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(54) Titre : UTILISATION D'UN PROMOTEUR SPECIFIQUE DE GRAINE POUR ENTRAINER L'EXPRESSION DE ODP1
DANS DES PLANTES OLEAGINEUSES CRUCIFERES POUR AUGMENTER LA TENEUR EN HUILE TOUT EN
CONSERVANT UNE GERMINATION NORMALE

(54) Title: USE OF A SEED SPECIFIC PROMOTER TO DRIVE ODP1 EXPRESSION IN CRUCIFEROUS OILSEED
PLANTS TO INCREASE OIL CONTENT WHILE MAINTAINING NORMAL GERMINATION

M.....S.....S.....R.....R.....	Consensus #1
10 20 30 40 50 60	
1 MERSQRQSPPPSPSSSSSSVSADTVLVPPGKRRAATAKAGAEPNKRIR-----	SEQ ID NO-37.pro
1 MRSFASCSSTSSV-----GFFATTEKRRP-----KHPRNNIKSQKC---	SEQ ID NO-39.pro
1 MRSFVSSTSSSSSSSCVGGGFDNNINLAAPRRPQSEKTKAKRRKRNQ-DDAKCEIE	SEQ ID NO-41.pro
1 MKKRLTTGTCSSSESSSVSSSTTSSPQSEAP--RP-----KRAKRAK-KSS----	SEQ ID NO-42.pro
.....RSS.YRGVTRHRWTGRFEAHLWDK.....KK.G.QVYLG	Consensus #1
70 80 90 100 110 120	
51 -----KDFAAAAAGKRSSVYRGVTRHRWTGRFEAHLWDKHCIAALHNKKKGRQVYLG	SEQ ID NO-37.pro
41 -----KONQTTTGGRSS-YRGVTRHRWTGRFEAHLWDKSSWNNIQSKK-GROVYLG	SEQ ID NO-39.pro
60 NNRGNNNNSNNNASSGRSS-YRGVTRHRWTGRFEAHLWDKSSWNSIQNKK-GROVYLG	SEQ ID NO-41.pro
46 -PSGKSHNPTS-EASTRSS-YRGVTRHRWTGRFEAHLWDKSSWNSIQNKK-GKQVYLG	SEQ ID NO-42.pro
AYD.EE.AA..YDLAALKYNG...LNFF.E.Y..E..EM..V..EEYLASLRR.SSGFS	Consensus #1
130 140 150 160 170 180	
103 AYDSEEAARAYDLAALKYNGPETLLNFFVEJYSSEMPEMEAV3REEYLASLRRSSSGFS	SEQ ID NO-37.pro
92 AYDTEESAARTYDLAALKYNGKDATLNFFJETYTELEEMDKV3REEYLASLRRQSSGFS	SEQ ID NO-39.pro
119 AYDNEEAARTYDLAALKYNGPGTLLNFFVESYRNEEEMRKVTKEEYLASLRRSSSGFS	SEQ ID NO-41.pro
103 AYDSEEAARHYDLAALKYNGPDTLLNFFHATYTKELEEMQVTKEEYLASLRRQSSGFS	SEQ ID NO-42.pro

(57) Abrégé/Abstract:

A recombinant DNA construct comprising a polynucleotide encoding an ODP1 polypeptide operably linked to a sucrose synthase 2 promoter where this construct can be used to increase oil content in the seeds of a cruciferous oilseed plant while maintaining normal germination is disclosed. A method for increasing oil content in the seeds of a cruciferous oilseed plant while maintaining normal germination using this construct is also disclosed.

PCT

- (57) Abstract:** A recombinant DNA construct comprising a polynucleotide encoding an ODP1 polypeptide operably linked to a sucrose synthase 2 promoter where this construct can be used to increase oil content in the seeds of a cruciferous oilseed plant while maintaining normal germination is disclosed. A method for increasing oil content in the seeds of a cruciferous oilseed plant while maintaining normal germination using this construct is also disclosed.

TITLE

USE OF A SEED SPECIFIC PROMOTER TO DRIVE ODP1 EXPRESSION IN
CRUCIFEROUS OILSEED PLANTS TO INCREASE OIL CONTENT WHILE
MAINTAINING NORMAL GERMINATION

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CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No.
61/165,548, filed April 1, 2009.

FIELD OF THE INVENTION

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This invention is in the field of biotechnology, in particular, this pertains to
increasing oil content while maintaining normal germination in a cruciferous oilseed
plant using a seed specific promoter to drive expression of ODP1.

BACKGROUND OF THE INVENTION

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Plant lipids have a variety of industrial and nutritional uses and are central to
plant membrane function and climatic adaptation. These lipids represent a vast
array of chemical structures, and these structures determine the physiological and
industrial properties of the lipid. Many of these structures result either directly or
indirectly from metabolic processes that alter the degree of unsaturation of the lipid.
Different metabolic regimes in different plants produce these altered lipids, and
either domestication of exotic plant species or modification of agronomically adapted
species is usually required to produce economically large amounts of the desired
lipid.

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There are serious limitations to using mutagenesis to alter fatty acid
composition and content. Screens will rarely uncover mutations that a) result in a
dominant ("gain-of-function") phenotype, b) are in genes that are essential for plant
growth, and c) are in an enzyme that is not rate-limiting and that is encoded by
more than one gene. In cases where desired phenotypes are available in mutant
corn lines, their introgression into elite lines by traditional breeding techniques is
slow and expensive, since the desired oil compositions are likely the result of
several recessive genes.

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Recent molecular and cellular biology techniques offer the potential for
overcoming some of the limitations of the mutagenesis approach, including the need
for extensive breeding. Some of the particularly useful technologies are seed-

specific expression of foreign genes in transgenic plants (see Goldberg et al (1989) *Cell* 56:149-160), and the use of antisense RNA to inhibit plant target genes in a dominant and tissue-specific manner (see van der Krol et al (1988) *Gene* 72:45-50). Other advances include the transfer of foreign genes into elite commercial varieties of commercial oilcrops, 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 generally bind DNA in a sequence-specific manner and either activate or repress transcription initiation. The specific mechanisms of these interactions remain to be fully elucidated. 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.

Several transcription factor families have been identified in plants. For example, nucleotide sequences encoding the following transcription factors families have been identified: Alfin-like, AP2 (APETALA2) and EREBPs (ethylene-responsive element binding proteins), ARF, AUX/IAA, bHLH, bZIP, C2C2 (Zn), C2C2 (Co-like), C2C2 (Dof), C2C2 (GATA), C2C2 (YABBY), C2H2 (Zn), C3H-type, CCAAT, CCAAT HAP3, CCAAT HAP5, CPP (Zn), DRAP1, E2F/DP, GARP, GRAS, HMG-BOX, HOMEO BOX, HSF, Jumanji, LFY, LIM, MADS Box, MYB, NAC, NIN-

like, Polycomb-like, RAV-like, SBP, TCP, TFIID, Transfactor, Trihelix, TUBBY, and WRKY.

WO 2005/075655 published on August 18, 2005 describes an AP2 domain transcription factor ODP2 (ovule development protein 2) and methods of use.

5 Patent No. 7,157,621 which issued on January 2, 2007, describes the alteration of oil traits in plants through controlled expression of selected genes in plants.

The AP2/ERF 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. The AP2/ERF proteins have been subdivided into two distinct subfamilies based on whether they contain one (ERF subfamily) or two (AP2 subfamily) DNA binding domains.

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. AP2/EREBP genes form a large multigene family, and they play a variety of roles throughout the plant life cycle. AP2/EREBP genes are key regulators of several developmental processes, including floral organ identity determination and leaf epidermal cell identity. In *Arabidopsis thaliana*, the homeotic gene APETALA2 (AP2) has been shown to control three salient processes during development: (1) the specification of flower organ identity throughout floral organogenesis (Jofuku et al., *Plant Cell* 6:1211-1225, 1994); (2) establishment of flower meristem identity (Irish and Sussex, *Plant Cell* 2:8:741-753, 1990); and (3) the temporal and spatial regulation of flower homeotic gene activity (Drews et al., *Cell* 65:6:991-1002, 1991). 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, an eighteen amino acid core region that is predicted to form an amphipathic α -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., *Proc. Natl. Acad. Sci. USA* 94:7076-7081, 1997) and in tobacco with the identification of the ethylene responsive element binding proteins (EREBPs) (Ohme-Takagi and Shinshi, *Plant Cell* 7:2:173-182,

1995). In Arabidopsis, these RAP2 (related to AP2) genes encode two distinct subfamilies of AP2 domain-containing proteins designated AP2-like and EREBP-like (Okamuro et al., *Proc. Natl. Acad. Sci. USA* 94:7076-7081, 1997). In vitro DNA binding has not been shown to date using the RAP2 proteins. Based upon the presence of two highly conserved motifs YRG and RAYD within the AP2 domain, it has been proposed that binding DNA binding occurs in a manner similar to that of AP2 proteins.

As was noted above, regulation of transcription of most eukaryotic genes is coordinated through sequence-specific binding of proteins to the promoter region located upstream of the gene. Many of these protein-binding sequences have been conserved during evolution and are found in a wide variety of organisms. One such feature is the "CCAAT" sequence element (Edwards et al, 1998, *Plant Physiol.* 117:1015-1022). CCAAT boxes are a feature of gene promoters in many eukaryotes including several plant gene promoters.

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., 1991, *Nucleic Acids Res.* 20:1087-1091).

WO 00/28058 published on May 18, 2000 describes HAP3-type CCAAT-box binding transcriptional activator polynucleotides and polypeptides, especially, the leafy cotyledon 1 transcriptional activator (LEC1) polynucleotides and polypeptides.

WO 99/67405 describes leafy cotyledon1 genes and their uses.

The human, murine and plant homologues of CCAAT-binding proteins have been isolated and characterized based on their sequence similarity with their yeast counterparts (Li et al., 1991, *Nucleic Acids Res.* 20:1087-1091). This high degree of sequence homology translates remarkably into functional interchangeability among orthologue proteins of different species (Sinha et al, 1995, *Proc. Natl. Acad. Sci. USA* 92:1624-1628). Unlike yeast, multiple forms of each HAP homolog have been identified in plants (Edwards et al, 1998, *Plant Physiol.* 117:1015-1022).

Molecular and genetic analysis revealed HAP members to be involved in the control of diverse and critical biological processes ranging from development and

cell cycle regulation to metabolic control and homeostasis (Lotan et al, 1998, *Cell* 93:1195-1205; Lopez et al, 1996, *Proc. Natl. Acad. Sci. USA* 93:1049-1053). In yeast, HAPs are involved in the transcriptional control of metabolic processes such as the regulation of catabolic derepression of *cyc1* and other genes involved in
 5 respiration (Becker et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:1968-1972).

In mammalian systems, several reports describe HAPs as direct or indirect regulators of several important genes involved in lipid biosynthesis such as fatty acid synthase (Roder et al, 1997, *Gene* 184:21-26), farnesyl diphosphate (FPP) synthase (Jackson et al, 1995, *J. Biol. Chem.* 270:21445-21448; Ericsson et al,
 10 1996, *J. Biol. Chem.* 271:24359-24364), glycerol-3-phosphate acyltransferase (GPA, Jackson et al, 1997), acetyl-CoA carboxylase (ACC, Lopez et al, 1996, *Proc. Natl. Acad. Sci. USA* 93:1049-1053) and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) synthase (Jackson et al, 1995, *J. Biol. Chem.* 270:21445-21448), among others.

15 In addition, other CCAAT-binding transcription factors have also been reported to be involved in different aspects of the control of lipid biosynthesis and adipocyte growth and differentiation in mammalian systems (see McKnight et al, 1989).

It appears that the currently available evidence to date points to a family of
 20 proteins of the CCAAT-binding transcription factors as important modulators of metabolism and lipid biosynthesis in mammalian systems. Such a determination has not been made for plant systems.

Other polypeptides that influence ovule and embryo development and stimulate cell growth, such as, *Lec1*, *Kn1*, *WUSCHEL*, *Zwille* and *Aintegumeta*
 25 (*ANT*) allow for increased transformation efficiencies when expressed in plants. See, for example, U.S. Application No. 2003/0135889.

In fact, a maize *Lec1* homologue of the *Arabidopsis* embryogenesis controlling gene *AtLEC1*, has been shown to increase oil content and transformation efficiencies in plants. See, for example, WO 03001902 and U.S. Patent No.
 30 6,512,165.

The putative AP2/EREBP transcription factor *WRINKLED1* (*WRI1*) is involved in the regulation of seed storage metabolism in *Arabidopsis* (Cermac 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.

Because 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. In the current invention, it has been found that use of a seed specific promoter, such as SUS2 from Arabidopsis, can drive expression of an ODP1 gene thereby increasing oil content in the seeds of a cruciferous oilseed plant without negatively affecting germination and seedling establishment.

SUMMARY OF THE INVENTION

In a first embodiment, the present invention concerns a recombinant DNA construct comprising a polynucleotide encoding an ODP1 polypeptide operably linked to a sucrose synthase 2 promoter wherein said construct increases oil content in the seeds of a cruciferous oilseed plant while maintaining normal germination and further wherein the amino acid sequence of said ODP1 polypeptide has at least 80%, at least 90%, at least 95% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NO:37, SEQ ID NO:39, and SEQ ID NO:41.

In another embodiment, the present invention concerns a recombinant construct comprising a sucrose synthase 2 promoter which comprises: (a) the nucleotide sequence of SEQ ID NO:43, SEQ ID NO:72 or SEQ ID NO:73, or (b) a nucleotide sequence comprising a functional fragment of the nucleotide sequence of SEQ ID NO:43, SEQ ID NO:72 or SEQ ID NO:73.

In another embodiment, the present invention concerns a transgenic cruciferous oilseed plant comprising in its genome the recombinant DNA construct of the invention. Also included are transgenic seeds obtained from such transgenic cruciferous oilseed plants, wherein the transgenic seed comprises in its genome the recombinant DNA construct of the invention.

In another embodiment, the present invention concerns a method for producing a transgenic cruciferous oilseed plant comprising transforming a cruciferous oilseed plant cell with the recombinant construct of the invention and regenerating a transgenic plant from the transformed plant cell, wherein the transgenic cruciferous oilseed plant
5 comprises in its genome the recombinant DNA construct of the invention.

In another embodiment, the present invention concerns a method for increasing oil content in seeds of a transgenic cruciferous oilseed plant while maintaining normal germination, said method comprising:

(a) transforming a cruciferous oilseed plant cell with a recombinant DNA
10 construct comprising a polynucleotide encoding an ODP1 polypeptide, wherein the amino acid sequence of said ODP1 polypeptide has at least 80%, at least 90% or at least 95% sequence identity with a sequence selected from the group consisting of SEQ ID NO:37, SEQ ID NO:39, and SEQ ID NO:41, said sequence being operably linked to a seed specific promoter;

15 (b) regenerating a transgenic cruciferous oilseed plant from the transformed cell of step (a), wherein said plant comprises the recombinant DNA construct;

(c) (c) obtaining a transgenic progeny plant derived from the transgenic cruciferous oilseed plant of step (b), wherein the transgenic progeny plant comprises in its genome the recombinant DNA construct;

20 (d) assaying the transgenic progeny plant obtained from step (c) for oil level and germination; and

(e) selecting those transgenic progeny plants having seeds with an increased level of oil and normal germination when compared to seeds obtained from a control cruciferous oilseed plant, wherein said control plant does not comprise the
25 recombinant DNA construct.

In another embodiment, the present invention concerns a method of the invention wherein the ODP1 polypeptide is a maize ODP1 polypeptide and, more specifically, the amino acid sequence of the ODP1 polypeptide comprises the sequence of SEQ ID NO:37. In addition, the seed specific promoter can be a
30 sucrose synthase 2 promoter and, more specifically, the nucleotide sequence of sucrose synthase 2 promoter comprises (a) the nucleotide sequence of SEQ ID NO:43, SEQ ID NO:72 or SEQ ID NO:73; or (b) a nucleotide sequence comprising

a functional fragment of the nucleotide sequence of SEQ ID NO:43, SEQ ID NO:72 or SEQ ID NO:73.

In another embodiment, the present invention concerns oil or by-products obtained from transgenic seed of the invention.

5 In another embodiment, the cruciferous oilseed plant or seed of any of the compositions or methods of the present invention can be canola or *Arabidopsis* or other plant species including but not limited to the following: *Barbarea vulgaris*, *Brassica campestris*, *Brassica carinata*, *Brassica elongate*, *Brassica fruticulosa*, *Brassica hirta*, *Brassica juncea*, *Brassica napus*, *Brassica narinosa*, *Brassica nigra*, *Brassica oleracea*,
10 *Brassica perviridis*, *Brassica rapa*, *Brassica rupestris*, *Brassica septiceps*, *Brassica tournefortii*, *Brassica verna*, *Camelina sativa*, *Crambe abyssinica*, *Lepidium campestre*, *Raphanus sativus*, *Sinapis alba*.

BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCE LISTING

15 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.

FIG. 1A-1C show a multiple alignment of the ODP1 polypeptides of *Zea mays* (SEQ ID NO:37), *Glycine max* (SEQ ID NO:39), *Momordica charantia* (SEQ ID
20 NO:41), and the WRINKLED1 gene from *Arabidopsis thaliana* (SEQ ID NO:42; NCBI GI NO. 32364685). The multiple alignment was assembled using the Clustal V method of alignment with the default parameters. Residues that match SEQ ID NO:37 exactly are enclosed in a box. Above the alignment is shown a consensus sequence. A residue is shown in the consensus sequence when all residues at that
25 position are identical.

