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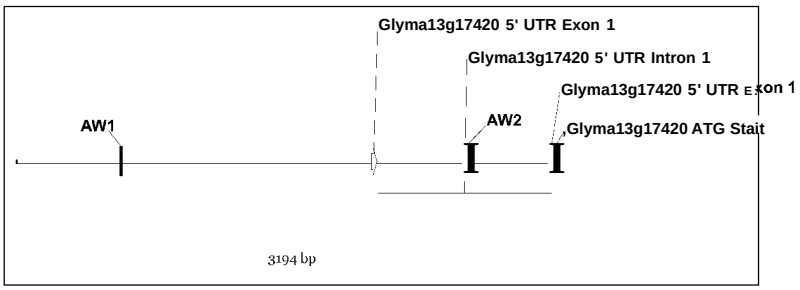
(54) Title: USE OF THE SOYBEAN SUCROSE SYNTHASE PROMOTER TO INCREASE PLANT SEED LIPID CONTENT

FIG. 1



glymal3g I7420 promoter for BB1927

(57) Abstract: Recombinant DNA constructs comprising the soybean sucrose synthase promoter operably linked to polynucleotides encoding transcription factors such as ODPl, Lecl and FUSCA3 are disclosed. These constructs are used for increasing oil content while maintaining normal germination in oilseed plants. Methods to increase oil content in the seeds of an oilseed plant using this construct are also disclosed herein.

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TITLE

USE OF THE SOYBEAN SUCROSE SYNTHASE PROMOTER TO INCREASE PLANT SEED LIPID CONTENT

FIELD OF THE INVENTION

This invention is in the field of biotechnology, in particular, this pertains to increasing oil content while maintaining normal germination in oilseed plants using the soybean sucrose synthase promoter to drive expression of transcription factors such as ODP1 , Lec1 and FUSCA3.

BACKGROUND OF THE INVENTION

Plant oil is a valuable renewable resource. Plant lipids have a variety of industrial and nutritional uses and are central to plant membrane function and climatic adaptation. Besides the nutritional uses, vegetable oils are gaining increasing interest as substitutes for petroleum-derived materials in fuels, lubricants, and specialty chemicals, especially as crude oil supplies decline. Oilseeds provide a unique platform for the production of high-value fatty acids that can replace non-sustainable petroleum products. (Cahoon et al. (2007) *Curr. Opin. Plant Biol.* 10:236-244). Methods to increase the content and to improve and alter the composition of plant oils are therefore desired.

Triacylglycerol (TAG) is the primary component of vegetable oil in plants; it is used by the seed as a stored form of energy to be used during seed germination. The quality and content of plant oil can be altered by various methods, by impinging on the enzymes involved directly or indirectly in TAG biosynthesis.

There are limitations to using conventional plant breeding to alter fatty acid composition and content. Molecular and cellular biology techniques offer the potential for overcoming some of the limitations of the conventional breeding approach. Some of the particularly useful technologies are seed-specific expression of foreign genes in transgenic plants (Goldberg et al. (1989) *Cell* 56:149-1 60), and the use of antisense RNA to inhibit plant target genes in a dominant and tissue-specific manner (van der Krol et al. (1988) *Gene* 72:45-50]. Other advances include the transfer of foreign genes into elite commercial varieties of commercial oilseed crops, such as soybean (Chee et al. (1989) *Plant Physiol.* 91:1212-1218; Christou et al. (1989) *Proc. Natl. Acad. Sci. U.S.A.* 86:7500-7504;

Hinchee et al. (1988) *Bio/Technology* 6:915-922; EPO publication 0 301 749 A2], rapeseed (De Block et al. (1989) *Plant Physiol.* 91:694-701), and sunflower (Everett et al. (1987) *Bio/Technology* 5:1201-1204), and the use of genes as restriction fragment length polymorphism (RFLP) markers in a breeding program, which makes introgression of recessive traits into elite lines rapid and less expensive (Tanksley et al. (1989) *Bio/Technology* 7:257-264). However, application of each of these technologies requires identification and isolation of commercially-important genes.

Transcription factors regulate transcription and orchestrate gene expression in plants and other organisms; control of transcription factor gene expression provides a powerful means for altering plant phenotype. The transformation of plants with transcription factors, however, can result in aberrant development based on the overexpression and/or ectopic expression of the transcription factor, and thus, tight control of timing, strength and location of transcription factor expression is crucial for optimal phenotype. Using strong seed-specific promoters or strong constitutive promoters can lead to aberrant phenotypes.

SUMMARY OF THE INVENTION

The present invention relates to the use of a seed-specific promoter of a soybean sucrose synthase gene or a *Medicago truncatula* sucrose synthase gene to drive expression of transcription factors such as soybean ODP1, Lec1 or FUSCA3 in the seeds of an oilseed plant, to increase oil content.

In one embodiment, a recombinant DNA construct comprising at least one heterologous polynucleotide encoding a polypeptide selected from the group consisting of: an ODP1 polypeptide, a Led polypeptide and a FUSCA3 polypeptide, wherein the at least one polynucleotide is operably linked to a soybean sucrose synthase promoter or a *Medicago truncatula* sucrose synthase promoter, wherein expression of said polypeptide in a transgenic soybean seed comprising the recombinant DNA construct results in an increased oil content in the transgenic soybean seed, when compared to a control soybean seed not comprising the recombinant DNA construct. The transgenic soybean seed comprising said recombinant DNA construct may have normal germination, when compared to a control soybean seed not comprising the recombinant DNA construct.

In another embodiment, a recombinant DNA construct as described herein, wherein the at least one polynucleotide is operably linked to a soybean sucrose synthase promoter, wherein the soybean sucrose synthase promoter comprises a nucleic acid sequence selected from the group consisting of: (a) the nucleic acid sequence of SEQ ID NO: 8; (b) a nucleic acid sequence with at least 95% sequence identity to the nucleic acid sequence of SEQ ID NO: 8; (c) a nucleic acid sequence that hybridizes to SEQ ID NO: 8 under stringent conditions; (d) a nucleic acid sequence that differs from SEQ ID NO: 8 in at least one way as described in FIG. 4; and (e) a nucleic acid sequence comprising a functional fragment of (a), (b), (c) or (d).

In another embodiment, a recombinant DNA construct as described herein, wherein the at least one polynucleotide is operably linked to a *Medicago truncatula* sucrose synthase promoter, wherein the *Medicago truncatula* sucrose synthase promoter comprises a nucleic acid sequence selected from the group consisting of: (a) the nucleic acid sequence of SEQ ID NO: 81 or SEQ ID NO: 85; (b) a nucleic acid sequence with at least 95% sequence identity to the nucleic acid sequence of SEQ ID NO: 81 or SEQ ID NO: 85; (c) a nucleic acid sequence that hybridizes to SEQ ID NO: 81 or SEQ ID NO: 85 under stringent conditions; and (d) a nucleic acid sequence comprising a functional fragment of (a), (b) or (c).

In another embodiment, a recombinant DNA construct as described herein, wherein the at least one heterologous polynucleotide encodes an ODP1 polypeptide, wherein the ODP1 polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95% or 100% identity to SEQ ID NO: 30 or SEQ ID NO: 70.

In another embodiment, a recombinant DNA construct as described herein, wherein the at least one heterologous polynucleotide encodes a Lec1 polypeptide, wherein the Led polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95% or 100% identity to SEQ ID NO: 17, 20, 25 or 65.

In another embodiment, a recombinant DNA construct as described herein, wherein the at least one heterologous polynucleotide encodes a FUSCA3 polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95% or 100% identity to SEQ ID NO: 32, 38, 45 or 49.

In another embodiment, a plant or a seed comprising any of the recombinant DNA constructs described above. The plant and the seed may be an oilseed plant and seed. The plant or seed may be a soybean plant or seed.

In another embodiment, a recombinant DNA construct as described herein, wherein the recombinant DNA construct further comprises a seed-specific promoter operably linked to a second heterologous polynucleotide encoding a DGAT polypeptide. The second heterologous polynucleotide may encode a DGAT1 polypeptide. The DGAT1 polypeptide may comprise an amino acid sequence with at least 80%, 85%, 90%, 95% or 100% sequence identity to SEQ ID NO: 55. The second heterologous polynucleotide may encode a DGAT2 polypeptide. The DGAT2 polypeptide may comprise an amino acid sequence with at least 80%, 85%, 90%, 95% or 100% sequence identity to SEQ ID NO: 60.

In another embodiment, a plant or a seed comprising the recombinant DNA constructs described above, wherein co-expression of said polypeptide and said DGAT polypeptide in a transgenic soybean seed comprising the recombinant DNA construct results in an increased oil content in the transgenic seed, when compared to a control seed that expresses said DGAT polypeptide from said seed-specific promoter by does not express said polypeptide selected from the group consisting of an ODP1 polypeptide, a Lec1 polypeptide and a FUSCA3 polypeptide. The plant and the seed may be an oilseed plant and seed. The plant or seed may be a soybean plant or seed.

In another embodiment, a plant comprising a first recombinant DNA construct comprising a soybean or a *Medicago truncatula* sucrose synthase promoter operably linked to a first heterologous polynucleotide encoding a first polypeptide selected from the group consisting of an ODP1 polypeptide, a Led polypeptide and a FUSCA3 polypeptide and a second recombinant DNA construct comprising a seed-specific promoter operably linked to a second heterologous polynucleotide encoding a DGAT polypeptide, wherein co-expression of said first polypeptide and said second polypeptide in a transgenic soybean seed comprising said first and said second recombinant DNA constructs results in an increased oil content in the transgenic seed, when compared to a control seed comprising only one, but not both, of the first and the second recombinant DNA constructs. The plant and the

seed may be an oilseed plant and seed. The plant and the seed may be a soybean plant and seed.

In another embodiment, a method of increasing oil content of a soybean seed, the method comprising the steps of: (a) introducing into a regenerable soybean cell any one of the recombinant DNA constructs described herein; (b) regenerating a transgenic plant from the regenerable soybean cell of (a) wherein the transgenic plant comprises the recombinant DNA construct; and (c) selecting a transgenic plant of step (b), or a transgenic progeny plant from the transgenic plant of step (b), wherein seed of the transgenic plant or the transgenic progeny plant comprises the recombinant construct and exhibits increased seed oil content while maintaining normal germination, when compared to a control soybean seed not comprising the DNA recombinant construct. The percent oil content of the transgenic soybean seed may be at least 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13% , 14% or 15%.

In another embodiment, a method of increasing oil content of a soybean seed, the method comprising the steps of: (a) introducing into a regenerable soybean cell a first recombinant DNA construct comprising a soybean or a *Medicago truncatula* sucrose synthase promoter operably linked to a first heterologous polynucleotide encoding a first polypeptide selected from the group consisting of an ODP1 polypeptide, a Lec1 polypeptide and a FUSCA3 polypeptide and a second recombinant DNA construct comprising a seed-specific promoter operably linked to a second heterologous polynucleotide encoding a DGAT polypeptide; (b) regenerating a transgenic plant from the regenerable soybean cell of (a) wherein the transgenic plant comprises the first and the second recombinant DNA constructs; and (c) selecting a transgenic plant of step (b), or a transgenic progeny plant from the transgenic plant of step (b), wherein seed of the transgenic plant or the transgenic progeny plant comprises the first and the second recombinant DNA constructs and wherein co-expression of said first polypeptide and said second polypeptide in a transgenic soybean seed comprising said first and said second recombinant DNA constructs results in an increased oil content in the transgenic soybean seed, when compared to a control soybean seed comprising only one, but not both, of the first and the second recombinant DNA constructs. The

percent oil content of the transgenic soybean seed may be at least 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13% , 14% or 15%.

In another embodiment, a transgenic plant obtained by any of the methods described herein, and transgenic seed of said transgenic plant.

In another embodiment, a vector, cell, plant, plant tissue or seed comprising any of the recombinant DNA constructs described herein.

BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCE LISTING

The invention can be more fully understood from the following detailed description and the accompanying drawings and Sequence Listing which form a part of this application. The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in Nucleic Acids Research 13:3021-3030 (1985) and in the Biochemical Journal 219 (No. 2): 345-373 (1984), which are herein incorporated by reference in their entirety. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. § 1.822.

FIG. 1 is a schematic diagram showing the promoter region and the 5' splice variants of GmSuS or Glyma13g17420 . The identified GmSus promoter region encodes the 5' UTR from the cDNA transcript as well as an intron which splits the 5' UTR. The positions of AW boxes AW1 and AW2 are also shown.

FIG. 2 shows an alignment comparing the amino acid sequences of Glyma17g00950 (SEQ ID NO: 17), Glyma07g39820 (SEQ ID NO: 20) and GmLec1 (SEQ ID NO: 25).

FIG. 3 shows an alignment comparing the amino acid sequences for Glyma16g05480 (SEQ ID NO: 32) and Glyma19g27340 (SEQ ID NO: 38), as predicted in the Glyma database, along with the predicted spliced sequence for GmFusca3-2 (SEQ ID NO: 45) and for GmFusca3-1 (SEQ ID NO: 49).

FIG. 4 shows the sequence diversity within different soybean lines of the genomic DNA region comprising the promoter, 5'-UTR and first intron of the Glyma13g17420 gene.

The sequence descriptions and Sequence Listing attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825. The Sequence Listing

contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in *Nucleic Acids Res.* 13:3021-3030 (1985) and in the *Biochemical J.* 219 (2):345-373 (1984) which are herein incorporated by reference. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. § 1.822.

SEQ ID NO: 1 is the nucleotide sequence of the *Arabidopsis* Sucrose Synthase 2 gene (AT5G49190), corresponding to the locus described previously in PCT Publication No. WO 2010/114989, and corresponding to GI NO. 30695613.

SEQ ID NO: 2 is the amino acid sequence encoded by the sequence set forth in SEQ ID NO: 1, and corresponds to GI NO. 332008397.

SEQ ID NO: 3 is the genomic sequence of the soybean Sucrose Synthase gene corresponding to the locus Glyma13g17420.

SEQ ID NO: 4 is the cDNA sequence of the soybean Sucrose Synthase gene corresponding to the locus Glymal 3g17420.

SEQ ID NO: 5 is the CDS (coding sequence) of the soybean Sucrose Synthase gene corresponding to the locus Glymal 3g17420. The soybean homolog to the *Arabidopsis* sucrose synthase 2 gene set forth in SEQ ID NO: 5 is called GmSuS.

SEQ ID NO: 6 is the amino acid sequence encoded by SEQ ID NO: 5, and is the sequence of soybean Sucrose Synthase polypeptide.

SEQ ID NO: 7 is the sequence for the 5' end of EST sdp3c.pk014.n18.

SEQ ID NO: 8 is the sequence of the genomic DNA upstream of the start codon of GmSuS (SEQ ID NO: 5), corresponding to the promoter for GmSuS.

SEQ ID NOS: 9 and 10 are the sequences of the oligonucleotides GmSuSyProm-5 and GmSuSyProm-3 respectively.

SEQ ID NO: 11 is the sequence of pLF284 construct.

SEQ ID NO: 12 is the sequence of the plasmid pKR1963.

SEQ ID NO: 13 is the sequence of the construct pKR1964.

SEQ ID NO: 14 is the sequence of the construct pKR1965.

SEQ ID NO: 15 is the sequence of the cDNA clone se2.1 1d12.

SEQ ID NO: 16 is the sequence of the soybean clone se2.1 1d1 2 from 38-71 8 bp, and is the coding sequence of Lec1b (Gl: 158525282) and corresponds to Glyma1 7g00950.

SEQ ID NO: 17 is the amino acid sequence encoded by the nucleotide sequence given in SEQ ID NO: 16.

SEQ ID NO: 18 is the full insert sequence of the cDNA clone se1 .pk0042.d8.

SEQ ID NO: 19 is the sequence from soybean cDNA clone se1 .pk0042.d8 with a corrected start site, corresponding to Glyma07g39820.

SEQ ID NO: 20 is the amino acid sequence encoded by the sequence given in SEQ ID NO: 19.

SEQ ID NOS: 2 1 and 22 are the sequences of the oligonucleotides SA275 and SA276 respectively.

SEQ ID NO: 23 is the sequence of the construct Glymal 7g00950/pCR8/GW/TOPO.

SEQ ID NO: 24 is the nucleotide sequence of GmLecl .

SEQ ID NO: 25 is the amino acid sequence encoded by the nucleotide sequence given in SEQ ID NO: 24.

SEQ ID NOS: 26 and 27 are the sequences of the oligonucleotides GmLec-5 and Gmlec-3 respectively.

SEQ ID NO: 28 is the sequence of pLF275 construct, containing GmLecl .

SEQ ID NO: 29 is the CDS of GmODPI .

SEQ ID NO: 30 is the amino acid sequence of GmODPI .

SEQ ID NO: 3 1 is the predicted CDS for Glymal 6g05480.

SEQ ID NO: 32 is the amino acid sequence for Glymal 6g05480.

SEQ ID NOS: 33 and 34 are the sequences of the oligonucleotides SA278 and SA279 respectively.

SEQ ID NO: 35 is the sequence of the plasmid Glymal 6g05480/pCR8/GW/TOPO.

SEQ ID NO: 36 is the sequence of the cDNA insert in the plasmid Glymal 6g05480/pCR8/GW/TOPO (SEQ ID NO: 35), determined by sequencing of the insert.

SEQ ID NO: 37 is the sequence of the predicted CDS of Glymal 9g27340 from the Glyma database.

SEQ ID NO: 38 is the sequence of the predicted amino acid sequence of Glyma1 9g27340 from the Glyma database.

SEQ ID NO: 39 is the genomic sequence from the soybean genome database, upstream of and including Glyma1 9g27340.

SEQ ID NOS: 40 and 41 are the sequences of the oligonucleotides GmFusca3-1 -5 and GmFusca3-3 respectively.

SEQ ID NO: 42 is the sequence of the construct pLF283.

SEQ ID NO: 43 is the sequence of the full length cDNA of the resulting PCR product for GmFusca3-2, amplified using the primers of SEQ ID NO: 40 and SEQ ID NO: 41.

SEQ ID NO: 44 is the sequence of the putative spliced CDS for GmFusca3-2.

SEQ ID NO: 45 is the sequence of the amino acid sequence for GmFusca3-2 encoded by SEQ ID NO: 44.

SEQ ID NO: 46 is the sequence of the oligonucleotide GmFusca3-2-5 used for amplifying GmFusca3-1 .

SEQ ID NO: 47 is the sequence of the construct pFL282.

SEQ ID NO: 48 is the full nucleotide sequence of GmFusca3-1 .

SEQ ID NO: 49 is the amino acid sequence of GmFusca3-1 .

SEQ ID NO: 50 is the sequence of the construct pKR1968.

SEQ ID NO: 51 is the sequence of the construct pKR1 971 .

SEQ ID NO: 52 is the sequence of the construct pKR1969.

SEQ ID NO: 53 is the sequence of the construct pKR1970.

SEQ ID NO: 54 is the CDS of GmDGATI cAII.

SEQ ID NO: 55 is the amino acid sequence of GmDGATI cAII.

SEQ ID NO: 56 is the sequence of the construct pKR2098.

SEQ ID NO: 57 is the sequence of the construct pKR21 00.

SEQ ID NO: 58 is the sequence of the construct pKR2099.

SEQ ID NO: 59 is the CDS of YLDGAT2.

SEQ ID NO: 60 is the amino acid sequence of YLDGAT2.

SEQ ID NO: 61 is the sequence of the construct pKR2082.

SEQ ID NO: 62 is the sequence of the construct pKR2084.

SEQ ID NO: 63 is the sequence of the construct pKR2083.

SEQ ID NO: 64 is the CDS of ZmLec1.

SEQ ID NO: 65 is the amino acid sequence of ZmLec1.

SEQ ID NOS: 66 and 67 are the sequences of the oligonucleotides oZLEC-1 and oZLEC-2 respectively.

SEQ ID NO: 68 is the sequence of the construct pKR21 15.

SEQ ID NO: 69 is the CDS of ZmODPL

SEQ ID NO: 70 is the amino acid sequence of ZmODPI .

SEQ ID NO: 71 is the sequence of the construct pKR21 21.

SEQ ID NO: 72 is the sequence of the construct pKR21 14.

SEQ ID NO: 73 is the sequence of the construct pKR21 23.

SEQ ID NO: 74 is the sequence of the construct pKR21 22.

SEQ ID NO: 75 is the sequence of the construct pKR2146.

SEQ ID NO: 76 is the sequence of the construct pKR2145.

SEQ ID NO: 77 is a conserved Lec1 sequence motif.

SEQ ID NO: 78 is the nucleotide sequence of the AW box.

SEQ ID NO: 79 is the nucleotide sequence of the predicted CDS for Medtr4g 124660.2.

SEQ ID NO: 80 is the amino acid sequence encoded by SEQ ID NO: 79.

SEQ ID NO: 81 is the predicted nucleotide sequence of the Medtr4g 124660.2 promoter region.

SEQ ID NO: 82 is the nucleotide sequence of the oMDSP-1 F forward primer.

SEQ ID NO: 83 is the nucleotide sequence of the oMDSP-I R reverse primer.

SEQ ID NO: 84 is the nucleotide sequence of construct pKR2434.

SEQ ID NO: 85 is the actual nucleotide sequence of the Medtr4g 124660.2 promoter region used in this study.

SEQ ID NO: 86 is the nucleotide sequence of construct pKR2446.

SEQ ID NO: 87 is the nucleotide sequence of construct pKR2457.

SEQ ID NO: 88 is the nucleotide sequence of construct pKR2461 .

SEQ ID NO: 89 is the nucleotide sequence of construct pKR2465.

SEQ ID NO: 90 is the nucleotide sequence of amiRNA GM-MFAD2-1 B.

SEQ ID NO: 91 is the nucleotide sequence of amiRNA Star Sequence 396b-GM-MFAD2-1 B.

SEQ ID NO: 92 is the nucleotide sequence of amiRNA GM-MFAD2-2.

SEQ ID NO: 93 is the nucleotide sequence of amiRNA Star Sequence 159-GM-MFAD2-2.

SEQ ID NO: 94 is the nucleotide sequence of the soy genomic miRNA precursor 159.

SEQ ID NO: 95 is the nucleotide sequence of the soy genomic miRNA precursor 396b.

SEQ ID NO: 96 is the nucleotide sequence of the amiRNA precursor 396b-fad2-1 b/1 59-fad2-2.

SEQ ID NO: 97 is the nucleotide sequence of construct pKR21 09.

SEQ ID NO: 98 is the nucleotide sequence of construct pKR21 18.

SEQ ID NO: 99 is the nucleotide sequence of construct pKR21 20.

SEQ ID NO: 100 is the nucleotide sequence of construct pKR21 19.

SEQ ID NO: 101 is the nucleotide sequence of nt 1857-1880 of SEQ ID NO: 81, which are deleted in SEQ ID NO: 85.

SEQ ID NO: 102 is the nucleotide sequence of a 25 bp insertion between nt 2224 and 2225 of SEQ ID NO: 81, which is present in SEQ ID NO: 85.

DETAILED DESCRIPTION

The disclosure of each reference set forth herein is hereby incorporated by reference in its entirety.

As used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "a plant" includes a plurality of such plants; reference to "a cell" includes one or more cells and equivalents thereof known to those skilled in the art, and so forth.

In the context of this disclosure, a number of terms and abbreviations are used. The following definitions are provided.

The terms "monocot" and "monocotyledonous plant" are used interchangeably herein. A monocot of the current invention includes the Gramineae.

The terms "dicot" and "dicotyledonous plant" are used interchangeably herein. A dicot of the current invention includes the following families: *Brassicaceae, Leguminosae,* and *Solanaceae.*

The terms "full complement" and "full-length complement" are used interchangeably herein, and refer to a complement of a given nucleotide sequence,

wherein the complement and the nucleotide sequence consist of the same number of nucleotides and are 100% complementary.

"Transgenic" refers to any cell, cell line, callus, tissue, plant part or plant, the genome of which has been altered by the presence of a heterologous nucleic acid, such as a recombinant DNA construct, including those initial transgenic events as well as those created by sexual crosses or asexual propagation from the initial transgenic event. The term "transgenic" as used herein does not encompass the alteration of the genome (chromosomal or extra-chromosomal) by conventional plant breeding methods or by naturally occurring events such as random cross-fertilization, non-recombinant viral infection, non-recombinant bacterial transformation, non-recombinant transposition, or spontaneous mutation.

"Genome" as it applies to plant cells encompasses not only chromosomal DNA found within the nucleus, but organelle DNA found within subcellular components (e.g., mitochondrial, plastid) of the cell.

"Plant" includes reference to whole plants, plant organs, plant tissues, plant propagules, seeds and plant cells and progeny of same. Plant cells include, without limitation, cells from seeds, suspension cultures, embryos, meristematic regions, callus tissue, leaves, roots, shoots, gametophytes, sporophytes, pollen, and microspores.

"Propagule" includes all products of meiosis and mitosis able to propagate a new plant, including but not limited to, seeds, spores and parts of a plant that serve as a means of vegetative reproduction, such as corms, tubers, offsets, or runners. Propagule also includes grafts where one portion of a plant is grafted to another portion of a different plant (even one of a different species) to create a living organism. Propagule also includes all plants and seeds produced by cloning or by bringing together meiotic products, or allowing meiotic products to come together to form an embryo or fertilized egg (naturally or with human intervention).

"Transgenic plant" includes reference to a plant which comprises within its genome a heterologous polynucleotide. For example, the heterologous polynucleotide is stably integrated within the genome such that the polynucleotide is passed on to successive generations. The heterologous polynucleotide may be integrated into the genome alone or as part of a recombinant DNA construct.

The commercial development of genetically improved germplasm has also advanced to the stage of introducing multiple traits into crop plants, often referred to as a gene stacking approach. In this approach, multiple genes conferring different characteristics of interest can be introduced into a plant. Gene stacking can be accomplished by many means including but not limited to co-transformation, retransformation, and crossing lines with different transgenes.

"Transgenic plant" also includes reference to plants which comprise more than one heterologous polynucleotide within their genome. Each heterologous polynucleotide may confer a different trait to the transgenic plant.

"Heterologous" with respect to sequence means a sequence that originates from a foreign species, or, if from the same species, is substantially modified from its native form in composition and/or genomic locus by deliberate human intervention.

"Progeny" comprises any subsequent generation of a plant.

"Polynucleotide", "nucleic acid sequence", "nucleotide sequence", or "nucleic acid fragment" are used interchangeably and is a polymer of RNA or DNA that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. Nucleotides (usually found in their 5'-monophosphate form) are referred to by their single letter designation as follows: "A" for adenylate or deoxyadenylate (for RNA or DNA, respectively), "C" for cytidylate or deoxycytidylate, "G" for guanylate or deoxyguanylate, "U" for uridylate, "T" for deoxythymidylate, "R" for purines (A or G), "Y" for pyrimidines (C or T), "K" for G or T, "H" for A or C or T, "I" for inosine, and "N" for any nucleotide.

"Polypeptide", "peptide", "amino acid sequence" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. The terms "polypeptide", "peptide", "amino acid sequence", and "protein" are also inclusive of modifications including, but not limited to, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation.

"Messenger RNA (mRNA)" refers to the RNA that is without introns and that can be translated into protein by the cell.

"cDNA" refers to a DNA that is complementary to and synthesized from an mRNA template using the enzyme reverse transcriptase. The cDNA can be single-stranded or converted into the double-stranded form using the Klenow fragment of DNA polymerase I.

"Coding region" refers to the portion of a messenger RNA (or the corresponding portion of another nucleic acid molecule such as a DNA molecule) which encodes a protein or polypeptide. "Non-coding region" refers to all portions of a messenger RNA or other nucleic acid molecule that are not a coding region, including but not limited to, for example, the promoter region, 5' untranslated region ("UTR"), 3' UTR, intron and terminator. The terms "coding region" and "coding sequence" are used interchangeably herein. The terms "non-coding region" and "non-coding sequence" are used interchangeably herein.

An "Expressed Sequence Tag" ("EST") is a DNA sequence derived from a cDNA library and therefore is a sequence which has been transcribed. An EST is typically obtained by a single sequencing pass of a cDNA insert.

"Mature" protein refers to a post-translationally processed polypeptide; i.e., one from which any pre- or pro-peptides present in the primary translation product has been removed.

"Precursor" protein refers to the primary product of translation of mRNA; i.e., with pre- and pro-peptides still present. Pre- and pro-peptides may be and are not limited to intracellular localization signals.

"Isolated" refers to materials, such as nucleic acid molecules and/or proteins, which are substantially free or otherwise removed from components that normally accompany or interact with the materials in a naturally occurring environment. Isolated polynucleotides may be purified from a host cell in which they naturally occur. Conventional nucleic acid purification methods known to skilled artisans may be used to obtain isolated polynucleotides. The term also embraces recombinant polynucleotides and chemically synthesized polynucleotides.

The terms "full complement" and "full-length complement" are used interchangeably herein, and refer to a complement of a given nucleotide sequence, wherein the complement and the nucleotide sequence consist of the same number of nucleotides and are 100% complementary.

"Recombinant" refers to an artificial combination of two otherwise separated segments of sequence, e.g., by chemical synthesis or by the manipulation of isolated segments of nucleic acids by genetic engineering techniques. "Recombinant" also includes reference to a cell or vector, that has been modified by the introduction of a heterologous nucleic acid or a cell derived from a cell so modified, but does not encompass the alteration of the cell or vector by naturally occurring events (e.g., spontaneous mutation, natural transformation/transduction/transposition) such as those occurring without deliberate human intervention.

The terms "recombinant construct", "expression construct", "chimeric construct", "construct", and "recombinant DNA construct" are used interchangeably herein. A recombinant construct comprises an artificial combination of nucleic acid fragments, e.g., regulatory and coding sequences that are not found together in nature. For example, a chimeric construct may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. Such a construct may be used by itself or may be used in conjunction with a vector.

This construct may comprise any combination of deoxyribonucleotides, ribonucleotides, and/or modified nucleotides. The construct may be transcribed to form an RNA, wherein the RNA may be capable of forming a double-stranded RNA and/or hairpin structure. This construct may be expressed in the cell, or isolated or synthetically produced. The construct may further comprise a promoter, or other sequences which facilitate manipulation or expression of the construct.

The term "conserved domain" or "motif means a set of amino acids conserved at specific positions along an aligned sequence of evolutionarily related proteins. While amino acids at other positions can vary between homologous proteins, amino acids that are highly conserved at specific positions indicate amino acids that are essential in the structure, the stability, or the activity of a protein. Because they are identified by their high degree of conservation in aligned sequences of a family of protein homologues, they can be used as identifiers, or "signatures", to determine if a protein with a newly determined sequence belongs to a previously identified protein family.

