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(54) **Delta 6-desaturases from primulaceae, expressing plants and PUFA-containing oils**

Delta 6-Desaturase aus Primulacaea, Pflanzen, exprimierende Pflanzen, und Öle, welche mehrfach ungesättigte Fettsäuren enthalten

Delta 6-désaturases de primulacées, plantes exprimant ces dernières et huiles contenant des acides gras polyinsaturés

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Description

[0001] The present invention relates to an flax oil comprising stearidonic acid obtainable by a method for the specific production of triglycerides having an increased content of unsaturated fatty acids, in particular ω-3 fatty acids having more than three double bonds.

[0002] Fatty acids and triglycerides have numerous applications in the food industry, animal nutrition, cosmetics and in the drug sector. Depending on whether they are free saturated or unsaturated fatty acids or are triglycerides having an increased content of saturated or unsaturated fatty acids, they are suitable for the most varied applications. Thus, for example, polyunsaturated fatty acids are added to infant formula to increase its nutria-tional value. The various fatty acids and triglycerides are mainly obtained from microorganisms such as Mortierella or from oil-producing plants such as soybean, oilseed rape, sunflower and others, and generally occur in the form of their triacylglycer-ides. However, they may also be obtained from animals, e.g. fish. The free fatty acids are advantageously produced by saponification.

[0003] Depending on application purpose oils containing saturated or unsaturated fatty acids are preferred, thus in human nutrition for example, lipids containing unsaturated fatty acids, especially polyunsaturated fatty acids, are preferred since they have a positive effect on the level of cholesterol in the blood and hence on the possibility of heart disease. They are employed in various dietary foods or medicinal drugs.

[0004] On account of their positive properties there has been no shortage of attempts in the past to make available genes which participate in the synthesis of fatty acids or triglycerides for the production of oils in various organisms having a modified content of unsaturated fatty acids. Thus, in WO 91/13972 and its US equivalent a Δ 9-desaturase is described. In WO 93/11245 a A 15-desaturase and in WO 94/11516 a Δ 12-desaturase is claimed. Other desaturases are described, for example, in EP-A-0 550 162, WO 94/18337, WO 97/30582, WO 97/21340, WO 95/18222, EP-A-0 794 250, Stukey et al., J. Biol. Chem., 265, 1990: 20144-20149, Wada et al., Nature 347, 1990: 200-203 or Huang et al., Lipids 34, 1999: 649-659. To date, however, the various desaturases have been only inadequately characterized biochemically since the enzymes in the form of membrane-bound proteins are isolable and characterizable only with very great difficulty (McKeon et al., Methods in Enzymol. 71, 1981: 12141-12147, Wang et al., Plant Physiol. Biochem., 26, 1988: 777-792). Generally, membrane-bound desaturases are characterized by introduction into a suitable organism which is then investigated for enzyme activity by means of analysis of starting materials and products. Δ 6-Desaturases are described in WO 93/06712, US 5,614,393, US 5614393, WO 96/21022, WO0021557 and WO 99/27111 and their application to production in transgenic organisms is also described, e.g. in WO 9846763, WO 9846764 and WO 9846765. At the same time the expression of various desaturases, as in WO 9964616 or WO 9846776, and the formation of polyunsaturated fatty acids is also described and claimed. With regard to the effectiveness of the expression of desaturases and their effect on the formation of polyunsaturated fatty acids it may be noted that through expression of a single desaturase as described to date only low contents of Δ 6 unsaturated fatty acids/lipids, such as by way of example gamma-linolenic acid and stearidonic acid, have been achieved. Furthermore, a mixture of ω-3 and ω-6 fatty acids was usually obtained since all A 6-desaturases described so far converted, for example, linoleic acid (ω-6 fatty acid) as well as α-linolenic acid (ω-3 fatty acid).

[0005] Accordingly, there is still a great demand for new and more suitable genes which encode enzymes which participate in the biosynthesis of unsaturated fatty acids and make it possible to produce certain fatty acids specifically on an industrial scale without unwanted byproducts forming. In the selection of genes for biosynthesis two characteristics above all are particularly important. On the one hand, there is as ever a need for improved processes for obtaining the highest possible contents of polyunsaturated fatty acids. On the other hand, the enzymes employed should be highly specific to a certain substrate since as far as possible unwanted byproducts must not be produced which might have negative or so far undiscovered physiological effects in humans or animals due to food/feed intake.

[0006] Accordingly, the present invention relates to an oil as defined in claim 1 or 2. . We have found that this object is achieved by the isolated nucleic acid sequences according to the invention which encode polypeptides having A 6-desaturase activity, wherein the A 6-desaturases encoded by the nucleic acid sequences specifically convert ω-3 fatty acids. The object was achieved by cloning isolated nucleic acid sequences , wherein the nucleic acid sequences encode a polypeptide having A 6-desaturase activity, wherein the Δ 6-desaturases encoded by the nucleic acid sequences specifically convert ω-3 fatty acids selected from the group:

a) a nucleic acid sequence having the sequence depicted in SEQ ID NO: 1 or SEQ ID NO: 3,

b) nucleic acid sequences which may be derived as a result of the degenerated genetic code from the encoding sequence contained in SEQ ID NO: 1 or SEQ ID NO: 3,

c) derivatives of the nucleic acid sequence depicted in SEQ ID NO: 1 or SEQ ID NO: 3 which encode polypeptides using the amino acid sequences depicted in SEQ ID NO: 2 or SEQ ID NO: 4 and have at least 75 % homology on the amino acid level with SEQ ID NO: 2 or SEQ ID NO: 4 and possess A 6-desaturase activity

[0007] The nucleic acid sequences encode polypeptides having a A 6-desaturase activity and originate from plants, advantageously Primulaceae such as Muscariodides or Aleuritia are specific for the conversion of ω-3 fatty acids and thus they preferably convert by way of example α-linolenic acid and not linoleic acid when they are expressed in a heterologous system and both fatty acids are available in the organism. By this means stearidonic acid, eicosapentaenoic acid or docosahexaenoic acid are produced in the host organisms such as plants or microorganisms without formation of arachidonic acid. This results in an advantageous synthesis of fatty acids of the ω-3 fatty acid family, while ω-6 fatty acids are scarcely formed if they occur at all. The Δ 6-desaturases according to the invention exhibit a higher activity towards ω-3 fatty acids as compared to ω-6 fatty acids by at least the factor 1.5, advantageously by at the least the factor 2, preferably by at least the factor 3, particularly preferably by at least the factor 4 and most particularly preferably by at least the factor 5. Due to this specificity the formation of unwanted fatty acids can be suppressed or completely prevented.

[0008] By derivative(s) of the sequences is meant, for example, functional homologues of the polypeptides or enzymes encoded by SEQ ID NO: 1 or SEQ ID NO: 3 which exhibit the same said specific enzymatic activity. This specific enzymatic activity allows advantageously the synthesis of unsaturated fatty acids having more than three double bonds in the fatty acid molecule. By unsaturated fatty acids is meant in what follows diunsaturated or polyunsaturated fatty acids which possess double bonds. The double bonds may be conjugated or nonconjugated. The said sequences encode enzymes which exhibit Δ 6-desaturase activity.

[0009] The enzyme, Δ 6-desaturase, advantageously introduces a cis double bond into fatty acid residues of glycerolipids at position C_6-C_7 (see SEQ ID NO: 1 and SEQ ID NO: 3). The enzymes additionally have a Δ 6-desaturase activity which advantageously introduces exclusively a cis double bond into fatty acid residues of glycerolipids at position C_6-C_7. This activity is also possessed by the enzymes having the sequences specified in SEQ ID NO: 1 and NO: 3 which are monofunctional A 6-desaturases.

[0010] The nucleic acid sequence(s) (for purposes of the application the singular encompasses the plural and vice versa) may advantageously be used for isolating other genomic sequences via homology screening.

[0011] The said derivatives may be isolated, for example, from other organisms, eukaryotic organisms such as plants, especially mosses, dinoflagellates or fungi.

[0012] Allele variants include in particular functional variants obtainable by deletion, insertion or substitution of nucleotides in the sequences depicted in SEQ ID NO: 1 or SEQ ID NO: 3, the enzymatic activity of the derived synthesized proteins being retained.

[0013] Starting from the DNA sequence described in SEQ ID NO: 1 and SEQ ID NO: 3 or parts of said sequences such DNA sequences can be isolated using, for example, normal hybridization methods or the PCR technique from other eukaryotes such as those identified above for example. These DNA sequences hybridize under standard conditions with the said sequences. For hybridization use is advantageously made of short oligonucleotides of the conserved regions, for example, which can be determined by comparisons with other desaturase genes in the manner known to those skilled in the art. The histidine box sequences are advantageously employed. However, longer fragments of the nucleic acids according to the invention or the complete sequences may also be used for hybridization. Depending on the nucleic acid employed: oligonucleotide, longer fragment or complete sequence, or depending on which type of nucleic acid, DNA or RNA, is used for hybridization these standard conditions vary. Thus, for example, the melting temperatures of DNA:DNA hybrids are approximately 10 °C lower than those of DNA:RNA hybrids of the same length.

