steps generally concern identifying the

transformed cells for further culturing and plant regeneration. In order to improve the ability to identify transformants, one may desire to employ a selectable or screenable marker gene with a transformation vector. In this case, one would then generally assay the potentially transformed cell population by exposing the cells to a selective agent or agents, or one would screen the cells for the desired marker gene trait.

[0069] In addition to direct transformation of a particular plant genotype with a construct, transgenic plants may be made by crossing a plant having a selected DNAto a second plant lacking the DNA. Plant breeding techniques may also be used to introduce multiple desaturases, for example Δ6, Δ12, and/or Δ15-desaturase(s) into a single plant. In this manner, the product of a Δ6-desaturase reaction can be effectively increased. By creating plants homozygous for a Δ6-desaturase gene and/or other desaturase genes (*e.g.*, Δ12- and/or Δ15-desaturase genes) beneficial metabolites can be increased in the plant.

[0070] As set forth above, a selected desaturase gene can be introduced into a particular plant variety by crossing, without the need for ever directly transforming a plant of that given variety. Therefore, a plant directly transformed or regenerated from cells which have been transformed, but also the progeny of such plants are contemplated. As used herein the term "progeny" denotes the offspring of any generation of a parent plant, wherein the progeny comprises a selected DNA construct. "Crossing" a plant to provide a plant line having one or more added transgenes or alleles relative to a starting plant line, as disclosed herein, is defined as the techniques that result in a particular sequence being introduced into a plant line by crossing a starting line with a donor plant line that comprises a transgene or allele of interest. To achieve this one could, for example, perform the following steps: (a) plant seeds of the first (starting line) and second (donor plant line that comprises a desired transgene or allele) parent plants; (b) grow the seeds of the first and second parent plants into plants that bear flowers; (c) pollinate a flower from the first parent plant with pollen from the second parent plant; and (d) harvest seeds produced on the parent plant bearing the fertilized flower.

[0071] Backcrossing is herein defined as the process including the steps of: (a) crossing a plant of a first genotype containing a desired gene, DNA sequence or element to a plant of a second genotype lacking said desired gene, DNA sequence or element; (b) selecting one or more progeny plant containing the desired gene, DNA sequence or element; (c) crossing the progeny plant to a plant of the second genotype; and (d) repeating steps (b) and (c) for the purpose of transferring a desired DNA sequence from a plant of a first genotype to a plant of a second genotype.

[0072] Introgression of a DNA element into a plant genotype is defined as the result of the process of backcross conversion. A plant genotype into which a DNA sequence has been introgressed may be referred to as a backcross converted genotype, line, inbred, or hybrid. Similarly a plant genotype lacking the desired DNA sequence may be referred to as an unconverted genotype, line, inbred, or hybrid.

EXAMPLES

[0073] The following examples are included to illustrate embodiments of the invention. Examples not covered by the scope of the claims are for illustrative purposes.

EXAMPLE 1

Vectors For Expression of Δ15- and Δ6-Desaturases in Corn

[0074] A binary vector was constructed to express a Δ15-desaturase and a Δ6-desaturase in corn embryo and aleurone tissue. This construct was prepared with the globulin promoter driving expression of a *Neurospora crassa* Δ15-desaturase mutagenized to increase expression in a monocot such as corn (SEQ ID NO:5) and of a *Mortierella alpina* Δ6 desaturase (SEQ ID NO:6, bp 71-1444) (U.S. Pat. No. 6,075,183). The *M. alpina* Δ6 desaturase was cloned into a shuttle vector containing the globulin promoter, pMON67624, resulting in pMON82809. The mutagenized *N. crassa* Δ15 desaturase was cloned into a shuttle vector containing the globulin promoter, pMON67624, resulting in pMON82810.

[0075] The two globulin desaturase expression cassettes were then cloned into the pMON30167 corn binary vector containing the CP4 marker gene for glyphosate resistance. The first expression cassette containing the *M. alpina* Δ6 desaturase was cloned into pMON30167, resulting in pMON82811. The second expression cassette containing the mutagenized *N. crassa* Δ15 desaturase was then cloned into pMON82811, resulting in corn transformation construct pMON82812 (FIG. 1). Transformed explants are obtained via *Agrobacterium tumefaciens*-mediated transformation. Plants are regenerated from transformed tissue. The greenhouse-grown plants are then analyzed for oil composition.

[0076] Another binary vector, pMON78175 was constructed to express a Δ15-desaturase and a Δ6-desaturase in corn embryo and aleurone tissue. To generate the binary vector, an expression cassette containing the *N. crassa* Δ15 desaturase (SEQ ID NO:5) under the control of the globulin promoter was PCR-amplified using pMON82812 as a template, cloned into a shuttle vector, and the cassette sequence-verified. The expression cassette containing the *P. juliae* Δ6-desaturase (SEQ ID NO: 7) driven by the globulin promoter was generated by PCR. As part of this process, the nucleotides immediately preceding the ATG-start codon of the *P. juliae* Δ6-desaturase were changed to CAGCC, to generate a

translation initiation region optimized for gene expression in monocotyledonous plants such as corn. Using standard restriction and ligation procedures that are well established in the art, the *P. juliae* Δ6-desaturase and the *N. crassa* Δ15 desaturase were subsequently cloned into a binary vector harboring a CP4 expression cassette as selectable marker to generate the corn transformation vector, pMON78175 (FIG 2). Transformed explants are obtained via *Agrobacterium tumefaciens*-mediated transformation. Plants are regenerated from transformed tissue. The greenhouse-grown plants are then analyzed for oil composition.

EXAMPLE 2

Vector for Expression of a Monocotyledonous Sequence-optimized *Primula juliae* Δ6 Desaturase in Corn

[0077] This example sets forth the design and construction of a *Primula juliae* Δ6 desaturase polynucleotide molecule modified for expression in monocotyledonous plants. It is well known in the art that non-endogenous protein-encoding sequences may not express well in plants (U.S. Patent No. 5,880,275). Therefore, using a native PjD6D polypeptide sequence (SEQ ID NO: 3), an artificial PjD6D protein-encoding polynucleotide sequence was designed and constructed by 1) using a codon usage bias similar to that of highly expressed monocot proteins, and by 2) removal of RNA destabilizing elements previously characterized and known to affect mRNA stability *in planta* (U.S. Patent No. 5,880,275). The resulting modified PjD6D polynucleotide sequence was designated PjD6Dnno (SEQ ID NO: 8) and encodes a polypeptide identical in sequence to the native PjD6D polypeptide (SEQ ID NO: 3).

