



HU000034217T2

(19) **HU****MAGYARORSZÁG****Szellemi Tulajdon Nemzeti Hivatala**(11) Lajstromszám: **E 034 217**(13) **T2**

EURÓPAI SZABADALOM SZÖVEGÉNEK FORDÍTÁSA

- (21) Magyar ügyszám: **E 11 000857**
(22) A bejelentés napja: **2005. 10. 07.**
- (96) Az európai bejelentés bejelentési száma:
EP 20050000857
- (97) Az európai bejelentés közzétételi adatai:
EP 2338328 A2 **2011. 06. 29.**
- (97) Az európai szabadalom megadásának meghirdetési adatai:
EP 2338328 B1 **2017. 09. 20.**
- (51) Int. Cl.: **A01H 1/00** (2006.01)
A01H 5/10 (2006.01)
C12P 7/64 (2006.01)
C12N 5/04 (2006.01)
C12N 15/82 (2006.01)
A01H 5/00 (2006.01)
C07C 57/00 (2006.01)

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- (54) **Meghatározott növények, amelyek magjában nincs telített zsírsav vagy a telített zsírsavak szintje csökkentett és a magból származó olaj**

Az európai szabadalom ellen, megadásának az Európai Szabadalmi Közlönyben való meghirdetésétől számított kilenc hónapon belül, felszólalást lehet benyújtani az Európai Szabadalmi Hivatalnál. (Európai Szabadalmi Egyezmény 99. cikk(1))

A fordítást a szabadalmas az 1995. évi XXXIII. törvény 84/H. §-a szerint nyújtotta be. A fordítás tartalmi helyességét a Szellemi Tulajdon Nemzeti Hivatala nem vizsgálta.



(11) **EP 2 338 328 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention
of the grant of the patent:
20.09.2017 Bulletin 2017/38

(51) Int Cl.:
A01H 1/00 (2006.01) **A01H 5/00** (2006.01)
A01H 5/10 (2006.01) **C12N 5/04** (2006.01)
C12N 15/82 (2006.01) **C12P 7/64** (2006.01)
C07C 57/00 (2006.01)

(21) Application number: **11000857.0**

(22) Date of filing: **07.10.2005**

(54) **Certain plants with no saturate or reduced saturate levels of fatty acids in seeds, and oil derived from the seeds**

Bestimmte Pflanzen mit keinem oder geringem Anteil an Fettsäuren in den Samen und aus den Samen gewonnenes Öl

Certaines plantes avec des niveaux non saturés ou réduits d'acides gras dans les graines et huile dérivée de ces graines

(84) Designated Contracting States:
**AT BE BG CH CY CZ DE DK EE ES FI FR GB GR
HU IE IS IT LI LT LU LV MC NL PL PT RO SE SI
SK TR**

(30) Priority: **08.10.2004 US 617532 P**

(43) Date of publication of application:
29.06.2011 Bulletin 2011/26

(62) Document number(s) of the earlier application(s) in
accordance with Art. 76 EPC:
05802517.2 / 1 799 027

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US-B1- 6 495 738 US-B2- 6 407 317

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Processing for Applications", 17 December 2003
(2003-12-17), CRC press, Boca Raton ISBN:
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DescriptionCross-Reference to Related Application

- 5 **[0001]** The subject application claims priority to U.S. provisional application serial number 60/617,532 filed on October 8, 2004.

Background of the Invention

- 10 **[0002]** Vegetable-derived oils have gradually replaced animal-derived oils and fats as the major source of dietary fat intake. However, saturated fat intake in most industrialized nations has remained at about 15% to 20% of total caloric consumption. In efforts to promote healthier lifestyles, the United States Department of Agriculture (USDA) has recently recommended that saturated fats make up less than 10% of daily caloric intake. To facilitate consumer awareness, current labeling guidelines issued by the USDA now require total saturated fatty acid levels be less than 1.0 g per 14 g serving to receive the "low-sat" label and less than 0.5 g per 14 g serving to receive the "no-sat" label. This means that the saturated fatty acid content of plant oils needs to be less than 7% and 3.5% to receive the "low sat" and "no sat" label, respectively. Since issuance of these guidelines, there has been a surge in consumer demand for "low-sat" oils. To date, this has been met principally with canola oil, and to a much lesser degree with sunflower and safflower oils.

- 15 **[0003]** The characteristics of oils, whether of plant or animal origin, are determined predominately by the number of carbon and hydrogen atoms, as well as the number and position of double bonds comprising the fatty acid chain. Most oils derived from plants are composed of varying amounts of palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3) fatty acids. Conventionally, palmitic and stearic acids are designated as "saturated" because their carbon chains are saturated with hydrogen atoms and hence have no double bonds; they contain the maximal number of hydrogen atoms possible. However, oleic, linoleic, and linolenic are 18-carbon fatty acid chains having one, two, and three double bonds, respectively, therein. Oleic acid is typically considered a mono-unsaturated fatty acid, whereas linoleic and linolenic are considered to be poly-unsaturated fatty acids. The U.S. Department of Agriculture defines "no saturates" or "no sat" products as a product having less than 3.5% by weight combined saturated fatty acids (as compared to the total amount of fatty acids).

- 20 **[0004]** While unsaturated fats (monounsaturated and polyunsaturated) are beneficial (especially when consumed in moderation), saturated and trans fats are not. Saturated fat and trans fat raise LDL cholesterol levels in the blood. Dietary cholesterol also raises LDL cholesterol and may contribute to heart disease even without raising LDL. Therefore, it is advisable to choose foods low in saturated fat, trans fat, and cholesterol as part of a healthful diet.

- 25 **[0005]** The health value of high levels of monounsaturates, particularly oleic acid, as the major dietary fat constituent has been established by recent studies. Such diets are thought to reduce the incidence of arteriosclerosis that results from diets high in saturated fatty acids. There is accordingly a need for an edible vegetable oil having a high content of monounsaturates. Seed mutagenesis has been used to produce a rapeseed oil with no more than 4% saturated fatty acid content (PCT International Patent Application publication Number WO 91/15578).

- 30 **[0006]** Over 13% of the world's supply of edible oil in 1985 was produced from the oilseed crop species Brassica, commonly known as rapeseed or mustard. Brassica is the third most important source of edible oil, ranking behind only soybean and palm. Because Brassica is able to germinate and grow at relatively low temperatures, it is also one of the few commercially important edible oilseed crops that can be cultivated in cooler agricultural regions, as well as serving as a winter crop in more temperate zones. Moreover, vegetable oils in general, and rapeseed oil in particular, are gaining increasing consideration for use in industrial applications because they have the potential to provide performance comparable to that of synthetic or mineral/naphthenic-based oils with the very desirable advantage of also being biodegradable.

- 35 **[0007]** Canola oil has the lowest level of saturated fatty acids of all vegetable oils. "Canola" refers to rapeseed (Brassica) which has an erucic acid (C22:1) content of at most 2 percent by weight based on the total fatty acid content of a seed (preferably at most 0.5 percent by weight and most preferably essentially 0 percent by weight) and which produces, after crushing, an air-dried meal containing less than 30 micromoles per gram of defatted (oil-free) meal. These types of rapeseed are distinguished by their edibility in comparison to more traditional varieties of the species.

- 40 **[0008]** Modification of vegetable oils may be effected chemically. This approach has been used to obtain a salad/cooking oil which contains saturated fatty acids of less than about 3% (U.S. Pat. No. 4,948,811); the oil may be formed by chemical reaction, or by physical separation of the saturated lipids. A general reference is made to using "genetic engineering" to achieve an oil of the desired characteristics (see column 3, line 58 *et seq.*). However, there is no detailed disclosure of how any particular oilseed plant could be so modified to provide a vegetable oil of the characteristics desired.

- 45 **[0009]** Typically, the fatty acid composition of vegetable oils has instead been modified through traditional breeding techniques. These techniques utilize existing germplasm as a source of naturally occurring mutations that affect fatty acid composition. Such mutations are uncovered and selected for by the use of appropriate screening, in conjunction

with subsequent breeding. For example, such an approach has been used to decrease the amount of the long chain fatty acid erucate in rapeseed oil (Stefansson, B. R. (1983) in High and Low Erucic Acid Rapeseed Oils, Kramer J. K. G. et al., eds; Academic Press, New York; pp. 144-161), and to increase the amount of the monounsaturated fatty acid oleate in corn oil (U.S. patent application, Ser. No. 07/554,526).

[0010] Recently, attempts have been made to increase the pool of available mutations from which to select desired characteristics through the use of mutagens. However, mutagens generally act by inactivation or modification of genes already present, resulting in the loss or decrease of a particular function. The introduction of a new characteristic through mutagenesis thus often depends on the loss of some trait already present. In addition, the achievement of desired goals with mutagens is generally uncertain. Only a few types of modified fatty acid compositions in vegetable oils have been achieved using this approach. One example of such a "created" mutation which affects fatty acid composition is the decrease of polyunsaturated fatty acids, in particular of linoleate and linolenate, in rapeseed oil, with a concomitant increase in the monounsaturated fatty acid oleate (Auld, M., et al., (1992) Crop Sci. in press). Another is the decrease of saturated fatty acids in rapeseed oil (PCT International Patent Application Publication Number WO 91/15578). However, the biochemistry of seed oil synthesis is complex, and not well understood; there may be several mechanisms which contribute to the changes in the fatty acid compositions observed in rapeseed oil (PCT International Patent Application Publication Number WO 91/15578). The use of mutagenesis to affect such changes is essentially random, and non-specific.

[0011] The possibility of modifying fatty acid composition through the use of genetic engineering would, in theory, allow the precise, controlled introduction of specific desirable genes, as well as the inactivation of specific undesirable genes or gene products. Thus, novel traits completely independent of genes already present could be introduced into plants, or preselected genes could be inactivated or modified. However, one predicate to making effective use of genetic engineering to modify fatty acid compositions is a reasonably accurate model of the mechanisms at work in the plant cell regulating fatty acid synthesis and processing.

[0012] U.S. Patent No. 6,495,738 (*see also* WO 99/50430) shows that the levels of saturated fatty acids in corn oil and tobacco seeds can be altered by expressing a fungal palmitate-CoA delta-9 desaturase within a plant cell. These proteins most likely enzymatically desaturate palmitate-CoA molecules, preferentially, by removing two hydrogen atoms and adding a double bond between the 9th and 10th carbon atoms from the CoA portion of the molecule, thus producing palmitoleic-CoA (16:1 delta-9). The palmitoleic-CoA is ultimately incorporated into seed oil thus lowering the total saturate levels of said oil. The total saturated fatty acid level of corn oil, averaging about 13.9%, does not meet the current labeling guidelines discussed above. Furthermore, corn is typically not considered to be an oil crop as compared to soybean, canola, sunflower, and the like. In fact, the oil produced and extracted from corn is considered to be a byproduct of the wet milling process used in starch extraction. Because of this, there has been little interest in modifying the saturate levels of corn oil.

[0013] It is postulated that, in oilseeds, fatty acid synthesis occurs primarily in the plastid, and that the newly synthesized fatty acids are exported from the plastid to the cytoplasm. In the cytoplasm they are utilized in the assembly of triglycerides, which occurs in the endoreticular membranes.

[0014] The major product of fatty acid synthesis is palmitate (16:0), which appears to be efficiently elongated to stearate (18:0). While still in the plastid, the saturated fatty acids may then be desaturated, by an enzyme known as delta-9 desaturase, to introduce one or more carbon-carbon double bonds. Specifically, stearate may be rapidly desaturated by a plastidial delta-9 desaturase enzyme to yield oleate (18:1). In fact, palmitate may also be desaturated to palmitoleate (16:1) by the plastidial delta-9 desaturase, but this fatty acid appears in only trace quantities (0-0.2%) in most vegetable oils.

[0015] Thus, the major products of fatty acid synthesis in the plastid are palmitate, stearate, and oleate. In most oils, oleate is the major fatty acid synthesized, as the saturated fatty acids are present in much lower proportions.

[0016] Subsequent desaturation of plant fatty acids outside the plastid in the cytoplasm appears to be limited to oleate, which may be desaturated to linoleate (18:2) and linolenate (18:3). In addition, depending on the plant, oleate may be further modified by elongation (to 20:1, 22:1, and/or 24:1), or by the addition of functional groups. These fatty acids, along with the saturated fatty acids palmitate and stearate, may then be assembled into triglycerides.

[0017] The plant delta-9 desaturase enzyme is soluble. It is located in the plastid stroma, and uses newly synthesized fatty acids esterified to ACP, predominantly stearyl-ACP, as substrates. This is in contrast to the yeast delta-9 desaturase enzyme, which is located in the endoplasmic reticular membrane (ER, or microsomal), uses fatty acids esterified to Co-A as substrates, and desaturates both the saturated fatty acids palmitate and stearate. U.S. Patent Nos. 5,723,595 and 6,706,950 relate to a plant desaturase.

[0018] The yeast delta-9 desaturase gene has been isolated from *Saccharomyces cerevisiae*, cloned, and sequenced (Stukey, J. E. et al., J. Biol. Chem. 264:16537-16544 (1989); Stukey, J. E. et al., J. Biol. Chem. 265:20144-20149 (1990)). This gene has also been used to transform the same yeast strain under conditions in which it is apparently overexpressed, resulting in increased storage lipid accumulation in the transformed yeast cells as determined by fluorescence microscopy using Nile Red as a stain for triglycerides (U.S. Pat. No. 5,057,419). The fatty acid composition was not characterized.

This reference contains a *general* discussion of using information from the isolated yeast delta-9 desaturase gene to first isolate other desaturase genes from yeast, or from other organisms, and then to re-introduce these genes into a yeast or plant under conditions. It is speculated that this could lead to high expression in order to modify the oil produced and its fatty acid composition.

[0019] Subsequently, it was reported that the yeast delta-9 desaturase gene had been introduced into tobacco leaf tissue (Polashcok, J. et al., FASEB J. 5:A1157 (1991) and was apparently expressed in this tissue. Further, this gene was expressed in tomato. See Wang et al., J. Agric Food Chem. 44:3399-3402 (1996); and C. Wang et al., Phytochemistry 58:227-232 (2001). While some increases in certain unsaturates and some decreases in some saturates were reported for both tobacco and tomato, tobacco and tomato are clearly not oil crops. This yeast gene was also introduced into *Brassica napus* (see U.S. Patent No. 5,777,201). Although a reduction in palmitate and stearate (saturates) and an increase in palmitoleate and oleate (unsaturates) was reported (see Tables 1a and 1b in Example 7 of that patent), this reference is discussed in more detail towards the beginning of the Detailed Description section, below. WO 00/11012 and U.S. Patent No. 6,825,335 relate to a synthetic yeast desaturase gene for expression in a plant, wherein the gene comprises a desaturase domain and a cyt b₅ domain. The Background section of these references discuss fatty acid synthesis in detail.

[0020] The performance characteristics, whether dietary or industrial, of a vegetable oil are substantially determined by its fatty acid profile, that is, by the species of fatty acids present in the oil and the relative and absolute amounts of each species. While several relationships between fatty acid profile and performance characteristics are known, many remain uncertain. Notwithstanding, the type and amount of unsaturation present in a vegetable oil have implications for both dietary and industrial applications.

[0021] Standard canola oil contains about 8-12% linolenic acid, which places it in a similar category as soybean oil with respect to oxidative, and hence flavor, stability. The oxidative stability of canola oil can be improved in a number of ways, such as by hydrogenating to reduce the amount of unsaturation, adding antioxidants, and blending the oil with an oil or oils having better oxidative stability. For example, blending canola oil with low linolenic acid oils, such as sunflower, reduces the level of 18:3 and thus improves the stability of the oil. However, these treatments necessarily increase the expense of the oil, and can have other complications; for example, hydrogenation tends to increase both the level of saturated fatty acids and the amount of trans unsaturation, both of which are undesirable in dietary applications.

[0022] High oleic oils are available, but, in addition to the possible added expense of such premium oils, vegetable oils from crops bred for very high levels of oleic acid can prove unsatisfactory for industrial uses because they retain fairly high levels of polyunsaturated fatty acids, principally linoleic and/or linolenic. Such oils may still be quite usable for dietary applications, including use as cooking oils, but have inadequate oxidative stability under the more rigorous conditions found in industrial applications. Even the addition of antioxidants may not suffice to bring these oils up to the levels of oxidative stability needed for industrial applications; this is probably due to the levels of linolenic acid, with its extremely high susceptibility to oxidation, found in these oils.

[0023] Oxidative stability is important for industrial applications to extend the life of the lubricant under conditions of heat and pressure and in the presence of chemical by-products. In such applications linolenic acid, and to a lesser extent linoleic acid, are again most responsible for poor oxidative stability.

[0024] Therefore, it would be desirable to obtain a variety of *Brassica napus* which is agronomically viable and produces seed oil having a level of oxidative stability sufficient to qualify it for use in dietary applications, and which would additionally be either sufficiently stable alone, or, depending on the precise application, sufficiently responsive to antioxidants, to find use in industrial applications.

[0025] European Patent Application EP 323753, U.S. Patent No. 5,840,946, and U.S. Patent No. 5,638,637 are directed to rapeseed oil having an oleic content of 80-90% (by weight, of total fatty acid content) and not more than 2% erucic acid. Mutagenesis was used to improve the oleic acid content. The claims of the '946 patent further specify that the oil also has an erucic acid content of no more than 2%, and alpha-linolenic acid content of less than 3.5%, and a saturated fatty acid content in the form of stearic and palmitic of no more than 7%. These patents relate to mutagenesis followed by selection.

[0026] U.S. Patent Nos. 5,387,758; 5,434,283; and 5,545,821 are directed to rapeseed having 2-4% combined stearic and palmitic acids (by weight), and an erucic acid content of no more than about 2% by weight. Mutagenesis was used to lower the stearic and palmitic acid content.

[0027] International Application WO 92/03919, and U.S. Patent Nos. 5,668,299; 5,861,187; and 6,084,157 are directed to rapeseed seeds, plants, and oils having altered fatty acid profiles. Several such profiles are mentioned, all of which contemplate a maximum erucic acid content of about 2%, combined with palmitic acid content of from about 7% to about 12%, linoleic content of about 14% to about 20%, stearic acid content of from about 0.8% to about 1.1%, and alpha-linolenic acid content of about 7% to about 9%, as well as certain ranges of FDA saturates. These patents define saturated fatty acids and "FDA saturates" as the sum of lauric (C12:0), myristic (C14:0), palmitic (C16:0), and stearic (C18:0) acids.

[0028] International Application WO 93/06714, and U.S. Patent Nos. 6,270,828; 6,562,397; 6,680,396; and 6,689,409 are directed to canola oil and seeds with reduced glucosinolates (and thus reduced sulfur), as well as an alpha-linolenic

acid content of about 2% to about 7%.

[0029] U.S. Patent No. 6,169,190 relates to oil from canola seed having an oleic fatty acid content of approximately 71-77% and a linolenic acid content of less than about 3%. Oleic:linolenic ratios between 34-55 are also claimed.

[0030] U.S. Patent Nos. 6,063,947 and 5,850,026 claim oil obtained from canola seeds, related canola plants, and methods of producing the oil, wherein the oil has an oleic acid content greater than about 80% (about 86-89%), a linoleic acid content of about 2% to about 6%, an alpha-linolenic acid content of less than 2.5% (about 1-2%), and an erucic acid content of less than about 2% (after hydrolysis). These patents relate to seed-specific inhibition of microsomal oleate desaturase (a delta-12 desaturase which converts oleic acid to linoleic acid) and microsomal linoleate desaturase (a delta-15 desaturase which converts linoleic acid to alpha-linolenic acid) gene expression.

[0031] U.S. Patent No. 5,952,544 claims fragments of a plant plastid or microsomal delta-15 fatty acid desaturase enzyme, which catalyzes a reaction between carbons 15 and 16.

[0032] U.S. Patent Nos. 4,627,192 and 4,743,402 relate to sunflower seeds and sunflower oil having an oleic acid content of approximately 80-94% (relative to the total fatty acid content thereof) and a ratio of linoleic to oleic of less than about 0.09. These sunflower plants were obtained by traditional breeding techniques.

[0033] WO 2003002751 relates to the use of kinase genes and the like to alter the oil phenotype of plants.

[0034] The ability of delta-9 desaturase genes to significantly (and desirably) affect the fatty acid profile of already-beneficial oil seed crops, particularly to decrease the levels of saturated fats without adversely affecting other aspects of the plant and oil, is unpredictable.

Brief Summary of the Invention

[0035] The subject invention provides "no sat" canola oil. The invention also relates in part to methods for reducing saturated fatty acids in certain plant seeds. These results were surprisingly achieved by the use of a delta-9 desaturase gene in canola (*Brassica*). Included in the subject invention are canola, capable of producing such oils and seeds. The subject invention also provides seeds and oils from said plants wherein the oils have particularly advantageous characteristics and fatty acid profiles, which were not heretofore attained. In some preferred embodiments, a preferred plant comprises at least two copies of a delta-9 desaturase gene. Seeds produced by such plants surprisingly do not exhibit effects of gene silencing but rather have further surprising reductions in levels of total saturates.

Brief Description of the Figures

[0036]

Figure 1 shows that a greater than 60% reduction of saturated fatty acids was achieved in *Arabidopsis*. This graph summarizes T2 and T3 seed data for a single *Arabidopsis* event.

Figure 2 shows a reduction in "sats" of up to 60-70% in T2 *Arabidopsis* seeds from 18 additional transformants. Data illustrated in this graph was a combination of the numerical data shown in **Table 8** and earlier numerical data.

Figure 3 shows that saturated fats were reduced by over 43% in Westar canola (and a 50% reduction was achieved when 24:0 was included).

Figure 4A shows a bar graph comparing total saturates of seeds from various canola plants comprising Event 36-11.19 compared to a control. **Figures 4B and 4C** present numerical data illustrated by the bar graph.

Figure 5A shows a bar graph comparing total saturates of seeds from various canola plants comprising Event 218-11.30 compared to a control. **Figures 5B and 5C** present numerical data illustrated by the bar graph.

Figures 6A-F show half-seed data from the T3 field trials. **Figures 6A and 6B** clearly show the reductions in C16:0 and increases in C16:1 in the transgenic events as compared to the nulls (events in which the transgene segregated out of the plant) and wild-type controls (non-transformed lines). **Figures 6C and 6D** clearly show the reductions in C18:0 and increases in C18:1 in the transgenic events as compared to the nulls and wild-type controls. **Figures 6E and 6F** clearly show the reductions in C20:0 and C22:0, respectively, in the transgenic events as compared to the nulls and wild-type controls.

Figures 6G and 6H clearly show shifts and reductions in C16:0, and shifts and increases in C16:1 in the transgenic events, as compared to the nulls and wild-type controls. **Figures 6I and 6J** clearly show shifts and reductions in C18:0, and shifts and increases in C18:1 in the transgenic events, as compared to the nulls and wild-type controls. **Figures 6K and 6L** show similar bar graphs for C18:2 and C18:3. **Figure 6M** further illustrates reductions in total saturates, as compared to already very good Nex 710 lines. **Figure 6N** shows distributions for 1000 seeds.

Figures 7A and 7B illustrate data obtained using the protocol of Example 16.

Figures 8 and 9 are pictures of two gels run with DNA from F3 plants, as discussed in Example 19.

Brief Description of the Sequences**[0037]**

SEQ ID NO:1 shows the nucleic acid sequence of the open reading frame for the plant-optimized, delta-9 desaturase gene used herein.

SEQ ID NO:2 shows the sequence of the ORF of SEQ ID NO:1 preceded by a Kozak sequence and a BamHI cloning site (residues 1-10), plus a translational terminator at the end of the ORF (residues 1379-1381).

SEQ ID NO:3 shows the nucleic acid sequence of the delta-9 forward B primer used to amplify the delta-9 gene.

SEQ ID NO:4 shows the nucleic acid sequence of the delta-9 reverse B primer used to amplify the delta-9 gene.

SEQ ID NO:5 shows the amino acid sequence encoded by SEQ ID NO:1.

Detailed Description of the Invention

[0038] The subject invention provides "no sat" canola oil. The invention also relates in part to methods for reducing saturated fatty acids in canola plant seeds. These results were surprisingly achieved through the use of a delta-9 desaturase gene to surprisingly produce "no sat" levels of fatty acids in canola (*Brassica*). The subject invention includes such plants and also provides seeds and oils from said plants wherein the oils have particularly advantageous characteristics and fatty acid profiles, which were not heretofore attained.

[0039] The *Aspergillus nidulans* microsomal delta-9-CoA desaturase gene is exemplified herein. This delta-9 desaturase is a membrane-bound enzyme and catalyzes the reaction of 16:0-CoA and 18:0-CoA to 16:1-CoA and 18:1-CoA (adding a double bond at the delta-9 location). The subject invention was further surprising in that the levels of other saturates, such as C20:0, C22:0, and C24:0, were also very surprisingly and advantageously reduced, while C16:1 and C18:1 unsaturates were increased (with little or no increases in C18:2 and C18:3, or even reductions of these relatively less stable polyunsaturates in some cases). Heretofore, it was unclear whether this would be a good enzyme (including whether the gene could be sufficiently expressed) in *Brassica* and other "good" oil seed plants, which already have a desirable (yet not optimal) fatty acid profile. (For example, it yielded only a 10% decrease in saturates in corn.)

[0040] As mentioned in the Background section, given the complex fatty acid profiles and metabolic pathways of different organisms and plants, and the different physical cell machinery thereof, even if this gene and enzyme could have an effect in *Brassica*, the effects could not be expected to be beneficial. As discussed in the Background section, increases in one or more types of desirable fatty acids often resulted in decreases of other desirable fatty acids, increases in undesirable fatty acids, and agronomic penalties (*i.e.*, other outright adverse effects on the modified plants). It was also surprising that the subject invention can be practiced without corresponding adverse effects to other valuable agronomic characteristics such as pod size, seed yield, seed size, oil yield, and the like. There were no adverse effects in plants homozygous for a simple transgenic insert. Some double homozygous stacks (made by crossing two transgenic events) exhibited decrease in pod number and seed set; the cause is yet unknown. However, Table 27 contains stacks (that is, apparently increased copy number events) having seed yields similar to non-transgenic controls *and also* 'no sat' composition.

[0041] Yet another reason for unpredictability arises because of differences between desaturases, and even between yeast, fungal, plant, and animal delta-9 desaturases. Differences in the desaturases can be attributed in part to differences in cell structures of the source organisms for the various desaturases. A yeast desaturase from U.S. Patent No. 5,777,201 is discussed above in the Background section. It is longer than the *Aspergillus* desaturase exemplified herein (510 amino acids vs. 455 amino acids). In addition, it has only about 52% identity over about 400 amino acids (as determined by both BLAST and BestFit, a Smith-Waterman program; both done in EMBOSS). Tables 1a and 1b of Example 7 of that patent show that the reductions in saturates achieved using the yeast desaturase were much weaker than those achieved according to the subject invention with the exemplified *Aspergillus* desaturase in canola. There are various factors that can be possible explanations for the relatively weaker performance of the yeast desaturase. For example, that protein might be inherently unstable in plants (while the subject desaturase is quite apparently very stable in canola). These can also be different in other enzyme properties, such as catalytic efficiencies, substrate affinities, cofactor affinities, and the like.

[0042] Compared to the safflower desaturase of U.S. Patent Nos. 5,723,595 and 6,706,950, the safflower desaturase is shorter (396 amino acids) than the presently exemplified *Aspergillus* desaturase (455 amino acids). The safflower desaturase is also found in the plastid, while the subject *Aspergillus* desaturase is found in the ER/microsomes/cytoplasmic compartment. Furthermore, the safflower desaturase uses acyl-ACP substrates found in the plastid, while the *Aspergillus* desaturase uses acyl-CoA substrates found in the cytoplasmic compartment. Thus, for the subject invention, it was not known if a substantial portion of the pool of acyl-CoA substrates would be available to the *Aspergillus* desaturase.

[0043] Thus, it was with great surprise that the subject delta-9 desaturase was found to be able to yield canola plants, seeds, and oil therefrom having excellent properties, particularly for improving food qualities of the oil. Very surprisingly,

a greater than 60% reduction of saturated fatty acids was achieved in Arabidopsis, and a greater than 43% reduction of saturated fatty acids was achieved in canola. Again, it is important to note that this was achieved in a plant that already yielded one of the best fatty acid profiles of any suitable plant. This invention was also used to achieve surprising and advantageous fatty acid profiles and ratios, as shown and discussed in more detail below. Although stearic acid is considered to be a saturated fatty acid, it has been found to have cholesterol-lowering effects. Thus, relatively higher levels of stearic acid can be beneficial. Similarly, relatively higher levels of arachidonic acid can be desirable. As shown in data herein, oil from seeds of the subject invention have advantageous profiles of these two fatty acids, together with desirable levels of vaccenic acid, for example. Also shown herein is that advantageous levels of these fatty acids and/or total saturates are present in combination with desirable plant height, yield, and other beneficial characteristics in the commercial-quality plants of the subject invention (as opposed to dwarf plants, for example). Again, exemplary data for such plants of the subject invention are presented herein.

[0044] It should be noted that the subject invention is not limited to the exemplified desaturase. Various desaturases and delta-9 desaturases are available in GENBANK, and sequence alignments can be performed, using standard procedures, to observe and compare differences in the sequences of the enzymes. Enzymes similar to that exemplified herein can be used according to the subject invention.

[0045] For example, the subject *Aspergillus* desaturase has two domains. The first domain (approximately the amino-terminal two-thirds of the molecule) is the desaturase domain, and the second domain (roughly the C-terminal third of the molecule) is a cytochrome b5 domain. Residues 62-279, for example, of SEQ ID NO:5 can be aligned with residues 4-233 of fatty acid desaturase gnl|CDD|25523 pfam00487, for example. Residues 332-407 of SEQ ID NO:5 can be aligned with residues 1-74 of gnl|CDD|22935 pfam00173 (cytochrome b5 domain). Residues 17-305 of SEQ ID NO:5 can be aligned with residues 3-288 of the lipid metabolism domain of fatty acid desaturase gnl|CDD|11113 COG1398 (OLE1). Residues 301-449 of SEQ ID NO:5 can be aligned with residues 11-163 of CYB5 (cytochrome b involved in lipid metabolism) of gnl|CDD|14396 COG5274. The desaturase domain of SEQ ID NO:5 lacking the cytochrome b5 could be functional, as this is the general structure of plant *plastidial* desaturases. There is also a published presumed microsomal pine desaturase (LOCUS AF438199) which uses acyl-CoA substrates found in the cytoplasmic compartment, and it lacks the cytb5 domain. It might also be possible to swap the *Aspergillus* cytochrome b5 domain with that of another organism, even one from a plant *cytoplasmic* desaturase. These domains, or segments encoding either or both of these domains, can be used as probes to define molecules of the subject invention, as discussed in more detail below.

[0046] Thus, the genes and proteins useful according to the subject invention include not only the specifically exemplified full-length sequences, but also portions, segments and/or fragments (including internal and/or terminal deletions compared to the full-length molecules) of these sequences, variants, mutants, chimerics, and fusions thereof. Proteins used in the subject invention can have substituted amino acids so long as they retain the characteristic enzymatic activity of the proteins specifically exemplified herein. "Variant" genes have nucleotide sequences that encode the same proteins or equivalent proteins having functionality equivalent to an exemplified protein. The terms "variant proteins" and "equivalent proteins" refer to proteins having the same or essentially the same enzymatic activity as the *Aspergillus nidulans* delta-9 desaturase of SEQ ID NO: 3. As used herein, reference to an "equivalent" sequence refers to sequences having amino acid substitutions, deletions, additions, or insertions that improve or do not adversely affect functionality. Fragments retaining functionality are also included in this definition. Fragments and other equivalents that retain the same or similar function, as a corresponding fragment of an exemplified protein are within the scope of the subject invention. Changes, such as amino acid substitutions or additions, can be made for a variety of purposes, such as increasing (or decreasing) protease stability of the protein (without materially/substantially decreasing the functionality of the protein).

[0047] Variations of genes may be readily constructed using standard techniques for making point mutations, for example. In addition, U.S. Patent No. 5,605,793, for example, describes methods for generating additional molecular diversity by using DNA reassembly after random fragmentation. Variant genes can be used to produce variant proteins; recombinant hosts can be used to produce the variant proteins. Using these "gene shuffling" techniques, equivalent genes and proteins can be constructed that comprise any 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 (for example) contiguous residues (amino acid or nucleotide) of any sequence exemplified herein.

[0048] Fragments of full-length genes can be made using commercially available exonucleases or endonucleases according to standard procedures. For example, enzymes such as *Bal31* or site-directed mutagenesis can be used to systematically cut off nucleotides from the ends of these genes. Also, genes that encode active fragments may be obtained using a variety of restriction enzymes. Proteases may be used to directly obtain active fragments of these proteins.

[0049] It is within the scope of the invention as disclosed herein that the subject proteins may be truncated and still retain functional activity. By "truncated protein" it is meant that a portion of a protein may be cleaved and yet still exhibit enzymatic activity after cleavage. Furthermore, effectively cleaved proteins can be produced using molecular biology techniques wherein the DNA bases encoding said protein are removed either through digestion with restriction endonucleases or other techniques available to the skilled artisan. After truncation, said proteins can be expressed in heterologous systems such as *Escherichia coli*, baculoviruses, plant-based viral systems, yeast and the like and then placed

in insect assays as disclosed herein to determine activity. It is well-known in the art that truncated proteins can be successfully produced so that they retain functional activity while having less than the entire, full-length sequence. It is well known in the art that *B.t.* toxins can be used in a truncated (core toxin) form. See, e.g., Adang et al., Gene 36:289-300 (1985), "Characterized full-length and truncated plasmid clones of the crystal protein of *Bacillus thuringiensis* subsp *kurstaki* HD-73 and their toxicity to *Manduca sexta*." There are other examples of truncated proteins that retain insecticidal activity, including the insect juvenile hormone esterase (U.S. Pat. No. 5,674,485 to the Regents of the University of California). As used herein, the term "toxin" is also meant to include functionally active truncations.

[0050] Proteins and genes for use according to the subject invention can be defined, identified, and/or obtained by using oligonucleotide probes, for example. These probes are detectable nucleotide sequences which may be detectable by virtue of an appropriate label or may be made inherently fluorescent as described in International Application No. WO 93/16094. The probes (and the polynucleotides of the subject invention) may be DNA, RNA, or PNA. In addition to adenine (A), cytosine (C), guanine (G), thymine (T), and uracil (U; for RNA molecules), synthetic probes (and polynucleotides) of the subject invention can also have inosine (a neutral base capable of pairing with all four bases; sometimes used in place of a mixture of all four bases in synthetic probes). Thus, where a synthetic, degenerate oligonucleotide is referred to herein, and "N" or "n" is used generically, "N" or "n" can be G, A, T, C, or inosine. Ambiguity codes as used herein are in accordance with standard IUPAC naming conventions as of the filing of the subject application (for example, R means A or G, Y means C or T, etc.).

[0051] As is well known in the art, if a probe molecule hybridizes with a nucleic acid sample, it can be reasonably assumed that the probe and sample have substantial homology/similarity/identity. Preferably, hybridization of the polynucleotide is first conducted followed by washes under conditions of low, moderate, or high stringency by techniques well-known in the art, as described in, for example, Keller, G.H., M.M. Manak (1987) DNA Probes, Stockton Press, New York, NY, pp. 169-170. For example, as stated therein, low stringency conditions can be achieved by first washing with 2x SSC (Standard Saline Citrate)/0.1% SDS (Sodium Dodecyl Sulfate) for 15 minutes at room temperature. Two washes are typically performed. Higher stringency can then be achieved by lowering the salt concentration and/or by raising the temperature. For example, the wash described above can be followed by two washings with 0.1x SSC/0.1% SDS for 15 minutes each at room temperature followed by subsequent washes with 0.1x SSC/0.1% SDS for 30 minutes each at 55° C. These temperatures can be used with other hybridization and wash protocols set forth herein and as would be known to one skilled in the art (SSPE can be used as the salt instead of SSC, for example). The 2x SSC/0.1% SDS can be prepared by adding 50 ml of 20x SSC and 5 ml of 10% SDS to 445 ml of water. 20x SSC can be prepared by combining NaCl (175.3 g/0.150 M), sodium citrate (88.2 g/0.015 M), and water, adjusting pH to 7.0 with 10 N NaOH, then adjusting the volume to 1 liter. 10% SDS can be prepared by dissolving 10 g of SDS in 50 ml of autoclaved water, then diluting to 100 ml.