[0014] By standard conditions is meant, for example, depending on the nucleic acid in question temperatures between 42 °C and 58 °C in an aqueous buffer solution having a concentration of between 0.1 and 5 x SSC (1 X SSC = 0.15 M NaCl, 15 mM sodium citrate, pH 7.2) or additionally in the presence of 50 % formamide, such as by way of example 42 °C in 5 x SSC, 50 % formamide. Hybridization conditions for DNA:DNA hybrids are advantageously 0.1 x SSC and temperatures between approximately 20 °C and 45 °C, preferably between approximately 30 °C and 45 °C. For DNA:RNA hybrids the hybridization conditions are advantageously 0.1 x SSC and temperatures between approximately 30 °C and 55 °C, preferably between approximately 45 °C and 55 °C. These specified temperatures for hybridization are melting temperature values calculated by way of example for a nucleic acid having a length of approximately 100 nucleotides and a G + C content of 50 % in the absence of formamide. The experimental conditions for DNA hybridization are described in relevant genetics textbooks such as by way of example Sambrook et al., -Molecular Cloning-, Cold Spring Harbor Laboratory, 1989, and may be calculated by formulae known to those skilled in the art, for example as a function of the length of the nucleic acids, the nature of the hybrids or the G + C content. Those skilled in the art may draw on the following textbooks for further information on hybridization: Ausubel et al. (eds), 1985, Current Protocols in Molecular Biology, John Wiley & Sons, New York; Hames and Higgins (eds), 1985, Nucleic Acids Hybridization: A Practical Approach, IRL Press at Oxford University Press, Oxford; Brown (ed), 1991, Essential Molecular Biology: A Practical Approach, IRL Press at Oxford University Press, Oxford.

[0015] Furthermore, by derivatives is meant homologues of the sequences SEQ ID NO: 1 and NO: 3, for example eukaryotic homologues, truncated sequences, single-stranded DNA of the encoding and nonencoding DNA sequence or RNA of the encoding and nonencoding DNA sequence.

[0016] In addition, by homologues of the sequences SEQ ID NO: 1 and SEQ ID NO: 3 is meant derivatives such as

by way of example promoter variants. These variants may be modified by one or more nucleotide exchanges, by insertion (s) and/or deletion(s) without, however, adversely affecting the functionality or efficiency of the promoters. Furthermore, the promoters can have their efficiency increased by altering their sequence or be completely replaced by more effective promoters even of foreign organisms.

[0017] By derivatives is also advantageously meant variants whose nucleotide sequence has been altered in the region from -1 to - 2000 ahead of the start codon in such a way that the gene expression and/or the protein expression is modified, preferably increased. Furthermore, by derivatives is also meant variants which have been modified at the 3' end.

[0018] By derivatives is also meant the antisense DNAs which may be employed for inhibiting protein biosynthesis of the proteins according to the invention. These antisense DNAs are numbered among the nonfunctional derivatives according to the invention such as derivatives which exhibit no enzymatic activity. Other methods known to those skilled in the art for the production of nonfunctional derivatives are what is known as cosuppression, the use of ribozymes and introns and the RNAi method. Ribozymes are catalytic RNA molecules having ribonuclease activity which can chop single-stranded nucleic acids, such as mRNA, with which they are complementary. In this way, using these ribozymes (Haselhoff and Gerlach, Nature, 334, 1988: 585-591) mRNA transcripts can be catalytically cleaved and, thus, the translation of this mRNA is suppressed. Ribozymes of this type can be specially tailored to their purpose (US 4,987,071; US 5,116,742 and Bartel et al., Science 261, 1993: 1411-1418). By this means, with the aid of the antisense DNA, fatty acids, lipids or oils having an increased content of saturated fatty acids can be produced.

[0019] The nucleic acid sequence which encodes a Δ6-desaturase may be produced by synthesis or obtained naturally or contain a mixture of synthetic and natural DNA components as well as consist of various heterologous Δ 6-desaturase gene segments from different organisms. In general, synthetic nucleotide sequences are produced with codons which are preferred by the corresponding host organisms, plants for example. This usually results in optimum expression of the heterologous gene. These codons preferred by plants may be determined from codons having the highest protein frequency which are expressed in most of the plant species of interest. An example concerning Corynebacterium glutamicum is provided in Wada et al. (1992) Nucleic Acids Res. 20:2111-2118). Such experiments can be carried out using standard methods and are known to the person skilled in the art.

[0020] Functionally equivalent sequences which encode the A 6-desaturase gene are those derivatives of the sequence according to the invention which despite differing nucleotide sequence still possess the desired functions, that is to say the enzymatic activity and specific selectivity of the proteins. Thus, functional equivalents include naturally occurring variants of the sequences described herein as well as artificial ones, e.g. artificial nucleotide sequences adapted to the codon use of a plant which have been obtained by chemical synthesis.

[0021] In addition, artificial DNA sequences are suitable, provided, as described above, they mediate the desired property, for example an increase in the content of Δ 6 double bonds in fatty acids, oils or lipids in organisms such as in a plant by overexpression of the Δ 6-desaturase gene in crop plants. Such artificial DNA sequences can exhibit Δ 6-desaturase activity, for example by back-translation of proteins constructed by means of molecular modeling, or be determined by in vitro selection. Possible techniques for in vitro evolution of DNA to modify or improve the DNA sequences are described in Patten, P.A. et al., Current Opinion in Biotechnology 8, 724-733(1997) or in Moore, J.C. et al., Journal of Molecular Biology 272, 336-347 (1997). Particularly suitable are encoding DNA sequences which are obtained by back-translation of a polypeptide sequence in accordance with the codon use specific to the host plant. Those skilled in the art familiar with the methods of plant genetics can easily determine the specific codon use by computer analyses of other known genes of the plant to be transformed.

[0022] Other suitable equivalent nucleic acid sequences which may be mentioned are sequences that encode fusion proteins, a component of the fusion protein being a Δ 6-desaturase polypeptide or a functionally equivalent part thereof. The second part of the fusion protein can be, for example, another polypeptide having enzymatic activity or an antigenic polypeptide sequence by means of which it is possible to demonstrate Δ 6-desaturase expression (e.g. myc tag or his tag). Preferably, however, this is a regulatory protein sequence, such as by way of example a signal sequence for the endoplasmic reticulum (= ER) which directs the Δ 6-desaturase protein to the desired point of action, or regulatory sequences which influence the expression of the nucleic acid sequence , such as promoters or terminators.

[0023] Advantageously, the Δ 6-desaturase genes may be combined with other genes for fatty acid biosynthesis in the method recited in the claims. Examples of such genes are the acetyl transferases, other desaturases or elongases such as A 4-, Δ 5-, Δ 6- or Δ 8-desaturases or ω-3- and/or ω-6-specific desaturases such as A 12 (for C_{18} fatty acids), Δ 15 (for C_{18} fatty acids) or A 19 (for C_{22} fatty acids) or such as Δ 5- or Δ 6-elongases. For in vivo and especially in vitro synthesis combination with e.g. NADH cytochrome B5 reductases which can take up or release reduction equivalents is advantageous.

[0024] By the amino acid sequences it is meant proteins which contain an amino acid sequence depicted in the sequences SEQ ID NO: 2 and SEQ ID NO: 4 or a sequence obtainable therefrom by substitution, inversion, insertion or deletion of one or more amino acid groups, the enzymatic activities of the proteins depicted in SEQ ID NO: 2 and NO: 4 being retained or not substantially reduced, that is they still possess the same enzymatic activity. By -not substantially

reduced- or -the same enzymatic activity- is meant all enzymes which still exhibit at least 10 %, preferably 20 %, particularly preferably 30 %, of the enzymatic activity of the initial enzyme obtained from the wild form of the said Primulaceae organism. In doing this, for example, certain amino acids may be replaced by others having similar physicochemical properties (space filling, basicity, hydrophobicity, etc.). For example, arginine residues are exchanged for lysine residues, valine residues for isoleucine residues or aspartic acid residues for glutamic acid residues. However, one or more amino acids may also be swapped in sequence, added or removed, or a plurality of these measures may be combined with one another.

[0025] By derivatives is also meant functional equivalents which in particular also contain natural or artificial mutations of an originally isolated sequence encoding Δ6-desaturase which continue to exhibit the desired function, that is the enzymatic activity and substrate selectivity thereof is not substantially reduced. Mutations comprise substitutions, additions, deletions, exchanges or insertions of one or more nucleotide residues. Thus, for example, the present invention also encompasses those nucleotide sequences which are obtained by modification of the Δ 6-desaturase nucleotide sequence. The aim of such a modification may be, e.g., to further bound the encoding sequence contained therein or also, e.g., to insert further restriction enzyme interfaces.

[0026] Functional equivalents also include those variants whose function by comparison with the initial gene or gene fragment is weakened (= not substantially reduced) or reinforced (= enzyme activity higher than the activity of the initial enzyme, that is activity is higher than 100 %, preferably higher than 110 %, particularly preferably higher than 130 %).

[0027] At the same time the nucleic acid sequence may, for example, advantageously be a DNA or cDNA sequence. Suitable encoding sequences for insertion into an expression cassette according to the invention include by way of example those which encode a Δ 6-desaturase with the sequences described above and lend the host the ability to overproduce fatty acids, oils or lipids having double bonds in the Δ6 position, it being advantageous when at the same time ω-3 fatty acids having at least four double bonds are produced. These sequences may be of homologous or heterologous origin.