FIG. 2 shows the percent sequence identity and divergence for each pair of polypeptides from the multiple alignment of FIG. 1A-1C.

SEQ ID NO:1 is the nucleotide sequence of vector pKS121/BS.

SEQ ID NO:2 is the nucleotide sequence of vector pDsRedxKS121/BS.

30 SEQ ID NO:3 is the nucleotide sequence of vector pKS332,

SEQ ID NO:4 is the nucleotide sequence of of PCR primer MWG345.

SEQ ID NO:5 is the nucleotide sequence of of PCR primer MWG346.

SEQ ID NO:6 is the nucleotide sequence of vector pKS336,

SEQ ID NO:7 is the nucleotide sequence of the T-DNA of the plant transformation vector pZBL120xKS336.

SEQ ID NO:8 is the nucleotide sequence of of PCR primer MWG339.

SEQ ID NO:9 is the nucleotide sequence of of PCR primer MWG340.

5 SEQ ID NO:10 is the nucleotide sequence of vector pKS333.

SEQ ID NO:11 is the nucleotide sequence of the T-DNA of the plant transformation vector pZBL120xKS333.

SEQ ID NO:12 is the nucleotide sequence of of PCR primer MWG341.

SEQ ID NO:13 is the nucleotide sequence of of PCR primer MWG342.

10 SEQ ID NO:14 is the nucleotide sequence of vector pKS334.

SEQ ID NO:15 is the nucleotide sequence of the T-DNA of the plant transformation vector pZBL120xKS334.

SEQ ID NO:16 is the nucleotide sequence of vector pKR132.

SEQ ID NO:17 is the nucleotide sequence of vector pKR627.

15 SEQ ID NO:18 is the nucleotide sequence of vector KS294.

SEQ ID NO:19 is the nucleotide sequence of vector pKR1142.

SEQ ID NO:20 is the nucleotide sequence of vector pKR1141.

SEQ ID NO:21 is the nucleotide sequence of of PCR primer SuSy-5.

SEQ ID NO:22 is the nucleotide sequence of of PCR primer SuSy-3.

20 SEQ ID NO:23 is the nucleotide sequence of vector pLF122.

SEQ ID NO:24 is the nucleotide sequence of vector pKR1155.

SEQ ID NO:25 is the nucleotide sequence of vector pKR1158.

SEQ ID NO:26 is the nucleotide sequence of vector pKR1167.

SEQ ID NO:27 is the nucleotide sequence of vector pKR92.

25 SEQ ID NO:28 is the nucleotide sequence of vector pKR1223.

SEQ ID NO:29 is the nucleotide sequence of vector pKR268.

SEQ ID NO:30 is the nucleotide sequence of vector pKR1143.

SEQ ID NO:31 is the nucleotide sequence of vector pKR1147.

SEQ ID NO:32 is the nucleotide sequence of vector pKR1220.

30 SEQ ID NO:33 is the nucleotide sequence of vector pKR1144.

SEQ ID NO:34 is the nucleotide sequence of vector pKR1149.

SEQ ID NO:35 is the nucleotide sequence of vector pKR1221.

SEQ ID NO:36 is the nucleotide sequence of the maize ODP1 coding region from cDNA clone cde1c.pk003.o22.

SEQ ID NO:37 is the amino acid sequence of the maize ODP1 encoded by SEQ ID NO:36. SEQ ID NO:37 is identical to SEQ ID NO:320 in U.S. Patent No.
5 7,157,621.

SEQ ID NO:38 is the nucleotide sequence of the soybean ODP1 coding region from cDNA clone se3.pk0003.f5.

SEQ ID NO:39 is the amino acid sequence of the soybean ODP1 encoded by SEQ ID NO:38. SEQ ID NO:39 is identical to SEQ ID NO:481 in U.S. Patent No.
10 7,157,621.

SEQ ID NO:40 is the nucleotide sequence of the *Momordica charantia* ODP1 coding region from cDNA clone fds1n.pk015.l15.

SEQ ID NO:41 is the amino acid sequence of the *Momordica charantia* ODP1 encoded by SEQ ID NO:40. SEQ ID NO:41 is identical to SEQ ID NO:477 in U.S.
15 Patent No. 7,157,621.

SEQ ID NO:42 is the amino acid sequence of WRINKLED1 (WRI1) from *Arabidopsis thaliana* and corresponds to NCBI GI NO. 32364685.

SEQ ID NO:43 is the nucleotide sequence of the sucrose synthase 2 (SUS2) promoter from *Arabidopsis thaliana* that is present in vector pKR1223.

20 SEQ ID NO:44 is the nucleotide sequence of the canola SUS2 homolog.

SEQ ID NO:45 is the amino acid sequence of the canola SUS2 homolog encoded by SEQ ID NO:44.

SEQ ID NO:46 is the nucleotide sequence of primer a.

SEQ ID NO:47 is the nucleotide sequence of primer b.

25 SEQ ID NO:48 is the nucleotide sequence of primer c.

SEQ ID NO:49 is the nucleotide sequence of primer d.

SEQ ID NO:50 is the nucleotide sequence of "Pvull rapa cons", a genomic sequence of canola variety NS1822BC that was generated with primers a and b.

30 SEQ ID NO:51 is the nucleotide sequence of "1,6 Dral gene cons", a genomic sequence of canola variety NS1822BC that was generated with primers c and d.

SEQ ID NO:52 is the nucleotide sequence of primer SA188.

SEQ ID NO:53 is the nucleotide sequence of primer SA189.

SEQ ID NO:54 is the nucleotide sequence of primer SA190.

SEQ ID NO:55 is the nucleotide sequence of primer SA191.

SEQ ID NO:56 is the nucleotide sequence of "BN SUS2 prom1/PCR blunt", which is derived from 1,6 Drai gene cons (SEQ ID NO:51).

5 SEQ ID NO:57 is the nucleotide sequence of "BN SUS2 prom2/PCR blunt", which is derived from PvuII rapa cons (SEQ ID NO:50).

SEQ ID NO:58 is the nucleotide sequence of vector KS427.

SEQ ID NO:59 is the nucleotide sequence of vector KS130.

SEQ ID NO:60 is the nucleotide sequence of vector KS432.

SEQ ID NO:61 is the nucleotide sequence of vector ARALO80,

10 SEQ ID NO:62 is the nucleotide sequence of primer D6 fwd.

SEQ ID NO:63 is the nucleotide sequence of primer D6 rev,

SEQ ID NO:64 is the nucleotide sequence of vector KS119.

SEQ ID NO:65 is the nucleotide sequence of vector KS430.

SEQ ID NO:66 is the nucleotide sequence of vector ARALO78.

15 SEQ ID NO:67 is the nucleotide sequence of vector KS428.

SEQ ID NO:68 is the nucleotide sequence of vector KS429.

SEQ ID NO:69 is the nucleotide sequence of vector ARALO77.

SEQ ID NO:70 is the nucleotide sequence of vector KS431.

SEQ ID NO:71 is the nucleotide sequence of vector ARALO79.

20 SEQ ID NO:72 is the nucleotide sequence of the sucrose synthase 2-1 (BnSUS2-1) promoter from *Brassica napus* that is present in BN SUS2 prom1/PCR blunt.

SEQ ID NO:73 is the nucleotide sequence of the sucrose synthase 2-2 (BnSUS2-2) promoter from *Brassica napus* that is present in BN SUS2 prom2/PCR
25 blunt.

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.

30 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 (No. 2):345-373 1984.

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.

DETAILED DESCRIPTION OF THE INVENTION

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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.

10

Units, prefixes, and symbols may be denoted in their SI accepted form. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxyl orientation, respectively. Numeric ranges recited within the specification are inclusive of the numbers defining the range and include each integer within the defined range. Amino acids may be referred to herein by either commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes. Unless otherwise provided for, software, electrical, and electronics terms as used herein are as defined in The New IEEE Standard Dictionary of Electrical and Electronics Terms (5th edition, 1993). The terms defined below are more fully defined by reference to the specification as a whole.

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

25

The term "ODP1" refers to an ovule development protein 1 that is involved with increasing oil content.

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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 "Arabidopsis sucrose synthase 2", "AtSuSy" and "AtSUS2") are used interchangeably herein. The Arabidopsis sucrose synthase 2 gene is from genomic locus At5g49190,

The term "germination" refers to the initial stages in the growth of a seed to form a seedling.

The term "recombinant" refers to an artificial combination of two otherwise separated segments of sequence, e.g., by chemical synthesis or by the
5 manipulation of isolated segments of nucleic acids by genetic engineering techniques.

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
10 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
15 be used in conjunction with a vector. If a vector is used, then the choice of vector is dependent upon the method that will be used to transform host cells as is well known to those skilled in the art. For example, a plasmid vector can be used. The skilled artisan is well aware of the genetic elements that must be present on the vector in order to successfully transform, select and propagate host cells comprising
20 any of the isolated nucleic acid fragments of the invention. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al., *EMBO J.* 4:2411-2418 (1985); De Almeida et al., *Mol. Gen. Genetics* 218:78-86 (1989)), and thus that multiple events must be screened in order to obtain lines displaying the desired expression
25 level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, immunoblotting analysis of protein expression, or phenotypic analysis, among others.

This construct may comprise any combination of deoxyribonucleotides, ribonucleotides, and/or modified nucleotides. The construct may be transcribed to
30 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.

As used herein, "encodes" or "encoding" refers to a DNA sequence which can be processed to generate an RNA and/or polypeptide.

As used herein, "expression" or "expressing" refers to production of a functional product, such as, the generation of an RNA transcript from an introduced construct, an endogenous DNA sequence, or a stably incorporated heterologous DNA sequence. The term may also refer to a polypeptide produced from an mRNA generated from any of the above DNA precursors. Thus, expression of a nucleic acid fragment may refer to transcription of the nucleic acid fragment (e.g., transcription resulting in mRNA or other functional RNA) and/or translation of RNA into a precursor or mature protein (polypeptide).

As used herein, "heterologous" with respect to a 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. For example, with respect to a nucleic acid, it can be a nucleic acid that originates from a foreign species, or is synthetically designed, or, if from the same species, is substantially modified from its native form in composition and/or genomic locus by deliberate human intervention. A heterologous protein may originate from a foreign species or, if from the same species, is substantially modified from its original form by deliberate human intervention.

"Plant" includes reference to whole plants, plant organs, plant tissues, 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.

The term "plant parts" includes differentiated and undifferentiated tissues including, but not limited to the following: roots, stems, shoots, leaves, pollen, seeds, tumor tissue and various forms of cells and culture (e.g., single cells, protoplasts, embryos and callus tissue). The plant tissue may be in plant or in a plant organ, tissue or cell culture.

The term "plant organ" refers to plant tissue or group of tissues that constitute a morphologically and functionally distinct part of a plant.

"Progeny" comprises any subsequent generation of a plant. Progeny will inherit, and stably segregate, genes and transgenes from its parent plant(s).

The term "introduced" means providing a nucleic acid (e.g., expression construct) or protein into a cell. Introduced includes reference to the incorporation of a nucleic acid into a eukaryotic or prokaryotic cell where the nucleic acid may be incorporated into the genome of the cell, and includes reference to the transient provision of a nucleic acid or protein to the cell. Introduced includes reference to stable or transient transformation methods, as well as sexually crossing. Thus, "introduced" in the context of inserting a nucleic acid fragment (e.g., a recombinant DNA construct/expression 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).

The term "genome" as it applies to a 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.

The term "isolated" refers to material, such as a nucleic acid or a protein, which is: (1) substantially or essentially free from components which normally accompany or interact with the material as found in its naturally occurring environment or (2) if the material is in its natural environment, the material has been altered by deliberate human intervention to a composition and/or placed at a locus in the cell other than the locus native to the material.

As used herein, "nucleic acid" means a polynucleotide and includes single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases. Nucleic acids may also include fragments and modified nucleotides. Thus, the terms "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 deoscytidylate, "G" for guanylate or deoxyguanylate, "U" for uridylate, "T" for deosythimidylate, "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.

The terms “subfragment that is functionally equivalent” and “functionally equivalent subfragment” are used interchangeably herein. These terms refer to a portion or subsequence of an isolated nucleic acid fragment in which the ability to alter gene expression or produce a certain phenotype is retained whether or not the fragment or subfragment encodes an active enzyme. For example, the fragment or subfragment can be used in the design of chimeric genes to produce the desired phenotype in a transformed plant. Chimeric genes can be designed for use in suppression by linking a nucleic acid fragment or subfragment thereof, whether or not it encodes an active enzyme, in the sense or antisense orientation relative to a plant promoter sequence.

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.

“Sequence identity” or “identity” in the context of nucleic acid or polypeptide sequences refers to the nucleic acid bases or amino acid residues in two sequences

that are the same when aligned for maximum correspondence over a specified comparison window.

Thus, "percentage of sequence identity" refers to the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the results by 100 to yield the percentage of sequence identity. Useful examples of percent sequence identities include, but are not limited to, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or any integer percentage from 50% to 100%. These identities can be determined using any of the programs described herein.

Sequence alignments and percent identity or similarity 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). Within the context of this application it will be understood that where sequence analysis software is used for analysis, that the results of the analysis will be based on the "default values" of the program referenced, unless otherwise specified. As used herein "default values" will mean any set of values or parameters that originally load with the software when first initialized.

The "Clustal V method of alignment" corresponds to the alignment method labeled Clustal V (described by Higgins and Sharp, *CABIOS*. 5:151-153 (1989); Higgins, D.G. et al. (1992) *Comput. Appl. Biosci.* 8:189-191) and found in the MEGALIGN™ program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). For multiple alignments, the default values correspond to GAP PENALTY=10 and GAP LENGTH PENALTY=10. Default parameters for pairwise alignments and calculation of percent identity of protein sequences using the Clustal 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 a "percent identity" by viewing the "sequence distances" table in the same program.

5 "BLASTN method of alignment" is an algorithm provided by the National Center for Biotechnology Information (NCBI) to compare nucleotide sequences using default parameters.

It is well understood by one skilled in the art that many levels of sequence identity are useful in identifying polypeptides, from other species, wherein such
10 polypeptides have the same or similar function or activity. Useful examples of percent identities include, but are not limited to, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or any integer percentage from 50% to 100%. Indeed, any integer amino acid identity from 50% to 100% may be useful in describing the present invention, such as 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%,
15 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%. Also, of interest is any full-length or partial complement of this isolated nucleotide fragment.

"Gene" refers to a nucleic acid fragment that expresses a specific protein,
20 including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "Native gene" refers to a gene as found in nature with its own regulatory sequences. "Chimeric gene" refers to any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory
25 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. A "foreign" gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into
30 a non-native organism, or chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

The term "genome" as it applies to a 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.

5 "Coding sequence" refers to a DNA sequence that codes for a specific amino acid sequence. "Regulatory sequences" 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
10 sequences, introns, polyadenylation recognition sequences, RNA processing sites, effector binding sites and stem-loop structures.

"Promoter" refers to a DNA sequence capable of controlling the expression of a coding sequence or functional RNA. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as
15 enhancers. Accordingly, an "enhancer" is a DNA sequence that can stimulate promoter activity, and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise
20 synthetic DNA segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of some
25 variation may have identical promoter activity. Promoters that cause a gene to be expressed in most cell types at most times are commonly referred to as "constitutive promoters". New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro, J. K., and Goldberg, R. B. *Biochemistry of Plants* 15:1-82 (1989).

30 "Functional variants" of the regulatory sequences (e.g., promoters) are also encompassed by the compositions of the present invention. Functional variants include, for example, the native regulatory sequences of the invention having one or more nucleotide substitutions, deletions or insertions. Functional variants of the

invention may be created by site-directed mutagenesis, induced mutation, or may occur as allelic variants (polymorphisms).

As used herein, a "functional fragment" of a regulatory sequence (e.g. a promoter) is a functional variant formed by one or more deletions from a larger regulatory element. For example, the 5' portion of a sequence with promoter activity may be deleted without abolishing promoter activity, as described by Zhu et al., *Plant Cell* 7:1681-1689 (1995). Such variants should retain promoter activity, particularly the ability to drive expression in seed or seed tissues. Activity can be measured by Northern blot analysis, reporter activity measurements when using transcriptional fusions, and the like. See, for example, Sambrook et al. (1989) *Molecular Cloning: A Laboratory Manual* (2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.).

"Translation leader sequence" refers to a polynucleotide sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner, R. and Foster, G. D., *Mol. Biotechnol.* 3:225-236 (1995)).

"3' non-coding sequences", "transcription terminator" or "termination sequences" refer to DNA sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht, I. L., et al. *Plant Cell* 1:671-680 (1989).

"RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript. A RNA transcript is referred to as the mature RNA when it is a RNA sequence derived from post-transcriptional processing of the primary transcript. "Messenger RNA" or "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, a mRNA template using the enzyme reverse transcriptase. The cDNA can be single-stranded or converted into double-stranded form using the Klenow fragment of DNA polymerase I. "Sense" RNA refers to RNA transcript that includes the mRNA and can be translated into protein within a cell or *in vitro*.

"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 gene (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. "Functional RNA" refers to antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes. The terms "complement" and "reverse complement" are used interchangeably herein with respect to mRNA transcripts, and are meant to define the antisense RNA of the message.