[0052] Detection of the probe provides a means for determining in a known manner whether hybridization has been maintained. Such a probe analysis provides a rapid method for identifying toxin-encoding genes of the subject invention. The nucleotide segments which are used as probes according to the invention can be synthesized using a DNA synthesizer and standard procedures. These nucleotide sequences can also be used as PCR primers to amplify genes of the subject invention.

[0053] Hybridization with a given polynucleotide is a technique that can be used to identify, find, and/or define proteins and genes useful according to the subject invention. As used herein, "stringent" conditions for hybridization refers to conditions which achieve the same, or about the same, degree of specificity of hybridization as the conditions described herein. Hybridization of immobilized DNA on Southern blots with ³²P-labeled gene-specific probes can be performed by standard methods (see, e.g., Maniatis, T., E.F. Fritsch, J. Sambrook [1982] Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). In general, hybridization and subsequent washes are carried out under conditions that allowed for detection of target sequences. For double-stranded DNA gene probes, hybridization can be carried out overnight at 20-25° C below the melting temperature (T_m) of the DNA hybrid in 6x SSPE, 5x Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature is described by the following formula (Beltz, G.A., K.A. Jacobs, T.H. Eickbush, P.T. Cherbas, and F.C. Kafatos [1983] Methods of Enzymology, R. Wu, L. Grossman and K. Moldave [eds.] Academic Press, New York 100:266-285):

$$T_m = 81.5^{\circ} \text{C} + 16.6 \log[\text{Na}^+] + 0.41(\% \text{G} + \text{C}) - 0.61(\% \text{formamide}) - 600/\text{length of duplex in base pairs}.$$

Washes are typically carried out as follows:

- 1) Twice at room temperature for 15 minutes in 1x SSPE, 0.1% SDS (low stringency wash).
- 2) Once at T_m-20° C for 15 minutes in 0.2x SSPE, 0.1 % SDS (moderate stringency wash).

[0054] For oligonucleotide probes, hybridization can be carried out overnight at 10-20° C below the melting temperature (T_m) of the hybrid in 6x SSPE, 5x Denhardt's solution, 0.1 % SDS, 0.1 mg/ml denatured DNA. T_m for oligonucleotide probes was determined by the following formula: T_m (° C) = 2(number T/A base pairs) + 4(number G/C base pairs) (Suggs, S.V., T. Miyake, E.H. Kawashime, M.J. Johnson, K. Itakura, and R.B. Wallace [1981] ICN-UCLA Symp. Dev. Biol Using Purified Genes, D.D. Brown [ed.], Academic Press, New York, 23:683-693).

[0055] Washes can be carried out as follows:

- 1) Twice at room temperature for 15 minutes 1x SSPE, 0.1% SDS (low stringency wash).
- 2) Once at the hybridization temperature for 15 minutes in 1x SSPE, 0.1% SDS (moderate stringency wash).

[0056] In general, salt and/or temperature can be altered to change stringency. With a labeled DNA fragment >70 or so bases in length, the following conditions can be used:

Low:	1 or 2x SSPE, room temperature
Low:	1 or 2x SSPE, 42° C
Moderate:	0.2x or 1x SSPE, 65° C
High:	0.1x SSPE, 65° C.

[0057] Duplex formation and stability depend on substantial complementarity between the two strands of a hybrid, and, as noted above, a certain degree of mismatch can be tolerated. Therefore, the probe sequences of the subject invention include mutations (both single and multiple), deletions, insertions of the described sequences, and combinations thereof, wherein said mutations, insertions and deletions permit formation of stable hybrids with the target polynucleotide of interest. Mutations, insertions, and deletions can be produced in a given polynucleotide sequence in many ways, and these methods are known to an ordinarily skilled artisan. Other methods may become known in the future.

[0058] Because of the degeneracy/redundancy of the genetic code, a variety of different DNA sequences can encode the amino acid sequences disclosed herein. It is well within the skill of a person trained in the art to create alternative DNA sequences that encode the same, or essentially the same, enzymes. These variant DNA sequences are within the scope of the subject invention.

[0059] The subject invention include, for example:

- 1) proteins obtained from wild type organisms;
- 2) variants arising from mutations;
- 3) variants designed by making conservative amino acid substitutions; and
- 4) variants produced by random fragmentation and reassembly of a plurality of different sequences that encode the subject TC proteins (DNA shuffling). See e.g. U.S. Patent No. 5,605,793.

[0060] The DNA sequences encoding the proteins useful according to the subject invention can be wild type sequences, mutant sequences, or synthetic sequences designed to express a predetermined protein. DNA sequences designed to be highly expressed in plants by, for example, avoiding polyadenylation signals, and using plant preferred codons, are particularly useful.

[0061] Certain proteins and genes have been specifically exemplified herein. As these proteins and genes are merely exemplary, it should be readily apparent that the subject invention comprises use of variant or equivalent proteins (and nucleotide sequences coding for equivalents thereof) having the same or similar functionality as the exemplified proteins. Equivalent proteins will have amino acid similarity (and/or homology) with an exemplified enzyme (or active fragment thereof). Preferred polynucleotides and proteins of the subject invention can be defined in terms of narrower identity and/or similarity ranges. The identity of the enzymatic protein is 80% or more, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% as compared to a sequence exemplified or suggested herein. Any number listed above can be used to define the upper and lower limits. For example, a protein of the subject invention can be defined as having 80-90% identity, for example, with an *Aspergillus nidulans* delta-9 desaturase of SEQ ID NO: 5.

[0062] Unless otherwise specified, as used herein, percent sequence identity and/or similarity of two nucleic acids is determined using the algorithm of Karlin and Altschul (1990), Proc. Natl. Acad. Sci. USA 87:2264-2268, modified as in Karlin and Altschul (1993), Proc. Natl. Acad. Sci. USA 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al. (1990), J. Mol. Biol. 215:402-410. BLAST nucleotide searches are performed with the NBLAST program, score = 100, wordlength = 12. Gapped BLAST can be used as described in Altschul et al. (1997), Nucl. Acids Res. 25:3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (NBLAST and XBLAST) are used. See NCBI/NIH website.

[0063] To obtain gapped alignments for comparison purposes, the AlignX function of Vector NTI Suite 8 (InforMax,

Inc., North Bethesda, MD, U.S.A.), can be used employing the default parameters. Typically these would be a Gap opening penalty of 15, a Gap extension penalty of 6.66, and a Gap separation penalty range of 8. Two or more sequences can be aligned and compared in this manner or using other techniques that are well-known in the art. By analyzing such alignments, relatively conserved and non-conserved areas of the subject polypeptides can be identified. This can be useful for, for example, assessing whether changing a polypeptide sequence by modifying or substituting one or more amino acid residues can be expected to be tolerated.

[0064] The amino acid homology/similarity/identity will typically (but not necessarily) be highest in regions of the protein that account for its activity or that are involved in the determination of three-dimensional configurations that are ultimately responsible for the activity. In this regard, certain amino acid substitutions are acceptable and can be expected to be tolerated. For example, these substitutions can be in regions of the protein that are not critical to activity. Analyzing the crystal structure of a protein, and software-based protein structure modeling, can be used to identify regions of a protein that can be modified (using site-directed mutagenesis, shuffling, etc.) to actually change the properties and/or increase the functionality of the protein.

[0065] Various properties and three-dimensional features of the protein can also be changed without adversely affecting the activity/functionality of the protein. Conservative amino acid substitutions can be expected to be tolerated/to not adversely affect the three-dimensional configuration of the molecule. Amino acids can be placed in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions whereby an amino acid of one class is replaced with another amino acid of the same type fall within the scope of the subject invention so long as the substitution is not adverse to the biological activity of the compound. The following list provides examples of amino acids belonging to each class.

Class of Amino Acid	Examples of Amino Acids
Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

[0066] In some instances, non-conservative substitutions can also be made. The critical factor is that these substitutions must not significantly detract from the functional/biological/enzymatic activity of the protein.

[0067] To obtain high expression of heterologous genes in plants, for example, it may be preferred to reengineer said genes so that they are more efficiently expressed in plant cells. Sequences can be designed for optimized expression in plants, generally, or they can be designed for optimized expression in a specific type of plant. Canola is one such plant where it may be preferred to re-design the heterologous gene(s) prior to transformation to increase the expression level thereof in said plant. Therefore, an additional step in the design of genes encoding a fungal protein, for example, is reengineering of a heterologous gene for optimal expression in a different type of organism. Guidance regarding the production of synthetic genes that are optimized for plant expression can be found in, for example, U.S. Patent No. 5,380,831. A sequence optimized for expression in plants is exemplified herein as **SEQ ID NO:1** (which encodes the *Aspergillus nidulans* protein, as shown in **SEQ ID NO:5**).

[0068] As used herein, reference to "isolated" polynucleotides and/or proteins, and "purified" proteins refers to these molecules when they are not associated with the other molecules with which they would be found in nature. Thus, reference to "isolated" and/or "purified" signifies the involvement of the "hand of man" as described herein. For example, a fungal polynucleotide (or "gene") of the subject invention put into a plant for expression is an "isolated polynucleotide." Likewise, a protein of the subject invention when produced by a plant is an "isolated protein."

[0069] A "recombinant" molecule refers to a molecule that has been recombined. When made in reference to a nucleic acid molecule, the term refers to a molecule that is comprised of nucleic acid sequences that are joined together by means of molecular biological techniques. The term "recombinant" when made in reference to a protein or a polypeptide refers to a protein molecule that is produced using one or more recombinant nucleic acid molecules.

[0070] The term "heterologous" when made in reference to a nucleic acid sequence refers to a nucleotide sequence that is ligated to, or is manipulated to become ligated to, a nucleic acid sequence to which it is not joined in nature, or to which it is joined at a different location in nature. The term "heterologous" therefore indicates that the nucleic acid molecule has been manipulated using genetic engineering, i.e. by human intervention. Thus, a gene of the subject invention can be operably linked to a heterologous promoter (or a "transcriptional regulatory region" which means a nucleotide sequence capable of mediating or modulating transcription of a nucleotide sequence of interest, when the transcriptional regulatory region is operably linked to the sequence of interest). Preferred heterologous promoters can be plant promoters. A promoter and/or a transcriptional regulatory region and a sequence of interest are "operably linked"

when the sequences are functionally connected so as to permit transcription of the sequence of interest to be mediated or modulated by the transcriptional regulatory region. In some embodiments, to be operably linked, a transcriptional regulatory region may be located on the same strand as the sequence of interest. The transcriptional regulatory region may in some embodiments be located 5' of the sequence of interest. In such embodiments, the transcriptional regulatory region may be directly 5' of the sequence of interest or there may be intervening sequences between these regions. The operable linkage of the transcriptional regulatory region and the sequence of interest may require appropriate molecules (such as transgenic activator proteins) to be bound to the transcriptional regulatory region, the invention therefore encompasses embodiments in which such molecules are provided, either *in vitro* or *in vivo*.

[0071] There are a number of methods for obtaining the proteins for use according to the subject invention. For example, antibodies to the proteins disclosed herein can be used to identify and isolate other proteins from a mixture. Specifically, antibodies may be raised to the portions of the proteins that are most constant and most distinct from other proteins. These antibodies can then be used to specifically identify equivalent proteins with the characteristic activity by immunoprecipitation, enzyme linked immunosorbent assay (ELISA), or immuno-blotting. Antibodies to the proteins disclosed herein, or to equivalent proteins, or to fragments of these proteins, can be readily prepared using standard procedures. Such antibodies are an aspect of the subject invention.

[0072] A protein "from" or "obtainable from" any of the subject isolates referred to or suggested herein means that the protein (or a similar protein) can be obtained from the exemplified isolate or some other source, such as another fungal or bacterial strain, or a plant (for example, a plant engineered to produce the protein). "Derived from" also has this connotation, and includes polynucleotides (and proteins) obtainable from a given type of fungus or bacterium, for example, wherein the polynucleotide is modified for expression in a plant, for example. One skilled in the art will readily recognize that, given the disclosure of a fungal gene and protein, a plant can be engineered to produce the protein. Antibody preparations, nucleic acid probes (DNA and RNA, for example), and the like may be prepared using the polynucleotide and/or amino acid sequences disclosed herein and used to screen and recover other protein genes from other (natural) sources.

[0073] Oils of the seeds of the subject invention retain a high degree of oxidative stability but contain lower levels of saturated fatty acids and higher levels of unsaturated fatty acids. Preferred oils of the subject invention have less than 3.5% total saturated fatty acid content, oleic content of at least 75% (and preferably and surprisingly less than 80%), and a poly unsaturated fatty acid content of less than 20% (and more preferably less than 15%, still more preferably less than 10%, and even more preferably less than 9%). The subject invention can also be used to achieve canola seed having total saturated fatty acid content (C:14, C:16, C:18, C:20, C:22, and C:24) of not more than (and preferably less than) 2.5% of the total fatty acid content, preferably with the oleic acid ranges as mentioned above). 18:2 and 18:3 levels, which contribute to oil instability, are not increased or are preferably reduced (for food applications). End points for ranges for any of these particular fatty acids, any combinations thereof, and particularly for either one or both of the C18 polyunsaturates, can be obtained from any of the Figures and Tables provided herein.

[0074] The subject invention can be used to provide agronomically elite canola seed that results in a refined/deodorized oil with less than 3.5% total saturates. Oil derived from these plants can be used to formulate various end products, or they can be used as stand-alone frying oil for "no sat" (or "low sat") products.

[0075] Unless indicated otherwise, the saturated fatty acid content of a given collection of canola seeds can be determined by standard procedures wherein the oil is removed from the seeds by crushing the seeds and is extracted as fatty acid methyl esters following reaction with methanol and sodium hydroxide. The resulting ester is then analyzed for fatty acid content by gas liquid chromatography using a capillary column which allows separation on the basis of the degree of unsaturation and chain length. This analysis procedure is described in, for example, J. K. Daun et al., J. Amer. Oil Chem. Soc. 60: 1751-1754 (1983).

[0076] The fatty acid composition of canola seed was determined as described below for either "half-seed" analysis, "single/whole seed" analysis, or "bulk seed" analyses. For "half-seed" analyses, a portion of cotyledonary tissue from the embryo was removed and analyzed; the remaining seed was then saved, and could be germinated if desired. Although the half-seed technique can be somewhat unreliable in selecting stable genetically controlled fatty acid mutations (and subsequent breeding and crosses), the subject invention demonstrates that preferred genes can be introduced and used to create stable lines. Unlike uncharacterized mutations, it is well known in the art that a gene can be introduced and stably maintained in plants. Thus, the analysis set forth herein demonstrates the utility of the subject genes and that canola oil having the indicated characteristics can be attained.

[0077] "No saturates" (*i.e.*, No Sat) levels of fatty acids were reached in seeds from transgenic lines derived from commercial Nexera 710 (canola) germplasm. The No Sat level is defined as less than 3.5% combined saturates. In addition, reduced saturates were seen in both the Westar canola line, and another Crucifer (*Arabidopsis*) with the same transformation construct. Notably, saturate levels in single seeds were down to 2.6 to 2.7% for some seeds. The subject invention can also be used to produce seeds with 2.5% or less total saturates. This is important because oil processing can add ~0.5-1% to the total saturate "score," meaning that the processed oil product can still measurably reach the FDA-defined No Sat level using standard testing procedures. Having this level of tolerance not only permits for some

levels of contamination (by higher saturate seeds) of testing equipment (especially if the plant operator does a poor job of keeping seed batches distinct), but also permits for some level of variation in field growth conditions (such as high temperatures, which tend to create more saturates) and cross-pollination by pollen drifting from unimproved canola in adjacent fields (which dilutes desirable genes).

[0078] The U.S. Food and Drug Administration defines "saturated fat" as "A statement of the number of grams of saturated fat in a serving defined as the sum of all fatty acids containing no double bonds." 21 CFR 101.9(c)(2)(i). Unless otherwise specified, this is the definition used herein for "total saturates" and "total saturated fat." A serving of a food product is considered to have "no saturated fat" if the product "contain[s] less than 0.5 gram of total fat in a serving." 21 CFR 101.9(c)(2)(i). "Total fat" is defined as "A statement of the number of grams of total fat in a serving defined as total lipid fatty acids and expressed as triglycerides." 21 CFR 101.9(c)(2). "Serving sizes" for various types of foods are defined in 21 CFR 101.12(b), which defines a serving of oil as 1 tablespoon or 15 ml. As used herein, this is understood to mean 14 grams. Thus, "no sat" canola oil (or canola oil comprising no saturated fat) is defined herein as canola oil having less than 0.5 grams of total saturated fat in a serving (14 grams of canola oil comprising 14 grams of fat). Stated another way, "no sat" canola oil comprises less than 3.57% total saturates (0.5 grams of total saturates divided by 14 grams of total fat). Unless specified otherwise, all percent fatty acids herein are percent by weight of the oil of which the fatty acid is a component.

[0079] As shown herein, the subject invention can surprisingly be used to obtain oil from canola seeds wherein said oil comprises less than 3.57% total saturates. Oil can be obtained from the subject seeds using procedures that are well-known in the art, as mentioned in the preceding paragraphs, and the oil can be assayed for content using well-known techniques, including the techniques exemplified herein. Unless otherwise specified, analysis that was used to generate half-seed oils data and field oils data used a base-catalyzed transesterification reaction (AOCS Ce 2-66, alternative method). The protocol is similar to the saponification/acid esterification protocol described herein, except the saponification/acid esterification protocol measure total lipids, of which the majority are the same fatty acids from triacylglycerides detected by the base-catalyzed transesterification reaction.

[0080] In the commercial Nexera 710 germplasm, levels of 18:3 fatty acids, those that contribute to oxidative instability, were relatively unchanged. Thus, the subject invention not only provides plants, seeds, and oils with lower saturated fat, but also plants, seeds, and oils that very surprisingly maintain other beneficial characteristics. That is, the plants of the subject invention and the genes useful according to the subject invention can surprisingly be used without adversely affecting other advantageous characteristics of the plants.

[0081] In preferred embodiments, the subject invention provides plants comprising more than one expressed copy of a delta-9 desaturase gene of the subject invention. Results presented herein show that expressing multiple copies of this gene surprisingly improved the fatty acid profile of canola plants (saturated fat levels were greatly reduced). This is surprising in part because the art was heretofore unpredictable regarding the expression of multiple copies of the same gene. "Gene silencing" is one known phenomenon that teaches against using multiple copies (inserted at different locations in the genome, for example) of a heterologous gene. It is also not ideal to attempt to obtain multiple transformation events. Thus, there was no motivation to produce plants comprising more than one (two, three, four, and the like) delta-9 desaturase event. There was also no expectation that such plants would actually have improved characteristics.

[0082] Two examples of Cruciferous plants are specifically exemplified herein: *Brassica napus* (canola) and *Arabidopsis*. However, as is known in the art, other *Brassica* species and other Crucifers can be used for, for example, breeding and developing desired traits in canola and the like. Other such plants that can thus be used according to the subject invention include *Brassica rapa*, *Brassica juncea*, *Brassica carinata*, *Brassica nigra*, and *Brassica oleracea*.

[0083] In preferred embodiments, delta-9 desaturase genes useful according to the subject invention are optimized for plant expression. Optimization exemplified herein included introducing preferred codons and a Kozak translational initiator region, and removing unwanted sequences. The gene was driven by the beta-phaseolin promoter (a strong dicot seed storage protein promoter).

[0084] Promoters for which expression coincides with oil synthesis (e.g. ACP, elongase) can be used to further reduce saturates, as expression occurs earlier than for storage proteins. (Prior tobacco constructs used the nos 3' UTR, and prior corn constructs used the constitutive maize Ubiquitin-1 promoter and nos 3' UTR.) Other dicot seed promoters can be used according to the subject invention, including vicilin, lectin, cruciferin, glycinin, and conglycinin promoters, plant seed promoters disclosed in US20030005485 A1, elongase promoters in US20030159173 A1, and the ACP promoter in U.S. Patent No. 5,767,363. See also, for example, US6100450A (seed specific, expresses in embryo, column 8 line 8); US20030159173A1 (section 0044 seed specific promoter; examples are USP, hordein, ACP, napin, FatB3, and FatB4); WO9218634A1 (introduction discusses seed-specific promoters from other patents pages 1 through 7; WO0116340 (page 7 line 13 provides a definition of a "seed specific" promoter, which typically expresses at less than 5% in other tissues; page 10 lines 19-29 discusses seed storage proteins like albumins, globulins, vicilin and legumin-like proteins, non-storage oleosins, promoters associated with fatty acid metabolism like ACP, saturases, desaturases, elongases); WO2003014347A2 (promoter definition p23-25: preferably 2x greater for seed-specific); US20030233677A1 (section 0033 provides "seed promoter" examples [napin, ACCase, 2S albumin, phaseolin, oleosin, zein, glutelin, starch

synthase, starch branching enzyme]); WO2003092361 (page 15 provides a definition for "promoter"; the top of page 17 provides promoter examples and patent references (storage proteins only) including zeins, 7S storage proteins, Brazil nut protein, phe-free protein, albumin, beta-conglycinin, 11S, alpha-hordothionin, arcelin, lectins, glutenin); US20030148300 A1 (see Claim 8, including the napin promoter, the phaseolin promoter, the soybean trypsin inhibitor promoter, the ACP promoter, stearyl-ACP desaturase promoter, the soy 7S promoter, the oleosin promoter, the conglycinin promoter, oleosin promoters, embryogenesis-abundant protein promoters, embryo globulin promoters, arcelin 5, the napin promoter, and the acid chitinase promoter); U.S. Patent No. 5,777,201 (column 6, lines 30-50, constitutive promoters, seed- and/or developmentally regulated promoters e.g. plant fatty acid lipid biosynthesis genes [ACPs, acyltransferases, desaturases, lipid transfer proteins] or seed promoters [napin, cruciferin, conglycinin, lectins] or inducible promoters [light, heat, wound inducers]).

[0085] The plastids of higher plants are an attractive target for genetic engineering. Chloroplast (a type of plastid) transformation has been achieved and is advantageous. See e.g. U.S. Patent Nos. 5,932,479; 6,004,782; and 6,642,053. See also U.S. Patent Nos. 5,693,507 and 6,680,426. Advantages of transformation of the chloroplast genome include: potential environmental safety because transformed chloroplasts are only maternally inherited and thus are not transmitted by pollen out crossing to other plants; the possibility of achieving high copy number of foreign genes; and reduction in plant energy costs because importation of proteins into chloroplasts, which is highly energy dependent, is reduced.

[0086] Plant plastids (chloroplasts, amyloplasts, elaioplasts, etioplasts, chromoplasts, etc.) are the major biosynthetic centers that, in addition to photosynthesis, are responsible for producing many industrially important compounds such as amino acids, complex carbohydrates, fatty acids, and pigments. Plastids are derived from a common precursor known as a proplastid; thus, the plastids in a given plant species all have the same genetic content.

[0087] Plastids of most plants are maternally inherited. Consequently, unlike heterologous genes expressed in the nucleus, heterologous genes expressed in plastids are not disseminated in pollen. Therefore, a trait introduced into a plant plastid will not be transmitted to wild-type relatives. This offers an advantage for genetic engineering of plants for tolerance or resistance to natural or chemical conditions, such as herbicide tolerance, as these traits will not be transmitted to wild-type relatives.

[0088] The plastid genome (plastome) of higher plants is a circular double-stranded DNA molecule of 120-160 kb which may be present in 1,900-50,000 copies per leaf cell (Palmer, 1991). In general, plant cells contain 500-10,000 copies of a small 120-160 kilobase circular genome, each molecule of which has a large (approximately 25 kb) inverted repeat. Thus, it is possible to engineer plant cells to contain up to 20,000 copies of a particular gene of interest; this can potentially result in very high levels of foreign gene expression.

[0089] Oils of the subject invention are applicable for, and can be specially tailored for, industrial as well as various food uses. Aside from cooking oil, itself, the subject invention also includes "no sat" products such as potato chips and the like (see U.S. Patent No. 6,689,409, which claims a fried food composition comprising potatoes and a canola oil; the subject invention, however, can be used to improve the compositions described in the '409 patent).

[0090] Plants of the subject invention can be crossed with other plants to achieve various desirable combinations of characteristics and traits. Even further improvements can be made by crossing the subject plants, using known breeding technique and other advantageous sources of germplasm such as other canola lines having additional or other beneficial traits and characteristics. Another example would be crosses with a line having a plastidial delta-9 desaturase.

[0091] Thus, the subject invention can be used to achieve less than 3.5% total saturated fatty acids in commercial oil under variable environmental conditions (and less than 3% total saturated fatty acids in seed oil in breeder seed). This can be accomplished with no reduction in the quality and quantity of storage proteins, with no increase in indigestible fiber in canola meal, and no negative impact on seed yield (or other desirable agronomic traits) per acre.

[0092] Following is a list of the common names of fatty acids, as used herein, together with their number of carbon atoms and double bonds. Saturated fats have zero double bonds.

Table 1.

Name	Number of Carbon Atoms Per Molecule	Number of Double Bonds Per Molecule
Lauric	12	0
Myristic	14	0
Palmitic	16	0
Palmitoleic	16	1
Stearic	18	0
Oleic*	18	1
Vaccenic**	18	1
Linoleic	18	2
Alpha-Linolenic	18	3

(continued)

Name	Number of Carbon Atoms Per Molecule	Number of Double Bonds Per Molecule
Arachidonic	20	0
5 Eicosenoic (or Arachidic)	20	1
Behenic	22	0
Erucic	22	1
Lignoceric	24	0
10 Nervonic	24	1
* = double bond at delta-9 position		
** = double bond at delta-11 position		

[0093] Unless specifically indicated or implied, the terms "a", "an", and "the" signify "at least one" as used herein.

[0094] Following are examples that illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 - Delta-9 desaturase gene rebuilding

[0095] A delta-9 desaturase gene of the subject invention was redesigned for plant expression through a combination of changing *Aspergillus nidulans* sequence to plant-preferred translational codons, introducing unique restriction enzyme sites, and removing unwanted sequences and some secondary structure. The redesigned gene was synthesized by Operon, Inc. The sequence of the open reading frame for this polynucleotide is provided here as **SEQ ID NO:1**. The sequence of the ORF preceded by a Kozak sequence and a BamHI cloning site (caps), plus a translational terminator at the end of the ORF (caps), is provided in **SEQ ID NO:2**.

Example 2 - Delta-9 desaturase plant transformation vector construction

[0096] The BamHI-BstEII gene fragment was cloned into a vector between the *Pv* beta-phaseolin promoter and *Pv* beta-phaseolin 3' UTR (pPhas-UTR). This construct was named pOIL. The promoter-gene-UTR fragment was excised from pOIL by digestion with NotI, blunted, and cloned into the blunt PmeI site of vector pOEA1. The final vector was named pPD9-OEA1.

Example 3-Plant transformation with pPD9-OEA1

[0097] Plasmid vector pPD9-OEA1 was transformed into *Agrobacterium tumefaciens* [strain C58GV3101 (C58C1RifR) pMP90 (GmR). Koncz and Schell, Mol. Gen. Genet (1986)]. The delta 9-desaturase plants were then obtained by *Agrobacterium tumefaciens* mediated plant transformation

[0098] *Arabidopsis* was transformed with the "dip method," a procedure well known in the art. Plants were selfed, and dried seed was collected for FAME (fatty acid methyl ester) analysis.

[0099] The protocol used for canola transformation was as described by Katavic [Katavic, Campbell, L., Friesen, L., Palmer, D., Keller, W., and Taylor, D.C. (1996), "Agrobacterium-mediated genetic transformation of selected high erucic acid *B. napus* cultivars," 4th Canadian Plant Tissue Culture and Genetic Engineering Conference, Saskatoon, SK, June 1-4, 1996], with modifications for DAS's Nexera line. Hypocotyl sections were isolated from 6-day-old seedlings of *B. napus*, cv Westar or Nexera 710 and were cultured on callus initiation medium prior to transformation. On the day of transformation, the hypocotyls were coincubated with an *Agrobacterium* culture containing the plasmid pPD9-OEA1 with the trait gene such that a fragment of plasmid DNA including the delta 9-desaturase gene was incorporated into the cell chromosome. After a co-cultivation period, the hypocotyls were transferred to callus initiation medium containing glutofosinate ammonium as the selection agent. Healthy, resistant callus tissue was obtained and repeatedly transferred to fresh selection medium for approximately 12 to 16 weeks. Plants were regenerated and transferred to Convirons growth chambers. Plants were selfed to obtain seed. If transgenic plants were sterile, they were crossed with pollen from unmodified Nexera 710 lines. Dry seed was harvested for FAME analysis.

Example 4 - Canola Event Sorting Process, and Summary of Canola Results

[0100] Unless otherwise specified, the following procedures were used to obtain the Nex 710/Delta-9 canola seeds,

the data for which is presented in subsequent Examples.

[0101] In general, there were four main steps for developing, selecting, or sorting events: sorting initial transgenic events, sorting T1 and T2 seed, sorting T1 or T2 plants, and sorting events by field performance. Transformed callus was first regenerated to T0 plants.

[0102] For the initial sorting, 107 putative transgenics were screened by agronomics and by southern blotting (simple or complex, i.e., more than 3 copies). Multiple seeds per event were screened. T1 seed saturates were determined, C16:1/C16:0 ratios were determined (to infer catalytic efficiency), and segregation of biochemical phenotypes were determined. Based on these data, and seed availability (timing, amount), a limited subset of seed was advanced.

[0103] For sorting T1 and T2 seed (a few events were advanced by one generation), half-seed analysis was conducted, and possible homozygotes were identified. Based on this data, half-seeds from segregating populations were selected for greenhouse growth.

[0104] The next main step was sorting T1 or T2 plants (a few events were advanced one generation). Southern blots were conducted to determine transgene integration complexity. Zygosity was also determined by INVADER assays (Third Wave Technologies, Inc.). These data were used to select seed for field trials.

[0105] For T1 greenhouse studies, 30 seeds per event were subjected to half-seed analysis. Nex 710 canola was used as a commercial check. All individuals within the event were zygosity sampled to determine allele copy number for Delta-9 and PAT. This was followed by PCR and indoleacetamide hydrolase (IAAH; a negative scoreable or screenable marker) analysis. All individuals within the event were leaf painted to determine the PAT segregation ratio. Southern analysis was then conducted on those individuals approaching a "no sat" profile, positive IAAH, and homozygous for PAT and Delta-9.

[0106] These were then used for T2 analysis (100 seeds from each of the above plants were half-seed analyzed). INVADER was used to verify copy number and to see if the event was segregating for D9 and PAT. Leaf painting was used to see if lines are segregating for PAT. 10 seed bulk fatty acid data was collected from plants based on half-seed data, INVADER results, LP, and IAAH-positive.

[0107] The fourth main step was sorting events by field performance (plant T2 or T3 seed, analyze T2 or T3 plants, then T3 or T4 seed). There was a wide sampling of transgenic events. Agronomics, Southern blots, and zygosity were analyzed. Batch seed oils analysis was also conducted. Based on these data, events were selected for crossing to increase gene dosage.

[0108] The following selection criteria was used to advance lines to field studies. 224 lines (including nulls) from 23 events (6 reps/entry) were evaluated in replicated nurseries at 3 locations. 6T3 and 17T2 events were planted. Selection of lines/event going to field was based on insert number and half-seed analysis followed by a 10-seed bulk fatty acid analysis of seed from each plant. The percent total sat range of selected lines was in the approximate range of 3.3-4.5%. The following T3 events were selected for further development:

Table 2A.

Event	Copy Number	# of Lines Field Tested
218-11.30	2 D9:2 PAT	45
36-11.19	2 D9:2 PAT	7
31a-3.30.01	1 D9:1 PAT	15
146-11.19	3 D9:1 + partial PAT	23
159a-11.19	2 D9:2 PAT	19
69-11.19	2 D9:2 PAT	20

[0109] The following T2 events were selected for further development:

Table 2B.

Event	Copy Number	# of Lines Field Tested
146-11.19	nd (not determined)	6
149-11.30	nd	8
15-11.19	nd	4
224-11.30	3 D9:2.5 PAT	10
226-11.30	nd	4
230-11.30	nd	2
250-11.19	nd	5
267-11.19	nd	5

EP 2 338 328 B1

(continued)

	Event	Copy Number	# of Lines Field Tested
5	284-11.19	nd	8
	309-11.30	nd	2
	32-11.30	nd	3
	324-11.30	nd	8
	43-11.19	nd	5
10	43b-11.30	2 D9:2 PAT	10
	57-11.30	nd	5
	68-11.30	2 D9:2 PAT	8
	96a-6.15	nd	2

[0110] Field tests were conducted as follows. Agronomic assessments were taken as discussed in a subsequent example to confirm that no agronomic penalty was associated with the Delta-9 (D9). 15 INVADER leaf tissue samples were collected from 28 of the most promising T3 lines (plus sib-nulls) for further copy number verification and to determine if lines were segregating for PAT. The D9 lines were chosen based on having less than 3.5% total saturates.

[0111] 10 Southern tissue samples were taken from the 3 most promising T2 lines, which were chosen based on having less than 3.5% total saturates. All tissue-sampled plants were self-pollinated. Fatty acid analysis was determined based on 10 seed bulk from selfed plants (455 samples), and 1 gram of bulk seed sample from OP rows (1445).

[0112] The "best" T4 events are as follows:

Table 3A.

	Event	Copy Number	# of Lines Field Tested
25	218-11.30	2 D9:2 PAT	9
	36-11.19	2 D9:2 PAT	2
	146-11.19	3 D9:1 + partial PAT	4
30	159a-11.19	2 D9:1 PAT	1
	69-11.19	2 D9:2 PAT	3

[0113] The "best" T3 events are as follows:

Table 3B.

	Event	Copy Number	# of Lines Field Tested
35	149-11.30	nd (not determined)	3
	43b-11.30	2 D9:2 PAT	1
40	57-11.30	nd	2
	284-11.19	nd	2

[0114] Generally, no major agronomic penalty associated with Delta-9 was observed, and some lines exhibited an approximately 10% increase in seed weight. Agronomic results are discussed in more detail below in **Example 18**. General observations regarding distributions of individual fatty acid components are discussed below in **Examples 11-13**. Summaries of mean TSAT data, % changes in TSATs, and % changes for certain fatty acid components for events 218-11.30, 36-11.19, and 69-11.19 are presented in **Tables 4-7**. Generally, ~30% to ~40% reductions in TSATs were observed, relative to the sib-null and wild type. However, even further improvements are discussed in more detail below and can be made with further crosses, for example.

