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DESCRIPTION

Field of the invention

[0001] The present disclosure relates to plants, seeds and products derived thereof, in particular to Brassica plants, seeds products derived thereof, that have mutant sequences conferring high oleic acid profile to the seed oil.

[0002] More particularly, the present disclosure relates to mutant delta-12 fatty acid desaturase sequences, also referred to herein as mutant FAD2 sequences, in such plants which confer high oleic acid profile on the seed oil.

Background

[0003] Delta-12 fatty acid desaturase (also known as oleic desaturase or oleate desaturase) is involved in the enzymatic conversion of oleic acid to linoleic acid.

[0004] Varieties with high level of oleic acid (possibly combined with low level of linolenic acid) are sought for many different applications (food applications, health applications, biodiesel applications and many others).

[0005] Mutant seeds providing an oil exhibiting a high oleic acid content (oleic acid content higher that 70 wt.% based upon the total weight of fatty acids present in the oil) previously reported in the literature had very poor agronomic value and/or bad root characteristics, and/or very low yield capacity.

[0006] There is still a need for material having stable, high oleic acid content (possibly combined with stable low linolenic acid content) across locations and across years, with also good agronomic performances and with normal oilseed rape morphology. In particular, the plants should have no fasciation and should have normal root development.

Summary of the invention

[0007] The present invention is defined in the claims.

[0008] A plant or a plant part or a seed according to the invention can be obtained by a mutagenesis treatment, more particularly by an Ethyl Methane Sulfonate (EMS) treatment.

[0009] regenerated plants comprise a nucleic acid sequence of at least 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO 9, wherein the nucleotide corresponding to position 1421 is deleted (i.e, further deleted of the nucleotide corresponding to position 1421), or a nucleic acid sequence of at least 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO 10, wherein the nucleotide corresponding to position 1453 is deleted (i.e. further deleted of the nucleotide corresponding to position 1453).

[0010] Preferably, said regenerated plants comprise a nucleic acid sequence of SEQ ID NO 9 wherein the nucleotide 1421 is deleted (i.e. of SEQ ID NO 9 further deleted of the nucleotide corresponding to position 1421), or a nucleic acid sequence of SEQ ID NO 10 wherein the nucleotide 1453 is deleted (i.e. of SEQ ID NO 10 further deleted of the nucleotide corresponding to position 1453), or a nucleic acid sequence of SEQ ID NO 3.

[0011] Step c) can comprise any method known in the art for identifying said mutation(s) (DEL.1421, DEL.1453, DEL.215 and possibly SNP1590), in particular step c) can comprise the use of restriction fragment length polymorphism (RFLP), random amplification polymorphism detection (RAPD), or polymerase chain reaction (PCR) method.

[0012] vegetable oil obtained from the seeds of the invention, comprises more than (about) 72%, 75%, 80%, or 85% of oleic acid based upon the total weight of the fatty acids present in said oil.

[0013] Preferably, the vegetable oil further comprises less than (about) 4%, 3,5%, 3%, 2%, 1% or 0,5% of linolenic acid.

Brief description of the figure

[0014]

Figure 1 corresponds to the list of sequences of the present disclosure.

Figure 2 corresponds to the notifications of acceptance of deposits of MSP05, 28DHS.086 and MSP12 varieties.

Detailed description of the invention

[0015] The present disclosure relates to plants, more particularly to Brassica plants, preferably to *Brassica napus* varieties, which provide an oil having a oleic acid content higher than 70 wt.%, based upon the total weight of fatty acids present in the oil.

[0016] More particularly, a plant of the disclosure has at least one mutated FAD2 gene according to the invention.

[0017] Preferably, said mutated FAD2 gene confers high oleic acid content (i.e. a oleic acid content higher than 70 wt.%, based upon the total weight of fatty acids present in the oil) to seeds of said plants and to oil extracted from said seeds.

[0018] The present disclosure relates also to any part
or any product of said plant bearing said at least one mutated FAD2 gene.

[0019] In the context of the present disclosure, a part
or product of a plant is meant to encompass a leaf, cotyledon, stem, petiole, stalk, seed or any other tissue or fragment of tissue of said plant.

[0020] The present disclosure relates also to any
progeny of said plant bearing said at least one mutated FAD2 gene of the invention.

[0021] In the context of the present disclosure, the
term "progeny" refers to direct and indirect descendants, offspring and derivatives of a plant or plants of the invention and includes the first, second, third and/or subsequent generations, which may be produced by self crossing, crossing with plants with the same or different genotypes, and may be modified by range of suitable genetic engineering techniques.

[0022] The present invention also relates to said mutated FAD2 genes that confer high oleic acid content in seeds when present in a plant.

[0023] In particular, the invention relates to novel isolated nucleic acid molecules corresponding to novel variant forms of FAD2 genes having a deleted nucleotide at or corresponding to position 1421 relative to a wild type FAD2 gene, such as the wild type FAD2 gene represented by SEQ ID NO 9.

[0024] Said deletion alters the functionality of the resulting FAD2 gene product, whereby the level of oleic acid is increased, in plant expressing the mutant sequence(s), compared to the corresponding level in plant expressing the wild-type sequence(s).

[0025] In particular, a nucleic acid molecule of the invention can comprise (or consist of) a nucleotide sequence having at least 95%, 96%, 97%, 98% or 99% identity with any of SEQ ID NO 9, or with the complementary form or RNA form thereof, wherein the nucleotide at or corresponding to position 1421 is deleted (i.e. further deleted of the nucleotide corresponding to position 1421).

[0026] A nucleic acid molecule of the invention containing said deletion at or corresponding to position 1421 (also referred to as DEL.1421) can be derived from *Brassica napus* varieties, such as CONTACT, CABRIOLET, 28DHS.086 and/or MSP12 varieties.

[0027] The term "at position 1421" is to be understood as designating the nucleotide position 1421 in a wild-type FAD2 gene represented by SEQ ID NO 9, but also as referring to the nucleotide corresponding to said position in a wild-type or variant FAD2 gene that would have a different nucleic acid sequence due to deletions or additional nucleotides in the sequence.

[0028] The term "corresponding to position" as used herein means that a position is not only determined by the number of the

preceding nucleotides. The position of a given nucleotide in accordance with the present invention may vary due to deletions or additional nucleotides in the nucleic acid sequence. Thus, under a "corresponding position" in accordance with the present invention it is to be understood that the nucleotide referred to may differ in the indicated number but still has similar neighbouring nucleotides in the linear sequence. For example, position 1453 in SEQ ID NO 10 or position 215 in SEQ ID NO 1 or 5 is such a position.

[0029] Similarly, the term "at position 118" is to be understood as designating the amino acid position 118 in a wild-type FAD2 protein represented by SEQ ID NO 2 or 6, but also as referring to the amino acid corresponding to said position in a wild-type or variant FAD2 protein that would have a different amino acid sequence due to deletions or additional amino acids in the polypeptide.

[0030] The term "corresponding to position" as used herein means that a position is not only determined by the number of the preceding amino acids. The position of a given amino acid in accordance with the present invention may vary due to deletions or additional amino acids in the polypeptide. Thus, under a "corresponding position" in accordance with the present invention it is to be understood that the amino acid(s) referred to may differ in the indicated number but still has (have) similar neighbouring amino acids in the linear sequence.

[0031] A nucleic acid molecule having mutation(s) resulting in said amino acid substitution at position 118 can be derived from *Brassica napus* varieties, such as 28DHS.086 variety and/or MSP12 variety.

[0032] More particularly, a nucleic acid molecule may have a mutation at position 1590 (also referred to as SNP1590) of the acid nucleic sequence of SEQ ID NO 10, which causes a change in genetic codon from CTT to TTT, resulting in a substitution of an amino acid at position 118 relative to the wild-type amino acid sequence, such as the wild-type FAD2 protein represented by SEQ ID NO 6.

[0033] An isolated nucleic acid molecule containing said SNP1590 mutation, resulting in a substitution of phenylalanine for leucine at position 118, alters the functionality of the resulting FAD2 gene product, whereby the level of oleic acid is increased in plant expressing the mutant sequence, compared to the corresponding level in plant expressing the wild-type sequence.

