

Regulatory nucleic acid molecules for enhancing seed-specific gene expression in plants promoting enhanced polyunsaturated fatty acid synthesis

The invention in principle pertains to the field of recombinant manufacture of fatty acids. It provides novel nucleic acid molecules comprising nucleic acid sequences encoding fatty acid desaturases, elongases, acyltransferases, terminator sequences and high expressing seed-specific promoters operatively linked to the said nucleic acid sequences wherein nucleic acid expression enhancing nucleic acids (NEENAs) are functionally linked to said promoters.

The invention also provides recombinant expression vectors containing the nucleic acid molecules, host cells or host cell cultures into which the expression vectors have been introduced, and methods for large-scale production of long chain polyunsaturated fatty acids (LCPUFAs), e.g. arachidonic acid (ARA), eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA).

Description of the Invention

Expression of transgenes in plants is strongly affected by various external and internal factors resulting in a variable and unpredictable level of transgene expression. Often a high number of transformants have to be produced and analyzed in order to identify lines with desirable expression strength. As transformation and screening for lines with desirable expression strength is costly and labor intensive there is a need for high expression of one or more transgenes in a plant. This problem is especially pronounced, when several genes have to be coordinately expressed in a transgenic plant in order to achieve a specific effect as a plant has to be identified in which each and every gene is strongly expressed.

For example, expression of a transgene can vary significantly, depending on construct design and positional effects of the T-DNA insertion locus in individual transformation events. Strong promoters can partially overcome these challenges. However, availability of suitable promoters showing strong expression with the desired specificity is often limited. In order to ensure availability of sufficient promoters with desired expression specificity, the identification and characterization of additional promoters can help to close this gap. However, natural availability of promoters of the respective specificity and strength and the time consuming characterization of promoter candidates impedes the identification of suitable new promoters.

In order to overcome these challenges, diverse genetic elements and/or motifs have been shown to positively affect gene expression. Among these, some introns have been recognized as genetic elements with a strong potential for improving gene expression. Although the mechanism is largely unknown, it has been shown that some introns positively affect the steady state amount of mature mRNA, possibly by enhanced transcriptional activity, improved mRNA maturation, enhanced nuclear mRNA export and/or improved translation initiation (e.g. Huang and Gorman, 1990; Le Hir et al., 2003; Nott et al., 2004). Since only selected introns were shown to increase expression, splicing as such is likely not accountable for the observed effects.

The increase of gene expression observed upon functionally linking introns to promoters is called intron mediated enhancement (IME) of gene expression and has been shown in various monocotyledonous (e.g. Callis et al., 1987; Vasil et al., 1989; Bruce et al., 1990; Lu et al., 2008) and dicotyledonous plants (e.g. Chung et al., 2006; Kim et al., 2006; Rose et al., 2008). In this respect, the position of the intron in relation to the translational start site (ATG) was shown to be crucial for intron mediated enhancement of gene expression (Rose et al., 2004).

Next to their potential for enhancing gene expression, few introns were shown to also affect the tissue specificity in their native nucleotide environment in plants. Reporter gene expression was found to be dependent on the presence of genomic regions containing up to two introns (Sieburth et al., 1997; Wang et al., 2004). 5' UTR introns have also been reported to be of importance for proper functionality of promoter elements, likely due to tissue specific gene control elements residing in the introns (Fu et al., 1995a; Fu et al., 1995b; Vitale et al., 2003; Kim et al., 2006). However, these studies also show that combination of introns with heterologous promoters can have strong negative impacts on strength and/or specificity of gene expression (Vitale et al., 2003; Kim et al., 2006, WO2006/003186, WO2007/098042). For example the strong constitutive Cauliflower Mosaic Virus CaMV35S promoter is negatively affected through combination with the sesame SeFAD2 5' UTR intron (Kim et al., 2006). In contrast to these observations, some documents show enhanced expression of a nucleic acid by IME without affecting the tissue specificity of the respective promoter (Schünemann et al., 2004). Introns or NEENAs that enhance seed-specific expression when functionally linked to a heterologous promoter have not been shown in the art.

In the present application further nucleic acid molecules are described that enhance the expression of said promoters without affecting their specificity upon functionally linkage to seed-specific promoters. These nucleic acid molecules are in the present application described as "nucleic acid expression enhancing nucleic acids" (NEENA). Introns have the intrinsic feature to be spliced out of the respective pre-mRNA. In contrast to that the nucleic acids presented in the application at hand, do not necessarily have to be included in the mRNA or, if present in the mRNA, have not necessarily to be spliced out of the mRNA in order to enhance the expression derived from the promoter the NEENAs are functionally linked to.

Detailed description of the Invention

A first embodiment of the invention pertains to a polynucleotide that promotes enhancing of polyunsaturated fatty acid synthesis, therefore it pertains in generally in the recombinant manufacture of polyunsaturated fatty acids.

Fatty acids are carboxylic acids with long-chain hydrocarbon side groups that play a fundamental role in many biological processes. Fatty acids are rarely found free in nature but, rather, occur in esterified form as the major component of lipids. As such, lipids/ fatty acids are

sources of energy (e.g., β -oxidation). In addition, lipids/ fatty acids are an integral part of cell membranes and, therefore, are indispensable for processing biological or biochemical information.

5 Fatty acids can be divided into two groups: saturated fatty acids formed of single carbon bonds and the unsaturated fatty acids which contain one or more carbon double bonds in *cis*-configuration. Unsaturated fatty acids are produced by terminal desaturases that belong to the class of nonheme-iron enzymes. Each of these enzymes are part of an electron-transport system that involves one or two other proteins, namely cytochrome b_5 and NADH-cytochrome b_5
10 reductase. The cytochrome b_5 functionality can also be *n*-terminally fused to the desaturase moiety of one single protein. Specifically, such enzymes catalyze the formation of double bonds between the carbon atoms of a fatty acid molecule, for example, by catalyzing the oxygen-dependent dehydrogenation of fatty acids (Sperling et al., 2003). Human and other mammals have a limited spectrum of desaturases that are required for the formation of particular double
15 bonds in unsaturated fatty acids and thus, have a limited capacity for synthesizing essential fatty acids, e.g., long chain polyunsaturated fatty acids (LCPUFAs). Thus, humans have to take up some fatty acids through their diet. Such essential fatty acids include, for example, linoleic acid (C18:2), linolenic acid (C18:3). In contrast, insects, microorganisms and plants are able to synthesize a much larger variety of unsaturated fatty acids and their derivatives. Indeed, the
20 biosynthesis of fatty acids is a major activity of plants and microorganisms.

Long chain polyunsaturated fatty acids (LCPUFAs) such as docosahexaenoic acid (DHA, 22:6(4,7,10,13,16,19)) are essential components of cell membranes of various tissues and organelles in mammals (nerve, retina, brain and immune cells). For example, over 30% of fatty
25 acids in brain phospholipid are 22:6 (n-3) and 20:4 (n-6) (Crawford, M.A., et al., (1997) *Am. J. Clin. Nutr.* 66:1032S-1041S). In retina, DHA accounts for more than 60% of the total fatty acids in the rod outer segment, the photosensitive part of the photoreceptor cell (Giusto, N.M., et al. (2000) *Prog. Lipid Res.* 39:315-391). Clinical studies have shown that DHA is essential for the growth and development of the brain in infants, and for maintenance of normal brain function in
30 adults (Martinetz, M. (1992) *J. Pediatr.* 120:S129-S138). DHA also has significant effects on photoreceptor function involved in the signal transduction process, rhodopsin activation, and rod and cone development (Giusto, N.M., et al. (2000) *Prog. Lipid Res.* 39:315-391). In addition, some positive effects of DHA were also found on diseases such as hypertension, arthritis, atherosclerosis, depression, thrombosis and cancers (Horrocks, L.A. and Yeo, Y.K. (1999)
35 *Pharmacol. Res.* 40:211-215). Therefore, appropriate dietary supply of the fatty acid is important for human health. Because such fatty acids cannot be efficiently synthesized by infants, young children and senior citizens, it is particularly important for these individuals to adequately intake these fatty acids from the diet (Spector, A.A. (1999) *Lipids* 34:S1-S3).