Table 4. Mean TSAT For All Lines from Event 218-11.30 Across 3 Sites

	Event	N	C16:0 %Total	C16:1 %Total	C18:0 %Total	C18:1 %Total	C18:2 %Total	C18:3 %Total	%Total Saturates
55	Selfs								
	Null	94	3.75	0.38	1.67	76.57	11.65	2.50	6.62
	#218-11.30	217	3.10	1.40	0.68	79.11	10.89	2.29	4.37
	%Wt		86%	385%	38%	101%	105%	98%	66%

EP 2 338 328 B1

(continued)

Event	N	C16:0 %Total	C16:1 %Total	C18:0 %Total	C18:1 %Total	C18:2 %Total	C18:3 %Total	%Total Saturates
	%Null	83%	365%	40%	103%	93%	92%	66%
Open Pollinated								
Null	3	3.70	0.47	1.65	78.07	10.65	2.40	6.45
Wt Control	88	3.60	0.36	1.79	78.19	10.37	2.34	6.60
#218-11.30	123	3.07	1.23	0.74	80.17	10.15	2.22	4.42
	%Wt	85%	338%	41%	103%	98%	95%	67%
	%Null	83%	263%	45%	103%	95%	92%	68%

Table 5. % Changes in TSATS C16:0, C16:1, C18:0 And C18:1 VS Nulls and WT Control Nex 710

							% TSATvs		% C16:0	% C16:1	% C18:0
Event	Line	C16:0	C16:1	C18:0	C18:1	TSAT	Null	WT	VsN	VsN	VsN
218-11.30(TS)	1361	2.87	1.31	0.64	80.23	4.04	39	37	22	317	61
218-11.30(TS)	1319	2.88	1.41	0.62	79.53	4.09	38	37	20	248	62
218-11.30(TS)	1304	2.95	1.33	0.60	79.16	4.10	38	36	18	230	63
218-11.30(TS)	1500	2.95	1.31	0.63	79.58	4.11	38	36	18	224	61
218-11.30(TS)	1405	3.04	1.40	0.60	80.44	4.17	37	35	16	245	63
218-11.30(TS)	1370	3.02	1.38	0.66	80.24	4.24	36	34	17	240	59
218-11.30(TS)	1369	3.03	1.30	0.65	79.44	4.25	36	34	16	220	59
218-11.30(T)	1370	2.96	1.20	0.77	80.28	4.31	31	33	18	196	53
218-11.30(T)	1405	3.01	1.19	0.71	78.65	4.31	31	33	17	193	56
218-11.30(N)	1299	3.62	0.41	1.61	78.10	6.26	.	.	.	.	.
218-11.30(NS)	1299	3.70	0.32	1.65	77.56	6.52	.	.	.	.	.
Nex 710	.	3.58	0.35	1.75	77.87	6.45	.	.	.	.	.

Table 6. % Changes in TSATS C16:0, C16:1, C18:0 And C18:1 VS Nulls and WT Control Nex 710

							% TSAT		% C16:0	% C16:1	% C18:0
Event	Line	C16:0	C16:1	C18:0	C18:1	TSAT	WT	VsN	VsN	VsN	VsN
36-11.19(T)	1099	2.93	1.23	0.77	78.85	4.30	32	33	16	322	55
36-11.19(N)	1127	3.49	0.29	1.70	77.14	6.36	.	.	.	.	.
Nex 710	.	3.58	0.35	1.75	77.87	6.45	.	.	.	.	.

Table 7. % Changes in TSATS C16:0, C16:1, C18:0 And C18:1 VS Nulls and WT Control Nex 710

							% TSAT		% C16:0	% C16:1	% C18:0
Event	Line	C16:0	C16:1	C18:0	C18:1	TSAT	WT	VsN	VsN	VsN	VsN
69-11.19(T)	1538	3.05	1.05	0.71	80.70	4.21	34	35	15	223	59
69-11.19(T)	1529	3.02	1.02	0.72	80.61	4.24	33	34	16	213	58
69-11.19(T)	1534	3.09	1.02	0.73	80.45	4.29	32	34	14	213	57
69-11.19(N)	1604	3.58	0.33	1.72	78.45	6.34	.	.	.	.	.
Nex 710	.	3.58	0.35	1.75	77.87	6.45	.	.	.	.	.

Example 5 - Molecular characterization of plants

[0115] Leaf samples were taken for DNA analysis to verify the presence of the transgenes by PCR and Southern analysis, and occasionally to confirm expression of PAT protein by ELISA.

5A. Protocol for PCR analysis for delta-9 desaturase.

[0116] The following two primers were used:

Delta-9 forward B:

5' TGA GTT CAT CTC GAG TTC ATG 3' (SEQ ID NO:3)

Delta-9 reverse B:

5' GAT CCA ACA ATG TCT GCT CC 3' (SEQ ID NO:4)

[0117] This primer pair yields a ~1380 bp fragment after amplifying the delta-9 gene.

[0118] The following cycling protocol was used in this screen with an MJ Tetrad thermal cycler:

1. 94° C, 2 minutes
2. 94° C, 1 minutes
3. 50° C, 2 minutes
4. 72° C, 3 minutes, + 5 seconds / cycle extension
5. repeat Steps 2-4 25 times
6. 4° C until ready for analysis, or at least 2 minutes

5B. Protocol for the extraction of plant genomic DNA for Southern analysis.

[0119] The DNeasy Plant Maxi Kit from Qiagen was used. The protocol in the booklet was used with the following changes to the elution part. Buffer AE was diluted 1:10 with DNA grade water (Fisher No. BP561-1). Two elutions were performed using 0.75ml of the diluted AE buffer pre-warmed to 65° C. DNA was precipitated with isopropanol and washed with 70% ethanol. The DNA pellet was resuspended in 100 µl of 1X TE buffer. DNA concentration was quantitated. 6 µg of DNA was aliquoted and adjusted to a final volume of 40 µl. Samples were stored at -20° C.

5C. FAME analysis (Direct FAME Synthesis from Seeds with Methanolic H₂SO₄)

[0120] The protocol for FAME analysis was as follows.

GC Specs

[0121] Gas Chromatograph: Hewlett-Packard 6890 with dual injection ports and dual flame ionization detectors.

[0122] Data System: HP Chemstation, Leap Technologies, Carrboro, NC 27510, PAL System.

[0123] Column: J&W capillary column, DB-23, 60M x 0.25mm i.d. with 0.15 microfilm thickness, maximum operating temperature 250° C. Catalog number: 122-2361.

[0124] Temperature profile: Equilibration time: 1 minute. Initial temperature: 50° C. Initial time: 3 minutes. Increase rate: 40° C/minute. Final temperature: 240° C. Final time: 7.25 minute.

[0125] FAME procedures for Arabidopsis. Add 100 µl (50 µg) **15:0 Standard** into a clean 16x125 mm glass tube (Internal Standard stock solution: 500 µg/ml of C15:0 TAG in 2:1 chloroform:isopropanol). Dry Standard under nitrogen in evaporation water bath at 55° C.

[0126] When dry add 2 ml 1N methanolic H₂SO₄ with 2% DMP (for 100 ml: 95,22 ml methanol, 2.772 ml H₂SO₄, 2 ml DMP = 2,2-dimethoxypropane). Heat tube to 85° C.

[0127] Weigh ~5 mg Arabidopsis seeds into clean 16x125 mm glass tube and record exact weight.

[0128] To destroy lipases, add hot meth. H₂SO₄ with Standard to tube with seeds, incubate for 15 minutes at 85° C.

[0129] Cool vial down to ~50° C, then crush seeds with glass pestle in mini grinder.

[0130] Transfer sample back into tube and incubate for at least one hour under nitrogen at 85° C. Cool vial on ice. Add first 0.5 ml 0.9% NaCl, then 250 µl **17:0 Standard** in hexane (0.1335 mg/ml methyl ester stock solution). Vortex, centrifuge at 1000g for 5 minutes.

[0131] Transfer 100-200 µl with Pasteur pipette into 1.5 ml vial with conical insert (0.5 ml).

[0132] Inject 5 µl into GC.

[0133] FAME procedures for Canola. Add 100 µl (50 µg) **15:0 Standard** into a clean 16x125 mm glass tube (Internal Standard stock solution: 500 µg/ml of C15:0 TAG in 2:1 chloroform:isopropanol). Dry Standard under nitrogen in evaporation water bath at 55° C.

EP 2 338 328 B1

[0134] When dry add 2 ml 1N methanolic H₂SO₄ with 2% DMP (for 100 ml: 95,22 ml methanol, 2.772 ml H₂SO₄, 2 ml DMP = 2,2-dimethoxypropane). Heat tube to 85° C.

[0135] Weigh one canola seed in clean tube and record exact weight.

[0136] To destroy lipases, add seed sample to tube containing Standard with hot methanol H₂SO₄, incubate for 15 minutes at 85° C.

[0137] Cool vials down to ~50° C, then crush seeds with glass pestle.

[0138] Incubate for at least 1 hour under N₂ at 85° C.

[0139] Cool vial on ice. Add first 0.5 ml 0.9% NaCl, then 250 µl **17:0 Standard** in hexane (0.1335 mg/ml methyl ester stock solution). Vortex, centrifuge at 1000g for 5 minutes.

[0140] Transfer 100-200 µl with Pasteur pipette into 1.5 ml vial with conical insert (0.5 ml).

[0141] Inject 5 µl into GC.

Example 6 - Arabidopsis Results

[0142] Initial results are illustrated in **Figure 1**, showing that a greater than 60% reduction of saturated fatty acids was achieved. Also, more 16:1 (5.9% for example) than 16:0 (4.4%) was achieved.

No Sat Oil via Δ9-CoA-Desaturase Approach

FAME analysis

[0143] T2 seeds from approximately 18 additional transformants were analyzed. This data (see **Table 8** and **Figure 2**) show a reduction in "sats" of up to 60-70%. This is an even stronger reduction in saturated fatty acids than the initial data (see **Figure 1**) indicated. It is important to note that the T2 generation is still segregating; thus, even better performing lines in following generations are expected. This point is true for all T1, T2, T3, and other initial generations (including canola lines) as reported elsewhere herein, until the trait is fixed and the line is homozygous for the transgene. (Stable lines and plants where the traits are fixed were produced and are described in subsequent Examples.)

Table 8.

	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1?	?	?	?	22:0	22:1	24:0	Tot Sats
WT1	7.8	0.2	3.2	11.2	27.3	21.4	2.8	20.1	2.3	0.7	0.0	0.4	2.4	0.1	14.4
TF-21	7.2	0.3	3.3	14.4	27.9	18.0	2.5	21.4	2.1	0.5	0.0	0.4	2.0	0.0	13.3
WT1-3	7.2	0.2	3.0	12.1	26.5	21.9	2.6	20.8	2.3	0.7	0.0	0.4	2.3	0.0	13.2
WT1-2	6.9	0.2	2.9	13.7	27.5	20.9	2.5	20.2	2.1	0.6	0.0	0.4	2.2	0.0	12.7
TF-10	5.5	3.0	2.0	19.5	28.7	18.4	1.3	17.9	1.6	0.4	0.0	0.3	1.3	0.0	9.1
TF-18	6.1	1.9	1.4	18.0	29.0	19.0	0.9	19.6	1.9	0.5	0.0	0.2	1.5	0.0	8.7
TF-13	4.7	2.2	1.6	19.9	27.9	19.1	1.3	19.2	1.8	0.4	0.0	0.3	1.5	0.0	7.9
TF-22	4.6	2.6	1.6	20.2	27.9	18.6	1.3	19.4	1.7	0.4	0.0	0.3	1.5	0.0	7.8
TF-12	5.5	2.1	1.2	18.6	28.4	20.1	0.8	19.4	1.8	0.5	0.0	0.2	1.5	0.0	7.6
TF-17	3.4	1.1	1.9	19.5	27.5	17.3	1.8	22.8	2.0	0.5	0.0	0.5	1.8	0.0	7.5
TF-14	5.0	2.1	1.4	18.8	27.3	21.4	1.0	19.2	1.7	0.5	0.0	0.0	1.6	0.0	7.4
TF-8	3.2	0.9	1.6	19.0	25.3	18.7	1.8	24.7	2.0	0.5	0.0	0.0	2.2	0.0	6.6
TF-19	4.1	2.7	1.0	20.7	28.4	19.9	0.8	18.8	1.7	0.4	0.0	0.2	1.4	0.0	6.0
TF-20	4.4	2.8	0.6	20.2	28.8	19.7	0.5	19.3	1.7	0.4	0.0	0.1	1.4	0.0	5.7
TF-7	5.0	2.9	0.0	20.8	27.5	25.8	0.0	15.3	1.3	0.0	0.0	0.0	1.4	0.0	5.0
TF-6	3.4	1.6	0.7	22.4	27.9	17.9	0.7	21.4	1.9	0.4	0.0	0.0	1.7	0.0	4.8
TF-11	3.4	2.7	0.6	22.4	28.8	19.7	0.5	18.5	1.6	0.4	0.0	0.2	1.3	0.0	4.7

Values are from a single sample prep and GC run (not averages)

TF-21 behaves as a wild-type (non-transformed) plant; possible explanations include gene silencing or non-transgenic escape (inadequate selection with glufosinate herbicide)

"?" indicates that identity of the peak on the GC chromatogram is questionable, or unknown

Example 7 - Westar data

[0144] Protocols similar to those described in Example 5C were applied to canola lines derived from well-known

"Westar" canola. As illustrated in **Figure 3**, the indicated saturated fats were reduced by over 43%, and a 50% reduction was achieved when 24:0 was included.

Example 8 - Exemplary Nexera 710 data

[0145] Protocols similar to those described elsewhere herein were applied to canola lines derived from well-known "Nexera 710" commercially elite canola. Total saturates were calculated used methodology discussed herein and as specified below.

[0146] Total saturates are derived from the sum of 16:0 + 18:0 + 20:0 + 22:0 + 24:0 fatty acids. Some notable saturate levels in single seeds are presented in Table 9. Oil profiles are presented as mol% values. The mol% value incorporates the formula weight of each specific fatty acid into the calculation. It uses the mass of a given fatty acid species (peak area, or the same value used to directly calculate % fatty acid), divided by the formula weight for that fatty acid species.

Table 9.	
Seeds	Saturate Level
Event 5 11.19 seed #6	3.1%
Event 5 11.19 #8	2.7%
Event 113a 11.19 #4	3.4%
Event 113a 11.19 #8	3.2%
Event 147 11.19 #3	3.0%
Event 147 11.19 #7	2.6%
Event 36a 11.19 seed	2.7%
Event (9)3 11.30	3.3%

[0147] Profiles from the seeds with the lowest total saturates were analyzed (seeds 113a 11.19 #4, 113a 11.19 #8, 5 11.19 #6 and 5 11.19 #8). Unmodified Nexera 710 germplasm values came from the same FAME analysis run. Plants were grown in the Conviron growth chamber, so actual mol% values may differ from field grown seed. In general, transgenic plants show a reduction in 16:0, 18:0 and 20:0, and increases in 16:1. 18:0 levels generally fell from an average of 1.4% (upper 2.08%, lower 0.81%) in Nexera 710 to 0.1% average (upper 0.6%, lower 0%) in select transgenic material. Also, 16:0 levels fell from an average of 4.6% (upper 5.12% to lower 4%) to 3.0% (upper 3.41% to lower 2.63%). Likewise, the 20:0 levels dropped from 0.5% average in Nexera 710 to 0% in the selected transgenics. The 16:1 levels were undetectable in Nexera 710, increasing to an average of 2.3% (upper 2.71% to lower 1.72%) in transgenics. The average 18:3 levels were slightly increased in the small transgenic population, but the range of values overlapped with the unmodified Nexera 710 samples. These results can be summarized as follows:

Table 10.		
Fatty Acid	Nexera 710	Select Transgenic Material
20:0	0.5% average	0%
18:0	1.4% average (upper 2.08%, lower 0.81%)	0.1% average (upper 0.6%, lower 0%)
16:0	4.6% average (upper 5.12% to lower 4%)	3.0% average (upper 3.41% to lower 2.63%)
16:1	Undetectable	2.3% average (upper 2.71% to lower 1.72%)

Example 9- Further Canola Data

[0148] Protocols similar to those described elsewhere herein were applied to additional canola lines derived from well-known "Nexera 710" commercially elite canola. Total saturates and the weight percent of the individual types of fatty acids, as indicated below, were calculated using methodology discussed herein.

[0149] **Figures 4A-C and 5A-C** show representative results, from Events 36-11.19 and 218-11.30 respectively, that demonstrate reduced saturated fatty levels that are obtainable by practicing the subject invention. By making further manipulations according to the subject invention, the saturated fat levels exemplified here can be even further reduced.

EP 2 338 328 B1

All of this data were obtained from selfed transgenic canola plants as indicated.

[0150] In summary, for Event 36-11.19, T2 half seed analysis from greenhouse-grown plants had total saturates as low as 2.57%. Total saturates for T3 whole seeds, from greenhouse-grown plants, were as low as 3.66%. Results are shown graphically in **Figure 4A** (numerical data are in **Figures 4B and 4C**). For Event 218-11.30 greenhouse-grown plants, T2 half seed analysis revealed total saturates to be as low as 2.71%. T3 whole seeds had total saturates as low as 3.37%. Results are shown graphically in **Figure 5A** (numerical data are in **Figures 5B and 5C**). For reference, NATREON has 6.5% total saturates, on average, under field conditions.

[0151] By making further improvements according to the subject invention (such as additional rounds of selfing, crosses with other superior lines, increasing desaturase gene copy number [either by additional transformation, by further breeding crosses, and the like], changing timing of desaturase expression, and mutagenesis), even greater levels of reduction of total saturated fat levels can be achieved.

Example 10 - Analysis of Further Canola Data - Percent reduction of total saturated fats

[0152] The data presented in Example 9 can be used in various calculations to illustrate various aspects of the subject invention. For example, percent reduction of total saturated fats can be calculated by first dividing the total saturates of a given plant by the total saturates of the control line, and then subtracting from 100%. Examples of such reductions, provided by the subject invention, are illustrated below. Results can be approximated by rounding to the closest whole (non-decimal) number.

Table 11.

Event (& generation)	TotalSats(TS)	Control TS	% Reduction
218-11.30 (T2)	2.71	6.36	57.4%
36-11.19 (T2)	2.57	6.44	~60%

[0153] Any number shown on any of the graphs, figures, tables, or otherwise discussed herein can be used as an endpoint to define the metes and bounds of the subject invention. Likewise, any calculations using any of these numbers, such as those shown above and those discussed in more detail below, can be used to define the metes and bounds of the subject invention. **Tables 12-14** show further representative results and calculations, for Lines with Events 218-11.30, 36-11.19, and 69-11.19.

Table 12. Event 218-11.30 HS Selections for Crossing

Line	C16:0	C16:1	C18:0	C18:1	TSAT	% ↓TSAT VS		% ↓C16:0	% tC16:1	% ↓C18:0
						Null	WT		Vs N	Vs N
2193HS50	2.05	1.54	0.33	81.34	2.66	54	53	43	611	75
2193HS9	2.27	1.76	0.27	75.02	2.81	51	50	37	709	79
2193HS22	2.31	1.31	0.29	81.16	2.87	50	49	36	505	78
2193HS23	2.29	1.47	0.28	77.17	2.89	50	49	36	577	79
2195(N)	3.60	0.22	1.32	76.25	5.75	.	.	.	.	.
Nex 710	3.33	0.18	1.51	77.39	5.61	.	.	.	.	.

Table 13. Event 36-11.19 HS Selections for Crossing

Line	C16:0	C16:1	C18:0	C18:1	TSAT	% ↓TSAT VS		% ↓C16:0	% ↑C16:1	% ↓C18:0
						Null	WT		Vs N	Vs N
1099HS3	1.95	1.76	0.52	81.92	2.92	51	48	45	1018	61
1099HS8	2.19	2.05	0.40	77.81	2.97	50	47	39	1201	70
1099HS17	2.11	1.73	0.42	80.16	2.99	50	47	41	1000	69
1099HS11	2.13	1.73	0.45	79.30	3.00	49	47	40	998	66
1099HS43	2.18	1.72	0.41	77.88	3.01	49	46	39	991	69
1127(N)	3.57	0.16	1.34	74.21	5.92	.	.	.	.	.
Nex 710	3.33	0.18	1.51	77.39	5.61	.	.	.	.	.

Table 14. Event 69-11.19 HS Selections for Crossing

Line	C16:0	C16:1	C18:0	C18:1	TSAT	% ↓ TSAT VS		% ↓ C16:0		% ↓ C16:1		% ↓ C18:0	
						Null	WT	VsN	VsN	VsN	VsN	VsN	VsN
1538HS23	1.88	2.20	0.34	79.56	2.64	55	53	49	1101	74			
1538HS26	2.18	1.60	0.29	77.55	2.81	52	50	41	776	78			
1538HS4	2.20	1.71	0.35	80.17	2.90	51	48	41	831	73			
1538HS36	2.17	1.49	0.37	78.24	2.93	50	48	41	713	72			
1604(N)	3.70	0.18	1.29	78.12	5.88	.	.	.	.	.	.	.	.
Nex 710	3.33	0.18	1.51	77.39	5.61	.	.	.	.	.	.	.	.

Example 11 - Analysis of Further Canola Data - Detailed fatty acid profiles

[0154] Again, **Figures 4A-C and 5A-C** show some representative results that show fatty acid profiles of various plants having events 218-11.30 and 36-11.19. Generally, these results demonstrate that not only are the 16:0 and 18:0 levels greatly reduced (with a resulting increase in corresponding unsaturated levels), but the 20:0, 22:0, and 24:0 levels are also advantageously, and surprisingly and unexpectedly, reduced. In some cases, 18:2 and 18:3 levels can also be reduced, which enhances the oxidative stability of the improved oil. Furthermore, any of the ratios suggested above (such as 16:0-16:1, 18:0-18:1, and, for example, 18:0-[20:0+22:0+24:0]) can be used to define advantageous results of practicing the subject invention. Combined percent reductions in total C20:0 + C22:0 + C24:0 are also surprisingly achieved according to the subject invention. Thus, the subject invention provides plants have advantageous and improved fatty acid profiles, as exemplified herein. By making further improvements according to the subject invention, even better reductions in saturates, increases in "no sats," and better ratios can be achieved.

[0155] For example, various calculations, using the following data from **Figure 5C** and **Figure 4B**, can be used to illustrate accomplishments of the subject invention. Sample data from **Figure 5C** and **Figure 4B** are presented in the following Table. The amount of each indicated fatty acid is indicated for each event and in parentheses for the relevant control plant(s).

Table 15.

Event (& generation)	C20:0 (control)	C22:0 (control)	C24:0 (control)
218-11.30 (T2)	0.11 (0.62)	0.12 (0.32)	0.03 (0.14)
36-11.19 (T3)	0.32 (0.64)	0.15 (0.42)	0.04 (0.21)

[0156] Looking at the 218-11.30 event, the total contribution to saturates by the C20:0, C22:0, and C24:0 components is 1.08% in the control, but these components are advantageously decreased to 0.26% in a canola line of the subject invention. This represents an over 4-fold decrease in these saturates. Likewise, each component can be considered individually. Again looking at the 218-11.30 event, the C20:0 component is 0.62% in the control / wild-type, while it is reduced about 5.6 times in the plant line of the subject invention (down to 0.11%). The C22:0 component is reduced about 2 2/3 times: 0.12% in the d-9 desaturase plant line, which is down from 0.32% in the control line that lacks the desaturase gene. C24:0 is reduced about 4 2/3 times, from 0.14% down to 0.03 %.

[0157] For Event 36 (or 36-11.19), two lines have C20:0, C22:0, and C24:0 content of 0.32, 0.15, and 0.04, and 0.30, 0.14, and 0.07, respectively. Compared to the control having 0.64, 0.42, and 0.21 respectively, these lines have about half the C20:0, and at least about a three-fold reduction in C22:0 and C24:0. The first line mentioned above actually exhibits an over 5X reduction in C24:0.

[0158] It will quickly become apparent that a great number of similar calculations can be made for any of the other lines of the subject invention, for any of these preferred fatty acid components. These illustrations should not be construed as limiting, and any such novel reductions and ratios can be used to define the subject invention.

Example 12 - Further Half-Seed Data of Subsequent Generations

[0159] Further half-seed FAME analysis is set forth in **Table 16**. This Figure shows total saturates as low as 2.64% in a T3 generation and 2.66% in a T4 generation. **Table 17** shows the copy number of D-9 desaturase genes present in the respective lines (see Sample ID in **Table 16** and ID column in **Table 17**). Effects of copy number are discussed in more detail below in Examples 14 and 19.

Table. 16.

Table. 16.												
			HALF-SEED FAME ANALYSIS - % of Total Oil									
EVENT	Generation	Sample ID:	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3		
69-11.19 (HL)	T3	03TGH01538HS23	nd	0.04	1.88	2.20	0.34	79.56	9.43	3.29		
218-11.30 (HL)	T4	03TGH02193HS50	nd	0.05	2.05	1.54	0.33	81.34	9.85	2.77		
69-11.19 (HL)	T3	03TGH01538HS26	nd	0.05	2.18	1.60	0.29	77.55	11.78	4.01		
218-11.30 (HL)	T4	03TGH02193HS9	nd	nd	2.27	1.76	0.27	75.02	14.44	3.08		
218-11.30 (HL)	T4	03TGH02193HS22	0.01	0.05	2.31	1.31	0.29	81.16	10.30	2.72		
218-11.30 (HL)	T4	03TGH02193HS23	0.01	0.06	2.29	1.47	0.28	77.17	13.57	3.15		
69-11.19 (HL)	T3	03TGH01538HS4	nd	0.03	2.20	1.71	0.35	80.17	10.19	3.07		
36-11.19 (HL)	T3	03TGH01099HS3	nd	0.04	1.95	1.76	0.52	81.92	8.61	3.03		
69-11.19 (HL)	T3	03TGH01538HS36	nd	0.04	2.17	1.49	0.37	78.24	11.54	3.39		
218-11.30 (HL)	T4	03TGH02193HS2	nd	0.06	2.31	1.60	0.30	77.39	13.23	2.98		
69-11.19 (HL)	T3	03TGH01538HS40	nd	0.03	2.26	1.46	0.38	79.75	10.51	3.44		
36-11.19 (HL)	T3	03TGH01099HS8	nd	0.05	2.19	2.05	0.40	77.81	12.22	2.86		
36-11.19 (HL)	T3	03TGH01099HS17	nd	0.06	2.11	1.73	0.42	80.16	10.37	2.73		
36-11.19 (HL)	T3	03TGH01099HS11	nd	0.06	2.13	1.73	0.45	79.30	10.98	3.04		
36-11.19 (HL)	T3	03TGH01099HS43	nd	0.06	2.18	1.72	0.41	77.88	12.09	3.06		
218-11.30 (HL)	T4	03TGH02194HS37	nd	0.06	2.25	1.34	0.44	83.00	8.52	2.50		
218-11.30 (HL)	T4	03TGH02194HS27	nd	0.08	2.31	1.26	0.41	79.74	11.23	3.22		
218-11.30 (HL)	T4	03TGH02194HS17	nd	0.06	2.30	1.34	0.40	79.75	11.15	3.19		
218-11.30 (HL)	T4	03TGH02194HS2	nd	0.06	2.25	1.22	0.44	81.50	9.84	2.79		
218-11.30 (HL)	T4	03TGH02194HS15	nd	0.07	2.35	1.32	0.40	78.85	12.00	3.29		
218-11.30 (N)	T4	03TGH02195HS3	0.01	0.06	3.60	0.23	1.37	76.94	12.52	2.69		
218-11.30 (N)	T4	03TGH02195HS9	nd	0.05	3.33	0.17	1.35	77.76	12.01	2.86		
218-11.30 (N)	T4	03TGH02195HS13	nd	0.06	3.76	0.21	1.13	76.48	13.23	2.62		
218-11.30 (N)	T4	03TGH02195HS16	0.01	0.05	3.31	0.20	1.35	75.89	13.05	3.82		

(continued)

Table. 16.													
			HALF-SEED FAME ANALYSIS - % of Total Oil										
EVENT	Generation	Sample ID:	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3			
218-11.30 (N)	T4	03TGH02195HS19	nd	0.06	4.02	0.27	1.43	74.20	14.43	2.90			
36-11.19 (N)	T3	03TGH01127HS1	nd	0.05	3.46	0.18	1.42	74.06	14.50	3.69			
36-11.19 (N)	T3	03TGH01127HS2	nd	0.06	3.53	0.14	1.30	73.14	15.34	3.37			
36-11.19 (N)	T3	03TGH01127HS4	nd	0.06	3.74	0.16	1.29	71.92	16.49	3.38			
36-11.19 (N)	T3	03TGH01127HS8	nd	0.04	3.59	0.14	1.28	75.09	13.69	2.67			
36-11.19 (N)	T3	03TGH01127HS18	nd	0.04	3.55	0.18	1.41	76.85	12.00	2.67			
WT Control		M94S010HS6	nd	0.06	3.36	0.17	1.45	76.60	12.52	3.36			
WT Control		M94S010HS12	nd	0.06	3.19	0.21	1.58	74.56	13.88	4.31			
WT Control		M94S010HS15	nd	0.03	3.37	0.16	1.62	78.68	10.07	3.26			
WT Control		M94S010HS17	nd	0.05	3.21	0.19	1.54	77.37	12.21	2.97			
WT Control		M94S010HS18	nd	0.07	3.54	0.18	1.37	79.73	9.91	2.35			
69-11.19(N)	T3	03TGH01604HS2	nd	0.06	3.77	0.15	1.19	80.15	9.04	2.60			
69-11.19(N)	T3	03TGH01604HS6	nd	0.05	3.74	0.24	1.22	75.57	13.27	3.23			
69-11.19(N)	T3	03TGH01604HS8	nd	0.05	3.66	0.11	1.35	80.52	8.74	2.64			
69-11.19(N)	T3	03TGH01604HS9	nd	0.04	3.88	0.21	1.32	77.33	11.40	2.67			
69-11.19(N)	T3	03TGH01604HS15	nd	0.05	3.48	0.21	1.37	77.05	12.00	3.38			
Table 16 (continued).													
			HALF-SEED FAME ANALYSIS - % of Total Oil										
EVENT	C20:0	C20:1	C20:2	C22:0	C22:1	C24:0	C24:1	TOTSAT	Selected	Leaf Paint Data			
69-11.19 (HL)	0.18	0.81	0.03	0.10	nd	0.10	0.05	2.64	Selected	Resistant			
218-11.30 (HL)	0.19	0.82	0.04	0.04	nd	nd	nd	2.66	Selected	Resistant			
69-11.19 (HL)	0.20	0.84	0.06	0.08	nd	0.02	0.01	2.81	Selected	Resistant			
218-11.30 (HL)	0.18	0.78	0.10	0.05	nd	0.04	0.04	2.81	Selected	Resistant			

(continued)

			HALF-SEED FAME ANALYSIS - % of Total Oil										
EVENT	C20:0	C20:1	C20:2	C22:0	C22:1	C24:0	C24:1	TOTSAT	Selected	Leaf Paint Data			
218-11.30 (HL)	0.15	0.77	0.04	0.06	nd	0.02	0.02	2.87	Selected	Resistant			
218-11.30 (HL)	0.17	0.73	0.06	0.06	nd	0.02	nd	2.89	Selected	Resistant			
69-11.19 (HL)	0.18	0.80	0.05	0.06	nd	0.09	0.05	2.90	Selected	Resistant			
36-11.19 (HL)	0.26	0.85	0.06	0.11	nd	0.04	nd	2.92	Selected	Resistant			
69-11.19 (HL)	0.19	0.85	0.08	0.12	nd	0.04	nd	2.93	Selected	Resistant			
218-11.30 (HL)	0.17	0.84	0.06	0.06	nd	0.05	nd	2.95	Selected	Resistant			
69-11.19 (HL)	0.21	0.84	0.04	0.08	nd	nd	0.03	2.96	Selected	Resistant			
36-11.19 (HL)	0.22	0.77	0.08	0.11	nd	0.01	0.02	2.97	Selected	Resistant			
36-11.19 (HL)	0.23	0.83	0.08	0.13	nd	0.04	0.09	2.99	Selected	Resistant			
36-11.19 (HL)	0.22	0.79	0.06	0.12	nd	0.02	0.07	3.00	Selected	Resistant			
36-11.19 (HL)	0.21	0.82	0.10	0.13	nd	0.01	0.07	3.01	Selected	Resistant			
218-11.30 (HL)	0.20	0.83	0.04	0.07	nd	nd	nd	3.03	Selected	Resistant			
218-11.30 (HL)	0.18	0.77	0.04	0.06	nd	0.01	nd	3.04	Selected	Resistant			
218-11.30 (HL)	0.19	0.76	0.05	0.07	nd	0.02	nd	3.05	Selected	Resistant			
218-11.30 (HL)	0.20	0.82	0.05	0.09	nd	0.03	nd	3.06	Selected	Resistant			
218-11.30 (HL)	0.18	0.75	0.04	0.06	nd	0.02	nd	3.08	Selected	Resistant			
218-11.30 (N)	0.52	1.16	0.05	0.23	nd	0.12	nd	5.90	Selected null	Susceptible			
218-11.30 (N)	0.42	1.21	0.05	0.18	nd	0.07	0.03	5.39	Selected null	Susceptible			
218-11.30 (N)	0.42	1.21	0.06	0.22	nd	0.08	nd	5.67	Selected null	Susceptible			
218-11.30 (N)	0.42	1.13	0.05	0.18	nd	0.07	0.03	5.39	Selected null	Susceptible			
218-11.30 (N)	0.52	1.23	0.06	0.27	nd	0.11	0.05	6.40	Selected null	Susceptible			
36-11.19 (N)	0.47	1.17	0.07	0.23	0.01	0.07	0.05	5.71	Selected null	Susceptible			
36-11.19 (N)	0.53	1.36	0.09	0.29	nd	0.13	0.07	5.84	Selected null	Susceptible			

(continued)

Table 16 (continued).											
EVENT	HALF-SEED FAME ANALYSIS - % of Total Oil										
	C20:0	C20:1	C20:2	C22:0	C22:1	C24:0	C24:1	TOTSAT	Selected	Leaf Paint Data	
36-11.19 (N)	0.50	1.35	0.08	0.27	0.02	0.09	0.06	5.96	Selected null	Susceptible	
36-11.19 (N)	0.59	1.45	0.07	0.36	0.04	0.15	0.09	6.01	Selected null	Susceptible	
36-11.19 (N)	0.61	1.41	0.08	0.35	nd	0.14	0.07	6.10	Selected null	Susceptible	
WT Control	0.45	1.13	0.05	0.18	nd	0.06	0.06	5.54	Selected null	Susceptible	
WT Control	0.41	0.97	0.06	0.15	nd	nd	0.04	5.39	Selected null	Susceptible	
WT Control	0.51	1.09	0.07	0.22	nd	0.05	nd	5.80	Selected null	Susceptible	
WT Control	0.46	1.17	0.07	0.18	nd	0.06	nd	5.50	Selected null	Susceptible	
WT Control	0.52	1.31	0.05	0.29	nd	0.06	0.02	5.85	Selected null	Susceptible	
69-11.19(N)	0.51	1.39	0.04	0.29	nd	0.12	nd	5.93	Selected null	Susceptible	
69-11.19(N)	0.46	1.22	0.06	0.21	nd	0.08	0.08	5.74	Selected null	Susceptible	
69-11.19(N)	0.53	1.29	0.05	0.28	nd	0.09	0.10	5.96	Selected null	Susceptible	
69-11.19(N)	0.54	1.36	0.07	0.31	0.02	0.08	0.07	6.16	Selected null	Susceptible	
69-11.19(N)	0.45	1.12	0.06	0.21	nd	0.05	0.04	5.61	Selected null	Susceptible	

Table 17.

southern copy #					
ID	Project	Event	Generation	D-9	PAT
03TGH02193HS50	TG03D9-62	218-11.30 (HL)	T4	1	1.5 or 2
03TGH02193HS9	TG03D9-62	218-11.30 (HL)	T4	1	1.5 or 2
03TGH02193HS22	TG03D9-62	218-11.30 (HL)	T4	1	1.5 or 2
03TGH02193HS23	TG03D9-62	218-11.30 (HL)	T4	1	1.5 or 2
03TGH02193HS2	TG03D9-62	218-11.30 (HL)	T4	1	1.5 or 2
03TGH02194HS37	TG03D9-62	218-11.30 (HL)	T4	1	1.5 or 2
03TGH02194HS27	TG03D9-62	218-11.30 (HL)	T4	1	1.5 or 2
03TGH02194HS17	TG03D9-62	218-11.30 (HL)	T4	1	1.5 or 2
03TGH02194HS2	TG03D9-62	218-11.30 (HL)	T4	1	1.5 or 2
03TGH02194HS15	TG03D9-62	218-11.30 (HL)	T4	1	1.5 or 2
03TGH02195HS9	TG03D9-62	218-11.30 (N)	T4	0	0
03TGH02195HS13	TG03D9-62	218-11.30 (N)	T4	0	0
03TGH02195HS16	TG03D9-62	218-11.30 (N)	T4	0	0
M94S010HS15	TG03D9-62	WT Control		0	0
M94S010HS15	TG03D9-62	WT Control		0	0
03TGH01099HS3	TG03D9-62	36-11.19 (HL)	T3	2	1.5
03TGH01099HS8	TG03D9-62	36-11.19 (HL)	T3	2	1.5
03TGH01099HS17	TG03D9-62	36-11.19 (HL)	T3	2	1.5
03TGH01099HS11	TG03D9-62	36-11.19 (HL)	T3	2	1.5
03TGH01099HS43	TG03D9-62	36-11.19 (HL)	T3	2	1.5
03TGH01127HS2	TG03D9-62	36-11.19 (N)	T3	0	0
03TGH01127HS4	TG03D9-62	36-11.19 (N)	T3	0	0
03TGH01127HS8	TG03D9-62	36-11.19 (N)	T3	0	0
03TGH01538HS23	TG03D9-62	69-11.19 (HL)	T3	2	1.5
03TGH01538HS26	TG03D9-62	69-11.19 (HL)	T3	2	1.5
03TGH01538HS4	TG03D9-62	69-11.19 (HL)	T3	2	1.5
03TGH01538HS36	TG03D9-62	69-11.19 (HL)	T3	2	1.5
03TGH01538HS40	TG03D9-62	69-11.19 (HL)	T3	2	1.5
03TGH01604HS6	TG03D9-62	69-11.19(N)	T3	0	0
03TGH01604HS8	TG03D9-62	69-11.19(N)	T3	0	0
03TGH01604HS9	TG03D9-62	69-11.19(N)	T3	0	0
M94S010HS15	TG03D9-62	WT Control		0	0
M94S010HS15	TG03D9-62	WT Control		0	0

Example 13 - Further Analysis of Half-Seed Data from Example 12, and Further Data Showing Fatty Acid Shifts Increases in Unsaturates, and Decreases in Saturates

[0160] Half-seed data from the T3 field trials was plotted to illustrate various comparisons of the fatty acid contents to

the "total sat" data. **Figures 6A and 6B** clearly show the reductions in C16:0 and increases in C16:1 in the transgenic events as compared to the nulls (events with a non-functional insert) and wild-type controls (non-transformed lines). **Figures 6C and 6D** clearly show the reductions in C18:0 and increases in C18:1 in the transgenic events as compared to the nulls and wild-type controls. **Figures 6E and 6F** clearly show the reductions in C20:0 and C22:0, respectively, in the transgenic events as compared to the nulls and wild-type controls.