[0078] A binary vector, pMON78171 was constructed to express a Δ15-desaturase and the sequence-modified Δ6-desaturase in corn embryo and aleurone tissue. To generate the binary vector, an expression cassette containing the *N. crassa* Δ15 desaturase (SEQ ID NO:5) under the control of the globulin promoter was PCR-amplified using pMON82812 as a template, cloned into a shuttle vector, and the cassette sequence-verified. The expression cassette containing the *P. juliae* Δ6-desaturase (SEQ ID NO:9) driven by the globulin promoter was generated by PCR. As part of this process, the nucleotides immediately preceding the ATG-start codon of the *P. juliae* Δ6-desaturase were changed to CAGCC, to generate a translation initiation region optimized for gene expression in monocotyledonous plants, such as corn. Using standard restriction and ligation procedures that are well established in the art the *P. juliae* Δ6-desaturase and the *N. crassa* Δ15 desaturase were subsequently cloned into a binary vector harboring a CP4 expression cassette as selectable marker to generate the corn transformation vector, pMON78171 (FIG 3). Transformed explants are obtained via *Agrobacterium tumefaciens*-mediated transformation. Plants are regenerated from transformed tissue. The greenhouse-grown plants are then analyzed for oil composition.

EXAMPLE 3

Fatty Acid Analysis

[0079] Fatty acid composition of mature kernels expressing pMON82812 was determined by grinding corn kernels and extracting the homogenate with heptane. The heptane extract was treated with toluene containing triheptadecanoin at 0.25mg/ml and sodium methoxide in methanol (0.6 N). The reaction was stopped with aqueous sodium chloride (10% wt/vol). After partitioning at room temperature, the organic phase was analyzed by GLC (Hewlett Packard model 6890 (120volt) equipped with a split/splitless capillary inlet (250 °C) and a flame ionization detector (270 °C). The column was a Supelco 24077 (0.25 mm od. x 15 m length) with a 0.25 μm bonded polyethylene glycol stationary phase. The fatty acid methyl esters are identified by retention time comparison to commercial standards. Qualitative weight percent compositions are calculated as area percents of identified peaks. The results of the analysis for kernels that exhibited SDA and GLA are given in Table 1. Partial null kernels containing only GLA were not found. Overall, more than two thirds of the kernels analyzed contained GLA and SDA. The analysis of a mature kernel from event ZM_103111:@, which was transformed with pMON82812, demonstrated 9.68% SDA.

TABLE 1: Fatty Acid Analysis of Single Mature Corn Kernels Expressing SDA and/or GLA

Pedigree	Gen	Oleic (18:1)	LA (18:2)	GLA (18:3)	ALA (18:3)	SDA (18:4)
ZM_S103111:@.	R1	26.08	13.62	0.91	26.44	9.68
ZM_S103432:@.	R1	23.63	14.87	0.85	30.17	8.56
ZM_S103121:@.	R1	27.39	16.2	1.14	27.61	5.8
ZM_S103432:@.	R1	21.44	14.11	0.68	36.19	5.79
ZM_S103111:@.	R1	25.5	16.36	0.61	30.56	5.19

(continued)

Pedigree	Gen	Oleic (18:1)	LA (18:2)	GLA (18:3)	ALA (18:3)	SDA (18:4)
ZM_S103435/LH244	F1	22.3	16.98	0.99	33.28	4.81
ZM_S103435/LH244	F1	23.85	19.52	0.84	29.91	4.21
ZM_S103121:@.	R1	26.2	15.07	1.11	32.29	3.93
ZM_S103432:@.	R1	20.38	18.37	0.85	35.49	3.84
ZM_S103121:@.	R1	25.07	17.73	0.77	30.96	3.78
ZM_S103110:@.	R1	21.57	18.99	0.68	34.07	3.69
ZM_S103432:@.	R1	19.99	19.61	0.78	34.71	3.69
ZM_S103110:@.	R1	22.17	18.57	0.58	34.81	3.67
ZM_S103427:@.	R1	18.42	23.76	1.01	32.27	3.61
ZM_S103435/LH244	F1	20.83	19.3	0.68	34.71	3.53
ZM_S103427:@.	R1	19.17	25.22	1.53	30.79	3.53
ZM_S103110:@.	R1	22.12	17.73	0.79	34.38	3.48
ZM_S103099/LH244	F1	20.26	21.88	0.75	32.13	3.4
ZM_S103432:@.	R1	16.98	18.61	0.8	40.32	3.27
ZM_S103432:@.	R1	21.18	19.65	0.73	34.34	3.25
ZM_S103111:@.	R1	24.59	19.08	0.35	30.79	3.23
ZM_S103111:@.	R1	21.29	19.93	0.71	33.77	3.23
ZM_S103099/LH244	F1	21.11	23.29	0.79	30.95	3.21
ZM_S103432:@.	R1	18.2	18.81	0.61	38.94	3.19
ZM_S103435/LH244	F1	21.27	19.75	0.7	34.33	3.13
ZM_S103432:@.	R1	20.8	21.47	0.76	33.59	3.12
ZM_S103121:@.	R1	23.71	18.96	0.75	31.97	3.1
ZM_S103121:@.	R1	23.81	17.28	0.98	33.75	3.07
ZM_S103099/LH244	F1	19.64	21.46	0.7	34.49	2.99
ZM_S103121:@.	R1	23.83	16.72	1.01	34.5	2.93
ZM_S103427:@.	R1	16.68	26.92	1.03	30.59	2.87
ZM_S103168/LH244	F1	18.58	23.81	1.34	32.42	2.87
ZM_S103432:@.	R1	17.94	18.89	0.72	39.8	2.84
ZM_S103110:@.	R1	20.14	19.32	0.58	36.65	2.77
ZM_S103111:@.	R1	20.57	19.13	0.34	36.1	2.7
ZM_S103168/LH244	F1	20.04	25.44	1.26	30.32	2.69
ZM_S103110:@.	R1	21.6	19.78	0.61	35.15	2.66
ZM_S103099/LH244	F1	21.06	23.54	0.67	31.49	2.58
ZM_S103435/LH244	F1	19.26	22.53	0.9	34.92	2.53
ZM_S103433/LH244	F1	22.95	20.01	0.39	33.51	2.47
ZM_S103168/LH244	F1	19.39	26.31	1.23	31.06	2.4
ZM_S103110:@.	R1	18.05	22.96	0.65	35.53	2.39
ZM_S103110:@.	R1	18.99	21.92	0.65	35.22	2.38

(continued)