The terms "homology", "homologous", "substantially similar" and "corresponding substantially" are used interchangeably herein. They refer to nucleic acid fragments wherein changes in one or more nucleotide bases do not affect the ability of the nucleic acid fragment to mediate gene expression or produce a certain phenotype. These terms also refer to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotides that do not substantially alter the functional properties of the resulting nucleic acid fragment relative to the initial, unmodified fragment. It is therefore understood, as those skilled in the art will appreciate, that the invention encompasses more than the specific exemplary sequences.

"Regulatory sequences" or "regulatory elements" are used interchangeably and refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include, but are not limited to, promoters, translation leader sequences, introns, and polyadenylation recognition sequences. The terms "regulatory sequence" and "regulatory element" are used interchangeably herein.

"Promoter" refers to a nucleic acid fragment capable of controlling transcription of another nucleic acid fragment.

"Promoter functional in a plant" is a promoter capable of controlling transcription in plant cells whether or not its origin is from a plant cell.

Promoters that cause a gene to be expressed in most cell types at most times are commonly referred to as "constitutive promoters".

High level, constitutive expression of the candidate gene under control of the 35S or UBI promoter may have pleiotropic effects, although candidate gene efficacy may be estimated when driven by a constitutive promoter. Use of tissue-specific and/or stress-specific promoters may eliminate undesirable effects but retain the ability to enhance drought tolerance. This effect has been observed in *Arabidopsis* (Kasuga et al. (1999) Nature Biotechnol. 17:287-91).

"Tissue-specific promoter" and "tissue-preferred promoter" are used interchangeably to refer to a promoter that is expressed predominantly but not

necessarily exclusively in one tissue or organ, but that may also be expressed in one specific cell.

"Developmentally regulated promoter" refers to a promoter whose activity is determined by developmental events.

Inducible promoters selectively express an operably linked DNA sequence in response to the presence of an endogenous or exogenous stimulus, for example by chemical compounds (chemical inducers) or in response to environmental, hormonal, chemical, and/or developmental signals. Examples of inducible or regulated promoters include, but are not limited to, promoters regulated by light, heat, stress, flooding or drought, pathogens, phytohormones, wounding, or chemicals such as ethanol, jasmonate, salicylic acid, or safeners.

A minimal or basal promoter is a polynucleotide molecule that is capable of recruiting and binding the basal transcription machinery. One example of basal transcription machinery in eukaryotic cells is the RNA polymerase II complex and its accessory proteins.

Plant RNA polymerase II promoters, like those of other higher eukaryotes, are comprised of several distinct "cis-acting transcriptional regulatory elements," or simply "cis-elements," each of which appears to confer a different aspect of the overall control of gene expression. Examples of such cis-acting elements include, but are not limited to, such as TATA box and CCAAT or AGGA box. The promoter can roughly be divided in two parts: a proximal part, referred to as the core, and a distal part. The proximal part is believed to be responsible for correctly assembling the RNA polymerase II complex at the right position and for directing a basal level of transcription, and is also referred to as "minimal promoter" or "basal promoter". The distal part of the promoter is believed to contain those elements that regulate the spatio-temporal expression. In addition to the proximal and distal parts, other regulatory regions have also been described, that contain enhancer and/or repressors elements The latter elements can be found from a few kilobase pairs upstream from the transcription start site, in the introns, or even at the 3' side of the genes they regulate (Rombauts, S. et al. (2003) *Plant Physiology* 132:1 162-1 176, Nikolov and Burley, (1997) *Proc Natl Acad Sci* USA 94: 15-22), Tjian and Maniatis (1994) Ce// 77: 5-8; Fessele et al., 2002 *Trends Genet* 18: 60-63, Messing et al.,

(1983) *Genetic Engineering of Plants: an Agricultural Perspective,* Plenum Press, NY, pp 211-227).

When operably linked to a heterologous polynucleotide sequence, a promoter controls the transcription of the linked polynucleotide sequence.

Operably linked" refers to the association of nucleic acid fragments in a single fragment so that the function of one is regulated by the other. For example, a promoter is operably linked with a nucleic acid fragment when it is capable of regulating the transcription of that nucleic acid fragment.

An intron sequence can be added to the 5' untranslated region, the protein-coding region or the 3' untranslated region to increase the amount of the mature message that accumulates in the cytosol. Inclusion of a spliceable intron in the transcription unit in both plant and animal expression constructs has been shown to increase gene expression at both the mRNA and protein levels up to 1000-fold. Buchman and Berg, *Mol. Cell Biol.* 8:4395-4405 (1988); Callis et al., *Genes Dev.* 1:1183-1200 (1987).

"Expression" refers to the production of a functional product. For example, expression of a nucleic acid fragment may refer to transcription of the nucleic acid fragment (e.g., transcription resulting in mRNA or functional RNA) and/or translation of mRNA into a precursor or mature protein.

"Overexpression" refers to the production of a gene product in transgenic organisms that exceeds levels of production in a null segregating (or non-transgenic) organism from the same experiment.

"Phenotype" means the detectable characteristics of a cell or organism.

"Introduced" in the context of inserting a nucleic acid fragment (e.g., a recombinant DNA construct) into a cell, means "transfection" or "transformation" or "transduction" and includes reference to the incorporation of a nucleic acid fragment into a eukaryotic or prokaryotic cell where the nucleic acid fragment may be incorporated into the genome of the cell (e.g., chromosome, plasmid, plastid or mitochondrial DNA), converted into an autonomous replicon, or transiently expressed (e.g., transfected mRNA).

A "transformed cell" is any cell into which a nucleic acid fragment (e.g., a recombinant DNA construct) has been introduced.

"Transformation" as used herein refers to both stable transformation and transient transformation.

"Stable transformation" refers to the introduction of a nucleic acid fragment into a genome of a host organism resulting in genetically stable inheritance. Once stably transformed, the nucleic acid fragment is stably integrated in the genome of the host organism and any subsequent generation.

"Transient transformation" refers to the introduction of a nucleic acid fragment into the nucleus, or DNA-containing organelle, of a host organism resulting in gene expression without genetically stable inheritance.

"Allele" is one of several alternative forms of a gene occupying a given locus on a chromosome. When the alleles present at a given locus on a pair of homologous chromosomes in a diploid plant are the same that plant is homozygous at that locus. If the alleles present at a given locus on a pair of homologous chromosomes in a diploid plant differ that plant is heterozygous at that locus. If a transgene is present on one of a pair of homologous chromosomes in a diploid plant that plant is hemizygous at that locus.

The term "crossed" or "cross" means the fusion of gametes via pollination to produce progeny (e.g., cells, seeds or plants). The term encompasses both sexual crosses (the pollination of one plant by another) and selfing (self-pollination, e.g., when the pollen and ovule are from the same plant). The term "crossing" refers to the act of fusing gametes via pollination to produce progeny.

A "favorable allele" is the allele at a particular locus that confers, or contributes to, a desirable phenotype, e.g., increased cell wall digestibility, or alternatively, is an allele that allows the identification of plants with decreased cell wall digestibility that can be removed from a breeding program or planting ("counterselection"). A favorable allele of a marker is a marker allele that segregates with the favorable phenotype, or alternatively, segregates with the unfavorable plant phenotype, therefore providing the benefit of identifying plants.

"Suppression DNA construct" is a recombinant DNA construct which when transformed or stably integrated into the genome of the plant, results in "silencing" of a target gene in the plant. The target gene may be endogenous or transgenic to the plant. "Silencing," as used herein with respect to the target gene, refers generally to the suppression of levels of mRNA or protein/enzyme expressed by the target gene,

and/or the level of the enzyme activity or protein functionality. The terms "suppression", "suppressing" and "silencing", used interchangeably herein, include lowering, reducing, declining, decreasing, inhibiting, eliminating or preventing. "Silencing" or "gene silencing" does not specify mechanism and is inclusive, and not limited to, anti-sense, cosuppression, viral-suppression, hairpin suppression, stem-loop suppression, RNAi-based approaches, and small RNA-based approaches.

A suppression DNA construct may comprise a region derived from a target gene of interest and may comprise all or part of the nucleic acid sequence of the sense strand (or antisense strand) of the target gene of interest. Depending upon the approach to be utilized, the region may be 100% identical or less than 100% identical (e.g., at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical) to all or part of the sense strand (or antisense strand) of the gene of interest.

Suppression DNA constructs are well-known in the art, are readily constructed once the target gene of interest is selected, and include, without limitation, cosuppression constructs, antisense constructs, viral-suppression constructs, hairpin suppression constructs, stem-loop suppression constructs, double-stranded RNA-producing constructs, and more generally, RNAi (RNA interference) constructs and small RNA constructs such as siRNA (short interfering RNA) constructs and miRNA (microRNA) constructs.

"Antisense inhibition" refers to the production of antisense RNA transcripts capable of suppressing the expression of the target gene or gene product. "Antisense RNA" refers to an RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target isolated nucleic acid fragment (U.S. Patent No. 5,107,065). The complementarity of an antisense RNA may be with any part of the specific gene transcript, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence.

"Cosuppression" refers to the production of sense RNA transcripts capable of suppressing the expression of the target gene or gene product. "Sense" RNA refers to RNA transcript that includes the mRNA and can be translated into protein within a cell or *in vitro.* Cosuppression constructs in plants have been previously designed

by focusing on overexpression of a nucleic acid sequence having homology to a native mRNA, in the sense orientation, which results in the reduction of all RNA having homology to the overexpressed sequence (see Vaucheret et al., *Plant J.* 16:651-659 (1998); and Gura, *Nature* 404:804-808 (2000)).

Another variation describes the use of plant viral sequences to direct the suppression of proximal mRNA encoding sequences (PCT Publication No. WO 98/36083 published on August 20, 1998).

RNA interference refers to the process of sequence-specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs) (Fire et al., *Nature* 391:806 (1998)). The corresponding process in plants is commonly referred to as post-transcriptional gene silencing (PTGS) or RNA silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes and is commonly shared by diverse flora and phyla (Fire et al., *Trends Genet.* 15:358 (1999)).

Small RNAs play an important role in controlling gene expression. Regulation of many developmental processes, including flowering, is controlled by small RNAs. It is now possible to engineer changes in gene expression of plant genes by using transgenic constructs which produce small RNAs in the plant.

Small RNAs appear to function by base-pairing to complementary RNA or DNA target sequences. When bound to RNA, small RNAs trigger either RNA cleavage or translational inhibition of the target sequence. When bound to DNA target sequences, it is thought that small RNAs can mediate DNA methylation of the target sequence. The consequence of these events, regardless of the specific mechanism, is that gene expression is inhibited.

MicroRNAs (miRNAs) are noncoding RNAs of about 19 to about 24 nucleotides (nt) in length that have been identified in both animals and plants (Lagos-Quintana et al., *Science* 294:853-858 (2001), Lagos-Quintana et al., *Curr. Biol.* 12:735-739 (2002); Lau et al., *Science* 294:858-862 (2001); Lee and Ambros, *Science* 294:862-864 (2001); Llave et al., *Plant Cell* 14:1 605-161 9 (2002); Mourelatos et al., *Genes. Dev.* 16:720-728 (2002); Park et al., *Curr. Biol.* 12:1484-1495 (2002); Reinhart et al., *Genes. Dev.* 16:1 6 16-1626 (2002)). They are

processed from longer precursor transcripts that range in size from approximately 70 to 200 nt, and these precursor transcripts have the ability to form stable hairpin structures.

MicroRNAs (miRNAs) appear to regulate target genes by binding to complementary sequences located in the transcripts produced by these genes. It seems likely that miRNAs can enter at least two pathways of target gene regulation: (1) translational inhibition; and (2) RNA cleavage. MicroRNAs entering the RNA cleavage pathway are analogous to the 21-25 nt short interfering RNAs (siRNAs) generated during RNA interference (RNAi) in animals and posttranscriptional gene silencing (PTGS) in plants, and likely are incorporated into an RNA-induced silencing complex (RISC) that is similar or identical to that seen for RNAi.

Transcription factors are proteins that generally bind DNA in a sequence-specific manner and either activate or repress transcription initiation. At least three types of separate domains have been identified within transcription factors. One is necessary for sequence-specific DNA recognition, one for the activation/repression of transcriptional initiation, and one for the formation of protein-protein interactions (such as dimerization). Studies indicate that many plant transcription factors can be grouped into distinct classes based on their conserved DNA binding domains (Katagiri F and Chua N H, 1992, *Trends Genet.* 8:22-27; Menkens A E, Schindler U and Cashmore A R, 1995, *Trends in Biochem Sci.* 13:506-510; Martin C and Paz-Ares J, 1997, *Trends Genet.* 13:67-73). Each member of these families interacts and binds with distinct DNA sequence motifs that are often found in multiple gene promoters controlled by different regulatory signals.

Ovule Development Proteins (ODP) are transcription factors containing two AP2 domains. AP2 transcription factors (herein referred to interchangeably as "AP2 domain transcription factors", "AP2 proteins", "AP2/ EREBP transcription factors", or "AP2 transcription factor proteins") such as ODP activate several genes in the oil or TAG biosynthetic pathway in the plant cell.

The term "ODP1 " refers to an ovule development protein 1 that is involved with increasing oil content. ODP1 is a member of the APETALA2 (AP2) family of proteins that play a role in a variety of biological events including, but not limited to, oil content.

US Patent Application No. 61/165,548 describes the use of an ODP1 gene for alteration of oil traits in plants. US Patent No. 7,579,529 describes an AP2 domain transcription factor and methods of its use. US Patent No. 7,157,621 discloses the use of ODP1 transcription factor for increasing oil content in plants. DuPont patent application WO 2010/114989 describes the use of an *Arabidopsis* Sus2 promoter to drive ODP1 (WRI1) expression in *Arabidopsis.*

The putative AP2/EREBP transcription factor WRINKLED1 (WRI1) is involved in the regulation of seed storage metabolism in *Arabidopsis* (Cernac and Benning(2004) *Plant J.* 40:575-585). Expression of the WRI1 cDNA under the control of the CaMV 35S promoter led to increased seed oil content. Oil-accumulating seedlings, however, showed aberrant development consistent with a prolonged embryonic state. Nucleic acid molecules encoding WRINKLED1-LIKE polypeptides and methods of use are also described in International Publication No. WO 2006/00732 A2.

The AP2/EREBP family of proteins is a plant-specific class of putative transcription factors that have been shown to regulate a wide-variety of developmental processes and are characterized by the presence of an AP2/ERF DNA binding domain. Specifically, AP2 (APETALA2) and EREBPs (ethylene-responsive element binding proteins) are the prototypic members of a family of transcription factors unique to plants, whose distinguishing characteristic is that they contain the so-called AP2 DNA-binding domain. DNA sequence analysis suggests that AP2 encodes a theoretical polypeptide of 432 aa, with a distinct 68 aa repeated motif termed the AP2 domain. This domain has been shown to be essential for AP2 functions and contains within the 68 aa motif an eighteen amino acid core region that is predicted to form an amphipathic a-helix (Jofuku et al., Plant Cell 6:1211-1225, 1994). AP2-like domain-containing transcription factors have been also been identified in both *Arabidopsis thaliana* (Okamuro et al.,(1997) *Proc. Natl. Acad. Sci. USA* 94:7076-7081,) and in tobacco with the identification of the ethylene responsive element binding proteins (EREBPs) (Ohme-Takagi and Shinshi, (1995) *Plant Cell* 7:2:173-182,).

HAP proteins constitute a large family of transcription factors first identified in yeast. They combine to form a heteromeric protein complex that activates transcription by binding to CCAAT boxes in eukaryotic promoters. The orthologous

Hap proteins display a high degree of evolutionary conservation in their functional domains in all species studied to date (Li et al. (1992) *Nucleic Acids Res* 20:1087-1091).

Leafy cotyledon 1 (Lec1 or Lec1/Hap3) is a transcription factor that is a key regulator of seed development in plants. Led is a CCAAT-binding factor (CBF)-type transcription factor. The terms "leafy cotyledon 1", "Led ", and "Hap3/Lec1 " are used interchangeably herein. LEC1 polypeptide is homologous to the HAP3 subunit of the CBF class of eukaryotic transcriptional activators that includes NF-Y, CP1, and HAP2/3/4/5 (Lotan et al. (1998) *Cell,* Vol. 93, 1195-1205, June 26).

The leafy cotyledonl (LEC1) gene controls many distinct aspects of embryogenesis. The *lec1* mutation is pleiotropic, which suggest that LEC1 has several roles in late embryo development. For example, LEC1 is required for specific aspects of seed maturation, inhibiting premature germination and plays a role in the specification of embryonic organ identity. Finally, LEC1 appears to act only during embryo development.

US Patent No. 6235975 describes leafy cotyledonl genes and their uses. A pending US patent application (US Application No. 11/899370) relates to isolated nucleic acid fragments encoding Led related transcription factors. US Patent Nos. 7294759, US7157621, US 7888560, US6825397 describe the use of Led genes for altering oil content in plants.

In *Arabidopsis,* Led has been shown to regulate the expression of fatty acid biosynthetic genes and Led has also been shown to be involved in embryo development (Mu et al., *Plant Physiology* (2008) 148: 1042-1054; Lotan et al. (1998) *Cell,* Vol. 93, 1195-1205, June 26; PCT publication number WO/1998037184 & US Patent Nos. 6235975, 6320102, 6545201; PCT publication no. WO/2001064022 & US patent 6781035, Braybrook, S.A. and Harada, J.J. (2008) *Trends Plant Sci* 13(12): 1360-1385).

WO 99/67405 describes leafy cotyledonl genes and their uses. A maize Led homologue of the *Arabidopsis* embryogenesis controlling gene AtLECI has been shown to increase oil content and transformation efficiencies in plants. See, for example, WO 03001902 and U.S. Patent No. 6,512,165.

Other polypeptides that influence ovule and embryo development and stimulate cell growth, such as, Led , Kn1, WUSCHEL, Zwille and Aintegumeta

(ANT) allow for increased transformation efficiencies when expressed in plants. See, for example, U.S. Application No. 2003/0135889, herein incorporated by reference. In fact, a maize Lec1 homologue of the *Arabidopsis* embryogenesis controlling gene AtLECI , has been shown to increase oil content and transformation efficiencies in plants. See, for example, WO 03001 902 and U.S. Patent No. 6,51 2,1 65.

Led homologs may be further identified by using conserved sequence motifs, such as the following amino acid sequence (given in single letter code, with "x" representing any amino acid) (US application number 60/301 ,91 3). Underlined amino acids in the following sequence are those that are conserved in Led but not found in Led -related proteins:

REQDxxMPxANVxRIMRxxLPxxAKISDDAKExIQECVSExISFxTxEANxRCxxxx RKTxxxE (SEQ ID NO:77)

The terms "FUS3", "FUSCA3" are used interchangeably herein. FUSCA3 is a transcription factor with a conserved VP1/ABI3-like B3 domain which is of functional importance for the regulation of seed maturation in *Arabidopsis thaliana.* It controls developmental timing in *Arabidopsis* through the hormones gibberellin and abscisic acid and is itself regulated by the Led transcription factor (Luerssen et al. (1998) *Plant J* (1998) 15 (6): 755-7; Stone et al. (2001) *Proc Natl Acad Sci* 98 (20): 11806-1 1811; Lee et al. (2003) *Proc Natl Acad Sci* 100 (4): 2152-2156, US Patent Nos. US751 1190 and US7446241 , PCT Publication No. WO1 998021 336, PCT Publication No. WO2008 157226, Braybrook, S.A. and Harada, J.J. (2008) *Trends Plant Sci* 13(12): 1360-1385). US Patent No. US7612253 describes methods of modulating cytokinin related processes in a plant using B3 domain proteins with a number of fusca3 homologs.

"Diacylglycerol acyltransferase" or "DGAT" (also known as "acyl-CoA-diacylglycerol acyltransferase" or "diacylglycerol O-acyltransferase") (EC 2.3.1 .20) is an integral membrane protein that catalyzes the final enzymatic step in the production of triacylglycerols in plants, fungi and mammals. This enzyme is responsible for transferring an acyl group from acyl-coenzyme-A to the sn-3 position of 1,2-diacylglycerol ("DAG") to form triacylglycerol ("TAG"). DGAT is associated with membrane and lipid body fractions in plants and fungi, particularly, in oilseeds where it contributes to the storage of carbon used as energy reserves. DGAT is

known to regulate TAG structure and direct TAG synthesis. Furthermore, it is known that the DGAT reaction is specific for oil synthesis (Lardizabal et al., *J. Biol. Chem.* 276(42):38862-28869 (2001)).

Two different families of DGAT proteins have been identified. The first family of DGAT proteins ("DGAT1") is related to the acyl-coenzyme A: cholesterol acyltransferase ("ACAT") and has been described in U.S. Patent Nos. 6,100,077 and 6,344,548. A second family of DGAT proteins ("DGAT2") is unrelated to the DGAT1 family and is described in PCT Patent Publication WO 2004/011671 published February 5, 2004. Other references to DGAT genes and their use in plants include PCT Publication No. WO1998/055,631 and US Patent No. 6822141.

"DGAT" and "diacylglycerol acyltransferase" are used interchangeably herein and refer to any member, or combination, of the DGAT1 or DGAT2 family of proteins.

Plant and fungal DGAT genes have been described previously (US Patent Nos. 7198937 and 7465565, US Publication No. 20080295204, US Application Nos. 12/470569 and 12/470517).

The term "fatty acids" refers to long chain aliphatic acids (alkanoic acids) of varying chain length, from about C_{12} to C_{22} (although both longer and shorter chain-length acids are known). The predominant chain lengths are between C_{16} and C22-The structure of a fatty acid is represented by a simple notation system of "X:Y", where X is the total number of carbon (C) atoms in the particular fatty acid and Y is the number of double bonds.

Generally, fatty acids are classified as saturated or unsaturated. The term "saturated fatty acids" refers to those fatty acids that have no "double bonds" between their carbon backbone. In contrast, "unsaturated fatty acids" have "double bonds" along their carbon backbones (which are most commonly in the cis-configuration). "Monounsaturated fatty acids" have only one "double bond" along the carbon backbone (e.g., usually between the 9th and 10th carbon atom as for palmitoleic acid (16:1) and oleic acid (18:1)), while "polyunsaturated fatty acids" (or "PUFAs") have at least two double bonds along the carbon backbone (e.g., between the 9th and 10th, and 12th and 13th carbon atoms for linoleic acid (18:2); and between the 9th and 10th, 12th and 13th, and 15th and 16th for a-linolenic acid (18:3)).

"Lipid bodies" refer to lipid droplets that usually are bounded by specific proteins and a monolayer of phospholipid. These organelles are sites where most organisms transport/store neutral lipids. Lipid bodies are thought to arise from microdomains of the endoplasmic reticulum that contain TAG-biosynthesis enzymes; and, their synthesis and size appear to be controlled by specific protein components.

"Neutral lipids" refer to those lipids commonly found in cells in lipid bodies as storage fats and oils and are so called because at cellular pH, the lipids bear no charged groups. Generally, they are completely non-polar with no affinity for water. Neutral lipids generally refer to mono-, di-, and/or triesters of glycerol with fatty acids, also called monoacylglycerol, diacylglycerol or TAG, respectively (or collectively, acylglycerols). A hydrolysis reaction must occur to release free fatty acids from acylglycerols.

The term "oil" refers to a lipid substance that is liquid at 25° C. and usually polyunsaturated. In contrast, the term "fat" refers to a lipid substance that is solid at 25° C. and usually saturated.

The terms "triacylglycerol", "oil" and "TAGs" are used interchangeably herein, and refer to neutral lipids composed of three fatty acyl residues esterified to a glycerol molecule (and such terms will be used interchangeably throughout the present disclosure herein). Such oils can contain long chain PUFAs (polyunsaturated fatty acids), as well as shorter saturated and unsaturated fatty acids and longer chain saturated fatty acids. Thus, "oil biosynthesis" generically refers to the synthesis of TAGs in the cell (PCT Publication Nos. WO2005063988, WO2007087492, WO20071 01273 and WO20071 03738, US Patent No. US781 221 6).

Oil and protein content in seeds can be determined using Near Infrared Spectroscopy by methods familiar to one skilled in the art (Agelet, et al. (201 2) Journal of Agricultural and Food Chemistry, 60(34): 8314-8322). An apparatus and methods for NIR analysis of single seeds and multiple seeds has been described in US Patent No. 7,508,51 7, herein incorporated by reference. Additional methods for the analysis of seed composition are provided in US Patent No. 8,143,473, herein incorporated by reference.

Medicago truncatula is a small legume native to the Mediterranean region that is used in genomic research. This species has been used as a model organism for legume biology because it has a small diploid genome, is self-fertile, has a rapid generation time and prolific seed production, and is amenable to genetic transformation.

The term "sucrose synthase" (SUS) refers to an enzyme used in carbohydrate metabolism that catalyzes the reversible conversion of sucrose and uridine diphosphate (UDP) to UDP-glucose and fructose in vitro. The terms "Soybean sucrose synthase 2" and "GmSuS" are used interchangeably herein. The Soybean sucrose synthase gene is from genomic locus Glyma1 3g1 7420.

The term "germination" refers to the process by which a dormant seed begins to sprout and grow into a seedling.

"Normal germination", as used herein, refers to a germination rate for seed of a transgenic plant comprising the recombinant DNA construct that is within at least 85%, 86%, 87%, 88%, 89%, 90%, 9 1%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of the observed germination rate, under the same conditions, for seed of a corresponding control plant that does not comprise the recombinant DNA construct.

In an embodiment of the present invention, the "cis-acting transcriptional regulatory elements" from the promoter sequence disclosed herein can be operably linked to "cis-acting transcriptional regulatory elements" from any heterologous promoter. Such a chimeric promoter molecule can be engineered to have desired regulatory properties. In an embodiment of this invention a fragment of the disclosed promoter sequence that can act either as a cis-regulatory sequence or a distal-regulatory sequence or as an enhancer sequence or a repressor sequence, may be combined with either a cis-regulatory or a distal regulatory or an enhancer sequence or a repressor sequence or any combination of any of these from a heterologous promoter sequence.

In a related embodiment, a cis-element of the disclosed promoter may confer a particular specificity such as conferring enhanced expression of operably linked polynucleotide molecules in certain tissues and therefore is also capable of regulating transcription of operably linked polynucleotide molecules. Consequently, any fragments, portions, or regions of the promoter comprising the polynucleotide

sequence shown in SEQ ID NO: 3 can be used as regulatory polynucleotide molecules.

Promoter fragments that comprise regulatory elements can be added, for example, fused to the 5' end of, or inserted within, another promoter having its own partial or complete regulatory sequences (Fluhr et al., Science 232:1 106-1 112, 1986; Ellis et al., EMBO J. 6:1 1-16, 1987; Strittmatter and Chua, Proc. Nat. Acad. Sci. USA 84:8986-8990, 1987; Poulsen and Chua, Mol. Gen. Genet. 214:16-23, 1988; Comai et al., Plant Mol. Biol. 15:373-381 , 1991 ; 1987; Aryan et al., Mol. Gen. Genet. 225:65-71 , 1991).

Cis elements can be identified by a number of techniques, including deletion analysis, i.e., deleting one or more nucleotides from the 5' end or internal to a promoter; DNA binding protein analysis using DNase I footprinting; methylation interference; electrophoresis mobility-shift assays, in vivo genomic footprinting by ligation-mediated PCR; and other conventional assays; or by sequence similarity with known cis element motifs by conventional sequence comparison methods. The fine structure of a cis element can be further studied by mutagenesis (or substitution) of one or more nucleotides or by other conventional methods (see for example, *Methods in Plant Biochemistry and Molecular Biology,* Dashek, ed., CRC Press, 1997, pp. 397-422; and *Methods in Plant Molecular Biology,* Maliga et al., eds., Cold Spring Harbor Press, 1995, pp. 233-300).

Cis elements can be obtained by chemical synthesis or by cloning from promoters that include such elements, and they can be synthesized with additional flanking sequences that contain useful restriction enzyme sites to facilitate subsequent manipulation. Promoter fragments may also comprise other regulatory elements such as enhancer domains, which may further be useful for constructing chimeric molecules.

Methods for construction of chimeric and variant promoters of the present invention include, but are not limited to, combining control elements of different promoters or duplicating portions or regions of a promoter (see for example, 4990607USA U.S. Patent No. 4,990,607; 5110732USA U.S. Patent No. 5,1 10,732; and 5097025USA U.S. Patent No. 5,097,025). Those of skill in the art are familiar with the standard resource materials that describe specific conditions and procedures for the construction, manipulation, and isolation of macromolecules

(e.g., polynucleotide molecules and plasmids), as well as the generation of recombinant organisms and the screening and isolation of polynucleotide molecules.

In an embodiment of the present invention, the soy sucrose synthase promoter disclosed herein can be modified. Those skilled in the art can create promoters that have variations in the polynucleotide sequence. The polynucleotide sequence of the promoter of the present invention as shown in SEQ ID NO: 8 may be modified or altered to enhance their control characteristics. As one of ordinary skill in the art will appreciate, modification or alteration of the promoter sequence can also be made without substantially affecting the promoter function. The methods are well known to those of skill in the art. Sequences can be modified, for example by insertion, deletion, or replacement of template sequences in a PCR-based DNA modification approach.

The present invention encompasses functional fragments and variants of the promoter sequence disclosed herein.

A "functional fragment "herein is defined as any subset of contiguous nucleotides of the promoter sequence disclosed herein, that can perform the same, or substantially similar function as the full length promoter sequence disclosed herein. A "functional fragment" with substantially similar function to the full length promoter disclosed herein refers to a functional fragment that retains largely the same level of activity as the full length promoter sequence and exhibits the same pattern of expression as the full length promoter sequence. A "functional fragment" of the promoter sequence disclosed herein exhibits constitutive expression.

An embodiment of this invention is a functional fragment of SEQ ID NO: 8, that comprises at least 50, 100, 200, 300, 400, 500, 1000,1500, 2000, 2500 or 3000 contiguous nucleotides from the 3' end of the polynucleotide sequence of SEQ ID NO: 8, SEQ ID NO: 81 or SEQ ID NO: 85.