40 Currently the major sources of DHA are oils from fish and algae. Fish oil is a major and traditional source for this fatty acid, however, it is usually oxidized by the time it is sold. In addition, the supply of fish oil is highly variable, particularly in view of the shrinking fish

populations. Moreover, the algal source of oil is expensive due to low yield and the high costs of extraction.

EPA and ARA are both $\Delta 5$ essential fatty acids. They form a unique class of food and feed constituents for humans and animals. EPA belongs to the n-3 series with five double bonds in the acyl chain. EPA is found in marine food and is abundant in oily fish from North Atlantic. ARA belongs to the n-6 series with four double bonds. The lack of a double bond in the ω -3 position confers on ARA different properties than those found in EPA. The eicosanoids produced from ARA have strong inflammatory and platelet aggregating properties, whereas those derived from EPA have anti-inflammatory and anti-platelet aggregating properties. ARA can be obtained from some foods such as meat, fish and eggs, but the concentration is low.

Gamma-linolenic acid (GLA) is another essential fatty acid found in mammals. GLA is the metabolic intermediate for very long chain n-6 fatty acids and for various active molecules. In mammals, formation of long chain polyunsaturated fatty acids is rate-limited by $\Delta 6$ desaturation. Many physiological and pathological conditions such as aging, stress, diabetes, eczema, and some infections have been shown to depress the $\Delta 6$ desaturation step. In addition, GLA is readily catabolized by the oxidation and rapid cell division associated with certain disorders, e.g., cancer or inflammation. Therefore, dietary supplementation with GLA can reduce the risks of these disorders. Clinical studies have shown that dietary supplementation with GLA is effective in treating some pathological conditions such as atopic eczema, premenstrual syndrome, diabetes, hypercholesterolemia, and inflammatory and cardiovascular disorders.

A large number of beneficial health effects have been shown for DHA or mixtures of EPA/DHA. DHA is a n-3 very long chain fatty acid with six double bonds.

Although biotechnology offers an attractive route for the production of specialty fatty acids, current techniques fail to provide an efficient means for the large scale production of unsaturated fatty acids. Accordingly, there exists a need for an improved and efficient method of producing unsaturated fatty acids, such as DHA, EPA and ARA.

Thus, the present invention relates to a polynucleotide comprising:

- a) at least one nucleic acid sequence encoding a polypeptide having desaturase or elongase activity;
- b) at least one seed-specific and/or a seed-preferential plant promoter operatively linked to the said nucleic acid sequence;
- c) at least one terminator sequence operatively linked to the said nucleic acid sequence and
- d) one or more nucleic acid expression enhancing nucleic acid (NEENA) molecule functionally linked to said promoter and which is/are heterologous to said promoter and to said polypeptide defined in a).

In one embodiment the term “ polynucleotide” as used in accordance with the present invention relates to a polynucleotide comprising a nucleic acid sequence which encodes a polypeptide having desaturase or elongase activity. Preferably, the polypeptide encoded by the polynucleotide of the present invention having desaturase, or elongase activity upon expression in a plant shall be capable of increasing the amount of PUFA and, in particular, LCPUFA in, e.g., seed oils or the entire plant or parts thereof. Such an increase is, preferably, statistically significant when compared to a LCPUFA producing transgenic control plant which expresses the the present state of the art set of desaturases and elongases required for LCPUFA synthesis but does not express the polynucleotide of the present invention. Whether an increase is significant can be determined by statistical tests well known in the art including, e.g., Student’s t-test. More preferably, the increase is an increase of the amount of triglycerides containing LCPUFA of at least 5%, at least 10%, at least 15%, at least 20% or at least 30% compared to the said control. Preferably, the LCPUFA referred to before is a polyunsaturated fatty acid having a C-20 or C-22 fatty acid body, more preferably, ARA, EPA or DHA. Suitable assays for measuring the activities mentioned before are described in the accompanying Examples.

The term “ desaturase” or “ elongase” as used herein refers to the activity of a desaturase, introducing a double bond into the carbon chain of a fatty acid, preferably into fatty acids with 18, 20 or 22 carbon molecules, or an elongase, introducing two carbon molecules into the carbon chain of a fatty acid, preferably into fatty acids with 18, 20 or 22 carbon molecules

Preferred polynucleotides are those having a nucleic acid sequence as shown in SEQ ID NOs: 95, 96, 97, 98, 99, 100 or 101 encoding for polypeptides exhibit desaturase or elongase activity (see table 3)

Other preferred polynucleotides are those having a nucleic acid sequence are shown in SEQ ID NOs: 102 or 103 encoding a polypeptide having desaturase or elongase activity (see table 4, also), that are especially used in addition to the polynucleotides listed in table 3 for synthesis of 22:6n-3 (DHA), i.e. in rapeseed.

A preferred seed-specific promoter as meant herein is selected from the group consisting of Napin, USP, Conlinin, SBP, Fae, Arc and LuPXR.. Other most preferred seed-specific promoter as meant herein are encoded by a nucleic acid sequence as shown in SEQ ID NOs: 25, 26, 27, 28, 29 or 30. A person skilled in the art is aware of methods for rendering a unidirectional to a bidirectional promoter and of methods to use the complement or reverse complement of a promoter sequence for creating a promoter having the same promoter specificity as the original sequence. Such methods are for example described for constitutive as well as inducible promoters by Xie et al. (2001) “ Bidirectionalization of polar promoters in plants” (Nature Biotechnology 19, pages 677 – 679). The authors describe that it is sufficient to add a minimal

promoter to the 5' prime end of any given promoter to receive a promoter controlling expression in both directions with same promoter specificity.

The term "NEENA" as described below is used for the expression "nucleic acid expression enhancing nucleic acid" referring to a sequence and/or a nucleic acid molecule of a specific sequence having the intrinsic property to enhance expression of a nucleic acid under the control of a promoter to which the NEENA is functionally linked. Hence a high expression promoter functionally linked to a NEENA as claimed is functional in complement or reverse complement and therefore the NEENA is functional in complement or reverse complement too.