Pedigree	Gen	Oleic (18:1)	LA (18:2)	GLA (18:3)	ALA (18:3)	SDA (18:4)
ZM_S103111:@.	R1	17.14	22.59	0.71	36.95	2.32
ZM_S103433/LH244	F1	23.64	19.84	0.38	33.12	2.29
ZM_S103436/LH244	F1	20.71	26.64	1.07	28.11	2.26
ZM_S103435/LH244	F1	21.89	21.12	0.6	33.35	2.24
ZM_S103110:@.	R1	22.59	28.35	0.52	25.05	2.15
ZM_S103168/LH244	F1	19.41	27.76	1.19	28.96	2.14
ZM_S103168/LH244	F1	17.81	28.33	1.28	31.34	2.1
ZM_S103097/LH244	F1	18.61	25.34	1.19	31.88	2.09
ZM_S103168/LH244	F1	20.08	28.05	1.27	28.28	2.06
ZM_S103433/LH244	F1	20.11	19.18	0.38	38.29	2.04
ZM_S103427:@.	R1	18.38	30.32	1.19	26.79	1.98
ZM_S103427:@.	R1	20.06	29.56	1.13	27.23	1.95
ZM_S103436/LH244	F1	19.82	28.13	0.89	28.57	1.94
ZM_S103110:@.	R1	18.74	22.83	0.72	35.29	1.91
ZM_S103433/LH244	F1	21.69	21.09	0.4	34.71	1.9
ZM_S103430/LH244	F1	23.25	25.64	0.92	27.64	1.89
ZM_S103099/LH244	F1	17.77	25.43	0.61	33.61	1.88
ZM_S103111:@.	R1	21.04	22.99	0.29	31.6	1.86
ZM_S103168/LH244	F1	18.19	27.7	1.18	31.55	1.86
ZM_S103099/LH244	F1	18.24	23.16	0.65	35.78	1.85
ZM_S103435/LH244	F1	21.02	27.67	0.88	28.27	1.83
ZM_S103433/LH244	F1	21.7	21.08	0.39	34.49	1.8
ZM_S103097/LH244	F1	20.11	26.32	1.08	29.94	1.8
ZM_S103427:@.	R1	16.95	30.23	1.08	30.11	1.8
ZM_S103437/LH244	F1	23.93	26.23	1.12	25.86	1.78
ZM_S103437/LH244	F1	23.5	26.49	0.99	26.32	1.77
ZM_S103168/LH244	F1	19.4	27.81	1.06	30.29	1.74
ZM_S103427:@.	R1	17.94	30.11	1.17	29.42	1.74
ZM_S103103/LH244	F1	21.32	31.31	1.16	24.36	1.66
ZM_S103433/LH244	F1	20.48	21.06	0.41	35.4	1.64
ZM_S103437/LH244	F1	19.71	26.4	1.06	31.7	1.6
ZM_S103433/LH244	F1	18.98	21.89	0.36	37.18	1.59
ZM_S103430/LH244	F1	21.41	26.76	0.91	27.06	1.56
ZM_S103437/LH244	F1	18.67	28.15	1.07	30.71	1.56
ZM_S103097/LH244	F1	19.97	28.13	1.18	28.16	1.55
ZM_S103436/LH244	F1	19.29	31.27	0.79	25.74	1.53
ZM_S103430/LH244	F1	22.43	25.58	0.81	28.41	1.53
ZM_S103430/LH244	F1	18.48	27.25	1.05	31.73	1.53

(continued)

Pedigree	Gen	Oleic (18:1)	LA (18:2)	GLA (18:3)	ALA (18:3)	SDA (18:4)
ZM_S103103/LH244	F1	21.25	31.91	1.13	24.06	1.53
ZM_S103435/LH244	F1	20.92	27.87	0.82	27.48	1.51
ZM_S103121:@.	R1	20	24.11	0.99	33.48	1.5
ZM_S103103/LH244	F1	20.9	31.44	1.08	25.09	1.5
ZM_S103103/LH244	F1	20.06	32.22	1.04	25.4	1.4
ZM_S103103/LH244	F1	20.02	33.05	1.09	24.5	1.39
ZM_S103097/LH244	F1	18.78	28.78	1.03	29.82	1.31
ZM_S103111:@.	R1	19.23	27.51	0.62	29.58	1.25
ZM_S103436/LH244	F1	18.53	30.87	0.66	27.55	1.22
LH244/ZM S103431	F1	20.88	23.22	0.34	32.46	1.19
LH244/ZM_S103431	F1	20.07	25.05	0.35	33.01	1.19
ZM_S103436/LH244	F1	20.39	31.62	0.69	26.02	1.16
ZM_S103111:@.	R1	20.48	24.18	0.47	32.33	1.11
ZM_S103435/LH244	F1	20.4	26.7	0.52	31.26	1.09
ZM_S103436/LH244	F1	19.35	31.69	0.71	27.3	1.08
LH244/ZM_S103431	F1	19.75	23.35	0.26	33.86	0.98
ZM_S103436/LH244	F1	20.11	32.54	0.71	26.25	0.96
ZM_S103430/LH244	F1	18.87	29.25	0.7	30.17	0.95
LH244/ZM_S103431	F1	21.18	25.86	0.2	29.33	0.87
LH244/ZM_S103098	F1	21.77	24.64	0.15	32.57	0.81
LH244/ZM_S103105	F1	17.72	32.84	0.41	27.61	0.68
ZM_S103434/LH244	F1	20.34	26.19	0.3	31.48	0.6
ZM_S103434/LH244	F1	21.59	26.44	0.28	29.99	0.58
ZM_S103434/LH244	F1	20.22	27.13	0.31	30.47	0.58
LH244/ZM_S103098	F1	19.29	27.08	0.19	33.6	0.52
ZM_S103434/LH244	F1	19.24	28.24	0.26	31.45	0.51
LH244/ZM_S103105	F1	17.73	34.46	0.44	27.24	0.5
LH244/ZM_S103431	F1	19.12	31.08	0.24	27.77	0.47
ZM_S103434/LH244	F1	17.63	29.39	0.24	32.47	0.38
LH244/ZM_S103105	F1	18.37	36.34	0.36	24.68	0.33
LH244/ZM_S103105	F1	18.62	38.05	0.34	22.84	0.27
ZM_ S103110:@.	R1	18.35	57.16	0	2.25	0
LH244/ZM_S103098	F1	19.18	58.95	0	1.78	0
LH244/ZM_S103098	F1	19.35	58.56	0	1.79	0
LH244/ZM_S103098	F1	19.17	59.15	0	1.8	0
LH244/ZM_S103098	F1	16.76	62.23	0	1.81	0
LH244/ZM_S103098	F1	18.39	59.37	0	1.88	0
LH244/ZM_S103098	F1	18.26	59.91	0	1.96	0

(continued)

Pedigree	Gen	Oleic (18:1)	LA (18:2)	GLA (18:3)	ALA (18:3)	SDA (18:4)
LH244/ZM_S103098	F1	17.14	61.34	0	2.06	0
LH244/ZM_S103098	F1	16.65	61.17	0	2.39	0

[0080] Fatty acid analysis of events generated by transformation with pMON78171 are shown in Table 2 below. Ten mature R1 or F1 seed were analyzed for their fatty acid composition as above, and average fatty acid composition was calculated from those numbers, excluding the nulls. The best performing event obtained with vector pMON78171 contained on average 28.6% SDA, and 2.2% GLA. The best performing single corn seed contained 32.9% SDA and 3.5% GLA.