[0028] By the expression cassette (= nucleic acid construct or fragment or gene construct) it is meant the sequences specified in SEQ ID NO: 1 and SEQ ID NO:3 which result from the genetic code and/or functional or nonfunctional derivatives thereof which are functionally linked with one or more regulation signals advantageously to increase the gene expression and which control the expression of the encoding sequence in the host cell. These regulatory sequences should allow the selective expression of the genes and the protein expression. Depending on the host organism this may mean, for example, that the gene is expressed and/or overexpressed only after induction or that it is expressed and/or overexpressed immediately. Examples of these regulatory sequences are sequences to which inductors or repressors bind and in this way regulate the expression of the nucleic acid. In addition to these new regulation sequences or instead of these sequences the natural regulation of these sequences ahead of the actual structural genes may still be present and optionally have been genetically modified so that natural regulation was switched off and the expression of the genes increased. However, the gene construct can also be built up more simply, that is no additional regulation signals have been inserted ahead of the nucleic acid sequence or derivatives thereof and the natural promoter with its regulation has not been removed. Instead of this the natural regulation sequence was mutated in such a way that no further regulation ensues and/or the gene expression is heightened. These modified promoters in the form of part sequences (= promoter containing parts of the nucleic acid sequences according to the invention) can also be brought on their own ahead of the natural gene to increase the activity. In addition, the gene construct may advantageously also contain one or more so-called enhancer sequences functionally linked to the promoter which allow enhanced expression of the nucleic acid sequence. At the 3' end of the DNA sequences additional advantageous sequences may also be inserted, such as further regulatory elements or terminators. The Δ 6-desaturase gene may be present in one or more copies in the expression cassette (= gene construct).

[0029] As described above, the regulatory sequences or factors can preferably positively influence and so increase the gene expression of the introduced genes. Thus, reinforcement of the regulatory elements advantageously on the transcription level may be effected by using powerful transcription signals such as promoters and/or enhancers. However, in addition reinforcement of translation is also possible, for example by improving the stability of the mRNA.

[0030] Suitable promoters in the expression cassette are in principle all promoters which can control the expression of foreign genes in organisms such as microorganisms like protozoa such as ciliates, algae such as green, brown, red or blue algae, bacteria such as gram-positive or gram-negative bacteria, yeasts such as Saccharomyces, Pichia or Schizosaccharomyces or fungi such as Mortierella, Traustochytrium or Schizochytrium, advantageously in plants or fungi. Use is preferably made in particular of plant promoters or promoters derived from a plant virus. Advantageous regulation sequences for the method recited in the claims are found for example in promoters such as cos, tac, trp, tet, trp-tet, lpp, lac, lpp-lac, $lacI^{q-}$, T7, T5, T3, gal, trc, ara, SP6, λ-P_R or in λ-P_L promoters which are employed advantageously in gram-negative bacteria. Other advantageous regulation sequences are found, for example, in the gram-positive promoters amy and SPO2, in the yeast or fungal promoters ADC1, MFα, AC, P-60, CYC1, GAPDH, TEF, rp28, ADH or in the plant promoters CaMV/35S [Franck et al., Cell 21(1980) 285-294], SSU, OCS, lib4, STLS1, B33, nos (= Nopalin Synthase Promoter) or in the ubiquintin promoter. The expression cassette may also contain a chemically inducible

promoter by means of which the expression of the exogenous Δ 6-desaturase gene in the organisms can be controlled advantageously in the plants at a particular time. Advantageous plant promoters of this type are by way of example the PRP1 promoter [Ward et al., Plant.Mol. Biol.22(1993), 361-366], a promoter inducible by benzenesulfonamide (EP 388186), a promoter inducible by tetracycline (Gatz et al., (1992) Plant J. 2,397-404), a promoter inducible by salicylic acid (WO 95/19443), a promoter inducible by abscisic acid (EP335528) and a promoter inducible by ethanol or cyclohexanone (WO93/21334). Other examples of plant promoters which can advantageously be used are the promoter of cytosolic FBPase from potato, the ST-LSI promoter from potato (Stockhaus et al., EMBO J. 8 (1989) 2445-245), the promoter of phosphoribosyl pyrophosphate amidotransferase from Glycine max (see also gene bank accession number U87999) or a nodiene-specific promoter as described in EP 249676. Particularly advantageous are those plant promoters which ensure expression in tissues or plant parts/organs in which fatty acid biosynthesis or the precursor stages thereof occurs, as in endosperm or in the developing embryo for example. Particularly noteworthy are advantageous promoters which ensure seed-specific expression such as by way of example the USP promoter or derivatives thereof, the LEB4 promoter, the phaseolin promoter or the napin promoter. The particularly advantageous USP promoter cited according to the invention or its derivatives mediate very early gene expression in seed development (Baeumlein et al., Mol Gen Genet, 1991, 225 (3): 459-67). Other advantageous seed-specific promoters which may be used for monocotylodonous or dicotylodonous plants are the promoters suitable for dicotylodons such as napin gene promoters, likewise cited by way of example, from oilseed rape (US 5,608,152), the oleosin promoter from Arabidopsis (WO 98/45461), the phaseolin promoter from Phaseolus vulgaris (US 5,504,200), the Bce4 promoter from Brassica (WO 91/13980) or the leguminous B4 promoter (LeB4, Baeumlein et al., Plant J., 2, 2, 1992: 233 - 239) or promoters suitable for monocotylodons such as the promoters of the lpt2 or lpt1 gene in barley (WO 95/15389 and WO 95/23230) or the promoters of the barley hordeine gene, the rice glutelin gene, the rice oryzin gene, the rice prolamin gene, the wheat gliadin gene, the white glutelin gene, the corn zein gene, the oats glutelin gene, the sorghum kasirin gene or the rye secalin gene which are described in WO99/16890.

[0031] Furthermore, particularly preferred are those promoters which ensure the expression in tissues or plant parts in which, for example, the biosynthesis of fatty acids, oils and lipids or the precursor stages thereof takes place. Particularly noteworthy are promoters which ensure a seed-specific expression. Noteworthy are the promoter of the napin gene from oilseed rape (US 5,608,152), the USP promoter from Vicia faba (USP = unknown seed protein, Baeumlein et al., Mol Gen Genet, 1991, 225 (3): 459-67), the promoter of the oleosin gene from Arabidopsis (WO98/45461), the phaseolin promoter (US 5,504,200) or the promoter of the legumin B4 gene (LeB4; Baeumlein et al., 1992, Plant Journal, 2 (2): 233-9). Other promoters to be mentioned are that of the lpt2 or lpt1 gene from barley (WO95/15389 and WO95/23230) which mediate seed-specific expression in monocotyledonous plants.

[0032] As described above, the expression construct (= gene construct, nucleic acid construct) may contain yet other genes which are to be introduced into the organisms. These genes can be subject to separate regulation or be subject to the same regulation region as the Δ6-desaturase gene. These genes are by way of example other biosynthesis genes, advantageously for fatty acid biosynthesis, which allow increased synthesis. Examples which may be mentioned are the genes for Δ 15-, Δ 12-, Δ 9-, A 6-, Δ 5-, Δ 4-desaturase, β-ketoacyl reductases, β-ketoacyl synthases, elongases or the various hydroxylases and acyl-ACP thioesterases. The desaturase genes are advantageously used in the nucleic acid construct.

[0033] In principle all atural promoters with their regulation sequences can be used like those named above for the expression cassette and the method referred to in the claims. Over and above this, synthetic promoters may also advantageously be used.

[0034] In the preparation of an expression cassette various DNA fragments can be manipulated in order to obtain a nucleotide sequence which usefully reads in the correct direction and is equipped with a correct reading raster. To connect the DNA fragments (= nucleic acids according to the invention) to one another adaptors or linkers may be attached to the fragments.

[0035] The promoter and the terminator regions can usefully be provided in the transcription direction with a linker or polylinker containing one or more restriction points for the insertion of this sequence. Generally, the linker has 1 to 10, mostly 1 to 8, preferably 2 to 6, restriction points. In general the size of the linker inside the regulatory region is less than 100 bp, frequently less than 60 bp, but at least 5 bp. The promoter may be both native or homologous as well as foreign or heterologous to the host organism, for example to the host plant. In the '-3' transcription direction the expression cassette contains the promoter, a DNA sequence which encodes a Δ 6-desaturase gene and a region for transcription termination. Different termination regions can be exchanged for one another in any desired fashion.

[0036] Furthermore, manipulations which provide suitable restriction interfaces or which remove excess DNA or restriction interfaces can be employed. Where insertions, deletions or substitutions, such as transitions and transversions, come into consideration, *in vitro* mutagenesis, primer repair, restriction or ligation may be used. In suitable manipulations such as restriction, chewing back or filling of overhangs for blunt ends complementary ends of the fragments can be provided for the ligation.

[0037] For an advantageous high expression the attachment of the specific ER retention signal SEKDEL inter alia can

be of importance (Schouten, A. et al., Plant Mol. Biol. 30 (1996), 781-792). In this way the average expression level is tripled or even quadrupled. Other retention signals which occur naturally in plant and animal proteins located in the ER may also be employed for the construction of the cassette.

[0038] Preferred polyadenylation signals are plant polyadenylation signals, preferably those which substantially correspond to T-DNA polyadenylation signals from Agrobacterium tumefaciens, in particular gene 3 of the T-DNA (octopin synthase) of the Ti plasmid pTiACH5 (Gielen et al., EMBO J.3 (1984), 835 et seq.) or corresponding functional equivalents.

[0039] An expression cassette is produced by fusion of a suitable promoter with a suitable A 6-desaturase DNA sequence together with a polyadenylation signal by common recombination and cloning techniques as described, for example, in T. Maniatis, E.F. Fritsch and J. Sambrook, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989) as well as in T.J. Silhavy, M.L. Berman and L.W. Enquist, Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1984) and in Ausubel, F.M. et al., Current Protocols in Molecular Biology, Greene Publishing Assoc. and Wiley-Interscience (1987).

[0040] In the preparation of an expression cassette various DNA fragments can be manipulated to produce a nucleotide sequence which usefully reads in the correct direction and is equipped with a correct reading raster. Adapters or linkers can be attached to the fragments for joining the DNA fragments.