In principal the NEENA may be functionally linked to any promoter such as tissue specific, inducible, developmental specific or constitutive promoters. The respective NEENA will lead to an enhanced seed-specific expression of the heterologous nucleic acid under the control of the respective promoter to which the one or more NEENA is functionally linked to. The enhancement of expression of promoters other than seed-specific promoters, for example constitutive promoters or promoters with differing tissue specificity, will influence the specificity of these promoters. Expression of the nucleic acid under control of the respective promoter will be significantly increased in seeds, where the transcript of said nucleic acid may have not or only weakly been detected without the NEENA functionally linked to its promoter. Hence, tissue- or developmental specific or any other promoter may be rendered to seed-specific promoters by functionally linking one or more of the NEENA molecules as described above to said promoter. Preferred NEENAs as for the present invention are encoded by the sequences shown in SEQ ID NOs: 11, 12, 13, 14, 15, 16, 17, 18, 19, 10, 21, 22, 23 or 24. More preferred NEENAs as for the present invention are encoded by the sequences shown in SEQ ID NOs: 6, 7, 8, 9 or 10 . Also (i) nucleic acid molecule having a sequence with an identity of 80% or more to any of the sequences as defined by SEQ ID NO: 6 to 24, preferably, the identity is 85% or more, more preferably the identity is 90% or more, even more preferably, the identity is 95% or more, 96% or more, 97% or more, 98% or more or 99% or more, in the most preferred embodiment, the identity is 100% to any of the sequences as defined by SEQ ID NO: 6 to 24 or (ii) a fragment of 100 bases or more consecutive bases, preferably 150 or more consecutive bases, more preferably 200 consecutive bases or more even more preferably 250 or more consecutive bases of a nucleic acid molecule of i) or ii) which has an expressing enhancing activity, for example 65% or more, preferably 70% or more, more preferably 75% or more, even more preferably 80% or more, 85% or more or 90% or more, in a most preferred embodiment it has 95% or more of the expression enhancing activity as the corresponding nucleic acid molecule having the sequence of any of the sequences as defined by SEQ ID NO: 6 to 24, or iii) a nucleic acid molecule which is the complement or reverse complement of any of the previously mentioned nucleic acid molecules under i) to ii) or iv) a nucleic acid molecule which is obtainable by PCR using oligonucleotide primers as shown in Table 6 or v) a nucleic acid molecule of 100 nucleotides or more, 150 nucleotides or more, 200 nucleotides or more or 250 nucleotides or more, hybridizing under conditions equivalent to hybridization in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 2 X SSC, 0.1% SDS at 50°C or

65°C, preferably 65°C to a nucleic acid molecule comprising at least 50, preferably at least 100, more preferably at least 150, even more preferably at least 200, most preferably at least 250 consecutive nucleotides of a transcription enhancing nucleotide sequence described by SEQ ID NO: 6 to 24 or the complement thereof are encompassed by the present invention. Preferably, said nucleic acid molecule is hybridizing under conditions equivalent to hybridization in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 1 X SSC, 0.1% SDS at 50°C or 65°C, preferably 65°C to a nucleic acid molecule comprising at least 50, preferably at least 100, more preferably at least 150, even more preferably at least 200, most preferably at least 250 consecutive nucleotides of a transcription enhancing nucleotide sequence described by SEQ ID NO: 6 to 24 or the complement thereof, more preferably, said nucleic acid molecule is hybridizing under conditions equivalent to hybridization in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 0,1 X SSC, 0.1% SDS at 50°C or 65°C, preferably 65°C to a nucleic acid molecule comprising at least 50, preferably at least 100, more preferably at least 150, even more preferably at least 200, most preferably at least 250 consecutive nucleotides of a transcription enhancing nucleotide sequence described by any of the sequences as defined by SEQ ID NO:1 to 15 or the complement thereof.

As described above under iv) the nucleic acid molecule obtainable by PCR using oligonucleotides shown in table 6 is obtainable for example from genomic DNA from Arabidopsis plants such as *A. thaliana* using the conditions as described in Example 3.2 below.

Preferably, the one or more NEENA is functionally linked to seed-specific promoters and will enhance expression of the nucleic acid molecule under control of said promoter. Seed-specific promoters to be used in any method of the invention may be derived from plants, for example monocotyledonous or dicotyledonous plants, from bacteria and/or viruses or may be synthetic promoters. Seed specific promoters to be used functionally linked to a NEENA are in a preferred embodiment the seed-specific promoter linked to NEENAs shown in SEQ ID NOs: 1, 2, 3, 4 or 5, table 5.

The high expression seed-specific promoters functionally linked to a NEENA may be employed in any plant comprising for example moss, fern, gymnosperm or angiosperm, for example monocotyledonous or dicotyledonous plant. In a preferred embodiment said promoter of the invention functionally linked to a NEENA may be employed in monocotyledonous or dicotyledonous plants, preferably crop plant such as corn, soy, canola, cotton, potato, sugar beet, rice, wheat, sorghum, barley, musa, sugarcane, miscanthus and the like. In a preferred embodiment of the invention, said promoter which is functionally linked to a NEENA may be employed in monocotyledonous crop plants such as corn, rice, wheat, sorghum, barley, musa, miscanthus or sugarcane. In an especially preferred embodiment the promoter functionally linked to a NEENA may be employed in dicotyledonous crop plants such as soy, canola, cotton or potato.

A high expressing seed-specific promoter as used in the application means for example a promoter which is functionally linked to a NEENA causing enhanced seed-specific expression of the promoter in a plant seed or part thereof wherein the accumulation of RNA or rate of synthesis of RNA in seeds derived from the nucleic acid molecule under the control of the
5 respective promoter functionally linked to a NEENA is higher, preferably significantly higher than the expression in seeds caused by the same promoter lacking a NEENA of the invention. Preferably the amount of RNA of the respective nucleic acid and/or the rate of RNA synthesis and/or the RNA stability in a plant is increased 50% or more, for example 100% or more, preferably 200% or more, more preferably 5 fold or more, even more preferably 10 fold or more,
10 most preferably 20 fold or more for example 50 fold compared to a control plant of same age grown under the same conditions comprising the same seed-specific promoter the latter not being functionally linked to a NEENA of the invention.

When used herein, significantly higher refers to statistical significance the skilled person is
15 aware how to determine, for example by applying statistical tests such as the t-test to the respective data sets.

Methods for detecting expression conferred by a promoter are known in the art. For example, the promoter may be functionally linked to a marker gene such as GUS, GFP or luciferase and
20 the activity of the respective protein encoded by the respective marker gene may be determined in the plant or part thereof. As a representative example, the method for detecting luciferase is described in detail below. Other methods are for example measuring the steady state level or synthesis rate of RNA of the nucleic acid molecule controlled by the promoter by methods known in the art, for example Northern blot analysis, qPCR, run-on assays or other methods
25 described in the art, or detecting the encoded protein using specific antibodies by methods known in the art, e.g. Western Blot and/or enzyme-linked immunosorbent assay (ELISA).

A skilled person is aware of various methods for functionally linking two or more nucleic acid molecules. Such methods may encompass restriction/ligation, ligase independent cloning,
30 recombineering, recombination or synthesis. Other methods may be employed to functionally link two or more nucleic acid molecules.

The term "heterologous" with respect to a nucleic acid molecule or DNA refers to a nucleic acid molecule which is operably linked to, or is manipulated to become operably linked to, a
35 second nucleic acid molecule to which it is not operably linked in nature, or to which it is operably linked at a different location in nature. For example, a NEENA of the invention is in its natural environment functionally linked to its native promoter, whereas in the present invention it is linked to another promoter which might be derived from the same organism, a different organism or might be a synthetic promoter. It may also mean that the NEENA of the present
40 invention is linked to its native promoter but the nucleic acid molecule under control of said promoter is heterologous to the promoter comprising its native NEENA. It is in addition to be understood that the promoter and/or the nucleic acid molecule under the control of said

promoter functionally linked to a NEENA of the invention are heterologous to said NEENA as their sequence has been manipulated by for example mutation such as insertions, deletions and the forth so that the natural sequence of the promoter and/or the nucleic acid molecule under control of said promoter is modified and therefore have become heterologous to a NEENA of the invention. It may also be understood that the NEENA is heterologous to the nucleic acid to which it is functionally linked when the NEENA is functionally linked to its native promoter wherein the position of the NEENA in relation to said promoter is changed so that the promoter shows higher expression after such manipulation.