TABLE 2: Fatty Acid Analysis of Mature Corn Kernels

Pedigree	Gen	Oleic	LA	GLA	ALA	SDA
ZM_S126797:@.	R1	21.34	12.92	3.53	13.49	32.92
ZM_S126797:@.	R1	22.11	12.12	3.3	14.12	32.58
ZM_S126797:@.	R1	21.91	12.87	3.5	13.1	32.28
ZM_S127034:@.	R1	23.26	9.86	1.22	17.05	31.2
ZM_S126797:@.	R1	24.07	14.98	3.24	13.06	28.74
ZM_S128026/LH244	F1	21.96	16.99	3.24	12.69	28.5
ZM_S127034:@.	R1	24.15	11.3	1.2	18.06	28.19
ZM_S129919:@.	R1	21.79	17.34	2.43	14.1	28.19
ZM_S127034:@.	R1	27.39	9.56	1.28	16.03	28.04
ZM_S127034:@.	R1	29.01	9.8	1.19	15.19	27.41
ZM_S128026/LH244	F1	22.35	17.95	3.58	12.5	26.93
ZM_S129919:@.	R1	20.77	19.48	3.02	14.79	26.51
ZM_S127034:@.	R1	25.09	12.21	1.29	17.85	26.4
ZM_S127034:@.	R1	22.99	15.22	1.3	18.47	25.87
ZM_S126797:@.	R1	21.95	19.77	4.66	11.88	25.81
ZM_S127034:@.	R1	26.95	13.34	1.35	15.95	25.62
ZM_S126797:@.	R1	20.94	20.76	4.4	13.5	24.7
ZM_S126790/LH244	F1	24.46	19.05	2.71	13.05	24.57
ZM_S128026/LH244	F1	23.44	19.46	3.71	12.28	24.24
ZM_S126797:@.	R1	21.41	22.02	4.87	11.41	23.95
ZM_S126808:@.	R1	25.61	18.44	2.38	14.16	23.81
ZM_S126797:@.	R1	22.74	20.52	4.23	12.86	23.8
ZM_S126797:@.	R1	20.95	22.77	4.76	11.94	23.74
ZM_S126808:@.	R1	22	19.57	2.76	16.21	23.64
ZM_S129919:@.	R1	21.49	23.45	2.95	13.33	22.4
ZM_S128026/LH244	F1	23.12	22.18	3.87	12.33	22.34
ZM_S126797:@.	R1	21.51	24.35	4.56	12	21.82
ZM_S126790/LH244	F1	25.99	20.48	2.46	13.48	21.71
ZM_S126995/LH244	F1	24.4	22.45	2.12	14.26	20.03

(continued)

Pedigree	Gen	Oleic	LA	GLA	ALA	SDA
ZM_S126790/LH244	F1	24.33	23.6	2.87	13.24	19.76
ZM_S126790/LH244	F1	24.24	24.08	2.88	12.74	19.44
ZM_S126995/LH244	F1	29.51	20.1	1.96	12.44	19.17
ZM_S126800/LH244	F1	26.18	24.62	3.01	11.52	18.9
ZM_S126800/LH244	F1	24.35	26.28	3.26	10.89	18.56
ZM_S129919:@.	R1	21.87	27.84	2.77	12.76	18.28
ZM_S126995/LH244	F1	26.78	24.15	2.09	11.97	18.26
ZM_S129919:@.	R1	21.22	28.87	3	12.33	18.18
ZM_S126800/LH244	F1	23.23	27.22	3.54	11.35	17.98
ZM_S126790/LH244	F1	22.01	28.63	3.36	12.63	17.78
ZM_S126995/LH244	F1	26.48	23.9	1.84	12.93	17.78
ZM_S126800/LH244	F1	26.6	25.01	2.72	11.7	17.54
ZM_S126995/LH244	F1	25.83	25.52	2.12	12.59	17.15
ZM_S129919:@.	R1	21.8	30	2.79	11.51	16.89
ZM_S129919:@.	R1	20.77	31.81	3.03	11.33	16.65
ZM_S126808:@.	R1	22.22	30.61	2.64	11.91	16.07
ZM_S126808:@.	R1	22.6	30.58	2.5	11.72	15.54
ZM_S126800/LH244	F1	23.86	31.07	3.13	11.37	15.07
ZM_S126800/LH244	F1	26.73	29.67	2.92	9.6	14.26
ZM_S126995/LH244	F1	31.78	22.68	1.87	12.32	14.07
ZM_S126808:@.	R1	22.94	33.24	2.81	10.51	13.79
ZM_S126808:@.	R1	21.28	34.77	3.15	10.62	13.65
ZM_S126808:@.	R1	25.73	32.94	2.19	9.26	11.95
ZM_S128026/LH244	F1	18.81	62.08	0.09	1.61	0.7
ZM_S126790/LH244	F1	19.23	62.98	0	1.36	0.06
ZM_S126790/LH244	F1	19.62	63.27	0	1.18	0
ZM_S126790/LH244	F1	19.56	63.19	0	1.38	0
ZM_S126790/LH244	F1	19.88	61.96	0	1.3	0
ZM_S126790/LH244	F1	20.43	61.28	0	1.37	0
ZM_S126800/LH244	F1	22.09	59.61	0	1.22	0
ZM_S126800/LH244	F1	20.09	62.11	0	1.32	0
ZM_S126800/LH244	F1	21.8	59.52	0	1.34	0
ZM_S126800/LH244	F1	22.55	59.63	0	1.28	0
ZM_S126808:@.	R1	20.9	60.65	0	1.43	0
ZM_S126808:@.	R1	20.57	60.95	0	1.54	0
ZM_S126808:@.	R1	18.75	62.61	0	1.45	0
ZM_S126995/LH244	F1	20.03	63.35	0	1.22	0
ZM_S126995/LH244	F1	21.55	59.59	0	1.1	0

(continued)

Pedigree	Gen	Oleic	LA	GLA	ALA	SDA
ZM_S126995/LH244	F1	24.63	56.02	0	1.12	0
ZM_S126995/LH244	F1	20.12	60.75	0	1.23	0
ZM_S126998:@.	R1	21.51	60	0	1.62	0
ZM_S126998:@.	R1	22.17	27.15	0	34.29	0
ZM_S126998:@.	R1	19.77	62.76	0	1.61	0
ZM_S126998:@.	R1	20.73	28.41	0	33.75	0
ZM_S126998:@.	R1	20.59	33.96	0	29.47	0
ZM_S126998:@.	R1	20.8	33.48	0	28.9	0
ZM_S126998:@.	R1	22.15	33.67	0	27.92	0
ZM_S126998:@.	R1	19.86	30.92	0	32.91	0
ZM_S126998:@.	R1	21.47	30.99	0	31.39	0
ZM_S126998:@.	R1	21.37	31.16	0	30.85	0
ZM_S127034:@.	R1	19.76	62.38	0	1.36	0
ZM_S127034:@.	R1	19.99	62.51	0	1.26	0
ZM_S127034:@.	R1	20.76	61.27	0	1.25	0
ZM_S128026/LH244	F1	20.86	59.51	0	1.63	0
ZM_S128026/LH244	F1	18.57	63.07	0	1.38	0
ZM_S128026/LH244	F1	19.7	62.41	0	1.13	0
ZM_S128026/LH244	F1	19.8	61.53	0	1.2	0
ZM_S128026/LH244	F1	17.96	65.06	0	1.32	0
ZM_S129919:@.	R1	20.7	60.71	0	1.29	0
ZM_S129919:@.	R1	20.04	61.49	0	1.45	0
ZM_S129919:@.	R1	19.53	61.77	0	1.37	0

[0081] Fatty acid analysis of events generated by transformation with pMON78175 are shown in Table 3 below. Ten mature R1 or F1 seed were analyzed for their fatty acid composition as above. The best performing single corn seed contained 12.4% SDA and 0% GLA.