[0041] The promoter and the terminator regions can usefully be provided in the transcription direction with a linker or polylinker containing one or more restriction points for the insertion of this sequence. Generally, the linker has 1 to 10, mostly 1 to 8, preferably 2 to 6, restriction points. In general the size of the linker inside the regulatory region is less than 100 bp, frequently less than 60 bp, but at least 5 bp. The promoter may be both native or homologous as well as foreign or heterologous to the host organism, for example to the host plant. In the 5'-3' transcription direction the expression cassette contains the promoter, a DNA sequence which encodes a Δ 6-desaturase gene and a region for transcription termination. Different termination regions can be exchanged for one another in any desired fashion.

[0042] In the preparation of an expression cassette various DNA fragments can be manipulated to produce a nucleotide sequence which usefully reads in the correct direction and is equipped with a correct reading raster. Adapters or linkers can be attached to the fragments for joining the DNA fragments.

[0043] The DNA sequences encoding two Δ 6-desaturases from Muscariodides vialii and Aleuritia farinosa contain all the sequence characteristics needed to achieve correct localization of the site of fatty acid, lipid or oil biosynthesis. Accordingly, no further targeting sequences are needed per se. However, such a localization may be desirable and advantageous and hence artificially modified or reinforced so that such fusion constructs are also a preferred advantageous embodiment of the invention.

[0044] Particularly preferred are sequences which ensure targeting in plastids. Under certain circumstances targeting into other compartments (reported in: Kermode, Crit. Rev. Plant Sci. 15, 4 (1996), 285-423) may also be desirable, e.g. into vacuoles, the mitochondrium, the endoplasmic reticulum (ER), peroxisomes, lipid structures or due to lack of corresponding operative sequences retention in the compartment of origin, the cytosol. Advantageously, the nucleic acid sequences or the gene construct together with at least one reporter gene are cloned into an expression cassette which is introduced into the organism via a vector or directly into the genome. This reporter gene should allow easy detection via a growth, fluorescence, chemical, bioluminescence or resistance assay or via a photometric measurement. Examples of reporter genes which may be mentioned are antibiotic- or herbicide-resistance genes, hydrolase genes, fluorescence protein genes, bioluminescence genes, sugar or nucleotide metabolic genes or biosynthesis genes such as the Ura3 gene, the Ilv2 gene, the luciferase gene, the β-galactosidase gene, the gfp gene, the 2-desoxyglucose-6-phosphate phosphatase gene, the β-glucuronidase gene, β-lactamase gene, the neomycin phosphotransferase gene, the hygromycin phosphotransferase gene or the BASTA (= gluphosinate-resistance) gene. These genes permit easy measurement and quantification of the transcription activity and hence of the expression of the genes. In this way genome positions may be identified which exhibit differing productivity.

[0045] Also an expression cassette disclosed is that comprises upstream, i.e. at the 5' end of the encoding sequence, a promoter and downstream, i.e. at the 3' end, a polyadenylation signal and optionally other regulatory elements which are operably linked to the intervening encoding sequence for Δ 6-desaturase and/or Δ 6-desaturase DNA sequence. By an operable linkage is meant the sequential arrangement of promoter, encoding sequence, terminator and optionally other regulatory elements in such a way that each of the regulatory elements can fulfill its function in the expression of the encoding sequence in due manner. The sequences preferred for operable linkage are targeting sequences for ensuring subcellular localization in plastids. However, targeting sequences for ensuring subcellular localization in the mitochondrium, in the endoplasmic reticulum (= ER), in the nucleus, in oil corpuscles or other compartments may also be employed as well as translation promoters such as the 5' lead sequence in tobacco mosaic virus (Gallie et al., Nucl. Acids Res. 15 (1987), 8693 -8711).

[0046] An expression cassette may, for example, contain a constitutive promoter or a tissue-specific promoter (preferably the USP or napin promoter) the gene to be expressed and the ER retention signal. For the ER retention signal the KDEL amino acid sequence (lysine, aspartic acid, glutamic acid, leucine) or the KKX amino acid sequence (lysine-lysine-X-stop, wherein X means every other known amino acid) is preferably employed.

[0047] For expression in a prokaryotic or eukaryotic host organism, for example a microorganism such as a fungus or a plant the expression cassette is advantageously inserted into a vector such as by way of example a plasmid, a phage or other DNA which allows optimum expression of the genes in the host organism. Examples of suitable plasmids are: in E. coli pLG338, pACYC184, pBR series such as e.g. pBR322, pUC series such as pUC18 or pUC19, M113mp series, pKC30, pRep4, pHS1, pHS2, pPLc236, pMBL24, pLG200, pUR290, pIN-III^{113}-B1, λgt11 or pBdCI; in Streptomyces pIJ101, pIJ364, pIJ702 or pIJ361; in Bacillus pUB110, pC194 or pBD214; in Corynebacterium pSA77 or pAJ667; in fungi pALS1, pIL2 or pBB116; other advantageous fungal vectors are described by Romanos, M.A. et al., [(1992) -Foreign gene expression in yeast: a review-, Yeast 8: 423-488] and by van den Hondel, C.A.M.J.J. et al. [(1991) -Heterologous gene expression in filamentous fungi- as well as in More Gene Manipulations in Fungi [J.W. Bennet & L.L. Lasure, eds., pp. 396-428: Academic Press: San Diego] and in - Gene transfer systems and vector development for filamentous fungi- [van den Hondel, C.A.M.J.J. & Punt, P.J. (1991) in: Applied Molecular Genetics of Fungi, Peberdy, J.F. et al., eds., pp. 1-28, Cambridge University Press: Cambridge]. Examples of advantageous yeast promoters are 2∝M, pAG-1, YEp6, YEp13 or pEMBLYe23. Examples of algal or plant promoters are pLGV23, pGHlac^{+}, pBIN19, pAK2004, pVKH or pDH51 (see Schmidt, R. and Willmitzer, L., 1988). The vectors identified above or derivatives of the vectors identified above are a small selection of the possible plasmids. Further plasmids are well known to those skilled in the art and may be found, for example, in the book Cloning Vectors (Eds. Pouwels P.H. et al. Elsevier, Amsterdam-New York-Oxford, 1985 , ISBN 0 444 904018). Suitable plant vectors are described inter alia in -Methods in Plant Molecular Biology and Biotechnology- (CRC Press), Ch. 6/7, pp. 71-119. Advantageous vectors are known as shuttle vectors or binary vectors which replicate in E. coli and Agrobacterium.

[0048] By vectors is meant with the exception of plasmids all other vectors known to those skilled in the art such as by way of example phages, viruses such as SV40, CMV, baculovirus, adenovirus, transposons, IS elements, phasmids, phagemids, cosmids, linear or circular DNA. These vectors can be replicated autonomously in the host organism or be chromosomally replicated, chromosomal replication being preferred.

[0049] Also, in the vector the expression cassette may also advantageously be introduced into the organisms in the form of a linear DNA and be integrated into the genome of the host organism by way of heterologous or homologous recombination. This linear DNA may be composed of a linearized plasmid or only of the expression cassette as vector or the nucleic acid sequences according to the invention.

[0050] Moreovecr the nucleic acid sequence can also be advantageous the nucleic acid sequence can also be introduced into an organism on its own.

[0051] If in addition to the nucleic acid sequence further genes are to be introduced into the organism, all together with a reporter gene in a single vector or each single gene with a reporter gene in a vector in each case can be introduced into the organism, whereby the different vectors can be introduced simultaneously or successively.

[0052] The vector advantageously contains at least one copy of the nucleic acid sequences according to the invention and/or the expression cassette (= gene construct) according to the invention.

[0053] By way of example the plant expression cassette can be installed in the pRT transformation vector ((a) Toepfer et al., 1993, Methods Enzymol., 217: 66-78; (b) Toepfer et al. 1987, Nucl. Acids. Res. 15: 5890 ff.).

[0054] Alternatively, a recombinant vector (= expression vector) can also be transcribed and translated in vitro, e.g. by using the T7 promoter and the T7 RNA polymerase.

[0055] The introduction of the nucleic acids according to the invention, the expression cassette or the vector into flax can in principle be done by all of the methods known to those skilled in the art. The introduction of the nucleic acid sequences gives rise to recombinant or transgenic organisms.

[0056] The transfer of foreign genes into the genome of a plant is called transformation. In doing this the methods described for the transformation and regeneration of plants from plant tissues or plant cells are utilized for transient or stable transformation. Suitable methods are protoplast transformation by poly(ethylene glycol)-induced DNA uptake, the -biolistic- method using the gene cannon - referred to as the particle bombardment method, electroporation, the incubation of dry embryos in DNA solution, microinjection and gene transfer mediated by Agrobacterium. Said methods are described by way of example in B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, eds. S.D. Kung and R. Wu, Academic Press (1993) 128-143 and in Potrykus Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991) 205-225). The construct to be expressed is preferably cloned into a vector which is suitable for transforming Agrobacterium tumefaciens, for example pBin19 (Bevan et al., Nucl. Acids Res. 12 (1984) 8711). Agrobacteria transformed by such a vector can then be used in known manner for the transformation of plants, in particular of crop plants such as by way of example tobacco plants, for example by bathing bruised leaves or chopped leaves in an agrobacterial solution and then culturing them in suitable media. The transformation of plants by means of Agrobacterium tumefaciens is described, for example, by Höfgen and Willmitzer in Nucl. Acid Res. (1988) 16, 9877 or is known inter alia from F.F. White, Vectors for Gene Transfer in Higher Plants; in Transgenic Plants, Vol. 1, Engineering and Utilization, eds. S.D. Kung and R. Wu, Academic Press, 1993, pp. 15-38.