A plant exhibiting enhanced seed-specific expression of a nucleic acid molecule as meant herein means a plant having a higher, preferably statistically significant higher seed-specific expression of a nucleic acid molecule compared to a control plant grown under the same conditions without the respective NEENA functionally linked to the respective nucleic acid molecule. Such control plant may be a wild-type plant or a transgenic plant comprising the same promoter controlling the same gene as in the plant of the invention wherein the promoter is not linked to a NEENA of the invention.

In generally the NEENA may be heterologous to the nucleic acid molecule which is under the control of said promoter to which the NEENA is functionally linked or it may be heterologous to both the promoter and the nucleic acid molecule under the control of said promoter.

The term "elongase activity" as meant by the present invention refers to the activity of the entire elongation complex as defined in the passage below and it is also be understood as the activity of the first component of the elongation complex with beta-ketoacyl-CoA synthase activity, which determines the substrate specificity of the entire elongation complex. By understanding the elongase activity as synthase activity only, the polypeptide of the of the present invention needs also comprising:

- e) at least one nucleic acid sequence encoding a polypeptide having beta-ketoacyl reductase activity;
 - f) at least one nucleic acid sequence encoding a polypeptide having dehydratase activity or
 - g) at least one nucleic acid sequence encoding a polypeptide having enoyl-CoA reductase activity
- , wherein the nucleic acid sequences defined in e) to g) are heterologous to said polypeptide having desaturase or elongase activity.

Preferably, the polynucleotide of the present invention comprises nucleic acid sequence encoding fatty acid dehydratase/enoyl-CoA reductase (nECR) protein having an activity of catalyzing the dehydration and reduction of fatty acid elongated intermediates.

Fatty acid elongation is catalyzed in four steps, represented by four enzymes: KCR (β -keto-acyl-CoA-synthase), KCR (β -keto-acyl-CoA reductase), DH (dehydratase) and ECR (enoyl-

CoA-reductase) forming the entire elongation complex. In the first step a fatty acid-CoA ester is condensed with malonyl-CoA producing a β -keto-acyl-CoA intermediate, which is elongated by to carbon atoms, and CO_2 . The keto-group of the intermediate is then reduced by the KCR to a hydroxyl-group. In the next step the DH cleaves of the hydroxyl-group (H_2O is produced), forming a 2-acylen-CoA ester. In the final step the double bound at position 2, 3 is reduced by the ECR forming the elongated acyl-CoA ester (Buchanan, Grissem, Jones (2000) Biochemistry & Molecular biology of plants, American Society of Plant Physiologists). DH and ECR activity might also be conferred by one single protein being a natural or artificial fusion of a DH-mojety and a ECR mojety, referred to as novel enoyl-CoA-reductase (nECR) in the present invention. In the current invention either all nucleic acid sequences defined in e) to f) could be comprised in the polynucleotide or only at least one of these nucleic acid sequences defined in e) to f) could be comprised in the polynucleotide in any combination occurred from different organisms.

A polynucleotide comprising a fragment of any of the aforementioned nucleic acid sequences is also encompassed as a nucleic acid molecule of the present invention. The fragment shall encode a polypeptide which still has nECR activity as specified above. Accordingly, the polypeptide may comprise or consist of the domains of the polypeptide of the present invention conferring the said biological activity. A fragment as meant herein, preferably, comprises at least 15, at least 20, at least 50, at least 100, at least 250 or at least 500 consecutive nucleotides of any one of the aforementioned nucleic acid sequences or encodes an amino acid sequence comprising at least 5, at least 10, at least 20, at least 30, at least 50, at least 80, at least 100 or at least 150 consecutive amino acids of any one of the aforementioned amino acid sequences.

The variant nucleic acid molecule or fragments referred to above, preferably, encode polypeptides retaining at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% or at least 90% of the nECR activity exhibited by the polypeptide encoded by the nucleotide sequences.

The term " polynucleotide" as used in accordance with the present invention also relates to a polynucleotide comprising a nucleic acid sequence which encodes a polypeptide havingacyltransferase activity. Preferably, the polypeptide encoded by the polynucleotide of the present invention having acyltransferaes activity upon expression in a plant shall be capable of increasing the amount of PUFA and, in particular, LCPUFA esterified to triglycerides in, e.g., seed oils or the entire plant or parts thereof. Such an increase is, preferably, statistically significant when compared to a LCPUFA producing transgenic control plant which expresses the minimal set of desaturases and elongases required for LCPUFA synthesis but does not express the polynucleotide of the present invention. Such a transgenic plant may, preferably, express desaturases and elongases comprised by the vector LJB765 listed in table 11 of example 5 in WO2009/016202 or a similar set of desaturases and elongases required for DHA synthesis. Whether an increase is significant can be determined by statistical tests well known in the art including, e.g., Student's t-test. More preferably, the increase is an increase of the

amount of triglycerides containing LCPUFA of at least 5%, at least 10%, at least 15%, at least 20% or at least 30% compared to the said control. Preferably, the LCPUFA referred to before is a polyunsaturated fatty acid having a C-20, C-22 or C24 fatty acid body, more preferably, EPA or DHA, most preferably, DHA. Suitable assays for measuring the activities mentioned before are described in the accompanying Examples. Variant nucleic acid molecules as referred above may be obtained by various natural as well as artificial sources. For example, nucleic acid molecules may be obtained by in vitro and in vivo mutagenesis approaches using the above mentioned specific nucleic acid molecules as a basis. Moreover, nucleic acid molecules being homologs or orthologs may be obtained from various animal, plant or fungus species. Preferably, they are obtained from plants such as algae, for example *Isochrysis*, *Mantoniella*, *Ostreococcus* or *Cryptocodinium*, algae/diatoms such as *Phaeodactylum*, *Thalassiosira* or *Thraustochytrium*, mosses such as *Physcomitrella* or *Ceratodon*, or higher plants such as the *Primulaceae* such as *Aleuritia*, *Calendula stellata*, *Osteospermum spinescens* or *Osteospermum hyoseroides*, microorganisms such as fungi, such as *Aspergillus*, *Phytophthora*, *Entomophthora*, *Mucor* or *Mortierella*, bacteria such as *Shewanella*, yeasts or animals. Preferred animals are nematodes such as *Caenorhabditis*, insects or vertebrates. Among the vertebrates, the nucleic acid molecules may, preferably, be derived from *Euteleostomi*, *Actinopterygii*; *Neopterygii*; *Teleostei*; *Euteleostei*, *Protacanthopterygii*, *Salmoniformes*; *Salmonidae* or *Oncorhynchus*, more preferably, from the order of the *Salmoniformes*, most preferably, the family of the *Salmonidae*, such as the genus *Salmo*, for example from the genera and species *Oncorhynchus mykiss*, *Trutta trutta* or *Salmo trutta fario*. Moreover, the nucleic acid molecules may be obtained from the diatoms such as the genera *Thalassiosira* or *Phaeodactylum*.