TABLE 3: Fatty Acid Analysis of Mature Corn Kernels

Pedigree	Gen	Oleic acid	Linoleic acid	GLA	ALA	SDA
ZM_S130139:@.	R1	26.57	8.39	0	34.39	12.36
ZM_S130134:@.	R1	30.59	9.04	0	30.31	11.86
ZM_S130139:@.	R1	26.39	9.41	0	34.91	11.12
ZM_S130135:@.	R1	25.09	12.98	0.12	34.81	9.93
ZM_S130133:@.	R1	30.62	10.85	0	30.93	9.63
ZM_S130136:@.	R1	30.55	11.99	0	32.28	7.63
ZM_S130136:@.	R1	28.27	13.53	0	33.41	7.45
ZM_S130136:@.	R1	29.14	10.61	0	34.59	7.44
ZM_S130134:@.	R1	25.43	14.32	0	35.68	7.3

(continued)

Pedigree	Gen	Oleic acid	Linoleic acid	GLA	ALA	SDA
ZM_S130134:@.	R1	23.94	16.92	0	35.7	6
ZM_S130133:@.	R1	22.93	17.14	0.02	37.21	5.88
ZM_S130136:@.	R1	28.28	11.89	0	35.63	5.86
ZM_S130140:@.	R1	23.47	15.34	0	37.85	5.68
ZM_S130133:@.	R1	21.75	17.83	0	37.43	5.59
ZM_S130072/LH244	F1	23.17	25.78	0.2	28.61	5.39
ZM_S130140:@.	R1	22.75	17.26	0	37.98	4.83
ZM_S130161:@.	R1	28.54	13.26	0	37.6	2.88
ZM_S130140:@.	R1	20.82	23.51	0	35.48	2.88
ZM_S130155/LH244	F1	20.87	59.43	0	2.2	0

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Claims

1. A transgenic corn seed comprising a seed oil having a stearidonic acid (18:4 n-3) content of from 20 % to 33 %, comprising gamma-linolenic acid in a content of less than 5 %, and comprising a polynucleotide sequence encoding a *Neurospora crassa* Δ15 desaturase mutagenized to increase expression in a monocot such as corn and a polynucleotide sequence encoding a Δ6 desaturase.

2. The transgenic corn seed of claim 1, wherein the seed oil

   (i) has a stearidonic acid content selected from: from 20 % to 30 %, and from 25 % to 30 %; or
   (ii) comprises gamma-linolenic acid in a content of less than 3 %; or
   (iii) has a ratio of stearidonic acid to gamma-linolenic acid selected from: from 1:1 to 10:1, from 2:1 to 10:1, from 3:1 to 5:1 and at least 3:1; or
   (iv) has a ratio of omega-3 to omega-6 fatty acids selected from: from 0.5:1 to 10:1, from 5:1 to 10:1, and at least 5:1.

3. The transgenic corn seed of claims 1 or 2, defined as an inbred seed or as a hybrid seed.

4. An endogenous corn seed oil having a stearidonic acid (18:4 n-3) content of from 20 % to 33 %, comprising gamma-linolenic acid in a content of less than 5 %, and being obtainable from the transgenic corn seed of claim 1 or 2.

5. A transgenic corn plant that produces a seed oil having a stearidonic acid (18:4 n-3) content of from 20 % to 33 %, comprising gamma-linolenic acid in a content of less than 5 %, and comprising a polynucleotide sequence encoding a *Neurospora crassa* Δ15 desaturase mutagenized to increase expression in a monocot such as corn and a polynucleotide sequence encoding a Δ6 desaturase.

6. The transgenic corn plant of claim 5, defined as an inbred plant or as a hybrid plant.

7. A transgenic plant transformed with a recombinant construct comprising a polynucleotide sequence encoding a *Neurospora crassa* Δ15 desaturase mutagenized to increase expression in a monocot such as corn and a polynucleotide sequence encoding a Δ6 desaturase, wherein the plant produces the corn seed oil of claim 1.

8. A method of producing seed oil, comprising growing the transgenic plant of claim 5 under plant growth conditions until the transgenic plant produces said seed oil.

9. A method of increasing the nutritional value of an edible product for human or non-human animal consumption, comprising adding the corn seed oil of claim 4 to the edible product.

10. The method of claim 9, wherein

    (i) the edible product is selected from human food, animal feed and a food supplement; or

(ii) the corn seed oil increases the stearidonic acid content of the edible product; or
(iii) the corn seed oil increases the ratio of omega-3 to omega-6 fatty acids of the edible product; or
(iv) the edible product lacks stearidonic acid prior to adding the corn seed oil.

11. A method of manufacturing food and/or feed, comprising adding the corn seed oil of claim 4 to starting ingredients to produce the food and/or feed.

12. Food or feed made by the method of claim 11.

13. A composition for providing stearidonic acid to a human or non-human animal, said composition comprising the corn seed oil of claim 4.

14. The composition of claim 13, which is an edible composition, preferably

(i) the edible composition is food or feed, most preferably the food comprises beverages, infused foods, sauces, condiments, salad dressings, fruit juices, syrups, desserts, icings and fillings, soft frozen products, confections or intermediate moisture food, or is food or feed for a companion animal; or
(ii) the edible composition is substantially a liquid or a solid; or
(iii) the edible composition is a food supplement and/or a nutraceutical.

15. The composition of claim 13, which

(i) is suitable to be administered to a human; or
(ii) is suitable to be administered to a non-human animal, preferably to livestock or poultry.

Patentansprüche

1. Transgenes Maiskorn, umfassend ein Maissamenöl mit einem Stearidonsäure(18:4 n-3)-Gehalt von 20 bis 33%, das gamma-Linolensäure in einem Gehalt von weniger als 5% umfasst, und umfassend eine Polynucleotidsequenz, die eine *Neurospora-crassa*-Δ15-Desaturase codiert, welche so mutagenisiert ist, dass sie die Expression in einer einkeimblättrigen Pflanze, wie Mais, erhöht, und eine Polynucleotidsequenz, die eine Δ6-Desaturase codiert.

2. Transgenes Maiskorn gemäß Anspruch 1, wobei das Maissamenöl

(i) einen Stearidonsäuregehalt aufweist, der aus 20% bis 30% und 25% bis 30% ausgewählt ist; oder
(ii) gamma-Linolensäure in einem Gehalt von weniger als 3% umfasst; oder
(iii) ein Verhältnis von Stearidonsäure zu gamma-Linolensäure aufweist, das aus 1:1 bis 10:1, 2:1 bis 10:1, 3:1 bis 5:1 und wenigstens 3:1 ausgewählt ist; oder
(iv) ein Verhältnis von omega-3- zu omega-6-Fettsäuren aufweist, das aus 0,5:1 bis 10:1, 5:1 bis 10:1 und wenigstens 5:1 ausgewählt ist.