A "variant" , as used herein, is the sequence of the promoter or the sequence of a functional fragment of a promoter containing changes in which one or more nucleotides of the original sequence is deleted, added, and/or substituted, while substantially maintaining promoter function. One or more base pairs can be inserted, deleted, or substituted internally to a promoter. In the case of a promoter fragment, variant promoters can include changes affecting the transcription of a

minimal promoter to which it is operably linked. Variant promoters can be produced, for example, by standard DNA mutagenesis techniques or by chemically synthesizing the variant promoter or a portion thereof. Variant polynucleotides also encompass sequences derived from a mutagenic and recombinogenic procedure such as DNA shuffling. With such a procedure, one or more cis-elements for the promoter can be manipulated to create a new enhancer domain. In this manner, libraries of recombinant polynucleotides are generated from a population of related sequence polynucleotides comprising sequence regions that have substantial sequence identity and can be homologously recombined *in vitro* or *in vivo.* Strategies for such DNA shuffling are known in the art. See, for example, Stemmer (1994) *Proc. Natl. Acad. Sci. USA 91A* 0747-1 0751 ; Stemmer (1994) *Nature* 370:389-391 ; Crameri *et al.* (1997) *Nature Biotech.* 75:436-438; Moore *et al.* (1997) *J. Mol. Biol.* 272:336-347; Zhang *et al. (1997) Proc. Natl. Acad. Sci. USA* 94:4504-4509; Crameri *et al.* (1998) *Nature* 397:288-291 ; and U.S. Patent Nos. 5,605,793 and 5,837,458.

Substitutions, deletions, insertions or any combination thereof can be combined to produce a final construct.

For polynucleotides, naturally occurring variants can be identified with the use of well-known molecular biology techniques, such as, for example, with polymerase chain reaction (PCR) and hybridization techniques as outlined herein. Generally, variants of a particular polynucleotide of the invention will have at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to that particular polynucleotide as determined by sequence alignment programs and parameters described elsewhere herein. A biologically active variant of a polynucleotide of the invention may differ from that sequence by as few as 1-15 nucleic acid residues, as few as 1-10, such as 6-10, as few as 10, 9, 8, 7, 6, 5, 4, 3, 2, or even 1 nucleic acid residue.

The promoter of the present invention may also be a promoter which comprises a nucleotide sequence hybridizable under stringent conditions with the complementary strand of the nucleotide sequence of SEQ ID NO: 8, SEQ ID NO: 81 or SEQ ID NO: 85.

Hybridization of such sequences may be carried out under stringent conditions. The terms "stringent conditions" and "stringent hybridization conditions"

as used herein refer to conditions under which a probe will hybridize to its target sequence to a detectably greater degree than to other sequences (e.g., at least 2-fold over background). Stringent conditions are sequence-dependent and will be different in different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences that are 100% complementary to the probe can be identified (homologous probing). Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that lower degrees of similarity are detected (heterologous probing).

The term "under stringent conditions" means that two sequences hybridize under moderately or highly stringent conditions. More specifically, moderately stringent conditions can be readily determined by those having ordinary skill in the art, e.g., depending on the length of DNA. The basic conditions are set forth by Sambrook et al., Molecular Cloning: A Laboratory Manual, third edition, chapters 6 and 7, Cold Spring Harbor Laboratory Press, 2001 and include the use of a prewashing solution for nitrocellulose filters 5xSSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization conditions of about 50% formamide, 2xSSC to 6xSSC at about 40-50 °C (or other similar hybridization solutions, such as Stark's solution, in about 50% formamide at about 42 °C) and washing conditions of, for example, about 40-60 °C, 0.5-6xSSC, 0.1 % SDS. Preferably, moderately stringent conditions include hybridization (and washing) at about 50 °C and 6xSSC. Highly stringent conditions can also be readily determined by those skilled in the art, e.g., depending on the length of DNA.

Generally, such conditions include hybridization and/or washing at higher temperature and/or lower salt concentration (such as hybridization at about 65 °C, 6xSSC to 0.2xSSC, preferably 6xSSC, more preferably 2xSSC, most preferably 0.2xSSC), compared to the moderately stringent conditions. For example, highly stringent conditions may include hybridization as defined above, and washing at approximately 65-68 °C, 0.2xSSC, 0.1 % SDS. SSPE (IxSSPE is 0.1 5 M NaCI, 10 mM NaH2PO4, and 1.25 mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.1 5 M NaCI and 15 mM sodium citrate) in the hybridization and washing buffers; washing is performed for 15 minutes after hybridization is completed.

Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide.

It is also possible to use a commercially available hybridization kit which uses no radioactive substance as a probe. Specific examples include hybridization with an ECL direct labeling & detection system (Amersham). Stringent conditions include, for example, hybridization at 42 °C for 4 hours using the hybridization buffer included in the kit, which is supplemented with 5% (w/v) Blocking reagent and 0.5 M NaCl, and washing twice in 0.4% SDS, 0.5xSSC at 55 °C for 20 minutes and once in 2xSSC at room temperature for 5 minutes.

Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1.0 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a final wash in 0.1X SSC at 60 to 65°C for a duration of at least 30 minutes. Duration of hybridization is generally less than about 24 hours, usually about 4 to about 12 hours. The duration of the wash time will be at least a length of time sufficient to reach equilibrium.

Specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the T_m (thermal melting point) can be approximated from the equation of Meinkoth and Wahl (1984) *Anal. Biochem. 138:267-284:* $T_m = 81.5°C + 16.6 (\log M) + 0.41 (\%GC) - 0.61 (\%\ form) - 500/L$; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. The T_m is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. T_m is reduced by about 1°C for each 1% of mismatching; thus, T_m, hybridization, and/or wash conditions can be adjusted to

hybridize to sequences of the desired identity. For example, if sequences with ≥90% identity are sought, the T_m can be decreased 10°C. Generally, stringent conditions are selected to be about 5°C lower than the T_m for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3, or 4°C lower than the T_m; moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9, or 10°C lower than the T_m; low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15, or 20°C lower than the T_m. Using the equation, hybridization and wash compositions, and desired T_m, those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a T_m of less than 45°C (aqueous solution) or 32°C (formamide solution), it is preferred to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Acid Probes,* Part I, Chapter 2 (Elsevier, New York); and Ausubel *et al.,* eds. (1995) *Current Protocols in Molecular Biology,* Chapter 2 (Greene Publishing and Wiley-Interscience, New York). See also Sambrook.

In an embodiment of the current invention, isolated sequences that have seed-specific promoter activity and which hybridize under stringent conditions to the soybean sucrose synthase promoter sequence disclosed herein, or to fragments thereof, are encompassed by the present invention. Generally, stringent conditions are selected to be about 5°C lower than the T_m for the specific sequence at a defined ionic strength and pH. However, stringent conditions encompass temperatures in the range of about 1°C to about 20°C lower than the T_m, depending upon the desired degree of stringency as otherwise qualified herein.

It is well understood by those skilled in the art that different terminator sequences may be used for the constructs described in the current invention. Terminators include, but are not limited to, bean phaseolin 3' terminator (WO 2004/071467), *Glycine max* Myb2 3' (US Application No. 12/486793), *Glycine max* kunitz trypsin inhibitor 3' (WO 2004/071467), *Glycine max* BD30 (also called P34) 3' (WO 2004/071467), *Pisum sativum* legumin A2 3' (WO 2004/071467), and *Glycine max* albumin 2S 3' (WO 2004/071467).

In addition, WO 2004/071467 and US Patent No. 7,129,089 describe the further linking together of individual promoter/gene/transcription terminator cassettes in unique combinations and orientations, along with suitable selectable marker cassettes, in order to obtain the desired phenotypic expression. Although this is done mainly using different restriction enzymes sites, one skilled in the art can appreciate that a number of techniques can be utilized to achieve the desired promoter/gene/transcription terminator combination or orientations. In so doing, any combination and orientation of embryo-specific promoter/gene/transcription terminator cassettes can be achieved. One skilled in the art can also appreciate that these cassettes can be located on individual DNA fragments or on multiple fragments where co-expression of genes is the outcome of co-transformation of multiple DNA fragments.

Sequence alignments and percent identity calculations may be determined using a variety of comparison methods designed to detect homologous sequences including, but not limited to, the Megalign® program of the LASERGENE® bioinformatics computing suite (DNASTAR® Inc., Madison, WI). Unless stated otherwise, multiple alignment of the sequences provided herein were performed using the Clustal V method of alignment (Higgins and Sharp (1989) *CABIOS.* 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments and calculation of percent identity of protein sequences using the Clustal V method are KTUPLE=1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. For nucleic acids these parameters are KTUPLE=2, GAP PENALTY=5, WINDOW=4 and DIAGONALS SAVED=4. After alignment of the sequences, using the Clustal V program, it is possible to obtain "percent identity" and "divergence" values by viewing the "sequence distances" table on the same program; unless stated otherwise, percent identities and divergences provided and claimed herein were calculated in this manner.

Alternatively, the Clustal W method of alignment may be used. The Clustal W method of alignment (described by Higgins and Sharp, *CABIOS.* 5:151-153 (1989); Higgins, D. G. et al., *Comput. Appl. Biosci.* 8:189-191 (1992)) can be found in the MegAlign™ v6.1 program of the LASERGENE® bioinformatics computing suite (DNASTAR® Inc., Madison, Wis.). Default parameters for multiple alignment

correspond to GAP PENALTY=10, GAP LENGTH PENALTY=0.2, Delay Divergent Sequences=30%, DNA Transition Weight=0.5, Protein Weight Matrix=Gonnet Series, DNA Weight Matrix=IUB. For pairwise alignments the default parameters are Alignment=Slow-Accurate, Gap Penalty=10.0, Gap Length=0.10, Protein Weight Matrix=Gonnet 250 and DNA Weight Matrix=IUB. After alignment of the sequences using the Clustal W program, it is possible to obtain "percent identity" and "divergence" values by viewing the "sequence distances" table in the same program.

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook, J., Fritsch, E.F. and Maniatis, T. *Molecular Cloning: A Laboratory Manual;* Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter "Sambrook").

Compositions:

A composition of the present invention is a plant comprising in its genome any of the recombinant DNA constructs (including any of the suppression DNA constructs) of the present invention (such as any of the constructs discussed above). Compositions also include any progeny of the plant, and any seed obtained from the plant or its progeny, wherein the progeny or seed comprises within its genome the recombinant DNA construct (or suppression DNA construct). Progeny includes subsequent generations obtained by self-pollination or out-crossing of a plant. Progeny also includes hybrids and inbreds.

In hybrid seed propagated crops, mature transgenic plants can be self-pollinated to produce a homozygous inbred plant. The inbred plant produces seed containing the newly introduced recombinant DNA construct (or suppression DNA construct). These seeds can be grown to produce plants that would exhibit altered oil content or used in a breeding program to produce hybrid seed, which can be grown to produce plants that would exhibit such altered oil content.

The modified seed and grain of the invention can also be obtained by breeding with transgenic plants, by breeding between independent transgenic events, by breeding of plants with one or more alleles (including mutant alleles) of genes encoding the proteins of the invention. Breeding, including introgression of transgenic and mutant loci into elite breeding germplasm and adaptation (improvement) of breeding germplasm to the expression of transgenes and mutant alleles, can be facilitated by methods such as by marker assisted selected breeding.

Embodiments of the current invention include:

In one embodiment, a recombinant DNA construct comprising at least one heterologous polynucleotide encoding a polypeptide selected from the group consisting of an ODP1 polypeptide, a Lec1 polypeptide and a FUSCA3 polypeptide, wherein the at least one polynucleotide is operably linked to a soybean or a *Medicago truncatula* sucrose synthase promoter, wherein expression of said polypeptide in a transgenic soybean seed comprising said recombinant DNA construct results in an increased oil content in the transgenic soybean seed, when compared to a control soybean seed not comprising the recombinant DNA construct.

In another embodiment, a recombinant DNA construct comprising at least one heterologous polynucleotide encoding a polypeptide selected from the group consisting of an ODP1 polypeptide, a Led polypeptide and a FUSCA3 polypeptide, wherein the at least one polynucleotide is operably linked to a seed-specific sucrose synthase promoter from a plant, wherein expression of said polypeptide in a transgenic soybean seed comprising said recombinant DNA construct is expressed in developing seeds in synchrony with oil and protein accumulation, and results in an increased oil content in the transgenic soybean seed, when compared to a control soybean seed not comprising the recombinant DNA construct. The seed-specific sucrose synthase promoter may be from an oilseed plant. The seed-specific sucrose synthase promoter may be from a legume plant.

In another embodiment, said transgenic soybean seed comprising said recombinant DNA construct has normal germination, when compared to a control soybean seed not comprising the recombinant DNA construct.

In another embodiment, said transgenic soybean seed comprising said recombinant DNA construct has a germination rate that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of the observed germination rate, under the same conditions, when compared to a control soybean seed not comprising the recombinant DNA construct.

In another embodiment, the soybean sucrose synthase promoter comprises a nucleic acid sequence selected from the group consisting of: (a) the nucleic acid sequence of SEQ ID NO: 8, (b) a nucleic acid sequence with at least 95% sequence identity to the nucleic acid sequence of SEQ ID NO: 8, (c) a nucleic acid sequence

that hybridizes to SEQ ID NO: 8 under stringent conditions; and (d) a nucleic acid sequence comprising a functional fragment of (a), (b) or (c).

In another embodiment, the soybean sucrose synthase promoter is an allele of SEQ ID NO: 8.

In another embodiment, the soybean sucrose synthase promoter differs from SEQ ID NO: 8 in at least one way as described in FIG. 4.

In another embodiment, the *Medicago truncatula* sucrose synthase promoter comprises a nucleic acid sequence selected from the group consisting of: (a) the nucleic acid sequence of SEQ ID NO: 81 or SEQ ID NO: 85, (b) a nucleic acid sequence with at least 95% sequence identity to the nucleic acid sequence of SEQ ID NO: 81 or SEQ ID NO:85, (c) a nucleic acid sequence that hybridizes to SEQ ID NO: 81 or SEQ ID NO:85 under stringent conditions; and (d) a nucleic acid sequence comprising a functional fragment of (a), (b) or (c).

In another embodiment, the *Medicago truncatula* sucrose synthase promoter is an allele of SEQ ID NO: 81 or SEQ ID NO: 85.

In another embodiment, the *Medicago truncatula* sucrose synthase promoter differs from SEQ ID NO:81 in at least one of the following ways: nt 67 is a T, nt 489 is a C, nts 553-555 (TTG) are deleted, nt 629 is an A, nt 649 is a C, nt 715 is an A, nt 784 is a C, nt 800 is a G, nt 893 is a G, nt 1166 is an A, nt 1535 is deleted (T), nt 1700 is a G, nt 1718 is a C, nt 1857-1880 are deleted (ATTTTAGAATATGCAATAAAATTG ; SEQ ID NO: 101), nt 1953 is a G, nt 2038 is deleted (A), there is a 25 bp insertion between nt 2224 and 2225 (AGGCTTGAGGAATAAGATAAGACTTGT; SEQ ID NO: 102),an A is inserted between nt 2225 and 2226, nt 2421 is a G, a C is inserted between nt 2734 and 2735 and nt 2881 is a T.

In another embodiment, the ODP1 polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95% or 100% identity to SEQ ID NO: 30 or SEQ ID NO: 70.

In another embodiment, the ODP1 polypeptide is an allele of SEQ ID NO: 30 or SEQ ID NO: 70.

In another embodiment, the ODP1 polypeptide comprises two APETALA2 (AP2) domains.

ODP1 sequences have also been disclosed in PCT Publication Number WO2010114989, US patent number US7157621, and US20100242138, each of which are incorporated herein by reference.

In one embodiment, the Lec1 polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95% or 100% identity to SEQ ID NO: 17, 20, 25 or 65.

In another embodiment, the Lec1 polypeptide is an allele of SEQ ID NO: 17, 20, 25 or 65.

In another embodiment, the Led polypeptide comprises the amino acid sequence of SEQ ID NO:77.

Led sequences have also been disclosed in the following: US Patent No. US7294754; US Patent No. US6825397; US Patent No. US7812216; US Publication Numbers US20100319086, US20110162101, US20110099665 and US20080313770; and US Patent No. US7317146; each of which is incorporated herein by reference.

In one embodiment, the FUSCA3 polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95% or 100% identity to SEQ ID NO: 32, 38, 45 or 49.

In another embodiment, the FUSCA3 polypeptide is an allele of SEQ ID NO: 32, 38, 45 or 49.

In another embodiment, the recombinant construct further comprises a second heterologous polynucleotide encoding a DGAT polypeptide operably linked to a seed-specific promoter. In one embodiment, the second polynucleotide is a DGAT1 polypeptide. In one embodiment, the DGAT1 polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95% or 100% sequence identity to SEQ ID NO: 55.

In another embodiment, the DGAT1 polypeptide is an allele of SEQ ID NO: 55.

In one embodiment, the second polynucleotide is a DGAT2 polypeptide. In one embodiment, the DGAT2 polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95% or 100% sequence identity to SEQ ID NO: 60.

In another embodiment, the DGAT2 polypeptide is an allele of SEQ ID NO: 60.

DGAT sequences have also been described in the following: US Publication Numbers US20080295204, US200902931 52, US200902931 5 1, US200901 58460, US200902931 50 and US20090291479; US Patent Numbers. US7273746 and US7267976; and PCT Publication No. WO201 1062748; each of which is incorporated herein by reference.

In one embodiment, a plant comprising a first recombinant DNA construct comprising a soybean or a *Medicago truncatula* sucrose synthase promoter operably linked to a first heterologous polynucleotide encoding a first polypeptide selected from the group consisting of an ODP1 polypeptide, a Lec1 polypeptide and a FUSCA3 polypeptide and a second recombinant DNA construct comprising a seed-specific promoter operably linked to a second heterologous polynucleotide encoding a DGAT polypeptide, wherein co-expression of said first polypeptide and said second polypeptide in a transgenic soybean seed comprising said first and said second recombinant DNA constructs results in an increased oil content in the transgenic seed, when compared to a control seed comprising only one, but not both, of the first and the second recombinant DNA constructs. The plant and the seed may be an oilseed plant and seed. The plant and the seed may be a soybean plant and seed.

One embodiment of the invention is a method of increasing oil content of a soybean seed, the method comprising the steps of: (a) introducing into a regenerable soybean cell one or more recombinant DNA constructs as described herein; (b) regenerating a transgenic plant from the regenerable soybean cell of (a) wherein the transgenic plant comprises the recombinant DNA construct; and (c) selecting a transgenic plant of step (b), or a transgenic progeny plant from the transgenic plant of step (b), wherein seed of the transgenic plant or the transgenic progeny plant comprises the recombinant DNA construct and wherein expression of said one or more polypeptides in the transgenic soybean seed comprising said recombinant DNA construct results in an increased oil content in the transgenic soybean seed, when compared to a control soybean seed not comprising said one or more recombinant DNA constructs. The percent oil content of the transgenic soybean seed may be at least 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14% or 15%.

One embodiment of the invention is a method of increasing oil content of a soybean seed, the method comprising the steps of: (a) introducing into a regenerable soybean cell a first recombinant DNA construct comprising a soybean or a *Medicago truncatula* sucrose synthase promoter operably linked to a first heterologous polynucleotide encoding a first polypeptide selected from the group consisting of an ODP1 polypeptide, a Lec1 polypeptide and a FUSCA3 polypeptide and a second recombinant DNA construct comprising a seed-specific promoter operably linked to a second heterologous polynucleotide encoding a DGAT polypeptide; (b) regenerating a transgenic plant from the regenerable soybean cell of (a) wherein the transgenic plant comprises the first and the second recombinant DNA constructs; and (c) selecting a transgenic plant of step (b), or a transgenic progeny plant from the transgenic plant of step (b), wherein seed of the transgenic plant or the transgenic progeny plant comprises the first and the second recombinant DNA constructs and wherein co-expression of said first polypeptide and said second polypeptide in a transgenic soybean seed comprising said first and said second recombinant DNA constructs results in an increased oil content in the transgenic soybean seed, when compared to a control soybean seed comprising only one, but not both, of the first and the second recombinant DNA constructs. The percent oil content of the transgenic soybean seed may be at least 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13% , 14% or 15%.

One embodiment of the invention is a method of increasing oil content of a soybean seed, the method comprising the steps of: (a) introducing into a first regenerable soybean cell a first recombinant DNA construct comprising a soybean or a *Medicago truncatula* sucrose synthase promoter operably linked to a first heterologous polynucleotide encoding a first polypeptide selected from the group consisting of an ODP1 polypeptide, a Lec1 polypeptide and a FUSCA3 polypeptide; (b) regenerating a first transgenic plant from the first regenerable soybean cell of (a) wherein the transgenic plant comprises the first recombinant DNA construct; (c) introducing into a second regenerable soybean cell a second recombinant DNA construct comprising a seed-specific promoter operably linked to a second heterologous polynucleotide encoding a DGAT polypeptide; (d) regenerating a second transgenic plant from the second regenerable soybean cell of (c) wherein the transgenic plant comprises the second recombinant DNA construct; (e) crossing

the first transgenic plant with the second transgenic plant; and (f) selecting a third transgenic plant from the cross of step (e), wherein seed of the third transgenic plant comprises the first and the second recombinant DNA constructs and wherein co-expression of said first polypeptide and said second polypeptide in said transgenic soybean seed results in an increased oil content in the transgenic soybean seed, when compared to a control soybean seed comprising only one, but not both, of the first and the second recombinant DNA constructs. The percent oil content of the transgenic soybean seed may be at least 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13% , 14% or 15%.

One embodiment of the invention is a method of increasing oil content of a soybean seed, the method comprising the steps of:

(a) crossing the following:

(i) a first transgenic soybean plant comprising a first recombinant DNA construct comprising a soybean or a *Medicago truncatula* sucrose synthase promoter operably linked to a first heterologous polynucleotide encoding a first polypeptide selected from the group consisting of an ODP1 polypeptide, a Lec1 polypeptide and a FUSCA3 polypeptide; with

(ii) a second transgenic soybean plant comprising a second recombinant DNA construct comprising a seed-specific promoter operably linked to a second heterologous polynucleotide encoding a DGAT polypeptide; and

(b) selecting a third transgenic plant from the cross of step (a), wherein seed of the third transgenic plant comprises the first and the second recombinant DNA constructs and wherein co-expression of said first polypeptide and said second polypeptide in said transgenic soybean seed results in an increased oil content in the transgenic soybean seed, when compared to a control soybean seed comprising only one, but not both, of the first and the second recombinant DNA constructs. The percent oil content of the transgenic soybean seed may be at least 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13% , 14% or 15%.

In one embodiment, a transgenic soybean seed comprising a recombinant DNA construct comprising a soybean or a *Medicago truncatula* sucrose synthase promoter operably linked to a heterologous polynucleotide encoding a polypeptide selected from the group consisting of an ODP1 polypeptide, a Lec1 polypeptide and a FUSCA3 polypeptide, wherein expression of said polypeptide in said transgenic

soybean seed comprising said recombinant DNA construct results in an increased oil content in the transgenic seed, when compared to a control soybean seed not comprising the recombinant DNA construct.

In one embodiment, the percent increase in oil content is at least 10%. In additional embodiments, the percent increase is at least 20%, 30%, 40%, 50%, 60%, 70% or 80%.

In one embodiment, a transgenic soybean seed comprising a first recombinant DNA construct comprising a soybean or a *Medicago truncatula* sucrose synthase promoter operably linked to a first heterologous polynucleotide encoding a first polypeptide selected from the group consisting of an ODP1 polypeptide, a Lec1 polypeptide and a FUSCA3 polypeptide and a second recombinant DNA construct comprising a seed-specific promoter operably linked to a second heterologous polynucleotide encoding a DGAT polypeptide, wherein co-expression of said first polypeptide and said second polypeptide in a transgenic soybean seed comprising said first and said second recombinant DNA constructs results in an increased oil content in the transgenic seed, when compared to a control soybean seed comprising only one, but not both, of the first and the second recombinant DNA constructs.

In one embodiment, the percent increase in oil content is at least 10%. In additional embodiments, the percent increase is at least 20%, 30%, 40%, 50%, 60%, 70% or 80%.

In the above embodiments, the control seed comprising only one, but not both, of the first and the second recombinant DNA constructs may be either: (a) a control seed comprising the first recombinant DNA construct but not comprising the second recombinant DNA construct, or (b) a control seed comprising the second recombinant DNA construct but not comprising the first recombinant DNA construct.

Additional embodiments include a vector, cell, plant, or seed comprising one or more of the recombinant DNA constructs described in the present invention.

The invention also encompasses regenerated, mature and fertile transgenic plants comprising one or more of the recombinant DNA constructs described above, transgenic seeds produced therefrom, T1 and subsequent generations. The transgenic plant cells, tissues, plants, and seeds may comprise at least one recombinant DNA construct of interest.

In another embodiment, the plant or seed comprising the recombinant DNA construct described herein may be at least one selected from the group consisting of: a dicotyledonous plant or seed; a legume plant or seed; an oilseed plant or seed; and a soybean plant or seed.

In another embodiment, the transgenic soybean seeds of the invention may be processed to yield soy oil, soy products and/or soy by-products. Soy products and by-products are described in US Patent No. 8,143,473, herein incorporated by reference.

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook, J., Fritsch, E.F. and Maniatis, T. *Molecular Cloning: A Laboratory Manual;* Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter "Sambrook").

EXAMPLES

The present invention is further illustrated in the following Examples, in which parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

EXAMPLE 1

Identification and Cloning of the Soy Sucrose Synthase Promoter

The *Arabidopsis* Sucrose Synthase 2 gene has been described previously (PCT Publication No. WO 201 0/1 14989) and the nucleotide and amino acid sequences are set forth in SEQ ID NO: 1 and SEQ ID NO: 2, respectively. A soybean homolog of the *Arabidopsis* Sucrose Synthase 2 gene was identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al., *J. Mol. Biol.* 215:403-410 (1993)) searches for similarity to sequences contained in the Soybean Genome Project, DoE Joint Genome Institute "Glymal .01" gene set. Specifically,

the *Arabidopsis* Sucrose Synthase 2 amino acid sequence (SEQ ID NO: 2) was used with the TBLASTN algorithm provided by National Center for Biotechnology Information (NCBI) with default parameters except the Filter Option was set to OFF.

The soybean homolog to the *Arabidopsis* Sucrose Synthase 2 gene identified corresponded to Glyma1 3g17420 and the predicted genomic, cDNA, CDS and corresponding amino acid sequences from Glyma are set forth in SEQ IDs NO: 3-6, respectively.

Soybean cDNA libraries from developing soybean (e.g. cDNA library sdp3c) were prepared, clones sequenced and sequence was analyzed as described in U.S. Patent No. 7,157,621 (the contents of which are herein incorporated by reference). A similar TBLASTN search against sequences from these soybean cDNA libraries identified a cDNA (EST sdp3c.pk014.n1 8) with a 5' end that differed from that predicted in the Glyma1 3g17420 cDNA sequence (SEQ ID NO: 4) in that the intron was splice differently. The sequence for the 5' end of EST sdp3c.pk014.n1 8 that was sequenced is set forth in SEQ ID NO: 7. The CDS from sdp3c.pk014.n18 appears to be the same as that for Glyma1 3g17420 (SEQ ID NO: 5). The soybean homolog to the *Arabidopsis* sucrose synthase 2 gene set forth in SEQ ID NO: 5 was named GmSus.

A region of genomic DNA upstream of the start codon of GmSus (SEQ ID NO: 5) was identified from the Glyma database by conducting BLAST searches as a promoter region and the sequence is set forth in SEQ ID NO: 8. FIG. 1 shows a schematic of the GmSus promoter region.

The identified GmSus promoter region encodes the 5' UTR from the cDNA transcript (bp 2101 to 3191 from SEQ ID NO: 8) as well as an intron (bp 2134 to 3168 from SEQ ID NO: 8). The 5' UTR region and intron was included as part of the promoter region as it contained an AW box (AW2 in Fig. 1) from bp 2662 to 2675 of SEQ ID NO: 8 within the intron. Another AW box (AW1 in Fig. 1) occurs from bp 616 to bp 629 of SEQ ID NO: 8. AW boxes consist of the nucleotide sequence [CnTnG](n)7[CG] (SEQ ID NO:78), where n is any nucleotide, and AW boxes are important binding sites for transcription factors such as wril in *Arabidopsis* (Maeo, K et al. (2009) *Plant Journal* 60(3): 476-487).

Genomic DNA was isolated from leaves of approximately 4 week old soy 93B86 plants using the DNEASY® Plant Mini Kit (Qiagen, Valencia, CA) and

following the manufacture's protocol. The GmSus promoter region (SEQ ID NO:8) was PCR-amplified from 93B86 genomic DNA using oligonucleotides GmSuSyProm-5 (SEQ ID NO:9) and GmSuSyProm-5 (SEQ ID NO:1 0) with the PHUSION™ High-Fidelity DNA Polymerase (Cat. No. F553S, Finnzymes Oy, Finland), following the manufacturer's protocol. The resulting DNA fragment was cloned into the pCR®-BLUNT® cloning vector using the ZERO BLUNT® PCR Cloning Kit (Invitrogen Corporation), following the manufacturer's protocol, to produce pLF284 (SEQ ID NO:1 1).

The EcoRI fragment of pLF284 (SEQ ID NO: 11), containing the GmSus promoter region (called GmSusPro), was cloned into the EcoRI site of pNEB1 93 (New England BioLabs, Beverly, MA) to produce pKR1 963 (SEQ ID NO: 12).

Plasmid pKR1543, which was previously described in PCT Publication No. WO 201 1/079005 (published on June 30, 201 1, the contents of which are herein incorporated by reference), was digested with NotI/XbaI and the fragment containing the Leg terminator, previously described in PCT Publication No. WO 2004/071467 (published on August 26, 2004, the contents of which are herein incorporated by reference) was cloned into the NotI/XbaI fragment of pKR1 963 (SEQ ID NO: 12), containing the GmSusPro, to produce pKR1 964 (SEQ ID NO: 13).

The BsiWI fragment of pKR1964 (SEQ ID NO: 13), containing the GmSusPro, was cloned into the BsiWI site of pKR325, previously described in PCT Publication No. WO 2004/071467, to produce pKR1 965 (SEQ ID NO: 14). Plasmid pKR1 965 contains a NotI site flanked by the GmSusPro and the Leg terminator as well as the hygromycin B phosphotransferase gene [Gritz, L. and Davies, J. (1983) *Gene* 25:1 79-188], flanked by the T7 promoter and transcription terminator, a bacterial origin of replication (ori) for selection and replication in *E. coli* and the hygromycin B phosphotransferase gene, flanked by the 35S promoter [Odell et al., (1985) *Nature* 3 13:81 0-81 2] and NOS 3' transcription terminator [Depicker et al., (1982) *J. Mol. Appl. Genet.* 1:561 :570] (35S/hpt/NOS3' cassette) for selection in soybean. In this way, polynucleotides (e.g., protein-coding regions) flanked by NotI sites can be cloned into the NotI site of pKR1 965 (SEQ ID NO: 14) and expressed in soy.

EXAMPLE 2

Cloning Lec1, Fusca3 and ODP1 Homologs from Soybean

GmLec1 from cDNA:

Soybean cDNA library se2, derived from developing soybean seeds (Glycine max L.) harvested at 13 days after flowering (DAF) was prepared, cDNA clones were sequenced and the sequence was analyzed as described in U.S. Patent No. 7,157,621.