[0161] Similar results, using data obtained as discussed in Example 4, are also illustrated with bar graphs. **Figures 6G and 6H** clearly show shifts and reductions in C16:0, and shifts and increases in C16:1 in the transgenic events, as compared to the nulls and wild-type controls. **Figures 6I and 6J** clearly show shifts and reductions in C18:0, and shifts and increases in C18:1 in the transgenic events, as compared to the nulls and wild-type controls. **Figures 6K and 6L** show similar bar graphs for C18:2 and C18:3.

[0162] The plots discussed above and **Figure 6M** also clearly illustrate the very surprising reduction in total saturates, as compared to already very good Nex 710 lines. **Figure 6N** shows distributions for 1000 seeds.

Example 14 - Decreasing saturated fat levels with multiple Delta-9 desaturase genes

[0163] Results from greenhouse increases and field trials suggest that there is a relationship between the reduction in total saturates and *Aspergillus delta-9 desaturase* copy number.

14A: FAME Analysis Protocol for Event Sorting and Effect of Transgene Copy Number

Sample preparation

[0164]

1. Obtain weight of individual seeds and place into plastic mother plate.
2. Add 2, 1/8" balls to each well
3. Take mother plate to liquid handler Hamilton:

add 400 μ L of heptane with IS (C11:0) and surrogate (C15:0 FAEE) then add 100 μ L of sodium methoxide (.5N)

4. Cap inserts with strips caps and Geno-grind for 5 minutes (1x @ 500)
5. Replace strip cap
6. Add plate lid with a rubber mat (extra sealing). Tape the lid with black electrical tape and remove bottom of mother plate to expose vials
7. Place the plate onto vortex/heater for 15 min. at 37°C / 60 rpm (the vortex wells are filled with sand)
8. Centrifuge plate @ 3500 rpm for 2 min.
9. Place the bottom lid back and remove lid/strip cap. Transfer 350 μ l of top layer into the daughter plate using Hamilton.
10. Then add 400 μ L of heptane with IS and surrogate to the extraction plate.
11. Repeat steps 5 to 10 two more times (for a total of 3 transfers)
12. Keep the extract plate on the Hamilton after last transfer. Transfer 50 μ l of extract into glass insert mounted in aluminum block containing 450 μ l heptane with C11
13. Inject on GC

GC/FID analysis

GC parameters:

[0165]

Injection 1 μ l splitless per sample

Column-

DB23, 15 meters, 0.25 mm I.D. and 0.15 μ m film thickness

GC parameters-

Oven temperature program-

70°C hold for 2.15min (splitless)

70°C - 150° @ 25°C min.,

150°C -180° @ 5°C min.,

EP 2 338 328 B1

180°C-220° @ 25°C min.,
 220°C hold for 2 min.
 Injector temp. - 230°C
 Detector temp. - 240°C
 Make-up gas - Nitrogen @ 25mL/ min.
 FID fuel - air @ 400mL/ min.,
 hydrogen @ 40mL/ min
 front injector: purge time 1 min.
 purge flow 35 ml/min
 back injector: purge time 2.15 min.
 purge flow 35 ml/min.
 front inlet pressure: 1.0 mL/min - constant flow
 back inlet pressure: 1.0 mL/min - constant flow
 Run time - 14.95 minutes,
 Flow rates - helium constant flow @ 1 mL/ min

Acquisition sequence

[0166] 96 samples are split between front and back column to minimize the build on the liner. The sample list is built with 5 injection methods corresponding to the type of sample being injected. The first 5 samples injected are always:

1. Matrix
2. Matrix
3. Standard1
4. Canola Positive Control
5. Reagent Blank
- 6-27. Canola samples
33. Standard2
- 34-54. Canola samples
55. Standard3

[0167] Each list comprises 3 events of 16 samples (48 samples).

[0168] Standard contains 25 ppm FAMES total distributed as follow:

Table 18.		
FAMES	Concentration (ppm)	Added to calibration
C14:0	0.25	Yes
C16:0	1	Yes
C18:0	0.75	Yes
C18:1	15	Yes
C18:2	3	Yes
C18:3	1.25	Yes
C20:0	0.75	Yes
C20:1	0.25	Yes
C22:0	0.75	Yes
C22:1	1.25	No
C24:0	0.75	Yes

14B: Preliminary Southern Blot Analysis to Approximate Copy Number

[0169] The DNA preparation protocol used for these purposes was as follows. Approximately 6 micrograms of DNA

EP 2 338 328 B1

was digested with HindIII, and digested DNA was run on a 0.75% agarose gel. Blotting onto positively charged nylon membrane and hybridization followed typical protocols (Maniatis, Roche Applied Science, Inc.). The probe consisted of a DIG-labeled (kit from Roche Applied Science, Inc.) PCR product derived from the *Aspergillus delta-9 desaturase* gene. Washes were done twice for 5 minutes in room-temperature 2XSSC/0.1% SDS, then twice in 65°C 0.1XSSC/0.1% SDS. Hybridized bands were visualized with the DIG-Luminescent Detection Kit according to manufacturer's guidelines (Roche Applied Science, Inc.). Hybridizing bands were counted, and transgenic samples were initially described as 'Simple' if they displayed 1 to 3 bands, or "Complex" if more than 3 bands.

14C: Comparison of Transgene Copy Number and Reduction in Saturated Fatty Acids in Transgenic Events

[0170] The following definitions apply to the Table:

# GC/FID analysis	number of individual seeds that has been analyzed
# seed C16:1 WT ratio <10%	number of seeds with a ratio C16:1/C16:0*100 less than 10%
# seed C16:1 intermediate ratio	number of seeds with a ratio C16:1/C16:0*100 more than 10% (interpreted as hemizygote based on FAME phenotype)
# seed C16:1 highest ratio	number of seed with the highest ratio C16:1/C16:0*100 (interpreted as homozygote based on FAME phenotype)
Ratio Sat in WT	ratio of saturated FA in the wild type seed for that particular event. If not available (interpreted as complex event based on FAME phenotype), the null average of 7.5% was used. WT: individual seeds whose sat:unsat ratio is <10%, which is similar to Null event seeds
Ratio Sat in transgenic	ratio of saturated FA in the homozygote seed for that particular event. If not available (complex event) used the average.
Sat reduction (%)	(saturated FA in wild type - saturated FA in transgenic) / saturated FA in wild type of that particular event. If saturated FA in wild type is not available (complex event) used the null average (7.5%).
S	'Simple' event, 1 to 3 transgene copies
PS	Probably a 'Simple' event
C	'Complex' event with more than 3 transgene copies
N	'Negative', no transgenes detected

Table 19.

Plant ID	Preliminary Southern Analysis	# seeds for GC FAME analysis	# seed with C16:1 WT ratio <10%	# seed with C16:1 intermediate ratio	# seed with C16:1 highest ratio	Ratio sat in WT	Ratio sat in transgenic	Ratio sat reduction
152-11.30	C	16	2	12	2	9.6	3.4	64.6
230-11.30	C	16	2	13	1	9.8	3.5	64.4
235-11.19	C	16	0	16	0	7.5	3.4	54.9
75-11.30	S	16	5	9	2	9.0	4.1	54.6
147-11.19	C	15	3	8	4	8.1	3.8	53.4
68-11.30	C	16	1	15	0	9.1	4.4	51.5
222-11.30	C	16	3	11	2	8.5	4.2	50.8
57-11.30	C	16	5	10	1	8.4	4.2	50.0

EP 2 338 328 B1

(continued)

Table 19.

Plant ID	Preliminary Southern Analysis	# seeds for GC FAME analysis	# seed with C16:1 WT ratio <10%	# seed with C16:1 intermediate ratio	# seed with C16:1 highest ratio	Ratio sat in WT	Ratio sat in transgenic	Ratio sat reduction
284-11.19	S	16	3	8	5	7.1	3.5	49.9
108-11.30	S	16	0	16	0	7.5	3.9	48.5
151b-11.30	S	16	3	9	4	7.1	3.7	48.3
87b-11.19	C	15	7	5	3	7.7	4.1	47.6
171-11.30	C	16	0	16	0	7.5	4.0	46.5
43b-11.30	S	16	1	14	1	6.9	3.7	46.4
32-11.30	pS	16	1	12	3	9.5	5.1	46.1
145-11.19	S	16	0	16	0	7.5	4.1	45.6
232-11.30	C	16	0	16	0	7.5	4.1	45.1
96a-6.15	pS	16	2	11	3	9.1	5.0	45.0
115-11.30	C	16	9	4	4	8.1	4.5	44.5
250-11.19	S	16	7	7	2	6.2	3.5	43.5
52-(2)-11.30	pS	16	0	16	0	7.5	4.3	43.1
226-11.30	C	16	1	12	3	5.8	3.3	42.8
224-11.30	C	16	2	10	4	6.8	3.9	42.7
149-11.30	S	16	7	7	2	6.0	3.5	42.6
309-11.30	C	16	5	7	4	6.8	4.0	41.5
159a-11.19	S	16	8	8	0	8.0	4.7	41.3
114-11.30	C	16	0	16	0	7.5	4.5	40.4
5-11.19	C	16	1	15	0	7.3	4.4	40.0
294-11.19	C	16	0	16	0	7.5	4.5	40.0
(9)-3-11.30	S	16	6	7	3	7.9	4.8	39.8
210-11.19	S	16	7	4	4	7.8	4.8	39.1
15-11.19	pS	16	6	10	0	7.5	4.6	38.7
162a-11.30	S	16	10	6	0	7.9	4.9	38.6
72-11.19	S	16	13	3	0	7.0	4.3	37.8
324-11.30	S	16	1	9	6	6.1	3.8	37.2
162c-11.30	S	16	2	14	0	7.4	4.7	37.2
102-11.19	pS	16	4	10	2	7.6	4.8	36.1
126-11.30	S	16	4	9	3	8.3	5.3	35.7
322-11.30	S	16	1	9	6	5.7	3.8	32.6
249 (294)-11.30	S	16	2	10	4	8.0	5.5	31.9
330-11.19	pS	16	3	12	1	6.2	4.2	31.8

EP 2 338 328 B1

(continued)

Table 19.

Plant ID	Preliminary Southern Analysis	# seeds for GC FAME analysis	# seed with C16:1 WT ratio <10%	# seed with C16:1 intermediate ratio	# seed with C16:1 highest ratio	Ratio sat in WT	Ratio sat in transgenic	Ratio sat reduction
138-11.30	pS	15	5	7	3	7.5	5.1	31.8
35b-11.30	S	16	1	12	3	7.1	4.9	31.5
218-11.30	S	16	0	16	0	7.5	5.2	30.9
146-11.19	S	16	2	8	6	6.4	4.4	30.9
175-11.30	S	16	6	8	2	7.2	5.1	28.7
69-11.19	S	16	4	9	3	7.0	5.0	28.3
311-11.30	C	16	0	16	0	7.5	5.4	28.3
162-11.19	pS	16	9	7	0	6.6	4.8	27.4
350-11.19	pS	16	3	12	1	6.7	4.9	27.0
36-11.19	S	15	1	9	5	6.8	5.0	26.8
320-11.30	C	16	7	9	0	7.1	5.3	24.9
245-11.30	C	16	0	16	0	7.5	5.7	24.7
326-11.19	S	16	5	1	10	5.7	4.5	20.6
213-11.30	S	16	8	8	0	6.2	5.1	18.0
26-11.30	PS	16	8	7	1	7.8	7.4	5.0
209-11.19	S	16	16	0	0	7.9	7.9	0.0
5a-6.15	N	16	16	0	0	9.2	9.2	0.0
14a-6.15	N	13	13	0	0	8.2	8.2	0.0
14b-6.15	N	16	16	0	0	8.0	8.0	0.0
63.6.15	N	16	16	0	0	9.3	9.3	0.0
99a-6.15	N	16	16	0	0	9.2	9.2	0.0
99c-6.15	N	16	16	0	0	8.2	8.2	0.0
87a-11.19	N	16	16	0	0	7.9	7.9	0.0
35a-11.30	N	16	16	0	0	7.1	7.1	0.0
43a-11.30	N	15	15	0	0	7.4	7.4	0.0
76.11.30	N	16	16	0	0	7.5	7.5	0.0

[0171] The "Ratio of Saturate Reduction" was used to rank events because it generally used seed from the same transgenic event. This direct comparison helps reduce variability between plants caused by tissue culture and growing plants at different times.

[0172] The above data shows an apparent gene dosage effect; more copies of the transgene tend to cause a more effective reduction in saturated fatty acids. For example, there are 57 "non-control" plants represented above. These 57 plants can be divided into three groups of 19 plants. The top set of plants (exhibiting the best reductions in saturates and the lowest levels of saturates) have 11 of 19 "complex" events (more than 3 copies of the desaturase gene). This set contained 8 of 19 events characterized as 'Simple' or 'probably Simple.' The middle set of 19 plants had only 6 of 19 "complex" events (13 of 19 "simple" or "probably simple" events). Still further, the third set of 19 plants (showing, relatively, the least reductions in saturates) contained only 3 complex events, with 16 of the 19 events being "simple" or

"probably simple." Thus, plants with cells having more than 3 copies of the desaturase appear to show a better reduction in saturates than plants with cells having only 1 copy of the gene.

Example 15 - Segregation of Oleic and Vaccenic Acids

[0173] Vaccenic acid is a C18:1 with the double bond in the delta-11 position. Vaccenic acid is formed by elongating delta-9 C16:1 outside of the plastid. The following is important because other analytical methods discussed herein combined oleic and vaccenic acid peaks together into a single percent composition that was labeled as "oleic." That is, there was no separation of the two unless otherwise indicated. By subtracting out the vaccenic acid contribution, it is presently demonstrated that the percent contribution of oleic acid is, preferably and advantageously (and surprisingly), maintained at less than 80% while still achieving a reduction (to "no sat" or "low sat" levels) of overall total saturates. Two types of analyses were used to demonstrate this, as set forth in the following two Examples.

Example 16 - Canola Delta-9 Seed Extraction and Analysis for Vaccenic Acid SOP

[0174] **Figures 7A and 7B** illustrate data obtained using the following protocol.

Sample preparation (same as before)

[0175]

1. Obtain weight of individual seeds and place into plastic mother plate.
2. Add 2, 1/8" balls to each well
3. Take mother plate to liquid handler Hamilton
- add 400 μ L of heptane with IS (C 11:0) and surrogate (C15:0FAEE) then add 100 μ L of sodium methoxide (.5N)
4. Cap inserts with strips caps and Geno-grind for 5 minutes (1x @ 500)
5. Replace strip cap
6. Add plate lid with a rubber mat (extra sealing). Tape the lid with black electrical tape and remove bottom of mother plate to expose vials
7. Place the plate onto vortex/heater for 15 min. at 37° C / 60 rpm (the vortex wells are filled with glass beads)
8. Centrifuge plate @ 3500 rpm for 2 min.
9. Place the bottom lid back and remove lid/strip cap. Transfer 350 μ l of top layer into the daughter plate using Hamilton.
10. Then add 400 μ L of heptane with IS and surrogate to the extraction plate.
11. Repeat steps 5 to 10 two more times (for a total of 3 transfers)
12. Keep the extract plate on the Hamilton after last transfer. Transfer 50 μ l of extract into glass insert mounted in aluminum block containing 450 μ l heptane with IS C11
13. Inject on GC

GC/FID analysis (different from FAMES profile)

GC parameters:

[0176]

Injection 1 μ l splitless per sample

Column-

BPX 70 from SGE, 15 meters, 0.25 mm I.D. and 0.25 μ m film thickness

GC parameters-

Oven temperature program-

70°C hold for 2.15min (splitless)

70°C -140° @ 25°C min.,

140°C hold for 14 min

140°C -180° @ 10°C min.,

180°C hold for 3 min

180°C - 220° @ 25°C min.,

220°C hold for 3 min.

Injector temp. - 230°C

EP 2 338 328 B1

Detector temp. - 240°C
 Make-up gas - Nitrogen @ 25mL/ min.
 FID fuel - air @ 400mL/ min.,
 hydrogen @ 40mL/ min
 front injector: purge time 1 min.\
 purge flow 35 ml/min
 back injector: purge time 2.15 min.
 purge flow 35 ml/min.
 front inlet pressure: 1.0 mL/min - constant flow
 back inlet pressure: 1.0 mL/min - constant flow
Run time - 30.55 minutes,
Flow rates - helium constant flow @ 1 mL/ min

Acquisition sequence

[0177] 96 samples are split between front and back column to minimize the build on the liner. The sample list is built with 5 injection methods corresponding to the type of sample being injected. The first 5 samples injected are always:

1. Matrix
2. Matrix
3. Standard1
4. Reagent Blank
- 5-32. Canola samples
33. Standard2
- 34-54. Canola samples
55. Standard3

[0178] Each list comprises 6 events of 8 samples (1 seed = 1 sample) (48 samples).

[0179] Standard contains 200 ppm FAMES total distributed as follow:

Table 20.		
FAMES	Concentration (ppm)	Added to calibration
C14:0	2	yes
C16:0	8	yes
C18:0	6	yes
C18:1	120	yes
C18:2	24	yes
C18:3	10	yes
C20:0	6	yes
C20:1	2	yes
C22:0	6	yes
C22:1	10	No
C24:0	6	Yes

Example 17 - Analysis of Vaccenic Acid Contribution Using Gas Chromatography/Mass Spectrometry/Time of Flight

[0180] **Table 21** shows data obtained using the following protocol. In **Table 21**, for the T4, percent lipid was not reduced; it was maintained at 40.8% in the transgenic line (the same as the control line).

Table 21.

Event	Generation	D-9	PAT		C14:0	C16:0	C16:1	C18:0	C18:1	Vacc 18:1	C18:2	C18:3
69-11.19 (HL)	T3	2	1.5	average	0*	2.3	1.6	0.4	78.6	3.1	9.2	3.4
69-11.19(N)	T3	0	0	average	0.0	3.7	0.3	1.9	75.2	3.2	10.1	3.2
218-11.30 (HL)	T4	1	1.5 or 2	average	0.0	2.8	1.2	0.7	78.2	2.9	9.9	3.1
218-11.30 (N)	T4	0	0	average	0.0	3.9	0.3	1.8	74.4	3.1	10.8	3.3

Table 21. (continued)

Event	Generation	D-9	PAT		C20:0	C20:1	C22:0	C24:0	Percent Lipid	Seed weight	Total oil (mg)	% saturated FA
69-11.19 (HL)	T3	2	1.5	average	0.2	0.8	0.1	0.1	35.1	5.3	1.9	3.2
69-11.19(N)	T3	0	0	average	0.7	1.2	0.4	0.2	42.6	4.0	1.7	6.9
218-11.30 (HL)	T4	1	1.5 or 2	average	0.3	0.8	0.1	0.0	40.8	4.2	1.7	4.0
218-11.30 (N)	T4	0	0	average	0.7	1.2	0.4	0.2	40.8	4.0	1.6	6.9

*: if less than detection limit by processing method the default was 0

Acquisition and processing using HP Chemserver package

Instruments description

- [0181]** Time Of Flight mass spectrometer Pegasus III from Leco interfaced with a gas chromatograph-HP 6890.
- [0182]** Combi Pal autoinjector from CTC Analytics technology mounted on the HP 6890 with a 10 µl syringe.

EP 2 338 328 B1

GC method

GC parameters:

5 **[0183]**

Injection 1 to 3 µl splitless per sample

Column-

SolGel Wax, 30 meters, 0.25 mm I.D. and 0.25µm film thickness

10 GC parameters-

Oven temperature program-

70°C -175° @ 25°C min., (splitless)

175°C hold for 25 min.

175°C - 230° @ 50°C min.,

15 230°C hold for 3 min.

Injector temp. - 230°C

Transfer line. - 300°C

back injector: purge time 30 seconds.

purge flow 20 ml/min.

20 back inlet pressure: 2 mL/min - constant flow

Run time - 33.3 minutes,

Flow rates - helium constant flow @ 2 mL/ min

Mass spectrometer

25

Mass selection:

[0184]

30 Collected mass from 50 to 600 amu

Filament bias: -70 V

Ion source: 225°C

Detector:

35

[0185]

Detector voltage: 1600 V

Acquisition rate: 10 spectra/sec

40 Solvent delay 100 seconds

Fragmentation

45 **[0186]** ChromaTOF Software compiling fragmentation and deconvoluting co migrating peaks for better separation and interpretation.

[0187] Identification of fatty acids is performed by retention time and fragmentation match based on Standard solution (see below) injected in the same conditions and/or good match with NIST/EPA/NIH database included in software described above.

50 **[0188]** Vaccenic acid methyl ester also known as cis 11-octadecenoic acid methyl ester was identified from the canola seed extract by running a standard made from Vaccenic acid from Sigma (CAS:506-17-2). The retention time is 1207 seconds and produce a 853 match with standard and 814 with library describe above.

[0189] Composition of the standard injected:

55

Table 22.		
FAMES	Concentration (ppm)	Added to calibration
C14:0	2	Yes

(continued)

Table 22.		
FAMES	Concentration (ppm)	Added to calibration
CC16:0	8	Yes
C18:0	6	Yes
C18:1	120	Yes
C18:2	24	Yes
C18:3	10	Yes
C20:0	6	Yes
C20:1	2	Yes
C22:0	6	Yes
C22:1	10	No
C24:0	6	Yes

Vaccenoate methyl ester process:

[0190] The methylated product was obtain after methylation of 100 mg of the acid:

- 100 mg dissolve in 5 ml of MeOHCl 0.5 N (Supelco) in a 30 ml glass vial
- heated 1 hour at 70°C under nitrogen
- after cooling at room temperature added 5 ml of water containing 0.9% NaCl
- partitioned the ester in hexane in three consecutive hexane extractions (15 ml)
- neutralize the acid residue by mixing the organic phase with 15 ml of water containing HNaCO₃ at 2.5%
- evaporate the organic layer under N₂ to obtain an oily transparent liquid at RT corresponding to a vaccenoate methyl ester

Example 18 - Achieving "No Sats" While Maintaining Superior Agronomic Traits

[0191] Agronomic measurements were made at the various field sites, comparing the transgenics and controls. The conclusion was that overall, the transgenic plants behaved like and exhibited similar traits (aside from much-improved saturate levels) the Nex 710 controls. Thus, the subject transgene(s) has no consistent negative effect on plant health. A summary of the traits and rating system is provided in **Table 23**. Various criteria were used at various field site locations.

[0192] **Tables 24-27** numerically illustrate some representative results. The following abbreviations are used in those figures:

DTF = Days to Flower	EOF = Days to End of Flower
HT = Height	SC = Sterile Counts
DTM = Days to Maturity	LSV = Late Season Vigor
LOD = Lodging	SD WT = Seed Weight

[0193] **Table 24** shows agronomic data for lines from Event #218-11.30. **Table 25** shows agronomic data for lines from Event #36-11.19. **Table 26** shows agronomic data for lines from Event 69-11.19. The foregoing demonstrates that the subject invention, *i.e.* achieving "no sat" canola, can be practiced, surprisingly, without adversely affecting other important agronomic traits.

Table 23.

Field Ratings

Rating	Timing	Scale	Details
Vigor/Establishment	3-4 leaf	1 to 5	1=excellent emergence, healthy stand; 5=very poor emergence and/or seedling health

EP 2 338 328 B1

(continued)

Field Ratings

Rating	Timing	Scale	Details
5 Herbicide tolerance 1	4-7 days after spraying	0 to 100%	1-5 barely detectable, 6-10 detectable by trained eye, 11-15 noticeable by a grower, 15+ likely a grower complaint, 100=all plants in plot are dead
Herbicide tolerance 2	10-14 days after spraying	0 to 100%	same as details for Herbicide tolerance 1
10 Days to flower (DTF)	rated every 2-3 days	# days after seeding	10 % of plants have at least 1 flower open
Days to end of flower (EOF)	rated every 2-3 days	# days after seeding	95% of plants in plot have finished flowering
15 Height	late pod fill	cm	height of perfectly erect plants, gather bunch of plants from centre of plot and stretch up to measure
Days to maturity (DTM)	rated every 2-3 days	# days after seeding	30% pod turn, or optimal time of commercial swathing. However, several pods per plot (from middle of the main raceme) should be opened to determine whether seed maturity correlates to pod colour (ie. Some varieties may appear green, but seeds are mature.)
20 Lodging resistance (LODGE_RES)	at maturity	1 to 5	1=perfectly erect, 5=horizontal
25 Yield		grams per plot	sample and weigh system should be used to standardize yield for varying moisture content; if system is not available, samples should be dried to constant weight prior to recording yield.
30 Late Season Vigor (1-5): Sterile Counts	just before maturity Flowering	100 % bloom	A visual assessment of the general agronomic performance of the line (i.e. potential yielding ability of the line, branching pattern, silique length and size) was used. A rating of 1-5 was used with 1 = best and 5 being the worst in agronomic appearance counting ten plants/plot
35 Notes	throughout season		note any plot or plots with poor emergence or plant stand, flooding, or any other factors that might affect accuracy of ratings.

Table 24. Agronomic Data Summary of Best Performing Lines from Event #218-11.30

											1000 SEED WT	
	Line	Event	DTF	EOF	HT	SC	DTF	LSV	LOD	SD WT	% NULL	%NEX 710
45	1361(T3)	#218-11.30	45	70	100	0	90	2	2	3.20	98	93
	1319(TS)	#218-11.30	44	70	98	0	90	2	1	3.23	98	94
	1304(TS)	#218-11.30	46	72	110	0	90	2	1	3.45	105	101
	1500(TS)	#218-11.30	45	71	103	0	91	3	1	3.12	95	91
	1405(TS)	#218-11.30	45	72	109	0	91	2	1	3.05	93	89
50	1370(TS)	#218-11.30	44	70	106	0	90	2	1	3.41	104	99
	1369(TS)	#218-11.30	44	70	102	0	91	2	1	3.33	102	97
	1370(T)	#218-11.30	44	70	106	0	90	2	1	3.41	104	99
	1405(T)	#218-11.30	45	72	109	0	91	2	1	3.05	93	89
	1299(N)	#218-11.30	43	69	98	0	90	1	1	3.28	.	96
55	Nex 710	.	42	67	100	0	88	2	1	3.43	.	.

Table 25. Agronomic Data Summary of Best Performing Lines from Event 36-11.19

1000 SEED WT										
Line	Event	DTF	EOF	HT	SC	DTM	LSV	LOD	SD WT	% NULL %NEX 710
1099(T)	36-11.19	45	70	106	0	90	2	1	3.78	98 110
1127(N)	36-11.19	44	68	102	0	89	2	1	3.85	. 112
Nex 710	.	42	67	100	0	88	2	1	3.43	. .

Table 26. Agronomic Data Summary of Best Performing Lines from Event 69-11.19

1000 SEED WT										
Line	Event	DTF	EOF	HT	SC	DTM	LSV	LOD	SD WT	% NULL %NEX 710
1538	69-11.19	45	72	103	0	91	4	1	3.17	94 92
1529	69-11.19	45	71	112	0	91	3	1	3.41	101 99
1534	69-11.19	46	71	109	0	91	3	1	3.24	96 94
1604(N)	69-11.19	43	68	102	0	89	2	1	3.38	. 99
Nex 710	.	42	67	100	0	88	2	1	3.43	. .

Example 19 - Dose Effect: Further Lowering of Saturates by Insertion of Multiple Copies of 8-9 Desaturase Genes

[0194] This Example further shows that "stacking" multiple copies of δ -9 desaturase genes has a surprising dose effect, which can be used to even further reduce saturates in oil seed plants such as canola. The following is greenhouse data measured using FAME procedures unless otherwise indicated.

[0195] Table 27 reports F3 10 seed bulk fatty acid data from native Nex 710, the simple events (218, 36, 69), and from each of the F3 seed packages tracing back to F2 plants that were selected on the basis of InVader assays. (The last two columns of Table 27 show approximate average total saturates for the respective plants, and approximate average reduction in total saturates, as compared to the Nex 710 control.) At that generation, Southern data was not used. Thus, based on saturate expression and InVader assays, a selection of suspected stacks and suspected parental siblings as well as nulls was made to be reconfirmed by growing out F3 plants and re-testing using Southern.

[0196] For example, while not statistically analyzed, samples from the 41 "stack" lines have an average total saturates of 3.5%. The 21 "single" lines have an average total saturates of 3.84%.

[0197] Table 28 contains a summary of the suspected F3 stacks, suspected parental siblings, and nulls that were replanted for confirmation of copy number and zygosity. Lines named TDN0400141, TDN0400142, TDN0400145, TDN0400155, TDN0400158, and TDN0400160 were suspected to be homozygous stacks. Lines TDN0400189, TDN0400143, TDN0400197, TDN0400167, and TDN0400184 were suspected to be parental siblings out of the stacks. Lines TDN0400198, TDN0400199 were advanced as nulls selfed out of the stacks. TDN0400202, TDN0400204, and TDN0400208 are the simple events. This material is also currently in the field or recently harvested. Thus, field data is not yet available.

[0198] Figures 8 and 9 are pictures of two gels run with DNA from F3 plants. Similar issues with isolating DNA for Southern analysis were encountered at this step, so not all 9 of the plants submitted appeared in gels. Based on the gels, lines TDN0400141, TDN0400142, and possibly TDN0400158 (only 2 plants) are homozygous stacks. TDN0400145 is still segregating for event 36, and TDN0400155 is still segregating for event 69. Line TDN0400160 appears to have an odd segregation of bands which may indicate that it is segregating for all 3 simple events. Follow-up will be conducted TDN0400160. Plant #8 of from this cross has a low sat level of 2.6% which is consistent with being a triple stack of high zygosity. Additional molecular analysis could confirm this. Not all of the lines suspected to be parental siblings appear in the gels (Lines TDN0400184, TDN0400189, and TDN0400197 highlighted in the Event column of Table 29, discussed below). Based on the single plant fatty acid data, it appears that the 9 plants from TDN0400184 (suspected of being a sibbed-out simple event 218) have as low sat levels as the suspected stacks. That is, the trend in the data is that stacks consistently show a reduction in saturates compared to "sibbed-out" events (single transgenic events recovered from crosses of two transgenic events).

[0199] Table 29 contains 10 seed bulk fatty acid data from Nex 710, the simple events, and each single F4 plant. Nine plants of each suspected stack, null, simple event, and the like were regrown, tissue sampled for molecular analysis, and kept through to seed set for fatty acid analysis.

Table 27.

Name	Source	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2
TDN0400210	NEX 710	0.0	0.0	4.1	0.3	1.4	76.5	11.6	2.9	0.6	1.5	0.1
TDN0400211	NEX 710	0.0	0.1	4.4	0.3	1.5	73.9	13.5	2.9	0.7	1.5	0.1
TDN0400212	NEX 710	0.0	0.0	4.3	0.3	1.6	76.2	11.5	2.8	0.7	1.5	0.0
TDN0400201	TDN04-123	0.0	0.0	3.0	1.5	0.5	79.3	11.0	2.8	0.3	0.9	0.0
TDN0400202	TDN04-123	0.0	0.0	3.0	1.6	0.5	79.3	10.9	2.8	0.3	0.9	0.0
TDN0400203	TDN04-123	0.0	0.0	2.8	1.6	0.4	79.3	10.8	3.1	0.2	0.8	0.0
TDN0400204	TDN04-128	0.0	0.1	2.7	1.9	0.5	78.8	11.0	3.0	0.3	0.8	0.0
TDN0400205	TDN04-128	0.0	0.0	2.7	1.9	0.5	80.3	9.8	2.8	0.3	0.8	0.0
TDN0400206	TDN04-128	0.0	0.0	2.8	1.9	0.6	79.4	10.6	2.7	0.3	0.8	0.0
TDN0400207	TDN04-132	0.0	0.1	2.6	1.8	0.5	79.4	10.8	2.7	0.3	0.9	0.0
TDN0400208	TDN04-132	0.0	0.1	2.5	1.9	0.6	79.4	10.7	2.5	0.3	1.0	0.0
TDN0400209	TDN04-132	0.0	0.1	2.7	1.9	0.6	79.7	10.6	2.4	0.3	0.9	0.0
TDN0400198	TDN04-133/P-116	0.0	0.0	4.0	0.3	1.2	75.3	12.8	3.4	0.5	1.5	0.1
TDN0400199	TDN04-134/P11	0.0	0.0	4.2	0.3	1.2	73.7	14.2	3.4	0.5	1.5	0.1
TDN0400200	TDN04-135/P125	0.0	0.0	4.0	0.3	1.5	75.6	12.6	3.1	0.6	1.5	0.1
TDN0400136	TDN04-133/P14	0.0	0.0	2.4	2.2	0.4	79.9	9.7	3.0	0.3	0.9	0.1
TDN0400137	TDN04-133/P25	0.0	0.0	2.4	2.1	0.6	79.8	9.2	3.1	0.4	1.1	0.1
TDN0400138	TDN04-133/P29	0.0	0.0	2.5	2.3	0.4	81.5	8.5	2.6	0.3	0.8	0.0
TDN0400139	TDN04-133/P37	0.0	0.0	2.3	2.4	0.4	80.1	9.9	3.0	0.6	0.8	nd
TDN0400140	TDN04-133/P40	0.0	0.0	2.4	2.1	0.5	81.4	8.3	2.9	0.3	0.9	0.0
TDN0400141	TDN04-133/P70	0.0	0.0	2.2	2.4	0.3	80.3	9.3	3.2	0.3	0.9	0.0
TDN0400142	TDN04-133/P114	0.0	0.0	2.2	2.4	0.3	79.9	9.8	3.2	0.3	0.9	nd
TDN0400143	TDN04-133/P121	0.0	0.0	2.8	1.7	0.6	79.6	10.7	2.5	0.3	0.9	0.0
TDN0400144	TDN04-133/P122	0.0	0.0	2.7	1.9	0.5	80.6	9.5	2.8	0.3	0.9	0.0
TDN0400145	TDN04-133/P129	0.0	0.0	2.3	2.1	0.4	81.2	9.1	2.7	0.1	0.9	nd
TDN0400146	TDN04-133/P139	0.0	0.0	2.8	1.7	0.6	79.7	10.1	3.2	0.3	0.9	nd
TDN0400147	TDN04-133/P1	0.0	0.0	2.7	2.0	0.5	80.9	8.8	2.7	0.4	1.0	0.0
TDN0400148	TDN04-133/P11	0.0	0.0	2.6	2.2	0.8	79.7	9.1	3.0	0.4	1.0	nd
TDN0400149	TDN04-133/P54	0.0	0.0	2.5	2.0	0.5	82.2	8.0	2.5	0.3	0.9	nd
TDN0400150	TDN04-133/P57	0.0	0.0	2.8	1.7	0.5	79.0	11.1	2.9	0.3	0.9	0.0
TDN0400151	TDN04-133/P67	0.0	0.0	2.1	2.2	0.4	80.4	9.4	3.0	0.3	1.0	0.0

Table 27.