The term "operably linked" refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is regulated by the other. For example, a promoter is operably linked with a coding sequence when it is capable of regulating the expression of that coding sequence (i.e., the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in a sense or antisense orientation. In another example, the complementary RNA regions of the invention can be operably linked, either directly or indirectly, 5' to the target mRNA, or 3' to the target mRNA, or within the target mRNA, or a first complementary region is 5' and its complement is 3' to the target mRNA.

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: Cold Spring Harbor, NY (1989). Transformation methods are well known to those skilled in the art and are described *infra*.

"PCR" or "polymerase chain reaction" is a technique for the synthesis of large quantities of specific DNA segments and consists of a series of repetitive cycles (Perkin Elmer Cetus Instruments, Norwalk, CT). Typically, the double-stranded DNA is heat denatured, the two primers complementary to the 3' boundaries of the

target segment are annealed at low temperature and then extended at an intermediate temperature. One set of these three consecutive steps is referred to as a "cycle".

The terms "plasmid", "vector" and "cassette" refer to an extra chromosomal element often carrying genes that are not part of the central metabolism of the cell, and usually in the form of circular double-stranded DNA fragments. Such elements may be autonomously replicating sequences, genome integrating sequences, phage or nucleotide sequences, linear or circular, of a single- or double-stranded DNA or RNA, derived from any source, in which a number of nucleotide sequences have been joined or recombined into a unique construction which is capable of introducing a promoter fragment and DNA sequence for a selected gene product along with appropriate 3' untranslated sequence into a cell. "Transformation cassette" refers to a specific vector containing a foreign gene and having elements in addition to the foreign gene that facilitates transformation of a particular host cell. "Expression cassette" refers to a specific vector containing a foreign gene and having elements in addition to the foreign gene that allow for enhanced expression of that gene in a foreign host (i.e., to a discrete nucleic acid fragment into which a nucleic acid sequence or fragment can be moved.)

The term "expression", as used herein, refers to the production of a functional end-product (e.g., a mRNA or a protein [either precursor or mature]).

"Stable transformation" refers to the transfer of a nucleic acid fragment into a genome of a host organism, including both nuclear and organellar genomes, resulting in genetically stable inheritance. In contrast, "transient transformation" refers to the transfer of a nucleic acid fragment into the nucleus, or DNA-containing organelle, of a host organism resulting in gene expression without integration or stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" organisms.

As used herein, "transgenic" refers to a plant or a cell which comprises within its genome a heterologous polynucleotide. Preferably, 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 an expression construct. Transgenic is used herein to include any cell, cell line, callus, tissue, plant part or plant, the

genotype of which has been altered by the presence of heterologous nucleic acid including those transgenics initially so altered as well as those created by sexual crosses or asexual propagation from the initial transgenic. 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.

The present invention concerns a recombinant DNA construct comprising a polynucleotide encoding an ODP1 polypeptide operably linked to a sucrose synthase 2 promoter wherein said construct increases oil content in the seeds of a cruciferous oilseed plant while maintaining normal germination and further wherein the amino acid sequence of said ODP1 polypeptide has at least 80% sequence identity to a sequence selected from the group consisting of SEQ ID NO:37, SEQ ID NO:39, and SEQ ID NO:41.

In another embodiment, the sequence identity can be at least 90% or 95%.

In another embodiment the ODP1 polypeptide comprises a sequence selected from the group consisting of SEQ ID NO:37, SEQ ID NO:39, and SEQ ID NO:41.

In another embodiment, the sucrose synthase 2 promoter comprises: (a) the nucleotide sequence of SEQ ID NO:43, SEQ ID NO:72 or SEQ ID NO:73; or (b) a nucleotide sequence comprising a functional fragment of the nucleotide sequence of SEQ ID NO:43, SEQ ID NO:72 or SEQ ID NO:73.

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. The AP2/ERF 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. The AP2/ERF proteins have been subdivided into two distinct subfamilies based on whether they contain one (ERF subfamily) or two (AP2 subfamily) DNA binding domains.

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. AP2/EREBP genes form a large multigene family, and they play a variety of roles throughout the plant life cycle. AP2/EREBP genes are key regulators of several developmental processes, including floral organ identity determination and leaf epidermal cell identity. In *Arabidopsis thaliana*, the homeotic gene *APETALA2* (AP2) has been shown to control three salient processes during development: (1) the specification of flower organ identity throughout floral organogenesis (Jofuku et al., *Plant Cell* 6:1211-1225, 1994); (2) establishment of flower meristem identity (Irish and Sussex, *Plant Cell* 2:8:741-753, 1990); and (3) the temporal and spatial regulation of flower homeotic gene activity (Drews et al., *Cell* 65:6:991-1002, 1991). 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, an eighteen amino acid core region that is predicted to form an amphipathic α -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., *Proc. Natl. Acad. Sci. USA* 94:7076-7081, 1997) and in tobacco with the identification of the ethylene responsive element binding proteins (EREBPs) (Ohme-Takagi and Shinshi, *Plant Cell* 7:2:173-182, 1995). In *Arabidopsis*, these RAP2 (related to AP2) genes encode two distinct subfamilies of AP2 domain-containing proteins designated AP2-like and EREBP-like (Okamuro et al., *Proc. Natl. Acad. Sci. USA* 94:7076-7081, 1997). In vitro DNA binding has not been shown to date using the RAP2 proteins. Based upon the presence of two highly conserved motifs YRG and RAYD within the AP2 domain, it has been proposed that binding DNA binding occurs in a manner similar to that of AP2 proteins.

In another embodiment, the present invention concerns a transgenic cruciferous oilseed plant comprising in its genome the recombinant DNA construct of the invention. Also of interest is a transgenic seed obtained from a transgenic plant as described herein, wherein said seed comprises in its genome a recombinant DNA construct of the invention.

In still another aspect, the present invention concerns a method for producing a transgenic cruciferous oilseed plant comprising transforming a cruciferous oilseed plant

cell with a recombinant construct of the invention and regenerating a transgenic plant from the transformed plant cell.

This invention concerns a transgenic seed obtained from a transgenic plant made by a method of the invention, wherein said seed comprises in its genome a recombinant
5 DNA construct of the invention.

In another aspect, the present invention concerns a method for increasing oil content in seeds of a transgenic cruciferous oilseed plant while maintaining normal germination, said method comprising:

(a) transforming a cruciferous oilseed plant cell with a recombinant DNA
10 construct comprising a polynucleotide encoding an ODP1 polypeptide, wherein the amino acid sequence of said ODP1 polypeptide has at least 80%, at least 90% or at least 95% sequence identity with a sequence selected from the group consisting of SEQ ID NO:37, SEQ ID NO:39, and SEQ ID NO:41, said sequence being operably linked to a seed specific promoter;

15 (b) regenerating a transgenic cruciferous oilseed plant from the transformed cell of step (a), wherein said plant comprises the recombinant DNA construct;

(c) obtaining a transgenic progeny plant derived from the transgenic cruciferous oilseed plant of step (b), wherein the transgenic progeny plant comprises in its genome the recombinant DNA construct;

20 (d) assaying the transgenic progeny plant obtained from step (c) for oil level and germination; and

(e) selecting those transgenic progeny plants having seeds with an increased level of oil and normal germination when compared to seeds obtained from a control cruciferous oilseed plant, wherein said control plant does not comprise the
25 recombinant DNA construct.

Preferably, the ODP1 polypeptide is a maize ODP1 polypeptide and, more preferably, the amino acid sequence of the ODP1 polypeptide comprises the sequence of SEQ ID NO:37.

With respect to the seed specific promoter, it can be a sucrose synthase 2
30 promoter and preferably, the nucleotide sequence of sucrose synthase 2 promoter comprises: (a) the nucleotide sequence of SEQ ID NO:43, SEQ ID NO:72 or SEQ ID NO:73; or (b) a nucleotide sequence comprising a functional fragment of the nucleotide sequence of SEQ ID NO:43, SEQ ID NO:72 or SEQ ID NO:73.

The transgenic cruciferous oil seeds described herein of the invention can be processed to yield oil and/or seed by-products.

In another embodiment, the present invention concerns a recombinant DNA construct comprising a polynucleotide encoding a heterologous polypeptide
5 operably linked to a sucrose synthase 2 promoter, wherein the sucrose synthase 2 promoter comprises: (a) the nucleotide sequence of SEQ ID NO:43, SEQ ID NO:72 or SEQ ID NO:73; (b) a nucleotide sequence comprising a functional fragment of the nucleotide sequence of SEQ ID NO:43, SEQ ID NO:72 or SEQ ID NO:73; or (c)
10 a nucleotide sequence with at least 80%, at least 90% or at least 95% sequence identity to the nucleotide sequence of SEQ ID NO:43, SEQ ID NO:72 or SEQ ID NO:73; wherein the nucleotide sequence of (a), (b) or (c) has seed-specific promoter activity in a plant. The invention also concerns a transgenic plant, plant cell and seed comprising the recombinant DNA construct. The transgenic plant may be a transgenic cruciferous plant.

15 The nucleotide and deduced amino acid sequence of the canola SUS2 homolog transcript model are set forth as SEQ ID NO:44 and SEQ ID NO:45, respectively.

NCBI GI NO. 150912532 is the nucleotide sequence of the 5'-end of a *Brassica oleracea* cDNA.

20 SEQ ID NO:72 is the nucleotide sequence of the sucrose synthase 2-1 (BnSUS2-1) promoter from *Brassica napus* that is present in BN SUS2 prom1/PCR blunt. Comparison of SEQ ID NO:72 with SEQ ID NO:44 and NCBI GI NO. 150912532 indicate that nucleotide 427 is at or near the beginning of the 5'-Untranslated region of the canola SUS2 gene. Consequently, a fragment
25 comprising nucleotides 1-426 of SEQ ID NO:72 would be expected to have seed-specific promoter activity in a plant.

SEQ ID NO:73 is the nucleotide sequence of the sucrose synthase 2-2 (BnSUS2-2) promoter from *Brassica napus* that is present in BN SUS2 prom2/PCR blunt. Comparison of SEQ ID NO:73 with SEQ ID NO:44 and NCBI GI NO.
30 150912532 indicate that nucleotide 1766 is at or near the beginning of the 5'-Untranslated region of the canola SUS2 gene. Consequently, a fragment comprising nucleotides 1-1765 of SEQ ID NO:73 would be expected to have seed-specific promoter activity in a plant.

The cruciferous oilseed plant (or seed) of any of the compositions or methods of the present invention can be canola or Arabidopsis or other plant species including but not limited to the following: Barbarea vulgaris, Brassica campestris, Brassica carinata, Brassica elongate, Brassica fruticulosa, Brassica hirta, Brassica juncea, Brassica napus, Brassica narinosa, Brassica nigra, Brassica oleracea, Brassica perviridis, Brassica rapa, Brassica rupestris, Brassica septiceps, Brassica tournefortii, Brassica verna, Camelina sativa, Crambe abyssinica, Lepidium campestre, Raphanus sativus, Sinapis alba.

Methods of isolating seed oils are well known in the art: (Young et al., Processing of Fats and Oils, In *The Lipid Handbook*, Gunstone et al., eds., Chapter 5 pp 253-257; Chapman & Hall: London (1994)). Seed by-products include but are not limited to the following: meal, lecithin, gums, free fatty acids, pigments, soap, stearine, tocopherols, sterols and volatiles.

The production of edible vegetable oils including canola oil involves two overall processes, mechanical pressing and extraction, and further processing to remove impurities. The techniques used are similar for most vegetable oils produced from the seeds of plants. The crushing and extraction processes utilized by the canola industry today produce very little change to the fatty acid profile of the oil and the nutritional qualities of the meal.

For example, canola seeds are crushed into two component parts, oil and meal, which are then further manufactured into a wide variety of products. Further manufacturing, called refining, improves the color, flavor and shelf life of canola oil.

Canola oil is extracted in several stages. The first stage in processing canola is to roll or flake the seed. This ruptures cells and makes the oil easier to extract. Next the flaked or rolled seeds are cooked and subjected to a mild pressing process which removes some of the oil and compresses the seeds into large chunks called "cake fragments." The cake fragments undergo further processing to remove most of the remaining oil. The oil extracted during each step is combined. The oil is then subjected to processing according to the end product requirements. Different treatments are used to process salad oils, margarines, and shortenings.

Specifically, canola seed is cleaned by a number of different methods including air aspiration, indent cylinder cleaning, sieve screening, or a combination

of these. Cleaning ensures that the seed is free of extraneous plant and other foreign material which is referred to in the industry as "dockage". Seed generally contains less than 2.5% dockage following the cleaning process. Seed that has been cleaned is ready for subsequent crushing into canola oil and meal.

5 Seed which will be processed for oil and meal is preconditioned using mild heat treatment, and moisture is then adjusted to improve subsequent oil extraction. Following preconditioning, canola seed is next crushed and flaked and then heated slightly. These processes help to maximize oil recovery. The canola flakes are then "prepressed" in screw presses or expellers to reduce the oil content from about 42%
10 in-the seed (on an 8% moisture basis) to between 16-20%. Screw pressing also compresses the flakes into more dense cakes (called "press cake") which facilitates oil extraction.

 Press cake which results from seed processing is next subjected to one of two types of oil extraction to remove much of the remaining oil. Oil may be
15 extracted using either hexane ("solvent") extraction or by "cold-pressing" (also referred to as "expeller pressing"). The end-market into which the oil is sold generally dictates which form of extraction will be used. Hexane is the extraction medium used for the bulk of canola oil which is sold into the commodity grocery chain market as well as to the food industry. Cold-pressed canola oil represents a
20 much smaller volume sold to consumers and is generally marketed in specialty food stores. Both extraction processes result in an oil essentially bland in taste, light yellow in color, and with excellent nutritional and stability properties.

 Hexane extraction reduces the oil content of the press cake to very low levels. Oil recovery from canola seed is approximately 96% when this form of
25 extraction is used. This is accomplished by maximizing contact of the hexane with the press cake through a series of soakings or washings. Residual hexane in the extracted press cake and oil is easily removed by evaporation at low temperature. Solvent residues in oils and meals, when produced in accordance with good manufacturing practice, can be said to be truly insignificant.

30 The oil which is produced during the extraction process is referred to as "crude oil" because it contains various compounds which must be removed to ensure a product with good stability and shelf-life. These impurities include phospholipids, mucilaginous gums, free fatty acids, color pigments and fine meal

particles. Different methods are used to remove these by-products including water precipitation or organic acids in combination with water. Once removed, these by-products are added to the canola meal fraction in order to increase its feeding value (energy) and make it an even more nutritious product.

5 Following water precipitation and/or organic acid processing, the oil will still contain color compounds which, if not removed would make it unattractive to the consumer and also reduce its stability. These compounds are extracted through a process called bleaching. In contrast to what may be implied by the term, bleaching does not involve the use of harsh chemicals. Instead, during the bleaching process,
10 the oil is moved through a natural, diatomaceous clay to remove color compounds and other by-products.

Deodorization is the final step in the refining of all vegetable oils, including canola. Deodorization involves the use of steam distillation with the objective being the removal of any residual compounds which, if retained, could impart an adverse
15 odor and taste to the oil. The oil produced is referred to as "refined oil".

In still another embodiment, this invention concerns a transgenic progeny plant obtained from the plant of claim 7 or 12, wherein said transgenic progeny plant comprises the recombinant DNA construct.

There are a variety of methods for the regeneration of plants from plant
20 tissue. The particular method of regeneration will depend on the starting plant tissue and the particular plant species to be regenerated. The regeneration, development and cultivation of plants from single plant protoplast transformants or from various transformed explants is well known in the art (Weissbach and Weissbach, In: Methods for Plant Molecular Biology, (Eds.), Academic: San Diego,
25 CA (1988)). This regeneration and growth process typically includes the steps of selection of transformed cells and culturing those individualized cells through the usual stages of embryonic development through the rooted plantlet stage. Transgenic embryos and seeds are similarly regenerated. The resulting transgenic rooted shoots are thereafter planted in an appropriate plant growth medium such as
30 soil. Preferably, the regenerated plants are self-pollinated to provide homozygous transgenic plants. Otherwise, pollen obtained from the regenerated plants is crossed to seed-grown plants of agronomically important lines. Conversely, pollen from plants of these important lines is used to pollinate regenerated plants. A

transgenic plant of the present invention containing a desired polypeptide is cultivated using methods well known to one skilled in the art.

Normal germination of transgenic plant seed is defined as germination frequency that is very similar to the germination frequency of seed of the untransformed variety under produced under identical conditions.

In addition to the above discussed procedures, practitioners are familiar with the standard resource materials which describe specific conditions and procedures for: the construction, manipulation and isolation of macromolecules (e.g., DNA molecules, plasmids, etc.); the generation of recombinant DNA fragments and recombinant expression constructs; and, the screening and isolating of clones. See, for example: Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor: NY (1989); Maliga et al., *Methods in Plant Molecular Biology*, Cold Spring Harbor: NY (1995); Birren et al., *Genome Analysis: Detecting Genes*, Vol.1, Cold Spring Harbor: NY (1998); Birren et al., *Genome Analysis: Analyzing DNA*, Vol.2, Cold Spring Harbor: NY (1998); *Plant Molecular Biology: A Laboratory Manual*, eds. Clark, Springer: NY (1997).

Examples of cruciferous oilseed plants that can be used to practice the invention include, but are not limited to, *Brassica* species, and *Arabidopsis thaliana*.