Thus the present invention also relates to a polynucleotide comprising at least one nucleic acid sequence encoding a polypeptide having acyltransferase activity additionally to the above-mentioned polypeptides exhibit desaturase, elongase or beta-ketoacyl reductase, dehydratase or enoyl-CoA reductase activity. Therefore the polynucleotide of the present invention also comprising at least one nucleic acid sequence encoding a polypeptide having acyltransferase activity, wherein the nucleic acid sequence is heterologous to said polypeptide having desaturase, elongase, beta-ketoacyl reductase, dehydratase or enoyl-CoA reductase activity and wherein at least one seed-specific plant promoter and at least one terminator sequence are operatively linked to the said nucleic acid sequence and wherein one or more nucleic acid expression enhancing nucleic acid (NEENA) molecule is/are functionally linked to said promoter and which is/are heterologous to said promoter.

The term "acyltransferase activity" or "acyltransferase" as used herein encompasses all enzymatic activities and enzymes which are capable of transferring or are involved in the transfer of PUFA and, in particular; LCPUFA from the acyl-CoA pool or the membrane phospholipids to the triglycerides, from the acyl-CoA pool to membrane lipids and from membrane lipids to the acyl-CoA pool by a transesterification process. It will be understood that this acyltransferase activity will result in an increase of the LCPUFA esterified to triglycerides in, e.g., seed oils. In

particular, it is envisaged that these acyltransferases are capable of producing triglycerides having esterified EPA or even DHA, or that these acyltransferases are capable of enhancing synthesis of desired PUFA by increasing the flux for specific intermediates of the desired PUFA between the acyl-CoA pool (the site of elongation) and membrane lipids (the predominant site of desaturation). Specifically, acyltransferase activity as used herein pertains to lysophospholipid acyltransferase (LPLAT) activity, preferably, lysophosphatidylcholine acyltransferase (LPCAT) or Lysophosphophatidylethanolamine acyltransferase (LPEAT) activity, lysophosphosphatidic acid acyltransferase (LPAAT) activity, phospholipid:diacylglycerol acyltransferase (PDAT) activity, glycerol-3-phosphate acyltransferase (GPAT) activity or diacylglycerol acyltransferase (DGAT), and, more preferably, to PLAT, LPAAT, DGAT, PDAT or GPAT activity.

A polynucleotide encoding a polypeptide having a acyltransferase activity as specified above could be obtained for example from *Phytophthora infestans*. Polynucleotides encoding a polypeptide having desaturase or elongase activity as specified above could be obtained in accordance with the present invention from *Thraustochytrium* ssp. for example. Preferred acyltransferases which shall be present in the host cell are at least one enzyme selected from the group consisting of: LPLATs, LPAATs, DGATs, PDATs and GPATs. Especially preferred are the LPLATs LPLAT(Ce) from *Caenorhabditis elegans* (WO2004076617), LPCAT(Ms) from *Mantoniella squamata* (WO2006069936) and LPCAT(Ot) from *Ostreococcus tauri* (WO2006069936), pLPLAT_01332(Pi) (SEQ-ID No.:104 encoding the polypeptide SEQ-ID No.:125) pLPLAT_01330(Pi) (SEQ-ID No.:105 encoding the polypeptide SEQ-ID No.:126), pLPLAT_07077(Pi) (SEQ-ID No.:106 encoding the polypeptide SEQ-ID No.:127), LPLAT_18374(Pi) (SEQ-ID No.:107 encoding the polypeptide SEQ-ID No.:128), pLPLAT_14816(Pi) (SEQ-ID No.:108 encoding the polypeptide SEQ-ID No.:129), LPCAT_02075(Pi) (SEQ-ID No.:111 encoding the polypeptide SEQ-ID No.:132), pLPAAT_06638(Pi) (SEQ-ID No.:112 encoding the polypeptide SEQ-ID No.:133) from *Phytophthora infestans*, the LPAATs LPAAT(Ma)1.1 from *Mortierella alpina* (WO2004087902), LPAAT(Ma)1.2 from *Mortierella alpina* (WO2004087902), the LPAAT_13842(Pi) (SEQ-ID No.:109 encoding the polypeptide SEQ-ID No.:130), pLPAAT_10763(Pi) (SEQ-ID No.:110 encoding the polypeptide SEQ-ID No.:131) from *Phytophthora infestans*, the DGATs DGAT2(Cc) from *Cryptocodinium cohnii* (WO2004087902), pDGAT1_12278(Pi) (SEQ-ID No.:113 encoding the polypeptide SEQ-ID No.:134), DGAT2_03074(Pi) (SEQ-ID No.:114 encoding the polypeptide SEQ-ID No.:135), pDGAT2_08467(Pi) (SEQ-ID No.:115 encoding the polypeptide SEQ-ID No.:136), DGAT2_08470(Pi) (SEQ-ID No.:116 encoding the polypeptide SEQ-ID No.:137), pDGAT2_03835-mod(Pi) (SEQ-ID No.:117 encoding the polypeptide SEQ-ID No.:138), DGAT2_11677-mod(Pi) (SEQ-ID No.:118 encoding the polypeptide SEQ-ID No.:139), DGAT2_08432-mod(Pi) (SEQ-ID No.:119 encoding the polypeptide SEQ-ID No.:140), pDGAT2_08431(Pi) (SEQ-ID No.:120 encoding the polypeptide SEQ-ID No.:141), DGAT_13152-mod(Pi) (SEQ-ID No.:121 encoding the polypeptide SEQ-ID No.:142), the PDAT pPDAT_11965-mod(Pi) (SEQ-ID No.:122 encoding the polypeptide SEQ-ID No.:143) and the GPATs pGPAT-PITG_18707 (SEQ-ID No.:123 encoding the polypeptide SEQ-ID No.:144) and pGPAT-PITG_03371 (SEQ-ID No.:124 encoding the polypeptide SEQ-ID No.:145).

However, orthologs, paralogs or other homologs may be identified from other species. Preferably, they are obtained from plants such as algae, for example *Isochrysis*, *Mantoniella*, *Ostreococcus* or *Cryptocodinium*, algae/diatoms such as *Phaeodactylum* or *Thalassiosira* or *Thraustochytrium*, mosses such as *Physcomitrella* or *Ceratodon*, or higher plants such as the *Primulaceae* such as *Aleuritia*, *Calendula stellata*, *Osteospermum spinescens* or *Osteospermum hyoseroides*, microorganisms such as fungi, such as *Aspergillus*, *Phytophthora*, *Entomophthora*, *Mucor* or *Mortierella*, bacteria such as *Shewanella*, yeasts or animals. Preferred animals are nematodes such as *Caenorhabditis*, insects or vertebrates. Among the vertebrates, the nucleic acid molecules may, preferably, be derived from *Euteleostomi*, *Actinopterygii*; *Neopterygii*; *Teleostei*; *Euteleostei*, *Protacanthopterygii*, *Salmoniformes*; *Salmonidae* or *Oncorhynchus*, more preferably, from the order of the *Salmoniformes*, most preferably, the family of the *Salmonidae*, such as the genus *Salmo*, for example from the genera and species *Oncorhynchus mykiss*, *Trutta trutta* or *Salmo trutta fario*. Moreover, the nucleic acid molecules may be obtained from the diatoms such as the genera *Thalassiosira* or *Phaeodactylum*.