Name	Source	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2
TDN0400152	TDN04-133/P76	0.0	0.0	2.5	1.7	0.5	81.2	8.6	3.2	0.3	1.0	0.0
TDN0400153	TDN04-133/P77	0.0	0.0	2.7	1.6	0.5	81.6	8.8	2.7	0.3	1.0	0.0
TDN0400154	TDN04-134/P-27	0.0	0.0	2.5	2.1	0.4	82.0	7.9	2.7	0.3	0.9	0.0
TDN0400155	TDN04-134/P-32	0.0	0.0	2.4	2.3	0.4	79.6	10.1	3.4	0.1	0.8	nd
TDN0400156	TDN04-134/P-33	0.0	0.0	2.8	2.0	0.5	80.0	9.5	3.0	0.3	0.9	nd
TDN0400157	TDN04-134/P-34	0.0	0.0	2.8	1.9	0.6	80.3	9.5	2.7	0.3	0.9	0.0
TDN0400158	TDN04-134/P-38	0.0	0.0	2.2	2.4	0.3	80.1	9.4	3.4	0.3	0.8	0.0
TDN0400159	TDN04-134/P-42	0.0	0.0	2.6	2.1	0.5	79.7	10.0	3.1	0.3	0.9	0.0
TDN0400160	TDN04-134/P-48	0.0	0.0	2.2	2.3	0.3	81.9	8.5	3.0	0.0	0.8	nd
TDN0400161	TDN04-134/P-52	0.0	0.0	2.5	2.2	0.4	80.6	9.2	3.1	0.0	0.8	0.0
TDN0400162	TDN04-134/P-57	0.0	0.0	2.2	2.4	0.3	80.3	9.0	3.2	0.3	0.9	0.0
TDN0400163	TDN04-134/P-77	0.0	0.0	2.6	1.8	0.5	80.0	9.8	3.2	0.3	0.9	0.0
TDN0400164	TDN04-134/P-82	0.0	0.0	2.7	1.9	0.5	79.6	9.8	3.3	0.3	0.9	0.0
TDN0400165	TDN04-134/P-98	0.0	0.0	2.8	1.9	0.6	77.2	11.2	3.7	0.4	1.0	0.1
TDN0400166	TDN04-134/P-118	0.0	0.0	2.5	2.3	0.4	80.6	9.3	2.8	0.3	0.8	nd
TDN0400167	TDN04-134/P-119	0.0	0.0	2.6	1.9	0.5	80.5	9.4	2.8	0.3	0.9	nd
TDN0400168	TDN04-134/P-142	0.0	0.0	2.4	2.2	0.5	80.4	9.1	3.0	0.2	0.9	nd
TDN0400169	TDN04-134/P-40	0.0	0.0	3.2	1.3	0.7	79.0	10.6	2.8	0.4	1.0	0.0
TDN0400170	TDN04-134/P-41	0.0	0.0	2.5	2.0	0.4	81.0	8.9	2.9	0.3	0.9	0.0
TDN0400171	TDN04-134/P-60	0.0	0.0	2.5	2.0	0.4	80.3	9.5	3.1	0.3	0.9	0.0
TDN0400172	TDN04-134/P-66	0.0	0.1	3.1	1.2	0.7	79.8	10.1	3.0	0.3	1.0	0.0
TDN0400173	TDN04-135/P-23	0.0	0.0	2.6	2.2	0.5	80.6	9.3	2.7	0.3	0.8	0.0
TDN0400174	TDN04-135/P-24	0.0	0.0	2.5	2.5	0.5	81.2	8.9	2.7	0.0	0.7	0.0
TDN0400175	TDN04-135/P-25	0.0	0.0	2.2	2.3	0.4	82.0	8.2	2.5	0.3	0.8	0.0
TDN0400176	TDN04-135/P-36	0.0	0.1	2.3	2.3	0.4	81.2	8.6	2.7	0.3	0.9	0.0
TDN0400177	TDN04-135/P-41	0.0	0.0	2.2	2.1	0.7	83.2	7.1	2.3	0.4	0.9	0.0
TDN0400178	TDN04-135/P-45	0.0	0.0	2.4	2.2	0.6	81.8	7.8	2.7	0.4	0.9	0.0
TDN0400179	TDN04-135/P-47	0.0	0.0	2.3	2.5	0.5	81.8	8.0	2.5	0.3	0.8	0.0
TDN0400180	TDN04-135/P-48	0.0	0.0	2.3	2.3	0.6	83.2	6.8	2.2	0.4	0.9	0.0
TDN0400181	TDN04-135/P-64	0.0	0.1	2.2	2.0	0.6	81.7	8.7	2.3	0.4	0.9	0.0
TDN0400182	TDN04-135/P-86	0.0	0.1	2.3	2.4	0.5	80.2	9.5	2.7	0.3	0.8	0.0
TDN0400183	TDN04-135/P-108	0.0	0.0	2.3	2.1	0.5	82.0	8.2	2.5	0.3	0.9	0.0

Table 27.

Name	Source	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2
TDN0400184	TDN04-135/P-112	0.0	0.1	2.4	2.3	0.4	81.3	8.9	2.7	0.3	0.8	0.0
TDN0400185	TDN04-135/P-120	0.0	0.1	2.5	1.9	0.5	81.3	9.1	2.7	0.3	0.8	0.0
TDN0400186	TDN04-135/P-123	0.0	0.1	2.8	2.1	0.7	79.8	10.1	2.3	0.4	0.8	0.0
TDN0400187	TDN04-135/P-124	0.0	0.1	2.7	2.9	0.4	80.7	8.3	2.7	0.3	0.7	nd
TDN0400188	TDN04-135/P-13	0.0	0.1	2.4	1.8	0.5	80.1	9.9	2.8	0.3	0.9	0.0
TDN0400189	TDN04-135/P-31	0.0	0.0	2.7	1.6	0.6	80.3	10.1	2.8	0.3	0.9	0.0
TDN0400190	TDN04-135/P-46	0.0	0.1	2.7	2.1	0.7	78.9	9.6	2.6	0.4	1.2	0.0
TDN0400191	TDN04-135/P-51	0.0	0.0	2.1	1.5	0.8	83.1	7.9	2.1	0.4	1.0	0.1
TDN0400192	TDN04-135/P-55	0.0	0.0	2.3	1.6	0.7	82.0	8.6	2.5	0.3	0.9	0.0
TDN0400193	TDN04-135/P-56	0.0	0.1	2.7	1.6	0.6	79.5	10.9	2.6	0.3	0.9	0.0
TDN0400194	TDN04-135/P-57	0.0	0.1	2.7	1.3	0.8	80.4	10.1	2.4	0.4	1.0	0.0
TDN0400195	TDN04-135/P-74	0.0	0.0	3.0	1.5	0.6	79.7	10.2	2.8	0.3	0.8	0.0
TDN0400196	TDN04-135/P-75	0.0	0.0	2.9	1.7	0.6	80.0	10.0	2.7	0.3	0.8	0.0
TDN0400197	TDN04-135/P-88	0.0	0.1	2.7	1.6	0.5	79.1	10.8	3.1	0.3	0.9	0.0

Table 27 (continued).

Name	C22:0	C22:1	C24:0	C24:1	TOTSAT	Seed Weight	F2 Invader + Southern	Pedigree
TDN0400210	0.4	nd	0.0	0.0	6.6	7.1	Nex 710	
TDN0400211	0.4	nd	0.1	0.1	7.1	7.3	Nex 710	
TDN0400212	0.4	0.0	0.1	0.0	7.2	7.7	Nex 710	6.96
TDN0400201	0.0	nd	0.1	nd	3.9	8.2	69-11.19	
TDN0400202	0.0	nd	0.1	nd	3.9	7.6	69-11.19	
TDN0400203	0.1	nd	0.1	nd	3.8	7.3	69-11.19	3.84 0.4005729
TDN0400204	0.1	nd	0.2	0.0	3.7	9.1	218-11.30	
TDN0400205	0.0	nd	0.1	nd	3.6	9.2	218-11.30	
TDN0400206	0.0	0.0	0.1	0.0	3.9	9.2	218-11.30	3.75 0.4139063
TDN0400207	0.0	0.0	0.2	nd	3.7	10.7	36-11.19	
TDN0400208	0.1	nd	0.1	nd	3.7	9.8	36-11.19	
TDN0400209	0.0	nd	0.2	nd	3.8	8.5	36-11.19	3.72 0.4191146
TDN0400198	0.3	nd	0.0	0.0	6.2	10.2	69-11.19/36-11.19(null)	6.16

Table 27 (continued).

Name	C22:0	C22:1	C24:0	C24:1	TOTSAT	Seed Weight	F2 Invader + Southern	Pedigree	
TDN0400199	0.3	0.0	0.1	0.1	6.4	8.6	null	69-11.19/218-11.30(null)	6.43
TDN0400200	0.3	nd	0.1	0.0	6.5	11.3	null	218-11.30/36-11.19(null)	6.52
TDN0400136	0.0	nd	0.2	nd	3.4	5.3	stack	69-11.19/36-11.19	0.45
TDN0400137	0.0	nd	0.3	nd	3.7	3	stack	69-11.19/36-11.19	0.40
TDN0400138	0.1	0.0	0.2	nd	3.5	7.6	stack	69-11.19/36-11.19	0.43
TDN0400139	0.0	nd	0.2	nd	3.5	4.2	stack	69-11.19/36-11.19	0.43
TDN0400140	0.1	nd	0.2	nd	3.5	2.4	stack	69-11.19/36-11.19	0.43
TDN0400141	0.1	nd	0.2	nd	3.1	1.3	stack	69-11.19/36-11.19	0.50
TDN0400142	0.0	nd	0.2	nd	3.0	4.8	stack	69-11.19/36-11.19	0.51
TDN0400143	0.1	nd	0.2	nd	4.0	8.5	stack	69-11.19/36-11.19	0.35
TDN0400144	0.0	nd	0.2	nd	3.7	8.5	stack	69-11.19/36-11.19	0.40
TDN0400145	0.0	nd	0.2	nd	3.1	5.8	stack	69-11.19/36-11.19	0.51
TDN0400146	0.1	nd	0.2	nd	4.0	8.9	stack	69-11.19/36-11.19	0.36
TDN0400147	0.1	nd	0.2	nd	3.9	6.6	single	69-11.19/36-11.19	0.37
TDN0400148	0.0	nd	0.3	nd	4.2	2.8	single	69-11.19/36-11.19	0.32
TDN0400149	0.1	nd	0.2	nd	3.6	6	single	69-11.19/36-11.19	0.43
TDN0400150	0.0	nd	0.2	nd	3.9	9.7	single	69-11.19/36-11.19	0.38
TDN0400151	0.1	nd	0.2	nd	3.2	1.7	single	69-11.19/36-11.19	0.48
TDN0400152	0.0	nd	0.2	nd	3.6	1.6	single	69-11.19/36-11.19	0.42
TDN0400153	0.0	nd	0.2	nd	3.7	6.4	single	69-11.19/36-11.19	0.40
TDN0400154	0.1	0.0	0.3	nd	3.5	4.1	stack	69-11.19/218-11.30	0.45
TDN0400155	0.0	0.0	0.2	nd	3.1	3.2	stack	69-11.19/218-11.30	0.51
TDN0400156	0.0	nd	0.2	nd	3.8	2.7	stack	69-11.19/218-11.30	0.40
TDN0400157	0.1	0.0	0.2	nd	4.0	7.7	stack	69-11.19/218-11.30	0.37
TDN0400158	0.1	nd	0.2	nd	3.1	1.4	stack	69-11.19/218-11.30	0.52
TDN0400159	0.0	nd	0.2	nd	3.6	6.4	stack	69-11.19/218-11.30	0.44
TDN0400160	0.0	nd	0.2	0.0	2.8	1.2	stack	69-11.19/218-11.30	0.56
TDN0400161	0.0	nd	0.2	nd	3.3	4.5	stack	69-11.19/218-11.30	0.49
TDN0400162	0.1	nd	0.3	nd	3.2	3	stack	69-11.19/218-11.30	0.49
TDN0400163	0.0	nd	0.2	nd	3.5	1.9	stack	69-11.19/218-11.30	0.45
TDN0400164	0.1	nd	0.2	nd	3.8	2.4	stack	69-11.19/218-11.30	0.40
TDN0400165	0.1	0.0	0.2	nd	4.1	1.1	stack	69-11.19/218-11.30	0.36

Table 27 (continued).

Name	C22:0	C22:1	C24:0	C24:1	TOTSAT	Seed Weight	F2 Invader + Southern	Pedigree
TDN0400166	0.0	nd	0.2	nd	3.5	6.1	stack	69-11.19/218-11.30
TDN0400167	0.1	nd	0.2	nd	3.8	7.2	stack	69-11.19/218-11.30
TDN0400168	0.0	nd	0.2	nd	3.4	1.6	stack	69-11.19/218-11.30
TDN0400169	0.2	nd	0.2	nd	4.6	6.7	single	69-11.19/218-11.30
TDN0400170	0.1	nd	0.2	nd	3.5	1.8	single	69-11.19/218-11.30
TDN0400171	0.0	nd	0.2	nd	3.4	5	single	69-11.19/218-11.30
TDN0400172	0.1	nd	0.2	nd	4.5	8.5	single	69-11.19/218-11.30
TDN0400173	0.0	0.0	0.2	nd	3.6	7.3	stack	218-11.30/36-11.19
TDN0400174	0.1	nd	0.2	nd	3.3	6.9	stack	218-11.30/36-11.19
TDN0400175	0.0	nd	0.2	0.0	3.2	6.1	stack	218-11.30/36-11.19
TDN0400176	0.0	0.0	0.2	nd	3.4	4.5	stack	218-11.30/36-11.19
TDN0400177	0.1	nd	0.2	0.0	3.5	5.9	stack	218-11.30/36-11.19
TDN0400178	0.1	nd	0.2	nd	3.7	3.8	stack	218-11.30/36-11.19
TDN0400179	0.0	nd	0.3	nd	3.4	4.2	stack	218-11.30/36-11.19
TDN0400180	0.1	nd	0.3	nd	3.7	2.8	stack	218-11.30/36-11.19
TDN0400181	0.0	nd	0.2	nd	3.5	6.4	stack	218-11.30/36-11.19
TDN0400182	0.1	nd	0.2	0.0	3.5	8.3	stack	218-11.30/36-11.19
TDN0400183	0.0	nd	0.2	nd	3.5	6.7	stack	218-11.30/36-11.19
TDN0400184	0.1	nd	0.2	nd	3.4	9	stack	218-11.30/36-11.19
TDN0400185	0.0	nd	0.1	nd	3.4	10.1	stack	218-11.30/36-11.19
TDN0400186	0.0	nd	0.1	0.0	4.0	8.3	stack	218-11.30/36-11.19
TDN0400187	0.0	nd	0.2	0.0	3.7	5.2	stack	218-11.30/36-11.19
TDN0400188	0.1	nd	0.2	0.0	3.6	5.8	single	218-11.30/36-11.19
TDN0400189	0.0	nd	0.1	nd	3.7	8.1	single	218-11.30/36-11.19
TDN0400190	0.0	nd	0.2	nd	4.0	0.1	single	218-11.30/36-11.19
TDN0400191	0.2	nd	0.2	nd	3.7	5.4	single	218-11.30/36-11.19
TDN0400192	0.0	nd	0.2	0.0	3.5	6.4	single	218-11.30/36-11.19
TDN0400193	0.0	0.0	0.2	nd	3.8	7.6	single	218-11.30/36-11.19
TDN0400194	0.1	nd	0.1	0.0	4.1	7.5	single	218-11.30/36-11.19
TDN0400195	0.1	nd	0.1	nd	4.2	9.4	single	218-11.30/36-11.19
TDN0400196	0.1	nd	0.1	nd	4.1	7.3	single	218-11.30/36-11.19
TDN0400197	0.1	nd	0.2	0.0	3.9	8	single	218-11.30/36-11.19

Table 28.

Name	Pedigree	Source	Gener- ation	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	TOTSAT	Follow-up done to confirm:	Seed (g)
TDN0400211	Nex 710	NEX 710		4.37	0.34	1.46	73.94	13.53	2.91	7.08		10.2
TDN0400202	69-11.19	TDN04-123	T7	2.97	1.59	0.47	79.3	10.86	2.85	3.86		8.6
TDN0400204	218-11.30	TDN04-128	T6	2.66	1.9	0.49	78.85	10.98	3.01	3.73		1.3
TDN0400208	36-11.19	TDN04-132	T6	2.51	1.95	0.58	79.45	10.73	2.52	3.69		4.8
TDN0400198	69-11.19/36-11.19(null)	TDN04-133/P-116	F3	4.05	0.29	1.2	75.27	12.77	3.42	6.16		5.8
TDN0400199	69-11.19/218-11.30(null)	TDN04-134/P11	F3	4.16	0.29	1.24	73.67	14.17	3.4	6.43		3.2
TDN0400141	69-11.19/36-11.19	TDN04-133/P70	F3	2.24	2.36	0.32	80.3	9.3	3.23	3.1	homo Stack	1.4
TDN0400142	69-11.19/36-11.19	TDN04-133/P114	F3	2.15	2.38	0.3	79.89	9.77	3.17	3.02	homo Stack	1.2
TDN0400145	69-11.19/36-11.19	TDN04-133/P129	F3	2.34	2.09	0.37	81.23	9.12	2.7	3.07	homo Stack	8.1
TDN0400155	69-11.19/218-11.30	TDN04-134/P-32	F3	2.38	2.31	0.42	79.55	10.15	3.4	3.12	homo Stack	8.5
TDN0400158	69-11.19/218-11.30	TDN04-134/P-38	F3	2.2	2.36	0.29	80.14	9.44	3.36	3.05	homo Stack	8.0
TDN0400160	69-11.19/218-11.30	TDN04-134/P-48	F3	2.23	2.27	0.29	81.88	8.47	2.97	2.81	homo Stack	7.2
TDN0400189	218-11.30/36-11.19	TDN04-135/P-31	F3	2.69	1.65	0.55	80.3	10.07	2.78	3.7	homo 36	9.0
TDN0400143	69-11.19/36-11.19	TDN04-133/P121	F3	2.82	1.66	0.62	79.6	10.65	2.46	4.04	homo 69	
TDN0400197	218-11.30/36-11.19	TDN04-135/P-88	F3	2.75	1.64	0.54	79.11	10.78	3.06	3.89	homo 218	
TDN0400167	69-11.19/218-11.30	TDN04-134/P-119	F3	2.64	1.94	0.51	80.54	9.4	2.83	3.8	homo 218	
TDN0400184	218-11.30/36-11.19	TDN04-135/P-112	F3	2.4	2.26	0.42	81.28	8.89	2.7	3.4	homo 218	

Table 29.

Name	Generation	Source Pop	Event Name	QA Lab ID	C12:0	C14:0	C16:0	C16:1
Nex 710	DH	Polo/SVO95-09	Natreon	05-147-0001	0.0	0.1	3.5	0.2
Nex 710	DH	Polo/SVO95-09	Natreon	05-147-0002	0.0	0.1	3.7	0.2
Nex 710	DH	Polo/SVO95-09	Natreon	05-147-0003	0.0	0.1	3.7	0.2
Nex 710	DH	Polo/SVO95-09	Natreon	05-147-0004	0.0	0.1	3.6	0.2
Nex 710	DH	Polo/SVO95-09	Natreon	05-147-0005	0.0	0.1	3.7	0.2
Nex 710	DH	Polo/SVO95-09	Natreon	05-147-0006	0.0	0.0	3.7	0.2
Nex 710	DH	Polo/SVO95-09	Natreon	05-147-0007	0.0	0.1	3.7	0.3
Nex 710	DH	Polo/SVO95-09	Natreon	05-147-0008	0.0	0.0	3.7	0.2
Nex 710	DH	Polo/SVO95-09	Natreon	05-147-0009	0.0	0.1	3.8	0.2
TDN0400141-1	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0010	0.0	0.0	2.3	1.8
TDN0400141-2	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0011	0.0	0.0	2.3	2.3
TDN0400141-3	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0012	0.0	0.0	2.2	2.3
TDN0400141-4	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0013	0.0	0.1	2.2	2.2
TDN0400141-5	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0014	0.0	0.0	2.2	2.1
TDN0400141-6	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0015	0.0	0.1	2.2	2.3
TDN0400141-7	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0016	0.0	0.1	2.2	2.5
TDN0400141-8	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0017	0.0	0.0	2.1	2.3
TDN0400141-9	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0018	0.0	0.1	2.1	2.2
TDN0400142-1	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0019	0.0	0.1	2.0	2.1
TDN0400142-2	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0020	0.0	0.0	2.0	2.3
TDN0400142-3	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0021	0.0	0.0	2.1	2.0
TDN0400142-4	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0022	0.0	0.0	2.0	2.4
TDN0400142-5	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0023	0.0	0.1	2.1	2.3
TDN0400142-6	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0024	0.0	0.0	2.1	2.1
TDN0400142-7	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0025	0.0	0.0	2.0	2.1
TDN0400142-8	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0026	0.0	0.1	2.0	2.1
TDN0400142-9	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0027	0.0	0.0	2.1	2.0
TDN0400143-1	F4	03TGH01538/03TGH01099	36.11.19 (from 69.11.19::36.11.19)	05-147-0028	0.0	0.0	2.4	1.7
TDN0400143-2	F4	03TGH01538/03TGH01099	36.11.19 (from 69.11.19::36.11.19)	05-147-0029	0.0	0.1	2.3	1.5
TDN0400143-3	F4	03TGH01538/03TGH01099	36.11.19 (from 69.11.19::36.11.19)	05-147-0030	0.0	0.1	2.5	1.6
TDN0400143-4	F4	03TGH01538/03TGH01099	36.11.19 (from 69.11.19::36.11.19)	05-147-0031	0.0	0.1	2.6	1.4

Table 29.

Name	Gener- ation	Source Pop	Event Name	QA Lab ID	C12:0	C14:0	C16:0	C16:1
TDN0400143-5	F4	03TGH01538/03TGH01099	36.11.19 (from 69.11.19::36.11.19)	05-147-0032	0.0	0.1	2.4	1.8
TDN0400143-6	F4	03TGH01538/03TGH01099	36.11.19 (from 69.11.19::36.11.19)	05-147-0033	0.0	0.0	2.3	1.7
TDN0400143-7	F4	03TGH01538/03TGH01099	36.11.19 (from 69.11.19::36.11.19)	05-147-0034	0.0	0.1	2.6	1.3
TDN0400143-8	F4	03TGH01538/03TGH01099	36.11.19 (from 69.11.19::36.11.19)	05-147-0035	0.0	0.0	2.7	1.6
TDN0400143-9	F4	03TGH01538/03TGH01099	36.11.19 (from 69.11.19::36.11.19)	05-147-0036	0.0	0.1	2.3	1.7
TDN0400145-1	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0037	0.0	0.1	2.4	1.6
TDN0400145-2	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0038	0.0	0.0	2.7	1.4
TDN0400145-3	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0039	0.0	0.1	2.3	1.7
TDN0400145-4	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0040	0.0	0.1	2.3	1.9
TDN0400145-5	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0041	0.0	0.1	2.4	1.6
TDN0400145-6	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0042	0.0	0.1	2.2	2.0
TDN0400145-7	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0043	0.0	0.0	2.3	2.0
TDN0400145-8	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0044	0.0	0.0	2.2	1.9
TDN0400145-9	F4	03TGH01538/03TGH01099	69.11.19::36.11.19	05-147-0045	0.0	0.0	2.1	2.2
TDN0400155-1	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0046				
TDN0400155-2	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0047				
TDN0400155-3	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0048	0.0	0.0	2.5	1.8
TDN0400155-4	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0049	0.0	0.1	2.2	2.0
TDN0400155-5	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0050				
TDN0400155-6	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0051	0.0	0.0	2.4	2.0
TDN0400155-7	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0052	0.0	0.1	2.6	1.8
TDN0400155-8	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0053	0.0	0.0	2.2	2.1
TDN0400155-9	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0054	0.0	0.1	2.4	2.0
TDN0400158-1	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0055	0.0	0.0	2.2	2.1
TDN0400158-2	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0056	0.0	0.0	2.6	1.4
TDN0400158-3	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0057	0.0	0.0	2.2	1.9
TDN0400158-4	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0058	0.0	0.0	2.3	1.8
TDN0400158-5	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0059				
TDN0400158-6	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0060	0.0	0.0	2.1	2.4
TDN0400158-7	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0061				
TDN0400158-8	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0062				
TDN0400158-9	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0063	0.0	0.0	2.1	2.5

Table 29.

Name	Gener- ation	Source Pop	Event Name	QA Lab ID	C12:0	C14:0	C16:0	C16:1
TDN0400160-1	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0064	0.0	0.0	2.2	1.9
TDN0400160-2	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0065	0.0	0.0	2.2	2.0
TDN0400160-3	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0066	0.0	0.0	2.2	2.0
TDN0400160-4	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0067	0.0	0.0	2.6	1.6
TDN0400160-5	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0068	0.0	0.0	2.2	2.2
TDN0400160-6	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0069	0.0	0.0	2.2	2.1
TDN0400160-7	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0070	0.0	0.0	2.1	2.3
TDN0400160-8	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0071	0.0	0.1	2.1	2.2
TDN0400160-9	F4	03TGH01538/03TGH02193	69.11.19::218.11.30	05-147-0072				
TDN0400167-1	F4	03TGH01538/03TGH02193	218.11.30 (from 69.11.19::218.11.30)	05-147-0073	0.0	0.0	2.5	1.7
TDN0400167-2	F4	03TGH01538/03TGH02193	218.11.30 (from 69.11.19::218.11.30)	05-147-0074	0.0	0.1	2.4	1.8
TDN0400167-3	F4	03TGH01538/03TGH02193	218.11.30 (from 69.11.19::218.11.30)	05-147-0075	0.0	0.1	2.5	1.7
TDN0400167-4	F4	03TGH01538/03TGH02193	218.11.30 (from 69.11.19::218.11.30)	05-147-0076	0.0	0.0	2.5	1.7
TDN0400167-5	F4	03TGH01538/03TGH02193	218.11.30 (from 69.11.19::218.11.30)	05-147-0077	0.0	0.0	2.5	1.7
TDN0400167-6	F4	03TGH01538/03TGH02193	218.11.30 (from 69.11.19::218.11.30)	05-147-0078	0.0	0.0	2.5	1.6
TDN0400167-7	F4	03TGH01538/03TGH02193	218.11.30 (from 69.11.19::218.11.30)	05-147-0079	0.0	0.0	2.4	1.9
TDN0400167-8	F4	03TGH01538/03TGH02193	218.11.30 (from 69.11.19::218.11.30)	05-147-0080	0.0	0.0	2.5	1.7
TDN0400167-9	F4	03TGH01538/03TGH02193	218.11.30 (from 69.11.19::218.11.30)	05-147-0081	0.0	0.1	2.5	1.7
TDN0400184-1	F4	03TGH02193/03TGH01099	218.11.30 (from 218.11.30::36-11.19)	05-147-0082	0.0	0.1	2.3	1.8
TDN0400184-2	F4	03TGH02193/03TGH01099	218.11.30 (from 218.11.30::36-11.19)	05-147-0083	0.0	0.1	2.4	1.8
TDN0400184-3	F4	03TGH02193/03TGH01099	218.11.30 (from 218.11.30::36-11.19)	05-147-0084	0.0	0.0	2.2	2.0
TDN0400184-4	F4	03TGH02193/03TGH01099	218.11.30 (from 218.11.30::36-11.19)	05-147-0085	0.0	0.1	2.1	2.2
TDN0400184-5	F4	03TGH02193/03TGH01099	218.11.30 (from 218.11.30::36-11.19)	05-147-0086	0.0	0.1	2.3	2.1
TDN0400184-6	F4	03TGH02193/03TGH01099	218.11.30 (from 218.11.30::36-11.19)	05-147-0087	0.0	0.1	2.3	2.0
TDN0400184-7	F4	03TGH02193/03TGH01099	218.11.30 (from 218.11.30::36-11.19)	05-147-0088	0.0	0.1	2.3	2.0
TDN0400184-8	F4	03TGH02193/03TGH01099	218.11.30 (from 218.11.30::36-11.19)	05-147-0089	0.0	0.1	2.2	2.1
TDN0400184-9	F4	03TGH02193/03TGH01099	218.11.30 (from 218.11.30::36-11.19)	05-147-0090	0.0	0.0	2.4	2.0
TDN0400189-1	F4	03TGH02193/03TGH01099	36-11.19 (from 218.11.30::36-11.19)	05-147-0091	0.0	0.1	2.8	1.5
TDN0400189-2	F4	03TGH02193/03TGH01099	36-11.19 (from 218.11.30::36-11.19)	05-147-0092	0.0	0.0	2.8	1.0
TDN0400189-3	F4	03TGH02193/03TGH01099	36-11.19 (from 218.11.30::36-11.19)	05-147-0093	0.0	0.1	2.5	1.6
TDN0400189-4	F4	03TGH02193/03TGH01099	36-11.19 (from 218.11.30::36-11.19)	05-147-0094	0.0	0.0	2.7	1.1
TDN0400189-5	F4	03TGH02193/03TGH01099	36-11.19 (from 218.11.30::36-11.19)	05-147-0095	0.0	0.0	2.4	1.7

Table 29.

Name	Gener- ation	Source Pop	Event Name	QA Lab ID	C12:0	C14:0	C16:0	C16:1
TDN0400189-6	F4	03TGH02193/03TGH01099	36-11.19 (from 218.11.30::36-11.19)	05-147-0096	0.0	0.1	2.5	1.8
TDN0400189-7	F4	03TGH02193/03TGH01099	36-11.19 (from 218.11.30::36-11.19)	05-147-0097	0.0	0.0	2.7	1.5
TDN0400189-8	F4	03TGH02193/03TGH01099	36-11.19 (from 218.11.30::36-11.19)	05-147-0098	0.0	0.0	2.6	1.2
TDN0400189-9	F4	03TGH02193/03TGH01099	36-11.19 (from 218.11.30::36-11.19)	05-147-0099	0.0	0.1	2.7	1.3
TDN0400197-1	F4	03TGH02193/03TGH01099	218.11.30 (from 218.11.30::36-11.19)	05-147-0100	0.0	0.1	3.0	1.5
TDN0400197-2	F4	03TGH02193/03TGH01099	218.11.30 (from 218.11.30::36-11.19)	05-147-0101	0.0	0.1	2.5	1.9
TDN0400197-3	F4	03TGH02193/03TGH01099	218.11.30 (from 218.11.30::36-11.19)	05-147-0102	0.0	0.0	2.6	1.7
TDN0400197-4	F4	03TGH02193/03TGH01099	218.11.30 (from 218.11.30::36-11.19)	05-147-0103	0.0	0.1	2.5	1.8
TDN0400197-5	F4	03TGH02193/03TGH01099	218.11.30 (from 218.11.30::36-11.19)	05-147-0104	0.0	0.1	2.7	1.7
TDN0400197-6	F4	03TGH02193/03TGH01099	218.11.30 (from 218.11.30::36-11.19)	05-147-0105	0.0	0.1	2.8	1.5
TDN0400197-7	F4	03TGH02193/03TGH01099	218.11.30 (from 218.11.30::36-11.19)	05-147-0106	0.0	0.1	2.8	2.5
TDN0400197-8	F4	03TGH02193/03TGH01099	218.11.30 (from 218.11.30::36-11.19)	05-147-0107	0.0	0.1	2.5	1.4
TDN0400197-9	F4	03TGH02193/03TGH01099	218.11.30 (from 218.11.30::36-11.19)	05-147-0108	0.0	0.1	2.7	1.7
TDN0400198-1	F4	03TGH01538/03TGH01099	null (from 69-11.19::36-11.19)	05-147-0109	0.0	0.1	3.7	0.3
TDN0400198-2	F4	03TGH01538/03TGH01099	null (from 69-11.19::36-11.19)	05-147-0110	0.0	0.1	3.9	0.3
TDN0400198-3	F4	03TGH01538/03TGH01099	null (from 69-11.19::36-11.19)	05-147-0111	0.0	0.1	3.7	0.3
TDN0400198-4	F4	03TGH01538/03TGH01099	null (from 69-11.19::36-11.19)	05-147-0112	0.0	0.1	3.6	0.3
TDN0400198-5	F4	03TGH01538/03TGH01099	null (from 69-11.19::36-11.19)	05-147-0113	0.0	0.1	3.7	0.3
TDN0400198-6	F4	03TGH01538/03TGH01099	null (from 69-11.19::36-11.19)	05-147-0114	0.0	0.1	3.8	0.3
TDN0400198-7	F4	03TGH01538/03TGH01099	null (from 69-11.19::36-11.19)	05-147-0115	0.0	0.1	3.7	0.3
TDN0400198-8	F4	03TGH01538/03TGH01099	null (from 69-11.19::36-11.19)	05-147-0116	0.0	0.1	3.7	0.3
TDN0400198-9	F4	03TGH01538/03TGH01099	null (from 69-11.19::36-11.19)	05-147-0117	0.0	0.1	3.5	0.3
TDN0400199-1	F4	03TGH01538/03TGH02193	null (from 69-11.19/218-11.30)	05-147-0118	0.0	0.1	3.7	0.3
TDN0400199-2	F4	03TGH01538/03TGH02193	null (from 69-11.19/218-11.30)	05-147-0119	0.0	0.0	3.7	0.3
TDN0400199-3	F4	03TGH01538/03TGH02193	null (from 69-11.19/218-11.30)	05-147-0120	0.0	0.1	3.6	0.3
TDN0400199-4	F4	03TGH01538/03TGH02193	null (from 69-11.19/218-11.30)	05-147-0121	0.0	0.1	3.6	0.2
TDN0400199-5	F4	03TGH01538/03TGH02193	null (from 69-11.19/218-11.30)	05-147-0122	0.0	0.0	3.7	0.3
TDN0400199-6	F4	03TGH01538/03TGH02193	null (from 69-11.19/218-11.30)	05-147-0123	0.0	0.0	3.9	0.3
TDN0400199-7	F4	03TGH01538/03TGH02193	null (from 69-11.19/218-11.30)	05-147-0124	0.0	0.1	3.7	0.3
TDN0400199-8	F4	03TGH01538/03TGH02193	null (from 69-11.19/218-11.30)	05-147-0125	0.0	0.0	3.7	0.3
TDN0400199-9	F4	03TGH01538/03TGH02193	null (from 69-11.19/218-11.30)	05-147-0126	0.0	0.1	3.6	0.3
TDN0400202-1	T8	02TGH00038	69.11.19	05-147-0127	0.0	0.0	2.5	1.6

Table 29.