Assays for gene expression based on the transient expression of cloned nucleic acid constructs have been developed by introducing the nucleic acid molecules into plant cells by polyethylene glycol treatment, electroporation, or particle bombardment (Marcotte et al., *Nature* 335:454-457 (1988); Marcotte et al., *Plant Cell* 1:523-532 (1989); McCarty et al., *Cell* 66:895-905 (1991); Hattori et al., *Genes Dev.* 6:609-618 (1992); Goff et al., *EMBO J.* 9:2517-2522 (1990)).

Transient expression systems may be used to functionally dissect gene constructs (see generally, Maliga et al., *Methods in Plant Molecular Biology*, Cold Spring Harbor Press (1995)). It is understood that any of the nucleic acid molecules of the present invention can be introduced into a plant cell in a permanent or transient manner in combination with other genetic elements such as vectors, promoters, enhancers etc.

In addition to the above discussed procedures, practitioners are familiar with the standard resource materials which describe specific conditions and procedures for the construction, manipulation and isolation of macromolecules (e.g., DNA

molecules, plasmids, etc.), generation of recombinant organisms and the screening and isolating of clones, (see for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press (1989); Maliga et al., *Methods in Plant Molecular Biology*, Cold Spring Harbor Press (1995); Birren et al., *Genome Analysis: Detecting Genes*, 1, Cold Spring Harbor, New York (1998); Birren et al., *Genome Analysis: Analyzing DNA*, 2, Cold Spring Harbor, New York (1998); Plant Molecular Biology: A Laboratory Manual, eds. Clark, Springer, New York (1997)).

EXAMPLES

The present invention is further defined 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 preferred embodiments of the invention, are given by way of illustration only. The scope of the claims should not be limited by the preferred embodiments set forth in the examples, but should be given the broadest interpretation consistent with the description as a whole.

The meaning of abbreviations is as follows: "sec" means second(s), "min" means minute(s), "h" means hour(s), "d" means day(s), "μL" means microliter(s), "mL" means milliliter(s), "L" means liter(s), "μM" means micromolar, "mM" means millimolar, "M" means molar, "mmol" means millimole(s), "μmole" mean micromole(s), "g" means gram(s), "μg" means microgram(s), "ng" means nanogram(s), "U" means unit(s), "bp" means base pair(s) and "kB" means kilobase(s).

EXAMPLE 1

Construction of Vector pZBL120xKS336 for Expression of a *Zea mays* ODP1 Under Control of a Beta-Conglycinin Promoter:

Plasmid pKS332 was constructed via a number of different intermediate vectors. The *Ascl* cassette containing Kti3 Promoter::*NotI*::Kti3 Terminator from pKS121 (PCT Application No. WO 02/00904) was blunt-end cloned into the *NotI* (filled-in) site on pBLUESCRIPT® II SK+ (Stratagene) to give pKS121/BS (Seq ID

NO:1). The *NcoI*/*NotI* fragment from expression vector pDsRed-Express (Clontech) was blunt-end cloned into the *NotI* (filled-in) site of pKS121/BS to give pDsRedxKS121/BS (SEQ ID NO:2). The *Bam*HI cassette containing Kti3 Promoter::DsRed::Kti3 Terminator in pDS-REDxKS121/BS (SEQ ID NO:1) was ligated into the *Bam*HI site of pKS123 (PCT Application No. WO 02/08269) to give pKS332 (SEQ ID NO:3). A DNA fragment encoding the ODP1 polypeptide from maize, Zm-ODP1, described in U.S. Patent No. 7,157,621, was synthesized by PCR with primers to introduce *NotI* sites at both ends. Applicant's cDNA clone cde1c.pk003.o22 (SEQ ID NO:319 in U.S. Patent No. 7,157,621) was used as template in a PCR reaction using primers MWG345 (SEQ ID NO:4) and MWG346 (SEQ ID NO:5). The resulting PCR product was digested with *NotI* restriction enzyme and ligated into the *NotI* site of pKS332 to give pKS336 (SEQ ID NO:6). Plasmid pKS336 contains the ZM-ODP1 protein-coding region of cDNA clone cde1c.pk003.o22 fused at its 5' terminus with the promoter of the soybean gene for the α' -subunit of β -conglycinin (Beachy et al. (1985) EMBO J. 4:3047-3053) and at its 3' end with the terminator sequence from the phaseolin gene of common bean, *Phaseolus vulgaris* (Doyle et al. (1986) J. Biol. Chem. 261:9228-9238). The β -conglycinin promoter directs strong seed-specific expression of transgenes in transformed plants.

A 5.9 kb DNA fragment containing the ZM-ODP1 and DsRed expression cassettes was excised from KS336 using the restriction enzyme *AscI* and the ends were filled-in with T4 DNA polymerase (Promega, Madison, USA). This fragment was ligated to linearized DNA of the *Agrobacterium tumefaciens* binary vector pZBL120, which had been linearized with *EcoRI* and *Bam*HI and the ends filled-in, to give pZBL120xKS336. The T-DNA of the plant transformation vector pZBL120xKS336 is set forth as SEQ ID NO:7.

It is noted that the binary vector pZBL120 is identical to the pZBL1 binary vector (American Type Culture Collection Accession No. 209128) described in U.S. Patent No. 5,968,793, except the NOS promoter was replaced with a 963 bp 35S promoter (NCBI Accession No. V00141; also known as NCBI General Identifier No. 58821) from nucleotide 6494 to 7456 in the NOS Promoter::nptII::OCS Terminator cassette. The new 35S Promoter::nptII::OCS Terminator cassette serves as a kanamycin (Kan) resistance plant selection marker in pZBL120.

EXAMPLE 2Generation and Analysis of Oil Content of Transgenic Arabidopsis Lines Containing a Beta-Conglycinin Promoter::ZM-ODP1::Phaseolin Terminator ExpressionCassette

5 Plasmid DNA of pZBL120xKS336, containing the beta-conglycinin promoter::ZM-ODP1::phaseolin terminator expression cassette, was introduced into *Agrobacterium tumefaciens* NTL4 (Luo et al, *Molecular Plant-Microbe Interactions* (2001) 14(1):98-103) by electroporation. Briefly, 1 µg plasmid DNA was mixed with 100 µL of electro-competent cells on ice. The cell suspension was transferred to a 10 100 µL electroporation cuvette (1 mm gap width) and electroporated using a BIORAD electroporator set to 1 kV, 400Ω and 25 µF. Cells were transferred to 1 mL LB medium and incubated for 2 h at 30 °C. Cells were plated onto LB medium containing 50 µg/mL kanamycin. Plates were incubated at 30 °C for 60 h. Recombinant *Agrobacterium* cultures (500 mL LB, 50 µg/mL kanamycin) were 15 inoculated from single colonies of transformed *agrobacterium* cells and grown at 30 °C for 60 h. Cells were harvested by centrifugation (5000xg, 10 min) and resuspended in 1 L of 5 % (W/V) sucrose containing 0.05 % (V/V) Silwet. *Arabidopsis* plants were grown in soil at a density of 30 plants per 100 cm² pot in METRO-MIX® 360 soil mixture for 4 weeks (22 °C, 16 h light/8 h dark, 100 µE m⁻²s⁻¹). Plants were repeatedly dipped into the *Agrobacterium* suspension harboring the binary vector pZBL120xKS336 and kept in a dark, high humidity environment for 24 h. Plants were grown for three to four weeks under standard plant growth conditions described above and plant material was harvested and dried for one week at ambient temperatures in paper bags. Seeds were harvested using a 25 0.425 mm mesh brass sieve.

Cleaned *Arabidopsis* seeds (2 grams, corresponding to about 100,000 seeds) were sterilized by washes in 45 mL of 80 % ethanol, 0.01 % TRITON® X-100, followed by 45 mL of 30 % (V/V) household bleach in water, 0.01 % TRITON® X-100 and finally by repeated rinsing in sterile water. Aliquots of 20,000 seeds were 30 transferred to square plates (20 x 20 cm) containing 150 mL of sterile plant growth medium comprised of 0.5 x MS salts, 0.53 % (W/V) sorbitol, 0.05 MES/KOH (pH 5.8), 200 µg/mL TIMENTIN®, and 50 µg/mL kanamycin solidified with 10 g/L

agar. Homogeneous dispersion of the seed on the medium was facilitated by mixing the aqueous seed suspension with an equal volume of melted plant growth medium. Plates were incubated under standard growth conditions for ten days. Kanamycin-resistant seedlings were transferred to plant growth medium without selective agent and grown for one week before transfer to soil. Plants were grown to maturity and T2 seeds were harvested and plated on selective media containing kanamycin. Approximately 100 events were generated in this manner. Wild-type (WT) control plants were grown in the same flat containing pZBL120xKS336 T1 plants. T2 seed were harvested and oil content was measured by NMR as follows.

10 *NMR based analysis of seed oil content:*

Seed oil content was determined using a Maran Ultra NMR analyzer (Resonance Instruments Ltd, Whitney, Oxfordshire, UK). Samples (e.g., batches of Arabidopsis seed ranging in weight between 5 and 200 mg) were placed into pre-weighed 2 mL polypropylene tubes (Corning Inc, Corning NY, USA; Part no. 430917) previously labeled with unique bar code identifiers. Samples were then placed into 96 place carriers and processed through the following series of steps by an ADEPT COBRA 600™ SCARA robotic system:

1. pick up tube (the robotic arm was fitted with a vacuum pickup devise);
2. read bar code;
- 20 3. expose tube to antistatic device (ensured that Arabidopsis seed were not adhering to the tube walls);
4. weigh tube (containing the sample), to 0.0001 g precision;
5. take NMR reading; measured as the intensity of the proton spin echo 1 msec after a 22.95 MHz signal had been applied to the sample (data was collected for 32 NMR scans per sample);
- 25 6. return tube to rack; and
7. repeat process with next tube.

Bar codes, tubes weights and NMR readings were recorded by a computer connected to the system. Sample weight was determined by subtracting the polypropylene tube weight from the weight of the tube containing the sample.

Seed oil content (on a % seed weight basis) of Arabidopsis seed was calculated as follows:

$$\text{mg oil} = (\text{NMR signal} - 2.1112)/37.514;$$

$$\% \text{ oil} = [(\text{mg oil})/1000]/[\text{g of seed sample weight}] \times 100.$$

Prior to establishing this formula, Arabidopsis seed oil was extracted as follows. Approximately 5 g of mature Arabidopsis seed (cv Columbia) were ground to a fine powder using a mortar and pestle. The powder was placed into a 33 x 94 mm paper thimble (Ahlstrom # 7100-3394; Ahlstrom, Mount Holly Springs, PA, USA) and the oil extracted during approximately 40 extraction cycles with petroleum ether (BP 39.9 – 51.7 °C) in a Soxhlet apparatus. The extract was allowed to cool and the crude oil was recovered by removing the solvent under vacuum in a rotary evaporator. Calibration parameters were determined by precisely weighing 11 standard samples of partially purified Arabidopsis oil (samples contained 3.6, 6.3, 7.9, 9.6, 12.8, 16.3, 20.3, 28.2, 32.1, 39.9 and 60 mg of partially purified Arabidopsis oil) weighed to a precision of 0.0001 g) into 2 mL polypropylene tubes (Corning Inc, Corning NY, USA; Part no. 430917) and subjecting them to NMR analysis. A calibration curve of oil content (% seed weight basis) to NMR value was established.

Seed oil content of most transgenic lines was increased when compared to oil content of seed collected from wild-type control plants grown in the same flat. The phenotype of two representative transgenic lines, C00536 and C00576, are described below in detail. Kanamycin-resistant T2 seedlings were transferred from selective growth media to soil. For C00536, thirteen T2 plants were grown with four wild-type (WT) control plants. For C00576 ten T2 plants were grown with seven WT control plants. Plants were grown to maturity, T3 seed were harvested from individual plants and subjected to oil quantitation by NMR.

Data are summarized in Table 1. Presence of the pZBL120xKS336 transgene is associated with an increase in oil content in transgenic T3 seed when compared Arabidopsis plants of identical genetic background that lack the transgene.

TABLE 1

Oil Content of T3 Seed of pZBL120xKS336 Transgenics

Exp	Event ID	Plant #	% Oil	Exp	Event ID	Plant #	% Oil
1	C00536	1	45.7	2	C00576	1	48.0
1	C00536	2	45.1	2	C00576	2	47.9
1	C00536	3	45.0	2	C00576	3	45.9
1	C00536	4	44.6	2	C00576	4	45.3
1	C00536	5	44.0	2	C00576	5	44.5
1	C00536	6	43.7	2	C00576	6	43.7

1	C00536	7	43.5	2	C00576	7	43.6
1	C00536	8	42.8	2	C00576	8	42.1
1	C00536	9	42.7	2	C00576	9	41.9
1	C00536	10	42.0	2	C00576	10	41.0
1	C00536	11	42.0	2	C00576	AVG	44.4
1	C00536	12	41.9	2	WT	1	42.2
1	C00536	13	39.9	2	WT	2	40.9
1	C00536	AVG	43.3	2	WT	3	40.4
1	WT	1	39.5	2	WT	4	39.3
1	WT	2	37.5	2	WT	5	38.7
1	WT	3	37.0	2	WT	6	38.0
1	WT	4	34.7	2	WT	7	37.8
1	WT	AVG	37.2	2	WT	AVG	39.6

Transgenic T3 seed selections that no longer segregated for the DsRed marker gene were identified by visual inspection using a suitable light source. For both events non-segregating transgenic seed were planted in soil alongside
5 untransformed WT plants.

T4 seed were harvested from individual T3 plants and WT controls. Oil content was measured by NMR (Table 2). Presence of the pZBL120xKS336 transgene is associated with an increase in oil content in transgenic T4 seed when compared to Arabidopsis plants of identical genetic background that lack the
10 transgene.

TABLE 2
Oil Content of T4 Seed of pZBL120xKS336 Transgenics

Exp	Event ID	Plant #	% Oil	Exp	Event ID	Plant #	% Oil
1	C00536	1	46.5	3	C00576	1	45.3
1	C00536	2	46.5	3	C00576	2	44.8
1	C00536	3	46.4	3	C00576	3	44.7
1	C00536	4	46.3	3	C00576	4	44.7
1	C00536	5	46.3	3	C00576	5	44.4
1	C00536	6	46.2	3	C00576	6	44.2
1	C00536	7	46.2	3	C00576	7	44.2
1	C00536	8	46.2	3	C00576	8	44.2
1	C00536	9	46.2	3	C00576	9	44.2
1	C00536	10	46.1	3	C00576	10	44.0
1	C00536	11	46.0	3	C00576	11	43.8
1	C00536	12	45.8	3	C00576	12	43.3
1	C00536	13	45.2	3	C00576	13	43.1
1	C00536	14	45.1	3	C00576	14	43.0
1	C00536	15	45.1	3	C00576	15	41.8
1	C00536	16	44.5	3	C00576	16	41.1
1	C00536	17	43.5	3	C00576	AVG	43.8

1	C00536	18	43.4	3	WT	1	43.8
1	C00536	AVG	45.6	3	WT	2	42.9
1	WT	1	44.8	3	WT	3	42.4
1	WT	2	44.6	3	WT	4	41.9
1	WT	3	42.3	3	WT	5	41.6
1	WT	4	42.1	3	WT	6	40.3
1	WT	5	42.0	3	WT	7	37.5
1	WT	AVG	43.2	3	WT	8	41.1
2	C00536	1	45.7	3	WT	AVG	41.4
2	C00536	2	45.6	4	C00576	1	46.6
2	C00536	3	45.6	4	C00576	2	46.4
2	C00536	4	45.4	4	C00576	3	46.3
2	C00536	5	45.4	4	C00576	4	46.2
2	C00536	6	45.4	4	C00576	5	46.2
2	C00536	7	45.4	4	C00576	6	46.2
2	C00536	8	45.4	4	C00576	7	46.2
2	C00536	9	45.4	4	C00576	8	45.7
2	C00536	10	45.1	4	C00576	9	45.7
2	C00536	11	45.1	4	C00576	10	45.6
2	C00536	12	45.0	4	C00576	11	45.6
2	C00536	13	44.8	4	C00576	12	45.4
2	C00536	14	44.7	4	C00576	13	45.4
2	C00536	15	44.6	4	C00576	14	45.1
2	C00536	16	44.5	4	C00576	15	45.0
2	C00536	17	43.5	4	C00576	16	44.3
2	C00536	18	43.1	4	C00576	17	44.2
2	C00536	AVG	45.0	4	C00576	AVG	45.7
2	WT	1	43.8	4	WT	1	44.7
2	WT	2	43.3	4	WT	2	44.6
2	WT	3	42.3	4	WT	3	44.4
2	WT	4	41.8	4	WT	4	43.7
2	WT	5	41.5	4	WT	5	43.5
2	WT	6	40.2	4	WT	6	42.2
2	WT	AVG	42.1	4	WT	AVG	43.9

A total of five flats were planted using WT seed and homozygous T4 seed of C00536 and C00576. Twenty-four transgenic T4 plants were grown alongside twelve WT plants. Plants were grown to maturity. From each flat WT and transgenic seed were bulk-harvested. Oil content of bulk seed samples was measured by NMR (Table 3). Presence of the pZBL120xKS336 transgene is associated with an increase in oil content in transgenic T5 seed when compared to Arabidopsis plants of identical genetic background that lack the transgene.