[0057] Agrobacteria transformed by an expression vector according to the invention may likewise be used in known manner for the transformation of plants such as test plants like Arabidopsis or crop plants such as cereal crops, corn,

oats, rye, barley, wheat, soybean, rice, cotton, sugar beet, canola, sunflower, flax, hemp, potatoes, tobacco, tomatoes, carrots, paprika, oilseed rape, tapioca, cassava, arrowroot, tagetes, alfalfa, lettuce and the various tree, nut and vine species, in particular of oil-containing crop plants such as soybean, peanut, castor oil plant, sunflower, corn, cotton, flax, oilseed rape, coconut, oil palm, safflower (Carthamus tinctorius) or cocoa bean, e.g. by bathing bruised leaves or chopped leaves in an agrobacterial solution and then culturing them in suitable media. For the production of PUFAs, for example stearidonic acid, eicosapentaenoic acid and docosahexaenoic acid, borage or Primulaceae are advantageously suitable.

[0058] The genetically modified plant cells may be regenerated by all of the methods known to those skilled in the art. Appropriate methods can be found in the publications referred to above by S.D. Kung and R. Wu, Potrykus or Höfgen and Willmitzer.

[0059] A Suitable organism or host organism for the nucleic acid, expression cassette or vector is Hax.

[0060] Within the framework of the present invention, increasing the content of fatty acids, oils or lipids possessing Δ 6 double bonds means, for example, the artificially acquired trait of increased biosynthetic performance due to functional overexpression of the Δ 6-desaturase gene in the organisms according to the invention, advantageously in the transgenic plants according to the invention, by comparison with the nongenetically modified initial plants at least for the duration of at least one plant generation.

[0061] The preferred locus of biosynthesis, of fatty acids, oils or lipids for example, is generally the seed or cell layers of the seed so that a seed-specific expression of the Δ 6-desaturase gene is appropriate. It is, however, obvious that the biosynthesis of fatty acids, oils or lipids need not be limited to the seed tissue but rather can also occur in tissue-specific manner in all other parts of the plant - in epidermis cells or in the nodules for example.

[0062] A constitutive expression of the exogenous Δ 6-desaturase gene is, moreover, advantageous. On the other hand, however, an inducible expression may also appear desirable.

[0063] The efficiency of the expression of the Δ 6-desaturase gene can be determined, for example, *in vitro* by shoot meristem propagation. In addition, an expression of the Δ 6-desaturase gene modified in nature and level and its effect on fatty acid, oil or lipid biosynthesis performance can be tested on test plants in greenhouse trials.

[0064] The present disclosure comprises transgenic organisms such as transgenic plants transformed by an expression cassette containing a Δ 6-desaturase gene sequence or DNA sequences hybridizing therewith, as well as transgenic cells, tissue, parts and reproduction material of such plants. Particular preference is given in this case to . .

[0065] Disclosed herein are futhermore:

- A method for the transformation of a plant comprising the introduction of expression cassettes according to the invention containing a Δ6-desaturase gene sequence derived from Primulaceae or DNA' sequences hybridizing therewith into a plant cell, into callus tissue, an entire plant or protoplasts of plants.

- A method for producing PUFAs, wherein the method comprises the growing of a transgenic organism comprising a nucleic acid as claimed in claims 1 to 4, a gene construct as claimed in claim 6 or a vector as claimed in claim 7 encoding a Δ 6-desaturase which specifically desaturates ω-3 fatty acids, and wherein due to the activity of the Δ 6-desaturase PUFAs are formed in the organism which exhibit an increased content of ω-3 fatty acids. In this method ω-3 fatty acids such as stearidonic acid, eicosapentaenoic acid or docosahexaenoic acid are advantageously produced.

- Use of a Δ 6-desaturase DNA gene sequence or DNA sequences hybridizing therewith for the production of plants having an increased content of fatty acids, oils or lipids having Δ 6 double bonds due to the expression of said Δ 6-desaturase DNA sequence in plants.

- Use of a Δ 6-desaturase DNA gene sequence DNA sequences hybridizing therewith for the production of plants having an increased content of fatty acids, oils or lipids having Δ 6 double bonds, particularly of ω-3 fatty acids, due to the expression of said Δ6-desaturase DNA sequence in plants.

- Proteins containing the amino acid sequences depicted in SEQ ID NO: 2 or NO: 4.

- Use of said proteins having the sequences SEQ ID NO: 2 or NO: 4 for producing unsaturated fatty acids.

[0066] A method for producing triglycerides having an increased content of unsaturated fatty acids comprising: introducing at least one said nucleic acid sequence according to the invention or at least one expression cassette according to the invention into an oil-producing organism; growing said organism; and isolating oil contained in said organism; is applied for the production of the oil of the invention as claimed in claims 1 and 2

[0067] The methods identified above advantageously allow the synthesis of triglycerides having an increased content of fatty acids containing Δ 6 double bonds, wherein the substrate used for the reaction of the A 6-desaturase is preferably

α-linolenic acid. In this way the method identified above advantageously allows the synthesis of fatty acids derived from stearidonic acid ($C_{18:4}^{\Delta 6, 9, 12, 15}$) such as by way of example eicosapentaenoic acid and docosahexaenoic acid.

[0068] Organism for the said method is that.

[0069] After transformation plants are first of all regenerated as described above and then cultured or cultivated as normal.

[0070] After growth the lipids are isolated from the organisms in the usual way. For this purpose, after harvesting the organisms may first of all be digested or used directly. The lipids are advantageously extracted using suitable solvents such as apolar solvents like hexane or ethanol, isopropanol or mixtures such as hexane/isopropanol, phenol/chloroform/isoamyl alcohol at temperatures of between 0 °C and 80 °C, preferably between 20 °C and 50°C. The biomass is usually extracted with an excess of solvent, for example an excess of solvent to biomass of 1:4. The solvent is then removed, for example by distillation. Extraction can also be done using supercritical CO_2. After extraction the remaining biomass may be removed, for example by filtration.

[0071] The crude oil isolated in this way can then be further purified, for example by removing cloudiness by treatment with polar solvents such as acetone or chloroform and then filtration or centrifugation. Further purification through columns is also possible.

[0072] The further object of the invention comprises unsaturated fatty triglycerides having an increased content of unsaturated fatty acids produced by the methods identified above can be used for producing foods, animal feeds, cosmetics or pharmaceuticals. For this purpose the latter are added in customary quantities to the foods, the animal feed, the cosmetics or pharmaceuticals.

[0073] Said triglycerides having an increased content of unsaturated fatty acids produced by the methods identified above are the result of the expression of the nucleic acids according to the invention in the various host organisms. This results overall in a modification of the composition of the compounds in the host cell containing unsaturated fatty acids by comparison with the original starting host cells which do not contain the nucleic acids. These modifications are more marked in host organisms, for example plant cells, which naturally do not contain the proteins or enzymes encoded by the nucleic acids than in host organisms which naturally do contain the proteins or enzymes encoded by the nucleic acids. This gives rise to host organisms containing oils, lipids, phospholipids, sphingolipids, glycolipids, triacylglycerols and/or free fatty acids having a higher content of PUFAs. For the purposes of the invention, by an increased content is meant that the host organisms contain at least 5 %, advantageously at least 10 %, preferably at least 20 %, particularly preferably at least 30 %, most particularly preferably at least 40 % more polyunsaturated fatty acids by comparison with the initial organism which does not contain the nucleic acids according to the invention. This is particularly the case for plants which do not naturally contain longer-chain polyunsaturated C_{20} or C_{22} fatty acids such as DHA, EPA or ARA.

[0074] The invention is explained in more detail by the following examples.

Examples

Example 1: General cloning methods

[0075] The cloning methods, such as by way of example restriction cleavages, agarose gel electrophoresis, purification of DNA fragments, transfer of nucleic acids to nitrocellulose and nylon membranes, linkage of DNA fragments, transformation of Escherichia coli cells, culture of bacteria and sequence analysis of recombinant DNA, were carried out as described in Sambrook et al. (1989) (Cold Spring Harbor Laboratory Press: ISBN 0-87969-309-6).

Example 2: Sequence analysis of recombinant DNA

[0076] Sequencing of recombinant DNA molecules was done using a laser fluorescence DNA sequencer from the ABI company by the method of Sanger (Sanger et al. (1977) Proc. Natl. Acad. Sci. USA74, 5463-5467). Fragments resulting from a polymerase chain reaction were sequenced and checked to prevent polymerase errors in the constructs to be expressed.

Example 3: Cloning of the Δ 6-desaturase from Muscariodides vialii (= SEQ ID NO: 3)

[0077] Total RNA from young Muscariodides vialii leaves was isolated with the aid of the RNAeasy kit from the Qiagen company (Valencia, CA, USA). With the aid of oligo-dT-cellulose poly-A+RNA (mRNA) was isolated from the total RNA (Sambrook et al., 1989). Using the Reverse Transcription System kit from Promega the RNA was reverse transcribed and the synthesized cDNA was used for PCR amplification of the A 6-desaturases. Degenerate primers were used for the amplification of the A 6-desaturase. The nucleotide sequence was derived from the first and the third histidine box motif of borage A 6-desaturase (Syanova et al., 1997, WO9621022).

Primer 1: GGITGGHTIGGICAYGAYKYIKSICA

Primer 2: GGRAAIAGRTGRTGYTCDATYTG

[0078] In the primers identified here and in the primer sequences set out below the symbols or letters in accordance with Wobble IUPAC-IUB have the following meaning:

R = A/G; Y = C/T; M = A/C ; K = G/T ; S = G/C; W = A/T; H = A/C/T; B = G/T/C; V = G/C/A; D = G/T/A und N = G/A/T/C.