[0034] In the framework of the invention, the term "SNP1590" refers to the single nucleotide polymorphism corresponding to said mutation at position 1590 of the nucleic acid of SEQ ID NO 10, and can refer also to the corresponding mutation in any nucleic acid molecule encoding a FAD2 protein of the invention having a substituted amino acid at position 118 (or corresponding to position 118) relative to the wild-type FAD2 protein, such as the wild-type FAD2 protein represented by SEQ ID NO 2 or 6.

[0035] Any fragment of a nucleic acid molecule of the invention of at least 20, 25, 50, 100 or more nucleotides and comprising at least one mutation resulting in a FAD2 protein according to the invention is contemplated.

[0036] In particular, a fragment of a nucleic acid molecule of the invention of at least 20, 25, 50, 100 or more nucleotides comprising said deletion DEL1421, DEL1453 or DEL215 or said SNP1590 is contemplated.

[0037] Also contemplated is a fragment of a nucleic acid molecule of the invention of at least 20, 25, 50, 100 or more nucleotides comprising said DEL1421 and said SNP1590.

[0038] Such fragments can be used as primers, as probes and/or as markers.

[0039] The nucleic acid fragments of the invention can be used as markers in plant genetic mapping.

[0040] In particular, the nucleic acid fragments of the invention can be used as markers in plant breeding programs.

[0041] Such markers may include restriction fragment length polymorphism (RFLP), random amplification polymorphism detection (RAPD), polymerase chain reaction (PCR) or self-sustained sequence replication (3SR) markers, for example.

[0042] Marker-assisted breeding techniques may be used to identify and follow a plant according to the invention or its progeny, also object of the invention, during the breeding process.

[0043] Marker-assisted breeding techniques may be used in addition to, or as an alternative to, other sorts of identification techniques.

[0044] An example of marker-assisted breeding is the use of PCR primers that specifically amplify a nucleic acid molecule of the invention.

[0045] The invention may be used in methods for segregation and selection analysis of genetic crosses involving plants having nucleic acid sequences of the invention.

[0046] Also object of the present invention is a nucleic acid molecule of at least 20, 25, 50, 100 or more nucleotides, that hybridizes under stringent conditions to any nucleic acid sequence of SEQ ID NO 1, 3, 5, 7, and 9 to 12, which contains (or further contains) a mutation according to the invention.

[0047] An example of stringent hybridization conditions is hybridization at about 50°C, or at about 60°C or higher, and 0.1xSSC (buffer of 0.15M NaCl, 0.015M trisodium citrate).

[0048] A method may for example involve determining the presence in a genome of particular FAD2 alleles containing at least said deletion at (or corresponding to) position 1421 relative to a wild type FAD2 gene, such as the wild type FAD2 gene represented by SEQ ID 9 and/or said substitution, SNP1590, (resulting in a substitution of phenylalanine for leucine) at (or corresponding to) position 118 relative to a wild type FAD2 protein, such as the wild type FAD2 protein represented by SEQ ID NO 2 or 6.

[0049] Such a determination may for example be achieved with a range of techniques, such as PCR amplification, DNA fingerprinting, RNA fingerprinting, gel blotting and RFLP analysis, nuclease protection assays, sequencing of the relevant nucleic acid fragment, the generation of antibodies (monoclonal or polyclonal), or alternative methods adapted to distinguish the protein produced by the relevant alleles from other variant/forms of that protein or from the wild-type.

[0050] More particularly, such fragments can be used in method of marker assisted selection for high oleic traits in plants, preferably in Brassica species, more particularly in *Brassica napus* varieties.

[0051] Another aspect of the invention is related to a vector comprising a nucleic acid molecule of the invention, possibly operably linked to one or more adjacent regulatory sequence(s) originating from homologous or from heterologous organisms.

[0052] In the present context "vector" is defined as any biochemical construct which may be used for the introduction of a nucleotide sequence (by transduction, transfection, transformation, infection, conjugation, etc.) into a cell.

[0053] Advantageously, a vector may be selected from the group consisting of plasmids (including replicative and integrative plasmids), viruses, phagemids, chromosomes, transposons, liposomes, cationic vesicles, or a mixture thereof. Said vector may already comprise one or more adjacent regulatory sequence(s), allowing the expression of said nucleic acid molecule and its transcription into a polypeptide of the invention.

[0054] The present invention also encompasses any peptide, which may still have a delta-12 oleate desaturase activity, resulting from the expression of a nucleic acid of the invention containing said deletion (DEL1421, DEL1453 or DEL215), such as the peptide of SEQ ID NO 4.

[0055] Nucleic acid molecules, recombinant nucleic acid molecules, and/or vectors disclosed herein are useful to transform target plants, and thereby confer altered FAD2 gene product, whereby the level of oleic acid is modified, preferably increased, in plant expressing a mutant FAD2 of the invention, compared to the corresponding level in plant expressing the wild-type sequence.

[0056] The present invention is also related to a transformed host cell, or recombinant host cell, containing (or having incorporated) one or more of the nucleotide sequences and/or vectors according to the invention having the deletion 1421.

[0057] In the present context, a "transformed host cell" or "recombinant cell", also referred to as "transformant", is a cell having incorporated one or more of the nucleotide sequences and/or vectors according to the invention. The transformed host cell may be a cell in which said vector(s) and/or said nucleotide sequence(s) is/are introduced by means of genetic transformation, preferably by means of homologous recombination, or by any other well known methods used for obtaining a recombinant organism.

[0058] Any method by which the novel sequence can be incorporated into the host genome is contemplated.

[0059] More particularly, any method by which the novel sequence can be incorporated into the host genome, and stably inherited by its progeny, is contemplated.

[0060] A broad range of known techniques currently exist for achieving direct or indirect transformation of higher plants with exogenous DNA.

[0061] Transformation of plant cells can be mediated by the use of vectors. A common method of achieving transformation is the use of *Agrobacterium tumefaciens* to introduce a foreign gene into the target plant cell.

[0062] Plant viruses also provide a possible means for transfer of exogenous DNA.

[0063] Direct uptake of plant cells can also be employed. Typically, protoplasts of the target plant are placed in culture in the presence of the nucleic acid molecules to be transferred, and an agent which promotes the uptake of said nucleic acid molecules by protoplast. Useful agents in this regard are polyethylene glycol or calcium phosphate.

[0064] Alternatively, nucleic acid molecules uptake can be stimulated by electroporation. In this method, an electrical pulse is used to open temporary pores in a protoplast cell membrane, and said nucleic acid molecules in the surrounding solution are then drawn into the cell through the pores. Similarly, microinjection can be employed to deliver said nucleic acid molecules directly into a cell, and preferably directly into the nucleus of the cell.

[0065] In these techniques, transformation occurs in a plant cell in culture. Subsequent to the transformation event, plant cells can be regenerated to whole plants.

[0066] Techniques for the regeneration of mature plants from callus or protoplast culture are well known.

[0067] Alternate methods are also available which do not necessarily require the use of isolated cells, and therefore, plant regeneration techniques, to achieve transformation. These are generally referred to as "ballistic" or "particle acceleration" methods, in which nucleic acid molecules coated metal particles are propelled into plant cells by either a gunpowder charge or electrical discharge. In this manner, plant cells in culture or plant reproductive organs or cells, e.g. pollen, can be stably transformed with the nucleic acid molecules of interest.

[0068] The present invention can be applied to transformation of virtually any type of plant, monocotyledons or dicotyledons.

[0069] Suitable plants to be transformed are preferably oil producing crops, such as sunflower, soybean, cotton, corn, etc., preferably Brassica species, more preferably *Brassica napus* varieties.

[0070] a plant or a plant part or a seed of the invention contains a first FAD2 gene having a deletion at or corresponding to position 1421 relative to a wild-type FAD2 gene, such as wild-type FAD2 gene of SEQ ID NO 9, and a second FAD2 gene encoding a FAD-2 protein having an amino acid substitution at or corresponding to position 118 relative to a wild-type FAD-2 protein, such as the wild-type FAD-2 protein of SEQ ID NO 2 or 6.