Seed oil content in a given plant is a highly variable trait that responds strongly to plant growth conditions (Li Y, Beisson F, Pollard M, Ohlrogge J (2006)

Oil content of Arabidopsis seeds: The influence of seed anatomy, light and plant-to-plant variation, Phytochemistry 67:904-915). It is therefore important that an increase in oil content associated with a particular strategy is observed in multiple environments, over several generations and under conditions that allow for maximal oil accumulation by isogenic control lines. The increase in oil content associated with presence of the pZBL120xKS336 transgene was consistently observed over three generations and in different growth chambers. The average oil increase associated with two different pZBL120xKS336 transgenic events was at least 2 % points and as high as 3.6 % points (i.e., an oil increase of as high as 8.5 % compared to untransformed WT seed). This oil increase was observed under growth conditions in which untransformed Arabidopsis seed produced the expected levels of oil, indicating that oil seed storage lipid accumulation was operating at maximum levels.

TABLE 3

Oil Content of T5 Seed of pZBL120xKS336 Transgenics

Flat ID	Event ID	Oil (%)	Δ Oil (%) Points)	Δ Oil (%)
A	C00576	45.1	1.7	3.9
	WT	43.5		
B	C00576	46.4	1.9	4.2
	WT	44.5		
C	C00576	44.8	2.3	5.5
	WT	42.5		
D	C00576	45.5	2.0	4.7
	WT	43.4		
E	C00576	44.6	2.0	4.7
	WT	42.6		
AVG	C00576		2.0	4.6
A	C00536	45.9	3.3	7.8
	WT	42.6		
B	C00536	45.8	3.4	8.1
	WT	42.4		
C	C00536	46.7	4.7	11.2
	WT	42.0		
D	C00536	44.7	3.9	9.6
	WT	40.8		
E	C00536	46.2	2.6	6.0
	WT	43.5		
AVG	C00536		3.6	8.5

EXAMPLE 3

Construction of Vector pZBL120xKS333 for Expression of a *Momordica charantia*

ODP1 Under Control of a Beta-Conglycinin Promoter:

An ODP1 protein-coding region from balsam pear (*Momordica charantia*)
5 described in detail in U.S. Patent No. 7,157,621 was synthesized by PCR with
primers to introduce *NotI* sites at both ends of the gene. Applicants cDNA clone
fds1n.pk015.115 was used as a template in the PCR reaction using primers MWG339
(SEQ ID NO:8) and MWG340 (SEQ ID NO:9). The resulting PCR product was
digested with *NotI* restriction enzyme and ligated into the *NotI* site of pKS332 to give
10 pKS333 (SEQ ID NO:10).

A 6.1 kb DNA fragment containing the MC-ODP1 and DsRed expression
cassettes was excised from KS333 using the restriction enzyme *Ascl*, the ends were
filled-in with T4 DNA polymerase (Promega, Madison, USA) and the fragment was
blunt-end ligated to DNA of the *Agrobacterium tumefaciens* binary vector pZBL120,
15 which had been linearized with *EcoRI* and *BamHI* and the ends filled-in. The
resulting plant transformation vector was designated pZBL120xKS333, and the T-
DNA of this vector is set forth as SEQ ID NO:11.

EXAMPLE 4

Construction of Vector pZBL120xKS334 for Expression of a *Glycine max* ODP1

Under Control of a Beta-Conglycinin Promoter:

An ODP1 protein-coding region from soybean described in detail in U.S.
Patent No. 7,157,621 was synthesized by PCR with primers to introduce *NotI* sites
at both ends of the gene. Applicants cDNA clone se3.pk0003.f5 was used as
template in the PCR reaction using primers MWG341 (SEQ ID NO:12) and
25 MWG342 (SEQ ID NO:13). The resulting PCR product was digested with *NotI*
restriction enzyme and ligated into the *NotI* site of pKS332 to give pKS334 (SEQ ID
NO:14).

A 6.1 kb DNA fragment containing the GM-ODP1 and DsRed expression
cassettes was excised from KS334 using the restriction enzyme *Ascl*, the ends were
30 filled-in with T4 DNA polymerase (Promega, Madison, USA) and the fragment was
blunt-end ligated to DNA of the *Agrobacterium tumefaciens* binary vector pZBL120,
which had been linearized with *EcoRI* and *BamHI* and the ends filled-in. The

resulting plant transformation vector was designated pZBL120xKS334, and the T-DNA of this vector is set forth as SEQ ID NO:15.

EXAMPLE 5

Generation of Arabidopsis Lines Transformed with *Momordica charantia* ODP1 or *Glycine max* ODP1 and Analysis of Seed Oil Content

Binary vector constructs pZBL120xKS333 (*Momordica charantia* ODP1) and pZBL120xKS334 (*Glycine max* ODP1) were used for Arabidopsis transformation using the floral dip method as described above. Transgenic lines were selected on plant growth media containing kanamycin. 75 and 190 lines were generated with pZBL120xKS333 and pZBL120xKS334, respectively. T1 plants of all lines were grown with 13 untransformed WT plants in the same growth chamber. Plants were grown to maturity. Seed were harvested from individual plants and oil content was measured by NMR (TABLE 4)

TABLE 4

Oil Content of T2 seed of pZBL120xKS333 and pZBL120xKS334 Transgenics

Arabidopsis Line	# of Plants	% Oil Range	Average % Oil
pZBL120xKS333	77	25.5-46.6	41.7
pZBL120xKS334	180	16.0-48.1	40.7
WT	13	31.9-43.2	39.1

T2 seed of two representative transgenic lines, 4445 (pZBL120xKS333) and 4485 (pZBL120xKS334), had an oil content of 45.1 % and 45.2 % respectively. T2 seed of these two lines were germinated on selective media, seedlings were transferred to soil, T2 plants were grown to maturity and T3 seed were harvested. After one more round of germination on selective media and seed production for each event five flats were planted with 24 kanamycin-resistant 4445 or 4485 seedlings and 12 WT seedlings. Plants were grown to maturity. From each flat WT and transgenic seed were bulk-harvested. Oil content of bulk seed samples was measured by NMR (Table 5). Presence of the pZBL120xKS333 or pZBL120xKS334 transgenes is associated with an increase in oil content in transgenic T5 seed when compared to Arabidopsis plants of identical genetic background that lack the transgene.

TABLE 5

Oil Content of T5 seed of pZBL120xKS333 and pZBL120xKS334 Transgenics

Flat ID	Construct	Event ID	Oil (%)	Δ Oil (% Points)	Δ Oil (%)
A	pZBL120xKS333	4445	44.9	0.7	1.5
		WT	44.2		
B	pZBL120xKS333	4445	45.3	1.8	4.0
		WT	43.6		
C	pZBL120xKS333	4445	46.0	2.4	5.4
		WT	43.7		
D	pZBL120xKS333	4445	44.6	1.4	3.2
		WT	43.2		
E	pZBL120xKS333	4445	43.2	-0.6	-1.4
		WT	43.8		
AVG	pZBL120xKS333			1.1	2.5
A	pZBL120xKS334	4485	45.4	2.8	6.7
		WT	42.5		
B	pZBL120xKS334	4485	44.4	1.3	3.1
		WT	43.1		
C	pZBL120xKS334	4485	44.5	1.7	4.0
		WT	42.8		
D	pZBL120xKS334	4485	45.1	1.5	3.3
		WT	43.7		
E	pZBL120xKS334	4485	45.4	1.6	3.8
		WT	43.8		
AVG	pZBL120xKS334			1.8	4.2

5 The oil increase associated with presence of the *Momordica charantia* ODP1 transgene (pZBL120xKS333) is 1.1 % points (i.e., an oil increase of 2.5 % compared to untransformed WT seed).

The oil increase associated with presence of the *Glycine max* ODP1 transgene (pZBL120xKS334) is 1.8 % points (i.e., an oil increase of 4.2 % compared to untransformed WT seed).

10

EXAMPLE 6

Compositional Analysis of Arabidopsis Seed Transformed with *Zea mays* ODP1, *Momordica charantia* ODP1 or *Glycine max* ODP1

15 T5 seed of Arabidopsis events C00536, 4445 and 4485 carrying pZBL120xKS336 (*Zea mays* ODP), pZBL120xKS333 (*Momordica charantia* ODP1) and pZBL120xKS334 (*Glycine max* ODP1) transgenes, respectively, and WT seed derived from plants grown alongside each of the transgenic events were subjected

to compositional analysis as described below. Seed weight was measured by determining the weight of 100 seed. This analysis was performed in triplicate.

Tissue preparation:

Arabidopsis seed (approximately 0.5g in a ½ x 2" polycarbonate vial) was
5 ground to a homogeneous paste in a GENOGRINDER® (3 x 30sec at 1400 strokes per minute, with a 15 sec interval between each round of agitation). After the second round of agitation the vials were removed and the Arabidopsis paste was scraped from the walls with a spatula prior to the last burst of agitation.

Determination of protein content:

10 Protein contents were estimated by combustion analysis on a Thermo FINNIGAN™ Flash 1112EA combustion analyzer running in the NCS mode (vanadium pentoxide was omitted) according to instructions of the manufacturer. Triplicate samples of the ground pastes, 4-8 mg, weighed to an accuracy of 0.001mg on a METTLER-TOLEDO® MX5 micro balance, were used for analysis.
15 Protein contents were calculated by multiplying % N, determined by the analyzer, by 6.25. Final protein contents were expressed on a % tissue weight basis.

Determination of non-structural carbohydrate content:

Sub-samples (30-35 mg) of the ground paste were weighed (to an accuracy of 0.1mg) into 13x100mm glass tubes; the tubes had TEFLON® lined screw-cap
20 closures. Three replicates were prepared for each sample tested.

Lipid extraction was performed by adding 2 ml aliquots of heptane to each tube. The tubes were vortex mixed and placed into an ultrasonic bath (VWR Scientific Model 750D) filled with water heated to 60 °C. The samples were sonicated at full-power (~360 W) for 15 min and were then centrifuged (5 min x 1700
25 g). The supernatants were transferred to clean 13x100mm glass tubes and the pellets were extracted 2 more times with heptane (2 ml, second extraction; 1 ml third extraction) with the supernatants from each extraction being pooled. After lipid extraction 1 ml acetone was added to the pellets and after vortex mixing, to fully disperse the material, they were taken to dryness in a Speedvac.

30 *Non-structural carbohydrate extraction and analysis:*

Two ml of 80% ethanol was added to the dried pellets from above. The samples were thoroughly vortex mixed until the plant material was fully dispersed in the solvent prior to sonication at 60 °C for 15 min. After centrifugation, 5 min x 1700

g, the supernatants were decanted into clean 13x100mm glass tubes. Two more extractions with 80% ethanol were performed and the supernatants from each were pooled. The extracted pellets were suspended in acetone and dried (as above). An internal standard β -phenyl glucopyranoside (100 μ l of a 0.5000 $\pm$ 0.0010g/100ml stock) was added to each extract prior to drying in a Speedvac. The extracts were maintained in a desiccator until further analysis.

The acetone dried powders from above were suspended in 0.9 ml MOPS (3-N[Morpholino]propane-sulfonic acid; 50mM, 5mM CaCl_2 , pH 7.0) buffer containing 100 U of heat-stable α -amylase (from *Bacillus licheniformis*; Sigma A-4551). Samples were placed in a heat block (90 $^{\circ}\text{C}$) for 75 min and were vortex mixed every 15 min. Samples were then allowed to cool to room temperature and 0.6 ml acetate buffer (285mM, pH 4.5) containing 5 U amyloglucosidase (Roche 110 202 367 001) was added to each. Samples were incubated for 15 –18 h at 55 $^{\circ}\text{C}$ in a water bath fitted with a reciprocating shaker; standards of soluble potato starch (Sigma S-2630) were included to ensure that starch digestion went to completion.

Post-digestion the released carbohydrates were extracted prior to analysis. Absolute ethanol (6 ml) was added to each tube and after vortex mixing the samples were sonicated for 15 min at 60 $^{\circ}\text{C}$. Samples were centrifuged (5 min x 1700 g) and the supernatants were decanted into clean 13x100mm glass tubes. The pellets were extracted 2 more times with 3 ml of 80% ethanol and the resulting supernatants were pooled. Internal standard (100ul β -phenyl glucopyranoside, as above) was added to each sample prior to drying in a Speedvac.

Sample preparation and analysis:

The dried samples from the soluble and starch extractions described above were solubilized in anhydrous pyridine (Sigma-Aldrich P57506) containing 30 mg/ml of hydroxylamine HCl (Sigma-Aldrich 159417). Samples were placed on an orbital shaker (300 rpm) overnight and were then heated for 1 hr (75 $^{\circ}\text{C}$) with vigorous vortex mixing applied every 15 min. After cooling to room temperature, 1ml hexamethyldisilazane (Sigma-Aldrich H-4875) and 100 μ l trifluoroacetic acid (Sigma-Aldrich T-6508) were added. The samples were vortex mixed and the precipitates were allowed to settle prior to transferring the supernatants to GC sample vials.

Samples were analyzed on an Agilent 6890 gas chromatograph fitted with a DB-17MS capillary column (15m x 0.32mm x 0.25um film). Inlet and detector temperatures were both 275 °C. After injection (2 µl, 20:1 split) the initial column temperature (150 °C) was increased to 180 °C at a rate of 3 °C/min and then at 25 °C/min to a final temperature of 320 °C. The final temperature was maintained for 10 min. The carrier gas was H₂ at a linear velocity of 51 cm/sec. Detection was by flame ionization. Data analysis was performed using Agilent ChemStation software. Each sugar was quantified relative to the internal standard and detector responses were applied for each individual carbohydrate (calculated from standards run with each set of samples). Final carbohydrate concentrations were expressed on a tissue weight basis.

TABLE 6

Composition Analysis of pZBL120xKS336, pZBL120xKS333 and pZBL120xKS334 Transgenic Seed and WT Control Seed

Construct	Event ID	Oil (% NMR)	Protein %	Seed Weight (µg)	fructose (µg mg ⁻¹ seed)
pZBL120xKS336	C00536	46.7	15.7	24	0.6
	WT	42	18.1	24	1
	Δ TG/WT %	11.2	-13.3	0.0	-40.0
Construct	Event ID	glucose (µg mg ⁻¹ seed)	sucrose (µg mg ⁻¹ seed)	raffinose (µg mg ⁻¹ seed)	stachyose (µg mg ⁻¹ seed)
pZBL120xKS336	C00536	8.5	17.2	0.4	2.1
	WT	12.1	29.2	0.8	3.1
	ΔTG/WT %	-29.8	-41.1	-50.0	-32.3
Construct	Event ID	Oil (% NMR)	Protein %	Seed Weight (µg)	fructose (µg mg ⁻¹ seed)
pZBL120xKS333	4445	46	15	21.7	1
	WT	43.7	14.8	20.7	1.2
	Δ TG/WT %	5.3	1.4	4.8	-16.7
Construct	Event ID	glucose (µg mg ⁻¹ seed)	sucrose (µg mg ⁻¹ seed)	raffinose (µg mg ⁻¹ seed)	stachyose (µg mg ⁻¹ seed)
pZBL120xKS333	4445	7.8	14.6	0.5	2
	WT	10.3	26.6	0.6	3.6
	Δ TG/WT %	-24.3	-45.1	-16.7	-44.4
Construct	Event ID	Oil (% NMR)	Protein %	Seed Weight (µg)	fructose (µg mg ⁻¹ seed)
pZBL120xKS334	4485	45.4	14.8	20.3	0.6

		WT	42.5	14.5	20.7	0.9
		Δ TG/WT %	6.8	2.1	-1.9	-33.3
			glucose	sucrose	raffinose	stachyose
			($\mu\text{g mg}^{-1}$	($\mu\text{g mg}^{-1}$	($\mu\text{g mg}^{-1}$	($\mu\text{g mg}^{-1}$
			seed)	seed)	seed)	seed)
Construct	Event ID					
pZBL120xKS334	4485		6.3	11.7	0.5	1.6
	WT		10.4	30.4	0.7	3.3
	Δ TG/WT %		-39.4	-61.5	-28.6	-51.5

Table 6 shows that a reduction of soluble carbohydrates is consistently associated with presence of the pZBL120xKS333, 334 and 336 transgenes. There is no consistent change in protein content or seed weight that can be attributed to the pZBL120xKS333, 334 and 336 transgenes.

EXAMPLE 7

Germination Assays of Arabidopsis Seed Transformed with *Zea mays* ODP1, *Momordica charantia* ODP1 or *Glycine max* ODP1

T5 seed of Arabidopsis events C00536, 4445 and 4485 carrying pZBL120xKS336 (*Zea mays* ODP1), pZBL120xKS333 (*Momordica charantia* ODP1) and pZBL120xKS334 (*Glycine max* ODP1) transgenes, respectively, were subjected to germination assays on standard Arabidopsis growth media (see above) containing either 10 g L⁻¹ sucrose or equimolar amounts of sorbitol (5.3 g L⁻¹). Seeds were surface-sterilized and homogeneous dispersion of the seed on the medium was facilitated by mixing the aqueous seed suspension with an equal volume of melted plant growth medium containing the either sucrose or sorbitol. Plates were incubated under standard conditions (22 °C, 16 h light/8 h dark, 100 $\mu\text{E m}^{-2}\text{s}^{-1}$) and germination rate and seedling phenotype was scored 14 days after plating (Table 7).