Thus, the term “ polynucleotide” as used in accordance with the present invention further encompasses variants of the aforementioned specific polynucleotides representing orthologs, paralogs or other homologs of the polynucleotide of the present invention. Moreover, variants of the polynucleotide of the present invention also include artificially generated muteins. Said muteins include, e.g., enzymes which are generated by mutagenesis techniques and which exhibit improved or altered substrate specificity, or codon optimized polynucleotides. The polynucleotide variants, preferably, comprise a nucleic acid sequence characterized in that the sequence can be derived from the aforementioned specific nucleic acid sequences shown in any one of SEQ ID NOs: 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123 or 124 by a polynucleotide encoding a polypeptide having an amino acid sequence (i.e. as shown in any one of SEQ ID NOs: 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144 or 145 as for acyltransferases) by at least one nucleotide substitution, addition and/or deletion, whereby the variant nucleic acid sequence shall still encode a polypeptide having a desaturase or elongase activity as specified above. Variants also encompass polynucleotides comprising a nucleic acid sequence which is capable of hybridizing to the aforementioned specific nucleic acid sequences, preferably, under stringent hybridization conditions. These stringent conditions are known to the skilled worker and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N. Y. (1989), 6.3.1-6.3.6. A preferred example for stringent hybridization conditions are hybridization conditions in $6 \times$ sodium chloride/sodium citrate (= SSC) at approximately 45°C, followed by one or more wash steps in $0.2 \times$ SSC, 0.1% SDS at 50 to 65°C. The skilled worker knows that these hybridization conditions differ depending on the type of nucleic acid and, for example when organic solvents are present, with regard to the temperature and concentration of the buffer. For example, under “ standard hybridization conditions” the temperature differs depending on the type of nucleic acid

between 42°C and 58°C in aqueous buffer with a concentration of 0.1 to 5 × SSC (pH 7.2). If organic solvent is present in the abovementioned buffer, for example 50% formamide, the temperature under standard conditions is approximately 42°C. The hybridization conditions for DNA: DNA hybrids are, preferably, 0.1 × SSC and 20°C to 45°C, preferably between 30°C and 45°C. The hybridization conditions for DNA:RNA hybrids are, preferably, 0.1 × SSC and 30°C to 55°C, preferably between 45°C and 55°C. The abovementioned hybridization temperatures are determined for example for a nucleic acid with approximately 100 bp (= base pairs) in length and a G + C content of 50% in the absence of formamide. The skilled worker knows how to determine the hybridization conditions required by referring to textbooks such as the textbook mentioned above, or the following textbooks: Sambrook et al., "Molecular Cloning" , Cold Spring Harbor Laboratory, 1989; Hames and Higgins (Ed.) 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. Alternatively, polynucleotide variants are obtainable by PCR-based techniques such as mixed oligonucleotide primer- based amplification of DNA, i.e. using degenerated primers against conserved domains of the polypeptides of the present invention. Conserved domains of the polypeptide of the present invention may be identified by a sequence comparison of the nucleic acid sequences of the polynucleotides or the amino acid sequences of the polypeptides of the present invention. Oligonucleotides suitable as PCR primers as well as suitable PCR conditions are described in the accompanying Examples. As a template, DNA or cDNA from bacteria, fungi, plants or animals may be used. Further, variants include polynucleotides comprising nucleic acid sequences which are at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the nucleic acid sequences shown in any one of SEQ ID NOs: 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123 or 124 preferably, encoding polypeptides retaining a desaturase, elongase, or acyltransferase activity as specified above. Moreover, also encompassed are polynucleotides which comprise nucleic acid sequences encoding a polypeptide having an amino acid sequences which are at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequences encoded by the nucleic acid sequences shown in any one of SEQ ID NOs: 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123 or 124 (i.e. as shown in any one of SEQ ID NOs: 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144 or 145 as for acyltransferases), wherein the polypeptide, preferably, retains desaturase, elongase or acyltransferase activity as specified above. The percent identity values are, preferably, calculated over the entire amino acid or nucleic acid sequence region. A series of programs based on a variety of algorithms is available to the skilled worker for comparing different sequences. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch algorithm (Needleman 1970, J. Mol. Biol. (48):444-453) which has been incorporated into the needle program in the EMBOSS software package (EMBOSS: The European Molecular Biology

Open Software Suite, Rice,P., Longden,I., and Bleasby,A, Trends in Genetics 16(6), 276-277, 2000), using either a BLOSUM 45 or PAM250 scoring matrix for distantly related proteins, or either a BLOSUM 62 or PAM160 scoring matrix for closer related proteins, and a gap opening penalty of 16, 14, 12, 10, 8, 6, or 4 and a gap extension penalty of 0.5, 1, 2, 3, 4, 5, or 6.

5 Guides for local installation of the EMBOSS package as well as links to WEB-Services can be found at <http://emboss.sourceforge.net>. A preferred, non-limiting example of parameters to be used for aligning two amino acid sequences using the needle program are the default parameters, including the EBLOSUM62 scoring matrix, a gap opening penalty of 10 and a gap extension penalty of 0.5. In yet another preferred embodiment, the percent identity between two

10 nucleotide sequences is determined using the needle program in the EMBOSS software package (EMBOSS: The European Molecular Biology Open Software Suite, Rice,P., Longden,I., and Bleasby,A, Trends in Genetics 16(6), 276-277, 2000), using the EDNAFULL scoring matrix and a gap opening penalty of 16, 14, 12, 10, 8, 6, or 4 and a gap extension penalty of 0.5, 1, 2, 3, 4, 5, or 6. A preferred, non-limiting example of parameters to be used in

15 conjunction for aligning two amino acid sequences using the needle program are the default parameters, including the EDNAFULL scoring matrix, a gap opening penalty of 10 and a gap extension penalty of 0.5. The nucleic acid and protein sequences of the present invention can further be used as a “ query sequence” to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed

20 using the BLAST series of programs (version 2.2) of Altschul et al. (Altschul 1990, J. Mol. Biol. 215:403-10). BLAST using nucleic acid sequences of the invention as query sequence can be performed with the BLASTn, BLASTx or the tBLASTx program using default parameters to obtain either nucleotide sequences (BLASTn, tBLASTx) or amino acid sequences (BLASTx) homologous to sequences encoded by the nucleic acid sequences of the invention. BLAST

25 using protein sequences encoded by the nucleic acid sequences of the invention as query sequence can be performed with the BLASTp or the tBLASTn program using default parameters to obtain either amino acid sequences (BLASTp) or nucleic acid sequences (tBLASTn) homologous to sequences of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST using default parameters can be utilized as described in

30 Altschul et al. (Altschul 1997, Nucleic Acids Res. 25(17):3389-3402).

The following block diagram shows the relation of sequence types of query and hit sequences for various BLAST programs

Input query sequence	Converted Query	Algorithm	Converted Hit	Actual Database	35
DNA		BLASTn		DNA	
PRT		BLASTp		PRT	
DNA	PRT	BLASTx		PRT	
PRT		tBLASTn	PRT	DNA	40
DNA	PRT	tBLASTx	PRT	DNA	

A polynucleotide comprising a fragment of any of the aforementioned nucleic acid sequences is also encompassed as a polynucleotide of the present invention. The fragment shall encode a polypeptide which still has desaturase and elongase activity as specified above. Accordingly, the polypeptide may comprise or consist of the domains of the polypeptide of the present invention conferring the said biological activity. A fragment as meant herein, preferably, comprises at least 50, at least 100, at least 250 or at least 500 consecutive nucleotides of any one of the aforementioned nucleic acid sequences or encodes an amino acid sequence comprising at least 20, at least 30, at least 50, at least 80, at least 100 or at least 150 consecutive amino acids of any one of the aforementioned amino acid sequences.