[0071] In particular, a plant or a plant part or a seed of the invention preferably contains a nucleic acid sequence corresponding to SEQ ID NO 1 or 5, wherein the nucleotide (C) at position 215 is deleted (i.e. corresponding to SEQ ID NO 1 or 5 further deleted of the nucleotide at position 215), or contains a (variant) nucleic acid sequence, of at least 80%, preferably at least 85%, more preferably at least 90% and even more preferably at least 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO 1 or 5, wherein the nucleotide corresponding to position 215 is deleted (i.e. with SEQ ID NO 1 or 5 further deleted of the nucleotide corresponding to position 215).

[0072] Examples of such plants are CONTACT and CABRIOLET varieties, which are registered varieties.

[0073] A preferred plant or a plant part or a seed according to the invention, further contains a nucleic acid sequence encoding a FAD-2 protein having an amino acid substitution at or corresponding to position 118 relative to a wild-type FAD-2 protein, such as the wild-type FAD-2 protein of SEQ ID NO 2 or 6. Preferably, said substituted amino acid at or corresponding to position 118 is phenylalanine.

[0074] Examples of such plants are 28DHS.086 and MSP12 varieties.

[0075] MSP05 variety is maintained as a Budapest Treaty patent deposit with NCIMB under accession number NCIMB 41233 made July 9, 2004.

[0076] 28DHS.086 variety is maintained as a Budapest Treaty patent deposit with NCIMB under accession number NCIMB 41365 made December 22, 2005.

[0077] MSP12 variety is maintained as a Budapest Treaty patent deposit with NCIMB under accession number NCIMB 41374 made February 10, 2006.

[0078] A mutated plant or a plant part or a seed according to the invention exhibits the surprising advantage of developing a good rooting system. More particularly, having regard to a mutated Brassica napus variety according to the invention, the principal root and the secondary rooting system have a length comparable (similar) to respectively the principal root and the secondary rooting system of wild-type varieties. In comparison, mutated Brassica napus obtained by carrying out the method described in WO98/56239 shows a principal root much smaller than the wild-types and a secondary rooting system severely impaired.

[0079] A plant or a plant part or a seed according to the invention can be obtained by a mutagenesis treatment, more particularly by an Ethyl Methane Sulfonate (EMS) treatment.

[0080] The seeds obtained from plants of the invention provide an oil having an oleic acid content of more than 70wt.%, more preferably of more than 75wt.%, based upon the total weight of fatty acid present the oil.

[0081] a vegetable oil obtained from at least one plant according to the invention, comprises more than (about) 70%, 72%, 75%, 80%, or 85% of oleic acid.

[0082] More particularly, a vegetable oil obtained from at least one Brassica species of the invention, more preferably from at least one *Brassica napus* variety according to the invention, comprises more than (about) 70%, 72%, 75%, 80%, or 85% of oleic acid. Said oil can further comprise less than (about) 4%, 3,5%, 3%, 2%, 1% or 0,5% of linolenic acid, based upon the total weight of the fatty acids present in the oil.

[0083] Preferably, said oil comprises more than (about) 70%, 72%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90%, preferably between (about) 70% and (about) 90%, more preferably between (about) 72% and (about) 89% of oleic acid. Said oil can further comprise less than (about) 4%, 3,5%, 3%, 2%, 1%, or 0,5%, preferably between (about) 4% and (about) 0,4% of linolenic acid, based upon the total weight of the fatty acids present in the oil.

[0084] According to a preferred embodiment, two double low winter rapeseed varieties (ENVOL and LIBERATOR) were submitted to an Ethyl Methane Sulfonate (EMS) treatment in 1992. The EMS treatment was performed at 2,5% and 5% for 4h or 8h.

[0085] The M1 generation was grown in a greenhouse after 8 weeks of vernalization in a growth chamber and then harvested in July 93.

[0086] M1 seeds were planted in the field in September 93, bagged at the beginning of flowering and M2 seeds harvested in July 94.

[0087] M2 seeds were planted in September 94, bagged at the beginning of flowering and M3 seeds harvested in July 95.

[0088] The progenies were then analysed for fatty acid composition using gas chromatography based analytical method, as commonly known in this area of technology.

[0089] All progenies showing an oleic content higher than 68% were kept.

[0090] Selected progeny was replanted in the field in September 1995, bagged in April then harvested in July 1996.

[0091] At this stage progenies were screened for good agronomic and morphological characteristics, such as good germination capacity, good autumn vigor, good winter hardiness, good rooting system, good blackleg and light leaf spot resistance as well as excellent lodging resistance.

[0092] Material which was too tall and too late was eliminated as well as material showing strong fasciation.

[0093] Analysis of the remaining progeny was again done by gas chromatography to select individuals with oleic acid levels higher than 68%. All of these individuals were planted in the field in September 1996-1997.

[0094] A progeny called MUT 152-96 looked particularly interesting in terms of agronomic and morphological characteristics, as well as for its oleic acid content. It was cultivated in isolation during the crop season of September 1996-1997. The most interesting progenies in terms of agronomic and morphological characteristics were selected for bagging and crossing.

[0095] Crossing was performed with double low winter oilseed rape varieties having a conventional fatty acid profile (i.e. oleic acid below 70%) or with low linolenic acid content (i.e. less than about 3.5%) in order to develop lines with a high oleic acid content associated with low linolenic acid content (HOLL).

[0096] The material was progressed into pedigree breeding, self pollination until at least the F7 generation.

[0097] At all generations strong selection pressure was applied against fasciation and for normal plant development and normal rooting system.

[0098] Fatty acid composition was monitored in each generation and only material with oleic acid content higher than 75% and linolenic acid content below 3.5% was kept.

[0099] Different HOLL varieties were obtained by this process such as for example MSP05.

[0100] And by crossing MSP05 variety and CABRIOLET variety, 28DHS.086 variety was obtained.

[0101] MSP12 was developed by the same breeding process than MSP05 but as among starting parent CONTACT was used instead of parents having a conventional fatty acid profile.

[0102] The double low varieties with conventional fatty acid profiles used in this work were BRISTOL, CAPITOL, CAPVERT, VIVOL and CAIMAN and these varieties have been multiplied or maintained using the same maintenance scheme as described here above for the HOLL lines (in accordance with the technical rules published by the "GNIS" and edited by SEDIS, e.g. see 2003 edition, vol. 1, pp.135-147 related to crop plants).

[0103] Basic seed was used for the determination of fatty acid content in trials - small research trials (6 to 12 m^2) or development trials (500 m^2) and for the sequencing work.

EXAMPLES

Example 1

[0104] The seeds were grinded in a first solution consisting of methanol (800ml), trimethyl-pentane (200ml) and 5g of Na OH. About 3 ml of solution was used for about 10g of seeds (in other words about 10 to 50 seeds for 1 ml of solution).

[0105] Extraction was performed during 20 minutes and thereafter a second solution, consisting of trimethylamine (900ml), and propanol, 2- (100ml), was added at the same volume as the first solution.

[0106] The resulting solution was vortexed and allowed to rest until formation of an upper phase.

[0107] The upper phase was sampled and transferred into viols.

[0108] One microliter of same was injected in a gas chromatograph (Fisons from thermo-electron with a columm DB3 -30 meter with a diameter of 0.25 mm and a thickness of 25 micrometer). Running time was about 4 min.

[0109] The oleic acid content results are summarized in table 1.

Table 1.

Varieties	Oleic acid content (wt.%)	Appreciation
CONTACT	71,8-75,2	High
CABRIOLET	73,2-76,8	High
28DHS086	80,3-83,1	Very high
MSP12	80,3-83,5	Very high
MSP05	78,1-81,9	Very high
BRISTOL	61,4-65,7	Normal
VIVOL	60,8-63,2	Normal
CAPVERT	58,9-65,9	Normal
CAIMAN	61,9-64,0	Normal
CAPITOL	59,7-64,6	Normal

[0110] The oleic acid content is based on the total weight of the fatty acid in the extracted oil.

Example 2

[0111] Plant materials used for sequencing are:

- mutant lines with higher oleic fatty acid content: CONTACT, CABRIOLET and 28DHS.086; and
- wild type varieties with normal oleic acid content: Bristol, Capitol, Vivol, Capvert and Caiman.

[0112] All these lines were grown in a growth chamber and the cotyledons and stems were collected from 7-day-old plants.

[0113] The plant tissues were freeze-dried and used for DNA extraction.

[0114] DNA was isolated with Qiagen Plant DNA kits (Qiagen INC.-USA, Valencia CA).