A cDNA clone (se2.11d12) was identified from cDNA library se2 with homology to transcription factor LEAFY COTYLEDON 1 (Lec1) (Lotan, T. et al. (1998) *Cell* 93(7): 1195-1205).

The cDNA clone was fully sequenced by methods described in U.S. Patent No. 7,157,621 and its sequence is set forth in SEQ ID NO: 15. This clone appears to have 2 separate cDNA clones inserted into it but the sequence from 38-718 bp is 100% identical to the coding sequence of led b (NCBI Accession # EU088289.1 GI:158525282) and to the CDS of Glymal 7g00950 based on a blast comparison. The coding sequence from clone se2.11d12, which corresponds to that of Glymal 7g00950, is shown in SEQ ID NO:16 and the encoded amino acid sequence is shown in SEQ ID NO:17.

A separate cDNA clone (se1.pk0042.d8) identified from cDNA library se1, derived from developing soybean seeds (Glycine max L.) harvested at 6-10 DAF and described in U.S. Patent No. 7,157,621, also contained a led homolog as determined by blast analysis. The full insert sequence of se1.pk0042.d8 is shown in SEQ ID NO:18. The sequence from cDNA clone se1.pk0042.d8 is 99% identical to the coding sequence of led a (NCBI Accession # EU088288.1 GI:158525280) and 100% identical to the CDS of Glyma07g39820 based on a blast comparison. The coding sequence from clone se1.pk0042.d8 appears to be 2 nt short of the ATG but is shown in SEQ ID NO: 19 with the correct start as compared to Glyma07g39820. The corresponding encoded amino acid sequence is shown in SEQ ID NO: 20.

DNA was also prepared from an aliquot of cDNA library se2 using the QIAprep® Spin Miniprep Kit (Qiagen Inc., Valencia, CA) following the manufacturer's protocol. The DNA from the cDNA library was used as template in a PCR reaction using oligonucleotides SA275 (SEQ ID NO: 21) and SA276 (SEQ ID NO: 22), using the "Platinum"-brand Taq DNA polymerase (Life Technologies),

following the manufacturer's protocol. The PCR fragment was cloned using the pCR®843W/TOPO® TA Cloning Kit (Invitrogen Corporation) to produce plasmid Glyma1 7g00950/pCR8/GW/TOPO (SEQ ID NO: 23). The CDS from the PCR product contained in Glyma17g00950/pCR8/GW/TOPO (SEQ ID NO: 23), named GmLecl , is set forth in SEQ ID NO: 24 and the corresponding amino acid sequence of GmLec1 is set forth in SEQ ID NO: 25. It should be noted that both the CDS and amino acid sequence of GmLec1 are different than those corresponding to either Glyma1 7g00950 or Glyma07g39820. An alignment comparing the amino acid sequences of Glyma17g00950 (SEQ ID NO: 17), Glyma07g39820 (SEQ ID NO: 20) and GmLec1 (SEQ ID NO: 25) is shown in FIG. 2.

GmLed gene was PCR-amplified from Glyma1 7g00950/pCR8/GW/TOPO (SEQ ID NO: 23) using oligonucleotides Gmlec-5 (SEQ ID NO:26) and Gmlec-3 (SEQ ID NO:27) with the PHUSION™ High-Fidelity DNA Polymerase (Cat. No. F553S, Finnzymes Oy, Finland), following the manufacturer's protocol. The PCR fragment was cloned into the pCR®-BLUNT® cloning vector using the ZERO BLUNT® PCR Cloning Kit (Invitrogen Corporation), following the manufacturer's protocol, to produce pLF275 (SEQ ID NO: 28).

NotI fragment containing GmODPI:

The soybean ODP (GmODPI) is described in U.S. Patent No. 7,1 57,621 . The cloning of GmODPI with flanking NotI sites into plasmid KS334 was previously described in PCT Publication No. WO 201 0/1 14989 (published on October 7, 201 0, the contents of which are herein incorporated by reference). It should be noted that there is a typo in the map of KS334 (SEQ ID NO: 14 in WO201 0/1 14989) and that there should be an additional 3 nucleotides (TGA) at position 1237 to form a stop codon and end the CDS in KS334. The CDS and amino acid sequence of GmODPI from WO201 0/1 14989 are set forth here in SEQ ID NO: 29 and SEQ ID NO: 30, respectively.

PCR GmFusca3-1 & GmFusca3-2 from cDNA:

Based on BLAST analysis of the soy genome sequence database, Glyma1 6g05480 was identified with homology to the Fusca3 transcription factor (Luerssen, H. et al. (1998) *Plant Journal,* 15(6): 755-764). The predicted CDS and amino acid sequence for Glyma16g05480 as predicted in the Glyma database are shown in SEQ ID NO: 31 and SEQ ID NO: 32, respectively.

DNA prepared from an aliquot of cDNA library se2 (described above) was used as template in a PCR reaction using oligonucleotides SA278 (SEQ ID NO: 33) and SA279 (SEQ ID NO: 34), using the "Platinum"-brand Taq DNA polymerase (Life Technologies), following the manufacturer's protocol. The PCR fragment was cloned using the pCR®843WTOPO® TA Cloning Kit (Invitrogen Corporation) to produce plasmid Glymal 6g05480/pCR8/GW/TOPO (SEQ ID NO: 35). The cDNA insert in Glymal 6g05480/pCR8/GW/TOPO (SEQ ID NO: 35) was sequenced and the sequence is set forth in SEQ ID NO: 36.

The cDNA insert (SEQ ID NO: 36) was analyzed by BLAST and was found to be different than what was predicted for Glymal 6g05480 (SEQ ID NO: 31). The sequence also did not code for a perfect CDS as early stop codons within were found. Comparison of the cDNA insert sequence to the genome sequence in Glyma revealed the 3' end of cDNA insert to be 100% identical to the predicted coding sequence of Glymal 9g27340. The predicted CDS and corresponding amino acid sequence of Glymal 9g27340 from the Glyma database are set forth in SEQ ID NO: 37 and SEQ ID NO: 38, respectively.

The cDNA insert is larger than the predicted CDS for Glyma 19g27340 (SEQ ID NO: 38) and has an additional 1193 bp at the 5' end. Further comparison of the cDNA insert to genomic sequence upstream of the CDS from Glymal 9g27340 (SEQ ID NO: 37) reveals 100% identity, with the exception of a single nucleotide coming from oligo SA278 (SEQ ID NO: 33). The full genomic DNA sequence, from the soy genome database, upstream of and including Glymal 9g27340 is set forth in SEQ ID NO: 39.

The cDNA insert (SEQ ID NO: 36) did not code for a complete CDS and it was determined that either an unspliced intron sequence was contained with the cDNA sequence or that an alternate start codon was present. The full length sequence from the cDNA insert (called GmFusca3-2), which may contain introns, was PCR-amplified using oligonucleotides GmFusca3-1 -5 (SEQ ID NO: 40) and GmFusca3-3 (SEQ ID NO: 41) with the PHUSION™ High-Fidelity DNA Polymerase (Cat. No. F553S, Finnzymes Oy, Finland), following the manufacturer's protocol.

The PCR fragment was cloned into the pCR®-BLUNT® cloning vector using the ZERO BLUNT® PCR Cloning Kit (Invitrogen Corporation), following the manufacturer's protocol, to produce pLF283 (SEQ ID NO: 42).

The full length cDNA of the resulting PCR product for GmFusca3-2 is shown in SEQ ID NO: 43 and is identical to the original cDNA (SEQ ID NO: 36) except that nucleotide 17 has been changed from C to T to agree with that predicted in Glyma1 9g27340 genomic DNA sequence. A putative spliced CDS as well as the corresponding encoded amino acid sequence for GmFusca3-2 is shown in SEQ ID NO: 44 and SEQ ID NO: 45, respectively.

A second shorter ORF sequence contained within the cDNA insert (SEQ ID NO: 36), called GmFusca3-1 , was PCR-amplified using oligonucleotides GmFusca3-2-5 (SEQ ID NO: 46) and GmFusca3-3 (SEQ ID NO: 41) with the PHUSION™ High-Fidelity DNA Polymerase (Cat. No. F553S, Finnzymes Oy, Finland), following the manufacturer's protocol.

The resulting PCR fragment containing Fusca3-1 was cloned into the pCR®-BLUNT® cloning vector using the ZERO BLUNT® PCR Cloning Kit (Invitrogen Corporation), following the manufacturer's protocol, to produce pLF282 (SEQ ID NO: 47).

The full sequence contains no unspliced introns and the coding sequence as well as the corresponding encoded amino acid sequence of GmFusca3-1 is shown in SEQ ID NO: 48 and 49, respectively.

An alignment comparing the amino acid sequences for Glyma1 6g05480 (SEQ ID NO: 32) and Glyma1 9g27340 (SEQ ID NO: 38), as predicted in the Glyma database, along with the predicted spliced sequence for GmFusca3-2 (SEQ ID NO: 45) and for GmFusca3-1 (SEQ ID NO: 49) is shown in Fig. 3.

EXAMPLE 3

Expressing GmLecl , GmODPI , GmFusca-3-1 and GmFusca3-2 in Soybean Embryos under Control of the GmSus Promoter

The NotI fragment of pLF275 (SEQ ID NO: 28), containing GmLec1, the NotI fragment of KS334, containing GmODPI , the NotI fragment of pLF282 (SEQ ID NO: 47), containing GmFusca3-1 , and the NotI fragment of pLF283 (SEQ ID NO: 42), containing GmFusca3-2 were cloned into the NotI site of pKR1 965 (SEQ ID NO: 14) to produce pKR1 968 (SEQ ID NO: 50), pKR1 971 (SEQ ID NO: 51), pKR1 969 (SEQ ID NO: 52) and pKR1 970 (SEQ ID NO: 53), respectively. In this way, the respective transcription factors could be expressed behind the soy sucrose synthase promoter (GmSusPro). Plasmid pKR278, previously described in PCT Publication No. WO

2008/147935 (published on October 13, 2009, the contents of which are incorporated by reference), and containing no transcription factor, but having the hygromycin selectable marker, was used as a negative control.

DNA from plasmids pKR1968 (SEQ ID NO: 50), pKR1971 (SEQ ID NO: 51), pKR1969 (SEQ ID NO: 52), pKR1970 (SEQ ID NO: 53) and pKR278 was prepared for particle bombardment into soybean embryogenic suspension culture and transformed exactly as described previously in PCT Publication No. WO 2008/147935. Soybean embryogenic suspension culture was initiated, grown, maintained and bombarded and events were selected and matured on SHaM media also exactly as described in PCT Publication No. WO 2008/147935. A summary of genes, plasmids and model system experiment ("MSE") numbers is shown in Table 1.

TABLE 1

Summary of Genes, Plasmids and Experiments

Experiment	Plasmid	Gene	SEQ ID NO	
			nt	aa
MSE 2863	pKR1968	GmLec1	24	25
MSE 2864	pKR1969	GmFusca3-1	48	49
MSE 2865	pKR1970	GmFusca3-2	44	45
MSE 2866	pKR1971	GmODP1	29	30
MSE 2867	pKR278	Empty Vector Control	-	-

Approximately 10-20 matured embryos from each of approximately 30 events per bombardment experiment were lyophilized, ground, oil content was measured by NMR and fatty acid profile was evaluated by FAME-GC analysis exactly as described in PCT Publication No. WO 2008/147935. The results for oil content and

fatty acid profile for each event as well as the average of all events (Avg.) and average for the top 5 events having highest oil content (Top5 Avg.) are shown in Table 2.

In Table 2, results are sorted based on oil content from highest to lowest. In Table 2, oil content is reported as a percent of total dry weight (% Oil) and fatty acid content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids.

TABLE 2

Summary of Oil Content and Fatty Acid Profiles for Events Expressing GmLecl , GmFusca3-1 , GmFusca3-2, GmODPI or Empty Vector Control

	% oil	16:0	18:0	18:%1	18:2	18:3
2863-3	9.9	15.6	7.0	17.9	48.3	11.2
2863-21	9.3	14.7	8.8	18.5	46.5	11.5
2863-24	8.6	15.5	7.9	17.1	46.1	13.4
2863-13	8.2	17.1	5.9	16.3	46.4	14.4
2863-6	7.7	15.3	8.6	18.9	44.0	13.3
2863-29	7.6	15.8	9.0	19.1	42.3	13.8
2863-1 1	7.4	15.8	8.1	18.4	44.2	13.5
2863-30	7.1	15.9	5.7	20.5	43.8	14.1
2863-23	7.1	16.5	6.3	21.0	42.1	14.1
2863-7	6.8	15.9	7.8	16.2	45.5	14.6
2863-22	6.6	15.7	7.7	18.4	43.9	14.3
2863-25	6.4	14.6	6.5	20.6	43.1	15.2
2863-5	6.4	16.7	6.2	19.0	43.2	15.0
2863-19	6.2	16.2	5.7	20.4	42.7	15.1
2863-8	6.1	15.9	9.7	18.7	41.6	14.2
2863-14	5.9	15.8	8.3	16.9	44.1	14.9
2863-10	5.8	17.2	7.1	17.4	43.9	14.5
2863-2	5.7	16.7	5.7	19.8	41.9	16.0
2863-1	5.6	17.0	6.1	20.1	41.9	14.9
2863-9	5.3	16.6	8.7	18.9	41.5	14.3
2863-26	5.3	15.2	8.3	16.4	43.9	16.2
2863-28	5.3	17.2	4.5	14.9	46.3	17.1
2863-27	5.0	17.5	5.6	12.9	48.1	16.0
2863-4	5.0	16.9	5.6	18.9	42.4	16.2
2863-20	4.9	16.3	6.0	20.1	42.4	15.2
2863-16	4.7	17.9	5.0	14.1	45.9	17.1
2863-17	4.2	18.1	4.1	12.7	46.1	19.1
2863-15	3.2	19.3	4.6	15.1	42.2	18.8
2863-12	3.2	17.6	5.1	15.3	43.5	18.5

2863-18	2.5	17.3	5.6	17.0	37.8	22.4
Avg.	6.1	16.5	6.7	17.7	43.9	15.3
Top5 Avg.	8.7	15.6	7.6	17.7	46.2	12.7
2864-10	7.6	14.9	6.2	16.4	46.5	15.9
2864-15	7.6	15.0	9.2	18.6	44.3	12.9
2864-25	7.5	15.9	5.5	20.3	44.1	14.2
2864-12	7.3	17.3	4.9	13.4	49.8	14.5
2864-18	7.2	15.2	8.6	18.1	44.5	13.6
2864-6	6.9	15.3	8.7	18.6	42.7	14.8
2864-26	6.8	16.2	7.3	16.9	45.1	14.5
2864-7	6.8	14.8	8.1	17.8	43.8	15.4
2864-28	6.2	17.6	4.5	11.2	50.4	16.4
2864-19	6.0	15.6	9.4	18.8	41.6	14.6
2864-1	5.9	17.1	6.8	14.7	46.3	15.2
2864-17	5.8	16.8	6.9	22.0	41.4	12.9
2864-2	5.8	16.6	5.0	20.7	43.4	14.5
2864-9	5.7	17.2	5.8	12.7	47.1	17.2
2864-22	5.6	16.6	6.3	13.8	47.3	16.0
2864-4	5.6	16.0	7.6	22.1	40.6	13.8
2864-27	5.0	15.8	10.0	20.8	39.2	14.3
2864-3	4.9	17.4	6.5	20.7	39.8	15.6
2864-1 1	4.6	15.4	5.3	17.4	44.2	17.8
2864-30	4.4	17.4	6.7	15.2	43.2	17.5
2864-29	4.1	17.2	6.8	15.5	42.0	18.5
2864-8	4.0	16.9	4.9	18.4	42.1	17.7
2864-31	3.8	18.1	4.9	13.5	44.4	19.1
2864-14	3.7	17.1	5.5	18.5	42.4	16.5
2864-24	3.6	17.4	5.8	18.8	39.7	18.4
2864-5	3.5	16.2	7.7	19.0	43.6	13.5
2864-21	3.3	16.4	4.6	14.4	44.2	20.4
2864-13	2.9	17.6	6.0	18.6	38.8	19.1
2864-23	2.6	18.4	5.1	13.3	41.7	21.5
2864-20	2.5	17.9	4.7	13.5	41.8	22.2
2864-16	2.1	16.0	6.2	13.2	43.9	20.6
Avg.	5.1	16.5	6.5	17.0	43.5	16.4
Top5 Avg.	7.5	15.7	6.9	17.3	45.9	14.2
2865-7	7.6	16.5	5.6	20.1	45.0	12.7
2865-24	5.9	17.6	4.1	13.9	50.5	13.9
2865-29	5.6	17.1	4.1	14.5	47.8	16.6
2865-14	5.1	16.1	6.2	19.6	42.5	15.6
2865-27	5.1	19.3	4.0	13.7	48.2	14.8
2865-23	5.0	18.9	4.1	15.8	45.9	15.3
2865-8	4.9	16.9	6.2	16.1	47.5	13.3
2865-25	4.8	18.3	4.1	15.2	46.6	15.8

2865-21	4.7	18.4	4.4	15.3	47.0	14.9
2865-1	4.5	18.9	4.2	14.4	46.8	15.8
2865-13	4.3	19.3	4.1	14.5	47.9	14.3
2865-12	4.3	17.1	4.8	15.8	43.0	19.3
2865-20	4.1	16.8	4.1	14.6	47.6	16.9
2865-28	3.6	18.4	5.6	20.2	42.1	13.7
2865-18	3.4	19.2	4.7	14.9	45.0	16.2
2865-1 1	3.3	16.8	5.5	18.2	45.1	14.5
2865-30	3.0	15.5	5.3	15.5	43.3	20.5
2865-6	2.9	17.2	5.5	18.1	41.2	18.1
2865-15	2.9	19.2	4.2	13.2	44.7	18.6
2865-5	2.8	18.6	4.6	12.2	44.1	20.5
2865-22	2.4	19.8	5.1	15.6	43.4	16.0
2865-10	2.3	18.0	5.4	19.2	42.8	14.6
2865-9	2.1	19.4	4.4	12.0	41.1	23.1
2865-2	2.0	18.7	4.4	13.3	43.8	19.8
2865-3	1.9	18.0	5.5	16.0	43.0	17.4
2865-19	1.6	17.9	5.3	14.0	42.7	20.1
2865-4	1.4	17.9	4.5	11.7	44.5	21.5
2865-16	1.3	18.2	5.5	12.9	41.0	22.3
2865-17	1.1	17.7	5.4	17.9	37.3	21.7
Avg.	3.6	18.0	4.9	15.5	44.5	17.2
Top5 Avg.	5.9	17.3	4.8	16.4	46.8	14.7
2866-10	9.8	19.0	6.3	19.8	44.6	10.3
2866-23	9.6	15.5	6.2	22.1	45.2	11.0
2866-12	8.4	13.5	7.0	23.3	45.1	11.1
2866-13	8.1	16.0	5.6	21.6	44.2	12.6
2866-5	8.1	16.7	5.7	24.3	42.5	10.8
2866-1	7.8	15.6	7.1	26.0	40.1	11.2
2866-9	6.6	15.5	8.5	29.6	36.0	10.4
2866-3	6.6	15.4	8.9	28.9	37.0	9.7
2866-7	6.6	15.7	8.9	20.0	42.2	13.1
2866-18	6.5	15.8	8.7	20.3	42.7	12.5
2866-6	6.3	16.0	7.7	18.7	43.2	14.4
2866-26	5.6	15.9	6.9	22.9	43.0	11.3
2866-29	5.6	16.4	6.3	22.9	40.7	13.7
2866-21	5.5	15.7	7.8	27.2	38.5	10.8
2866-20	5.4	16.4	7.3	25.0	38.6	12.7
2866-1 1	5.2	17.6	6.1	22.8	40.5	12.9
2866-4	4.7	16.6	6.5	22.7	40.0	14.2
2866-8	4.7	15.8	7.6	29.4	36.1	11.1
2866-16	4.6	14.5	9.2	30.6	35.2	10.5
2866-27	4.5	17.6	6.7	18.8	44.8	12.1
2866-15	4.5	17.0	6.2	24.2	37.8	14.8
2866-24	4.4	17.3	4.9	13.1	50.6	14.1
2866-30	3.7	16.7	5.8	18.5	46.1	12.9

2866-2	3.7	16.6	5.9	21.3	39.6	16.6
2866-31	3.6	18.1	4.8	14.6	48.6	14.0
2866-19	3.5	19.3	4.8	13.9	47.3	14.7
2866-28	3.5	17.1	6.7	19.9	42.8	13.5
2866-17	3.4	18.0	5.0	16.2	46.2	14.6
2866-14	3.3	18.7	5.3	15.0	45.1	15.8
2866-22	2.5	17.2	5.2	13.8	48.3	15.5
2866-25	2.0	17.8	5.3	17.1	43.8	16.1
Avg.	5.4	16.6	6.6	21.4	42.5	12.9
Top5 Avg.	8.8	16.2	6.2	22.2	44.3	11.2
2867-5	7.6	17.2	5.7	14.5	48.9	13.7
2867-24	6.2	17.9	5.1	13.1	48.6	15.3
2867-18	6.0	17.9	5.7	14.5	45.0	16.8
2867-19	5.7	16.1	7.1	18.1	43.2	15.5
2867-20	5.5	16.8	5.8	13.3	49.6	14.5
2867-29	5.4	16.2	6.4	22.4	40.3	14.7
2867-2	5.2	16.4	7.7	16.6	45.3	14.0
2867-15	5.1	16.8	5.8	20.0	43.1	14.4
2867-7	5.0	16.7	6.5	15.4	47.9	13.5
2867-28	4.9	16.9	6.6	14.2	46.7	15.6
2867-13	4.8	16.8	6.4	23.9	37.7	15.2
2867-26	4.8	16.2	7.4	17.8	46.2	12.5
2867-1	4.7	15.8	8.5	18.7	44.3	12.7
2867-16	4.7	16.1	7.7	18.2	43.4	14.7
2867-30	4.6	16.2	6.2	22.5	40.6	14.6
2867-1 1	4.6	17.5	6.4	21.6	40.4	14.1
2867-25	4.6	17.1	7.2	16.5	44.2	15.1
2867-23	4.4	16.5	7.0	15.5	46.7	14.4
2867-14	4.2	18.2	6.0	15.2	44.5	16.0
2867-6	4.2	16.1	6.5	25.8	37.5	14.2
2867-9	4.2	17.0	6.5	15.3	46.3	14.9
2867-8	4.1	16.2	5.2	18.7	42.1	17.9
2867-10	4.0	17.1	5.5	19.4	42.6	15.3
2867-27	4.0	17.1	6.6	26.4	35.6	14.4
2867-21	3.8	16.3	6.1	21.2	43.5	12.9
2867-17	3.4	17.7	6.6	15.9	43.8	16.0
2867-12	3.4	17.3	7.0	20.9	39.3	15.5
2867-31	3.4	16.5	7.4	17.9	43.5	14.7
2867-4	3.2	18.2	4.8	11.0	47.6	18.4
2867-22	3.0	16.9	6.3	22.0	39.2	15.6
2867-3	2.3	17.9	5.8	13.6	46.0	16.6
Avg.	4.5	16.9	6.4	18.1	43.7	14.9
Top5 Avg.	6.2	17.2	5.9	14.7	47.1	15.2

A summary comparing the average oil content and average fatty acid profile for all events in each experiment is shown in Table 3. In Table 3, average oil content is reported as a percent of total dry weight (Avg. Oil) and average fatty acid content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids. Table 3 also shows the change in oil content (Avg. % Inc.) as compared to the Control experiment where Avg. % Inc. is calculated as the Avg. Oil for that experiment minus the Avg. Oil for the control experiment divided by the Avg. Oil for the control experiment expressed as a percent.

TABLE 3

Summary of Average Oil Content and Fatty Acid Profiles for All Events Expressing GmLecl , GmFusca3-1 , GmFusca3-2, GmODPI or Empty Vector Control

MSE	Vector (Gene)	Avg. Oil	Avg. % Inc	16:0	18:0	18:1	18:2	18:3
2863	pKR1968 (GmLec1)	6.1	34%	16.5	6.7	17.7	43.9	15.3
2864	pKR1969 (GmFusca3-1)	5.1	13%	16.5	6.5	17.0	43.5	16.4
2865	pKR1970 (GmFusca3-2)	3.6	-21%	18.0	4.9	15.5	44.5	17.2
2866	pKR1971 (GmODP1)	5.4	19%	16.6	6.6	21.4	42.5	12.9
2867	pKR278 (Control)	4.5	0%	16.9	6.4	18.1	43.7	14.9

A summary comparing the average oil content and average fatty acid profile of the top 5 events having the highest oil content for each experiment is shown in Table 4. In Table 4, average oil for the 5 events having highest oil content is reported as a percent of total dry weight (Top5 Avg. Oil) and average fatty acid content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids. Table 4 also shows the change in oil content (Top5 Avg. % Inc.) as compared to the Control experiment where Avg. % Inc. is calculated as the Avg. Oil for that experiment minus the Avg. Oil for the control experiment divided by the Avg. Oil for the control experiment expressed as a percent.

TABLE 4

Summary of Average Oil Content and Fatty Acid Profiles for the Top5 Events Having Highest Oil Contents and Expressing GmLecl , GmFusca3-1 , GmFusca3-2, GmODPI or Empty Vector Control

MSE	Gene (Vector)	Avg. Oil	Avg. % Inc	16:0	18:0	18:1	18:2	18:3
2863	GmLec1 (pKR1968)	8.7	41%	15.6	7.6	17.7	46.2	12.7
2864	GmFusca3-1 (pKR1969)	7.5	21%	15.7	6.9	17.3	45.9	14.2
2865	GmFusca3-2 (pKR1970)	5.9	-5%	17.3	4.8	16.4	46.8	14.7
2866	GmODP1 (pKR1971)	8.8	43%	16.2	6.2	22.2	44.3	11.2
2867	Control (pKR278)	6.2	0%	17.2	5.9	14.7	47.1	15.2

Both Tables 3 and 4 demonstrate that expression of GmLecl , GmFusca3-1 and GmODPI lead to an increase in oil content in soy.

EXAMPLE 4

Co-Expressing GmLecl , GmODPI , GmFusca-3-1 and GmFusca3-2 with GmDGATI cAII In Soybean Embryos

Plasmid pKR1520 was previously described in PCT Publication No. WO 2009/143397 (published on November 26, 2009, the contents of which are incorporated by reference) and contains a modified soy DGAT1 (called GmDGATI cAII here and called GM-DGAT1c9c1 0c1 1 in WO 2009/143397) under control of the seed-specific, soy beta-conglycinin promoter. The CDS and amino acid sequence of GmDGATI cAII from PCT Publication No. WO 2009/143397 is set forth in SEQ ID NO: 54 and SEQ ID NO: 55, respectively.

The SbfI fragment of pKR1 968 (SEQ ID NO: 50), containing GmLec1, the SbfI fragment of pKR1 971 (SEQ ID NO: 5 1), containing GmODPI and the SbfI fragment of pKR1 969 (SEQ ID NO: 52), containing GmFusca3-1 , were cloned into the SbfI site of pKR1520 to produce pKR2098 (SEQ ID NO: 56), pKR21 00 (SEQ ID NO: 57) and pKR2099 (SEQ ID NO: 58), respectively. In this way, the respective transcription factors could be expressed behind the soy sucrose synthase promoter (GmSusPro) and co-expressed with GmDGATI cAII (SEQ ID NO: 54).

DNA from plasmids pKR2098 (SEQ ID NO: 56), pKR21 00 (SEQ ID NO: 57) and pKR2099 (SEQ ID NO: 58) and pKR1 520 was prepared for particle bombardment into soybean embryogenic suspension culture and transformed exactly as described previously in PCT Publication No. WO 2008/147935.

Soybean embryogenic suspension culture was initiated, grown, maintained and bombarded and events were selected and matured on SHaM media also exactly as described in PCT Publication No. WO 2008/147935. A summary of genes, plasmids and model system experiment numbers is shown in Table 5.

TABLE 5

Summary of Genes, Plasmids and Experiments

Experiment	Plasmid	Gene1[1,2]	Gene2	Gene2 SEQ ID NO	
				nt	aa
MSE 2984	pKR1520	GmDGAT1cAll	-	-	-
MSE 2985	pKR2098	GmDGAT1cAll	GmLec1	24	25
MSE 2986	pKR2099	GmDGAT1cAll	GmFusca3-1	48	49
MSE 2987	pKR2100	GmDGAT1cAll	GmODP1	29	30

[1]Gene1 nucleotide sequence of SEQ ID NO: 54

[2]Gene1 amino acid sequence of SEQ ID NO: 55

Approximately 10-20 matured embryos from each of approximately 30 events per bombardment experiment were lyophilized, ground, oil content was measured by NMR and fatty acid profile was evaluated by FAME-GC analysis exactly as described in PCT Publication No. WO 2008/147935. The results for oil content and fatty acid profile for each event as well as the average of all events (Avg.) and average for the top 5 events having highest oil content (Top5 Avg.) are shown in Table 6.

In Table 6, results are sorted based on oil content from highest to lowest. In Table 6, oil content is reported as a percent of total dry weight (% Oil) and fatty acid

content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids.