The variant polynucleotides or fragments referred to above, preferably, encode polypeptides retaining desaturase or elongase activity to a significant extent, preferably, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% or at least 90% of the desaturase and elongase activity exhibited by any of the polypeptide encoded by the nucleic acid sequences shown in any one of SEQ ID NOs: 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123 or 124 (i.e. as shown in any one of SEQ ID NOs: 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144 or 145 as for acyltransferases). The activity may be tested as described in the accompanying Examples.

The polynucleotides of the present invention either essentially consist of the aforementioned nucleic acid sequences or comprise the aforementioned nucleic acid sequences. Thus, they may contain further nucleic acid sequences as well. Preferably, the polynucleotide of the present invention may comprise in addition to an open reading frame further untranslated sequence at the 3' and at the 5' terminus of the coding gene region: at least 500, preferably 200, more preferably 100 nucleotides of the sequence upstream of the 5' terminus of the coding region and at least 100, preferably 50, more preferably 20 nucleotides of the sequence downstream of the 3' terminus of the coding gene region. Furthermore, the polynucleotides of the present invention may encode fusion proteins wherein one partner of the fusion protein is a polypeptide being encoded by a nucleic acid sequence recited above. Such fusion proteins may comprise as additional part other enzymes of the fatty acid or PUFA biosynthesis pathways, polypeptides for monitoring expression (e.g., green, yellow, blue or red fluorescent proteins, alkaline phosphatase and the like) or so called "tags" which may serve as a detectable marker or as an auxiliary measure for purification purposes. Tags for the different purposes are well known in the art and comprise FLAG-tags, 6-histidine-tags, MYC-tags and the like.

The polynucleotide of the present invention shall be provided, preferably, either as an isolated polynucleotide (i.e. purified or at least isolated from its natural context such as its natural gene locus) or in genetically modified or exogenously (i.e. artificially) manipulated form. An isolated polynucleotide can, for example, comprise less than approximately 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in the genomic DNA of the cell from which the nucleic acid is derived. The polynucleotide, preferably,

is provided in the form of double or single stranded molecule. It will be understood that the present invention by referring to any of the aforementioned polynucleotides of the invention also refers to complementary or reverse complementary strands of the specific sequences or variants thereof referred to before. The polynucleotide encompasses DNA, including cDNA and genomic DNA, or RNA polynucleotides.

However, the present invention also pertains to polynucleotide variants which are derived from the polynucleotides of the present invention and are capable of interfering with the transcription or translation of the polynucleotides of the present invention. Such variant polynucleotides include anti-sense nucleic acids, ribozymes, siRNA molecules, morpholino nucleic acids (phosphorodiamidate morpholino oligos), triple-helix forming oligonucleotides, inhibitory oligonucleotides, or micro RNA molecules all of which shall specifically recognize the polynucleotide of the invention due to the presence of complementary or substantially complementary sequences. These techniques are well known to the skilled artisan. Suitable variant polynucleotides of the aforementioned kind can be readily designed based on the structure of the polynucleotides of this invention.

Moreover, comprised are also chemically modified polynucleotides including naturally occurring modified polynucleotides such as glycosylated or methylated polynucleotides or artificial modified ones such as biotinylated polynucleotides.

In a preferred embodiment of the polynucleotide of the present invention, said polynucleotide further comprises an expression control sequence operatively linked to the said nucleic acid sequence.

The term “ expression control sequence” as used herein refers to a nucleic acid sequence which is capable of governing, i.e. initiating and controlling, transcription of a nucleic acid sequence of interest, in the present case the nucleic sequences recited above. Such a sequence usually comprises or consists of a promoter or a combination of a promoter and enhancer sequences. Expression of a polynucleotide comprises transcription of the nucleic acid molecule, preferably, into a translatable mRNA. Additional regulatory elements may include transcriptional as well as translational enhancers. The following promoters and expression control sequences may be, preferably, used in an expression vector according to the present invention. The cos, tac, trp, tet, trp-tet, lpp, lac, lpp-lac, lacIq, T7, T5, T3, gal, trc, ara, SP6, λ - PR or λ -PL promoters are, preferably, used in Gram-negative bacteria. For Gram-positive bacteria, promoters amy and SPO2 may be used. From yeast or fungal promoters ADC1, AOX1r, GAL1, MF α , AC, P-60, CYC1, GAPDH, TEF, rp28, ADH are, preferably, used. For animal cell or organism expression, the promoters CMV-, SV40-, RSV-promoter (Rous sarcoma virus), CMV-enhancer, SV40-enhancer are preferably used. From plants the promoters CaMV/35S (Franck 1980, Cell 21: 285-294], PRP1 (Ward 1993, Plant. Mol. Biol. 22), SSU, OCS, lib4, usp, STLS1, B33, nos or the ubiquitin or phaseolin promoter. Also preferred in this context are inducible promoters, such as the promoters described in EP 0 388 186 A1 (i.e. a

benzylsulfonamide-inducible promoter), Gatz 1992, Plant J. 2:397-404 (i.e. a tetracyclin-inducible promoter), EP 0 335 528 A1 (i.e. a abscisic-acid-inducible promoter) or WO 93/21334 (i.e. a ethanol- or cyclohexenol-inducible promoter). Further suitable plant promoters are the promoter of cytosolic FBPase or the ST-LSI promoter from potato (Stockhaus 1989, EMBO J. 8, 2445), the phosphoribosyl-pyrophosphate amidotransferase promoter from Glycine max (Genbank accession No. U87999) or the node-specific promoter described in EP 0 249 676 A1. Particularly preferred are promoters which enable the expression in tissues which are involved in the biosynthesis of fatty acids. Also particularly preferred are seed-specific promoters such as the USP promoter in accordance with the practice, but also other promoters such as the LeB4, DC3, phaseolin or napin promoters. Further especially preferred promoters are seed-specific promoters which can be used for monocotyledonous or dicotyledonous plants and which are described in US 5,608,152 (napin promoter from oilseed rape), WO 98/45461 (oleosin promoter from Arobidopsis, US 5,504,200 (phaseolin promoter from Phaseolus vulgaris), WO 91/13980 (Bce4 promoter from Brassica), by Baeumlein et al., Plant J., 2, 2, 1992:233-239 (LeB4 promoter from a legume), these promoters being suitable for dicots. The following promoters are suitable for monocots: lpt-2 or lpt-1 promoter from barley (WO 95/15389 and WO 95/23230), hordein promoter from barley and other promoters which are suitable and which are described in WO 99/16890. In principle, it is possible to use all natural promoters together with their regulatory sequences, such as those mentioned above, for the novel process. Likewise, it is possible and advantageous to use synthetic promoters, either additionally or alone, especially when they mediate a seed-specific expression, such as, for example, as described in WO 99/16890. In a particular embodiment, seed-specific promoters are utilized to enhance the production of the desired PUFA or LCPUFA. In a preferred embodiment of the present invention promoters encoded by the nucleic acid sequences shown in any one of SEQ ID NOs: 25, 26, 27, 28, 29 or 30 are used.

The term “ operatively linked” as used herein means that the expression control sequence and the nucleic acid of interest are linked so that the expression of the said nucleic acid of interest can be governed by the said expression control sequence, i.e. the expression control sequence shall be functionally linked to the said nucleic acid sequence to be expressed. Accordingly, the expression control sequence and, the nucleic acid sequence to be expressed may be physically linked to each other, e.g., by inserting the expression control sequence at the 5'end of the nucleic acid sequence to be expressed. Alternatively, the expression control sequence and the nucleic acid to be expressed may be merely in physical proximity so that the expression control sequence is capable of governing the expression of at least one nucleic acid sequence of interest. The expression control sequence and the nucleic acid to be expressed are, preferably, separated by not more than 500 bp, 300 bp, 100 bp, 80 bp, 60 bp, 40 bp, 20 bp, 10 bp or 5 bp.