Name	Generation	Source Pop	Event Name	QA Lab ID	C12:0	C14:0	C16:0	C16:1
TDN0400202-2	T8	02TGH00038	69.11.19	05-147-0128	0.0	0.0	2.6	1.5
TDN0400202-3	T8	02TGH00038	69.11.19	05-147-0129	0.0	0.0	2.6	1.4
TDN0400202-4	T8	02TGH00038	69.11.19	05-147-0130	0.0	0.0	2.5	1.5
TDN0400202-5	T8	02TGH00038	69.11.19	05-147-0131	0.0	0.0	2.4	1.8
TDN0400202-6	T8	02TGH00038	69.11.19	05-147-0132	0.0	0.0	2.6	1.4
TDN0400202-7	T8	02TGH00038	69.11.19	05-147-0133	0.0	0.1	2.5	1.4
TDN0400202-8	T8	02TGH00038	69.11.19	05-147-0134	0.0	0.0	2.4	1.5
TDN0400202-9	T8	02TGH00038	69.11.19	05-147-0135	0.0	0.0	2.5	1.5
TDN0400204-1	T7	02TGH00032	218.11.30	05-147-0136	0.0	0.0	2.3	1.8
TDN0400204-2	T7	02TGH00032	218.11.30	05-147-0137	0.0	0.0	2.4	1.8
TDN0400204-3	T7	02TGH00032	218.11.30	05-147-0138	0.0	0.0	2.3	1.8
TDN0400204-4	T7	02TGH00032	218.11.30	05-147-0139	0.0	0.0	2.4	1.7
TDN0400204-5	T7	02TGH00032	218.11.30	05-147-0140	0.0	0.0	2.4	1.5
TDN0400204-6	T7	02TGH00032	218.11.30	05-147-0141	0.0	0.0	2.4	1.7
TDN0400204-7	T7	02TGH00032	218.11.30	05-147-0142	0.0	0.0	2.5	1.6
TDN0400204-8	T7	02TGH00032	218.11.30	05-147-0143	0.0	0.0	2.5	1.6
TDN0400204-9	T7	02TGH00032	218.11.30	05-147-0144	0.0	0.0	2.4	1.7
TDN0400208-1	T7	02TGH00037	36.11.19	05-147-0145	0.0	0.1	2.1	1.7
TDN0400208-2	T7	02TGH00037	36.11.19	05-147-0146	0.0	0.1	2.1	1.9
TDN0400208-3	T7	02TGH00037	36.11.19	05-147-0147	0.0	0.1	2.2	1.8
TDN0400208-4	T7	02TGH00037	36.11.19	05-147-0148	0.0	0.1	2.2	1.8
TDN0400208-5	T7	02TGH00037	36.11.19	05-147-0149	0.0	0.0	2.5	1.6
TDN0400208-6	T7	02TGH00037	36.11.19	05-147-0150	0.0	0.0	2.4	1.5
TDN0400208-7	T7	02TGH00037	36.11.19	05-147-0151	0.0	0.0	2.2	1.3
TDN0400208-8	T7	02TGH00037	36.11.19	05-147-0152	0.0	0.1	2.1	1.8
TDN0400208-9	T7	02TGH00037	36.11.19	05-147-0153	0.0	0.0	2.1	1.8

Table 29 (continued).

Name	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C22:0	C22:1	C24:0	C24:1	% Sats	Seed Weight	Seed Weight Units
Nex 710	1.5	78.5	9.9	2.9	0.6	1.4	0.1	0.4	0.0	0.3	0.2	6.4	10.1	G
Nex 710	1.5	77.7	10.5	3.0	0.6	1.4	0.1	0.4	0.0	0.3	0.0	6.6	9.2	G
Nex 710	1.3	77.6	10.7	2.9	0.6	1.5	0.1	0.4	0.0	0.3	0.2	6.4	10.2	G
Nex 710	1.5	78.8	9.2	2.7	0.7	1.6	0.1	0.5	0.0	0.4	0.2	6.7	5.7	G
Nex 710	1.4	78.1	10.0	2.9	0.7	1.5	0.1	0.5	0.0	0.4	0.2	6.6	10.4	G
Nex 710	1.4	77.0	11.2	3.0	0.6	1.4	0.1	0.4	0.0	0.3	0.2	6.5	13.3	G
Nex 710	1.6	78.2	9.8	2.6	0.7	1.5	0.1	0.5	0.0	0.4	0.3	6.9	9.5	G
Nex 710	1.4	78.0	10.2	2.9	0.6	1.4	0.1	0.4	0.0	0.4	0.2	6.5	11.1	g
Nex 710	1.5	77.5	10.7	2.7	0.7	1.5	0.1	0.4	0.0	0.3	0.2	6.7	11.8	g
TDN0400141-1	0.5	82.9	8.0	2.3	0.2	0.8	0.0	0.1	0.0	0.3	0.0	3.4	7.3	g
TDN0400141-2	0.5	81.6	7.9	2.6	0.2	0.9	0.1	0.2	0.0	0.3	0.0	3.6	6.1	g
TDN0400141-3	0.4	82.3	7.8	2.6	0.2	0.9	0.0	0.1	0.0	0.3	0.0	3.2	3.4	g
TDN0400141-4	0.5	81.0	8.4	2.9	0.3	0.9	0.0	0.1	0.0	0.4	0.0	3.5	4.3	g
TDN0400141-5	0.4	82.3	7.8	2.7	0.2	0.9	0.0	0.1	0.0	0.3	0.0	3.3	5.7	g
TDN0400141-6	0.5	79.1	9.7	3.2	0.3	1.0	0.1	0.2	0.0	0.3	0.0	3.5	1.8	g
TDN0400141-7	0.5	81.9	7.4	2.6	0.3	0.9	0.0	0.1	0.0	0.4	0.0	3.5	2.2	g
TDN0400141-8	0.4	82.3	7.9	2.5	0.2	0.8	0.1	0.1	0.0	0.2	0.0	3.1	5.1	g
TDN0400141-9	0.5	80.8	8.9	3.1	0.2	1.0	0.1	0.1	0.0	0.1	0.0	3.1	3.1	g
TDN0400142-1	0.5	81.9	8.1	2.4	0.3	1.0	0.1	0.1	0.0	0.3	0.0	3.3	7.3	g
TDN0400142-2	0.4	81.3	8.8	2.8	0.2	0.9	0.0	0.1	0.0	0.3	0.0	3.0	7.8	g
TDN0400142-3	0.4	82.8	7.8	2.5	0.2	0.9	0.0	0.1	0.0	0.3	0.0	3.1	7.7	g
TDN0400142-4	0.4	82.1	7.7	2.8	0.2	0.9	0.1	0.1	0.0	0.2	0.0	3.0	4.9	g
TDN0400142-5	0.4	80.7	9.2	2.9	0.2	0.9	0.1	0.1	0.0	0.2	0.0	3.0	5	g
TDN0400142-6	0.4	81.5	9.0	2.6	0.2	0.9	0.0	0.1	0.0	0.2	0.0	3.1	9.7	g
TDN0400142-7	0.4	81.6	8.8	2.6	0.2	0.9	0.1	0.1	0.0	0.3	0.0	3.0	8.6	g
TDN0400142-8	0.4	81.2	9.0	2.8	0.2	0.9	0.1	0.0	0.0	0.3	0.0	2.9	7.7	g
TDN0400142-9	0.4	81.0	9.2	2.6	0.2	0.9	0.1	0.1	0.0	0.3	0.0	3.3	11.2	g
TDN0400143-1	0.5	81.7	9.0	2.4	0.2	0.9	0.1	0.2	0.0	0.2	0.0	3.6	8.5	g
TDN0400143-2	0.6	81.2	9.6	2.6	0.3	1.0	0.1	0.1	0.0	0.2	0.0	3.6	5.7	g
TDN0400143-3	0.6	81.2	9.3	2.3	0.3	0.9	0.1	0.2	0.0	0.3	0.0	3.9	10.8	g

Table 29 (continued).

Name	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C22:0	C22:1	C24:0	C24:1	% Sats	Seed Weight	Seed Weight Units
TDN0400143-4	0.6	82.4	8.5	2.4	0.3	0.9	0.0	0.2	0.0	0.2	0.0	3.9	6.2	g
TDN0400143-5	0.5	81.9	8.9	2.4	0.3	0.9	0.1	0.2	0.0	0.2	0.0	3.5	9.6	g
TDN0400143-6	0.5	81.7	9.1	2.4	0.3	0.9	0.0	0.2	0.0	0.2	0.0	3.6	10.2	g
TDN0400143-7	0.7	81.6	9.1	2.3	0.4	1.0	0.1	0.2	0.0	0.2	0.0	4.1	10.7	g
TDN0400143-8	0.5	80.1	9.9	2.9	0.2	0.9	0.1	0.1	0.0	0.2	0.0	3.8	3.6	g
TDN0400143-9	0.5	82.3	8.6	2.3	0.2	0.9	0.1	0.2	0.0	0.2	0.0	3.4	8.7	g
TDN0400145-1	0.6	80.7	9.9	2.5	0.3	0.9	0.1	0.2	0.0	0.2	0.0	3.7	8.9	g
TDN0400145-2	0.5	80.4	10.3	2.6	0.3	0.9	0.1	0.2	0.0	0.0	0.0	3.7	7.3	g
TDN0400145-3	0.5	81.8	8.9	2.7	0.2	0.9	0.1	0.1	0.0	0.2	0.0	3.4	8	g
TDN0400145-4	0.5	81.9	8.4	2.5	0.2	0.9	0.0	0.1	0.0	0.4	0.0	3.5	8.7	g
TDN0400145-5	0.5	83.1	7.9	2.2	0.3	0.9	0.0	0.1	0.0	0.2	0.0	3.6	8.3	g
TDN0400145-6	0.4	81.8	8.6	2.7	0.2	0.9	0.1	0.1	0.0	0.3	0.0	3.3	8.9	g
TDN0400145-7	0.4	82.9	7.5	2.4	0.2	0.9	0.1	0.1	0.0	0.3	0.0	3.3	7.8	g
TDN0400145-8	0.5	83.2	7.5	2.6	0.2	0.9	0.0	0.1	0.0	0.2	0.0	3.2	8.4	g
TDN0400145-9	0.4	82.4	7.8	2.7	0.2	0.8	0.1	0.1	0.0	0.3	0.0	3.1	8.3	g
TDN0400155-1													6.4	g
TDN0400155-2													0	g
TDN0400155-3	0.6	81.9	8.3	2.6	0.2	0.8	0.1	0.1	0.0	0.2	0.0	3.7	11.2	g
TDN0400155-4	0.5	82.5	7.8	2.7	0.2	0.8	0.0	0.1	0.0	0.3	0.0	3.3	10.2	g
TDN0400155-5													0	g
TDN0400155-6	0.4	81.9	8.8	2.6	0.3	0.8	0.0	0.0	0.0	0.0	0.0	3.2	7.5	g
TDN0400155-7	0.5	82.3	8.4	2.4	0.3	0.8	0.0	0.1	0.0	0.0	0.0	3.6	9	g
TDN0400155-8	0.5	84.2	6.4	2.3	0.3	0.8	0.0	0.1	0.0	0.2	0.0	3.3	7.5	g
TDN0400155-9	0.5	82.5	8.0	2.6	0.3	0.8	0.0	0.1	0.0	0.0	0.0	3.2	10	g
TDN0400158-1	0.4	83.4	7.4	2.5	0.3	0.8	0.0	0.1	0.0	0.0	0.0	3.0	9	g
TDN0400158-2	0.5	83.2	8.0	2.4	0.3	0.9	0.0	0.0	0.0	0.0	0.0	3.5	7.2	g
TDN0400158-3	0.5	82.5	8.0	2.7	0.3	0.8	0.0	0.1	0.0	0.0	0.0	3.1	9.7	g
TDN0400158-4	0.5	83.3	7.5	2.5	0.3	0.8	0.0	0.1	0.0	0.1	0.0	3.2	8.6	g
TDN0400158-5													0	g
TDN0400158-6	0.4	82.8	7.2	2.8	0.2	0.8	0.0	0.1	0.0	0.0	0.0	2.8	6.4	g

Table 29 (continued).

Name	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C22:0	C22:1	C24:0	C24:1	% Sats	Seed Weight	Seed Weight Units
TDN0400158-7													0 g	0 g
TDN0400158-8													0 g	0 g
TDN0400158-9	0.4	82.0	7.9	2.8	0.2	0.8	0.0	0.1	0.0	0.0	0.0	2.8	3.2 g	2.8 g
TDN0400160-1	0.4	82.4	8.1	2.8	0.3	0.8	0.0	0.1	0.0	0.1	0.0	3.1	7.8 g	3.1 g
TDN0400160-2	0.4	82.9	7.9	2.6	0.3	0.8	0.0	0.1	0.0	0.0	0.0	3.0	8.5 g	3.0 g
TDN0400160-3	0.4	83.3	7.4	2.6	0.3	0.8	0.0	0.1	0.0	0.0	0.0	3.0	6.5 g	3.0 g
TDN0400160-4	0.6	81.8	8.9	2.6	0.3	0.8	0.0	0.1	0.0	0.0	0.0	3.5	12 g	12 g
TDN0400160-5	0.4	82.3	7.7	2.7	0.3	0.8	0.0	0.1	0.0	0.1	0.0	3.1	4 g	4 g
TDN0400160-6	0.4	82.5	8.1	2.6	0.3	0.8	0.0	0.1	0.0	0.1	0.0	3.1	9.2 g	3.1 g
TDN0400160-7	0.4	83.0	7.3	2.6	0.3	0.8	0.0	0.0	0.0	0.1	0.0	3.0	6.5 g	3.0 g
TDN0400160-8	0.4	82.6	8.0	2.8	0.1	0.8	0.0	0.0	0.0	0.0	0.0	2.6	2.3 g	2.3 g
TDN0400160-9													0 g	0 g
TDN0400167-1	0.6	83.2	7.9	2.3	0.3	0.8	0.0	0.1	0.0	0.0	0.0	3.5	11.1 g	11.1 g
TDN0400167-2	0.5	82.7	8.1	2.4	0.3	0.8	0.0	0.1	0.0	0.1	0.0	3.4	9.6 g	9.6 g
TDN0400167-3	0.6	82.4	8.5	2.4	0.3	0.8	0.0	0.1	0.0	0.0	0.0	3.5	10.1 g	10.1 g
TDN0400167-4	0.5	82.0	8.9	2.5	0.3	0.8	0.0	0.1	0.0	0.0	0.0	3.4	9.4 g	9.4 g
TDN0400167-5	0.6	81.8	8.9	2.4	0.3	0.8	0.0	0.1	0.0	0.1	0.0	3.7	11.9 g	11.9 g
TDN0400167-6	0.6	82.3	8.7	2.4	0.3	0.8	0.0	0.1	0.0	0.0	0.0	3.5	10.3 g	10.3 g
TDN0400167-7	0.5	81.8	8.7	2.6	0.3	0.9	0.0	0.1	0.0	0.0	0.0	3.3	8.5 g	8.5 g
TDN0400167-8	0.6	82.1	8.9	2.5	0.3	0.8	0.0	0.1	0.0	0.0	0.0	3.5	9.2 g	9.2 g
TDN0400167-9	0.5	82.7	8.3	2.4	0.3	0.8	0.0	0.1	0.0	0.0	0.0	3.5	8.7 g	8.7 g
TDN0400184-1	0.5	82.9	8.1	2.5	0.3	0.8	0.0	0.1	0.0	0.0	0.0	3.1	10.2 g	10.2 g
TDN0400184-2	0.5	83.1	7.9	2.4	0.3	0.8	0.0	0.0	0.0	0.0	0.0	3.3	8.7 g	8.7 g
TDN0400184-3	0.5	83.5	7.3	2.3	0.3	0.8	0.0	0.1	0.0	0.0	0.0	3.1	8.6 g	8.6 g
TDN0400184-4	0.5	83.7	7.1	2.2	0.3	0.8	0.0	0.1	0.0	0.1	0.0	3.1	10.3 g	10.3 g
TDN0400184-5	0.5	83.3	7.1	2.3	0.3	0.8	0.0	0.1	0.0	0.1	0.0	3.3	6.9 g	6.9 g
TDN0400184-6	0.5	82.4	8.1	2.6	0.3	0.8	0.0	0.1	0.0	0.0	0.0	3.2	10.5 g	10.5 g
TDN0400184-7	0.5	84.0	6.9	2.1	0.3	0.8	0.0	0.1	0.0	0.1	0.0	3.3	9.6 g	9.6 g
TDN0400184-8	0.5	82.2	8.2	2.6	0.1	0.8	0.0	0.1	0.0	0.1	0.0	3.0	10.1 g	10.1 g
TDN0400184-9	0.5	82.0	8.5	2.4	0.3	0.8	0.0	0.1	0.0	0.1	0.0	3.5	8.1 g	8.1 g

Table 29 (continued).

Name	C:18:0	C:18:1	C:18:2	C:18:3	C20:0	C20:1	C20:2	C22:0	C22:1	C24:0	C24:1	% Sats	Seed Weight Units
TDN0400189-1	0.8	79.9	10.6	2.4	0.3	0.8	0.0	0.2	0.0	0.0	0.0	4.2	10 g
TDN0400189-2	0.7	80.9	9.8	2.6	0.3	0.9	0.0	0.2	0.0	0.0	0.0	4.1	8.5 g
TDN0400189-3	0.7	83.4	7.8	2.1	0.3	0.9	0.0	0.1	0.0	0.0	0.0	3.7	10.7 g
TDN0400189-4	0.6	79.5	11.0	2.9	0.3	1.0	0.0	0.1	0.0	0.0	0.0	3.8	8.8 g
TDN0400189-5	0.6	82.4	8.5	2.3	0.3	0.9	0.0	0.1	0.0	0.0	0.0	3.4	10.1 g
TDN0400189-6	0.6	82.2	8.5	2.5	0.3	0.8	0.0	0.1	0.0	0.0	0.0	3.5	11.6 g
TDN0400189-7	0.6	81.5	9.2	2.5	0.3	0.8	0.0	0.1	0.0	0.0	0.0	3.8	10.6 g
TDN0400189-8	0.7	82.2	8.8	2.3	0.3	0.9	0.0	0.2	0.0	0.0	0.0	3.8	11.2 g
TDN0400189-9	0.8	81.3	9.2	2.4	0.4	1.0	0.1	0.0	0.0	0.3	0.0	4.2	8.7 g
TDN0400197-1	0.7	79.6	10.3	2.6	0.3	0.9	0.0	0.1	0.0	0.1	0.0	4.3	9 g
TDN0400197-2		79.1	10.5	3.0	0.3	0.9	0.0	0.0	0.0	0.1	0.0	3.5	11 g
TDN0400197-3	0.5	81.5	8.8	2.7	0.3	0.9	0.0	0.1	0.0	0.3	0.0	3.8	11.6 g
TDN0400197-4	0.5	81.4	8.7	2.7	0.3	0.8	0.0	0.0	0.0	0.3	0.0	3.7	12.3 g
TDN0400197-5	0.5	79.3	10.6	3.0	0.3	0.9	0.1	0.1	0.0	0.2	0.0	3.8	10.6 g
TDN0400197-6	0.5	79.8	10.2	2.6	0.4	1.0	0.0	0.0	0.0	0.4	0.0	4.4	12.3 g
TDN0400197-7	0.7	77.2	11.3	3.3	0.3	0.9	0.0	0.0	0.0	0.3	0.0	4.0	6.9 g
TDN0400197-8	0.5	80.4	9.9	2.6	0.3	1.1	0.1	0.0	0.0	0.3	0.0	3.9	9.7 g
TDN0400197-9	0.7	80.2	9.8	3.1	0.3	0.8	0.0	0.0	0.0	0.2	0.0	3.8	11.1 g
TDN0400198-1	0.5	77.1	11.1	3.3	0.6	1.5	0.0	0.5	0.0	0.0	0.0	6.3	11.2 g
TDN0400198-2	1.4	77.4	11.3	3.0	0.6	1.6	0.1	0.1	0.0	0.0	0.0	5.9	11.6 g
TDN0400198-3	1.4	77.5	11.0	3.0	0.6	1.6	0.0	0.3	0.0	0.1	0.0	6.0	11.1 g
TDN0400198-4	1.3	78.0	10.2	3.1	0.6	1.5	0.0	0.3	0.0	0.6	0.0	6.5	12.8 g
TDN0400198-5	1.4	77.8	10.5	3.0	0.5	1.5	0.0	0.3	0.0	0.5	0.0	6.4	12.5 g
TDN0400198-6	1.3	77.3	11.1	3.1	0.6	1.5	0.0	0.4	0.0	0.2	0.0	6.3	6.7 g
TDN0400198-7	1.3	78.1	10.3	3.0	0.6	1.5	0.1	0.4	0.0	0.0	0.0	6.2	11.1 g
TDN0400198-8	1.4	78.5	10.1	2.9	0.6	1.6	0.0	0.2	0.0	0.0	0.0	6.0	13.8 g
TDN0400198-9	1.5	76.1	12.1	3.4	0.5	1.7	0.1	0.5	0.0	0.0	0.0	5.8	7.6 g
TDN0400199-1	1.2	77.6	10.7	3.1	0.6	1.5	0.0	0.1	0.0	0.1	0.0	6.0	11.3 g
TDN0400199-2	1.5	78.3	10.3	2.9	0.6	1.6	0.0	0.0	0.0	0.1	0.0	5.9	11.9 g
TDN0400199-3	1.4	77.5	11.0	3.0	0.6	1.6	0.0	0.2	0.0	0.0	0.0	5.9	11.2 g

Table 29 (continued).

Name	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C22:0	C22:1	C24:0	C24:1	% Sats	Seed Weight	Seed Weight Units
TDN0400199-4	1.4	79.3	9.8	2.7	0.6	1.6	0.0	0.3	0.0	0.0	0.0	5.9	8.4	g
TDN0400199-5	1.4	78.9	10.1	2.7	0.6	1.7	0.0	0.0	0.0	0.0	0.0	5.9	10	g
TDN0400199-6	1.5	78.4	10.2	2.7	0.7	1.6	0.0	0.3	0.0	0.0	0.0	6.4	8.2	g
TDN0400199-7	1.5	77.5	10.5	3.0	0.6	1.5	0.1	0.4	0.0	0.1	0.0	6.2	12.4	g
TDN0400199-8	1.4	78.8	10.0	2.7	0.6	1.5	0.1	0.2	0.0	0.1	0.0	6.1	8.6	g
TDN0400199-9	1.5	78.3	10.1	2.8	0.7	1.6	0.1	0.1	0.0	0.1	0.0	5.9	10.3	g
TDN0400202-1	1.4	81.9	8.3	2.7	0.3	0.9	0.0	0.1	0.0	0.2	0.0	3.6	14.3	g
TDN0400202-2	0.5	83.3	7.8	2.1	0.3	0.9	0.0	0.0	0.0	0.0	0.0	3.5	10.3	g
TDN0400202-3	0.6	82.3	8.6	2.6	0.2	0.8	0.0	0.0	0.0	0.0	0.0	3.3	9.5	g
TDN0400202-4	0.5	81.2	9.2	2.8	0.3	0.9	0.0	0.0	0.0	0.2	0.0	3.6	8.1	g
TDN0400202-5	0.5	82.9	7.8	2.5	0.3	0.9	0.0	0.0	0.0	0.0	0.0	3.1	6.5	g
TDN0400202-6	0.4	82.4	8.4	2.6	0.3	0.9	0.1	0.1	0.0	0.0	0.0	3.5	11.8	g
TDN0400202-7	0.5	82.5	8.4	2.6	0.2	0.9	0.0	0.0	0.0	0.1	0.0	3.4	8.2	g
TDN0400202-8	0.5	82.0	8.7	2.7	0.3	0.9	0.0	0.0	0.0	0.0	0.0	3.3	8.2	g
TDN0400202-9	0.5	82.5	8.3	2.5	0.3	0.9	0.0	0.0	0.0	0.2	0.0	3.5	10.6	g
TDN0400204-1	0.5	82.8	7.8	2.5	0.3	0.9	0.1	0.0	0.0	0.1	0.0	3.4	10.4	g
TDN0400204-2	0.5	82.4	8.0	2.5	0.3	0.9	0.0	0.1	0.0	0.1	0.0	3.5	11.6	g
TDN0400204-3	0.6	82.1	8.5	2.6	0.3	0.8	0.0	0.0	0.0	0.1	0.0	3.3	11.3	g
TDN0400204-4	0.5	81.8	8.9	2.6	0.3	0.8	0.0	0.0	0.0	0.0	0.0	3.2	10.1	g
TDN0400204-5	0.5	82.8	8.1	2.5	0.2	0.8	0.0	0.0	0.0	0.0	0.0	3.2	11	g
TDN0400204-6	0.5	82.8	7.8	2.6	0.3	0.8	0.0	0.0	0.0	0.1	0.0	3.4	9.4	g
TDN0400204-7	0.6	82.1	8.7	2.5	0.3	0.8	0.0	0.0	0.0	0.0	0.0	3.4	10.3	g
TDN0400204-8	0.5	82.4	8.6	2.4	0.2	0.8	0.1	0.0	0.0	0.1	0.0	3.3	7.7	g
TDN0400204-9	0.5	83.1	7.7	2.4	0.3	0.8	0.0	0.0	0.1	0.0	0.0	3.4	10.5	g
TDN0400208-1	0.6	82.2	8.7	2.2	0.4	1.0	0.0	0.0	0.0	0.0	0.0	3.2	10.3	g
TDN0400208-2	0.7	80.9	9.5	2.5	0.3	1.0	0.0	0.1	0.0	0.1	0.0	3.3	8	g
TDN0400208-3	0.6	82.4	8.3	2.2	0.4	1.0	0.1	0.0	0.0	0.0	0.0	3.3	8.6	g
TDN0400208-4	0.7	82.6	8.3	2.3	0.3	1.0	0.1	0.0	0.0	0.0	0.0	3.2	8.3	g
TDN0400208-5	0.6	81.1	9.5	2.4	0.3	0.9	0.0	0.0	0.0	0.1	0.0	3.6	12.6	g
TDN0400208-6	0.6	80.8	10.0	2.6	0.3	0.9	0.1	0.1	0.0	0.0	0.0	3.4	10.5	g

Table 29 (continued).

Name	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C22:0	C22:1	C24:0	C24:1	% Sats	Seed Weight Units
TDN0400208-7	0.6	81.6	9.6	2.2	0.3	1.0	0.0	0.1	0.0	0.0	0.0	3.4	10.1 g
TDN0400208-8	0.7	82.4	8.5	2.3	0.3	1.0	0.0	0.1	0.0	0.0	0.0	3.3	8.4 g
TDN0400208-9	0.7	81.8	8.6	2.4	0.4	1.0	0.1	0.0	0.0	0.0	0.0	3.2	8.2 g

1. A canola plant that produces seed having an oil fraction comprising less than 3.5 % total saturates and less than 80% oleic acid.

2. The plant of item 1 wherein said oil fraction comprises 70% to 78% oleic acid.

3. The plant of item 1 wherein said oil fraction comprises no more than 3% linolenic acid.

EP 2 338 328 B1

4. The plant of item 1 wherein said oil fraction comprises 70% to 78% oleic acid and no more than 3.5% linolenic acid.

5. Seed produced by the canola plant of item 1.

6. Canola oil comprising less than 3.5 % total saturates and less than 80% oleic acid.

7. A fried food composition comprising potato material and canola oil according to item 6.

8. The canola plant of item 1 wherein said oil fraction has no more than 2.7% total saturates.

9. The canola seed of item 5 having an oil fraction comprising no more than 2.7% total saturates.

10. The canola oil of item 6 having an oil fraction comprising no more than 2.7% total saturates.

11. A method of producing a fried food composition wherein said method comprises frying potato material in canola oil according to item 6.

12. A canola plant comprising at least one polynucleotide, stably incorporated in a genome of said plant, that encodes a delta-9 desaturase protein wherein the full complement of a nucleic acid molecule that encodes a protein of SEQ ID NO:5 maintains hybridization, after wash, with said polynucleotide, wherein said wash conditions are 2x SSC (Standard Saline Citrate) and 0.1% SDS (Sodium Dodecyl Sulfate) for 15 minutes at room temperature.

13. The plant of item 12 wherein said nucleic acid molecule comprises SEQ ID NO:1.

14. The plant of item 12 wherein said wash conditions are 0.1x SSC and 0.1% SDS for 15 minutes at room temperature.

15. The plant of item 12 wherein said wash conditions are 0.1x SSC and 0.1% SDS for 30 minutes at 55° C.

16. The plant of item 12 wherein said genome comprises two of said polynucleotides.

17. The plant of item 12 wherein said genome comprises three of said polynucleotides.

18. The plant of item 12 wherein said polynucleotide is operably linked to a seed-specific promoter.

19. A plant produced by the seed of item 5.

20. A method of reducing saturated fat in the oil fraction of at least one seed of a transgenic canola plant, as compared to a wild-type oil fraction of seeds of a corresponding wild-type canola plant, wherein said method comprises producing a canola plant that expresses a polynucleotide that encodes a delta-9 desaturase protein wherein a nucleotide molecule that encodes said protein hybridizes with the molecule of SEQ ID NO: 1.

21. The method of item 20 wherein the oil fraction comprises less than 80% oleic acid.

22. The method of item 20 wherein said protein causes a reduction in palmitic acid (16:0), a reduction in behenic acid (22:0), and an increase in palmitoleic acid (16:1) in the oil fraction of said seed of said transgenic canola plant relative to said corresponding wild-type canola plant.

23. The method of item 20 wherein said saturated fat is reduced by at least 60%.

24. A polynucleotide comprising a sequence of nucleotides shown in SEQ ID NO:1.

25. The plant of item 1 wherein said plant is at least 100cm in height with an average seed weight above 3 mg.

SEQUENCE LISTING

[0200]

EP 2 338 328 B1

<110> Dow AgroSciences LLC

<120> certain Plants with "No Saturate" or Reduced Saturate Levels of Fatty Acids in seeds, and Oil Derived from the seeds

<130> 502-20 T1

<140> EP 05 802 517.2

<141> 2005-10-07

<150> US 60/617,532

<151> 2004-10-08

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<170> PatentIn version 3.3

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EP 2 338 328 B1

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	gctcatcata ggtacaccga taccgacaag gaccatact ctggttaggaa gggacttctc	420
	tactctcacc ttggatggat ggtgatgaag cagaacccaa agaggatcgg aaggaccgac	480
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	gagttcccat ctgactacag gaacgctatc gagtggcacc agtacgatcc taccaagtgg	840
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	gagatcgaga agggaagggt tcaacaactt cagaagaagc ttgataggaa gagggctact	960
	cttgattggg gaaccccact tgatcaactt ccagtgatgg aatgggatga ctacgttgag	1020
30	caagctaaga acggaagggg acttgttgct atcgttgag ttgttcacga tgttaccgac	1080
	ttcatcaagg atcaccagg aggaaaggct atgatctctt ctggaatcgg aaaggatgct	1140
	accgctatgt tcaacggagg agtgtactac cactctaacg cagctcacia ccttcttagc	1200
35		
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	aaggagaacg ttgagtacgt tagggatgga tctggacaaa gggatgatcag ggctggagag	1320
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<212> DNA

45 <213> Artificial Sequence

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EP 2 338 328 B1

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EP 2 338 328 B1

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 35 40 45

Gly Cys Ile Gln Ala Phe Trp Val Pro Leu Gln Leu Lys Thr Ala Ile
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35 Trp Ala Val Ile Tyr Tyr Phe Phe Thr Gly Leu Gly Ile Thr Ala Gly
 65 70 75 80

40 Tyr His Arg Leu Trp Ala His Cys Ser Tyr Ser Ala Thr Leu Pro Leu
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45

50

55

EP 2 338 328 B1

Arg Ile Trp Leu₁₀₀ Ala Ala Val Gly Gly₁₀₅ Gly Ala Val Glu Gly₁₁₀ Ser Ile

5 Arg Trp Trp₁₁₅ Ala Arg Asp His Arg₁₂₀ Ala His His Arg Tyr₁₂₅ Thr Asp Thr

10 Asp Lys₁₃₀ Asp Pro Tyr Ser Val₁₃₅ Arg Lys Gly Leu Leu₁₄₀ Tyr Ser His Leu

Gly Trp Met Val Met Lys₁₅₀ Gln Asn Pro Lys Arg₁₅₅ Ile Gly Arg Thr Asp₁₆₀

15 Ile Ser Asp Leu Asn₁₆₅ Glu Asp Pro Val Val₁₇₀ Val Trp Gln His Arg₁₇₅ Asn

20 Tyr Leu Lys Val₁₈₀ Val Phe Thr Met Gly₁₈₅ Leu Ala Val Pro Met₁₉₀ Leu Val

Ala Gly Leu₁₉₅ Gly Trp Gly Asp Trp₂₀₀ Leu Gly Gly Phe Val₂₀₅ Tyr Ala Gly

25 Ile Leu Arg Ile Phe Phe Val₂₁₅ Gln Gln Ala Thr Phe₂₂₀ Cys Val Asn Ser

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Arg Asp His Val Ile₂₄₅ Thr Ala Leu Val Thr₂₅₀ Leu Gly Glu Gly Tyr₂₅₅ His

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40 His Gln Tyr₂₇₅ Asp Pro Thr Lys Trp₂₈₀ Ser Ile Trp Ala Trp₂₈₅ Lys Gln Leu

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55 Gly Val Val His Asp Val Thr Asp Phe Ile Lys Asp His Pro Gly Gly

355

360

365

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 385 390 395 400

Thr Met Arg Val Gly Val Ile Arg Gly Gly Cys Glu Val Glu Ile Trp
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Lys Arg Ala Gln Lys Glu Asn Val Glu Tyr Val Arg Asp Gly Ser Gly
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Gln Arg Val Ile Arg Ala Gly Glu Gln Pro Thr Lys Ile Pro Glu Pro
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Ile Pro Thr Ala Asp Ala Ala
 450 455

1

Claims

1. Canola oil obtainable from seeds of a canola plant comprising a polynucleotide, stably incorporated in a genome of said seeds, that encodes a delta-9 desaturase protein, wherein said oil comprises less than 3.5% total saturates and 70% to less than 80% oleic acid and wherein said total saturates are the sum of 16:0, 18:0, 20:0, 22:0 and 24:0 fatty acids.
2. Canola seeds from a canola plant, wherein said seeds comprise a polynucleotide, stably incorporated in a genome of said seeds, that encodes a delta-9 desaturase protein, said seeds comprising an oil of claim 1, wherein said delta-9 desaturase protein is the delta-9 desaturase protein of SEQ ID NO: 5 or a variant thereof, and wherein said variant has an amino acid sequence having 80% or more sequence identity to SEQ ID NO: 5 and has the same functionality as the delta-9 desaturase of SEQ ID NO: 5.
3. The seeds of claim 2 comprising 70% to 78% oleic acid.
4. The seeds of claim 2 comprising no more than 3% linolenic acid.
5. The seeds of claim 2 comprising 70% to 78% oleic acid and no more than 3.5% linolenic acid.
6. The seeds of claim 2 comprising no more than 2.7% total saturates.
7. A canola plant that produces seeds comprising a polynucleotide, stably incorporated in a genome of said seeds, that encodes a delta-9 desaturase protein, the seeds comprising an oil of claim 1, and wherein said delta-9 desaturase protein is the delta-9 desaturase protein of SEQ ID NO: 5 or a variant thereof, and wherein said variant has an amino acid sequence having 80% or more sequence identity to SEQ ID NO: 5 and has the same functionality as the delta-9 desaturase of SEQ ID NO: 5.
8. The seeds of claim 2 or the canola plant of claim 7 wherein the oil comprises less than 0.3% C20:0 plus C22:0 plus C24:0.
9. The seeds of claim 2 or the canola plant of claim 7 wherein the oil comprises a polyunsaturated fatty acid content of less than 20%, preferably less than 15%, preferably less than 10%, or preferably less than 9%.