[0115] PCR was performed with TaqGold protocol (AB Biosystem, Inc,).

[0116] Reaction mix includes 2.5 µl 10x buffer, 0.2 µl TaqGold, 0.2 µl dNTP(25mM), 2 µl primers (5uM) and 10 ul DNA template (2ng/ul) and 10.1 ul H_2O.

[0117] PCR cycles were as follows: 94°C 5 min; 8 cycles of 94°C 40sec, 62°C 40sec, 72°C 1min, 94°°C 40sec, 60°C 40sec, 72°C 1min, 94°C 40sec, 58°C 40sec, 72°C 1 min, 94°C 40 sec, 56°C 40sec, 72°C 1 min; 3 cycles of 94°C 40sec, 55°C 40sec, 72°C 1 min; hold at 72°C for 7 min.

[0118] PCR products were analyzed on 1% agarose gel.

[0119] For sequencing, 5 µl PCR products were removed to a new tube and 1 µl Exonuclease I (1:50 dilution) and 1 µl Shrimp Alkaline Phosphatase (1:5 dilution).

[0120] The mix was incubated at 37°C for 20 min and then 80°C for 15 min to inactivate the enzymes.

[0121] 40 µl H_2O was added and 6 µl were used as template with 1 µl sequencing primer.

[0122] Sequencing was done on 3730 DNA Analyzer (Applied Biosystems).

[0123] Sequences were assembled and aligned using SeqMan II program of the LaserGene (DNASTAR, INC, Madison. WI).

Example 3

[0124] Four *Brassica napus* delta-12 oleate desaturase (FAD2) gene sequences, 4684997, 46399190, 8705228 and 4092878, were downloaded from Genebank (NCBI). These sequences were used as queries to blast against Monsanto sequence database.

[0125] Using the "blastn" programs (NCBI), a number of high score hits were obtained. All the hit sequences were downloaded and reassembled with the SeqmanII program (DNASTAR Inc, Madison, Wisconsin, USA).

[0126] Two distinct transcripts were identified and designated as Fad2-1 (SEQ ID NO 11) and Fad2-2 (SEQ ID NO 12). Fad2-1 and Fad2-2 share a high sequence homology, with 97 % sequence identity.

[0127] To identify causative mutations associated with high oleic acid content in the mutant lines and their progenies, nested locus-specific primers were designed to cover the entire sequences.

[0128] The 3'end of a primer was always located at a nucleotide that differentiated Fad2-1 from Fad2-2 except those located at 5'and 3' ends of the consensus sequences where there was not differential nucleotide between the two genes.

[0129] The primers were also designed in such way that one amplicon would overlap with another to ensure full coverage of the entire sequence. These primers were arrayed and used to generate locus-specific amplicons on mutants and wild types. Sequencing results indicated that all the locus-specific PCR primers behaved as expected.

[0130] Sequences belonging to the same gene were assembled together using SeqManII program.

[0131] The consensus genomic sequences of the wild-type Fad2-1 and Fad2-2 genes are represented respectively by SEQ ID NO 9 and 10.

[0132] Table 2 summarizes the sequence features of both Fad2-1 and Fad2-2 genes.
Table 2:

Features	FAD2-1 position	FAD2-2 position
Gene	1 - 2601	1 - 2666
5'UTR	1 - 1206	1 - 1238
Exon	1 - 108	1 - 111
Intron	109 - 1202	112 - 1234
Exon	1207 - 2601	1235 - 2619
CDS	1207 - 2361	1239 - 2393
3'UTR	2362 - 2601	2394 - 2666

[0133] The features are based on the consensus genomic sequences from multiple reads on different genotypes.

[0134] Both Fad2-1 and Fad2-2 genes have one intron each.

[0135] The intron sizes are slightly different between two genes. For Fad2-1, intron spans 1105bp starting from position 109 to 1213, while for Fad2-2, intron consists of 1123bp starting from position 112 to 1234 on the consensus sequences.

[0136] The intron is located at 5'UTR region.

[0137] Putative translation initiation codons are located at 1207 and 1239 for Fad2-1 and Fad2-2 genes, respectively.

[0138] The translation termination codons are located at 2370-2372 and 2391-2393, respectively for Fad2-1 and Fad2-2.

[0139] 3'UTR sequences are 247 base pairs for Fad2-1 and 273 base pairs for Fad2-2 genes.

[0140] A deletion at position 1421 (called DEL.1421) of FAD2-1 gene caused a frame-shift in genetic codons, resulting in premature termination of the polypeptides.

[0141] A point mutation at position 1590 (SNP1590) of FAD2-2 gene (as represented by SEQ ID NO 7) caused an amino acid residue change from leucine (CTT) to phenylalanine (TTT).

[0142] Both leucine and phenylalanine are hydrophobic in nature and share some common amino acid properties, but phenylalanine contains a large rigid aromatic group on the side chain that causes some change in the function of the enzyme.

[0143] Moreover, in combination with DEL.215, this mutation causes more visible effect on the phenotype.

[0144] Combination of different alleles at these mutations created a gradient on oleic content as observed on different mutant lines (see table 1).

[0145] Two mutant lines, 28DHS.086 and MSP12, carried double mutations at DEL1421 and SNP1590. Since both mutations were missense mutations, the FAD2 gene functions are severely affected, resulting in the highest oleic content in the mutant line.

[0146] It is to be noted that Brassica napus varieties carrying only the SNP1590 mutation exhibits a normal oleic acid content (i.e. an oleic acid content equivalent to the oleic acid content of the wild-types).

[0147] Two mutant lines, CONTACT and CABRIOLET, carried a single point mutation at DEL.1421, resulting in an oleic content slightly below in comparison with the double mutants.

[0148] In summary, the sequence data strongly indicated that these mutations at Fad2-1 and Fad2-2 are associated with oleic contents on different mutant lines.

[0149] The identification of causative sequence variations is crucial to design diagnostic assays specifically for each mutant allele.

[0150] Knowledge of association between sequence variations and phenotypes can allow to design marker assays to accurately predict the oleic acid content in plants without the need of wet chemical analysis of the fatty acid content.

SEQUENCE LISTING

[0151]

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<130> MSTO.09B/WO

<160> 12

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Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe Lys Arg Ser
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Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser
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Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro
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ctc tcc tac ttc gcc tgg cct ctc tac tgg gcc tgc cag ggc tgc gtc      288
Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val
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cta acc ggc gtc tgg gtc ata gcc cac gag tgc ggc cac cac gcc ttc      336
Leu Thr Gly Val Trp Val Ile Ala His Glu Cys Gly His His Ala Phe
            100                 105                 110

agc gac tac cag tgg ctg gac gac acc gtc ggc ctc atc ttc cac tcc      384
Ser Asp Tyr Gln Trp Leu Asp Asp Thr Val Gly Leu Ile Phe His Ser
        115                 120                 125

ttc ctc ctc gtc cct tac ttc tcc tgg aag tac agt cat cga cgc cac      432
Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser His Arg Arg His
    130                 135                 140

cat tcc aac act ggc tcc ctc gag aga gac gaa gtg ttt gtc ccc aag      480
His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys
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Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu
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tac tta gcc ttc aac gtc tcg ggg aga cct tac gac ggc ggc ttc gct      624
Tyr Leu Ala Phe Asn Val Ser Gly Arg Pro Tyr Asp Gly Gly Phe Ala
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Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp Arg Glu Arg Leu
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Tyr Arg Tyr Ala Ala Val Gln Gly Val Ala Ser Met Val Cys Phe Tyr
                245                 250                 255

gga gtt cct ctt ctg att gtc aac ggg ttc tta gtt ttg atc act tac      816
Gly Val Pro Leu Leu Ile Val Asn Gly Phe Leu Val Leu Ile Thr Tyr
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ttg cag cac acg cat cct tcc ctg cct cac tat gac tcg tct gag tgg      864
Leu Gln His Thr His Pro Ser Leu Pro His Tyr Asp Ser Ser Glu Trp
        275                 280                 285

gat tgg ttg agg gga gct ttg gcc acc gtt gac aga gac tac gga atc      912
Asp Trp Leu Arg Gly Ala Leu Ala Thr Val Asp Arg Asp Tyr Gly Ile
    290                 295                 300

ttg aac aag gtc ttc cac aat atc acg gac acg cac gtg gcg cat cac      960
Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His
305                 310                 315                 320

ctg ttc tcg acc atg ccg cat tat cac gcg atg gaa gct acg aag gcg     1008
Leu Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala
                325                 330                 335

ata aag ccg ata ctg gga gag tat tat cag ttc gat ggg acg ccg gtg     1056
Ile Lys Pro Ile Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val
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Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser
    50                  55                  60

Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro
65                  70                  75                  80

Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val
                85                  90                  95

Leu Thr Gly Val Trp Val Ile Ala His Glu Cys Gly His His Ala Phe
            100                 105                 110

Ser Asp Tyr Gln Trp Leu Asp Asp Thr Val Gly Leu Ile Phe His Ser
        115                 120                 125

Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser His Arg Arg His
    130                 135                 140

His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys
145                 150                 155                 160

Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr Leu Asn Asn Pro Leu
                165                 170                 175

Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu
            180                 185                 190

Tyr Leu Ala Phe Asn Val Ser Gly Arg Pro Tyr Asp Gly Gly Phe Ala
        195                 200                 205

Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp Arg Glu Arg Leu
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Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu
225                 230                 235                 240

Tyr Arg Tyr Ala Ala Val Gln Gly Val Ala Ser Met Val Cys Phe Tyr
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Leu Gln His Thr His Pro Ser Leu Pro His Tyr Asp Ser Ser Glu Trp
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Asp Trp Leu Arg Gly Ala Leu Ala Thr Val Asp Arg Asp Tyr Gly Ile
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Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His
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Leu Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala
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Ile Lys Pro Ile Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val
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Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr Val Glu Pro
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Glu Thr Asp Asn Ile Lys Arg Val Pro Cys Glu Thr Pro Pro Phe Thr
            20                  25                  30

gtc gga gaa ctc aag aaa gca atc cca ccg cac tgt ttc aaa cgc tcg      144
Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe Lys Arg Ser
        35                  40                  45

atc cct cgc tct ttc tcc tac ctc atc tgg gac atc atc ata gcc tcc      192
Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser
    50                  55                  60


tgc ttc tac tac gtc gcc acc att act tcc ctc tcc tcc ctc acc ctc      240
Cys Phe Tyr Tyr Val Ala Thr Ile Thr Ser Leu Ser Ser Leu Thr Leu
65                  70                  75                  80

tct cct act tcg cct ggc ctc tct act ggg cct gcc agg gct gcg tcc      288
Ser Pro Thr Ser Pro Gly Leu Ser Thr Gly Pro Ala Arg Ala Ala Ser
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        35                  40                  45

Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser
    50                  55                  60

Cys Phe Tyr Tyr Val Ala Thr Ile Thr Ser Leu Ser Ser Leu Thr Leu
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1               5                   10                  15

gaa acc gac acc atc aag cgc gta ccc tgc gag aca ccg ccc ttc act       96
Glu Thr Asp Thr Ile Lys Arg Val Pro Cys Glu Thr Pro Pro Phe Thr
            20                  25                  30

gtc gga gaa ctc aag aaa gca atc cca ccg cac tgt ttc aaa cgc tcg      144
Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe Lys Arg Ser
        35                  40                  45

atc cct cgc tct ttc tcc tac ctc atc tgg gac atc atc ata gcc tcc      192
Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser
    50                  55                  60

tgc ttc tac tac gtc gcc acc act tac ttc cct ctc ctc cct cac cct      240
Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro
65                  70                  75                  80

ctc tcc tac ttc gcc tgg cct ctc tac tgg gcc tgc caa ggg tgc gtc      288
Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val
                85                  90                  95

cta acc ggc gtc tgg gtc ata gcc cac gag tgc ggc cac cac gcc ttc      336
Leu Thr Gly Val Trp Val Ile Ala His Glu Cys Gly His His Ala Phe
            100                 105                 110

agc gac tac cag tgg ctt gac gac acc gtc ggt ctc atc ttc cac tcc      384
Ser Asp Tyr Gln Trp Leu Asp Asp Thr Val Gly Leu Ile Phe His Ser
        115                 120                 125

ttc ctc ctc gtc cct tac ttc tcc tgg aag tac agt cat cga cgc cac      432
Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser His Arg Arg His
    130                 135                 140

cat tcc aac act ggc tcc ctc gag aga gac gaa gtg ttt gtc ccc aag      480
His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys
145                 150                 155                 160

aag aag tca gac atc aag tgg tac ggc aag tac ctc aac aac cct ttg      528
Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr Leu Asn Asn Pro Leu
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gga cgc acc gtg atg tta acg gtt cag ttc act ctc ggc tgg ccg ttg      576
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Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu
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Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp Arg Glu Arg Leu
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Phe Arg Tyr Ala Ala Ala Gln Gly Val Ala Ser Met Val Cys Phe Tyr
                245                 250                 255

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Gly Val Pro Leu Leu Ile Val Asn Gly Phe Leu Val Leu Ile Thr Tyr
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Leu Gln His Thr His Pro Ser Leu Pro His Tyr Asp Ser Ser Glu Trp
        275                 280                 285

gat tgg ttg agg gga gct ttg gct acc gtt gac aga gac tac gga atc      912
Asp Trp Leu Arg Gly Ala Leu Ala Thr Val Asp Arg Asp Tyr Gly Ile
    290                 295                 300

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Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His
305                 310                 315                 320

ctg ttc tcc acg atg ccg cat tat cac gcg atg gaa gct acc aag gcg     1008
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Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr Val Glu Pro
        355                 360                 365

gac agg caa ggt gag aag aaa ggt gtg ttc tgg tac aac aat aag tta     1152
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Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro
65                  70                  75                  80

Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val
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Leu Thr Gly Val Trp Val Ile Ala His Glu Cys Gly His His Ala Phe
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Ser Asp Tyr Gln Trp Leu Asp Asp Thr Val Gly Leu Ile Phe His Ser
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Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser His Arg Arg His
    130                 135                 140

His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys
145                 150                 155                 160

Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr Leu Asn Asn Pro Leu
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Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu
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Tyr Leu Ala Phe Asn Val Ser Gly Arg Pro Tyr Asp Gly Gly Phe Ala
        195                 200                 205

Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp Arg Glu Arg Leu
    210                 215                 220

Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu
225                 230                 235                 240

Phe Arg Tyr Ala Ala Ala Gln Gly Val Ala Ser Met Val Cys Phe Tyr
                245                 250                 255

Gly Val Pro Leu Leu Ile Val Asn Gly Phe Leu Val Leu Ile Thr Tyr

            260                 265                 270

Leu Gln His Thr His Pro Ser Leu Pro His Tyr Asp Ser Ser Glu Trp
        275                 280                 285

Asp Trp Leu Arg Gly Ala Leu Ala Thr Val Asp Arg Asp Tyr Gly Ile
    290                 295                 300

Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His
305                 310                 315                 320

Leu Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala
                325                 330                 335

Ile Lys Pro Ile Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val
            340                 345                 350

Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr Val Glu Pro
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Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr Asn Asn Lys Leu
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Glu Thr Asp Thr Ile Lys Arg Val Pro Cys Glu Thr Pro Pro Phe Thr
            20                  25                  30

gtc gga gaa ctc aag aaa gca atc cca ccg cac tgt ttc aaa cgc tcg      144
Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe Lys Arg Ser
        35                  40                  45

atc cct cgc tct ttc tcc tac ctc atc tgg gac atc atc ata gcc tcc      192
Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser
    50                  55                  60

tgc ttc tac tac gtc gcc acc act tac ttc cct ctc ctc cct cac cct      240
Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro
65                  70                  75                  80

ctc tcc tac ttc gcc tgg cct ctc tac tgg gcc tgc caa ggg tgc gtc      288

Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val
                85                  90                  95

cta acc ggc gtc tgg gtc ata gcc cac gag tgc ggc cac cac gcc ttc      336
Leu Thr Gly Val Trp Val Ile Ala His Glu Cys Gly His His Ala Phe
            100                 105                 110

agc gac tac cag tgg ttt gac gac acc gtc ggt ctc atc ttc cac tcc      384
Ser Asp Tyr Gln Trp Phe Asp Asp Thr Val Gly Leu Ile Phe His Ser
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ttc ctc ctc gtc cct tac ttc tcc tgg aag tac agt cat cga cgc cac      432
Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser His Arg Arg His
    130                 135                 140

cat tcc aac act ggc tcc ctc gag aga gac gaa gtg ttt gtc ccc aag      480
His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys
145                 150                 155                 160

aag aag tca gac atc aag tgg tac ggc aag tac ctc aac aac cct ttg      528
Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr Leu Asn Asn Pro Leu
                165                 170                 175

gga cgc acc gtg atg tta acg gtt cag ttc act ctc ggc tgg ccg ttg      576
Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu
            180                 185                 190

tac tta gcc ttc aac gtc tcg gga aga cct tac gac ggc ggc ttc gct      624
Tyr Leu Ala Phe Asn Val Ser Gly Arg Pro Tyr Asp Gly Gly Phe Ala
        195                 200                 205

tgc cat ttc cac ccc aac gct ccc atc tac aac gac cgc gag cgt ctc      672
Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp Arg Glu Arg Leu
    210                 215                 220

cag ata tac atc tcc gac gct ggc atc ctc gcc gtc tgc tac ggt ctc      720
Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu
225                 230                 235                 240

ttc cgt tac gcc gcc gcg cag gga gtg gcc tcg atg gtc tgc ttc tac      768
Phe Arg Tyr Ala Ala Ala Gln Gly Val Ala Ser Met Val Cys Phe Tyr
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gga gtc ccg ctt ctg att gtc aat ggt ttc ctc gtg ttg atc act tac      816
Gly Val Pro Leu Leu Ile Val Asn Gly Phe Leu Val Leu Ile Thr Tyr
            260                 265                 270

ttg cag cac acg cat cct tcc ctg cct cac tac gat tcg tcc gag tgg      864
Leu Gln His Thr His Pro Ser Leu Pro His Tyr Asp Ser Ser Glu Trp
        275                 280                 285

gat tgg ttg agg gga gct ttg gct acc gtt gac aga gac tac gga atc      912
Asp Trp Leu Arg Gly Ala Leu Ala Thr Val Asp Arg Asp Tyr Gly Ile
    290                 295                 300

ttg aac aag gtc ttc cac aat att acc gac acg cac gtg gcg cat cat      960
Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His
305                 310                 315                 320

ctg ttc tcc acg atg ccg cat tat cac gcg atg gaa gct acc aag gcg     1008
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Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro
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Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val
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Leu Thr Gly Val Trp Val Ile Ala His Glu Cys Gly His His Ala Phe
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Ser Asp Tyr Gln Trp Phe Asp Asp Thr Val Gly Leu Ile Phe His Ser
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Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser His Arg Arg His
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REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- WO9856239A [0079]

Non-patent literature cited in the description

- GNIS20030000vol. 1, 135-147 [0102]

Patentkrav

1. Nukleinsyremolekyle, der omfatter sekvensen ifølge SEQ ID NO 1 eller 5 yderligere deleteret for nukleotidet ved position 215, eller en (variant) nukleinsyresekvens, der koder for et FAD2-protein, der er mindst 95 % identisk med SEQ ID NO 1 eller 5, yderligere deleteret for nukleotidet svarende til position 215.

2. Nukleinsyremolekyle ifølge krav 1, der omfatter sekvensen ifølge SEQ ID NO 9 yderligere deleteret for nukleotidet ved position 1421, eller en (variant) nukleinsyresekvens, der koder for et FAD2-protein, der er mindst 95 % identisk med SEQ ID NO 9, yderligere deleteret for nukleotidet svarende til position 1421 eller mindst 95 % identisk med SEQ ID NO 10, yderligere deleteret for nukleotidet svarende til position 1453.

3. Nukleinsyremolekyle ifølge krav 1, der har sekvensen ifølge SEQ ID NO 3, dens komplementære form eller RNA-formen deraf.

4. Fragment på mindst 20 nukleotider af et nukleinsyremolekyle ifølge krav 3, hvilket fragment omfatter det muterede kodon, der er et resultat af deletionen.

5. Vektor, der omfatter et nukleinsyremolekyle ifølge et hvilket som helst af kravene 1 til 3.

6. Værtscelle, der omfatter en nukleinsyresekvens ifølge et hvilket som helst af kravene 1 til 3 eller en vektor ifølge krav 5.

7. Plante stabilt transformeret med en vektor ifølge krav 5.

8. Plante ifølge krav 7, hvor planten, der skal transformeres, er udvalgt fra gruppen bestående af oliefremstillende afgrøder.

9. Plante ifølge krav 8, hvor de oliefremstillende afgrøder er solsikker, sojabønner, bomuld, majs og/eller raps.

10. Polypeptid kodet for af et nukleinsyremolekyle ifølge et hvilket som helst af kravene 1 til 3.

11. Polypeptid ifølge krav 10, der har aminosyresekvensen ifølge SEQ ID NO 4.

12. Fremgangsmåde til øgning af oliesyreindholdet i en plante, der omfatter transformering af en plante med vektoren ifølge krav 5.

13. Anvendelse af et fragment på mindst 20 nukleotider ifølge krav 4 som en primer, sonde og/eller selektiv markør.

14. Fremgangsmåde til markørassisteret udvælgelse af planter, i særdeleshed *Brassica*-arter, ved anvendelse af et nukleinsyremolekyle ifølge et hvilket som helst af kravene 1 til 4.

15. Assaykit, der omfatter en første beholder, der indeholder et nukleinsyremolekyle ifølge et hvilket som helst af kravene 1 til 4.

16. Plante eller en plantedel eller et frø, der indeholder en nukleinsyresekvens svarende til SEQ ID NO 1 eller 5 yderligere deleteret for nukleotidet ved position 215, og som yderligere indeholder en anden nukleinsyresekvens, der koder for et FAD-2 protein, der har en aminosyresubstitution ved eller svarende til position 118 i forhold til et vildtype FAD-2-protein såsom vildtype FAD-2-proteinet ifølge SEQ ID NO 2 eller 6, hvor leucinresten ved eller svarende til position 118 i forhold til vildtype FAD-2-proteinet er substitueret af en phenylalaninrest.

17. Fremgangsmåde til fremstilling af plantelinjer med højt olieindhold omfattende:

(a) induktion af mutagenese i mindst nogle celler fra en plante, der er en *Brassica napus*-sort, der har et oliesyreindhold på mindre end 70 %;

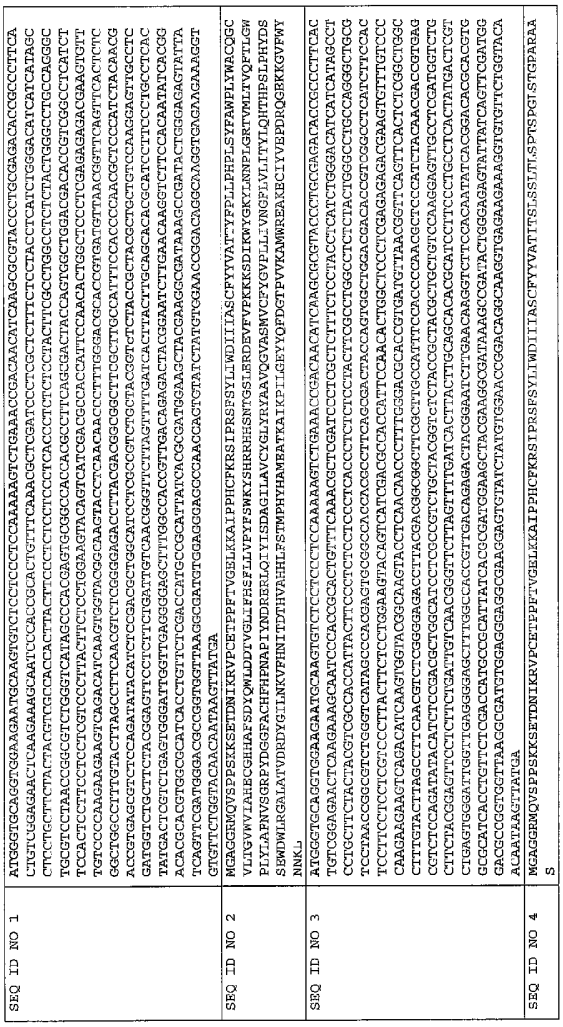
(b) regenerering af planter fra mindst én af de muterede celler; og

(c) udvælgelse af regenererede planter, der har et nukleinsyremolekyle, der omfatter sekvensen ifølge SEQ ID NO 1 eller 5 yderligere deleteret for nukleotidet ved position 215, eller en (variant) nukleinsyresekvens, der koder for et FAD2-protein, der er mindst 95 % identisk med SEQ ID NO 1 eller 5, yderligere deleteret for nukleotidet svarende til position 215.