PCR protocol

[0079]

Addition temperature: 1 min at 45 °C
Denaturing temperature: 1 min at 94 °C
Elongation temperature: 2 min at 72 °C
Number of cycles: 35

[0080] The PCR mixture was separated on an agarose gel and a 660 bp fragment was isolated. The PCR fragment was cloned in the pGEM-T easy vector (Promega) and the insert was then sequenced.

[0081] The missing 5' and 3' region of the isolated gene fragment from Muscariodides vialii was isolated with the aid of the Smart RACE cDNA kit (Clonetech) and then sequenced. Starting from 3' and 5' sequence primers were derived in order to isolate the complete clone. For this purpose primers were derived from the DNA regions around the start methionine and the stop codon. The PCR yielded a single band of the expected size. The cDNA was again cloned in the pGEM T easy vector and the now complete gene was sequenced.

Example 4: Cloning of the A 6-desaturase from Aleuritia farinosa (= SEQ ID NO: 1)

[0082] Total RNA from young Aleuritia farinose leaves was isolated with the aid of the RNAeasy kit from the Qiagen company (Valencia, CA, USA). With the aid of oligo-dT-cellulose poly-A+ RNA (mRNA) was isolated from the total RNA (Sambrook et al., 1989). Using the Reverse Transcription System kit from Promega the RNA was reverse transcribed and the synthesized cDNA was used for PCR amplification of the Δ 6-desaturases. Degenerate primers were used for the amplification of the A 6-desaturase. The nucleotide sequence was derived from the first and the third histidine box motif of borage A 6-desaturase (Syanova et al., 1997, WO9621022).

Primer 1: GGITGGHTIGGICAYGAYKYIKSICA
Primer 2: GGRAAIAGRTGRTGYTCDATYTG

PCR protocol

[0083]

Addition temperature: 1 min at 45 °C
Denaturing temperature: 1 min at 94 °C
Elongation temperature: 2 min at 72 °C
Number of cycles: 35

[0084] The PCR mixture was separated on an agarose gel and a 660 bp fragment was isolated. The PCR fragment was cloned in the pGEM-T easy vector (Promega) and the insert was then sequenced.

[0085] The missing 5' and 3' region of the isolated gene fragment from Aleuritia farinose was isolated with the aid of the Smart RACE cDNA kit (Clonetech) and then sequenced. Starting from 3' and 5' sequence primers were derived in order to isolate the complete clone. For this purpose primers were derived from the DNA regions around the start methionine and the stop codon. The PCR yielded a single band of the expected size. The cDNA was again cloned in the pGEM T easy vector and the now complete gene was sequenced.

Example 5: Cloning of expression plasmids for constitutive expression in plants

[0086] By means of appropriate primers at the 5' and 3' end of both new desaturases a CLAI and a XbaI interface was introduced.

[0087] Primer design for the A 6-desaturase from M. vialii:

atcgatatggctaacaaatctcccacc (ClaI)

tctagattagccgtgtgtgtggacggctt (XbaI)

[0088] Primer design for the A 6-desaturase from A. farinosa:

atcgatatggctaacaaatctcccacc (ClaI)

tctagatcacccgagagttttaagagct (XbaI)

[0089] The PCR products were separated in agarose gel, digested with ClaI/XbaI and ligated into the appropriately cut vector pSLJ4K1. The resultant plasmids contain 35S promoter (cauliflower mosaic virus; Franck et al. (1980) Cell 21, 285), the Δ6-desaturase from Muscariodides vialii or Aleuritia farinosa and the 35S terminator in the vector pSLJ4K1. Apart from said promoters or terminators all constitutive promoters or all plant virus promoters such as advantageously the nos promoter (Wilkinson et al., Journal of Experimental Botany, 48, 1997: 307 et seq.) or the ubiquintin promoter may be used. The promoters and terminators identified in the description may also advantageously be used in principle for expression.

[0090] The constructs were used for the transformation of Arabidopsis thaliana, oilseed rape, tobacco and linseed.

Example 5: Cloning of expression plasmids for seed-specific expression in plants

[0091] For the transformation of plants a further transformation vector based on pBin-USP containing the BamHI fragments of the A 6-desaturases from M. vialii or A. farinosa was produced. The BamHI interfaces were, as described in Example 4 [5? This is the second Example 5!], attached to the start ATGs or stop codons with the aid of appropriate primers by PCR.

[0092] Primer design for the A 6-desaturase from M. vialii:

ggatccatggctaacaaatctcccacc

ggatccttagccgtgtgtgtggacggctt

[0093] Primer design for the Δ 6-desaturase from A. farinosa:

ggatccatggctaacaaatctcccacc

ggatcctcacccgagagttttaagagct

[0094] pBin-USP is a derivative of the plasmid pBin19. pBin-USP was produced from pBin19 by inserting a USP promoter as an EcoRI-BaMHI fragment into pBin19 (Bevan et al. (1980) Nucl. Acids Res. 12, 8711). The polyadenylation signal is that of gene 3 of the T-DNA of the Ti plasmid pTiACH5 (Gielen et al., (1984) EMBO J. 3, 835), whereby nucleotides 11749-11939 were isolated as a PvuII-HindIII fragment and after addition of SphI linkers to the PvuII interface between the SpHI-HindIII interface of the vector were cloned. The USP promoter corresponds to nucleotides 1-684 (gene bank accession number X56240), wherein a part of the nonencoding region of the USP gene is contained in the promoter. The promoter fragment running to 684 base pairs was amplified by standard methods by means of commercial T7 standard primer (Stratagene) and using a synthesized primer through a PCR reaction. (Primer sequence: 5'-GTCGACCCGCGGACTAGTGGGCCCTCTAGACCCGGGGGATCC GGATCTGCTGGCTATGAA-3'). The PCR fragment was recut using EcoRI/SalI and inserted into the vector pBin19 with OCS terminator. The plasmid having the designation pBinUSP was obtained. The constructs were used for transforming Arabidopsis thaliana, oilseed rape, tobacco and linseed.

Example 6: Production of transgenic plants

[0095]

a) Production of transgenic plants (modified in accordance with Moloney et al., 1992, Plant Cell Reports, 8:238-242) To produce transgenic oilseed rape plants binary vectors in Agrobacterium tumefaciens C58C1:pGV2260 or Escherichia coli were used (Deblaere et al, 1984, Nucl. Acids. Res. 13, 4777-4788). For transforming oilseed rape plants (var. Drakkar, NPZ Nordeutsche Pflanzenzucht, Hohenlieth, Germany) a 1:50 dilution of an overnight culture

of a positively transformed agrobacteria colony in Murashige-Skoog medium (Murashige and Skoog 1962 Physiol. Plant. 15, 473) containing 3 % of saccharose (3MS medium) was used. Petioles or hypocotyledons of freshly germinated sterile rape plants (approx. 1 cm^2 each) were incubated in a Petri dish with a 1:50 agrobacteria dilution for 5-10 minutes. This was followed by 3-day concubation in darkness at 25 °C on 3MS medium containing 0.8 % of Bacto-Agar. After three days, culturing was continued with 16 hours of light / 8 hours of darkness and in a weekly cycle on MS medium containing 500 mg/l of Claforan (sodium cefotaxime), 50 mg/l of kanamycin, 20 microM of benzylaminopurine (BAP) and 1.6 g/l of glucose. Growing shoots were transferred onto MS medium containing 2 % of saccharose, 250 mg/l of Claforan and 0.8 % of Bacto-Agar. If after three weeks no roots had formed 2-indolylbutyric acid was added to the medium as a growth hormone for rooting purposes.
Regenerated shoots were obtained on 2MS medium using kanamycin and Claforan, transferred into soil after rooting and after culturing grown for two weeks in a climate-controlled chamber, brought to blossom and after harvesting of ripe seed investigated for Δ 6-desaturase expression by means of lipid analyses. Lines having increased contents of double bonds at the Δ 6 position were identified. In the stably transformed transgenic lines functionally expressing the transgene it was found that there is an increased content of double bonds at the Δ 6 position by comparison with untransformed control plants.
b) Transgenic flax plants may be produced, for example by the by the method Bell et al., 1999, In Vitro Cell. Dev. Biol.-Plant. 35(6):456-465, by means of particle bombardment. Agrobacteria-mediated transformations can be produced, for example, as described by Mlynarova et al. (1994), Plant Cell Report 13: 282-285.

Example 7: Lipid extraction from seed

[0096] Plant material was first of all mechanically homogenized by means of triturators in order to render it more amenable to extraction.

[0097] It was then heated to 100 °C for 10 min and after cooling on ice sedimented again. The cell sediment was hydrolyzed with 1 M methanolic sulfuric acid and 2 % dimethoxypropane for 1h at 90 °C and the lipids were transmethylated. The resultant fatty acid methyl esters (FAMEs) were finally extracted into petroleum ether. The extracted FAMEs were analyzed by gas-liquid chromatograph using a capillary column (Chrompack, WCOT fused silica, CP wax 52 CB, 25 m, 0.32 mm) and a temperature gradient of from 170 °C to 240 °C in 20 min and 5 min at 240 °C. The identity of the fatty acid methyl esters was confirmed by comparison with corresponding FAME standards (Sigma). The identity and the position of the double bond was further analyzed by means of GC-MS by suitable chemical derivatization of the FAME mixtures, e.g. to form 4,4-dimethoxyoxazoline derivatives (Christie, 1998). The GC analyses of the fatty acid methyl esters obtained from the transgenic rape seeds exhibiting seed-specific expression of the A 6-desaturase are presented in Table 1. The transgenic rape seeds contained up to 5 % of γ-linolenic acid in the seed.