TABLE 6

Summary of Oil Content and Fatty Acid Profiles for Events Expressing GmDGATI cAII with GmLec1. GmFusca3-1 or GmODPI

	% oil	16:0	18:0	18:1	18:2	18:3
2984-2	9.32	14.46	7.12	32.85	36.23	9.34
2984-29	8.43	14.33	8.26	31.62	36.64	9.15
2984-4	7.63	14.70	7.20	28.72	37.99	11.39
2984-24	6.86	15.52	6.84	26.74	41.07	9.83
2984-6	6.60	16.94	5.65	20.30	36.75	20.36
2984-8	6.46	14.45	7.54	32.53	36.10	9.38
2984-25	6.41	14.93	7.19	29.25	37.09	11.54
2984-11	5.86	15.32	6.32	26.67	37.50	14.20
2984-30	5.56	16.39	6.21	23.04	40.99	13.37
2984-12	5.34	15.83	6.18	24.45	38.38	15.16
2984-18	4.61	16.78	5.59	18.05	44.53	15.06
2984-19	4.56	15.38	6.88	29.28	35.27	13.19
2984-7	4.27	15.56	5.73	29.14	35.31	14.26
2984-16	4.25	16.44	5.84	21.69	40.16	15.87
2984-31	4.20	15.22	6.04	22.50	39.87	16.37
2984-28	4.19	15.76	6.15	26.96	36.72	14.41
2984-1	3.87	15.78	6.82	29.12	35.13	13.15
2984-27	3.75	16.05	6.67	25.82	36.68	14.78
2984-21	3.36	15.93	6.97	25.76	37.04	14.31
2984-5	3.25	16.04	5.34	21.85	38.82	17.95
2984-13	3.21	16.28	7.58	22.99	38.11	15.03
2984-3	3.20	16.80	5.81	23.71	36.80	16.88
2984-14	3.04	16.70	6.74	23.50	38.30	14.76
2984-20	3.00	16.68	6.75	21.83	38.83	15.92
2984-23	2.94	16.67	7.14	26.96	34.93	14.31
2984-15	2.71	16.89	5.36	17.26	40.57	19.92
2984-26	2.65	17.07	5.53	23.87	35.64	17.88
2984-10	2.58	17.16	5.07	19.58	39.15	19.05
2984-9	2.53	18.99	4.57	20.90	37.35	18.19
2984-22	2.52	17.24	5.35	18.79	40.42	18.21
2984-17	2.45	17.21	5.61	21.36	38.97	16.85
Avg.	4.50	16.11	6.32	24.74	37.98	14.84
Top5 Avg.	7.77	15.19	7.02	28.04	37.73	12.01
'2985-1	11.32	14.05	6.20	33.72	38.52	7.52
2985-9	10.54	13.39	8.11	35.06	35.71	7.73
2985-23	10.18	14.30	6.93	32.93	37.45	8.38

2985-28	9.87	13.71	6.71	37.57	34.84	7.1 8
2985-1 9	9.39	14.42	6.81	3 1.25	38.24	9.29
2985-1 7	9.1 1	14.57	6.32	28.39	40.70	10.01
2985-24	8.94	14.19	7.08	34.90	35.61	8.21
2985-1 1	8.04	14.90	7.1 3	3 1.07	37.27	9.63
2985-1 8	7.57	16.08	5.1 9	18.95	46.29	13.50
2985-29	7.29	15.24	7.14	28.32	38.60	10.70
2985-25	7.25	13.74	7.43	37.53	34.1 0	7.20
2985-14	6.88	15.20	6.96	3 1.79	36.42	9.62
2985-6	6.67	14.97	6.56	28.93	38.71	10.84
2985-30	6.46	15.96	6.53	16.84	45.97	14.70
2985-27	6.36	15.33	6.64	26.34	40.21	11.48
2985-5	6.25	15.60	5.96	24.88	40.29	13.26
2985-1 5	6.1 7	16.85	5.42	25.02	40.57	12.15
2985-26	5.94	15.84	6.33	27.64	38.09	12.10
2985-3	5.86	15.48	6.40	24.48	39.93	13.71
2985-2	5.12	16.34	5.90	22.1 8	40.69	14.90
2985-1 2	5.1 0	16.51	6.55	23.07	38.63	15.25
2985-1 3	5.05	16.32	6.07	18.51	45.20	13.89
2985-31	4.75	17.38	6.33	2 1.32	40.38	14.60
2985-4	4.41	17.06	5.1 0	18.20	42.54	17.1 0
2985-21	4.38	15.99	6.41	19.61	42.79	15.19
2985-22	4.28	17.00	6.07	23.1 5	40.43	13.36
2985-1 0	3.71	16.56	5.93	24.73	39.45	13.32
2985-1 6	3.29	16.62	5.38	20.23	38.80	18.97
2985-7	3.26	16.95	6.46	2 1.87	40.53	14.1 9
2985-8	2.84	16.88	5.26	19.34	39.99	18.54
2985-20	2.46	20.08	5.07	16.79	39.65	18.41
Avg.	6.41	15.73	6.33	25.95	39.57	12.42
Top5 Avg.	10.26	13.97	6.95	34.1 0	36.95	8.02
2986-1 3	12.08	14.1 1	7.29	29.76	40.57	8.26
2986-14	9.48	15.35	7.22	27.69	39.56	10.19
2986-21	8.96	14.52	6.68	3 1.53	38.85	8.42
2986-2	8.49	15.69	7.1 6	27.1 5	39.78	10.22
2986-7	8.22	14.73	6.70	37.98	32.64	7.96
2986-1 7	8.1 3	15.65	6.55	22.1 3	44.57	11.09
2986-1 2	7.93	16.01	5.59	25.79	4 1.51	11.10
2986-1	7.87	14.34	7.24	32.35	37.08	8.99
2986-5	7.56	15.06	6.1 2	33.97	36.01	8.85
2986-1 6	7.53	15.36	6.91	32.1 9	36.34	9.21
2986-3	7.43	15.21	5.1 6	17.26	46.98	15.39
2986-24	7.1 3	15.93	6.26	20.01	45.26	12.54
2986-1 8	6.79	15.97	6.1 3	20.41	44.98	12.50
2986-1 9	6.73	15.83	6.33	2 1.92	42.56	13.35
2986-6	6.48	13.40	8.25	44.98	27.01	6.36
2986-23	6.25	15.99	6.28	22.04	42.68	13.01
2986-1 5	6.04	16.04	6.23	23.80	4 1.36	12.57

2986-20	5.98	17.17	5.96	23.94	41.44	11.49
2986-25	5.94	16.05	6.56	19.97	43.82	13.61
2986-27	5.80	14.18	6.40	27.22	39.60	12.60
2986-29	5.51	16.00	5.04	21.20	43.39	14.37
2986-9	5.48	15.77	6.72	19.81	42.90	14.79
2986-4	5.42	16.95	5.97	19.96	44.57	12.56
2986-10	4.95	16.33	6.66	23.74	39.55	13.72
2986-30	4.65	16.25	6.37	21.89	42.77	12.73
2986-11	4.51	15.98	6.52	27.94	37.95	11.61
2986-8	4.36	17.29	5.63	20.77	40.92	15.40
2986-26	4.06	17.21	5.52	20.73	43.19	13.36
2986-22	3.96	16.46	6.26	28.71	37.50	11.08
2986-28	3.28	17.67	5.64	20.27	41.54	14.88
Avg.	6.57	15.75	6.38	25.57	40.56	11.74
Top5 Avg.	9.45	14.88	7.01	30.82	38.28	9.01
2987-20	12.17	14.93	6.81	34.83	36.56	6.87
2987-5	11.26	13.58	7.25	31.24	39.66	8.27
2987-29	10.88	15.09	7.40	36.20	34.60	6.71
2987-16	10.57	14.09	7.46	33.87	36.42	8.16
2987-23	8.79	15.14	7.81	35.32	33.79	7.94
2987-13	8.68	16.00	5.65	23.11	43.90	11.35
2987-2	8.53	15.23	7.36	33.83	34.58	9.01
2987-28	7.93	13.55	9.78	40.08	29.47	7.12
2987-19	7.92	15.16	6.44	19.87	46.41	12.13
2987-4	7.37	14.91	6.56	26.12	41.57	10.84
2987-27	6.45	15.89	7.07	25.71	39.42	11.91
2987-17	6.31	16.71	6.26	22.14	42.71	12.17
2987-22	6.29	15.56	6.52	23.53	42.86	11.53
2987-15	5.95	15.59	6.35	21.63	43.38	13.05
2987-9	5.93	15.88	5.83	22.21	41.06	15.02
2987-14	5.81	17.54	6.82	32.38	32.46	10.79
2987-1	5.67	16.70	5.59	20.52	44.56	12.64
2987-26	5.61	15.98	6.41	24.77	39.04	13.80
2987-30	5.53	15.96	6.26	23.42	40.36	13.99
2987-3	5.30	16.46	6.34	24.45	40.62	12.12
2987-10	4.79	15.82	7.19	26.35	39.72	10.92
2987-25	4.67	15.89	7.76	29.34	36.64	10.37
2987-6	4.66	15.68	6.62	27.99	36.93	12.80
2987-8	4.54	16.20	6.11	26.29	38.62	12.78
2987-21	4.52	14.91	8.32	35.11	32.32	9.34
2987-18	4.18	15.80	7.21	29.57	35.85	11.57
2987-24	3.73	15.11	6.88	24.86	40.85	12.30
2987-11	3.61	17.46	5.35	20.08	40.96	16.15
2987-7	3.51	15.53	6.22	30.82	34.50	12.93
2987-12	3.21	16.81	6.73	22.57	38.75	15.15
Avg.	6.48	15.64	6.81	27.61	38.62	11.32
Top5 Avg.	10.73	14.56	7.35	34.29	36.20	7.59

A summary comparing the average oil content and average fatty acid profile for all events in each experiment is shown in Table 7. In Table 7, average oil content is reported as a percent of total dry weight (Avg. Oil) and average fatty acid content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids. Table 3 also shows the change in oil content (Avg. % Inc.) as compared to the Control experiment where Avg. % Inc. is calculated as the Avg. Oil for that experiment minus the Avg. Oil for the control experiment divided by the Avg. Oil for the control experiment expressed as a percent.

TABLE 7

Summary of Average Oil Content and Fatty Acid Profiles for All Events Expressing GmDGATIcAII with GmLec1, GmFusca3-1or GmODPI

MSE	Vector (Gene)	Avg. Oil	Avg. % Inc	16:0	18:0	18:1	18:2	18:3
2984	pKR1520 (n/a)	4.5	0%	16.1	6.3	24.7	38.0	14.8
2985	pKR2098 (GmLec1)	6.4	42%	15.7	6.3	26.0	39.6	12.4
2986	pKR2099 (GmFusca3-1)	6.6	46%	15.7	6.4	25.6	40.6	11.7
2987	pKR2100 (GmODP1)	6.5	44%	15.6	6.8	27.6	38.6	11.3

A summary comparing the average oil content and average fatty acid profile of the top 5 events having the highest oil content for each experiment is shown in Table 8. In Table 8, average oil for the 5 events having highest oil content is reported as a percent of total dry weight (Top5 Avg. Oil) and average fatty acid content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids. Table 4 also shows the change in oil content (Top5 Avg. % Inc.) as compared to the Control experiment where Avg. % Inc. is calculated as the Avg. Oil for that experiment minus the Avg. Oil for the control experiment divided by the Avg. Oil for the control experiment expressed as a percent.

TABLE 8

Summary of Average Oil Content and Fatty Acid Profiles for the Top5 Events Having Highest Oil Contents and Expressing GmDGATI cAII with GmLecl , GmFusca3-1 or GmODPI

MSE	Vector (Gene)	Avg Oil	Avg % Inc	16:0	18:0	18:1	18:2	18:3
2984	pKR1520 (n/a)	7.8	0%	15.2	7.0	28.0	37.7	12.0
2985	pKR2098 (GmLec1)	10.3	32%	14.0	7.0	34.1	37.0	8.0
2986	pKR2099 (GmFusca3-1)	9.4	22%	14.9	7.0	30.8	38.3	9.0
2987	pKR2100 (GmODP1)	10.7	38%	14.6	7.3	34.3	36.2	7.6

Both Tables 7 and 8 demonstrate that expression of GmLec1, GmFusca3-1 and GmODPI with GmDGATI cAII lead to an increase in oil content in soy above that for GmDGATI cAII alone.

EXAMPLE 5

Co-Expressing GmLec1, GmODPI , GmFusca-3-1 and GmFusca3-2 With YLDGAT2 In Soybean Embryos

Plasmid pKR1256 was previously described in PCT Publication No. WO 2008/147935 and contains a Yarrowia lipolytica DGAT2 (called YLDGAT2 in WO 2008/147935) under control of the seed-specific, soy beta-conglycinin promoter. The CDS and aa sequence of YLDGAT2 from PCT Publication No. WO 2008/147935 is set forth in SEQ ID NO: 59 and SEQ ID NO: 60, respectively.

The Sbfl fragment of pKR1 968 (SEQ ID NO: 50), containing GmLed , the Sbfl fragment of pKR1 971 (SEQ ID NO: 5 1), containing GmODPI and the Sbfl fragment of pKR1 969 (SEQ ID NO: 52), containing GmFusca3-1 , were cloned into the Sbfl site of pKR1 256 to produce pKR2082 (SEQ ID NO: 6 1), pKR2084 (SEQ ID NO: 62) and pKR2083 (SEQ ID NO: 63), respectively. In this way, the respective transcription factors could be expressed behind the soy sucrose synthase promoter (GmSusPro) and co-expressed with YLDGAT2 (SEQ ID NO: 59).

DNA from plasmids pKR2082 (SEQ ID NO: 6 1), pKR2084 (SEQ ID NO: 62) and pKR2083 (SEQ ID NO: 63) and pKR1 256 was prepared for particle bombardment into soybean embryogenic suspension culture and transformed

exactly as described previously in PCT Publication No. WO 2008/147935. Soybean embryogenic suspension culture was initiated, grown, maintained and bombarded and events were selected and matured on SHaM media also exactly as described in PCT Publication No. WO 2008/147935. A summary of genes, plasmids and model system experiment numbers is shown in Table 9.

TABLE 9

Summary of Genes, Plasmids and Experiments

Experiment	Plasmid	Gene1[1,2]	Gene2	Gene2 SEQ ID NO	
				nt	aa
3017	pKR1256	YLDGAT2	-	-	-
3018	pKR2082	YLDGAT2	GmLec1	24	25
3019	pKR2083	YLDGAT2	GmFusca3-1	48	49
3020	pKR2084	YLDGAT2	GmODP	29	30

[1]Gβηβ1 nucleotide sequence of SEQ ID NO: 59

[2]Gene1 amino acid sequence of SEQ ID NO: 60

Approximately 10-20 matured embryos from each of approximately 30 events per bombardment experiment were lyophilized, ground, oil content was measured by NMR and fatty acid profile was evaluated by FAME-GC analysis exactly as described in PCT Publication No. WO 2008/147935. The results for oil content and fatty acid profile for each event as well as the average of all events (Avg.) and average for the top 5 events having highest oil content (Top5 Avg.) are shown in Table 10.

In Table 10, results are sorted based on oil content from highest to lowest. In Table 10, oil content is reported as a percent of total dry weight (% Oil) and fatty acid content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids.

TABLE 10

Summary of Oil Content and Fatty Acid Profiles for Events Expressing YLDGAT2 with GmLec1, GmFusca3-1 or GmODPI

	% oil	16:0	18:0	18:1	18:2	18:3
3017-13	13.72	12.08	6.15	29.99	44.30	7.48
3017-18	13.14	12.08	5.73	33.42	40.61	8.16
3017-25	12.64	14.47	5.31	17.82	51.29	11.11
3017-22	12.36	13.29	6.21	27.79	42.62	10.09
3017-32	11.14	13.46	6.07	27.14	44.74	8.59
3017-4	10.76	14.14	5.79	28.40	41.94	9.73
3017-9	10.70	14.87	5.23	22.81	46.72	10.38
3017-16	10.57	14.79	5.38	21.80	47.42	10.60
3017-8	10.57	14.81	6.29	25.54	43.89	9.48
3017-17	9.48	12.33	5.89	32.24	42.96	6.58
3017-19	9.41	14.20	5.91	23.85	44.80	11.25
3017-2	9.39	15.20	5.37	22.87	44.49	12.07
3017-23	9.03	12.09	8.97	39.60	32.75	6.59
3017-14	9.02	15.29	6.03	23.78	43.09	11.81
3017-5	8.89	14.78	7.68	24.09	41.71	11.74
3017-3	8.41	15.15	6.32	28.80	40.19	9.54
3017-1	8.40	15.50	6.15	21.90	42.45	14.00
3017-29	8.14	14.99	6.72	28.17	39.30	10.83
3017-15	8.01	14.83	6.92	25.24	41.34	11.66
3017-34	7.99	14.61	6.89	25.68	43.83	8.99
3017-10	7.93	14.62	7.49	27.24	40.62	10.03
3017-7	7.52	14.57	6.61	29.19	39.82	9.81
3017-30	7.50	14.61	7.04	26.97	42.70	8.68
3017-27	7.36	14.34	8.91	30.81	37.02	8.92
3017-21	7.25	14.12	8.58	30.87	37.73	8.69
3017-28	6.63	14.82	6.95	29.47	38.94	9.82
3017-24	5.99	14.96	9.85	31.34	35.56	8.29
3017-6	5.98	15.91	6.64	25.13	40.68	11.64
3017-20	5.86	14.84	6.67	26.23	42.46	9.80
3017-26	5.72	13.98	10.16	35.42	32.62	7.83
3017-11	5.58	13.20	7.63	37.58	34.02	7.57
3017-31	5.33	14.05	8.45	32.66	35.81	9.03
3017-33	4.70	14.90	8.12	32.46	34.61	9.91
3017-12	4.49	14.94	6.07	26.27	40.63	12.09
Avg.	8.52	14.32	6.89	28.02	40.99	9.79
Top5 Avg.	12.60	13.08	5.90	27.23	44.71	9.09
3018-29	16.95	11.61	5.42	32.58	43.67	6.72
3018-17	15.19	10.65	6.96	38.09	38.24	6.06
3018-22	14.87	9.66	7.05	48.08	30.24	4.98
3018-16	14.51	11.46	6.52	38.75	37.38	5.88

3018-27	14.00	11.39	6.00	39.98	36.40	6.23
3018-4	12.90	11.32	6.54	34.78	40.20	7.16
3018-19	12.26	13.06	5.28	31.71	42.04	7.90
3018-2	11.72	11.57	4.94	32.05	42.96	8.48
3018-20	11.65	10.89	5.08	38.25	37.85	7.93
3018-11	11.47	12.37	6.68	38.24	35.18	7.54
3018-13	10.84	11.85	7.36	41.64	33.08	6.06
3018-30	10.41	14.51	5.98	25.16	44.25	10.11
3018-7	10.03	10.84	7.56	46.85	29.72	5.03
3018-8	10.00	15.36	5.09	20.72	48.63	10.22
3018-15	9.81	12.34	8.07	39.27	32.70	7.63
3018-25	9.80	12.45	5.76	33.67	41.00	7.11
3018-9	9.32	14.09	5.71	22.46	49.20	8.54
3018-28	9.21	12.94	8.87	34.67	34.39	7.72
3018-12	9.21	15.40	5.47	24.61	43.40	11.11
3018-23	9.19	15.47	8.14	27.57	38.98	9.83
3018-24	9.06	14.64	7.51	27.12	41.56	9.17
3018-5	8.97	14.06	5.23	26.34	45.06	9.31
3018-18	8.95	12.56	6.73	37.59	34.39	8.73
3018-3	8.27	12.99	6.84	34.06	38.34	7.77
3018-26	8.00	15.82	5.74	22.39	45.62	10.43
3018-21	5.99	13.63	8.88	34.58	34.47	8.44
3018-1	5.98	15.00	8.98	30.75	35.25	10.01
3018-10	5.72	14.11	7.29	36.00	35.14	7.46
3018-6	5.49	14.13	6.87	27.10	41.60	10.29
3018-14	4.49	14.47	6.75	36.34	34.50	7.93
Avg.	10.14	13.02	6.64	33.38	38.85	8.06
Top5_Avg.	15.10	10.95	6.39	39.49	37.19	5.98
3019-27	11.11	15.22	4.66	23.96	46.19	9.97
3019-23	10.06	12.24	5.28	27.99	43.63	10.86
3019-4	9.83	11.43	6.94	43.16	32.24	6.23
3019-7	9.77	11.22	6.15	37.45	37.56	7.62
3019-15	9.16	12.50	6.60	39.08	34.52	7.30
3019-20	8.67	16.44	5.12	19.31	46.64	12.49
3019-12	8.22	12.27	7.06	38.86	33.71	8.10
3019-17	8.07	16.60	5.47	26.70	40.57	10.66
3019-11	7.78	13.40	6.26	31.75	38.36	10.22
3019-24	7.76	13.56	5.79	34.04	37.79	8.82
3019-19	7.21	15.81	5.83	21.60	43.54	13.23
3019-6	7.07	12.94	6.45	33.73	37.02	9.86
3019-13	7.07	14.26	5.42	35.78	36.24	8.30
3019-3	6.94	13.72	5.57	39.86	33.47	7.39
3019-2	6.84	13.36	6.58	30.96	38.13	10.97
3019-10	6.80	14.81	6.49	26.45	41.18	11.07
3019-5	6.73	14.48	4.78	28.73	40.26	11.76
3019-30	6.52	13.40	6.23	36.19	35.51	8.67
3019-21	6.47	15.74	7.75	24.42	40.60	11.49

3019-14	6.27	15.39	7.18	23.21	41.62	12.59
3019-1	5.93	15.61	7.27	23.55	41.13	12.44
3019-29	5.69	14.67	5.72	22.51	41.63	15.48
3019-18	5.54	14.58	4.85	36.76	35.78	8.04
3019-16	5.48	16.00	5.62	25.73	40.35	12.29
3019-22	4.63	16.81	6.03	20.42	43.23	13.51
3019-9	4.21	16.90	4.07	24.22	41.43	13.38
3019-8	3.87	16.96	5.46	20.23	40.10	17.23
3019-26	3.83	16.75	6.65	24.01	38.72	13.86
3019-28	3.44	16.98	5.19	21.93	42.09	13.81
3019-25	3.05	17.10	5.38	19.21	39.89	18.42
Avg.	6.80	14.71	5.93	28.73	39.44	11.20
Top5_Avg.	9.99	12.52	5.93	34.33	38.83	8.40
3020-4	18.24	11.66	5.14	42.44	35.63	5.13
3020-2	17.99	14.04	5.23	40.23	35.32	5.18
3020-16	15.32	14.60	4.66	32.03	41.59	7.12
3020-10	14.86	10.19	6.05	44.43	33.95	5.39
3020-28	14.26	10.64	6.90	41.20	36.44	4.81
3020-21	13.75	14.84	4.76	25.37	45.76	9.26
3020-11	13.00	11.26	6.37	35.10	39.89	7.39
3020-20	12.26	14.91	4.81	33.19	38.68	8.40
3020-24	12.06	13.49	4.95	39.62	34.81	7.13
3020-27	12.02	13.37	7.85	37.87	34.44	6.48
3020-14	11.70	13.88	5.89	42.81	31.65	5.78
3020-22	11.32	15.05	4.24	22.49	47.99	10.22
3020-30	11.08	14.99	5.43	26.34	43.96	9.28
3020-18	10.19	15.53	5.47	35.57	35.97	7.47
3020-23	9.71	12.39	6.38	45.44	29.30	6.49
3020-25	9.68	12.55	6.81	44.02	30.15	6.47
3020-1	9.37	12.21	6.23	39.89	34.65	7.02
3020-26	8.60	12.44	6.36	38.32	34.56	8.31
3020-12	8.48	14.01	6.49	37.51	34.00	8.00
3020-3	8.29	12.29	6.92	33.60	38.01	9.18
3020-17	8.17	14.81	5.14	23.98	44.24	11.83
3020-6	7.46	12.93	7.35	40.18	31.90	7.64
3020-13	7.39	15.19	6.69	24.53	41.62	11.98
3020-19	7.34	15.34	6.88	24.47	40.59	12.72
3020-8	6.50	15.65	7.96	25.19	39.40	11.79
3020-7	6.15	17.20	6.39	29.08	37.37	9.96
3020-15	5.63	15.85	7.51	27.81	36.66	12.17
3020-9	5.34	14.05	6.54	43.17	27.99	8.25
3020-29	4.63	18.01	6.17	32.09	33.33	10.39
3020-5	3.67	15.71	7.21	28.74	34.84	13.49
Avg.	10.15	13.97	6.16	34.56	36.82	8.49
Top5_Avg.	16.13	12.23	5.60	40.07	36.59	5.53

A summary comparing the average oil content and average fatty acid profile for all events in each experiment is shown in Table 11. In Table 11, average oil content is reported as a percent of total dry weight (Avg. Oil) and average fatty acid content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids. Table 11 also shows the change in oil content (Avg. % Inc.) as compared to the Control expermiment where Avg. % Inc. is calculated as the Avg. Oil for that experiment minus the Avg. Oil for the control experiment divided by the Avg. Oil for the control experiment expressed as a percent.

TABLE 11

Summary of Average Oil Content and Fatty Acid Profiles for All Events Expressing YLDGAT2 with GmLec1, GmFusca3-1or GmODPI

MSE	Vector (Gene)	Avg Oil	% Inc	16:0	18:0	18:1	18:2	18:3
3017	pKR1256 (n/a)	8.5	0%	14.3	6.9	28.0	41.0	9.8
3018	pKR2082 (GmLec1)	10.1	19%	13.0	6.6	33.4	38.8	8.1
3019	pKR2083 (GmFusca3-1)	6.8	-20%	14.7	5.9	28.7	39.4	11.2
3020	pKR2084 (GmODP1)	10.1	19%	14.0	6.2	34.6	36.8	8.5

A summary comparing the average oil content and average fatty acid profile of the top 5 events having the highest oil content for each experiment is shown in Table 12. In Table 12, average oil for the 5 events having highest oil content is reported as a percent of total dry weight (Top5 Avg. Oil) and average fatty acid content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids. Table 12 also shows the change in oil content (Top5 Avg. % Inc.) as compared to the Control experiment where Avg. % Inc. is calculated as the Avg. Oil for that experiment minus the Avg. Oil for the control experiment divided by the Avg. Oil for the control experiment expressed as a percent.

TABLE 12

Summary of Average Oil Content and Fatty Acid Profiles for the Top5 Events Having Highest Oil Contents and Expressing YLDGAT2 with GmLec1, GmFusca3-1 or GmODPI

MSE	Vector (Gene)	Avg. Oil	Avg. % Inc	16:0	18:0	18:1	18:2	18:3
3017	pKR1256 (n/a)	12.6	0%	13.1	5.9	27.2	44.7	9.1
3018	pKR2082 (GmLec1)	15.1	20%	11.0	6.4	39.5	37.2	6.0
3019	pKR2083 (GmFusca3-1)	10.0	-21%	12.5	5.9	34.3	38.8	8.4
3020	pKR2084 (GmODP)	16.1	28%	12.2	5.6	40.1	36.6	5.5

Both Tables 11 and 12 demonstrate that expression of GmLec1 and GmODPI with YLDGAT2 lead to an increase in oil content in soy above that for YLDGAT2 alone.

EXAMPLE 6

Cloning Led and ODP1 Homologs from Maize

ZmLec1 with flanking Not! sites:

The maize Led (ZmLed) is described in U.S. Patent No. 6,825,397. The CDS and aa sequences for ZmLed are set forth in SEQ ID NO: 64 and SEQ ID NO: 65, respectively.

ZmLed was PCR-amplified from a cDNA clone using oligonucleotides oZLEC-1 (SEQ ID NO: 66) and oZLEC-2 (SEQ ID NO: 67) with the PHUSION™ High-Fidelity DNA Polymerase (Cat. No. F553S, Finnzymes Oy, Finland), following the manufacturer's protocol. The PCR fragment was cloned into the pCR®-BLUNT® cloning vector using the ZERO BLUNT® PCR Cloning Kit (Invitrogen Corporation), following the manufacturer's protocol, to produce pKR21 15 (SEQ ID NO: 68).

ZmODPI with flanking Notl sites:

The maize ODP1 (ZmODPI) is described in U.S. Patent No. 7,157,621 . The cloning of ZmODPI with flanking Notl sites into plasmid KS336 was previously described in PCT Publication No. WO 201 0/1 14989 (published on October 7, 201 0, the contents of which are herein incorporated by reference). It should be noted that

there is a typo in the map of KS336 (SEQ ID NO: 6 in WO2010/114989) and that there should be an additional 3 nucleotides (TGA) at position 1192 to form a stop codon and end the CDS in KS336. The CDS and amino acid sequence of ZmODPI in KS336 from WO2010/114989 are set forth here in SEQ ID NO: 69 and SEQ ID NO: 70, respectively.

EXAMPLE 7

Expressing ZmLecl and ZmODPI in Soybean Embryos under Control of the GmSus Promoter

The NotI fragment of pKR2115 (SEQ ID NO: 68), containing ZmLec1 and the NotI fragment of KS336, containing ZmODPI were cloned into the NotI site of pKR1965 (SEQ ID NO: 14) to produce pKR2121 (SEQ ID NO: 71) and pKR2114 (SEQ ID NO: 72), respectively. In this way, the respective transcription factors could be expressed behind the soy sucrose synthase promoter (GmSusPro). Plasmid pKR278, containing no transcription factor, but having the hygromycin selectable marker, was used as a negative control.

DNA from plasmids pKR2121 (SEQ ID NO: 71), pKR2114 (SEQ ID NO: 72) and pKR278 was prepared for particle bombardment into soybean embryogenic suspension culture and transformed exactly as described previously in PCT Publication No. WO 2008/147935. Soybean embryogenic suspension culture was initiated, grown, maintained and bombarded and events were selected and matured on SHaM media also exactly as described in PCT Publication No. WO 2008/147935. A summary of genes, plasmids and model system experiment numbers is shown in Table 13.

TABLE 13

Summary of Genes, Plasmids and Experiments

Experiment	Plasmid	Gene	SEQ ID NO	
			nt	aa
MSE 3053	pKR2114	ZmODP1	69	70
MSE 3054	pKR2121	ZmLec1	64	65

MSE 3055	pKR278	Empty Vector Control	-	-

Approximately 10-20 matured embryos from each of approximately 30 events per bombardment experiment were lyophilized, ground, oil content was measured by NMR and fatty acid profile was evaluated by FAME-GC analysis exactly as described in PCT Publication No. WO 2008/147935. The results for oil content and fatty acid profile for each event as well as the average of all events (Avg.) and average for the top 5 events having highest oil content (Top5 Avg.) are shown in Table 14.

In Table 14, results are sorted based on oil content from highest to lowest. In Table 14, oil content is reported as a percent of total dry weight (% Oil) and fatty acid content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids.