18. Fremgangsmåde ifølge krav 17, hvor de regenererede planter endvidere indeholder en nukleinsyresekvens, der koder for et FAD-2-protein, der har en aminosyresubstitution ved eller svarende til position 118 i forhold til et vildtype FAD2-protein, såsom vildtype FAD2-proteinet ifølge SEQ ID NO 2 eller 6.

19. Fremgangsmåde ifølge krav 17 eller 18, hvor mutagenesen er induceret ved hjælp af EMS-behandling.

DRAWINGS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SEQ ID NO 5	ATGGGTGCAGGTGGAAGAATGCAAGTGTCTCCTCCCTCCAAGAAGTCTGAAACCGACACCATCAAGCGCGTACCCTGCGAGACACCGCCCTTCAC TGTCGGAGAACTCAAGAAAGCAATCCCACCGCACTGTTTCAAACGCTCGATCCCTCGCTCTTTCTCCTACCTCATCTGGGACATCATCATAGCCT CCTGCTTCTACTACGTCGCCACCACTTACTTCCCTCTCCTCCCTCACCCTCTCTCCTACTTCGCCTGGCCTCTCTACTGGGCCTGCCAAGGGTGC GTCCTAACCGGCGTCTGGGTCATAGCCCACGAGTGCGGCCACCACGCCTTCAGCGACTACCAGTGGCTTGACGACACCGTCGGTCTCATCTTCCA CTCCTTCCTCCTCGTCCCTTACTTCTCCTGGAAGTACAGTCATCGACGCCACCATTCCAACACTGGCTCCCTCGAGAGAGACGAAGTGTTTGTCC CCAAGAAGAAGTCAGACATCAAGTGGTACGGCAAGTACCTCAACAACCCTTTGGGACGCACCGTGATGTTAACGGTTCAGTTCACTCTCGGCTGG CCGTTGTACTTAGCCTTCAACGTCTCGGGAAGACCTTACGACGGCGGCTTCGCTTGCCATTTCCACCCCAACGCTCCCATCTACAACGACCGCGA GCGTCTCCAGATATACATCTCCGACGCTGGCATCCTCGCCGTCTGCTACGGTCTCTTCCGTTACGCCGCCGCGCAGGGAGTGGCCTCGATGGTCT GCTTCTACGGAGTCCCGCTTCTGATTGTCAATGGTTTCCTCGTGTTGATCACTTACTTGCAGCACACGCATCCTTCCCTGCCTCACTACGATTCG TCCGAGTGGGATTGGTTGAGGGGAGCTTTGGCTACCGTTGACAGAGACTACGGAATCTTGAACAAGGTCTTCCACAATATTACCGACACGCACGT GGCGCATCATCTGTTCTCCACGATGCCGCATTATCACGCGATGGAAGCTACCAAGGCGATAAAGCCGATACTGGGAGAGTATTATCAGTTCGATG GGACGCCGGTGGTTAAGGCGATGTGGAGGGAGGCGAAGGAGTGTATCTATGTGGAACCGGACAGGCAAGGTGAGAAGAAAGGTGTGTTCTGGTAC AACAATAAGTTATGA
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SEQ ID NO 7	ATGGGTGCAGGTGGAAGAATGCAAGTGTCTCCTCCCTCCAAGAAGTCTGAAACCGACACCATCAAGCGCGTACCCTGCGAGACACCGCCCTTCAC TGTCGGAGAACTCAAGAAAGCAATCCCACCGCACTGTTTCAAACGCTCGATCCCTCGCTCTTTCTCCTACCTCATCTGGGACATCATCATAGCCT CCTGCTTCTACTACGTCGCCACCACTTACTTCCCTCTCCTCCCTCACCCTCTCTCCTACTTCGCCTGGCCTCTCTACTGGGCCTGCCAAGGGTGC GTCCTAACCGGCGTCTGGGTCATAGCCCACGAGTGCGGCCACCACGCCTTCAGCGACTACCAGTGGTTTGACGACACCGTCGGTCTCATCTTCCA CTCCTTCCTCCTCGTCCCTTACTTCTCCTGGAAGTACAGTCATCGACGCCACCATTCCAACACTGGCTCCCTCGAGAGAGACGAAGTGTTTGTCC CCAAGAAGAAGTCAGACATCAAGTGGTACGGCAAGTACCTCAACAACCCTTTGGGACGCACCGTGATGTTAACGGTTCAGTTCACTCTCGGCTGG CCGTTGTACTTAGCCTTCAACGTCTCGGGAAGACCTTACGACGGCGGCTTCGCTTGCCATTTCCACCCCAACGCTCCCATCTACAACGACCGCGA GCGTCTCCAGATATACATCTCCGACGCTGGCATCCTCGCCGTCTGCTACGGTCTCTTCCGTTACGCCGCCGCGCAGGGAGTGGCCTCGATGGTCT GCTTCTACGGAGTCCCGCTTCTGATTGTCAATGGTTTCCTCGTGTTGATCACTTACTTGCAGCACACGCATCCTTCCCTGCCTCACTACGATTCG TCCGAGTGGGATTGGTTGAGGGGAGCTTTGGCTACCGTTGACAGAGACTACGGAATCTTGAACAAGGTCTTCCACAATATTACCGACACGCACGT GGCGCATCATCTGTTCTCCACGATGCCGCATTATCACGCGATGGAAGCTACCAAGGCGATAAAGCCGATACTGGGAGAGTATTATCAGTTCGATG GGACGCCGGTGGTTAAGGCGATGTGGAGGGAGGCGAAGGAGTGTATCTATGTGGAACCGGACAGGCAAGGTGAGAAGAAAGGTGTGTTCTGGTAC AACAATAAGTTATGA
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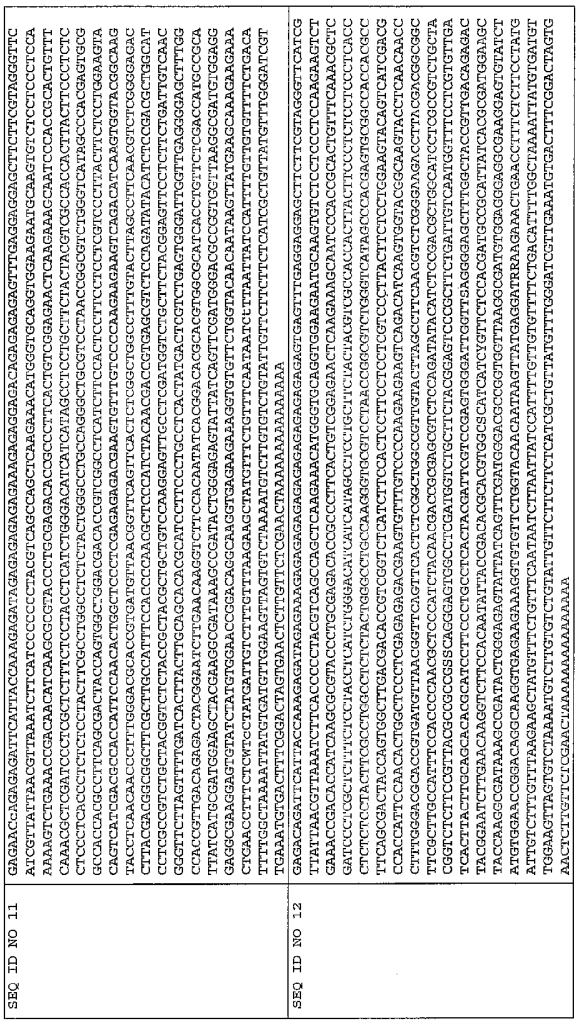