Example 8: Expression of A 6-desaturases from Primulaceae in yeast (Saccharomyces cerevisiae)

[0098] The open reading rasters of the A 6-desaturases obtained from Muscariodides vialii and Aleuritia farinosa were each cloned behind the galactose-inducible GAL1 promoter of the yeast expression vector pYES2 (Invitrogen). The open reading rasters were amplified by means of PCR. The interfaces used for cloning were KpnI and EcoRI.

[0099] Primer design for M. vialii:

ggtaccatggctaacaaatctcccacc (KpnI)

gaattcttagccgtgtgtgtggacggctt (EcoRI)

[0100] Primer design for A. farinosa:

ggtaccatggctaacaaatctcccacc (KpnI)

gaattctcacccgagagttttaagagct (EcoRI)

[0101] The vectors produced were used for expression in yeast. The substrate specificities were determined by feeding the transformed yeast strains with α-linolenic acid and linoleic acid. The methodology used is described, for example, in Napier and Michaelson, 2001,Lipids. 36(8):761-766; Sayanova et al., 2001, Journal of Experimental Botany. 52(360): 1581-1585, Sperling et al., 2001, Arch. Biochem. Biophys. 388(2):293-298 and Michaelson et al., 1998, FEBS Letters. 439(3):215-218.

[0102] In order to compare the specificities the A 6-desaturase from borage (Borago officinalis) was likewise expressed in yeast (Sayanova et al.,1999, Plant Physiol. 121(2):641-646).

Table 1: Expression in yeast. Comparison of substrate specificities of A 6-sesaturases from borage, A. farinosa and M. vialii.

Construct	pYES2	Borage	A. farinosa	M. vialii
Fatty Acid				
$C_{16:0}$	24.8	20	23.2	20.3
$C_{16:1}{}^{\Delta 9}$	22.5	20	18.2	21.7
$C_{18:0}$	6.1	6.2	5.9	4.7
$C_{18:1}{}^{\Delta 9}$	17.1	17.1	19.9	15.8
$C_{18:2}{}^{\Delta,9,12}$	13.8	11.5	12.8	15.3
$C_{18:3}{}^{\Delta 6,9,12}$	0	5.5	5	1.4
C18:3 $^{\Delta 9,12,15}$	15.6	15.5	12.2	13.9
$C_{18:4}{}^{\Delta 6,9,12,15}$	0	3.9	7.7	6.8

Table 2: Conversion of linoleic acid by A 6-desaturase from borage and the A 6-desaturases from Primulaceae by comparison with the conversion of α-linolenic acid.

Construct	pYES2	Borage	A. farinosa	M. vialii
% by wt. of Δ 6-desaturated fatty acids in the total fatty acids	nd	11.10%	14.10%	8.80%
% conversion of LA to Δ6 fatty acids	nd	32.30%	28.10%	8.40%
% conversion of ALA to A 6 fatty acids	nd	20.10%	38.70%	32.80%
Ratio n3:n6		0.71	1.54	4.8

LA = linoleic acid (= $C_{18:2}{}^{\Delta 9,12}$) ⇒ Δ 6-desaturation produces
ALA = α-linolenic acid (= $C_{18:3}{}^{\Delta 9,12,15}$) ⇒ Δ 6-desaturation produces $C_{18:4n}{}^{\Delta 6,9,12,15}$
nd = not determined

[0103] It may be gathered from the tables that the nucleic acid sequences according to the invention encode A 6-desaturases which are specific for ω-3 fatty acids.

SEQUENZPROTOKOLL

[0104]

<110> BASF Plant Science GmbH

<120> Delta-6-desaturases from Primulaceae, expressing plants and PUFA-containing oils

<130> 2002_54

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tca gac ctg aaa tcc cac aac aag gca ggt gac cta tgg ata tca atc   96
Ser Asp Leu Lys Ser His Asn Lys Ala Gly Asp Leu Trp Ile Ser Ile
             20                  25                  30

cac ggc caa gtc tac gac gtg tcc tct tgg gcc gcc ctt cat ccg ggg   144
His Gly Gln Val Tyr Asp Val Ser Ser Trp Ala Ala Leu His Pro Gly
         35                  40                  45

ggc act gcc cct ctc atg gcc ctt gca gga cac gac gtg acc gat gct   192
Gly Thr Ala Pro Leu Met Ala Leu Ala Gly His Asp Val Thr Asp Ala
     50                  55                  60

ttc ctc gcg tac cat ccc cct tcc act gcc cgt ctc ctc cct cct ctc   240
Phe Leu Ala Tyr His Pro Pro Ser Thr Ala Arg Leu Leu Pro Pro Leu
 65                  70                  75                  80

tct acc aac ctc ctt ctt caa aac cac tcc gtc tcc ccc acc tcc tca   288
Ser Thr Asn Leu Leu Leu Gln Asn His Ser Val Ser Pro Thr Ser Ser
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gcc agg ggc cac act gct tac gcc acc ttc gtc ttc atg ata gcg atg   384
Ala Arg Gly His Thr Ala Tyr Ala Thr Phe Val Phe Met Ile Ala Met
        115                 120                 125

ttt cta atg agc gtg act gga gtc ctt tgc agc gac agt gcg tgg gtc   432
Phe Leu Met Ser Val Thr Gly Val Leu Cys Ser Asp Ser Ala Trp Val
    130                 135                 140

cat ttg gct agc ggc gga gca atg ggg ttc gcc tgg atc caa tgc gga   480
His Leu Ala Ser Gly Gly Ala Met Gly Phe Ala Trp Ile Gln Cys Gly
145                 150                 155                 160

tgg ata ggt cac gac tct ggg cat tac cgg att atg tct gac agg aaa   528
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                165                 170                 175

tgg aac tgg ttc gcg caa atc cta agc aca aac tgc ctc cag ggg att   576
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            180                 185                 190

agt atc ggg tgg tgg aag tgg aac cat aat gcg cac cac atc gct tgc   624
Ser Ile Gly Trp Trp Lys Trp Asn His Asn Ala His His Ile Ala Cys
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aat agc ctg gat tac gac ccc gac ctc cag tat atc cct ttg ctc gtc   672
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    210                 215                 220

gtc tcc ccc aag ttc ttc aac tcc ctt act tct cgt ttc tac gac aag   720
Val Ser Pro Lys Phe Phe Asn Ser Leu Thr Ser Arg Phe Tyr Asp Lys
225                 230                 235                 240

aag ctg aac ttc gac ggc gtg tcg agg ttt ctg gtt tgc tac cag cac   768
Lys Leu Asn Phe Asp Gly Val Ser Arg Phe Leu Val Cys Tyr Gln His
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tgg acg ttt tat ccg gtc atg tgt gtc gct agg ctg aac atg ctc gcg   816
Trp Thr Phe Tyr Pro Val Met Cys Val Ala Arg Leu Asn Met Leu Ala
            260                 265                 270

cag tca ttt ata acg ctt ttc tcg agt agg gag gtg tgc cat agg gcg   864
Gln Ser Phe Ile Thr Leu Phe Ser Ser Arg Glu Val Cys His Arg Ala
        275                 280                 285
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    290                 295                 300

ctt tct tgt tta cct aat tgg ggc gag agg att atg ttt ttg ctt gcg   960
Leu Ser Cys Leu Pro Asn Trp Gly Glu Arg Ile Met Phe Leu Leu Ala
305                 310                 315                 320

agc tat tcc gtt acg ggg ata caa cac gtg cag ttc agc ttg aac cat   1008
Ser Tyr Ser Val Thr Gly Ile Gln His Val Gln Phe Ser Leu Asn His
                325                 330                 335

ttt tct tcg gac gtc tat gtg ggc ccg cca gta ggt aat gac tgg ttc   1056
Phe Ser Ser Asp Val Tyr Val Gly Pro Pro Val Gly Asn Asp Trp Phe
            340                 345                 350

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385                 390                 395                 400

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    50                  55                  60

Phe Leu Ala Tyr His Pro Pro Ser Thr Ala Arg Leu Leu Pro Pro Leu
65                  70                  75                  80

Ser Thr Asn Leu Leu Leu Gln Asn His Ser Val Ser Pro Thr Ser Ser
                85                  90                  95

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            100                 105                 110

Ala Arg Gly His Thr Ala Tyr Ala Thr Phe Val Phe Met Ile Ala Met
        115                 120                 125

Phe Leu Met Ser Val Thr Gly Val Leu Cys Ser Asp Ser Ala Trp Val
    130                 135                 140

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                165                 170                 175

Trp Asn Trp Phe Ala Gln Ile Leu Ser Thr Asn Cys Leu Gln Gly Ile
            180                 185                 190

Ser Ile Gly Trp Trp Lys Trp Asn His Asn Ala His His Ile Ala Cys
        195                 200                 205

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    210                 215                 220
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                245                 250                 255

Trp Thr Phe Tyr Pro Val Met Cys Val Ala Arg Leu Asn Met Leu Ala
            260                 265                 270

Gln Ser Phe Ile Thr Leu Phe Ser Ser Arg Glu Val Cys His Arg Ala
        275                 280                 285

Gln Glu Val Phe Gly Leu Ala Val Phe Trp Val Trp Phe Pro Leu Leu
    290                 295                 300

Leu Ser Cys Leu Pro Asn Trp Gly Glu Arg Ile Met Phe Leu Leu Ala
305                 310                 315                 320