TABLE 7

Germination Assays for pZBL120xKS336, pZBL120xKS333 and pZBL120xKS334 Transgenic Seeds

Line ID	Media Type	Total Seed (#)	Altered Seedling Morphology (#)	No Germination (#)	Healthy Seedlings (#)
C00536	sucrose	93	69	2	22
C00536	sucrose	84	50	3	31
C00536	sucrose	90	73	3	14

C00536	sorbitol	95	6	89	0
C00536	sorbitol	112	24	88	0
C00536	sorbitol	100	49	51	0
4445	sucrose	82	24	22	36
4445	sucrose	63	24	7	32
4445	sucrose	94	36	12	46
4445	sorbitol	106	70	36	0
4445	sorbitol	119	77	42	0
4445	sorbitol	106	97	9	0
4485	sucrose	98	50	48	0
4485	sucrose	109	37	70	2
4485	sucrose	129	80	39	10
4485	sorbitol	131	24	107	0
4485	sorbitol	128	25	103	0
4485	sorbitol	127	23	102	2
Line ID	Media Type		Altered Seedling Morphology (%)	No Germination (%)	Healthy Seedlings (%)
C00536	sucrose		74.2	2.2	23.7
C00536	sucrose		59.5	3.6	36.9
C00536	sucrose		81.1	3.3	15.6
		AVG	71.6	3.0	25.4
C00536	sorbitol		6.3	93.7	0.0
C00536	sorbitol		21.4	78.6	0.0
C00536	sorbitol		49.0	51.0	0.0
		AVG	25.6	74.4	0.0
4445	sucrose		29.3	26.8	43.9
4445	sucrose		38.1	11.1	50.8
4445	sucrose		38.3	12.8	48.9
		AVG	35.2	16.9	47.9
4445	sorbitol		66.0	34.0	0.0
4445	sorbitol		64.7	35.3	0.0
4445	sorbitol		91.5	8.5	0.0
		AVG	74.1	25.9	0.0
4485	sucrose		51.0	49.0	0.0
4485	sucrose		33.9	64.2	1.8
4485	sucrose		62.0	30.2	7.8
		AVG	49.0	47.8	3.2
4485	sorbitol		18.3	81.7	0.0
4485	sorbitol		19.5	80.5	0.0
4485	sorbitol		18.1	80.3	1.6
		AVG	18.7	80.8	0.5

It is evident that germination and/or seedling development is significantly affected in all events analyzed. Germination is improved in the presence of sucrose; however, in events carrying pZBL120xKS336 and pZBL120xKS334 the seed germinating on sucrose containing media gave rise to seedlings with altered morphology, namely the presence of leaf structures that fail to become green and which resemble non-photosynthetic cotyledon tissue.

Total fatty acid (FA) composition and content of seedling tissue of C00536, 4485 and WT seedlings were measured 14 days after plating on media containing 10 g L⁻¹ sucrose. Briefly, seedling tissue was frozen on dry ice or by incubation in a -80 °C freezer for two h followed by lyophilization for 48 h.

Dried seedling tissue was ground to a fine powder using a GENOGRINDER® vial (1/2"X2" polycarbonate) and a steel ball (SPEX Centriprep (Metuchen, NJ, U.S.A.)). Grinding time was 30 sec at 1450 oscillations per min. Ten mg of tissue were weighed into Eppendorf tubes. The tissue was extracted using 100 µL heptane at room temperature under continuous shaking for 2 h. Heptane extracts were cleared by centrifugation and 25 µL of extract was derivatized to fatty acid methyl esters as follows. One mL of a 25% sodium methoxide stock solution was added to 24 mL of HPLC grade methanol. Sodium methoxide was stored under an inert gas.

Five µL of a 17:0 TAG (Nu-Chek Prep, Elysian, MN, USA) stock solution (10 mg/mL) was combined with 25 µL of heptane tissue extract in a glass culture tube and 500 µL of 1% sodium methoxide was added. Samples were derivatized in a water bath at 50 °C for 15 min. Samples were allowed to cool to RT and 1 mL of 1M NaCl was added followed by brief mixing. FAMES were extracted into 1 mL of heptane and 4 µL sample were quantitated by GC analysis (Table 8).

TABLE 8

Fatty Acid Composition and Total Fatty Acid Content of Seedling Tissue of WT Plants and pZBL120xKS334 and pZBL120xKS336 Transgenic Plants Grown on Sucrose-Containing Media

Event ID	% Total FA									Total FA (% DW)
	16:0	18:0	18:1	18:2	18:3	20:0	20:1	20:2	22:0	
WT	13.5	10.0	42.3	15.2	15.6	1.0	0.9	0.0	1.5	4.3

4485	11.8	2.7	13.2	26.6	26.7	2.5	13.5	2.2	0.7	18.6
C00536	7.9	2.3	15.0	17.7	32.4	3.2	18.2	2.0	1.2	21.9

Table 8 demonstrates that seedling tissue of transgenic lines carrying pZBL120xKS334 and pZBL120xKS336 transgenes showed increased fatty acid content when compared to WT seedlings. Moreover, the fatty acid profile of transgenic seedling tissue is similar to that of Arabidopsis WT seed in that it contains significant levels (>15 %) of C20 fatty acids.

In summary, use of a strong heterologous seed storage protein promoter (soybean β -conglycinin promoter) for expression in Arabidopsis of ODP1 genes from a diverse range of plant species belonging to the families of Leguminosae, Cucurbitaceae and Poaceae, resulted in increased seed storage lipid accumulation at the expense of soluble carbohydrates. However, seed germination and seedling establishment was negatively affected in transgenic lines expressing ODP1 genes under control of a strong heterologous seed storage protein promoter.

EXAMPLE 8

Construction of Arabidopsis Expression Vector pKR1223 for Expression of *Zea mays* ODP Under Control of the Seed-Specific, Low Strength Arabidopsis Sucrose Synthase Promoter

The present example describes the synthesis of Arabidopsis expression vector pKR1223 which allows for expression of the *Zea mays* ODP gene under control of the promoter of an Arabidopsis sucrose synthase gene (At5g49190). Additionally, vector pKR1223 provides seed-specific expression of the DsRed gene in order to visualize positive transformants as well as constitutive expression of the npt gene for selection on kanamycin.

Plasmid pKR132 (SEQ ID NO:16) which is described in PCT Publication No. WO 2004/071467, was

digested with BamHI/Sall and the fragment containing the soy albumin promoter was cloned into the BamHI/XhoI fragment of the pCR-Blunt® cloning vector (Invitrogen Corporation) to produce the starting vector pKR627 (SEQ ID NO:17).

Plasmid KS294 (SEQ ID NO:18) contains a NotI site flanked by the SCP1 promoter and the phaseolin transcription terminator (SCP1Pro::NotI::PhasTerm). The SCP1 promoter is a synthetic constitutive promoter comprising a portion of the

CaMV 35S promoter (Odell et al. (1985) Nature 313:810-812) and the Rsyn7-Syn II Core synthetic consensus promoter (US Patent No. 6,072,050 and 6,555,673.

See also, for example,

US20030226166, Table 13.

- 5 Downstream of this element is the Tobacco Mosaic Virus (TMV) omega 5'-UTR translational enhancer element (Gallie et al. (1992) Nucleic Acid Research 20:4631-4638), followed by the NotI site and the 3' transcription termination region of the phaseolin gene (Doyle et al., (1986) *J. Biol. Chem.* 261:9228-9238). The XbaI fragment of KS294 (SEQ ID NO:18), containing the SCP1Pro::NotI::PhasTerm
10 cassette, was cloned into the XbaI site of pKR627 (SEQ ID NO:17) to produce pKR1142 (SEQ ID NO:19).

The BamHI fragment of KS334 (SEQ ID NO:14; Example 1), containing the Kti3Pro:DsRed:Kti3Term cassette, was cloned into the BamHI site of pKR278 (SEQ ID NO:20), which was previously described in U.S. Patent Publication No.

- 15 US20080095915, to produce vector pKR1141 (SEQ ID NO:20).

- Genomic DNA was isolated from 3 week-old wild-type Arabidopsis col-0 seedlings using the DNEASY® Plant Mini Kit (Qiagen, Valencia, CA) and following the manufacture's protocol. An Arabidopsis Sucrose Synthase ("AtSuSy";
20 "AtSUS2") promoter derived from gene At5g49190 was PCR-amplified from Arabidopsis genomic DNA using oligonucleotides SuSy-5 (SEQ ID NO:21) and SuSy-3 (SEQ ID NO:22) 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
25 the ZERO BLUNT® PCR Cloning Kit (Invitrogen Corporation), following the manufacturer's protocol, to produce pLF122 (SEQ ID NO:23).

The BamHI/NotI fragment of pLF122 (SEQ ID NO:23), containing the AtSuSy promoter, was cloned into the BamHI/NotI fragment of pKR1142 (SEQ ID NO:19), containing the phaseolin terminator, to produce pKR1155 (SEQ ID NO:24).

- 30 The Asp718/BsiWI fragment of pKR1155 (SEQ ID NO:24), containing the AtSuSy promoter, was cloned into the BsiWI site of pKR1141 (SEQ ID NO:20), to produce pKR1158 (SEQ ID NO:25).

The NotI fragment of KS336 (SEQ ID NO:6; Example 1), containing the corn ODP, was cloned into the NotI site of pKR1158 (SEQ ID NO:25), to produce pKR1167 (SEQ ID NO:26).

The Ascl fragment of pKR1167 (SEQ ID NO:26), containing the corn ODP gene, was cloned into the Ascl fragment of pKR92 (SEQ ID NO:27) which was previously described in WO2007/061845 (published on May 31, 2007),

to produce pKR1223 (SEQ ID NO:28).

EXAMPLE 9

Construction of Arabidopsis Expression Vector pKR1220 for Expression of the Corn ODP Under Control of the Seed-Specific, Medium-Strength Soy Annexin Promoter

The present example describes the synthesis of Arabidopsis expression vector pKR1220 which allows for seed-specific expression of the corn ODP gene under control of the soy annexin promoter. Additionally, vector pKR1220 provides seed-specific expression of the DsRed gene in order to visualize positive transformants and constitutive expression of the npt gene for selection on kanamycin.

The BsiWI fragment of pKR268 (SEQ ID NO:29; which is described in PCT Publication No. WO 04/07 1467,

containing the AnnexinPro::NotI::BD30Term cassette, was cloned into the BsiWI site of pKR1141 (SEQ ID NO:20) to give pKR1143 (SEQ ID NO:30).

The NotI fragment of KS336 (SEQ ID NO:6), containing the corn ODP1 gene, was cloned into the NotI site of pKR1143 (SEQ ID NO:30), to produce pKR1147 (SEQ ID NO:31).

The Ascl fragment of pKR1147 (SEQ ID NO:31), containing the corn ODP1 gene, was cloned into the Ascl fragment of pKR92 (SEQ ID NO:27) to produce pKR1220 (SEQ ID NO:32).

EXAMPLE 10

Construction of Arabidopsis Expression Vector pKR1221 for Expression of the Corn ODP Under Control of the Constitutive, Medium Strength SCP1 Promoter

The present example describes the synthesis of Arabidopsis expression vector pKR1221 which allows for constitutive expression of the corn ODP1 gene under control of the SCP1 promoter. Additionally, vector pKR1221 provides seed-

specific expression of the DSred gene in order to visualize positive transformants and constitutive expression of the npt gene for selection on kanamycin.

The Asp718/BsiVI fragment of pKR1142 (SEQ ID NO:19), containing the SCP1Pro::NotI::PhasTerm cassette, was cloned into the BsiVI site of pKR1141 (SEQ ID NO:20), to produce pKR1144 (SEQ ID NO:33).

The NotI fragment of KS336 (SEQ ID NO:6), containing the corn ODP1, was cloned into the NotI site of pKR1144 (SEQ ID NO:33), to produce pKR1149 (SEQ ID NO:34).

The AscI fragment of pKR1149 (SEQ ID NO:34), containing the corn ODP1 gene, was cloned into the AscI fragment of pKR92 (SEQ ID NO:27) to produce pKR1221 (SEQ ID NO:35).

EXAMPLE 11

Generation and Analysis of T2 Seed of Arabidopsis Lines Transformed with Corn ODP Under Control of the SCP1, Annexin or Sucrose Synthase Promoters

Plasmid DNA of pKR1220, pKR1221 and pKR1223 was introduced into *Agrobacterium tumefaciens* NTL4 (Luo et al, *Molecular Plant-Microbe Interactions* (2001) 14(1):98-103) by electroporation. Briefly, 1 µg plasmid DNA was mixed with 100 µL of electro-competent cells on ice. The cell suspension was transferred to a 100 µL electroporation cuvette (1 mm gap width) and electroporated using a BIORAD electroporator set to 1 kV, 400Ω and 25 µF. Cells were transferred to 1 mL LB medium and incubated for 2 h at 30 °C. Cells were plated onto LB medium containing 50 µg/mL kanamycin. Plates were incubated at 30 °C for 60 h. Recombinant *Agrobacterium* cultures (500 mL LB, 50 µg/mL kanamycin) were inoculated from single colonies of transformed *Agrobacterium* cells and grown at 30 °C for 60 h. Cells were harvested by centrifugation (5000xg, 10 min) and resuspended in 1 L of 5 % (W/V) sucrose containing 0.05 % (V/V) Silwet. Arabidopsis plants were grown in soil at a density of 30 plants per 100 cm² pot in METRO-MIX® 360 soil mixture for 4 weeks (22 °C, 16 h light/8 h dark, 100 µE m⁻²s⁻¹). Plants were repeatedly dipped into the *Agrobacterium* suspension harboring the relevant binary vector and kept in a dark, high humidity environment for 24 h. Plants were grown for three to four weeks under standard plant growth conditions described above and plant material was harvested and dried for one week at

ambient temperatures in paper bags. Seeds were harvested using a 0.425 mm mesh brass sieve.

Cleaned Arabidopsis seeds (2 grams, corresponding to about 100,000 seeds) were sterilized by washes in 45 mL of 80 % ethanol, 0.01 % TRITON® X-100, followed by 45 mL of 30 % (V/V) household bleach in water, 0.01 % TRITON® X-100 and finally by repeated rinsing in sterile water. Aliquots of 20,000 seeds were transferred to square plates (20 x 20 cm) containing 150 mL of sterile plant growth medium comprised of 0.5 x MS salts, 0.53 % (W/V) sorbitol, 0.05 MES/KOH (pH 5.8), 200 µg/mL TIMENTIN®, and 50 µg/mL kanamycin solidified with 10 g/L agar. Homogeneous dispersion of the seed on the medium was facilitated by mixing the aqueous seed suspension with an equal volume of melted plant growth medium. Plates were incubated under standard growth conditions for ten days. Kanamycin-resistant seedlings were transferred to plant growth medium without selective agent and grown for one week before transfer to soil. Plants were grown to maturity and T2 seeds were harvested and plated on selective media containing kanamycin. Approximately 100 events were generated in this manner. Wild-type control plants were grown in the same flat containing transgenic T1 plants. T2 seeds were harvested and oil content was measured by NMR (Tables 9 and 10).

TABLE 9

Data from Germination Assays for T2 Seed of pKR1220, pKR1221 and pKR1223 Transgenics on Selective Medium Containing Kanamycin and Sorbitol

Event ID	pKR	Total Seed (#)	Trans-genic Seed (#)	ASM* (#)	Kan ^S (#)	No Germination (#)	Healthy Seed-Lings (#)	Δ Oil % points
35634	1220	122	110	11	12	31	68	2.6
36062	1220	134	127	25	7	85	17	2.4
35637	1220	147	133	16	14	100	17	2.4
36066	1220	143	123	22	20	59	42	2
35636	1220	116	105	19	11	62	24	1.7
36059	1220	101	85	14	16	52	19	1.6
36104	1221	104	104	6	0	96	2	4.7
36078	1221	83	66	0	17	66	0	3
36087	1221	93	89	0	4	89	0	2
36090	1221	103	103	1	0	98	4	1.9
36101	1221	134	126	0	8	126	0	1.7
36122	1221	108	92	0	16	92	0	1.7
36162	1223	92	83	8	9	20	55	5.3
36210	1223	112	111	2	1	21	88	4.4

36151	1223	144	142	66	2	40	36	3.6
36194	1223	94	91	14	3	11	66	3.4
36157	1223	101	77	14	24	10	53	3.4
36181	1223	160	149	15	11	88	46	3.3
36199	1223	103	95	17	8	12	66	3.2
36208	1223	119	110	22	9	20	68	3.1
36161	1223	134	120	19	14	33	68	3
36200	1223	144	140	0	4	101	39	2.8
36154	1223	110	99	10	11	7	82	2.7
36209	1223	109	106	10	3	31	65	2.6
36179	1223	172	147	10	25	68	69	2.6
36180	1223	162	149	16	13	51	82	2.6
36213	1223	146	127	22	19	57	48	2.4
36206	1223	86	79	17	7	0	62	2.2

* "ASM" denotes Altered Seedling Morphology

TABLE 10

Results from Germination Assays for T2 Seed of pKR1220, pKR1221 and pKR1223

Transgenics on Selective Medium Containing Kanamycin and Sorbitol

Event ID	pKR	% ASM*	% No Germination	% Healthy Seedlings	Δ Oil % Points
35634	1220	10.0	28.2	61.8	2.6
36062	1220	19.7	66.9	13.4	2.4
35637	1220	12.0	75.2	12.8	2.4
36066	1220	17.9	48.0	34.1	2.0
35636	1220	18.1	59.0	22.9	1.7
36059	1220	16.5	61.2	22.4	1.6
	AVG	15.7	56.4	27.9	2.1
36104	1221	5.8	92.3	1.9	4.7
36078	1221	0.0	100.0	0.0	3.0
36087	1221	0.0	100.0	0.0	2.0
36090	1221	1.0	95.1	3.9	1.9
36101	1221	0.0	100.0	0.0	1.7
36122	1221	0.0	100.0	0.0	1.7
	AVG	1.1	97.9	1.0	2.5
36162	1223	9.6	24.1	66.3	5.3
36210	1223	1.8	18.9	79.3	4.4
36151	1223	46.5	28.2	25.4	3.6
36194	1223	15.4	12.1	72.5	3.4
36157	1223	18.2	13.0	68.8	3.4
36181	1223	10.1	59.1	30.9	3.3
36199	1223	17.9	12.6	69.5	3.2
36208	1223	20.0	18.2	61.8	3.1
36161	1223	15.8	27.5	56.7	3.0
36200	1223	0.0	72.1	27.9	2.8
36154	1223	10.1	7.1	82.8	2.7
36209	1223	9.4	29.2	61.3	2.6
36179	1223	6.8	46.3	46.9	2.6

36180	1223	10.7	34.2	55.0	2.6
36213	1223	17.3	44.9	37.8	2.4
36206	1223	21.5	0.0	78.5	2.2
	AVG	14.4	28.0	57.6	3.2

* "ASM" denotes Altered Seedling Morphology

EXAMPLE 12

Analysis of T3 and T4 Seed of Arabidopsis Plants Transformed with *Zea mays* ODP

Under Control of the Arabidopsis Sucrose Synthase Promoter

5 T2 seeds of pKR1223 transformation events 36162, 36180 and 36181 were germinated on selective media containing kanamycin. Twenty-four kanamycin-resistant seedlings were planted in soil along side twelve untransformed WT Arabidopsis plants. Plants were grown to maturity and T3 seed samples were harvested from individual T2 plants. A bulk seed sample was generated from all WT
10 plants in a given flat. Oil content was measured by NMR (Table 11).