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SEQ ID NO 12	GAGACAGATTCATTACCAAAGAGATAGAGAAAGAGAGGAGACAGAGAGAGTTGAGAGAGAGTCTTCGTAGGGTTCATCG TTATTAACGTTAAATCTTCACCCCTACGTCAGCCAGCTCAAGAAACATGGGTGCAGGTGGAAGAATGCAAGTGTCTCCTCCCCTCCAAGAAGTCT GAAACCGACACCATCAAGCGCGTACCCTGCGAGACACCGCCCTTCACTGTCGAGAACTCAAGAAAGCAATCCCACCGCACTGTTTCAAACGCTC GATCCCTCGCTCTTCTCCTACCTCATCTGGGACATCATAGCCTCCTGTCTTCACTAGTCGTCGCCACCACTTACTCCCTCTCCTCACC CTCTCTCCTACTTCGCCTGGCCTCTCTACTGGCCTCGCCAAGGGTGCGTCCTAACCGGCGTCTGGGTCATAGCCCACGAGTGCGCCACCACGCC TTCAGCGACTACCAGTGGCTTGACGACACCGTCGGTCTCATCTTCCACTCCTTCCCTCGTCCCTTACTTCTCCTGGAAGTACAGTCATCGACG CCACCATTCCAACACTGGCTCCCTCGAGAGACGAAGTGTTTGTCCCCCAAGAAGAAGTCAGACATCAAGTGGTACGGTACCTCAACAACCC CTTTGGGACGCACCGTGATGTTAACGGTTCATCTCTCGGCTGGCCGTTGTACTTACGCCTTCAACGTCCGGAGTCCGGAGACCTTACGACGGCGGC TTCGCTTGCCATTCCACCCCAACGCTCCCATCTACAACGACCGCGACGCGTCCCATCTCGCCGTCTGCTACGACGGCGGCTTCGCTTGCCATTTCCA CGGTCTCTTCCGTTACGCGCGCGSSCAGGGAGTGGCCTCGATGGTCTGCTTCTACGGAGTTCCCGCTTCTGATTGTCAATGGTTTCCTCGTGTTGA TCACTTACTTGCAGCACACGCATCCTTCCCTGCCTCACTACGATTCGTCCGAGTGGATTGGTTGAGGGAGCTTTGGCTACCGTTGACAGAGAC TACGGAATCTTGAACAAGGTCTTCCACAATATTACCGACACGCACGTGGCGCATCATCYGTTCTCCACGATGCCGCATTATCACGCGATGGAGC TACCRAGGCGATAAAGCCGATACTGGGAGAGTATTATCAGTTCGATGGGACGCCGGTGTTAAGGCGATGTGGAGGCAGGGAGTGTATCT ATGTGGAACCGGACAGGCAGAAGAAAGGTGTGTTCTGGTACAACAATAAGTTATGAAGCAAAGAAGAAACTCAACCTTTCTCTTCCTATG ATTGTCTTTGTTTAAGAAGCTATGTTTCTGTTTCAATAATCTTTAATTATCCATTTTGTGTGTTTCTGACATTTTGGCTAAAATTATGTGATGT TGGAAGTTAGTGTCTAAAATGTCTTGTGTCTGTATTGTTTCTTCTTCATCGCTGTTATGTTTGGGATCGTTGAAATGTGACTTTCGGACTAGTG AACTCTTGTTCTCGAACTAAAAAAAAAAAAAAA

Fig. 1

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Monsanto SAS
Centre de Recherche de Boissay
28310 Toury
France

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

NAME AND ADDRESS OF DEPOSITOR

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:

Brassica napus
CV Oleifera (METZG) MSP05

Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:

NCIMB 41235

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

[ ] a scientific description

[X] a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on 9 July 2004 (date of the original deposit)

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on

(date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: NCIMB Ltd.,

Address: 23 St Machar Drive
Aberdeen
AB24 3RY
Scotland, UK.

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorised official(s):

Date: 19 July 2004

Where Rule 6.4(d) applies, such date is the date on which the status of International Depositary Authority was acquired.

Form BP/4 (sole page)

Fig. 2a

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

Monsanto SAS
Centre de Recherche de Boissay
28310 Toury
France

INTERNATIONAL FORM

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified on the following page

NAME AND ADDRESS OF THE PARTY
TO WHOM THE VIABILITY STATEMENT
IS ISSUED

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: AS ABOVE Address:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NCIMB 41233 Date of the deposit or of the transfer[1]: 9 July 2004

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 9 July 2004 [2]. On that date, the said microorganism was:[3]

[X] viable

[ ] no longer viable

1 Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

2 In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

3 Mark with a cross the applicable box.

Form BP/9 (first page)

Fig. 2b

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED*

V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: NCIMB Ltd., Address: 23 St Machar Drive Aberdeen AB24 3RY Scotland	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorised official(s): Date: 19 July 2004

* Fill in if the information has been requested and if the results of the test were negative.

Form BP/9 (second and last page)

Fig. 2c

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

Monsanto S.A.S.
Centre de Recherche de Boissay
28310 Toury
France

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

NAME AND ADDRESS
OF DEPOSITOR

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
Brassica napus CV Oleifera (METZG) 28-DHS 086	NCIMB 41365

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

[ ] a scientific description

[X] a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on 22 December 2005 (date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: NCIMB Ltd.,
Ferguson Building
Craibstone Estate
Address: Bucksburn
Aberdeen,
AB21 9YA,
Scotland

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorised official(s):

Date: 4 January 2006

[1] Where Rule 6.4(d) applies, such date is the date on which the status of International Depositary Authority was acquired.

Form BP/4 (sole page)

Fig. 2d

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

Monsanto S.A.S
Centre de Recherche de Boissay
28310 Toury
France

INTERNATIONAL FORM

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified on the following page

NAME AND ADDRESS OF THE PARTY
TO WHOM THE VIABILITY STATEMENT
IS ISSUED

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: AS ABOVE	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
Address:	NCIMB 41365
	Date of the deposit or of the transfer[1]: 22 December 2005

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 5 January 2006 [2]. On that date, the said microorganism was:

[3] [X] viable

[3] [ ] no longer viable

[1] Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

[2] In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

[3] Mark with a cross the applicable box.

Form BP/9 (first page)

Fig. 2e

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: NCIMB Ltd., Ferguson Building Address: Craibstone Estate Bucksburn Aberdeen, AB21 9YA, Scotland.	Signature(s) of person(s) having the power to represent the International Depositary Date: 16 January 2006

[4] Fill in if the information has been requested and if the results of the test were negative.

Form BP/9 (second and last page)

Fig. 2f

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

Monsanto S.A.S
Centre de Recherche de Boissay
28310 Toury
France

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

NAME AND ADDRESS OF DEPOSITOR

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
Brassica napus CV Oleifera (METZG) MSP12	NCIMB 41374

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

[ ] a scientific description

[X] a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on 10 February 2006 (date of the original deposit)[1]

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: NCIMB Ltd.,
Ferguson Building
Craibstone Estate
Address: Bucksburn
Aberdeen,
AB21 9YA,
Scotland

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

Date: 27 February 2006

[1] Where Rule 6.4(d) applies, such date is the date on which the status of International Depositary Authority was acquired.

Form BP/4 (sole page)

Fig. 2g

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

Monsanto S.A.S
Centre de Recherche de Boissay
28310 Toury
France

NAME AND ADDRESS OF THE PARTY
TO WHOM THE VIABILITY STATEMENT
IS ISSUED

INTERNATIONAL FORM

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified on the following page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: AS ABOVE	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NCIMB 41374
Address:	Date of the deposit or of the transfer[1]: 10 February 2006

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 10 February 2006 [2]. On that date, the said microorganism was

[3] [X] viable

[3] [ ] no longer viable

1 Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

2 In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

3 Mark with a cross the applicable box.

Form BP/9 (first page)

Fig. 2h

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]
V. INTERNATIONAL DEPOSITARY AUTHORITY
Name: NCIMB Ltd., Ferguson Building Address: Craibstone Estate Bucksburn Aberdeen, AB21 9YA, Scotland.
Signature(s) of person(s) having the power to represent the International Depositary Date: 27 February 2006

[4] Fill in if the information has been requested and if the results of the test were negative.

Form BP/9 (second and last page)

Fig. 2i