10. The canola plant of claim 7 wherein said oil has no more than 2.7% total saturates.
11. The canola plant of claim 7 wherein said canola plant is at least 100 cm in height with an average seed weight above 3 mg.
12. A method of reducing saturated fat in the oil fraction of seeds of a transgenic canola plant by at least 43%, as compared to a wild-type oil fraction of seeds of a corresponding wild-type canola plant, wherein said method comprises producing a transgenic canola plant that expresses a polynucleotide that encodes a delta-9 desaturase protein wherein the full complement of a nucleic acid molecule that encodes said protein hybridizes with the nucleic acid molecule of SEQ ID NO: 1 under stringent conditions.
13. A method of reducing saturated fat in the oil fraction of seeds of a transgenic canola plant by at least 43%, as compared to a wild-type oil fraction of seeds of a corresponding wild-type canola plant, wherein said method comprises producing a transgenic canola plant that expresses a polynucleotide that encodes a delta-9 desaturase protein, wherein said delta-9 desaturase protein is the delta-9 desaturase protein of SEQ ID NO:5 or a variant thereof, and wherein said variant has an amino acid sequence having 80% or more sequence identity to SEQ ID NO:5 and has the same functionality as the delta-9 desaturase of SEQ ID NO:5.
14. The canola oil of claim 1 having an oil fraction comprising no more than 2.7% total saturates.
15. A fried food composition comprising potato material and canola oil according to claim 1.
16. A method of producing a fried food composition wherein said method comprises frying potato material in canola oil according to claim 1.

Patentansprüche

1. Rapsöl, erhältlich aus Samen einer Rapspflanze, umfassend ein Polynukleotid, das stabil in ein Genom besagte Samen eingebaut ist, das ein Delta-9-Desaturaseprotein kodiert, wobei besagtes Öl weniger als 3,5% Gesamtgehalt an gesättigten Fettsäuren und 70% bis weniger als 80% Ölsäure umfasst und wobei der besagte Gesamtgehalt die Summe von 16:0, 18:0, 20:0, 22:0 und 24:0 Fettsäuren ist.
2. Rapssamen von einer Rapspflanze, wobei besagte Samen ein Polynukleotid umfassen, das stabil in ein Genom besagter Samen eingebaut ist, das ein Delta-9-Desaturaseprotein kodiert, besagte Samen umfassen das Öl nach Anspruch 1.
3. Samen nach Anspruch 2, umfassend 70% bis 78% Ölsäure.
4. Samen nach Anspruch 2, umfassend nicht mehr als 3% Linolensäure.
5. Samen nach Anspruch 2, umfassend 70% bis 78% Ölsäure und nicht mehr als 3,5% Linolensäure.
6. Samen nach Anspruch 2, umfassend nicht mehr als 2,7% Gesamtgehalt an gesättigten Fettsäuren.
7. Rapspflanze, die Samen produziert, die ein Polynukleotid umfassen, das stabil in ein Genom besagter Samen eingebaut ist, das ein Delta-9-Desaturaseprotein kodiert, die Samen umfassen das Öl nach Anspruch 1.
8. Samen nach Anspruch 2 oder die Rapspflanze nach Anspruch 7, wobei das Öl weniger als 0,3% C20:0 plus C22:0 plus C24:0 umfasst.
9. Samen nach Anspruch 2 oder Rapspflanze nach Anspruch 7, wobei das Öl einen Gehalt an mehrfach ungesättigten Fettsäuren von weniger als 20%, vorzugsweise weniger als 15%, vorzugsweise weniger als 10% oder vorzugsweise weniger als 9% umfasst.
10. Rapspflanze nach Anspruch 7, wobei besagtes Öl nicht mehr als 2,7% Gesamtgehalt an gesättigten Fettsäuren umfasst.

11. Rapspflanze nach Anspruch 7, wobei die Rapspflanze mindestens 100 cm hoch ist und ein durchschnittliches Samengewicht über 3 mg aufweist.
12. Verfahren zur Reduzierung von gesättigtem Fett in der Ölfraction von Samen einer transgenen Rapspflanze um mindestens 43% im Vergleich zu einer Wildtyp-Ölfraction von Samen einer entsprechenden Wildtyp-Rapspflanze, wobei besagtes Verfahren das Herstellen einer transgenen Raps-Pflanze umfasst, die ein Polynukleotid exprimiert, das ein Delta-9-Desaturaseprotein codiert, wobei das vollständige Komplement eines Nukleinsäuremoleküls, das besagtes Protein codiert, mit dem Nukleinsäuremolekül der SEQ ID NO: 1 unter stringenten Bedingungen hybridisiert.
13. Verfahren zur Reduzierung von gesättigtem Fett in der Ölfraction von Samen einer transgenen Rapspflanze um mindestens 43% im Vergleich zu einer Wildtyp-Ölfraction von Samen einer entsprechenden Wildtyp-Rapspflanze, wobei besagtes Verfahren das Herstellen einer transgenen Raps-Pflanze umfasst, die ein Polynukleotid exprimiert, das ein Delta-9-Desaturaseprotein codiert, wobei besagtes Delta-9-Desaturaseprotein das Delta-9-Desaturaseprotein der SEQ ID NO: 5 oder eine Variante davon ist und wobei die Variante eine Aminosäuresequenz aufweist, die 80% oder mehr Sequenzidentität zu SEQ ID NO: 5 und die gleiche Funktionalität wie die Delta-9-Desaturase von SEQ ID NO: 5 hat.
14. Rapsöl nach Anspruch 1 mit einer Ölfraction, die nicht mehr als 2,7% Gesamtgehalt an gesättigten Fettsäuren umfasst.
15. Gebratene Nahrungsmittelzusammensetzung, umfassend Kartoffelmateriel und Rapsöl nach Anspruch 1.
16. Verfahren zur Herstellung einer gebratenen Nahrungsmittelzusammensetzung, wobei besagtes Verfahren das Braten von Kartoffelmateriel in Rapsöl nach Anspruch 1 umfasst.

Revendications

1. Huile de canola pouvant être obtenue à partir de graines d'une plante de canola comprenant un polynucléotide, incorporé de manière stable dans un génome desdites graines, qui code pour une protéine delta-9 désaturase, tandis que ladite huile comprend moins de 3,5% de composants saturés totaux et de 70% à moins de 80% d'acide oléique et tandis que lesdits composants saturés totaux sont la somme de 16:0, 18:0, 20:0, 22:0 et 24:0 acides gras.
2. Graines de canola provenant d'une plante de canola, dans lesquelles lesdites graines comprennent un polynucléotide, incorporé de manière stable dans un génome desdites graines, qui code pour une protéine delta-9 désaturase, lesdites graines comprenant une huile selon la revendication 1, tandis que ladite protéine delta-9 désaturase est la protéine delta-9 désaturase selon SEQ ID NO: 5 ou une variante de celle-ci, et tandis que ladite variante a une séquence d'acides aminés ayant 80% ou plus d'identité de séquences avec SEQ ID NO: 5 et possède la même fonctionnalité que la delta-9 désaturase selon SEQ ID NO: 5.
3. Graines selon la revendication 2 comprenant 70% à 78% d'acide oléique.
4. Graines selon la revendication 2 ne comprenant pas plus de 3% d'acide linoléique.
5. Graines selon la revendication 2 comprenant 70% à 78% d'acide oléique et pas plus de 3,5% d'acide linoléique.
6. Graines selon la revendication 2 ne comprenant pas plus de 2,7% de composants saturés totaux.
7. Plante de canola qui produit des graines comprenant un polynucléotide, incorporé de manière stable dans un génome desdites graines, qui code pour une protéine delta-9 désaturase, les graines comprenant une huile selon la revendication 1, et tandis que ladite protéine delta-9 désaturase est la protéine delta-9 désaturase selon SEQ ID NO: 5 ou une variante de celle-ci, et tandis que ladite variante a une séquence d'acides aminés ayant 80% ou plus d'identité de séquences vis-à-vis de SEQ ID NO: 5 et a la même fonctionnalité que la delta-9 désaturase selon SEQ ID NO: 5.
8. Graines selon la revendication 2 ou plante de canola selon la revendication 7 dans lesquelles l'huile comprend moins de 0,3% de C20:0 plus C22:0 plus C24:0.

EP 2 338 328 B1

9. Graines selon la revendication 2 ou plante de canola selon la revendication 7 dans lesquelles l'huile comprend une teneur en acide gras polyinsaturé inférieure à 20%, de préférence inférieure à 15%, de préférence inférieure à 10%, ou de préférence inférieure à 9%.

10. Plante de canola selon la revendication 7 dans laquelle ladite huile n'a pas plus de 2,7% de composants saturés totaux.

11. Plante de canola selon la revendication 7 dans laquelle ladite plante de canola a au moins 100 cm de hauteur avec un poids moyen de graines supérieur à 3 mg.

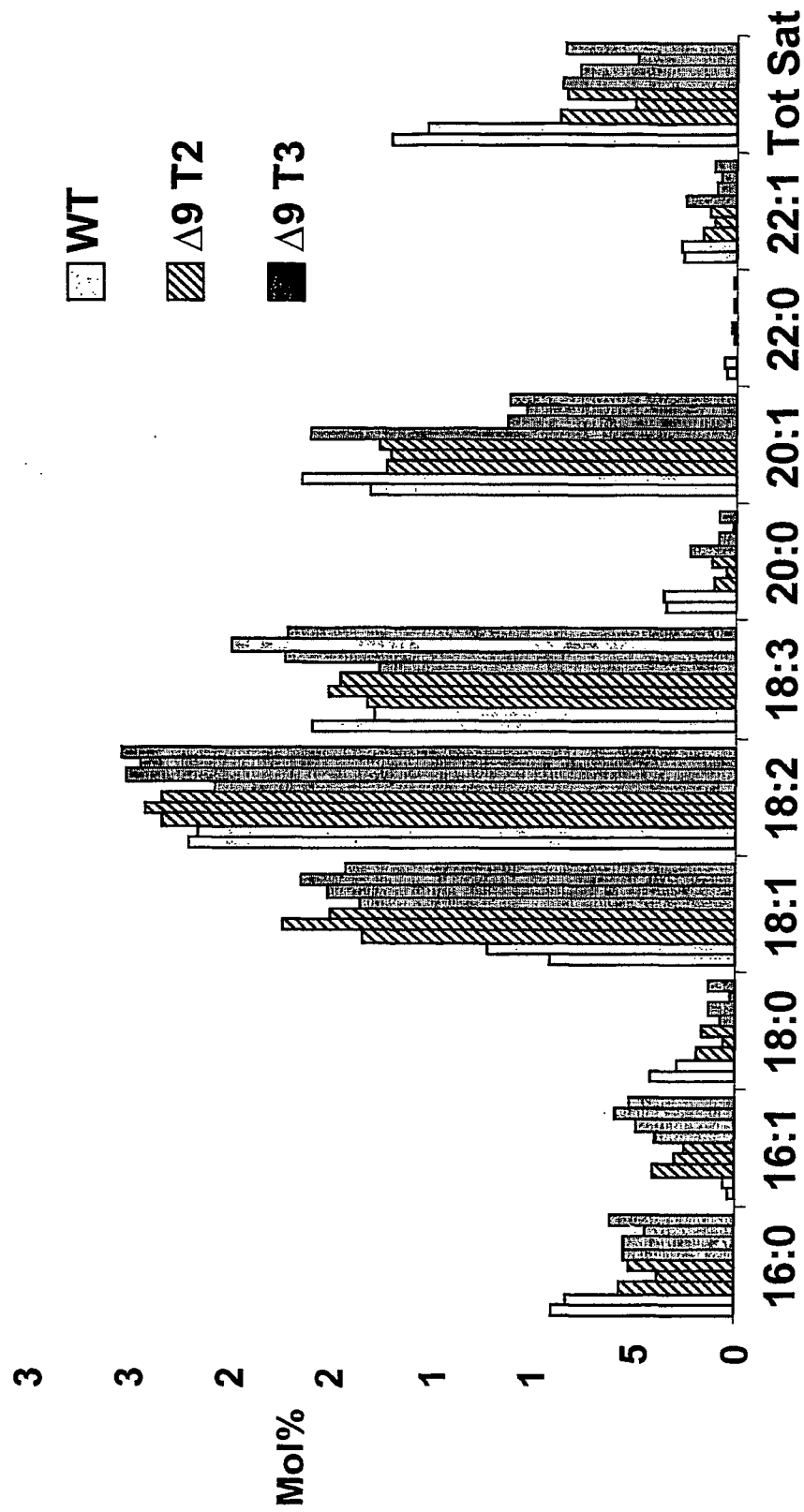
12. Procédé pour la réduction de la graisse saturée dans la fraction huile de graines d'une plante de canola transgénique d'au moins 43%, par comparaison avec une fraction huile de type sauvage de graines d'une plante de canola de type sauvage correspondante, tandis que ledit procédé comprend la production d'une plante de canola transgénique qui exprime un polynucléotide qui code pour une protéine delta-9 désaturase, tandis que le complément total d'une molécule d'acide nucléique qui code pour ladite protéine hybride avec la molécule d'acide nucléique selon SEQ ID NO: 1 dans des conditions stringentes.

13. Procédé pour la réduction de la graisse saturée dans la fraction huile de graines d'une plante de canola transgénique d'au moins 43%, par comparaison avec une fraction huile de type sauvage de graines d'une plante de canola de type sauvage correspondante, tandis que ledit procédé comprend la production d'une plante de canola transgénique qui exprime un polynucléotide qui code pour une protéine delta-9 désaturase, tandis que ladite protéine delta-9 désaturase est la protéine delta-9 désaturase selon SEQ ID NO: 5 ou une variante de celle-ci, et tandis que ladite variante a une séquence d'acides aminés ayant 80% ou plus d'identité de séquences vis-à-vis de SEQ ID NO: 5 et a la même fonctionnalité que la delta-9 désaturase selon SEQ ID NO: 5.

14. Huile de canola selon la revendication 1 ayant une fraction huile ne comprenant pas plus de 2,7% de composants saturés totaux.

15. Composition alimentaire frite comprenant une matière de pomme de terre et de l'huile de canola selon la revendication 1.

16. Procédé pour la production d'une composition alimentaire frite dans lequel ledit procédé comprend la friture d'une matière de pomme de terre dans de l'huile de canola selon la revendication 1.



> 60% reduction of saturated fatty acids.
 → In best case more 16:1 (5.9%) than 16:0 (4.4%).

FIG. 1

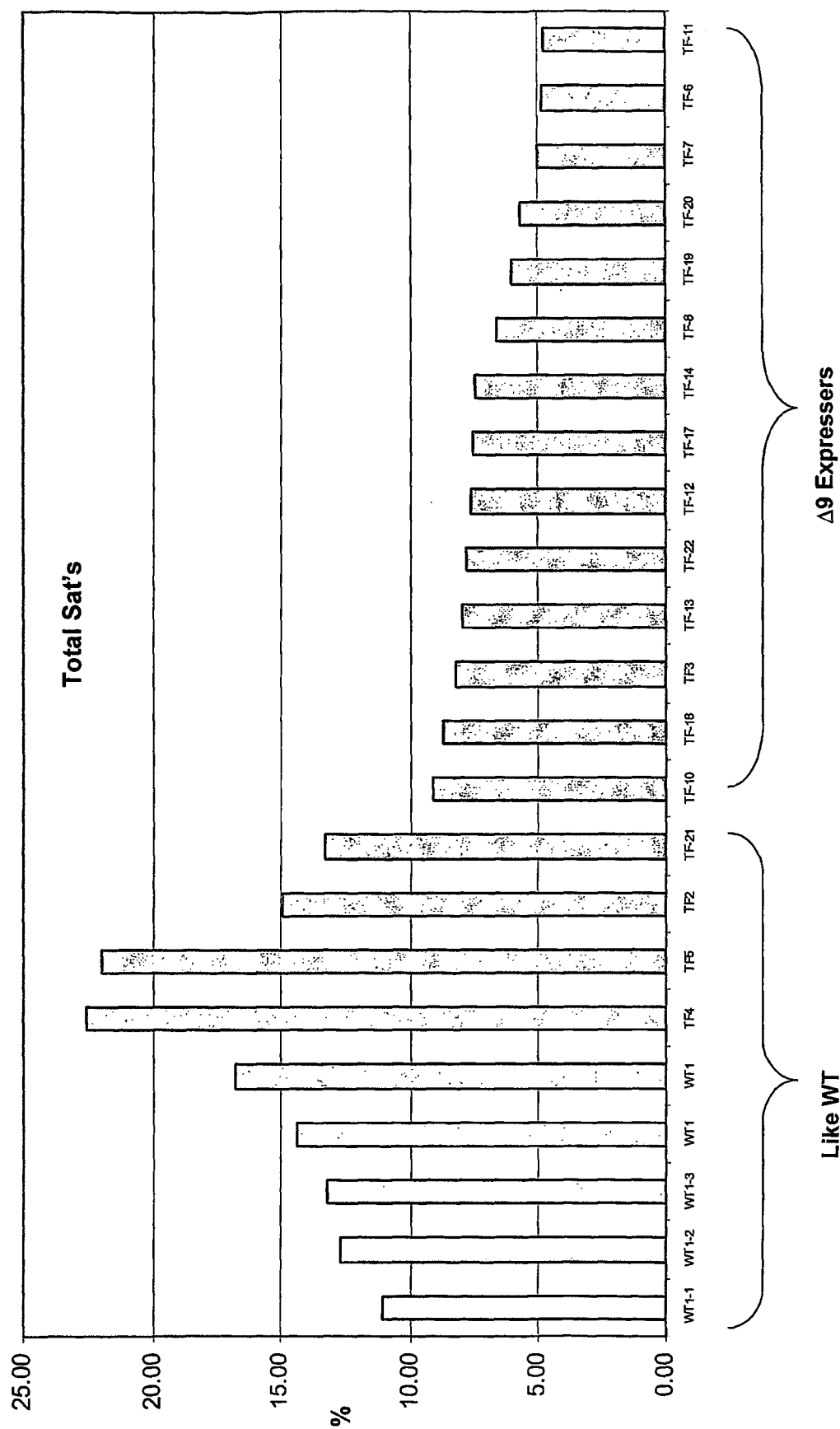
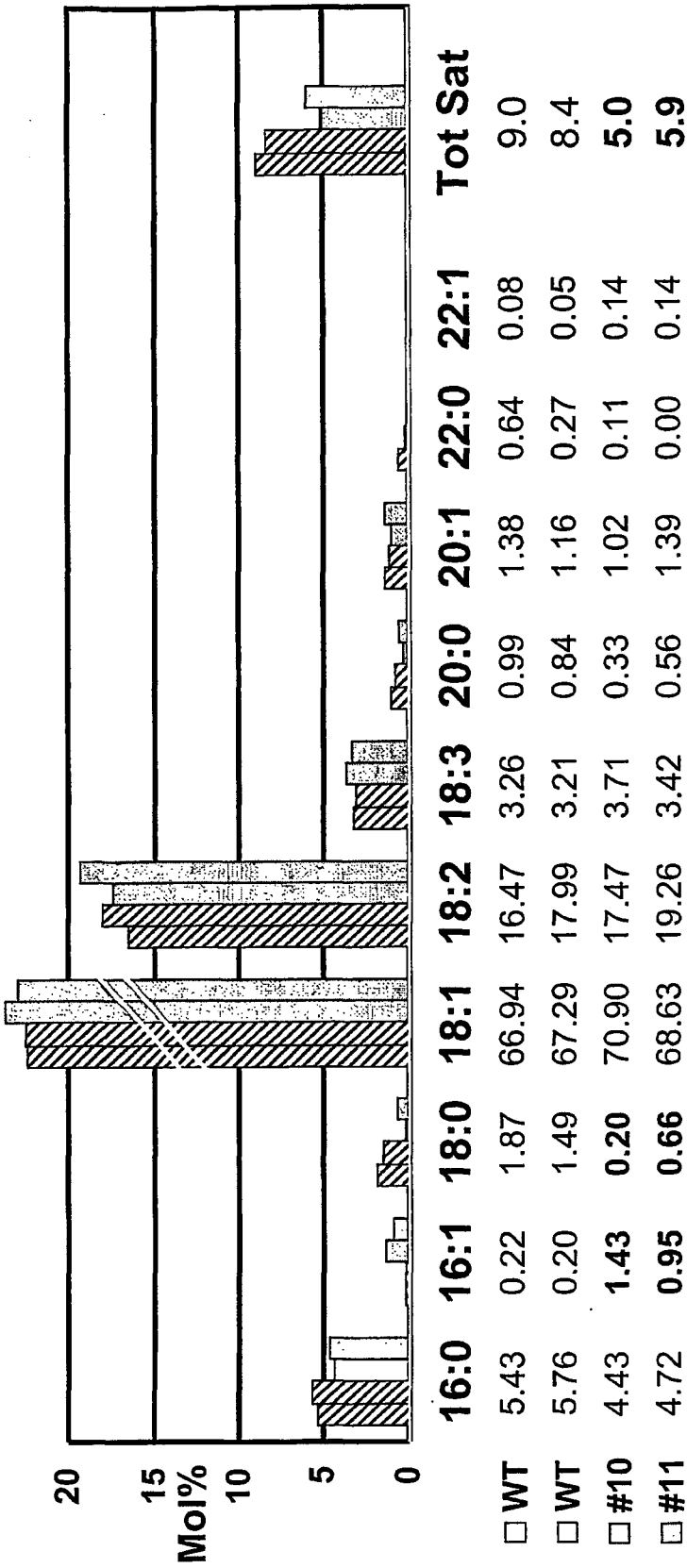


FIG. 2

- Three transgenic plants from one callus, all Southern positive, Southern pattern indicates one event.
- FAME analysis on single seeds of T1 generation ($\Rightarrow$ T2 in Arabidopsis).



In best case 43% reduction in Sats (50% when 24:0 included)

FIG. 3

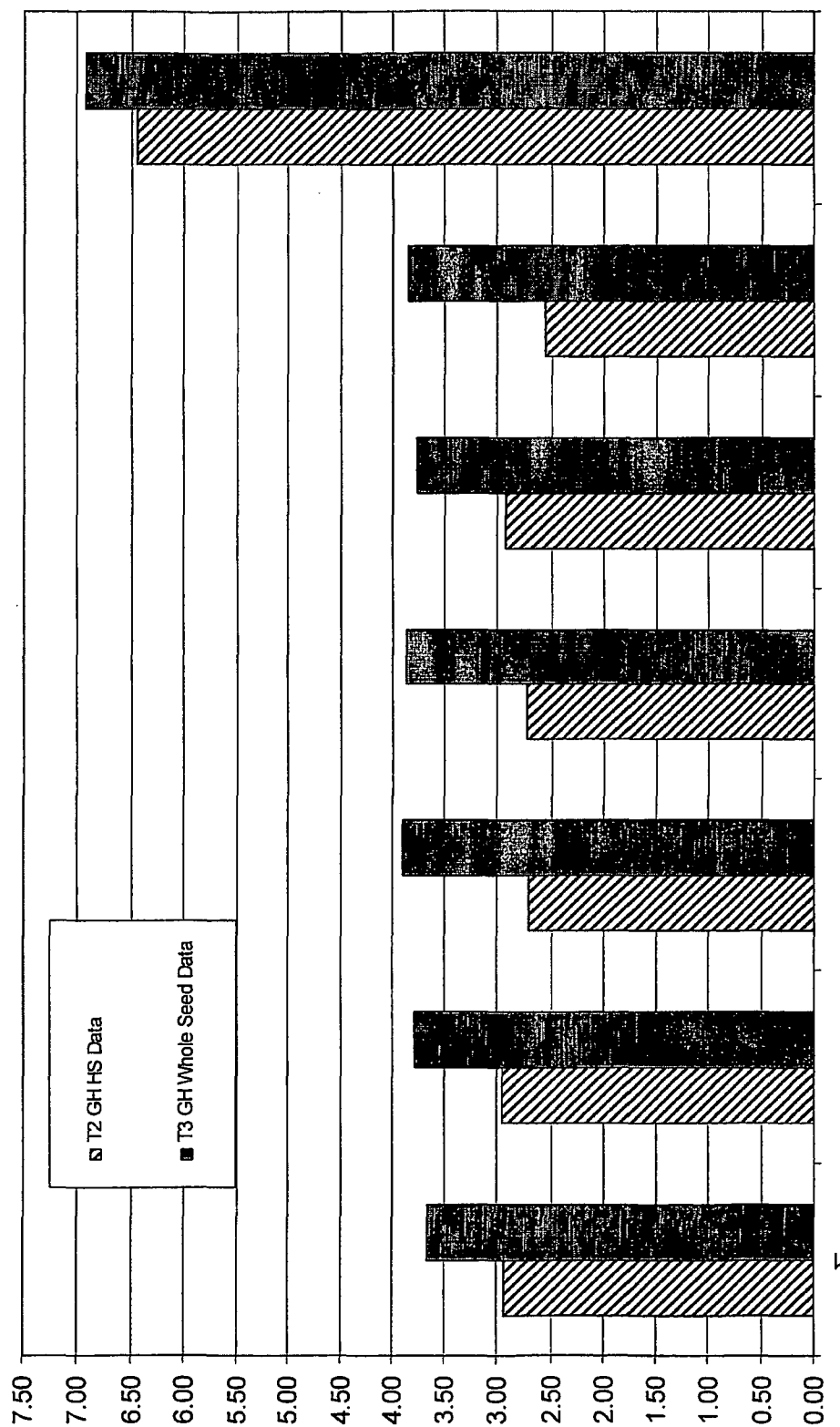


FIG. 4A

36-11.19 T3 Fatty Acid Data														
C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C22:0	C22:1	C24:0	C24:1	TOTSAT
0.05	2.52	1.83	0.59	80.27	9.71	2.51	0.32	0.90	0.04	0.15	nd	0.04	0.27	3.66
0.05	2.56	1.86	0.61	79.73	10.31	2.43	0.34	0.92	0.06	0.16	0.05	0.07	0.07	3.78
0.06	2.72	1.93	0.58	77.72	12.07	2.61	0.33	0.90	0.05	0.15	nd	0.06	0.10	3.89
0.05	2.66	2.00	0.56	77.65	11.80	2.61	0.33	0.91	0.04	0.16	nd	0.11	0.20	3.87
0.05	2.65	1.79	0.56	78.27	11.67	2.68	0.30	0.81	0.04	0.14	nd	0.07	0.23	3.76
0.06	2.56	1.85	0.63	78.48	11.42	2.48	0.35	0.94	0.05	0.16	nd	0.09	0.17	3.85
0.06	4.16	0.34	1.45	73.03	14.87	2.72	0.64	1.39	0.07	0.42	nd	0.21	0.18	6.93

FIG. 4B

36-11.19 T2 Fatty Acid Data					
C16:0	C16:1	C18:0	C18:1	C18:2	TOTSAT
2.16	1.68	0.36	79.63	11.99	2.82
2.16	1.69	0.40	78.95	12.72	2.73
1.98	1.85	0.36	82.20	9.73	2.58
1.95	1.80	0.34	79.21	12.08	3.17
2.15	1.74	0.36	80.66	11.35	2.45
1.96	1.80	0.28	77.36	13.73	3.62
nd	0.06	4.13	68.82	18.42	4.57
					6.44

FIG. 4C

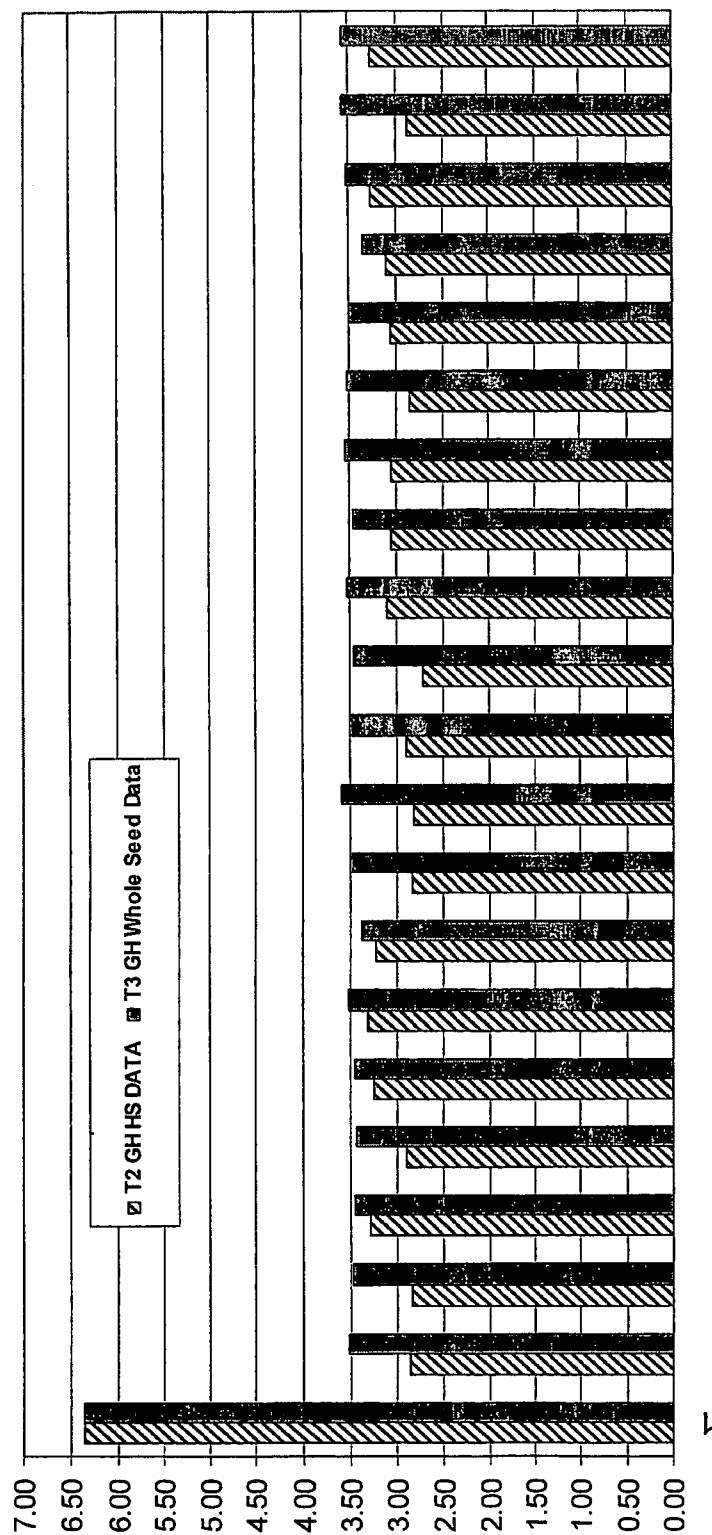


FIG. 5A

Seed Amount	218-11.30 T3 Fatty Acid				218-11.30 T3 Fatty Acid Data-10 seed Bulk																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
	C14:0	Data-10 seed Bulk			C16:1	C18:0C18:1C18:2C18:3C20:0C20:1C20:2C22:0C22:1C24:0C24:1TOT SAT																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											

FIG. 5B

218-11.30 T2 Fatty Acid Data-HS data

C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C22:0	C24:0	C24:1	TOTSAT
0.04	3.92	0.18	1.32	78.50	10.76	2.77	0.62	1.27	0.06	0.32	0.14	0.10	6.36
0.06	2.19	1.93	0.32	81.51	10.01	2.92	0.19	0.70	0.07	0.07	0.03	0.01	2.85
0.07	2.24	2.07	0.28	80.02	10.79	3.45	0.19	0.73	0.08	0.06	nd	0.03	2.84
0.05	2.44	1.80	0.44	82.08	9.32	2.57	0.23	0.82	0.05	0.10	0.04	0.05	3.30
0.06	2.21	1.97	0.33	83.22	8.46	2.63	0.19	0.76	0.06	0.08	0.03	0.01	2.91
0.05	2.27	2.09	0.50	83.32	8.01	2.50	0.27	0.71	0.05	0.12	0.04	0.07	3.24
0.05	2.43	1.93	0.47	79.71	11.02	3.24	0.24	0.69	0.04	0.08	0.04	0.06	3.31
0.05	2.34	1.67	0.44	80.81	10.33	3.09	0.26	0.77	0.05	0.12	0.03	0.05	3.23
0.05	2.25	1.94	0.28	79.50	11.57	3.37	0.20	0.71	0.04	0.03	0.02	0.02	2.84
0.05	2.24	1.91	0.26	77.77	12.80	3.94	0.21	0.71	0.05	0.04	0.02	0.01	2.82
0.05	2.26	1.95	0.34	78.65	12.13	3.52	0.11	0.76	0.06	0.12	0.03	0.02	2.91
0.05	2.03	1.94	0.34	79.83	11.69	2.82	0.16	0.86	0.07	0.08	0.04	0.08	2.71
0.05	2.29	1.77	0.38	81.32	9.85	3.01	0.23	0.77	0.04	0.09	0.08	0.13	3.10
0.05	2.36	1.88	0.33	81.69	9.51	2.99	0.23	0.76	0.05	0.08	0.02	0.05	3.08
0.06	2.37	2.06	0.34	80.82	10.19	3.02	0.22	0.73	0.05	0.08	nd	0.06	3.07
0.06	2.16	2.01	0.36	80.30	10.81	3.14	0.14	0.73	0.06	0.09	0.06	0.07	2.86
0.06	2.31	2.00	0.32	78.80	12.47	2.82	0.22	0.74	0.05	0.07	0.10	0.06	3.07
0.04	2.16	2.01	0.47	81.75	9.76	2.44	0.28	0.79	0.05	0.13	0.03	0.10	3.10
0.05	2.40	1.64	0.44	79.87	11.47	2.95	0.22	0.72	0.05	0.13	0.03	0.02	3.26
0.05	2.14	2.00	0.35	79.75	11.10	3.42	0.20	0.74	0.08	0.11	0.02	0.04	2.88
0.04	2.33	1.77	0.51	81.86	9.66	2.63	0.26	0.74	0.04	0.11	0.03	0.03	3.28