TABLE 11

Oil Content of T3 Seed of pKR1223 Transgenics

Event	Plant #	% oil	Event	Plant #	% oil	Event	Plant #	% oil
36162	1	44.6	36180	1	44.5	36181	1	47.2
36162	2	44.5	36180	2	44.3	36181	2	46.3
36162	3	44.4	36180	3	43.8	36181	3	46.2
36162	4	44.3	36180	4	43.8	36181	4	46.1
36162	5	44.3	36180	5	43.7	36181	5	45.9
36162	6	44.2	36180	6	43.6	36181	6	45.7
36162	7	44.2	36180	7	43.6	36181	7	45.4
36162	8	43.9	36180	8	43.6	36181	8	45.0
36162	9	43.8	36180	9	43.5	36181	9	45.0
36162	10	43.7	36180	10	43.4	36181	10	45.0
36162	11	43.7	36180	11	43.3	36181	11	45.0
36162	12	43.7	36180	12	43.3	36181	12	44.9
36162	13	43.7	36180	13	43.3	36181	13	44.9
36162	14	43.7	36180	14	43.3	36181	14	44.8
36162	15	43.6	36180	15	43.2	36181	15	44.7
36162	16	43.5	36180	16	43.2	36181	16	44.6
36162	17	43.5	36180	17	43.1	36181	17	44.5
36162	18	43.5	36180	18	43.1	36181	18	44.4
36162	19	43.4	36180	19	42.9	36181	19	44.4
36162	20	43.0	36180	20	42.9	36181	20	43.8
36162	21	42.8	36180	21	42.8	36181	21	43.8
36162	22	42.2	36180	22	42.8	36181	22	43.6
36162	23	41.8	36180	23	42.7	36181	23	43.3
36162	24	36.4	36180	24	42.6	36181	24	42.6
36162	AVG	43.4	36180	AVG	43.3	36181	AVG	44.9

WT in			WT in			WT in		
36162			36180			36181		
Exp.	AVG	41.8	Exp.	AVG	41.9	Exp.	AVG	41.9

Transgenic T3 seed selections of events 36180 and 36162 that no longer segregated for the DsRed marker gene were identified by visual inspection using a suitable light source. These T3 selections that were homozygous for the pKR1223 transgene were subjected to germination assays on plant growth media containing sucrose or sorbitol as described above (Table 12).

TABLE 12**Germination Assays for T3 Seed of pKR1223 Transgenics**

Event	Media Type	Total Seed (#)	ASM * (#)	No Germination (#)	Healthy Seedlings (#)
36180	sucrose	83	0	0	83
36180	sucrose	111	0	0	111
36180	sucrose	110	0	0	110
36180	sorbitol	121	0	0	121
36180	sorbitol	128	0	0	128
36180	sorbitol	118	0	0	118
36162	sucrose	88	0	0	88
36162	sucrose	111	1	1	109
36162	sucrose	90	0	0	90
36162	sorbitol	97	0	0	97
36162	sorbitol	103	0	0	103
36162	sorbitol	107	2	0	105

Event	Media Type		ASM * (%)	No Germination (%)	Healthy Seedlings (%)
36180	sucrose		0.0	0.0	100.0
36180	sucrose		0.0	0.0	100.0
36180	sucrose		0.0	0.0	100.0
36180	sucrose	AVG	0.0	0.0	100.0
36180	sorbitol		0.0	0.0	100.0
36180	sorbitol		0.0	0.0	100.0
36180	sorbitol		0.0	0.0	100.0
36180	sorbitol	AVG	0.0	0.0	100.0
36162	sucrose		0.0	0.0	100.0
36162	sucrose		0.9	0.9	98.2
36162	sucrose		0.0	0.0	100.0
36162	sucrose	AVG	0.3	0.3	99.4
36162	sorbitol		0.0	0.0	100.0
36162	sorbitol		0.0	0.0	100.0
36162	sorbitol		1.9	0.0	98.1
36162	sorbitol	AVG	0.6	0.0	99.4

* "ASM" denotes Altered Seedling Morphology

Transgenic T3 seed selections of events 36180 and 36162 that no longer segregated for the DsRed marker gene were identified by visual inspection using a suitable light source. In case of event 36181 no T3 seed selections could be identified that did not segregate for the DS red marker in a total of 24 progeny seed samples derived from 24 kanamycin-resistant T2 plants. Moreover, when T3 seed were plated on selective agarose media, 25% of seed failed to germinate and 25% of the seedlings were sensitive to kanamycin. It is concluded that the transgene insertion in event 36181 can only be maintained in the heterozygous state. The homozygous nature of T3 seed selections of events 36180 and 36162 suggests that the seed phenotype of event 36181 is related to the transgene insertion site and not the transgene itself. It is believed that a gene that is important for development of viable seed was disrupted by the transgene insertion.

T3 seed selections of events 36180 and 36162 that were homozygous for the transgene insertion and T3 seed selections of event 36181 that were heterozygous for the transgene insertion were germinated on selective media containing kanamycin. Three flats were planted for every transgenic event as follows: 24 seedlings were planted in each flat next to 12 WT seedlings at identical developmental stage. Plants were grown to maturity for approximately eight weeks and seed were harvested in bulk from all transgenic and WT plants in a given flat. Oil content of seed was measured by NMR as described in Example 1. Results are summarized in Table 13. In all three events presence of the pKR1223-derived transgene leads to an increase in oil content that ranges between 0.7 and 2.2% points (1.6 – 5.4%).

TABLE 13

Oil Content of T4 Seed of pKR1223 Transgenics

Flat ID	Event ID	Oil (%)	Δ Oil (%) Points)	Δ Oil (%)
A	36181	42.8	2.2	5.4
	WT	40.6		
B	36181	43.5	2.1	5.2
	WT	41.4		
C	36181	40.8	1.5	4.0
	WT	39.2		
	AVG		2.0	4.9

A	36180	44.5	1.8	4.2
	WT	42.7		
B	36180	43.6	1.9	4.6
	WT	41.7		
C	36180	43.2	1.2	2.8
	WT	42.0		
		AVG	1.6	3.9
A	36162	43.3	1.4	3.4
	WT	41.9		
B	36162	43.6	0.7	1.6
	WT	42.9		
C	36162	43.8	1.0	2.4
	WT	42.7		
		AVG	1.0	2.5

T4 seed of events 36162 and 36180 were subjected to compositional analysis as described in Example 6.

TABLE 14

5

Composition of pKR1223 Transgenic T4 Seed and WT Control Seed

Event	Oil (% NMR)	Protein (%)	Seed Weight (μg)	Fructose (μg mg ⁻¹ seed)
36162	43.3	14.94	20.33	2.13
WT	41.9	15.05	19	2.39
Δ TG/WT %	3.3	-0.7	7.0	-10.9
Event	Glucose (μg mg ⁻¹ seed)	Sucrose (μg mg ⁻¹ seed)	Raffinose (μg mg ⁻¹ seed)	Stachyose (μg mg ⁻¹ seed)
36162	4.82	11.32	0.56	1.52
WT	5.17	14.28	0.64	1.58
Δ TG/WT %	-6.8	-20.7	-12.5	-3.8
Event	Oil (% NMR)	Protein (%)	Seed Weight (μg)	Fructose (μg mg ⁻¹ seed)
36180	43.6	15.17	21	2.07
WT	41.7	15.16	21	2.45
Δ TG/WT %	4.6	0.1	0.0	-15.5
Event	Glucose (μg mg ⁻¹ seed)	Sucrose (μg mg ⁻¹ seed)	Raffinose (μg mg ⁻¹ seed)	Stachyose (μg mg ⁻¹ seed)
36180	4.49	11.14	0.5	1.46
WT	4.97	14.08	0.57	1.45
Δ TG/WT %	-9.7	-20.9	-12.3	0.7

A reduction of soluble carbohydrates (mainly sucrose) was consistently associated with the presence of the pKR1223 transgene in events 36162 and 36180. There was no consistent change in protein content or seed weight that can be attributed to the presence of the transgene.

5 In summary, use of a promoter of the *Arabidopsis* sucrose synthase (SUS2) gene (At5g49190) for expression of maize ODP1 resulted in increased seed storage lipid accumulation at the expense of soluble carbohydrates. Seed germination and seedling establishment was not affected.

EXAMPLE 13

10 IDENTIFICATION OF SEED SPECIFIC PROMOTERS TO DRIVE ODP1 EXPRESSION IN CRUCIFEROUS OILSEED PLANTS

The 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, Girke T, Benning
15 C and Ohlrogge JB (2002) Contrapuntal networks of gene expression during *Arabidopsis* seed filling. Plant Cell 14: 1191-1206; Baud S, Vaultier M-N and Rochat C (2004) Structure and expression profile of the sucrose synthase multigene family in *Arabidopsis*. J Exp Bot 55: 397-409; and Baud S and Graham IA (2006) A
spatiotemporal analysis of enzymatic activities associated with carbon metabolism
20 in wild-type and mutant embryos of *Arabidopsis* using in situ histochemistry. Plant J 46: 155-169). The current invention describes the unexpected utility of a promoter sequence of a specific gene family member, At5g49190, to direct expression of heterologous ODP1 genes in a manner that allows for increased accumulation of oil during seed development of cruciferous oil seed without affecting germination and
25 seedling establishment of the resulting seed. At5g49190 is expressed during seedling development in synchrony with accumulation of oil and protein (*supra*). Genes homologous to At5g49190 can be identified in other plant species based on sequence similarity to the At5g49190 gene product and expression pattern of the homolog during seed development. One skilled in the art will recognize that
30 promoter sequences of these genes will have utility for expression of ODP1 genes for increased oil biosynthesis in cruciferous oil seed which is accompanied by unaltered seed germination and seedling establishment.

EXAMPLE 14

IDENTIFICATION OF CANOLA PROMOTERS TO DRIVE ODP1 EXPRESSION IN CRUCIFEROUS OILSEED PLANTS

Public EST and genomic sequence collections of Canola were searched with
5 the deduced amino acid sequence of At5g49190 (AtSUS2). Several ESTs and
genomic sequences were identified and assembled into a single contiguous
sequence that represents a transcript model of the canola homolog of At5g49190.
The nucleotide and deduced amino acid sequence of the canola SUS2 homolog
transcript model are set forth as SEQ ID NO:44 and SEQ ID NO:45, respectively.

10 Primers a (SEQ ID NO:46) b (SEQ ID NO:47) c (SEQ ID NO:48) and d (SEQ
ID NO:49) were used in genome walking experiments according to manufacturer
instructions (Clontech, CA, USA). Briefly genomic DNA of Pioneer Hi-Bred
International, Inc., spring canola variety NS1822BC was isolated using standard
protocols and digested with PvuII or DraI. After adaptor ligation PCR PvuII and DraI-
15 digested genomic DNA was used as template in PCR reactions with Primer a (SEQ
ID:46) and Primer c (SEQ ID NO:48), respectively. PCR products generated with
primers a (SEQ ID NO: 46) and c (SEQ ID NO:48) were amplified with primers b
(SEQ ID NO:47) and d (SEQ ID NO:49), respectively. In both rounds of PCR
experiments adaptor specific primers were used with primers a-d. Use of primers a
20 and b generated PCR products of 2.1 kb. Primers c and d generated PCR products
of 0.7 kb. These PCR products were cloned using the PCR blunt cloning system
(Invitrogen, CA, USA) and sequenced.

SEQ ID NO:50 (PvuII rapa cons) is genomic sequence of canola variety
NS1822BC that was generated with primers a and b. It is comprised of 312 bp of a
25 canola SUS2 homolog and 1924 bp of sequence upstream of the inferred start
codon of the SUS2 gene. This 1924 bp sequence (including the 5' untranslated
region) is designated the BnSUS2-2 promoter (SEQ ID NO:73).

SEQ ID NO:51 (1,6 DraI gene cons) is genomic sequence of canola variety
NS1822BC that was generated with primers c and d. It is comprised of 37 bp of a
30 canola SUS2 gene and 586 bp of sequence upstream of the inferred start codon of
the SUS2 gene. This 586 bp sequence (including the 5' untranslated region) is
designated the BnSUS2-1 promoter (SEQ ID NO:72).

Plasmid DNA of clone #6 containing 1,6 Dral gene cons (SEQ ID NO:51) was used in a PCR reaction with primers SA188 (SEQ ID NO:52) and SA189 (SEQ ID NO:53) using PHUSION™ DNA polymerase (New England Biolabs, Inc.). Plasmid DNA of clone #45 containing Pvull rapa cons (SEQ ID NO:50) was used in a PCR
 5 reaction with primers SA190 (SEQ ID NO:54) and SA191 (SEQ ID NO:55). PCR products from both reactions were cloned into PCR blunt (Invitrogen, CA, USA) according to manufacturer instructions and sequenced. BN SUS2 prom1/PCR blunt is derived from 1,6 Dral gene cons (SEQ ID NO:51). It's sequence is set forth as SEQ ID NO:56. BN SUS2 prom2/PCR blunt is derived from Pvull rapa cons (SEQ
 10 ID NO:50). It's sequence is set forth as SEQ ID NO:57.

BN SUS2 prom1/PCR blunt (SEQ ID NO:56) was linearized with XbaI and NotI and ligated with a NotI-XbaI fragment from KS332 (SEQ ID NO:3) containing Phas terminator and Kti promoter DS red gene and Kti terminator cassette to give KS427 (SEQ ID NO:58). KS427 (SEQ ID NO:58) was linearized with NotI. A delta-
 15 6 desaturase gene of *Mortierella alpina* was excised from KS130 (SEQ ID NO:59) using NotI and ligated to NotI linearized KS427 (SEQ ID NO:58) to give KS432 (SEQ ID NO:60). Expression cassettes for DSred and delta-6 desaturase genes were excised as a single DNA fragment by digestion with AscI and inserted into AscI linearized pKR92 (SEQ ID NO:27) to give ARALO80 (SEQ ID NO:61). The
 20 ARALO80 vector contains the following expression unit: BnSUS2-1 promoter::*M. alpina* delta-6 desaturase::phaseolin terminator.

Prior to this KS130 (SEQ ID NO:59) was constructed as follows: Plasmid DNA of CGR-5, which is described in U.S. Patent No. 5,968,809, was used in a PCR reaction with primers D6 fwd (SEQ ID NO:62) and D6 rev (SEQ ID NO:63).
 25 The PCR product was digested with NotI and ligated to NotI-linearized and de-phosphorylated KS119 vector (SEQ ID NO:64) to give KS130 (SEQ ID NO:59). Vector KS119 (SEQ ID NO:64) is described in International Publication No. WO2004071467.

The maize ODP1 gene was excised from KS336 (SEQ ID NO:6) using NotI
 30 and ligated to NotI linearized KS427 (SEQ ID NO:58) to give KS430 (SEQ ID NO:65). Expression cassettes for DSred and maize ODP1 genes were excised as a single fragment by digestion with AscI and inserted into AscI linearized pKR92 (SEQ

ID NO:27) to give ARALO78 (SEQ ID NO:66). The ARALO78 vector contains the following expression unit: BnSUS2-1 promoter::ZM-ODP1::phaseolin terminator.