3. Transgenes Maiskorn gemäß Anspruch 1 oder 2, das als Inzuchtsamen oder als Hybridsamen definiert ist.

4. Endogenes Maissamenöl, mit einem Stearidonsäure(18:4 n-3)-Gehalt von 20 bis 33%, das gamma-Linolensäure in einem Gehalt von weniger als 5% umfasst und aus dem transgenen Maiskorn gemäß Anspruch 1 oder 2 erhältlich ist.

5. Transgene Maispflanze, die ein Maissamenöl mit einem Stearidonsäure(18:4 n-3)-Gehalt von 20 bis 33%, das gamma-Linolensäure in einem Gehalt von weniger als 5% umfasst, und eine Polynucleotidsequenz, die eine *Neurospora-crassa*-Δ15-Desaturase codiert, welche so mutagenisiert ist, dass sie die Expression in einer einkeimblättrigen Pflanze, wie Mais, erhöht, und eine Polynucleotidsequenz, die eine Δ6-Desaturase codiert, umfasst, erzeugt.

6. Transgene Maispflanze gemäß Anspruch 5, das als Inzuchtpflanze oder als Hybridpflanze definiert ist.

7. Transgene Pflanze, die mit einem rekombinanten Konstrukt transformiert ist, das eine Polynucleotidsequenz, die eine *Neurospora-crassa*-Δ15-Desaturase codiert, welche so mutagenisiert ist, dass sie die Expression in einer einkeimblättrigen Pflanze, wie Mais, erhöht, und eine Polynucleotidsequenz, die eine Δ6-Desaturase codiert, um-

fasst, wobei die Pflanze das Maissamenöl gemäß Anspruch 1 erzeugt.

8. Verfahren zur Herstellung von Samenöl, umfassend das Wachsenlassen der transgenen Pflanze gemäß Anspruch 5 unter Pflanzenwachstumsbedingungen, bis die transgene Pflanze das Samenöl erzeugt.

9. Verfahren zur Erhöhung des Nährwerts eines essbaren Produkts zum menschlichen oder nichtmenschlichen tierischen Verbrauch, umfassend das Hinzufügen des Maissamenöls von Anspruch 4 zu dem essbaren Produkt.

10. Verfahren gemäß Anspruch 9, wobei

(i) das essbare Produkt aus Lebensmitteln, Tierfutter und einem Nahrungsergänzungsstoff ausgewählt ist; oder
(ii) das Maissamenöl den Stearidonsäuregehalt des essbaren Produkts erhöht; oder
(iii) das Maissamenöl das Verhältnis von omega-3- zu omega-6-Fettsäuren des essbaren Produkts erhöht; oder
(iv) das essbare Produkt vor der Zugabe des Maissamenöls keine Stearidonsäure enthält.

11. Verfahren zur Herstellung von Lebensmittel und/oder Futter, umfassend das Hinzufügen des Maissamenöls gemäß Anspruch 4 zu Ausgangszutaten zur Herstellung des Lebensmittels und/oder Futters.

12. Lebensmittel oder Futter, hergestellt nach dem Verfahren gemäß Anspruch 11.

13. Zusammensetzung zum Bereitstellen von Stearidonsäure für einen Menschen oder ein nichtmenschliches Tier, wobei die Zusammensetzung das Maissamenöl gemäß Anspruch 4 umfasst.

14. Zusammensetzung gemäß Anspruch 13, bei der es sich um eine essbare Zusammensetzung handelt, wobei vorzugsweise

(i) es sich bei der essbaren Zusammensetzung um Lebensmittel oder Futter handelt, wobei am meisten bevorzugt das Lebensmittel Getränke, Aufgusslebensmittel, Soßen, Würzen, Salatdressings, Fruchtsäfte, Sirupe, Desserts, Glasuren und Füllungen, weiche gefrorene Produkte, Süßwaren oder ein mittelfeuchtes Lebensmittel umfasst, oder es sich um Nahrung oder Futter für ein Haustier handelt; oder
(ii) die essbare Zusammensetzung im Wesentlichen eine Flüssigkeit oder ein Feststoff ist; oder
(iii) die essbare Zusammensetzung ein Nahrungsergänzungsstoff und/oder ein Nutrazeutikum ist.

15. Zusammensetzung gemäß Anspruch 13, die

(i) für die Verabreichung an einen Menschen geeignet ist; oder
(ii) für die Verabreichung an ein nichthumanes Tier, vorzugsweise an Vieh oder Geflügel, geeignet ist.

Revendications

1. Graine de maïs transgénique comprenant de l'huile de graines ayant une teneur en acide stéaridonique (18:4 n-3) de 20 à 33 %, comprenant de l'acide gamma-linolénique à une teneur inférieure à 5 %, et comprenant une séquence de polynucléotides codant pour une Δ15 désaturase de *Neurospora crassa* ayant subi une mutagenèse pour accroître son expression chez les monocotyles comme le maïs et une séquence de polynucléotides codant pour une Δ6 désaturase.

2. Graine de maïs transgénique selon la revendication 1, dans laquelle l'huile de graines

(i) a une teneur en acide stéaridonique choisie parmi : de 20 à 30 % et de 25 à 30 % ; ou
(ii) comprend de l'acide gamma-linolénique à une teneur inférieure à 3 % ; ou
(iii) a un rapport acide stéaridonique à acide gamma-linolénique choisi parmi : de 1:1 à 1:10, de 2:1 à 10:1, de 3:1 à 5:1 et au moins de 3:1 ; ou
(iv) a un rapport acides gras oméga-3 à oméga-6 choisi parmi : de 0,5:1 à 10:1, de 5:1 à 10:1 et au moins de 5:1.

3. Graine de maïs transgénique selon les revendications 1 ou 2, définie comme une graine autofécondée ou une graine hybride.

4. Huile de graines de maïs endogène ayant une teneur en acide stéaridonique (18:4 n-3) de 20 à 33 %, comprenant de l'acide gamma-linolénique à une teneur inférieure à 5 %, et pouvant être obtenue à partir de la graine de maïs transgénique selon la revendication 1 ou 2.

5. Plant de maïs transgénique qui produit une huile de graines ayant une teneur en acide stéaridonique (18:4 n-3) de 20 à 33 %, comprenant de l'acide gamma-linolénique à une teneur inférieure à 5 %, et comprenant une séquence de polynucléotides codant pour une Δ15 désaturase de *Neurospora crassa* ayant subi une mutagenèse pour accroître son expression chez les monocotyles comme le maïs et une séquence de polynucléotides codant pour une Δ6 désaturase.

6. Plant de maïs transgénique selon la revendication 5, défini comme un plant autofécondé ou un plant hybride.

7. Plant transgénique transformé avec une construction recombinée comprenant une séquence de polynucléotides codant pour une Δ15 désaturase de *Neurospora crassa* ayant subi une mutagenèse pour accroître son expression chez les monocotyles comme le maïs et une séquence de polynucléotides codant pour une Δ6 désaturase, où le plant produit l'huile de graines de maïs selon la revendication 1.

8. Procédé de production d'huile de graines, comprenant la culture du plant transgénique selon la revendication 5 dans des conditions de croissance végétale jusqu'à ce que le plant transgénique produise ladite huile de graines.