FIG. 5C

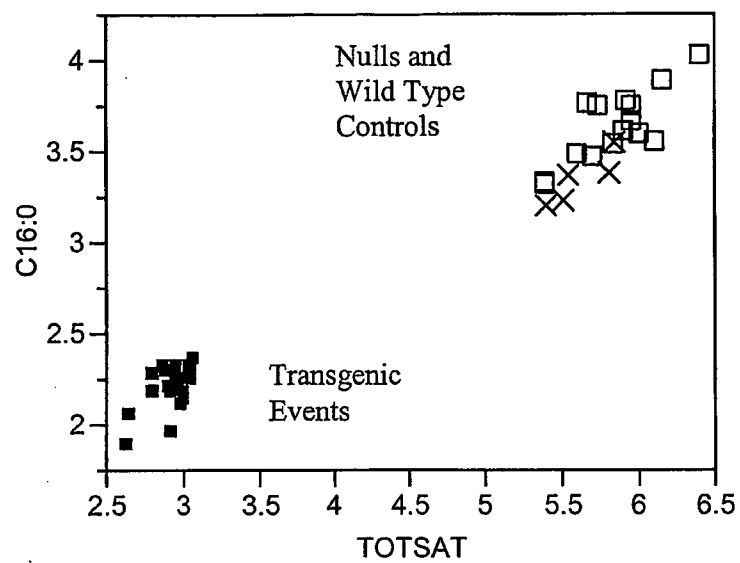


FIG. 6A

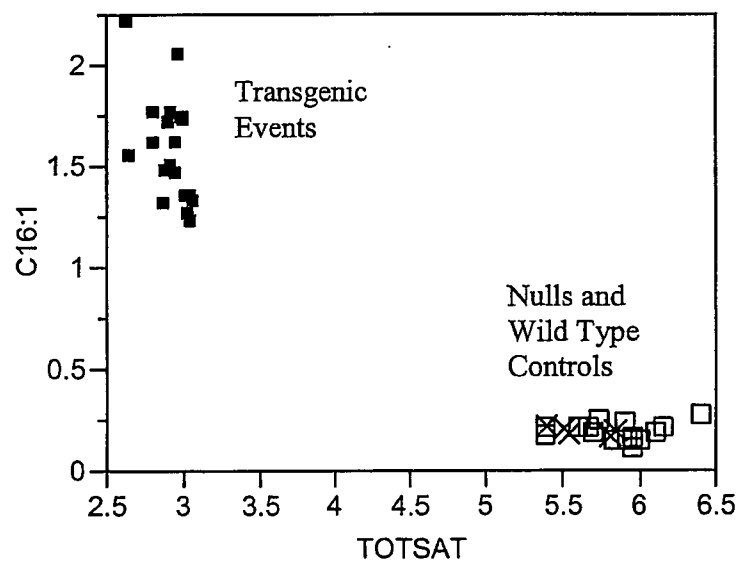


FIG. 6B

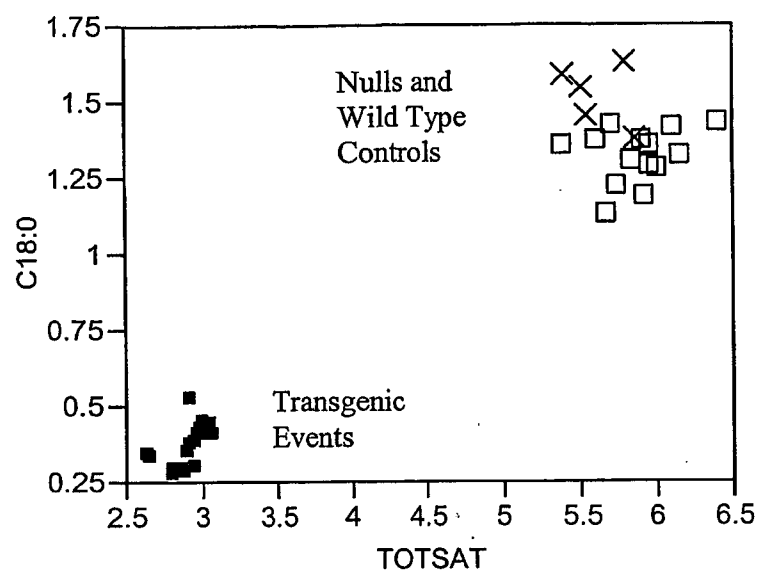


FIG. 6C

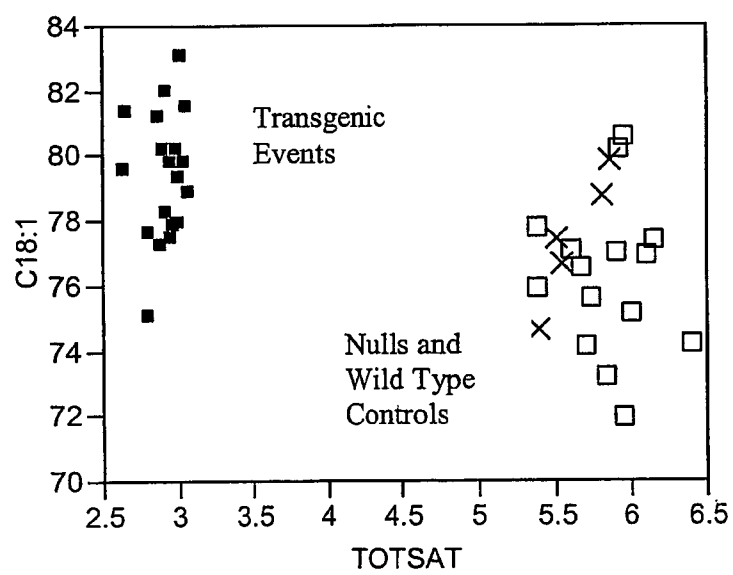


FIG. 6D

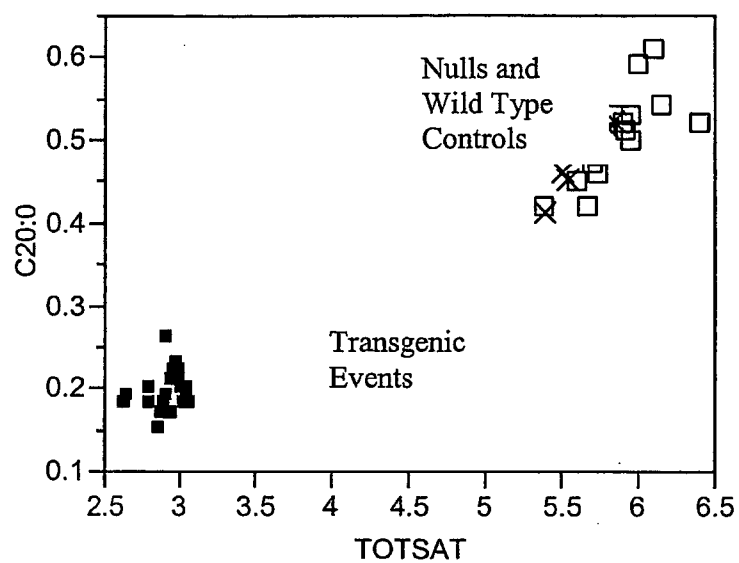


FIG. 6E

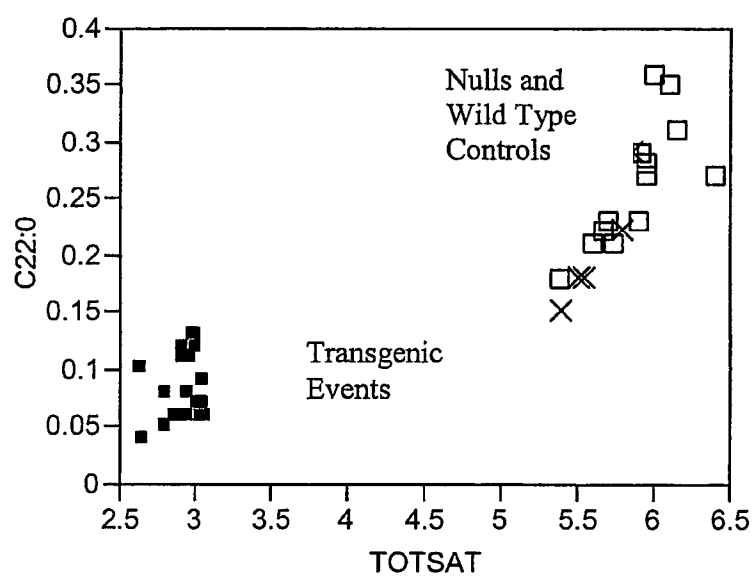


FIG. 6F

**Distributions
C16:0 % Total Fatty Acids**

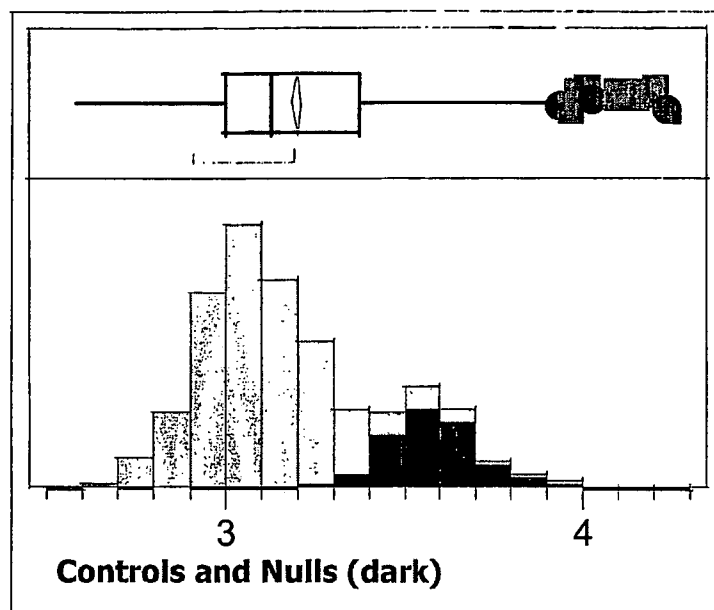


FIG. 6G

**Distributions
C16:1% Total Fatty Acids**

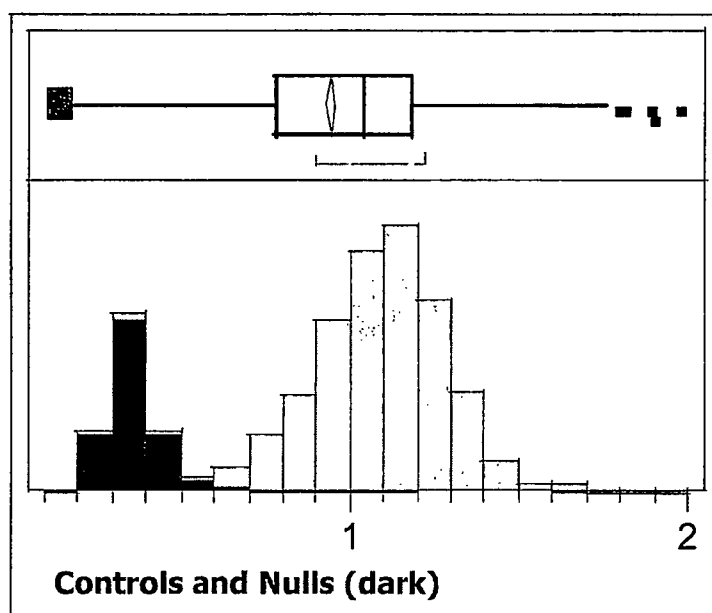


FIG. 6H

Distributions
C18:0 % Total Fatty Acids

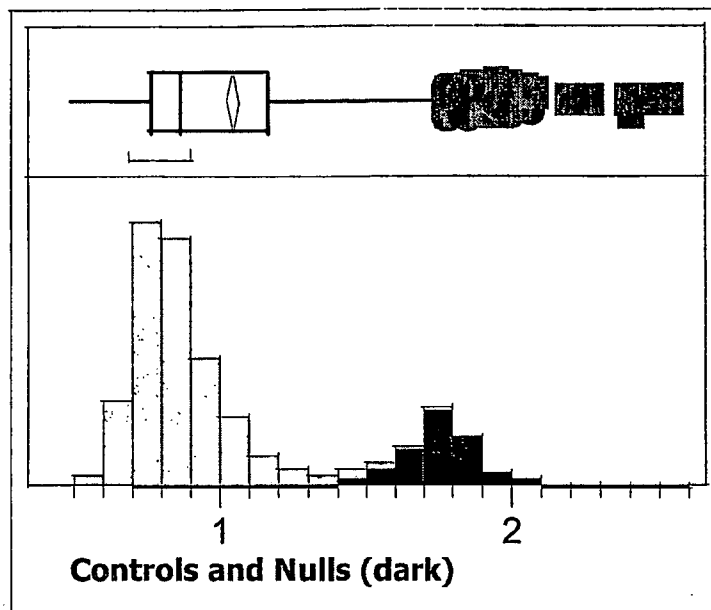


FIG. 6I

Distributions
C18:1 % Total Fatty Acids

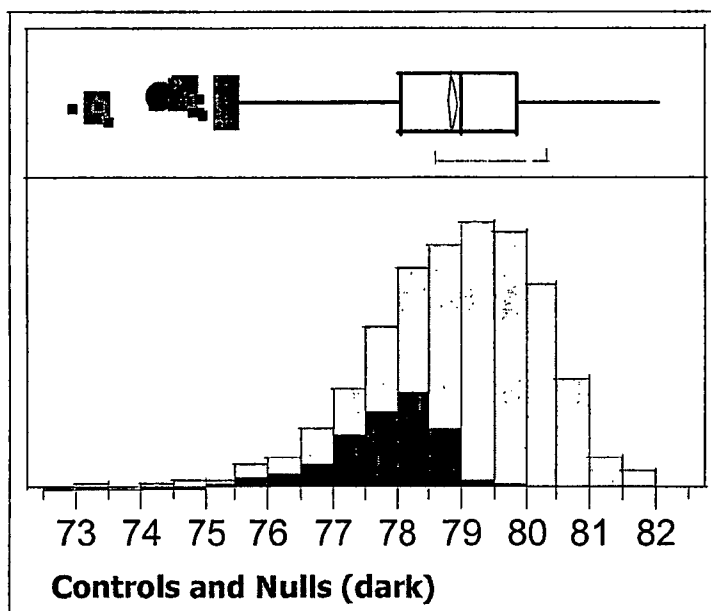


FIG. 6J

Distributions
C18:2 % Total Fatty Acids

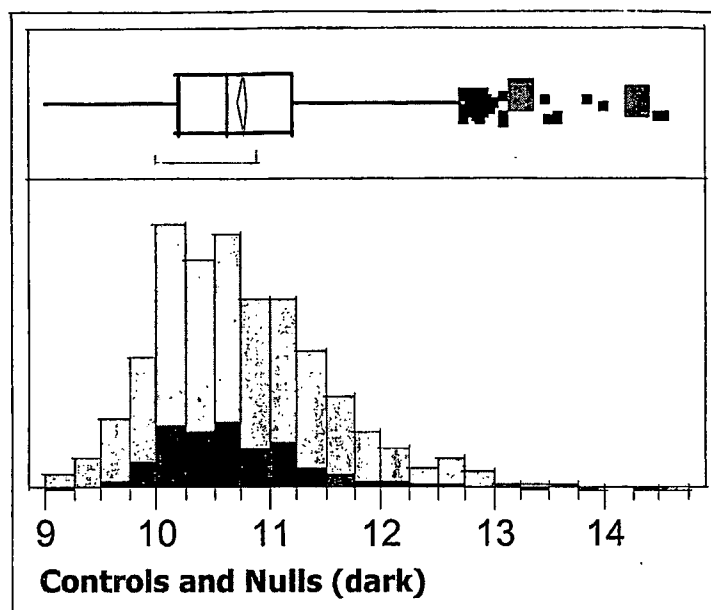


FIG. 6K

Distributions
C18:3 % Total Fatty Acids

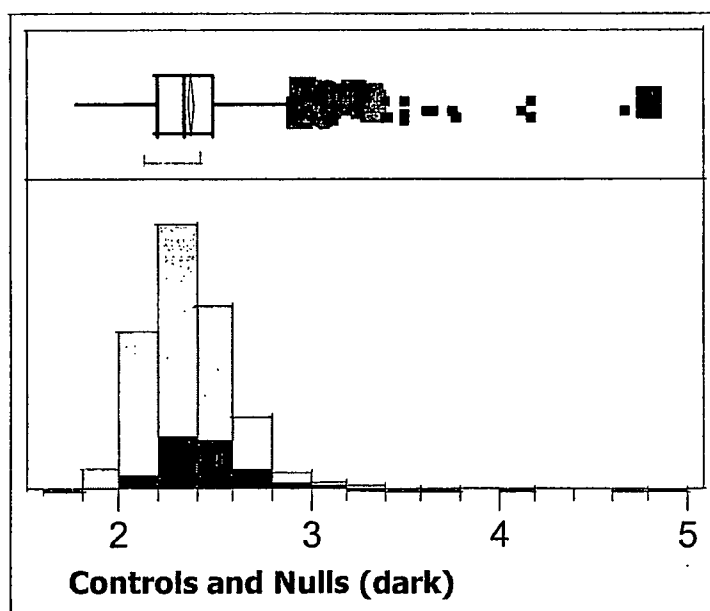


FIG. 6L

Distributions
% Total Saturated Fatty Acids

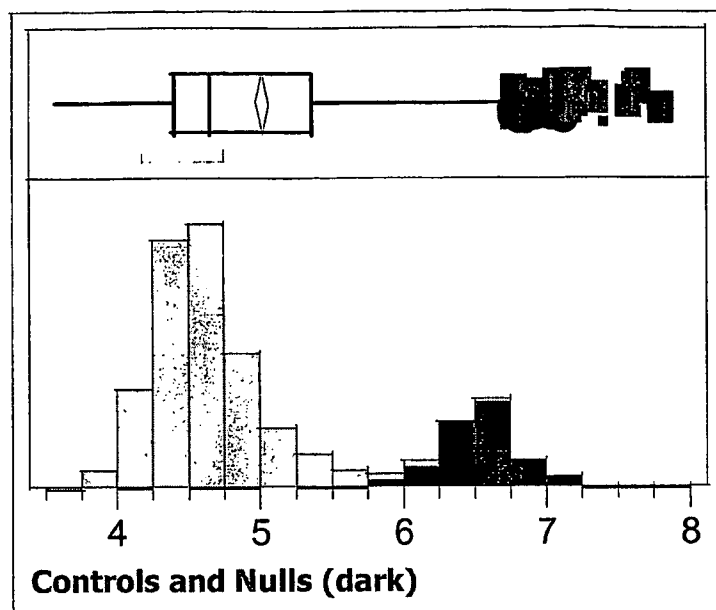


FIG. 6M

Distributions 1000 Seed Wt

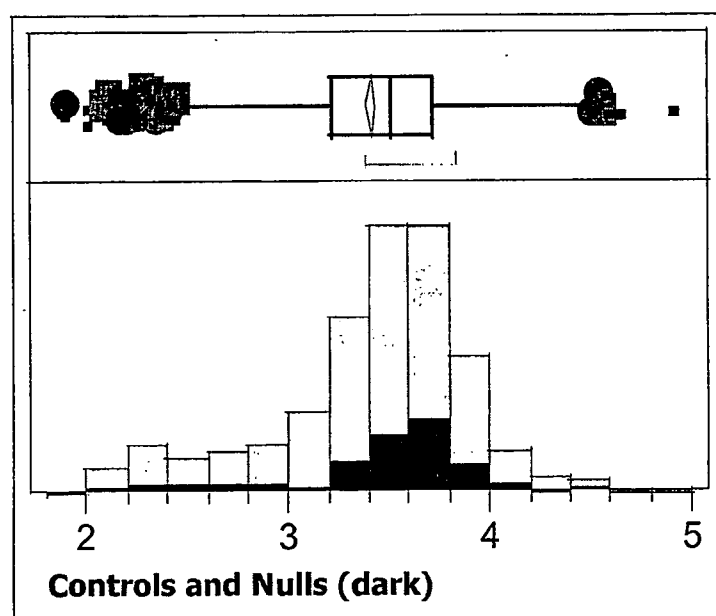


FIG. 6N

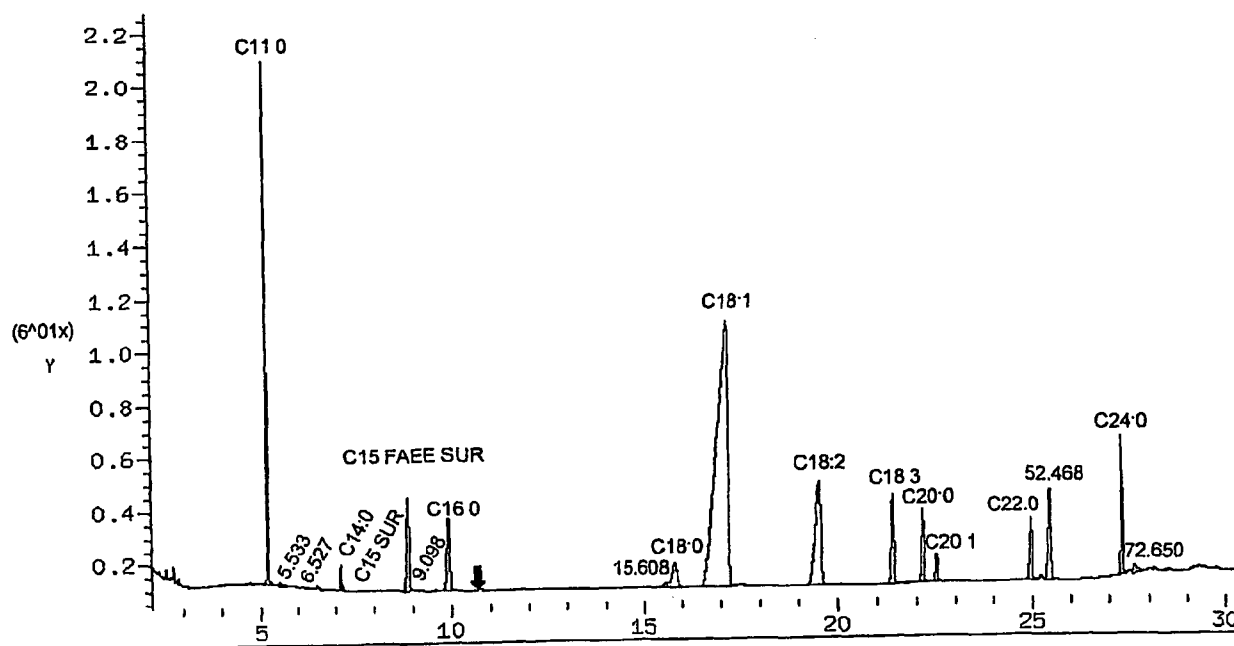


FIG. 7A

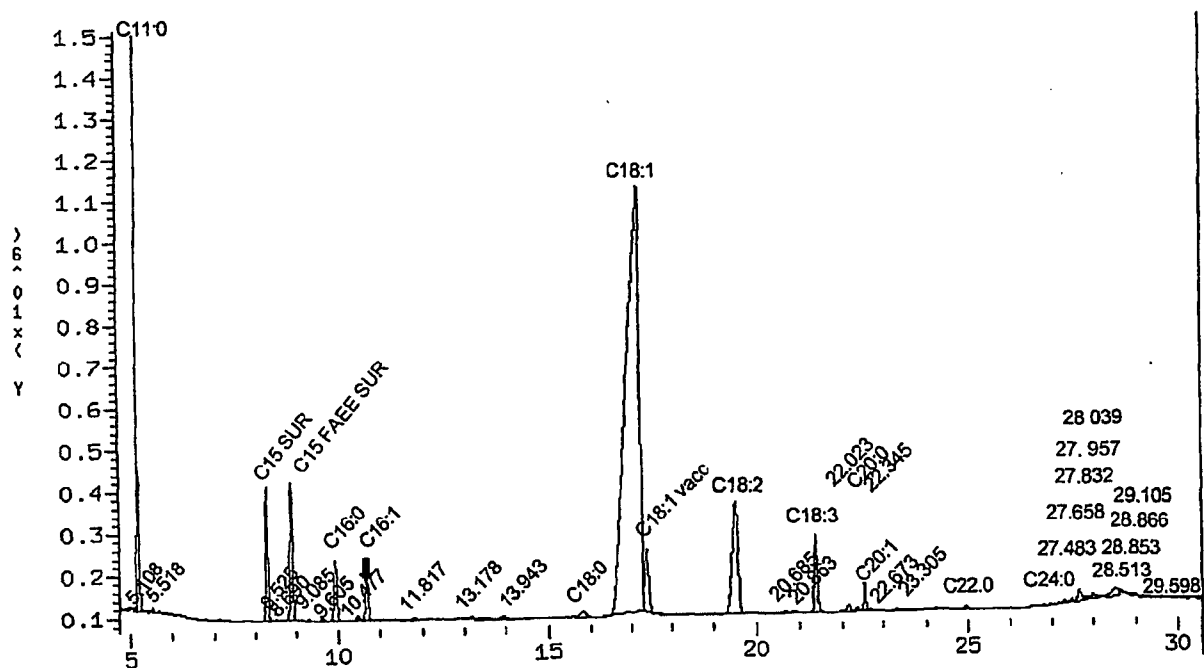


FIG. 7B

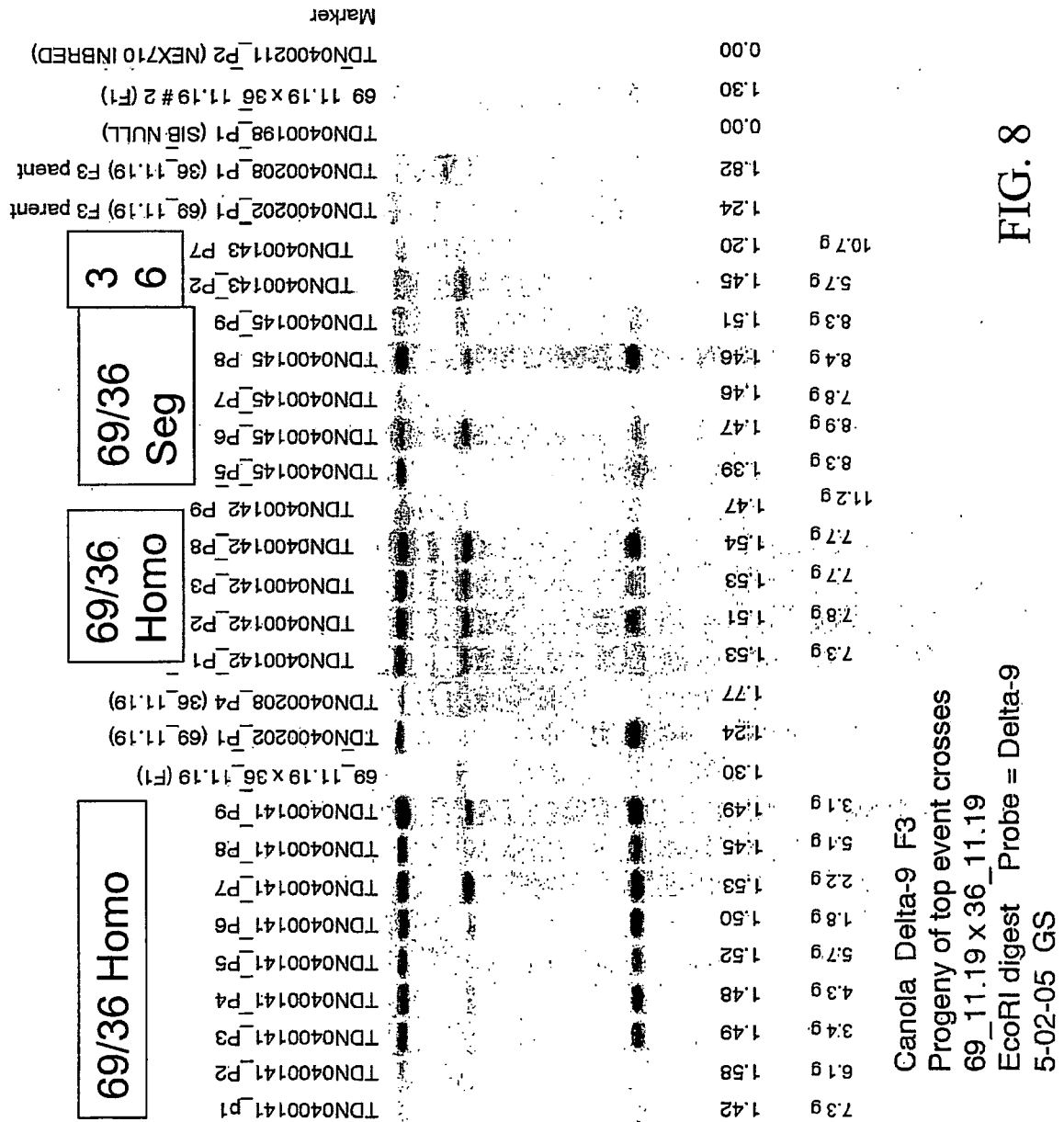
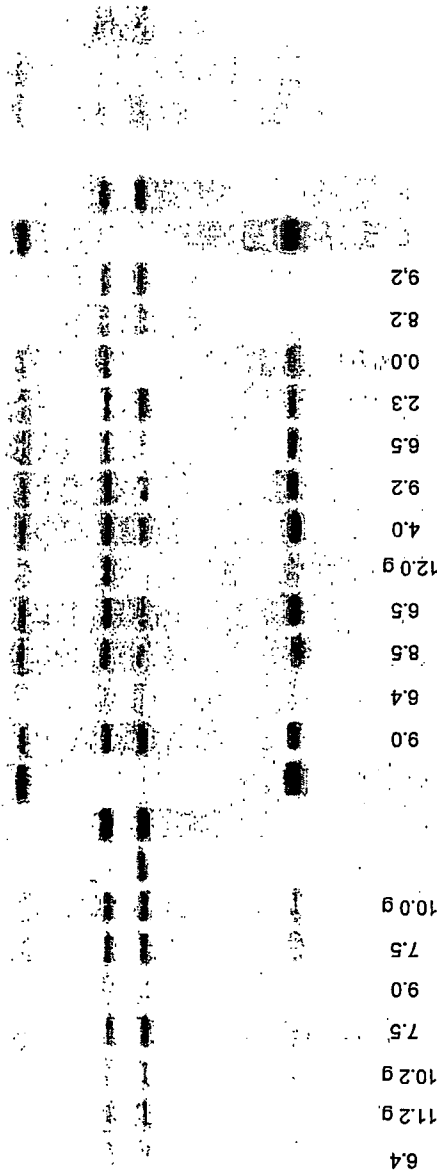
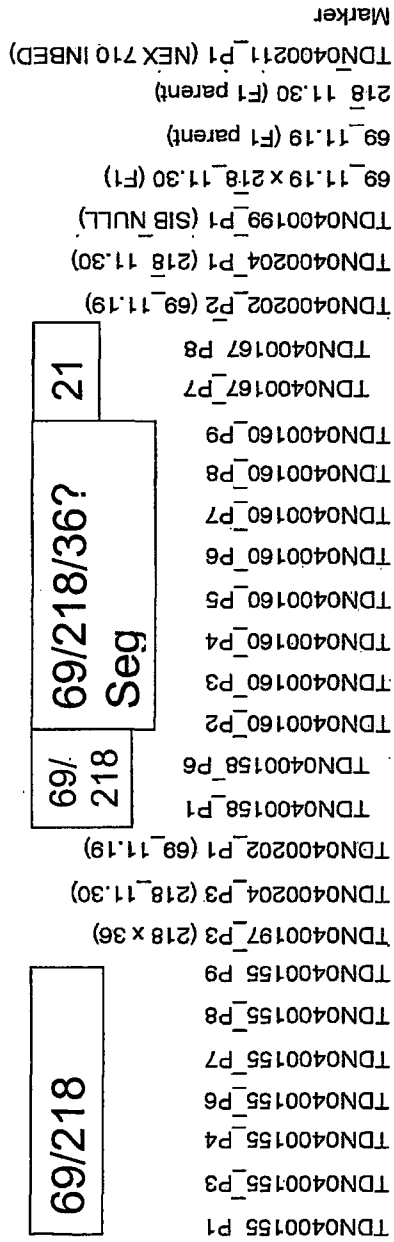


FIG. 8



Canola Delta-9 (F3)
Progeny of Top event crosses
69_11.19 x 218_11.30
EcoRI digest Probe = Delta9
5-2-05 GS

FIG. 9

REFERENCES CITED IN THE DESCRIPTION

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SZABADALMI IGÉNYPONTOK

1. Repceolaj, amely előállítható egy repce növény magjaiból, amely említett magok, a genomba stabilan beépülve tartalmazznak egy polinukleotidot, amely egy delta-9 deszaturáz proteint kódol, ahol az említett olaj kevesebb mint 3,5% összes telített zsírsavat és 70% - kevesebb mint 80% olajsavat tartalmaz, és ahol az említett összes telített zsírsav 16:0, 18:0, 20:0, 22:0 és 24:0 zsírsavak összessége.

2. Repce magok egy repce növényből, ahol az említett magok, a genomba stabilan beépülve tartalmazznak egy polinukleotidot, amely egy delta-9 deszaturáz proteint kódol, ahol az említett magok tartalmazzák az 1. igénypont szerinti olajat, ahol az említett delta-9 deszaturáz protein SEQ ID NO: 5 szerinti delta-9 deszaturáz protein vagy ennek variánsa, és ahol az említett variáns aminosav szekvenciája 80% vagy ennél nagyobb mértékben azonos a SEQ ID NO: 5 szekvenciával, és azonos funkcionálitással rendelkezik, mint a SEQ ID NO: 5 szerinti delta-9 deszaturáz.

3. A 2. igénypont szerinti magok, amelyek 70% - 78% olajsavat tartalmazznak.

4. A 2. igénypont szerinti magok, amelyek nem több mint 3% linolénsavat tartalmazznak.

5. A 2. igénypont szerinti magok, amelyek 70% - 78% olajsavat és nem több mint 3,5% linolénsavat tartalmazznak.

6. A 2. igénypont szerinti magok, amelyek nem több mint 2,7% összes telített zsírsavat tartalmazznak.

7. Repce növény, amely magokat termeszt, amelyek a genomba stabilan beépülve tartalmazznak egy polinukleotidot, amely egy delta-9 deszaturáz proteint kódol, ahol az említett magok tartalmazzák az 1. igénypont szerinti olajat, és ahol az említett delta-9 deszaturáz protein SEQ ID NO: 5 szerinti delta-9 deszaturáz protein vagy ennek variánsa, és ahol az említett variáns aminosav szekvenciája 80% vagy ennél nagyobb mértékben azonos a SEQ ID NO: 5 szekvenciával, és azonos funkcionálitással rendelkezik, mint a SEQ ID NO: 5 szerinti delta-9 deszaturáz.

8. A 2. igénypont szerinti magok vagy a 7. igénypont szerinti repce növény, ahol az olaj kevesebb mint 0,3% C20:0 plusz C22:0 plusz C24:0 zsírsavat tartalmaz.

9. A 2. igénypont szerinti magok vagy a 7. igénypont szerinti repce növény, ahol az olaj politelítetlen zsírsav tartalma kevesebb mint 20%, előnyösen kevesebb mint 15%, előnyösen kevesebb mint 10% vagy előnyösen kevesebb mint 9%.

10. A 7. igénypont szerinti repce növény, ahol az említett olaj nem több mint 2,7% összes telített zsírsavat tartalmaz.

11. A 7. igénypont szerinti repce növény, ahol az említett repce növény legalább 100 cm magas és a magok átlagos tömege 3 mg feletti.

12. Eljárás telített zsírsav csökkentésére transzgén repce növény magjai olaj frakciójában legalább 43% mértékben, megfelelő vad típusú repce növény magjai vad típusú olaj frakciójához viszonyítva, ahol az említett eljárás tartalmazza transzgén repce növény előállítását, amely delta-9 deszaturáz proteint kódoló polinukleotidot expresszál, ahol az említett proteint kódoló nukleinsav molekula teljes komplementere szigorú körülmények között hibridizál a SEQ ID NO: 1 szerinti nukleinsav molekulával.

13. Eljárás telített zsírsav csökkentésére transzgén repce növény magjai olaj frakciójában legalább 43% mértékben, megfelelő vad típusú repce növény magjai vad típusú olaj frakciójához viszonyítva, ahol az említett eljárás tartalmazza transzgén repce növény előállítását, amely delta-9 deszaturáz proteint kódoló polinukleotidot expresszál, ahol az említett delta-9 deszaturáz protein SEQ ID NO: 5 szerinti delta-9 deszaturáz protein vagy ennek variánsa, és ahol az említett variáns aminosav szekvenciája 80% vagy ennél nagyobb mértékben azonos a SEQ ID NO: 5 szekvenciával, és azonos funkcionalitással rendelkezik, mint a SEQ ID NO: 5 szerinti delta-9 deszaturáz.

14. Az 1. igénypont szerinti repceolaj, amely tartalmaz egy olaj frakciót, amely nem több mint 2,7% összes telített zsírsavat tartalmaz.

15. Sült élelmiszer készítmény, amely tartalmaz burgonya anyagot és az 1. igénypont szerinti repceolajat.

16. Eljárás sült élelmiszer készítmény előállítására, ahol az említett eljárás tartalmazza burgonya anyagnak az 1. igénypont szerinti repceolajban történő sütését.