BN SUS2 pro2/PCR blunt (SEQ ID NO:57) was linearized with XbaI and NotI and ligated with a NotI-XbaI fragment from KS332 (SEQ ID NO:3) containing Phas terminator and Kti promoter DS red gene and Kti terminator cassette to give KS428 (SEQ ID NO:67). KS428 (SEQ ID NO:67) was linearized with NotI. The delta-6 desaturase gene was excised from KS130 (SEQ ID NO:59) using NotI and ligated to NotI-linearized KS428 (SEQ ID NO:67) to give KS429 (SEQ ID NO:68). Expression cassettes for DSred and delta-6 desaturase genes were excised as a single DNA fragment by digestion with AscI and inserted into AscI linearized pKR92 (SEQ ID NO:27) to give ARALO77 (SEQ ID NO:69). The ARALO77 vector contains the following expression unit: BnSUS2-2 promoter::*M. alpina* delta-6 desaturase::phaseolin terminator.

The maize ODP1 gene was excised from KS336 (SEQ ID NO:6) using NotI and ligated to NotI-linearized KS428 (SEQ ID NO:67) to give KS431 (SEQ ID NO:70). Expression cassettes for DSred and maize ODP1 genes were excised by digestion with AscI and inserted into AscI linearized pKR92 (SEQ ID NO:27) to give ARALO79 (SEQ ID NO:71). The ARALO79 vector contains the following expression unit: BnSUS2-2 promoter::ZM-ODP1::phaseolin terminator.

Plasmid DNA of ARALO77, ARALO78, ARALO79 and ARALO80 were used for Agrobacterium-mediated transformation of Arabidopsis plants as described in Example 2.

EXAMPLE 15

Analysis of Progeny Seed of Arabidopsis Plants Transformed with

Zea mays ODP Under Control of Canola Sucrose Synthase Promoters

Oil content of progeny seed (e.g., T2 seed) of transgenic lines generated with ARALO78 and ARALO79 can be measured by NMR as described in Example 2. Progeny seed (e.g., T2 seed) of transgenic events generated with ARALO78 and ARALO79 are expected to show increased oil content when compared to seed of untransformed control plants grown alongside the transgenic events.

CLAIMS

What is claimed is:

1. A recombinant DNA construct comprising a polynucleotide encoding an ovule development protein 1 (ODP1) polypeptide operably linked to a sucrose synthase 2 promoter wherein said construct increases oil content in the seeds of a cruciferous oilseed plant while maintaining normal germination and further wherein the amino acid sequence of said ODP1 polypeptide has at least 80% sequence identity to a sequence selected from the group consisting of SEQ ID NO:37, SEQ ID NO:39, and SEQ ID NO:41.
2. The recombinant DNA construct of claim 1 wherein the amino acid sequence of said ODP1 polypeptide has at least 90% sequence identity to a sequence selected from the group consisting of SEQ ID NO:37, SEQ ID NO:39, and SEQ ID NO:41.
3. The recombinant DNA construct of claim 1 wherein the amino acid sequence of said ODP1 polypeptide has at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NO:37, SEQ ID NO:39, and SEQ ID NO:41.
4. The recombinant DNA construct of claim 1 wherein the amino acid sequence of said ODP1 polypeptide comprises a sequence selected from the group consisting of SEQ ID NO:37, SEQ ID NO:39 and SEQ ID NO:41.
5. The recombinant DNA construct of any one of claims 1-4 wherein the sucrose synthase 2 promoter comprises:
 - (a) the nucleotide sequence of SEQ ID NO:43, SEQ ID NO:72 or SEQ ID NO:73; or
 - (b) a nucleotide sequence comprising a functional fragment of the nucleotide sequence of SEQ ID NO:43, SEQ ID NO:72 or SEQ ID NO:73, which retains promoter activity.
6. The recombinant DNA construct of any one of claims 1-5 wherein the oilseed plant is canola or Arabidopsis.
7. A transgenic cruciferous oilseed plant cell comprising in its genome the recombinant DNA construct of any one of claims 1-6.

8. The transgenic cruciferous oilseed plant cell of claim 7 wherein the cruciferous oilseed plant cell is a canola cell or an Arabidopsis cell.
9. The plant cell of any one of claims 7 or 8, wherein the plant cell is a seed cell and wherein said seed cell comprises in its genome the recombinant DNA construct of any one of claims 1-6.
10. A method for producing a transgenic cruciferous oilseed plant comprising transforming a cruciferous oilseed plant cell with the recombinant DNA construct of any one of claims 1-6 and regenerating a transgenic cruciferous oilseed plant from the transformed cruciferous oilseed plant cell, wherein the transgenic cruciferous oilseed plant comprises in its genome the recombinant DNA construct of any one of claims 1-6.
11. A method for increasing oil content in seeds of a transgenic cruciferous oilseed plant while maintaining normal germination, said method comprising:
 - (a) transforming a cruciferous oilseed plant cell with a recombinant DNA construct comprising a polynucleotide encoding an ovule development protein 1 (ODP1) polypeptide, wherein the amino acid sequence of said ODP1 polypeptide has at least 80% sequence identity with a sequence selected from the group consisting of SEQ ID NO:37, SEQ ID NO:39, and SEQ ID NO:41, said sequence being operably linked to a sucrose synthase 2 promoter;
 - (b) regenerating a transgenic cruciferous oilseed plant from the transformed cell of step (a), wherein said plant comprises the recombinant DNA construct;
 - (c) obtaining a transgenic progeny plant derived from the transgenic cruciferous oilseed plant of step (b), wherein the transgenic progeny plant comprises in its genome the recombinant DNA construct;
 - (d) assaying the transgenic progeny plant obtained from step (c) for oil level and germination; and
 - (e) selecting those transgenic progeny plants having seeds with an increased level of oil and normal germination when compared to seeds obtained from a control cruciferous oilseed plant, wherein said control plant does not comprise the recombinant DNA construct.

12. The method of any one of claims 10-11 wherein the cruciferous oilseed plant is canola or Arabidopsis.
13. The method of any one of claims 11-12 wherein the ODP1 polypeptide is a maize ODP1 polypeptide.
14. The method of claim 13 wherein the amino acid sequence of the ODP1 polypeptide comprises the sequence of SEQ ID NO:37.
15. The method of any one of claims 11-14 wherein the nucleotide sequence of the sucrose synthase 2 promoter comprises:
 - (a) the nucleotide sequence of SEQ ID NO:43, SEQ ID NO:72 or SEQ ID NO:73; or
 - (b) a nucleotide sequence comprising a functional fragment of the nucleotide sequence of SEQ ID NO:43, SEQ ID NO:72 or SEQ ID NO:73, which retains promoter activity.
16. Use of the seed cell of claim 9 to prepare oil or by-products from said seed cell.
17. A method of preparing oil from the seed cell of claim 9 comprising crushing said seed cell and extracting said oil.
18. Use of the recombinant DNA construct as defined in any one of claims 1-6 for increasing the oil content in seeds of a cruciferous oilseed plant while maintaining normal germination.
19. A by-product obtained from the seed cell of claim 9, wherein the by-product comprises the seed cell of claim 9, and wherein the by-product is meal.

$\frac{1}{4}$

	M.....S.....	R.....R.....	R.....R.....	Consensus #1			
	10	20	30	40	50	60	
1	MERSQRQSPPPSPSS	-SSSVSADTVLVP	GKRRRAATAKA	GAEPNKRIR	-----		SEQ ID NO-37.pro
1	MKRSPASSCSSTSV	-----GFEAP	IEKRRP	-----KHPRRN	NLKSKC	---	SEQ ID NO-39.pro
1	MRRSPSVSTSSSS	CVGGGFDNNIN	LAAAPRRPQSEKT	GAKRRKRNQ	-DDAKCEIE		SEQ ID NO-41.pro
1	MKKRLTTSCTCSS	PSSSVSSSTTT	SSPIQSEAP--RP	-----KRAKRAK	-KSS----		SEQ ID NO-42.pro
	RSS.YRGVTRHRWTGR	FEAHLWDK.....	KK.G.QVYLG				Consensus #1
	70	80	90	100	110	120	
51	-----KDPA	AAAAGKRSSV	YRGVTRHRWTGR	FEAHLWDKHCL	AALHNKKKG	RQVYLG	SEQ ID NO-37.pro
41	-----KQNQT	TGGRRSSI	YRGVTRHRWTGR	FEAHLWDKSSWNNI	QSKE-GRQVYLG		SEQ ID NO-39.pro
60	NRNGNNSNNNA	SSGRSSI	YRGVTRHRWTGR	FEAHLWDKSSWNSIQNK	K-GRQVYLG		SEQ ID NO-41.pro
46	-PSGDKSHNPTS-P	ASTRSSI	YRGVTRHRWTGR	FEAHLWDKSSWNSIQNK	K-GKQVYLG		SEQ ID NO-42.pro
	AYD.EE.AA..YDLAALKYW	G...LNFP.E.Y..E..EM..V..EEYLASLR	.SSGFS				Consensus #1
	130	140	150	160	170	180	
103	AYDSEEAAARAYDLAALKYWG	PETLLNFPVEDYSSEMPEMEAVSRE	EYLASLRSSGFS				SEQ ID NO-37.pro
92	AYDT[EES]AARTYDLAALKYWG	KDATLNFP[ET]YTKEL[E]EMDKVSRE	EYLASLRQSSGFS				SEQ ID NO-39.pro
119	AYDN[E]EAAARTYDLAALKYWG	PGTTLNFPVESYRNEIEEMRKVTKEEY	LASLRSSGFS				SEQ ID NO-41.pro
103	AYDSEEAAAHITYDLAALKYWG	PDILNFPA[ET]YTKELEEMQORVTKEEY	LASLRQSSGFS				SEQ ID NO-42.pro

FIG. 1B

	RG.SKYRGVARHHHNGRWEARIGRV.G.KYLYLGT..TQEEAA.AYD.AAIEYRG.NAVT	Consensus #1
	190200210220230240	
163	RGVSKYRGVARHHHNGRWEARIGRVFGNKYLYLGTFTQEEAAKAYDLAAIEYRGVNAV	SEQ ID NO-37.pro
152	RGLSKYRGVARHHHNGRWEARIGRVGNKYLYLGTYKLTQEEAAVAYDMAAIEYRGVNAV	SEQ ID NO-39.pro
179	RGVSKYRGVARHHHNGRWEARIGRVFGSKYLYLGTYNLTQEEAAAYDMAAIEYRGVNAV	SEQ ID NO-41.pro
163	RGVSKYRGVARHHHNGRWEARIGRVFGNKYLYLGTYNLTQEEAAAYDMAAIEYRGANAV	SEQ ID NO-42.pro
	NFDIS.Y.....E.....	Consensus #1
	250260270280290300	
223	NFDISCYLDH-----PLFLAQLQQEPQVVPALNQ-----EPQPDQSETGTTEQE	SEQ ID NO-37.pro
212	NFDISNYMDKIKKKN-----DQTQQQTE--AQETVPNSSDSFEVEVEQQTTITTP	SEQ ID NO-39.pro
239	NFDISNYIGRLENKSSVFP-----AAEQP---LQPNC---S	SEQ ID NO-41.pro
223	NFDISNYIDRL-KKKGVFPFPVNVQANHQEGILVEAKQEVETREAKFEPRFEVKQQYVFE	SEQ ID NO-42.pro
	P.....L.W.....	Consensus #1
	310320330340350360	
267	PESSEAKTPDGS AEPDENAVP-----DDTAEP LTTVDDSI EEGL-WSPCMDY--	SEQ ID NO-37.pro
263	PPS-----ENLHMP PQQHQQYT--PHVSPREEESSLIITIMDHVLEQDL PWSF--MYTG	SEQ ID NO-39.pro
269	PASSSEEGEVVQQQQQTTFMAFSGPLQFPSMENSPTME-----EDHDLHWSF--LDTG	SEQ ID NO-41.pro
282	PQEEEEKKEEKAEQQQEAETVGYSEEA AVNCCIDSSTIMEMDRCGDNNELAWNFCMMDTG	SEQ ID NO-42.pro

FIG. 1C

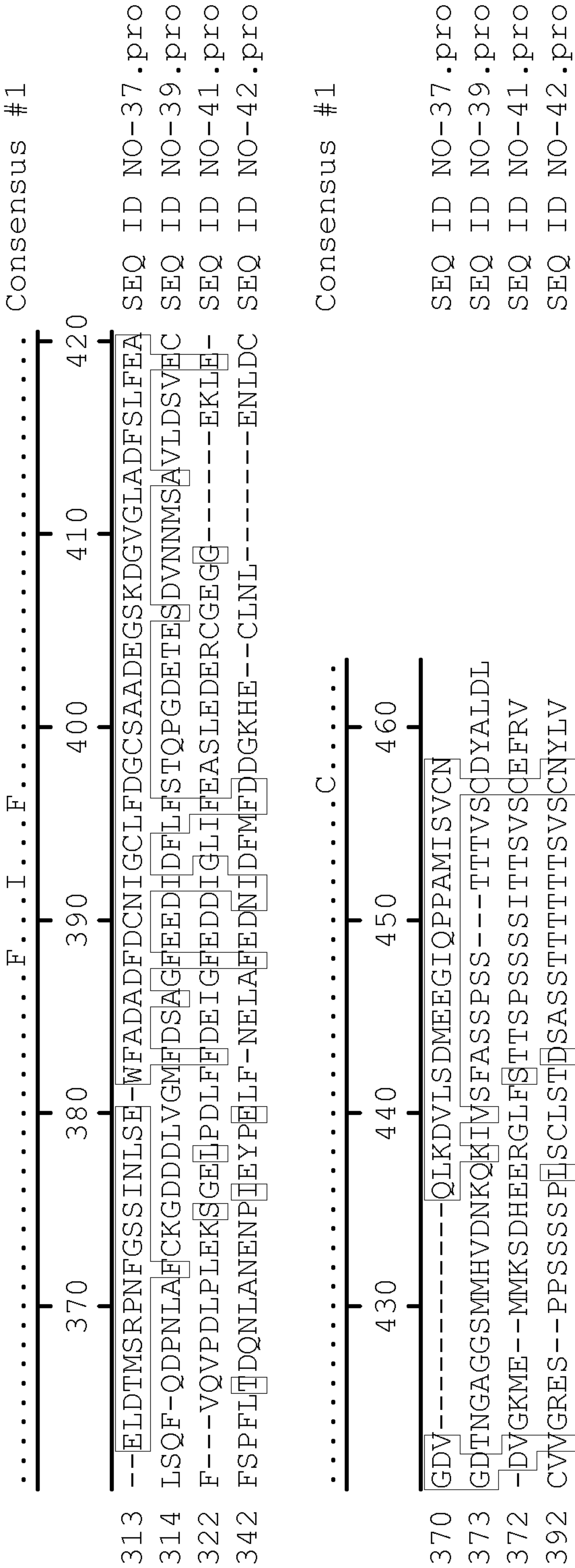


FIG. 2

		Percent Identity				
Divergence		1	2	3	4	
	1					SEQ ID NO-37.pro
	2		41.8	43.5	41.8	1
	3	79.5		46.5	45.4	2
	4	78.0	59.2		48.4	3
		85.0	72.3	59.1		4
		1	2	3	4	
						SEQ ID NO-42.pro

M.....S.....S.....R.....R..... Consensus #1

10 20 30 40 50 60

1	MERSQRQSPPPSPSSS	-SSSVSADTVLVPPGKRRRAATAKAGAEPNKRIR	-----	SEQ ID NO-37.pro
1	MKRS PASSCSSSTSSV	-----GFEAPIEKRRP	-----KHPRRNNLKSQKC---	SEQ ID NO-39.pro
1	MRRSPSVSTSSSSSSSS	CVGGGGFDSNNLNLAAPPRRPQSEKTGAKRRKRNQ	-DDAKCEIE	SEQ ID NO-41.pro
1	MKKRLTTSTCSSSPSSSV	SSSTTTSSPIQSEAP--RP	-----KRAKRAK-KSS-----	SEQ ID NO-42.pro

.....RSS.YRGVTRHRWTGRFEAHLWDK.....KK.G.QVYLG Consensus #1

70 80 90 100 110 120

51	-----KDPAAAAAGKRSSV	YRGVTRHRWTGRFEAHLWDKHCLAALHNKKKGRQVYLG	SEQ ID NO-37.pro
41	-----KQNQTTTGGRSS	IYRGVTRHRWTGRFEAHLWDKSSWNNIQSKK-GRQVYLG	SEQ ID NO-39.pro
60	NRNGNNNNSSNNNASS	GRSSIYRGVTRHRWTGRFEAHLWDKSSWNSIQNKK-GRQVYLG	SEQ ID NO-41.pro
46	-PSGDKSHNPTS-PAST	RSSIYRGVTRHRWTGRFEAHLWDKSSWNSIQNKK-GKQVYLG	SEQ ID NO-42.pro

AYD.EE.AA..YDLAALKYWG....LNFP.E.Y..E..EM..V..EEYLASLRR.SSGFS Consensus #1

130 140 150 160 170 180

103	AYDSEEAAARAYDLAALKYWG	PETLLNFPVEDYSSEMPEMEAVSREEYLASLRRRSSGFS	SEQ ID NO-37.pro
92	AYDTEESAARTYDLAALKYWG	KDATLNFPDET YTKELEEMDKVSREEYLASLRROSSGFS	SEQ ID NO-39.pro
119	AYDNEEAAARTYDLAALKYWG	PGTTLNFPVESYRNEIEEMRKVTKEEYLASLRRRSSGFS	SEQ ID NO-41.pro
103	AYDSEEAAAH TYDLAALKYWG	PDTILNFP AET YTKELEEMQRVTKEEYLASLRROSSGFS	SEQ ID NO-42.pro