9. Procédé d'accroissement de la valeur nutritionnelle d'un produit comestible pour la consommation humaine ou animale non humaine, comprenant l'ajout de l'huile de graines de maïs selon la revendication 4 au produit comestible.

10. Procédé selon la revendication 9, dans lequel

(i) le produit comestible est choisi parmi un aliment destiné à la consommation humaine, un aliment pour animaux et un complément alimentaire ;ou
(ii) l'huile de graines de maïs accroît la teneur en acide stéaridonique du produit comestible ; ou
(iii) l'huile de graines de maïs accroît le rapport acides gras oméga-3 à oméga-6 du produit comestible ; ou
(iv) le produit comestible est exempt d'acide stéaridonique avant ajout de l'huile de graines de maïs.

11. Procédé de préparation d'un aliment et/ou d'un aliment pour animaux, comprenant l'ajout de l'huile de graines de maïs selon la revendication 4 aux ingrédients de départ pour obtenir l'aliment et/ou l'aliment pour animaux.

12. Aliment ou aliment pour animaux préparé par le procédé selon la revendication 11.

13. Composition pour apporter de l'acide stéaridonique à un sujet humain ou à un animal non humain, ladite composition comprenant l'huile de graines de maïs selon la revendication 4.

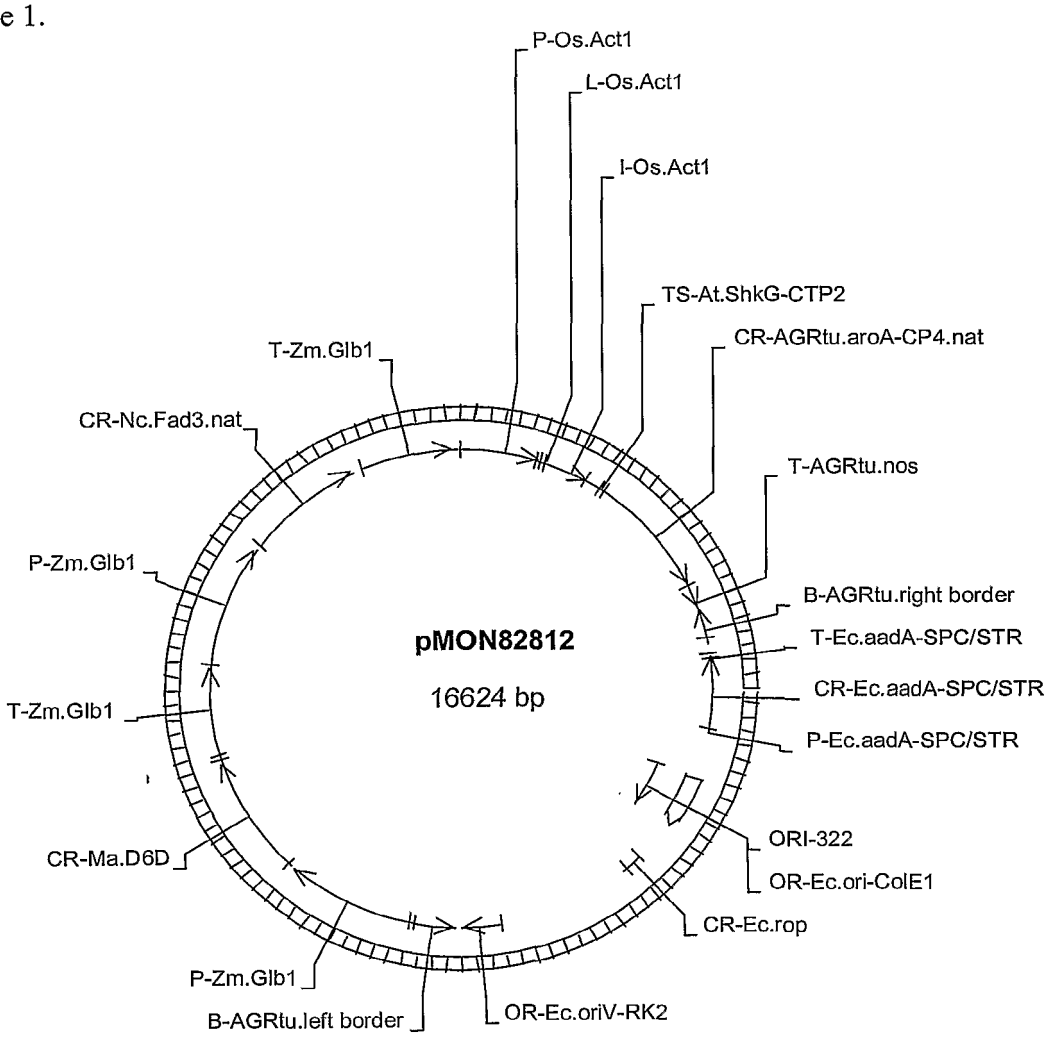
14. Composition selon la revendication 13, qui est une composition comestible, de préférence

(i) la composition comestible est un aliment ou aliment pour animaux, mieux encore l'aliment comprend les boissons, les aliments infusés, les sauces, les condiments, les vinaigrettes, les jus de fruits, les sirops, les desserts, les glaçages et les farces, les produits congelés, les friandises ou autres aliments ayant une teneur en humidité intermédiaire, ou est un aliment pour animal de compagnie ; ou
(ii) la composition comestible est essentiellement un liquide ou un solide ; ou
(iii) la composition comestible est un complément alimentaire et/ou un neutraceutique.

15. Composition selon la revendication 13, qui

(i) se prête à l'administration à un sujet humain ; ou
(ii) se prête à l'administration à un animal non humain, de préférence le bétail ou la volaille.