TABLE 14

Summary of Oil Content and Fatty Acid Profiles for Events Expressing ZmLecl , ZmODPI or Empty Vector Control

	% oil	16:0	18:0	18:1	18:2	18:3
3053-21	10.6	16.6	4.4	17.1	50.6	11.3
3053-1	9.8	17.0	4.8	18.0	48.8	11.4
3053-31	9.4	15.6	4.8	17.5	50.2	11.9
3053-25	9.2	16.1	4.8	20.6	47.3	11.3
3053-20	8.9	16.9	4.6	19.9	47.5	11.1
3053-7	8.6	16.4	4.4	19.6	45.9	13.6
3053-27	8.5	17.1	3.4	15.4	50.8	13.2
3053-18	8.3	15.6	5.6	17.1	49.2	12.5
3053-23	8.2	15.9	4.9	17.1	49.3	12.8
3053-11	8.1	16.8	5.1	21.1	44.9	12.1
3053-29	8.1	17.0	5.2	19.0	47.2	11.6
3053-12	8.0	16.6	6.1	21.5	43.2	12.5
3053-5	7.9	17.1	5.1	20.5	43.9	13.4
3053-2	7.8	15.8	3.8	16.9	49.8	13.7
3053-10	7.7	17.0	5.6	21.4	44.8	11.2
3053-13	7.6	17.4	4.8	19.2	45.3	13.3
3053-3	7.4	15.7	6.1	19.5	46.6	12.2
3053-15	7.3	15.5	5.5	19.1	46.6	13.2

3053-6	6.8	16.5	5.2	20.5	44.0	13.7
3053-17	6.8	16.7	5.8	24.7	41.9	10.9
3053-4	6.7	17.7	4.7	16.1	47.7	13.7
3053-24	6.7	16.3	7.1	24.6	39.8	12.2
3053-26	6.7	16.4	5.9	16.6	45.9	15.2
3053-16	6.5	17.3	5.3	19.5	44.8	13.1
3053-19	6.5	17.8	5.2	20.9	43.3	12.8
3053-9	6.3	18.2	5.1	20.8	43.4	12.5
3053-28	6.2	16.6	5.8	17.9	45.2	14.5
3053-14	6.0	16.8	6.4	25.0	39.9	11.8
3053-8	6.0	17.4	5.6	18.7	44.9	13.5
3053-30	5.7	17.2	6.7	26.7	38.3	11.1
3053-22	3.7	17.0	5.4	19.2	44.0	14.5
Avg.	7.5	16.7	5.3	19.7	45.6	12.6
Top5_Avg.	9.6	16.4	4.7	18.6	48.9	11.4
3054-11	9.1	15.9	5.4	21.9	45.3	11.5
3054-6	8.6	16.7	5.1	19.0	47.5	11.8
3054-25	8.3	16.2	5.7	21.0	44.4	12.7
3054-26	8.2	17.0	5.1	22.1	43.5	12.3
3054-7	7.8	15.6	6.8	17.6	48.0	12.0
3054-27	7.8	16.5	5.0	21.1	44.3	13.1
3054-10	7.4	15.9	3.4	15.5	50.0	15.3
3054-16	7.2	15.3	5.9	19.1	47.4	12.3
3054-17	7.1	16.3	4.9	21.8	42.5	14.4
3054-21	7.0	16.1	6.2	19.9	45.0	12.7
3054-4	6.9	15.8	5.3	18.6	46.9	13.4
3054-28	6.4	15.8	5.4	20.2	44.7	13.8
3054-19	6.4	16.1	5.8	18.1	45.9	14.1
3054-13	5.9	16.4	6.0	22.9	41.9	12.9
3054-9	5.7	16.2	5.1	18.3	46.4	14.0
3054-1	5.3	17.7	5.2	22.0	41.6	13.5
3054-24	5.1	16.2	5.7	21.6	42.7	13.8
3054-5	4.9	15.7	5.0	18.3	44.5	16.5
3054-14	4.9	15.5	5.2	25.7	39.2	14.4
3054-12	4.9	16.9	5.4	22.7	41.1	13.9
3054-22	4.5	16.6	6.5	32.2	33.4	11.3
3054-8	4.2	17.0	4.7	17.0	42.4	19.0
3054-23	4.2	18.3	5.3	21.8	40.4	14.1
3054-20	4.2	19.1	5.2	20.0	38.4	17.3
3054-18	4.1	15.8	7.7	26.9	38.9	10.7
3054-15	2.7	17.0	6.9	25.3	38.1	12.7
3054-2	2.6	17.7	6.5	26.6	36.5	12.8
3054-3	2.5	16.5	5.7	21.5	39.4	16.9
Avg.	5.9	16.5	5.6	21.4	42.9	13.7
Top5_Avg.	8.4	16.3	5.6	20.3	45.7	12.1
3055-29	6.4	16.3	6.9	17.3	46.2	13.3

3055-30	5.8	16.5	6.8	18.5	45.1	13.2
3055-3	5.7	16.2	7.6	17.8	44.5	13.8
3055-28	5.7	16.3	7.1	26.5	38.7	11.5
3055-1 2	5.5	17.0	5.9	17.1	45.3	14.7
3055-1 9	5.5	15.1	6.1	17.5	46.3	15.0
3055-1 5	5.3	17.2	7.1	18.0	43.4	14.3
3055-25	5.2	16.2	8.0	17.3	44.7	13.7
3055-1 3	5.2	16.5	7.3	16.7	45.1	14.5
3055-4	5.2	17.6	6.3	23.3	39.3	13.4
3055-20	4.7	16.9	6.0	16.8	44.5	15.8
3055-24	4.4	18.0	5.2	21.0	41.3	14.5
3055-1 1	4.2	18.5	5.4	20.8	39.9	15.4
3055-1 7	4.1	17.8	5.7	23.8	37.5	15.2
3055-7	4.1	17.8	5.0	18.8	42.9	15.4
3055-1 6	3.9	18.1	6.7	21.4	39.1	14.7
3055-27	3.8	17.3	6.7	17.7	42.6	15.7
3055-21	3.7	19.1	4.7	19.4	39.7	17.1
3055-22	3.6	18.0	5.0	19.6	41.6	15.8
3055-23	3.6	18.6	4.5	17.7	39.5	19.6
3055-1	3.6	17.9	5.8	16.0	42.6	17.8
3055-8	3.5	17.6	5.4	19.3	40.8	16.9
3055-5	3.4	18.9	5.7	24.8	36.9	13.6
3055-2	3.3	17.9	3.5	16.4	43.1	19.0
3055-6	3.3	18.6	5.5	21.5	38.9	15.5
3055-9	3.0	19.1	4.3	16.4	40.4	19.9
3055-14	2.5	18.1	4.8	20.9	37.3	18.8
3055-1 8	2.4	18.2	4.3	16.0	39.9	21.6
3055-1 0	2.2	19.1	4.6	18.3	37.1	21.0
3055-26	2.1	18.7	5.0	21.2	38.3	16.8
Avg.	4.2	17.6	5.8	19.3	41.4	15.9
Top5_Avg.	5.8	16.5	6.9	19.4	43.9	13.3

A summary comparing the average oil content and average fatty acid profile for all events in each experiment is shown in Table 15. In Table 15, average oil content is reported as a percent of total dry weight (Avg. Oil) and average fatty acid content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids. Table 15 also shows the change in oil content (Avg. % Inc.) as compared to the Control expermiment where Avg. % Inc. is calculated as the Avg. Oil for that experiment minus the Avg. Oil for the control experiment divided by the Avg. Oil for the control experiment expressed as a percent.

TABLE 15

Summary of Average Oil Content and Fatty Acid Profiles for All Events Expressing ZmLec1, ZmODPI or Empty Vector Control

MSE	Vector (Gene)	Avg. Oil	Avg. % Inc	16:0	18:0	18:1	18:2	18:3
3053	pKR2114 (ZmODP1)	7.5	80%	16.7	5.3	19.7	45.6	12.6
3054	pKR2121 (ZmLec1)	5.9	41%	16.5	5.6	21.4	42.9	13.7
3055	pKR278 (Control)	4.2	0%	17.6	5.8	19.3	41.4	15.9

A summary comparing the average oil content and average fatty acid profile of the top 5 events having the highest oil content for each experiment is shown in Table 16. In Table 16, average oil for the 5 events having highest oil content is reported as a percent of total dry weight (Top5 Avg. Oil) and average fatty acid content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids. Table 16 also shows the change in oil content (Top5 Avg. % Inc.) as compared to the Control experiment where Avg. % Inc. is calculated as the Avg. Oil for that experiment minus the Avg. Oil for the control experiment divided by the Avg. Oil for the control experiment expressed as a percent.

TABLE 16

Summary of Average Oil Content and Fatty Acid Profiles for the Top5 Events Having Highest Oil Contents and Expressing ZmLec1, ZmODPI or Empty Vector Control

MSE	Vector (Gene)	Avg. Oil	Avg. % Inc	16:0	18:0	18:1	18:2	18:3
3053	pKR2114 (ZmODP1)	9.6	65%	16.4	4.7	18.6	48.9	11.4
3054	pKR2121 (ZmLec1)	8.4	44%	16.3	5.6	20.3	45.7	12.1
3055	pKR278 (Control)	5.8	0%	16.5	6.9	19.4	43.9	13.3

Both Tables 15 and 16 demonstrate that expression of ZmLed and ZmODPI lead to an increase in oil content in soy.

EXAMPLE 8

Co-Expressing ZmLec1 and ZmODPI With GmDGATI cAII In Sov Embryos

The SbfI fragment of pKR21 2 1 (SEQ ID NO: 7 1), containing ZmLec1, and the SbfI fragment of pKR21 14 (SEQ ID NO: 72), containing ZmODPI , were cloned into the SbfI site of pKR1 520 to produce pKR21 23 (SEQ ID NO: 73) and pKR21 22 (SEQ ID NO: 74), respectively. In this way, the respective transcription factors could be expressed behind the soy sucrose synthase promoter (GmSusPro) and co-expressed with GmDGATI cAII (SEQ ID NO: 54).

DNA from plasmids pKR21 23 (SEQ ID NO: 73), pKR21 22 (SEQ ID NO: 74) and pKR1 520 was prepared for particle bombardment into soybean embryogenic suspension culture and transformed exactly as described previously in PCT Publication No. WO 2008/147935. Soybean embryogenic suspension culture was initiated, grown, maintained and bombarded and events were selected and matured on SHaM media also exactly as described in PCT Publication No. WO 2008/147935. A summary of genes, plasmids and model system experiment numbers is shown in Table 17.

TABLE 17

Summary of Genes, Plasmids and Experiments

				SEQ ID NO	
Experiment	**Plasmid**	**Gene1[1,2]**	**Gene2**	**nt**	**aa**
MSE 3006	pKR1520	GmDGAT1cAII	-	-	-
MSE 3009	pKR2122	GmDGAT1cAII	ZmODP1	69	70
MSE 3010	pKR2123	GmDGAT1cAII	ZmLec1	64	65

[1]Gene1 nucleotide sequence of SEQ ID NO: 54

[2]Gene1 amino acid sequence of SEQ ID NO: 55

Approximately 10-20 matured embryos from each of approximately 30 events per bombardment experiment were lyophilized, ground, oil content was measured by NMR and fatty acid profile was evaluated by FAME-GC analysis exactly as

described in PCT Publication No. WO 2008/147935. The results for oil content and fatty acid profile for each event as well as the average of all events (Avg.) and average for the top 5 events having highest oil content (Top5 Avg.) are shown in Table 18.

In Table 18, results are sorted based on oil content from highest to lowest. In Table 18, oil content is reported as a percent of total dry weight (% Oil) and fatty acid content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids.

TABLE 18

Summary of Oil Content and Fatty Acid Profiles for Events Expressing GmDGATI cAII with ZmLec1 or ZmODPI

	% oil	16:0	18:0	18:1	18:2	18:3
3006-28	15.46	12.83	5.81	34.01	40.95	6.41
3006-10	13.29	13.49	5.69	33.99	39.36	7.48
3006-19	13.12	13.84	4.51	27.42	44.84	9.38
3006-2	12.10	14.43	5.55	26.44	45.18	8.41
3006-3	11.99	13.03	5.65	32.35	40.09	8.88
3006-23	11.96	14.84	4.66	27.88	44.12	8.50
3006-24	11.49	13.02	7.30	33.49	38.56	7.64
3006-27	10.87	14.01	6.32	32.49	39.31	7.87
3006-1	10.85	13.82	6.53	31.04	40.49	8.12
3006-26	10.22	15.49	5.13	22.72	46.85	9.81
3006-20	10.19	15.49	4.65	21.58	47.28	11.01
3006-4	10.05	15.67	3.93	18.28	50.17	11.96
3006-25	10.04	14.35	7.08	27.96	41.52	9.09
3006-8	9.93	15.02	6.90	27.71	40.94	9.43
3006-6	9.51	17.52	4.38	17.94	48.66	11.51
3006-31	9.37	15.55	3.98	17.39	49.82	13.27
3006-7	9.27	16.20	5.90	23.30	43.50	11.10
3006-14	9.15	15.87	5.43	22.58	45.39	10.72
3006-21	8.75	15.23	5.32	20.46	47.62	11.38
3006-11	8.72	17.05	3.64	17.79	48.24	13.28
3006-15	8.65	13.41	8.25	39.07	32.68	6.60
3006-16	8.49	15.51	5.18	21.14	47.31	10.87
3006-30	8.48	14.77	6.08	23.92	44.56	10.66
3006-29	7.97	16.89	5.40	23.91	42.01	11.78
3006-18	7.43	15.84	5.42	21.80	45.40	11.55
3006-5	7.32	15.87	6.10	24.44	43.06	10.53
3006-12	6.59	17.85	6.26	27.20	38.06	10.62
3006-9	6.18	15.71	5.60	23.23	43.00	12.46

3006-17	6.14	15.66	6.81	24.98	41.52	11.03
3006-13	5.87	14.57	7.04	26.12	42.22	10.05
3006-22	3.13	15.44	7.76	28.15	37.39	11.26
Avg.	9.44	15.11	5.75	25.83	43.23	10.08
Top5_Avg.	13.19	13.52	5.44	30.84	42.08	8.11
3009-9	20.60	13.13	4.48	34.94	41.26	6.19
3009-8	17.21	13.31	6.15	30.24	43.29	7.01
3009-16	14.42	14.15	6.13	37.01	35.96	6.75
3009-6	14.40	11.74	5.79	33.69	42.37	6.41
3009-21	13.69	12.95	6.41	33.22	40.13	7.30
3009-3	12.99	13.56	7.47	30.41	40.69	7.88
3009-17	12.27	14.37	6.80	37.81	34.41	6.60
3009-13	11.12	13.78	8.03	37.56	33.72	6.91
3009-10	10.93	15.78	4.90	19.06	48.61	11.64
3009-28	10.85	14.55	4.65	19.63	49.88	11.29
3009-23	10.26	13.71	7.05	43.30	29.99	5.96
3009-26	9.92	15.60	5.79	27.33	41.87	9.40
3009-4	9.70	15.82	5.24	30.04	40.64	8.26
3009-29	9.49	14.37	6.20	25.89	43.74	9.79
3009-22	9.45	14.05	7.25	33.34	37.01	8.35
3009-18	9.39	14.78	5.41	22.88	46.23	10.70
3009-24	9.25	15.44	6.43	24.34	43.37	10.42
3009-5	9.18	14.95	4.74	20.21	48.01	12.10
3009-25	8.97	16.10	5.17	19.54	47.70	11.50
3009-7	8.86	15.62	5.05	18.50	49.05	11.77
3009-20	8.85	13.87	7.36	33.99	36.25	8.52
3009-1	8.19	15.06	5.35	21.07	45.91	12.61
3009-19	8.17	15.69	5.67	25.02	42.23	11.40
3009-2	8.02	15.11	4.98	20.67	46.58	12.66
3009-14	7.85	16.77	5.76	22.50	43.11	11.87
3009-31	7.61	14.88	6.38	26.16	42.38	10.21
3009-27	7.21	14.74	7.83	19.47	46.43	11.52
3009-30	7.14	15.23	6.04	23.66	44.16	10.90
3009-15	6.68	15.08	6.35	25.94	42.57	10.05
3009-11	6.55	16.25	5.89	25.36	40.89	11.61
3009-12	5.05	16.55	4.32	16.91	46.12	16.09
Avg.	10.14	14.74	5.97	27.09	42.41	9.80
Top5_Avg.	16.06	13.06	5.79	33.82	40.60	6.73
3010-18	16.30	12.38	4.54	30.86	44.74	7.48
3010-19	15.93	11.72	4.75	34.72	40.70	8.10
3010-2	15.70	12.48	4.09	32.28	42.54	8.61
3010-5	15.57	12.17	5.61	36.18	37.99	8.04
3010-30	15.40	12.66	4.52	33.89	41.29	7.64
3010-25	14.61	13.34	3.96	28.41	45.46	8.83
3010-3	13.94	12.74	5.10	31.91	40.89	9.36
3010-1	13.90	14.34	4.49	27.04	45.95	8.17

301 0-1 7	13.68	13.09	5.03	29.39	42.66	9.83
301 0-8	13.63	11.75	4.35	34.60	40.51	8.79
301 0-26	13.55	13.37	4.79	34.23	38.78	8.83
301 0-22	13.34	13.06	4.26	30.03	43.97	8.68
301 0-14	13.34	12.48	4.51	34.89	39.1 2	9.00
301 0-29	13.07	12.82	5.22	37.70	35.65	8.61
301 0-1 3	12.65	12.55	4.52	31.75	41.68	9.50
301 0-1 5	12.56	13.30	4.27	30.08	43.03	9.32
301 0-1 6	11.56	12.03	4.99	35.1 6	38.47	9.35
301 0-27	11.52	11.81	5.35	34.44	38.57	9.83
301 0-9	11.26	13.73	3.97	23.1 1	48.56	10.63
301 0-6	10.1 0	14.78	4.56	18.36	50.94	11.36
301 0-4	9.97	15.52	4.40	20.60	47.99	11.49
301 0-23	9.77	12.37	5.58	34.07	38.25	9.73
301 0-24	9.49	14.30	3.96	17.14	51.54	13.07
301 0-31	9.02	16.48	4.1 2	20.22	46.66	12.52
301 0-21	8.57	15.25	4.48	25.46	43.1 0	11.71
301 0-7	8.39	15.82	3.1 9	15.07	51.22	14.70
301 0-28	8.01	16.07	3.92	17.45	49.89	12.67
301 0-1 0	7.89	13.83	4.40	18.47	48.61	14.68
-1 1	7.60	18.93	3.83	18.45	44.69	14.1 0
301 0-1 2	7.58	16.09	5.28	21.85	44.01	12.77
301 0-20	6.35	13.92	5.1 3	17.60	49.14	14.20
Avg.	11.75	13.72	4.55	27.59	43.76	10.37
Top5 Avg.	15.78	12.28	4.70	33.59	41.45	7.98

A summary comparing the average oil content and average fatty acid profile for all events in each experiment is shown in Table 19. In Table 19, average oil content is reported as a percent of total dry weight (Avg. Oil) and average fatty acid content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids. Table 3 also shows the change in oil content (Avg. % Inc.) as compared to the Control experiment where Avg. % Inc. is calculated as the Avg. Oil for that experiment minus the Avg. Oil for the control experiment divided by the Avg. Oil for the control experiment expressed as a percent.

TABLE 19

Summary of Average Oil Content and Fatty Acid Profiles for All Events Expressing GmDGATI cAII with ZmLec1 or ZmODPI

MSE	Vector (Gene2)	Avg. Oil	Avg. % Inc	16:0	18:0	18:1	18:2	18:3
3006	pKR1520 (n/a)	9.4	0%	15.1	5.8	25.8	43.2	10.1

3009	pKR2122 (ZmODPI)	10.1	7%	14.7	6.0	27.1	42.4	9.8
3010	pKR2123 (ZmLed)	11.8	25%	13.7	4.6	27.6	43.8	10.4

A summary comparing the average oil content and average fatty acid profile of the top 5 events having the highest oil content for each experiment is shown in Table 20. In Table 20, average oil for the 5 events having highest oil content is reported as a percent of total dry weight (Top5 Avg. Oil) and average fatty acid content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids. Table 4 also shows the change in oil content (Top5 Avg. % Inc.) as compared to the Control experiment where Avg. % Inc. is calculated as the Avg. Oil for that experiment minus the Avg. Oil for the control experiment divided by the Avg. Oil for the control experiment expressed as a percent.

TABLE 20

Summary of Average Oil Content and Fatty Acid Profiles for the Top5 Events Having Highest Oil Contents and Expressing GmDGATI cAN with ZmLec1 or ZmODPI

MSE	Vector (Gene)	Avg. Oil	Avg. % Inc	16:0	18:0	18:1	18:2	18:3
3006	pKR1520 (n/a)	13.2	0%	13.5	5.4	30.8	42.1	8.1
3009	pKR2122 (ZmODP)	16.1	22%	13.1	5.8	33.8	40.6	6.7
3010	pKR2123 (ZmLec1)	15.8	20%	12.3	4.7	33.6	41.5	8.0

Both Tables 19 and 20 demonstrate that expression of ZmLed and ZmODPI with GmDGATI cAN lead to an increase in oil content in soy above that for GmDGATI cAN alone.

EXAMPLE 9

Co-Expressing ZmLed and ZmODPI with YLDGAT2 in Soy Embryos

The SbfI fragment of pKR2121 (SEQ ID NO: 71), containing ZmLed , and the SbfI fragment of pKR2114 (SEQ ID NO: 72), containing ZmODPI , were cloned into the SbfI site of pKR1256 to produce pKR2146 (SEQ ID NO: 75) and pKR2145 (SEQ ID NO: 76), respectively. In this way, the respective transcription factors could be

expressed behind the soy sucrose synthase promoter (GmSusPro) and co-expressed with YLDGAT2 (SEQ ID NO: 59).

DNA from plasmids pKR2146 (SEQ ID NO: 75), pKR2145 (SEQ ID NO: 76) and pKR1256 was prepared for particle bombardment into soybean embryogenic suspension culture and transformed exactly as described previously in PCT Publication No. WO 2008/147935. Soybean embryogenic suspension culture was initiated, grown, maintained and bombarded and events were selected and matured on SHaM media also exactly as described in PCT Publication No. WO 2008/147935. A summary of genes, plasmids and model system experiment numbers is shown in Table 21.

TABLE 21

Summary of Genes, Plasmids and Experiments

Experiment	Plasmid	Gene1[1, 2]	Gene2	Gene2 - SEQ ID NO	
				nt	aa
3073	pKR1256	YLDGAT2	-	-	-
3076	pKR2145	YLDGAT2	ZmODP1	69	70
3077	pKR2146	YLDGAT2	ZmLec1	64	65

[1]Gβηβ1 nucleotide sequence of SEQ ID NO: 59

[2]Gene1 amino acid sequence of SEQ ID NO: 60

Approximately 10-20 matured embryos from each of approximately 30 events per bombardment experiment were lyophilized, ground, oil content was measured by NMR and fatty acid profile was evaluated by FAME-GC analysis exactly as described in PCT Publication No. WO 2008/147935. The results for oil content and fatty acid profile for each event as well as the average of all events (Avg.) and average for the top 5 events having highest oil content (Top5 Avg.) are shown in Table 22.

In Table 22, results are sorted based on oil content from highest to lowest. In Table 22, oil content is reported as a percent of total dry weight (% Oil) and fatty

acid content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids.

TABLE 22

Summary of Oil Content and Fatty Acid Profiles for Events Expressing YLDGAT2 with ZmLec1 or ZmODPI

Event	% oil	16:0	18:0	18:1	18:2	18:3
3073-30	9.2	13.5	5.6	30.6	40.0	10.3
3073-28	7.8	17.0	3.7	18.8	45.8	14.8
3073-14	7.6	13.4	6.1	33.1	36.5	11.0
3073-15	6.9	16.0	5.7	22.3	42.1	13.9
3073-20	6.7	16.0	6.0	24.0	40.8	13.2
3073-1	6.6	14.2	6.5	32.6	36.1	10.6
3073-1 1	6.5	17.5	4.7	17.9	44.3	15.6
3073-10	6.4	14.1	6.6	27.9	38.3	13.1
3073-7	6.3	17.0	4.5	20.9	41.5	16.1
3073-24	6.2	14.7	6.1	28.7	38.0	12.5
3073-18	6.2	17.1	5.4	20.1	43.2	14.2
3073-29	6.1	17.3	5.3	20.4	41.0	16.0
3073-22	6.0	14.5	5.4	27.1	39.4	13.5
3073-5	6.0	14.1	5.2	18.1	45.0	17.6
3073-3	5.7	18.6	5.3	24.1	38.6	13.4
3073-2	5.7	16.5	5.5	21.5	41.3	15.1
3073-23	5.5	16.3	4.7	19.7	43.6	15.8
3073-6	5.5	17.1	6.0	24.7	38.9	13.4
3073-8	5.4	17.3	5.0	20.1	41.7	15.9
3073-17	5.3	15.4	5.2	22.3	43.6	13.4
3073-13	5.1	14.9	7.0	29.9	36.7	11.5
3073-16	4.6	16.8	6.4	24.7	38.1	14.0
3073-25	4.5	16.4	5.7	22.9	39.6	15.5
3073-4	4.4	15.7	5.1	29.8	35.6	13.8
3073-27	4.3	15.3	5.9	22.0	38.2	18.6
3073-19	4.3	16.6	6.5	23.5	38.9	14.5
3073-21	3.9	16.9	5.1	21.2	39.4	17.4
3073-26	3.8	17.1	4.7	18.8	39.5	19.8
3073-12	3.6	16.2	4.5	18.3	42.6	18.4
3073-9	3.0	17.5	4.9	21.4	38.6	17.6
Avg.	5.6	16.0	5.5	23.6	40.2	14.7
Top5 Avg.	7.6	15.2	5.4	25.7	41.0	12.6
3076-4	18.8	11.3	4.4	34.3	43.9	6.1
3076-2	15.4	12.3	6.7	34.0	40.5	6.5
3076-15	13.2	11.1	6.3	38.9	37.5	6.2

3076-12	12.1	11.2	7.6	32.5	41.3	7.4
3076-28	11.7	12.2	7.0	29.9	42.3	8.6
3076-5	11.4	13.4	6.9	29.0	41.6	9.0
3076-3	11.2	11.2	9.2	30.4	41.5	7.7
3076-13	11.0	11.7	5.3	33.7	41.4	7.9
3076-9	11.0	12.4	7.9	26.5	44.0	9.2
3076-26	10.5	13.9	5.3	38.1	36.0	6.8
3076-29	10.5	13.7	7.6	30.7	39.6	8.3
3076-10	10.2	14.1	6.0	29.8	41.2	9.0
3076-25	10.1	12.1	7.2	34.6	37.5	8.5
3076-27	9.2	13.7	6.1	34.0	39.3	7.0
3076-18	8.9	14.4	7.2	22.4	44.4	11.7
3076-24	8.9	13.7	7.8	26.8	42.1	9.7
3076-22	8.8	12.7	7.2	27.3	42.3	10.5
3076-8	8.8	14.1	7.0	26.1	41.6	11.1
3076-23	8.7	14.0	4.5	31.4	40.1	10.0
3076-1 1	8.3	15.1	6.6	17.9	47.5	13.0
3076-31	8.3	15.1	6.6	21.3	44.2	12.8
3076-21	8.1	13.4	6.6	32.2	39.9	7.9
3076-1	7.8	13.5	7.6	30.2	39.2	9.5
3076-17	7.7	15.5	4.8	17.9	47.4	14.4
3076-20	7.1	15.8	5.5	16.3	47.0	15.4
3076-16	6.8	14.9	5.6	23.8	43.2	12.4
3076-7	6.7	14.6	7.2	24.9	41.5	11.8
3076-14	6.2	15.8	5.4	19.1	45.3	14.5
3076-6	6.1	15.8	7.3	20.6	43.6	12.7
3076-19	4.6	15.9	6.0	20.4	44.1	13.5
3076-30	3.5	16.0	6.2	21.1	43.7	13.1
Avg.	9.4	13.7	6.5	27.6	42.1	10.1
Top5 Avg.	14.2	11.6	6.4	33.9	41.1	7.0
3076-16	15.5	11.5	6.7	35.0	39.4	7.3
3076-10	13.9	11.9	6.6	33.8	40.4	7.2
3076-21	12.6	10.2	8.2	41.9	33.0	6.7
3076-3	12.0	10.2	7.0	42.9	33.1	6.7
3076-23	11.5	11.7	8.0	37.1	36.9	6.2
3076-12	11.4	12.3	6.5	32.8	39.3	9.0
3076-26	10.9	12.2	5.6	30.5	42.0	9.7
3076-27	10.9	13.6	6.0	28.9	41.5	9.9
3076-22	10.7	11.8	6.4	38.3	35.3	8.2
3076-24	10.7	12.8	6.6	31.8	39.1	9.7
3076-5	10.4	11.0	4.1	37.1	40.6	7.2
3076-9	10.3	15.2	5.7	21.6	46.5	10.9
3076-17	10.0	13.3	6.8	34.7	36.8	8.5
3076-6	9.7	10.9	7.6	44.8	30.5	6.2
3076-13	9.6	15.1	5.8	20.8	47.5	10.8
3076-4	9.2	14.6	8.0	26.1	42.0	9.3
3076-15	8.9	13.7	4.6	33.1	36.7	12.0

3076-20	8.1	14.8	6.0	27.2	39.7	12.3
3076-1 1	7.5	12.7	6.3	36.7	35.1	9.2
3077-1	6.8	15.3	6.0	28.5	38.6	11.5
3076-25	6.7	15.8	5.2	22.8	43.0	13.3
3076-8	6.5	15.9	6.1	21.6	45.0	11.4
3076-7	5.3	17.1	7.4	28.9	36.6	10.1
3076-19	4.4	15.0	4.0	17.9	48.6	14.5
3076-28	4.3	14.0	3.6	26.7	42.2	13.4
3076-2	3.5	16.7	3.4	17.0	44.3	18.6
3076-18	3.1	15.4	3.6	21.7	41.2	18.0
3076-14	2.6	16.2	6.1	25.3	39.2	13.2
Avg.	8.8	13.6	6.0	30.2	39.8	10.4
Top5 Avg.	13.1	11.1	7.3	38.2	36.6	6.8

A summary comparing the average oil content and average fatty acid profile for all events in each experiment is shown in Table 23. In Table 23, average oil content is reported as a percent of total dry weight (Avg. Oil) and average fatty acid content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids. Table 3 also shows the change in oil content (Avg. % Inc.) as compared to the Control experiment where Avg. % Inc. is calculated as the Avg. Oil for that experiment minus the Avg. Oil for the control experiment divided by the Avg. Oil for the control experiment expressed as a percent.

TABLE 23

Summary of Average Oil Content and Fatty Acid Profiles for All Events Expressing YLDGAT2 with ZmLec1 or ZmODPI

MSE	Vector (Gene2)	Avg. Oil	Avg. % Inc	16:0	18:0	18:1	18:2	18:3
3073	pKR1256 (n/a)	5.6	0%	16.0	5.5	23.6	40.2	14.7
3076	pKR2145 (ZmODP1)	9.4	67%	13.7	6.5	27.6	42.1	10.1
3077	pKR2146 (ZmLec1)	8.8	57%	13.6	6.0	30.2	39.8	10.4

A summary comparing the average oil content and average fatty acid profile of the top 5 events having the highest oil content for each experiment is shown in Table 24. In Table 24, average oil for the 5 events having highest oil content is reported as a percent of total dry weight (Top5 Avg. Oil) and average fatty acid

content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids. Table 4 also shows the change in oil content (Top5 Avg. % Inc.) as compared to the Control experiment where Avg. % Inc. is calculated as the Avg. Oil for that experiment minus the Avg. Oil for the control experiment divided by the Avg. Oil for the control experiment expressed as a percent.