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            340                 345                 350

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        355                 360                 365

Asp Trp Phe His Gly Gly Leu Gln Phe Gln Val Glu His His Leu Phe
    370                 375                 380

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385                 390                 395                 400

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                405                 410                 415

Lys Ala Asn Val Phe Thr Leu Lys Thr Leu Arg Asn Thr Ala Ile Glu
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Ser Asp Leu Lys Gly His Asn Lys Ala Gly Asp Leu Trp Ile Ser Ile
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         35                  40                  45

ggc agt gcc ccc ctc atg gcc ctc gca gga cac gac gta acc gac gct  192
Gly Ser Ala Pro Leu Met Ala Leu Ala Gly His Asp Val Thr Asp Ala
     50                  55                  60

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Phe Leu Ala Tyr His Pro Pro Ser Thr Ala Arg Leu Leu Pro Pro Leu
 65                  70                  75                  80

tcc acc aac ctc ctc ctt caa aac cac tcc gtc tcc ccc acc tcc tct  288
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                 85                  90                  95

gac tac cgc aaa ctc ctc cac aac ttc cat aaa att ggt atg ttc cgc  336
Asp Tyr Arg Lys Leu Leu His Asn Phe His Lys Ile Gly Met Phe Arg
            100                 105                 110

gcc agg ggc cac act gct tac gcc acc ttc gtc atc atg ata gtg atg  384
Ala Arg Gly His Thr Ala Tyr Ala Thr Phe Val Ile Met Ile Val Met
        115                 120                 125

ttt cta acg agc gtg acc gga gtc ctt tgc agc gac agt gcg tgg gtc  432
Phe Leu Thr Ser Val Thr Gly Val Leu Cys Ser Asp Ser Ala Trp Val
    130                 135                 140

cat ctg gct agc ggc gca gca atg ggg ttc gcc tgg atc cag tgc gga  480
His Leu Ala Ser Gly Ala Ala Met Gly Phe Ala Trp Ile Gln Cys Gly
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            180                 185                 190

agt atc ggg tgg tgg aag tgg aac cat aac gcc cac cac att gct tgc   624
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225                 230                 235                 240

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tgg acg ttt tat cca gtc atg tgt gtc gct agg cta aac atg atc gca   816
Trp Thr Phe Tyr Pro Val Met Cys Val Ala Arg Leu Asn Met Ile Ala
            260                 265                 270

cag tcg ttt ata acg ctt ttc tcg agc agg gag gtg ggt cat agg gcg   864
Gln Ser Phe Ile Thr Leu Phe Ser Ser Arg Glu Val Gly His Arg Ala
        275                 280                 285

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            100                 105                 110

Ala Arg Gly His Thr Ala Tyr Ala Thr Phe Val Ile Met Ile Val Met
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Phe Leu Thr Ser Val Thr Gly Val Leu Cys Ser Asp Ser Ala Trp Val
    130                 135                 140

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            180                 185                 190

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Asn Ser Leu Asp Tyr Asp Pro Asp Leu Gln Tyr Ile Pro Leu Leu Val
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Val Ser Pro Lys Phe Phe Asn Ser Leu Thr Ser Arg Phe Tyr Asp Lys
225                 230                 235                 240

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                245                 250                 255

Trp Thr Phe Tyr Pro Val Met Cys Val Ala Arg Leu Asn Met Ile Ala
            260                 265                 270

Gln Ser Phe Ile Thr Leu Phe Ser Ser Arg Glu Val Gly His Arg Ala
        275                 280                 285

Gln Glu Ile Phe Gly Leu Ala Val Phe Trp Val Trp Phe Pro Leu Leu
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Phe Ser Ser Asp Val Tyr Val Gly Pro Pro Val Gly Asn Asp Trp Phe
            340                 345                 350

Lys Lys Gln Thr Ala Gly Thr Leu Asn Ile Ser Cys Pro Ala Trp Met
        355                 360                 365

Asp Trp Phe His Gly Gly Leu Gln Phe Gln Val Glu His His Leu Phe
    370                 375                 380

Pro Arg Met Pro Arg Gly Gln Phe Arg Lys Ile Ser Pro Phe Val Arg
385                 390                 395                 400

Asp Leu Cys Lys Lys His Asn Leu Pro Tyr Asn Ile Ala Ser Phe Thr
                405                 410                 415

Lys Ala Asn Val Leu Thr Leu Lys Thr Leu Arg Asn Thr Ala Ile Glu
            420                 425                 430

Ala Arg Asp Leu Ser Asn Pro Thr Pro Lys Asn Met Val Trp Glu Ala
        435                 440                 445

Val His Thr His Gly
    450
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Claims

1. Oil of flax produced by a method for producing PUFAs, wherein the method comprises growing a transgenic flax plant, which comprises a nucleic acid, which encode polypeptides having Δ-6-desaturase activity and isolating the oil contained in the flax plant, wherein the Δ-6-desaturases encoded by the nucleic acid sequences specifically convert w-3 fatty acids, and wherein said nucleic acid sequence is selected from the group:

   a) a nucleic acid sequence having the sequence depicted in SEQ ID NO: 1 or SEQ ID NO: 3,
   b) nucleic acid sequences which may be derived as a result of the degenerate genetic code from the encoding sequence contained in SEQ ID NO: 1 or SEQ ID NO: 3,
   c) derivatives of the nucleic acid sequence depicted in SEQ ID NO: 1 or SEQ ID NO: 3 which encode polypeptides using the amino acid sequences depicted in SEQ ID NO: 2 or SEQ ID NO: 4 and have at least 75 % homology on the amino acid level with SEQ ID NO: 2 or SEQ ID NO: 4 and possess Δ-6-desaturase activity

   and wherein due to the activity of said Δ-6 desaturase PUFAs are formed in said transgenic flax plant which exhibit an increased content of w-3 fatty acids, and wherein said oil of flax contains stearidonic acid.

2. Oil as claimed in claim 1, wherein the oil of flax contains eicosapentaenoic acid or docosahexaenoic acid.

Patentansprüche

1. Flachsöl, das durch ein Verfahren zur Herstellung von PUFAs hergestellt wird, wobei man bei dem Verfahren eine transgene Flachspflanze züchtet, die eine Nukleinsäure umfasst, die Polypeptide mit Δ-6-Desaturase-Aktivität kodiert, und das in der Flachspflanze enthaltene Öl isoliert, wobei die von den Nukleinsäuresequenzen kodierten Δ-6-Desaturasen spezifisch ω-3-Fettsäuren umwandeln und wobei die Nukleinsäuresequenz aus der folgenden Gruppe ausgewählt ist:

   a) einer Nukleinsäuresequenz mit der in SEQ ID NR: 1 oder SEQ ID NR: 3 dargestellten Sequenz,
   b) Nukleinsäuresequenzen, die infolge des degenerierten genetischen Codes von der in SEQ ID NR: 1 oder SEQ ID NR: 3 enthaltenen kodierenden Sequenz stammen können,
   c) Derivate der in SEQ ID NR: 1 oder SEQ ID NR: 3 dargestellten Nukleinsäuresequenz, die Polypeptide unter Verwendung der in SEQ ID NR: 2 oder SEQ ID NR: 4 dargestellten Aminosäuresequenzen kodieren und die mindestens 75% Homologie auf Aminosäure-Ebene zu SEQ ID NR: 2 oder SEQ ID NR: 4 aufweisen und Δ-6-Desaturase-Aktivität besitzen,

   und wobei aufgrund der Aktivität der Δ-6-Desaturase PUFAs in der transgenen Flachspflanze gebildet werden, die einen erhöhten Gehalt an ω-3-Fettsäuren aufweisen und wobei das Flachsöl Stearidonsäure enthält.

2. Öl nach Anspruch 1, wobei das Flachsöl Eicosapentaensäure oder Docosahexaensäure enthält.

Revendications

1. Huile de lin produite par un procédé pour produire des acides gras polyinsaturés (PUFA), où le procédé comprend la culture d'une plante de lin transgénique, qui comprend un acide nucléique, qui code pour des polypeptides ayant une activité de Δ-6-désaturase et l'isolement de l'huile contenue dans la plante de lin, où la Δ-6-désaturase codée par les séquences d'acides nucléiques convertit de manière spécifique des acides gras ω-3, et où ladite séquence d'acide nucléique est choisie dans le groupe de :

   a) une séquence d'acide nucléique ayant la séquence représentée dans SEQ ID NO : 1 ou SEQ ID NO : 3,
   b) des séquences d'acides nucléiques qui peuvent être dérivées, en tant que résultat du code génétique dégénéré, de la séquence codante contenue dans SEQ ID NO : 1 ou SEQ ID NO : 3,
   c) des dérivés de la séquence d'acide nucléique représentée dans SEQ ID NO : 1 ou SEQ ID NO : 3 qui codent pour des polypeptides utilisant les séquences d'acides aminés représentées dans SEQ ID NO : 2 ou SEQ ID NO : 4 et ont au moins 75 % d'homologie au niveau des acides aminés avec SEQ ID NO : 2 ou SEQ ID NO : 4 et possèdent une activité de Δ-6-désaturase,

   et où grâce à l'activité de ladite Δ-6-désaturase, des PUFA sont formés dans ladite plante de lin transgénique qui présentent une teneur augmentée en acides gras ω-3,
   et où ladite huile de lin contient de l'acide stéaridonique.

2. Huile selon la revendication 1, où l'huile de lin contient de l'acide eicosapentaénoïque ou de l'acide docosahexaénoïque.

REFERENCES CITED IN THE DESCRIPTION

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