Figure 1.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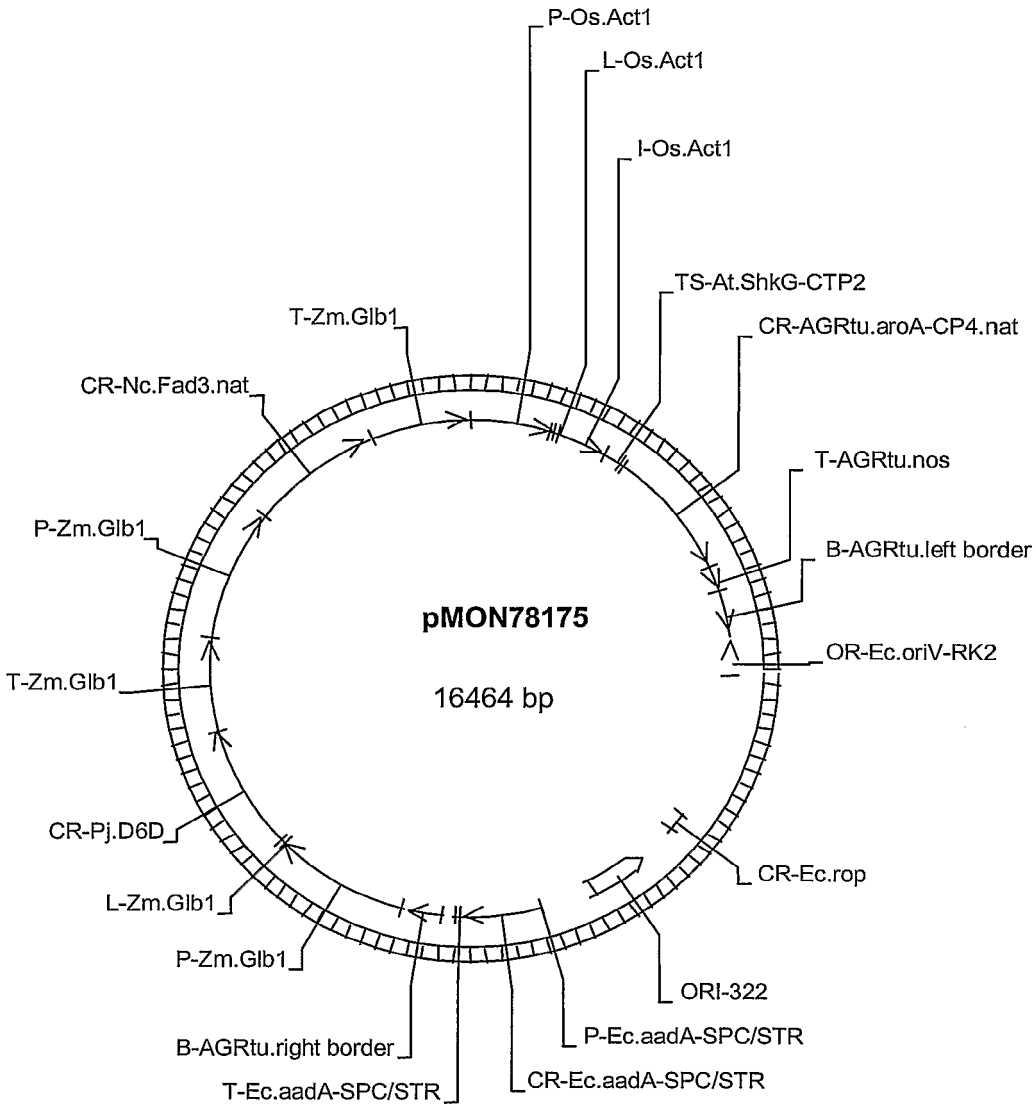
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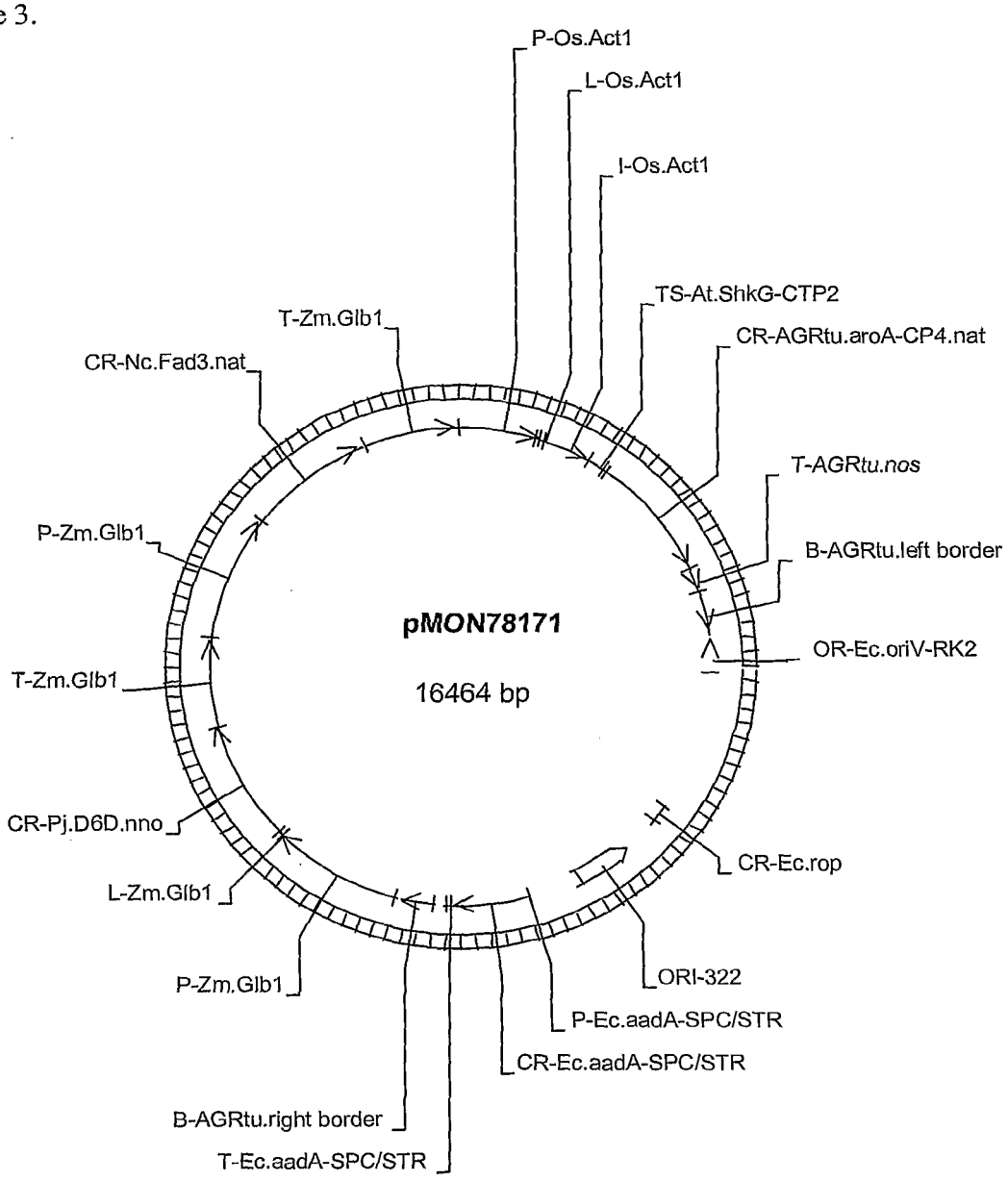
P-Zm.Glb1
L-Zm.Glb1
CR-Pj.D6D
T-Zm.Glb1
P-Zm.Glb1
CR-Nc.Fad3.nat
T-Zm.Glb1
pMON78175
16464 bp

Figure 3.


P-Os.Act1
L-Os.Act1
I-Os.Act1
TS-At.ShkG-CTP2
CR-AGRtu.aroA-CP4.nat
T-AGRtu.nos
B-AGRtu.left border
OR-Ec.oriV-RK2
CR-Ec.rop
ORI-322
P-Ec.aadA-SPC/STR
CR-Ec.aadA-SPC/STR
B-AGRtu.right border
T-Ec.aadA-SPC/STR
P-Zm.Glb1
L-Zm.Glb1
CR-Pj.D6D.nno
T-Zm.Glb1
P-Zm.Glb1
CR-Nc.Fad3.nat
T-Zm.Glb1
pMON78171
16464 bp

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