TABLE 24

Summary of Average Oil Content and Fatty Acid Profiles for the Top5 Events Having Highest Oil Contents and Expressing YLDGAT2 with ZmLec1 or ZmODPI

MSE	Vector (Gene2)	Avg. Oil	Avg. % Inc	16:0	18:0	18:1	18:2	18:3
3073	pKR1256 (n/a)	7.6	0%	15.2	5.4	25.7	41.0	12.6
3076	pKR2145 (ZmODP1)	14.2	86%	11.6	6.4	33.9	41.1	7.0
3077	pKR2146 (ZmLec1)	13.1	72%	11.1	7.3	38.2	36.6	6.8

Both Tables 23 and 24 demonstrate that expression of ZmLec1 and ZmODPI with YLDGAT2 lead to an increase in oil content in soy above that for YLDGAT2 alone.

EXAMPLE 10

Identification and Cloning of the *Medicago truncatula* Sucrose Synthase Promoter

The amino acid sequence of the soybean homolog (Glyma13g1 7420) to the *Arabidopsis* Sucrose Synthase 2 gene was identified (SEQ ID NO: 6).

A *Medicago truncatula* homolog of Glyma13g1 7420 (SEQ ID NO: 6) was identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al., *J. Mol. Biol.* 215:403-41 0 (1993)) searches for similarity to sequences contained in the *Medicago truncatula* Genome Project "Mt3.5.1 Release" gene set. Sequence information from the *Medicago truncatula* Genome Project is available at the J. Craig Venter Institute. Specifically, the Glyma13g 17420 amino acid sequence (SEQ ID NO: 6) was used with the TBLASTN algorithm provided by National Center for Biotechnology Information (NCBI) with default parameters except the Filter Option was set to OFF.

The *Medicago truncatula* homolog identified corresponded to Medtr4g 124660.2 and the predicted CDS and corresponding amino acid sequences for Medtr4g1 24660.2 are set forth in SEQ ID NO: 79 and SEQ ID NO: 80, respectively. The predicted amino acid sequence of Medtr4g 124660 shares 93.3 % sequence identity to the predicted amino acid sequence of Glyma1 3g 17420 in a CLUSTAL W alignment. *Medicago truncatula* gene expression data is available at the Bio-Array Resource for Plant Biology at the University of Toronto (Winter, D; et al. PLoS One (2007), 2(8):e71 8). Analysis of the *Medicago truncatula* gene expression data revealed that Medtr4g 124660 is expressed in developing seeds in synchrony with oil and protein accumulation.

A 3.3 kb promoter region of genomic DNA upstream of the start codon of Medtr4g 124660.2 was identified from the Medicago "Mt3.5.1 Release" and the sequence is set forth in SEQ ID NO: 8 1.

Medicago truncatula seeds were sterilized and germinated on plates using methods familiar to one skilled in the art. Genomic DNA was isolated from leaves of approximately 3 week old *Medicago truncatula* seedlings using the DNEASY® Plant Mini Kit (Qiagen, Valencia, CA) and following the manufacture's protocol. The Medtr4g 124660.2 promoter region (SEQ ID NO: 8 1) was PCR-amplified from the genomic DNA using forward primer oMDSP-1 F (SEQ ID NO: 82) and reverse primer oMDSP-1 R (SEQ ID NO: 83) with the PHUSION™ High-Fidelity DNA Polymerase (Cat. No. F553S, Finnzymes Oy, Finland), following the manufacturer's protocol. The resulting DNA fragment was cloned into the pCR®-BLUNT® cloning vector using the ZERO BLUNT® PCR Cloning Kit (Invitrogen Corporation), following the manufacturer's protocol, to produce pKR2434 (SEQ ID NO: 84).

The sequence of the promoter region sequence for multiple individual PCR products was determined from a number of clones and the actual sequence is set forth is SEQ ID NO: 85. The actual promoter sequence differs from SEQ ID NO: 8 1 in that nt 67 is a T, nt 489 is a C, nts 553-555 (TTG) are deleted, nt 629 is an A, nt 649 is a C, nt 7 15 is an A, nt 784 is a C, nt 800 is a G, nt 893 is a G, nt 1166 is an A, nt 1535 is deleted (T), nt 1700 is a G, nt 17 18 is a C, nt 1857-1 880 are deleted (ATTTTAG AATATGCAATAAAATTG ; SEQ ID NO: 10 1), nt 1953 is a G, nt 2038 is deleted (A), there is a 25 bp insertion between nt 2224 and 2225 (AGGCTTGAGGAATAAGATAAGACTTGT; SEQ ID NO: 102),an A is inserted

between nt 2225 and 2226, nt 2421 is a G, a C is inserted between nt 2734 and 2735 and nt 2881 is a T. These differences are likely due to a different cultivar of *Medicago truncatula* being used than that of used to determine the genome sequence.

The actual Medtr4g1 24660.2 promoter region (called MTSusPro; SEQ ID NO: 85) encodes the 5' UTR from nt 2495-3285 including an intron from nt 2524-3272.

Plasmid pKR1 964 (SEQ ID NO: 13) was digested with NotI/SalI and the fragment containing the Leg terminator was cloned into the NotI/XhoI fragment of pKR2434 (SEQ ID NO: 84), containing the MTSusPro, to produce pKR2446 (SEQ ID NO: 86).

The BsiWI fragment of pKR2446 (SEQ ID NO: 86), containing the MTSusPro, was cloned into the BsiWI site of pKR325 to produce pKR2457 (SEQ ID NO: 87). Plasmid pKR2457 contains a NotI site flanked by the MTSusPro and the Leg terminator as well as the hygromycin B phosphotransferase gene [Gritz, L. and Davies, J. (1983) *Gene* 25:1 79-1 88], flanked by the T7 promoter and transcription terminator, a bacterial origin of replication (ori) for selection and replication in *E. coli* and the hygromycin B phosphotransferase gene, flanked by the 35S promoter [Odell et al., (1985) *Nature* 313:810-812] and NOS 3' transcription terminator [Depicker et al., (1982) *J. Mol. Appl. Genet.* 1:561 :570] (35S/hpt/NOS3' cassette) for selection in soybean. In this way, polynucleotides (e.g., protein-coding regions) flanked by NotI sites can be cloned into the NotI site of pKR2457 (SEQ ID NO: 87) and subsequently expressed in soybean.

EXAMPLE 11

Expressing GmODPI in Soybean Embryos under Control of the *Medicago truncatula* Sucrose Synthase Promoter MTSusPro

The NotI fragment of KS334, containing GmODPI was cloned into the NotI site of pKR2457 (SEQ ID NO: 87) to produce pKR2461 (SEQ ID NO: 88). In this way, the GmODPI could be expressed behind the *Medicago truncatula* sucrose synthase promoter (MTSusPro).

Plasmid pKR278, previously described in PCT Publication No. WO 2008/147935, and containing no transcription factor, was used as a negative control.

DNA from plasmids pKR2461 (SEQ ID NO: 88) and pKR278 was prepared for particle bombardment into soybean embryogenic suspension culture and transformed exactly as described previously in PCT Publication No. WO 2008/147935. Soybean embryogenic suspension culture was initiated, grown, maintained and bombarded and events were selected and matured on SHaM media also exactly as described in PCT Publication No. WO 2008/147935. A summary of genes, plasmids and model system experiment ("MSE") numbers is shown in Table 25.

TABLE 25

Summary of Genes, Plasmids and Experiments

Experiment	Plasmid	Gene	SEQ ID NO	
			nt	aa
MSE 3405	pKR2461	GmODP1	29	30
MSE 3408	pKR278	Empty Vector Control	-	-

Approximately 10-20 matured embryos from each of approximately 30 events per bombardment experiment were lyophilized, ground, oil content was measured by NMR and fatty acid profile was evaluated by FAME-GC analysis exactly as described in PCT Publication No. WO 2008/147935. The results for oil content and fatty acid profile for each event as well as the average of all events (Avg.) and average for the top 5 events having highest oil content (Top5 Avg.) are shown in Table 26.

In Table 26, results are sorted based on oil content from highest to lowest. In Table 26, oil content is reported as a percent of total dry weight (%Oil) and fatty acid content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids.

TABLE 26

Summary of Oil Content and Fatty Acid Profiles for Events Expressing GmODPI or Empty Vector Control

	% oil	16:0	18:0	18:1	18:2	18:3
3405-6	8.75	16.15	4.56	19.73	47.20	12.35
3405-8	8.42	16.90	4.13	17.50	47.66	13.81
3405-28	7.82	14.81	4.74	17.99	48.88	13.57
3405-22	7.51	18.94	4.47	15.69	48.33	12.57
3405-10	7.45	15.90	6.32	23.41	42.44	11.94
3405-26	7.21	15.84	4.56	22.97	43.57	13.06
3405-18	7.20	14.51	6.66	21.47	44.01	13.35
3405-16	7.13	15.65	6.57	26.47	38.88	12.44
3405-17	7.03	13.38	5.55	27.10	42.71	11.25
3405-30	7.03	14.99	5.89	23.63	42.16	13.33
3405-23	7.00	16.99	6.17	25.64	39.15	12.05
3405-25	6.98	15.91	6.33	23.96	40.73	13.06
3405-15	6.71	16.58	4.53	19.49	44.44	14.96
3405-9	6.46	15.62	6.43	25.38	39.38	13.19
3405-5	6.33	15.53	6.65	26.24	37.94	13.64
3405-3	6.11	15.99	6.55	24.56	40.56	12.35
3405-12	6.03	16.60	6.28	21.03	42.76	13.32
3405-4	5.96	16.88	5.00	20.83	45.03	12.27
3405-14	5.39	17.58	5.60	23.24	38.95	14.64
3405-1	5.27	15.57	5.81	24.92	42.12	11.58
3405-29	5.13	15.38	6.49	29.95	36.53	11.65
3405-11	4.82	15.71	6.72	26.72	37.89	12.96
3405-13	4.46	16.99	4.21	14.27	46.23	18.30
3405-27	4.39	17.63	4.01	16.00	44.45	17.91
3405-2	4.26	17.24	5.13	18.15	43.89	15.59
3405-19	4.02	16.78	4.03	17.55	41.47	20.17
3405-7	3.80	17.47	5.41	19.24	39.73	18.15
3405-20	3.40	16.52	5.91	23.70	37.76	16.12
3405-21	3.17	15.01	5.54	19.70	42.96	16.79
3405-24	3.05	16.87	5.46	21.12	40.50	16.05
Avg.	5.94	16.20	5.52	21.92	42.28	14.08
Top5	7.99	16.54	4.85	18.87	46.90	12.85
3408-3	8.19	15.10	6.50	25.26	40.59	12.56
3408-6	6.36	15.50	5.91	22.56	43.40	12.62
3408-4	4.84	16.08	8.02	33.94	30.43	11.53
3408-2	4.61	16.26	5.09	15.84	44.05	18.76
3408-9	4.39	18.15	4.52	21.48	38.24	17.63
3408-7	4.23	16.44	6.11	26.28	34.96	16.22
3408-1	3.99	16.20	6.51	17.74	40.81	18.75
3408-10	3.62	17.37	6.26	23.12	35.29	17.96

Avg.	5.03	16.39	6.11	23.28	38.47	15.75
Top5	5.68	16.22	6.01	23.81	39.34	14.62

A summary comparing the average oil content and average fatty acid profile for all events in each experiment is shown in Table 27. In Table 27, average oil content is reported as a percent of total dry weight (Avg. Oil) and average fatty acid content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids. Table 27 also shows the change in oil content (Avg. % Inc.) as compared to the Control experiment where Avg. % Inc. is calculated as the Avg. Oil for that experiment minus the Avg. Oil for the control experiment divided by the Avg. Oil for the control experiment expressed as a percent.

TABLE 27

Summary of Average Oil Content and Fatty Acid Profiles for All Events Expressing GmODPI or Empty Vector Control

MSE	Vector (Gene)	Avg. Oil	Avg. % Inc	16:0	18:0	18:1	18:2	18:3
3405	pKR2461 (GmODP1)	5.94	18%	16.20	5.52	21.92	42.28	14.08
3408	pKR278 (Control)	5.03	0%	16.22	6.01	23.81	39.34	14.62

A summary comparing the average oil content and average fatty acid profile of the top 5 events having the highest oil content for each experiment is shown in Table 28. In Table 28, average oil for the 5 events having highest oil content is reported as a percent of total dry weight (Top5 Avg. Oil) and average fatty acid content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids. Table 28 also shows the change in oil content (Top5 Avg. % Inc.) as compared to the Control experiment where Avg. % Inc. is calculated as the Avg. Oil for that experiment minus the Avg. Oil for the control experiment divided by the Avg. Oil for the control experiment expressed as a percent.

TABLE 28

Summary of Average Oil Content and Fatty Acid Profiles for the Top5 Events Having Highest Oil Contents and Expressing GmODPI or Empty Vector Control

MSE	Gene (Vector)	Avg. Oil	Avg. % Inc	16:0	18:0	18:1	18:2	18:3
3405	GmODP1 (pKR2461)	7.99	41%	4.85	18.87	46.90	12.85	4.85
3408	Control (pKR278)	5.68	0%	16.22	6.01	23.81	39.34	14.62

Both Tables 27 and 28 demonstrate that expression of GmODPI , under control of the MTSusPro, leads to an increase in oil content in soy.

EXAMPLE 12

Co-Expressing GmODPI under Control of the MTSusPro with YLDGAT2 in Soybean Embryos

The SbfI fragment of pKR2461 (SEQ ID NO: 88), containing GmODPI was cloned into the SbfI site of pKR1256 to produce pKR2465 (SEQ ID NO: 89). In this way, the GmODPI could be expressed behind the *Medicago truncatula* sucrose synthase promoter (MtSusPro) and co-expressed with YLDGAT2 (SEQ ID NO: 59).

DNA from plasmid pKR2465 (SEQ ID NO: 89) was prepared for particle bombardment into soybean embryogenic suspension culture and transformed exactly as described previously in PCT Publication No. WO 2008/147935. Soybean embryogenic suspension culture was initiated, grown, maintained and bombarded and events were selected and matured on SHaM media also exactly as described in PCT Publication No. WO 2008/147935. A summary of genes, plasmids and model system experiment numbers is shown in Table 29.

TABLE 29

Summary of Genes, Plasmids and Experiments

Experiment	Plasmid	Gene1[1,2]	Gene2	Gene2 SEQ ID NO	
				nt	aa
3013	pKR1256	YLDGAT2	-	-	-

3410	pKR2465	YLDGAT2	GmODP	29	30

[1]ΘΘΠΘ1 nucleotide sequence of SEQ ID NO: 59

[2]Gene1 amino acid sequence of SEQ ID NO: 60

Approximately 10-20 matured embryos from each of approximately 30 events per bombardment experiment were lyophilized, ground, oil content was measured by NMR and fatty acid profile was evaluated by FAME-GC analysis exactly as described in PCT Publication No. WO 2008/147935. The results for oil content and fatty acid profile for each event as well as the average of all events (Avg.) and average for the top 5 events having highest oil content (Top5 Avg.) are shown in Table 30.

In Table 30, results are sorted based on oil content from highest to lowest. In Table 30, oil content is reported as a percent of total dry weight (% Oil) and fatty acid content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids.

TABLE 30

Summary of Oil Content and Fatty Acid Profiles for Events Expressing YLDGAT2 with GmODP1

	% oil	16:0	18:0	18:1	18:2	18:3
3410-13	12.84	14.00	7.52	38.62	33.00	6.86
3410-14	12.65	13.74	7.78	39.15	32.53	6.79
3410-10	10.91	12.35	7.43	39.29	33.65	7.28
3410-7	9.54	12.20	6.76	43.82	30.17	7.05
3410-12	9.24	13.10	6.50	31.48	38.65	10.27
3410-2	8.13	15.47	7.18	25.92	40.37	11.06
3410-1	7.71	15.31	7.93	26.95	38.07	11.74
3410-18	7.33	15.77	7.72	24.84	38.95	12.72
3410-20	7.21	15.86	6.26	24.01	40.70	13.17
3410-11	6.69	15.83	6.90	24.91	39.65	12.71
3410-22	6.00	19.18	7.02	21.20	38.22	14.38
3410-9	5.81	17.73	4.70	16.30	42.22	19.05
3410-3	5.60	16.69	6.26	22.27	38.26	16.51
3410-24	5.33	16.38	5.35	25.80	38.16	14.30
3410-6	5.21	12.97	6.87	31.30	37.10	11.77
3410-21	5.12	16.93	7.01	21.80	35.00	19.27
3410-8	5.04	15.87	6.20	24.22	39.68	14.03
3410-17	5.03	18.12	5.35	21.09	40.85	14.59

3410-16	4.96	15.07	6.42	23.73	38.66	16.12
3410-23	4.43	17.11	5.88	21.63	38.75	16.63
3410-4	3.46	17.68	5.71	17.57	42.30	16.72
3410-19	3.42	17.88	5.24	19.63	40.96	16.29
3410-15	3.39	15.10	4.93	18.06	40.91	21.00
3410-5	2.70	16.45	5.58	19.40	37.47	21.10
Avg.	6.57	15.70	6.44	25.96	38.10	13.81
Top5_Avg.	11.04	13.08	7.20	38.47	33.60	7.65
3413-17	9.79	12.44	4.66	37.55	35.95	9.40
3413-28	9.55	14.97	5.89	21.69	46.18	11.27
3413-29	9.00	13.79	5.32	33.06	37.80	10.03
3413-6	8.59	13.37	4.79	31.02	38.32	12.51
3413-27	7.50	14.37	7.30	30.67	36.18	11.47
3413-12	7.46	12.90	6.09	34.45	35.44	11.12
3413-13	7.03	13.39	6.70	29.70	36.93	13.28
3413-25	6.77	17.27	6.84	23.25	40.01	12.62
3413-26	6.76	16.17	4.52	23.89	39.80	15.62
3413-24	6.70	16.57	4.20	22.35	42.27	14.61
3413-19	6.33	15.79	6.91	26.12	38.09	13.09
3413-21	5.99	18.60	5.10	20.36	40.78	15.15
3413-9	5.71	14.86	3.99	24.64	39.24	17.28
3413-23	5.54	16.32	4.11	20.13	41.63	17.81
3413-2	5.39	15.11	4.09	24.74	39.50	16.56
3413-20	5.26	16.83	4.30	21.17	40.63	17.06
3413-11	5.23	15.29	5.65	26.43	37.27	15.35
3413-14	5.11	16.70	4.60	22.63	38.10	17.97
3413-18	4.61	16.73	3.82	18.75	41.48	19.21
3413-16	4.18	16.62	3.71	20.39	37.95	21.32
3413-15	4.12	16.87	4.46	19.87	41.60	17.20
3413-22	3.57	17.47	3.58	15.47	41.65	21.83
3413-5	3.56	16.90	3.88	17.62	39.90	21.71
3413-3	3.24	16.90	4.34	17.33	41.69	19.73
3413-7	2.97	16.31	5.25	18.53	37.52	22.39
3413-10	2.96	17.36	3.86	14.13	41.16	23.49
3413-8	2.93	16.62	5.51	23.68	39.11	15.09
3413-4	2.88	18.11	3.68	14.51	41.08	22.62
3413-1	2.28	16.97	5.10	20.71	38.28	18.94
Avg.	5.55	15.92	4.91	23.27	39.50	16.41
Top5_Avg.	8.89	13.79	5.59	30.80	38.89	10.93

A summary comparing the average oil content and average fatty acid profile for all events in each experiment is shown in Table 31. In Table 31, average oil content is reported as a percent of total dry weight (Avg.Oil) and average fatty acid content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1),

linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids. Table 31 also shows the change in oil content (Avg. %Inc.) as compared to the Control experiment where Avg. % Inc. is calculated as the Avg. Oil for that experiment minus the Avg. Oil for the control experiment divided by the Avg. Oil for the control experiment expressed as a percent.

TABLE 31

Summary of Average Oil Content and Fatty Acid Profiles for All Events Expressing YLDGAT2 with GmODPI

MSE	Vector (Gene)	Avg. Oil	Avg. % Inc	16:0	18:0	18:1	18:2	18:3
3413	pKR1256 (n/a)	5.55	0%	15.70	6.44	25.96	38.10	13.81
3410	pKR2465 (GmODP1)	6.57	18%	14.0	6.2	34.6	36.8	8.5

A summary comparing the average oil content and average fatty acid profile of the top 5 events having the highest oil content for each experiment is shown in Table 32. In Table 32, average oil for the 5 events having highest oil content is reported as a percent of total dry weight (Top5 Avg. Oil) and average fatty acid content for each fatty acid [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) & alpha-linolenic acid (18:3)] is reported as a weight % of total fatty acids. Table 12 also shows the change in oil content (Top5 Avg. % Inc.) as compared to the Control experiment where Avg. % Inc. is calculated as the Avg. Oil for that experiment minus the Avg. Oil for the control experiment divided by the Avg. Oil for the control experiment expressed as a percent.

TABLE 32

Summary of Average Oil Content and Fatty Acid Profiles for the Top5 Events Having Highest Oil Contents and Expressing YLDGAT2 with GmODPI

MSE	Vector (Gene)	Avg. Oil	Avg. % Inc	16:0	18:0	18:1	18:2	18:3
3413	pKR1256 (n/a)	8.89	0%	13.1	5.9	27.2	44.7	9.1
3410	pKR2465 (GmODP1)	11.04	24%	13.79	5.59	30.80	38.89	10.93

Both Tables 31 and 32 demonstrate that expression of GmODPI , under control of the MtSusPro, with YLDGAT2 lead to an increase in oil content in soy above that for YLDGAT2 alone.

EXAMPLE 13

Expressing GmLecl , GmODPI and GmFusca-3-1 in Soybean Seed under Control of the GmSus Promoter

Artificial microRNAs silencing fad2 genes as reporter for transgenic events:

The fatty acid desaturase 2-1 (Fad2-1) or 2-2 (fad2-2) gene families (Heppard, EP, et al. (1996) Plant Physiology, 110(1): 311-319), also known as delta-12 desaturase or omega-6 desaturase (US Patent Numbers US6872872B1 , US6919466B2 and US7105721 B2), convert oleic acid into linoleic acid. Effective silencing of the fad2-1 and fad2-2 gene families seed-specifically in soy results in seed oil having an increased oleic acid content which can be detected using methods known to one skilled in the art such as those described herein. This increased oleic acid content can be used as a reporter to identify transgenic seed in segregating seed populations from null seed.

The design and synthesis of artificial microRNAs (amiRNAs), and the respective STAR sequences that pair with amiRNAs, for silencing the soy fad2-1 and fad2-2 genes was previously described in US20090155910A1 (WO 2009/079532) (the contents of which are incorporated by reference) and the sequences are described in Table 33.

TABLE 33

amiRNA and Star Sequences For Sov fad2-1 and fad2-2

Gene Family	amiRNA	SEQ ID NO	STAR Sequence	SEQ ID NO
GmFad2-1	GM-MFAD2-1B	90	396b-GM-MFAD2-1B	91
GmFad2-2	GM-MFAD2-2	92	159-GM-MFAD2-2	93

The identification of the genomic miRNA precursor sequences 159 and 396b was described previously in US20090155910A1 (WO 2009/079532) and their sequences are set forth in SEQ ID NO: 94 and SEQ ID NO: 95, respectively.

Genomic miRNA precursor sequences 159 (SEQ ID NO: 94) and 396b (SEQ ID NO: 95) were converted to amiRNA precursors 396b-fad2-1 b and 159-fad2-2

using overlapping PCR as previously described in US20090155910A1 (WO 2009/079532).

amiRNA precursor 159-fad2-2 was cloned downstream of 396b-fad2-1 b to produce the amiRNA precursor 396b-fad2-1 b/1 59-fad2-2 (SEQ ID NO: 96).

The amiRNA precursor 396b-fad2-1 b/1 59-fad2-2 (SEQ ID NO: 96) is 1577 nt in length and is substantially similar to the deoxyribonucleotide sequence set forth in SEQ ID NO: 95 (from nt 1 to 574 of 396b-fad2-1 b/1 59-fad2-2) wherein nucleotides 196 to 216 of SEQ ID NO: 95 are replaced by GM-MFAD2-1 B amiRNA (SEQ ID NO: 90) and wherein nucleotides 262 to 282 of SEQ ID NO: 95 are replaced by 396b-GM-MFAD2-1 B Star Sequence (SEQ ID NO: 91). The amiRNA precursor 396b-fad2-1 b/1 59-fad2-2 (SEQ ID NO: 96) is also, substantially similar to the deoxyribonucleotide sequence set forth in SEQ ID NO: 94 (from nt 620 to 1577 of 396b-fad2-1 b/1 59-fad2-2) wherein nucleotides 276 to 296 of SEQ ID NO: 94 are replaced by GM-MFAD2-2 amiRNA (SEQ ID NO: 92) and wherein nucleotides 121 to 141 of SEQ ID NO: 94 are replaced by 159-GM-MFAD2-2 Star Sequence (SEQ ID NO: 93). In amiRNA precursor 396b-fad2-1 b/1 59-fad2-2, nt 575 to 610 are derived from cloning.

Construction of Soybean Expression Vector pKR2109:

Using standard PCR and cloning methods by one skilled in the art, the following DNA elements were assembled to produce the 8095 bp soybean expression vector pKR2109 (SEQ ID NO: 97) and having unique SbfI (nt 8093) and BsiWI (nt 1) restriction sites for cloning expression cassettes.

In pKR2109 (SEQ ID NO: 97), sequence 21-36 is a sequence of DNA comprising ORF stop codons in all 6 frames (ORFSTOP-A). Sequence 65-2578 is vector backbone containing the T7 promoter (sequence 1297-1394), the hygromycin phosphotransferase (hpt) gene coding region (sequence 1395-2435) and the T7 terminator (sequence 2436-2582). Sequence 2616-2632 is a sequence of DNA comprising ORF stop codons in all 6 frames (ORFSTOP-B). Sequence 2698-4006 is the constitutive soy SAMS promoter (U.S. Patent No. 7217858). Sequence 4011-4058 is a FLP recombinase recognition site *FRT*1 (U.S. Patent No. 8293533). Sequence 4068-5093 is the hygromycin phosphotransferase (hpt) gene coding region for selection in soy. Sequence 5102-5382 is the NOS 3' transcription terminator (Depicker et al., *J. Mol. Appl. Genet.* 1:561-570 (1982)). Sequence 5400-

6170 is the 776 bp fragment of the soy annexin promoter (described in Applicants' Assignee's US Patent No. 7,129,089). Sequence 6179-7756 is the amiRNA precursor 396b-fad2-1 b/1 59-fad2-2 (SEQ ID NO: 96). Sequence 7773-7988 is the soy BD30 transcription terminator (described in Applicants' Assignee's US Patent No. 8,084,074). Sequence 8021-8068 is a FLP recombinase recognition site *FR*787 (U.S. Patent No. 8293533).

Expressing GmLed, GmODPI and GmFusca3-1 in Soybean under Control of the GmSus Promoter:

The SbfI fragments of pKR1968 (SEQ ID NO: 50), containing GmLec1, pKR1971 (SEQ ID NO: 51), containing GmODPI and pKR1969 (SEQ ID NO: 52), containing GmFusca3-1 were cloned into the SbfI site of pKR2109 (SEQ ID NO: 97) to produce pKR2118 (SEQ ID NO: 98), pKR2120 (SEQ ID NO: 99) and pKR2119 (SEQ ID NO: 100), respectively.

Each experiment was given a name and a summary of the experiment name, construct used and genes expressed is shown in Table 34.

TABLE 34

Summary of Genes, Plasmids and Experiments

Experiment	Plasmid	Gene	Gene SEQ ID NO	
			nt	aa
Oil108	pKR2119	GmFusca3-1	48	49
Oil109	pKR2120	GmODP1	29	30
Oil110	pKR2118	GmLec1	24	25

DNA from these plasmids was prepared for particle bombardment into soybean embryogenic suspension culture and transformed exactly as described previously in PCT Publication No. WO 2008/147935. Soybean embryogenic suspension culture was initiated, grown and maintained and events were selected and matured exactly as described in PCT Publication No. WO 2008/147935. In this

case, hygromycin was used for selection. Events from each of the 3 experiments were screened at the embryo stage for fatty acid profile by methods described herein and those displaying an increased oleic acid phenotype were advanced.

Embryos from selected events were dried and germinated and T0 plants were grown and maintained exactly as described in PCT Publication No. WO 2008/147935.

Approximately 36 T1 seeds from T0 plants for each event were harvested and individual T1 seed were analyzed for oil and protein content using Near Infrared Spectroscopy by methods familiar to one skilled in the art [Agelet, et al. (2012) Journal of Agricultural and Food Chemistry , 60(34): 8314-8322].

Seeds were also analyzed for fatty acid profile in order to identify transgenic and null seed. Those seed having oleic acid contents higher than approximately 30%, resulting from expression of the amiRNA precursor 396b-fad2-1 b/1 59-fad2-2, were considered transgenic. Those with approximately less than 30% oleic acid content were considered null seed.

For each event, the average oil content of all transgenic seed and all null seed was determined. The average oil content of null seed was then subtracted from the average oil content of the transgenic seed and the difference is reported in Table 35 (Avg. Oil Delta %). The difference in average protein content between transgenic and null seed was similarly determined and is shown in Table 35 (Avg. Pro Delta %). The sum of the Avg. Oil Delta % and Avg. Pro Delta % (Avg. Proil Delta %) is also shown in Table 35. For a representative number of events of each construct at least 24 seeds were germinated in soil and germination rate was determined 10 days after planting.

In Table 35, the experiment name (Exp.), the gene being expressed (Gene) and the event name (Event) are also shown.

TABLE 35

Summary of Difference In Average Oil and Protein Contents Between Transgenic and Null T 1 Seed for Soybean Events Expressing GmLecl , GmFusca3-1 or GmODPI

Exp.	Gene	Event	Avg. Oil Delta %	Avg. Pro Delta %	Avg. Proil Delta %	Germi-nation %
Oil 108	GmFusca3-1	8798.1 0.3	1.3	2	3.3	78
Oil 108	GmFusca3-1	8798.4.1	1.2	1.5	2.7	71
Oil 108	GmFusca3-1	8798.1 .2	1	1.6	2.6	49
Oil 108	GmFusca3-1	8798.6.3	1	1.5	2.5	20
Oil 108	GmFusca3-1	8798.3.2	0.7	1.7	2.5	
Oil 108	GmFusca3-1	8798.4.3	1	1.3	2.3	57
Oil 108	GmFusca3-1	8798.8.1	-0.5	2.7	2.2	
Oil 108	GmFusca3-1	8798.1 .2	0.5	1.5	2	49
Oil 108	GmFusca3-1	8798.9.4	0.3	0.2	0.5	
Oil 109	GmODPI	881 0.5.1	1.9	2.4	4.3	99
Oil 109	GmODPI	8787.3.3	1.2	1.9	3.1	95
Oil 109	GmODPI	8787.1 2.2	0.4	2.4	2.8	90
Oil 109	GmODPI	87871 0.1	1.4	0.9	2.2	87
Oil 109	GmODPI	8787.4.1	0.7	1.4	2	
Oil 109	GmODPI	8787.8.4	1.1	0.8	1.9	
Oil 109	GmODPI	8787.1 0.5	-0.2	1.8	1.7	
Oil 109	GmODPI	8787.7.3	1.3	0.4	1.7	79
Oil 109	GmODPI	8787.3.2	0.3	0.8	1.1	
Oil 109	GmODPI	8787.1 .1	-0.2	1	0.8	85
Oil 109	GmODPI	8787.6.4	0.2	0.4	0.7	
Oil 109	GmODPI	8787.1 2.3	1.7	-1	0.6	95
Oil 109	GmODPI	8787.1 1.4	0	0.5	0.5	94
Oil 109	GmODPI	8787.6.3	-1.5	0.5	-1	83
Oil 110	GmLecl	8781 .6.1	1	2	2.9	33
Oil 110	GmLec1	8781 .2.2	0.9	1.8	2.8	91
Oil 110	GmLed	8781 .2.3	1.2	1.5	2.8	81
Oil 110	GmLec1	8781 .10.5	0.9	1.9	2.8	81
Oil 110	GmLed	8781 .3.6	0.8	1.5	2.3	32

Oil 110	GrnLecl	8781 .1 1.2	0.7	1.3	2	69
Oil 110	GrnLecl	8781 .1 1.1	0.3	0.5	0.7	

Table 35 shows that average oil and protein content is increased when GmFusca3-1 , GmODPI or GrnLecl is over-expressed in soybean under control of the GmSus promoter when compared to the average of null seed. Oil and protein are increased by as high as 2.9 to 4.3 points in these events. Table 35 also shows that T1 seed germination frequency of events with significant oil and protein increase due to expression of ODP1 , LEC1 and Fusca3 transcription factors can be as high as 99%, 91% and 78%, respectively.

T1 seed from events segregating as single copy (HiOleic Phenotype:Null = 3:1) were planted, plants were grown exactly as for TO plants and T2 seed were obtained. T2 seed from these events were analyzed for oleic acid, oil and protein content exactly as described herein and results are shown for Oil109 in Table 36.

For each event, the average oil content of all transgenic homozygous T2 seed and all null seed was determined. The average oil content of null seed was then subtracted from the average oil content of the homozygous T2 transgenic seed and the difference is reported in Table 36 (Avg. Oil Delta %). The difference in average protein content between T2 homozygous transgenic and null seed was similarly determined and is shown in Table 36 (Avg. Pro Delta %). The sum of the Avg. Oil Delta % and Avg. Pro Delta % (Avg. Proil Delta %) is also shown in Table 36.

TABLE 36

Summary of Difference In Average Oil and Protein Contents Between Homozygous Transgenic and Null T2 Seed for Soybean Events Expressing GmODPI

Exp.	Gene	Event	Avg. Oil Delta %	Avg. Pro Delta %	Avg. Proil Delta %
Oil 109	GmODP1	8787.10.1	1.8	2.8	4.7
Oil 109	GmODP1	8787.7.3	1.3	2.9	4.2
Oil 109	GmODP1	8810.5.1	1.5	1.5	3.0

Table 36 shows that average oil and protein content is increased when GmODPI is over-expressed in soybean under control of the GmSus promoter when compared to the average of null seed. Oil and protein are increased by as high as 3.0 to 4.7 points in these single copy events.

EXAMPLE 14

Identification of Seed Specific Promoters to Drive Expression of Transcription Factors in Leguminous Oilseed Plants

The Arabidopsis sucrose synthase gene family and the role of specific gene family members during seed development, specifically the mobilization of sucrose for seed storage compound biosynthesis, has been described (Ruuska SA, et al. (2002) Plant Cell 14: 1191-1206; Baud S, et al. (2004) J Exp Bot 55: 397-409; Baud S and Graham IA (2006) Plant J 46: 155-169; Angeles-Nunez, J G and Tiessen, A. (2010) Planta 232(3): 701-718; Angeles-Nunez, J G and Tiessen, A (2012) Plant Mol Biol 78(4-5): 377-392). The current invention describes the utility of a promoter sequence of a specific soybean sucrose synthase gene family member, Glyma13g17420, that is highly similar in deduced amino acid sequence to the At5g49190 gene product (PCT Publication No. WO 2010/114989 A1), to direct expression of native or heterologous transcription factor genes such as LEC1, FUSCA3 and ODP1 in a manner that allows for increased accumulation of protein and oil during seed development of leguminous oil seeds. Glyma13g17420 is expressed during soybean embryo maturation in synchrony with accumulation of oil and protein (Severin AJ, et al. (2010) BMC Plant Biology 10:160). Genes homologous to Glyma13g17420 can be identified in other leguminous plant species based on amino acid sequence similarity to the Glyma13g17420 gene product and expression pattern of the homolog during seed development. One skilled in the art will recognize that promoter sequences of these genes will have utility for expression of transcription factor genes for increased protein and oil accumulation in leguminous oil seeds.

EXAMPLE 15

Identification of Sequence Variability in the Glyma13g17420 Promoter and 5'-UTR in Glycine max Breeding Lines

Genomic DNA sequencing of a number of soybean lines was performed by next generation high throughput sequencing methods according to manufacturer

instructions (Illumina, San Diego, USA). Genomic sequence corresponding to the promoter, 5'-UTR and first exon of the Glymal 3g1 7420 gene (SEQ ID NO: 8) was assembled for each soybean line from the genomic sequencing reads. This region corresponds to the sequence Gm13:21,216,136-21,219,309 in the Soybean Genomic Assembly Glymal .01 (JGI). Short read sequencing data were extracted for this region from the soybean lines. Polymorphic variants and insertion/deletion variants were detected from the sequencing data and the alignments were visually inspected to ascertain whether the identified variants may have been caused by sequencing error.

The sequencing results are summarized in Figure 4 (lines w/o variants were not reported). The results indicate that significant diversity in the genomic DNA sequence that comprises the promoter, 5'-UTR and first intron of the Glymal 3g1 7420 gene exists within different soybean lines. One skilled in the art will recognize that regulatory sequences of the Glymal 3g1 7420 gene including promoter, 5'-UTR and first intron derived from divergent soybean (Glycine max) accessions will have utility for expression of transcription factor genes for increased protein and oil accumulation in leguminous oil seeds.

CLAIMS

We claim:

1. A recombinant DNA construct comprising at least one heterologous polynucleotide encoding a polypeptide selected from the group consisting of: an ODP1 polypeptide, a Lec1 polypeptide and a FUSCA3 polypeptide, wherein the at least one polynucleotide is operably linked to a soybean sucrose synthase promoter or a *Medicago truncatula* sucrose synthase promoter, wherein expression of said polypeptide in a transgenic soybean seed comprising the recombinant DNA construct results in an increased oil content in the transgenic soybean seed, when compared to a control soybean seed not comprising the recombinant DNA construct.

2. The recombinant DNA construct of claim 1, wherein said transgenic soybean seed comprising said recombinant DNA construct has normal germination, when compared to a control soybean seed not comprising the recombinant DNA construct.

3. The recombinant DNA construct of claim 1 or claim 2, wherein the at least one polynucleotide is operably linked to a soybean sucrose synthase promoter, wherein the soybean sucrose synthase promoter comprises a nucleic acid sequence selected from the group consisting of:

   a. the nucleic acid sequence of SEQ ID NO: 8;
   b. a nucleic acid sequence with at least 95% sequence identity to the nucleic acid sequence of SEQ ID NO: 8;
   c. a nucleic acid sequence that hybridizes to SEQ ID NO: 8 under stringent conditions;
   d. a nucleic acid sequence that differs from SEQ ID NO: 8 in at least one way as described in FIG. 4; and
   e. a nucleic acid sequence comprising a functional fragment of (a), (b), (c) or (d).

4. The recombinant DNA construct of claim 1 or claim 2, wherein the at least one polynucleotide is operably linked to a *Medicago truncatula* sucrose synthase promoter, wherein the *Medicago truncatula* sucrose synthase

promoter comprises a nucleic acid sequence selected from the group consisting of:

a. the nucleic acid sequence of SEQ ID NO: 81 or SEQ ID NO: 85;

b. a nucleic acid sequence with at least 95% sequence identity to the nucleic acid sequence of SEQ ID NO: 81 or SEQ ID NO: 85;

c. a nucleic acid sequence that hybridizes to SEQ ID NO: 81 or SEQ ID NO: 85 under stringent conditions; and

d. a nucleic acid sequence comprising a functional fragment of (a), (b) or (c).

5. The recombinant DNA construct of any one of claims 1-4, wherein the at least one heterologous polynucleotide encodes an ODP1 polypeptide, wherein the ODP1 polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95% or 100% identity to SEQ ID NO: 30 or SEQ ID NO: 70.

6. The recombinant DNA construct of any one of claims 1-4, wherein the at least one heterologous polynucleotide encodes a Lec1 polypeptide, wherein the Lec1 polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95% or 100% identity to SEQ ID NO: 17, 20, 25 or 65.

7. The recombinant DNA construct of any one of claims 1-4, wherein the at least one heterologous polynucleotide encodes a FUSCA3 polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95% or 100% identity to SEQ ID NO: 32, 38, 45 or 49.

8. The recombinant DNA construct of any of the claims 1-7, wherein the recombinant DNA construct further comprises a seed-specific promoter operably linked to a second heterologous polynucleotide encoding a DGAT polypeptide.

9. The recombinant DNA construct of claim 8 wherein the second heterologous polynucleotide encodes a DGAT1 polypeptide.

10. The recombinant DNA construct of claim 9 wherein the DGAT1 polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95% or 100% sequence identity to SEQ ID NO: 55.

11. The recombinant DNA construct of claim 8 wherein the second heterologous polynucleotide encodes a DGAT2 polypeptide.

12. The recombinant DNA construct of claim 11 wherein the DGAT2 polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95% or 100% sequence identity to SEQ ID NO: 60.

13. A plant or a seed comprising the recombinant DNA construct of any of the claims 1-12.

14. A plant or a seed comprising the recombinant DNA construct of any of the claims 8-12, wherein co-expression of said polypeptide and said DGAT polypeptide in a transgenic soybean seed comprising the recombinant DNA construct results in an increased oil content in the transgenic seed, when compared to a control seed that expresses said DGAT polypeptide from said seed-specific promoter by does not express said polypeptide selected from the group consisting of an ODP1 polypeptide, a Lec1 polypeptide and a FUSCA3 polypeptide.

15. A plant or a seed comprising the recombinant DNA construct of any of claims 1-7, wherein the plant or seed further comprises a second recombinant DNA construct comprising a seed-specific promoter operably linked to a second heterologous polynucleotide encoding a DGAT polypeptide, wherein co-expression of said polypeptide and said DGAT polypeptide in a transgenic soybean seed comprising said recombinant DNA construct and said second recombinant DNA construct results in an increased oil content in the transgenic seed, when compared to a control seed comprising only one, but not both, of the recombinant DNA construct and the second recombinant DNA construct.

16. The plant or seed of any of claims 13-15, wherein said plant or seed is a soybean plant or seed.

17. A method of increasing oil content of a soybean seed, the method comprising the steps of:

a. introducing into a regenerable soybean cell the recombinant DNA construct of any of claims 1-12;

b. regenerating a transgenic plant from the regenerable soybean cell of (a) wherein the transgenic plant comprises the recombinant DNA construct; and

c. selecting a transgenic plant of step (b), or a transgenic progeny plant from the transgenic plant of step (b), wherein seed of the transgenic

plant or the transgenic progeny plant comprises the recombinant construct and exhibits increased seed oil content while maintaining normal germination, when compared to a control soybean seed not comprising the DNA recombinant construct.

18. A method of increasing oil content of a soybean seed, the method comprising the steps of:

a. introducing into a regenerable soybean cell the recombinant DNA construct of any of claims 1-7 and a second recombinant DNA construct comprising a seed-specific promoter operably linked to a second heterologous polynucleotide encoding a DGAT polypeptide;

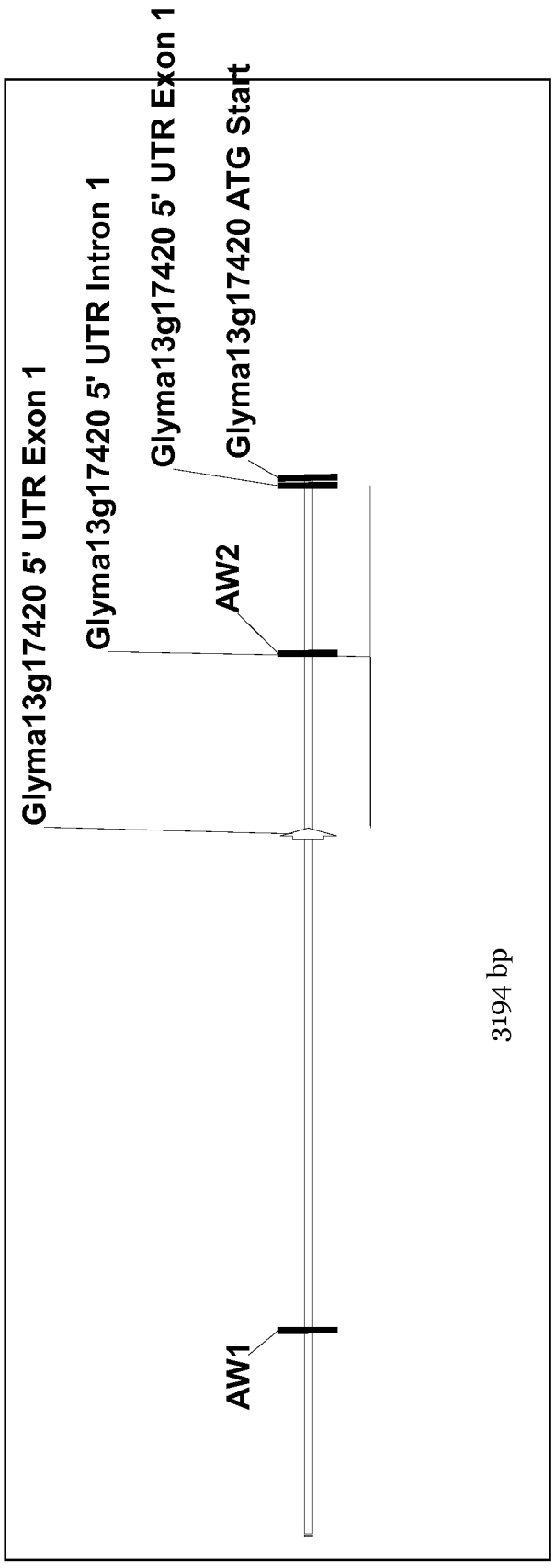
b. regenerating a transgenic plant from the regenerable soybean cell of (a) wherein the transgenic plant comprises the recombinant DNA construct and the second recombinant DNA construct; and

c. selecting a transgenic plant of step (b), or a transgenic progeny plant from the transgenic plant of step (b), wherein seed of the transgenic plant or the transgenic progeny plant comprises the recombinant DNA construct and the second recombinant DNA construct, and wherein co-expression of said polypeptide and said DGAT polypeptide in a transgenic soybean seed results in an increased oil content in the transgenic soybean seed, when compared to a control soybean seed comprising only one, but not both, of the recombinant DNA construct and the second recombinant DNA constructs.

19. A transgenic plant obtained by the method of claim 17 or claim 18.

20. A transgenic seed obtained from the transgenic plant of claim 19.

FIG. 1



glyma13g17420 promoter for BB1927

FIG. 2

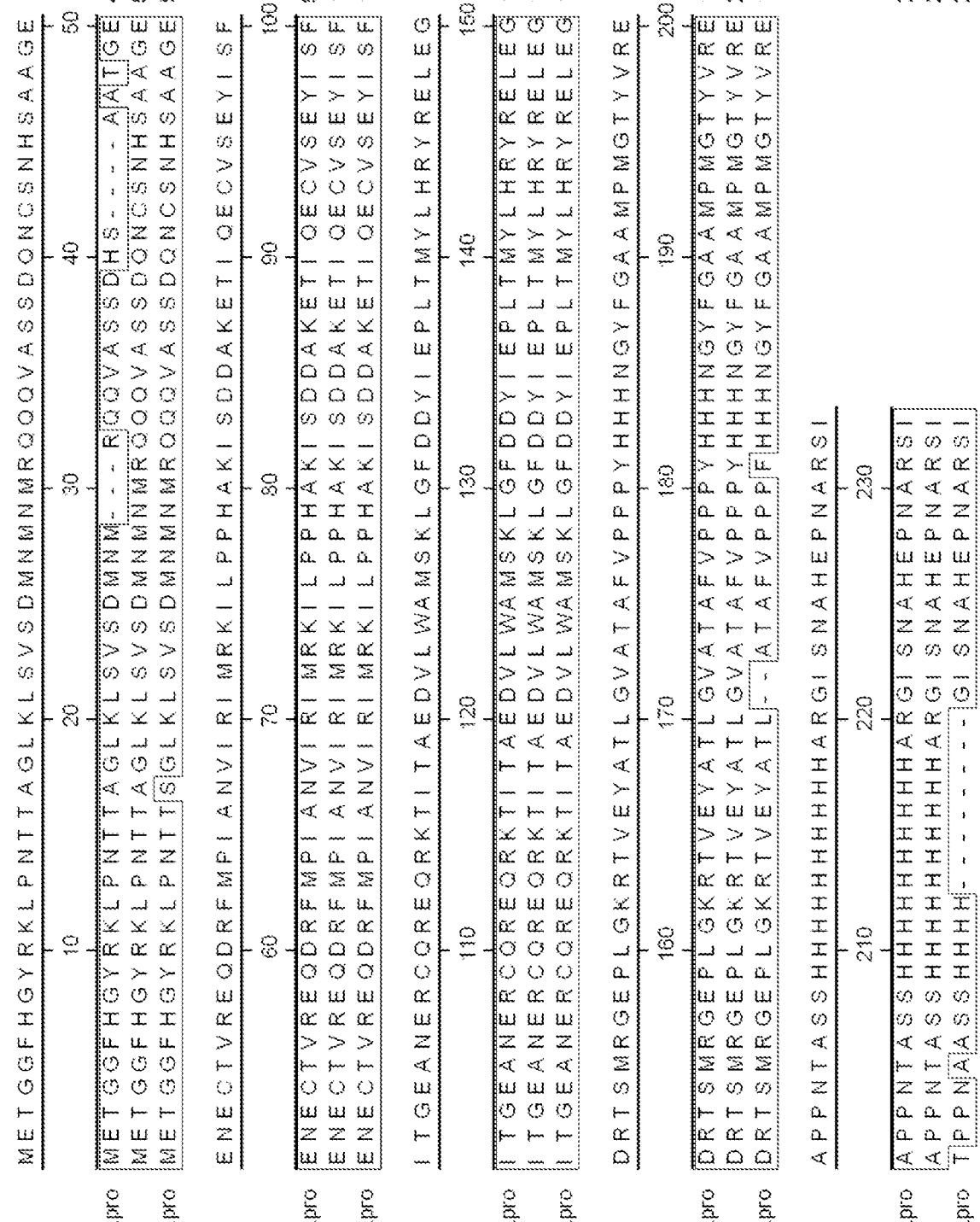


FIG. 3A

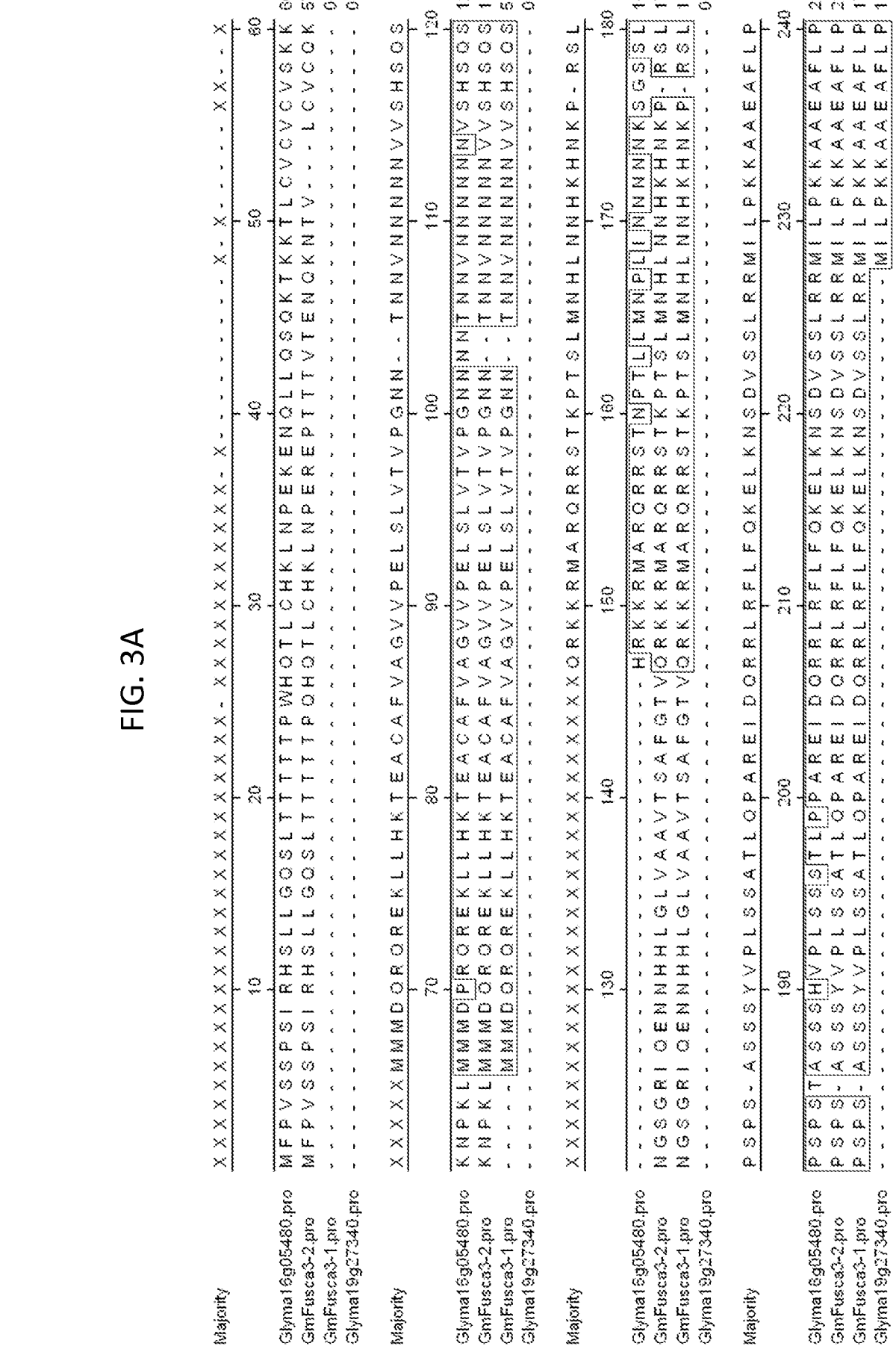


FIG. 3B

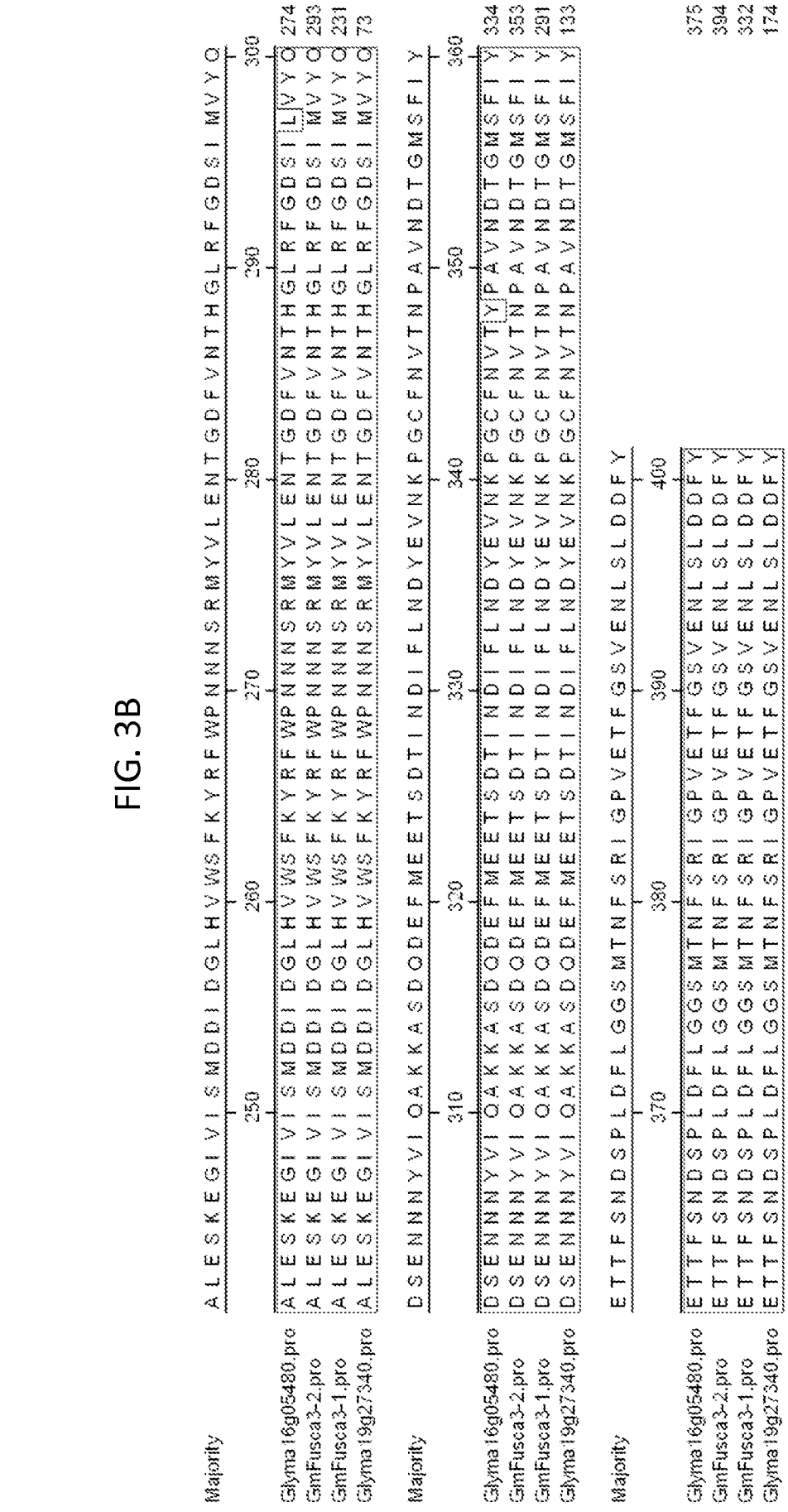

Majority
Glyma16g05480.pro
GmFusca3-2.pro
GmFusca3-1.pro
Glyma19g27340.pro
250
260
270
280
290
300
310
320
330
340
350
360
370
380
390
400
274
293
231
73
334
353
291
133
375
394
332
174

FIG. 4

Chromo -some	Position	Ref. Allele	Alternate Allele	Type	A	B	C	D	E	F	G	H
Gm13	21218639	T	G	SNP	Ref	Het	Alt	Ref	Ref	Ref	Ref	Ref
Gm13	21218079	T	A	SNP	Ref	Het	Alt	Ref	Ref	Ref	Ref	Het
Gm13	21219144	A	AA	INDEL	Het	Het	Het	Ref	Ref	Ref	Ref	Ref
Gm13	21219096	GT	GTCTAATTATT	INDEL	Het	Ref	Ref	Het	Het	Het	Ref	Ref
Gm13	21219095	TGT	TGTCTAATTAGT	INDEL	Het	Ref	Ref	Het	Het	Het	Ref	Ref
Gm13	21219097	T	TCTAATTATT	INDEL	Het	Ref	Ref	Het	Het	Het	Ref	Ref
Gm13	21216269	C	CTAATTATTGTTT	INDEL	Het	Ref	Ref	Het	Ref	Ref	Het	Ref
Gm13	21216986	GA	GAAA	INDEL	Ref	Ref	Ref	Het	Ref	Ref	Ref	Ref
Gm13	21216987	A	AAA	INDEL	Ref	Ref	Ref	Het	Ref	Ref	Ref	Ref
Gm13	21219102	AA	AAAGAA	INDEL	Het	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Gm13	21216434	G	GAATAAAG	INDEL	Ref	Ref	Ref	Het	Het	Het	Ref	Ref
Gm13	21217300	A	AATATATAC	INDEL	Het	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Gm13	21218374	T	TTTTG	INDEL	Het	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Gm13	21216174	C	CTAGA	INDEL	Ref	Ref	Ref	Het	Ref	Ref	Ref	Ref
Gm13	21216433	A	AATAAA	INDEL	Ref	Ref	Ref	Ref	Het	Het	Ref	Ref

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/070828

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/82 C12N9/10
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01H C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal , BIOSIS, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/114989 A1 (DU PONT [US] ; MEYER KNUT [US] ; DAMUDE HOWARD G [US] ; EVERARD JOHN D [U) 7 October 2010 (2010-10-07) exampl e 13	1-20

A	DATABASE EMBL [Onl i ne] 13 March 2009 (2009-03-13) , "Glyci ne max strai n Wi l l i ams 82 clone GM_WBc0099F23 , compl ete sequence .", XP002693656, retri eved from EBI accessi on no. EM_STD:AC235472 Database accessi on no. AC235472 For SEQ I D No 8 ; sequence	1-20

	-/- ·	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 March 2013	03/04/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Chakravarty, Ashok

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE EMBL [Online] 21 June 2002 (2002-06-21) , "Medicago truncatula chromosome 8 clone mth2-13h21 , complete sequence. ", XP002693657 , retrieved from EBI accession no. EM_STD:AC124967 Database accession no. AC124967 For SEQ ID No: 85; sequence -----	1-20

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2012/070828

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010114989 A1	07-10-2010	AU 2010232639 A1	15-09-2011
		CA 2755027 A1	07-10-2010
		EP 2414522 A1	08-02-2012
		US 2010257635 A1	07-10-2010
		WO 2010114989 A1	07-10-2010