In a further preferred embodiment of the polynucleotide of the present invention, said polynucleotide further comprises a terminator sequence operatively linked to the nucleic acid sequence. Preferably used terminators are encoded by the nucleotide sequences shown in

SEQ ID NOs: 36 or 37. More preferably used terminators are encoded by the nucleotide sequences shown in SEQ ID NOs: 31, 32, 33, 34 or 35

The term “ terminator” as used herein refers to a nucleic acid sequence which is capable of terminating transcription. These sequences will cause dissociation of the transcription machinery from the nucleic acid sequence to be transcribed. Preferably, the terminator shall be active in plants and, in particular, in plant seeds. Suitable terminators are known in the art and, preferably, include polyadenylation signals such as the SV40-poly-A site or the tk-poly-A site or one of the plant specific signals indicated in Loke et al. (Loke 2005, Plant Physiol 138, pp. 1457-1468), downstream of the nucleic acid sequence to be expressed.

The present invention also relates to a vector comprising the polynucleotide of the present invention.

The term “ vector” , preferably, encompasses phage, plasmid, viral vectors as well as artificial chromosomes, such as bacterial or yeast artificial chromosomes. Moreover, the term also relates to targeting constructs which allow for random or site- directed integration of the targeting construct into genomic DNA. Such target constructs, preferably, comprise DNA of sufficient length for either homologous or heterologous recombination as described in detail below. The vector encompassing the polynucleotide of the present invention, preferably, further comprises selectable markers for propagation and/or selection in a host. The vector may be incorporated into a host cell by various techniques well known in the art. If introduced into a host cell, the vector may reside in the cytoplasm or may be incorporated into the genome. In the latter case, it is to be understood that the vector may further comprise nucleic acid sequences which allow for homologous recombination or heterologous insertion. Vectors can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. The terms “ transformation” and “ transfection” , conjugation and transduction, as used in the present context, are intended to comprise a multiplicity of prior-art processes for introducing foreign nucleic acid (for example DNA) into a host cell, including calcium phosphate, rubidium chloride or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, natural competence, carbon-based clusters, chemically mediated transfer, electroporation or particle bombardment. Suitable methods for the transformation or transfection of host cells, including plant cells, can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) and other laboratory manuals, such as Methods in Molecular Biology, 1995, Vol. 44, Agrobacterium protocols, Ed.: Gartland and Davey, Humana Press, Totowa, New Jersey. Alternatively, a plasmid vector may be introduced by heat shock or electroporation techniques. Should the vector be a virus, it may be packaged in vitro using an appropriate packaging cell line prior to application to host cells.

Preferably, the vector referred to herein (VC-LJBXXX) is suitable as a cloning vector, i.e. replicable in microbial systems. Such vectors ensure efficient cloning in bacteria and, preferably,

yeasts or fungi and make possible the stable transformation of plants. Those which must be mentioned are, in particular, various binary and co-integrated vector systems which are suitable for the T-DNA-mediated transformation. Such vector systems are, as a rule, characterized in that they contain at least the vir genes, which are required for the Agrobacterium-mediated transformation, and the sequences which delimit the T-DNA (T-DNA border). These vector systems, preferably, also comprise further cis-regulatory regions such as promoters and terminators and/or selection markers with which suitable transformed host cells or organisms can be identified. While co-integrated vector systems have vir genes and T-DNA sequences arranged on the same vector, binary systems are based on at least two vectors, one of which bears vir genes, but no T-DNA, while a second one bears T-DNA, but no vir gene. As a consequence, the last-mentioned vectors are relatively small, easy to manipulate and can be replicated both in *E. coli* and in *Agrobacterium*. These binary vectors include vectors from the pBIB-HYG, pPZP, pBecks, pGreen series. Preferably used in accordance with the invention are Bin19, pBI101, pBinAR, pGPTV and pCAMBIA. An overview of binary vectors and their use can be found in Hellens et al, Trends in Plant Science (2000) 5, 446– 451. Furthermore, by using appropriate cloning vectors, the polynucleotides can be introduced into host cells or organisms such as plants or animals and, thus, be used in the transformation of plants, such as those which are published, and cited, in: Plant Molecular Biology and Biotechnology (CRC Press, Boca Raton, Florida), chapter 6/7, pp. 71-119 (1993); F.F. White, Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, 15-38; B. Jené et al., Techniques for Gene Transfer, in: Transgenic Plants, vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press (1993), 128-143; Potrykus 1991, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42, 205-225.

More preferably, the vector of the present invention is an expression vector. In such an expression vector, i.e. a vector which comprises the polynucleotide of the invention having the nucleic acid sequence operatively linked to an expression control sequence (also called “ expression cassette”) allowing expression in prokaryotic or eukaryotic cells or isolated fractions thereof. Suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pCDM8, pRc/CMV, pcDNA1, pcDNA3 (Invitrogen) or pSPORT1 (GIBCO BRL). Further examples of typical fusion expression vectors are pGEX (Pharmacia Biotech Inc; Smith 1988, Gene 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ), where glutathione S-transferase (GST), maltose E-binding protein and protein A, respectively, are fused with the recombinant target protein. Examples of suitable inducible nonfusion *E. coli* expression vectors are, inter alia, pTrc (Amann 1988, Gene 69:301-315) and pET 11d (Studier 1990, Methods in Enzymology 185, 60-89). The target gene expression of the pTrc vector is based on the transcription from a hybrid trp-lac fusion promoter by host RNA polymerase. The target gene expression from the pET 11d vector is based on the transcription of a T7-gn10-lac fusion promoter, which is mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is provided by the host strains BL21 (DE3) or HMS174 (DE3) from a resident λ -prophage which harbors a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter. The skilled worker is familiar with

other vectors which are suitable in prokaryotic organisms; these vectors are, for example, in *E. coli*, pLG338, pACYC184, the pBR series such as pBR322, the pUC series such as pUC18 or pUC19, the M113mp series, pKC30, pRep4, pHS1, pHS2, pPLc236, pMBL24, pLG200, pUR290, pIN-III113-B1, λ gt11 or pBdCl, in *Streptomyces* pIJ101, pIJ364, pIJ702 or pIJ361, in *Bacillus* pUB110, pC194 or pBD214, in *Corynebacterium* pSA77 or pAJ667. Examples of vectors for expression in the yeast *S. cerevisiae* comprise pYep Sec1 (Baldari 1987, *Embo J.* 6:229-234), pMFa (Kurjan 1982, *Cell* 30:933-943), pJRY88 (Schultz 1987, *Gene* 54:113-123) and pYES2 (Invitrogen Corporation, San Diego, CA). Vectors and processes for the construction of vectors which are suitable for use in other fungi, such as the filamentous fungi, comprise those which are described in detail in: van den Hondel, C.A.M.J.J., & Punt, P.J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: *Applied Molecular Genetics of fungi*, J.F. Peberdy et al., Ed., pp. 1-28, Cambridge University Press: Cambridge, or in: *More Gene Manipulations in Fungi* (J.W. Bennett & L.L. Lasure, Ed., pp. 396-428: Academic Press: San Diego). Further suitable yeast vectors are, for example, pAG-1, YEpl3 or pEMBLYe23. As an alternative, the polynucleotides of the present invention can be also expressed in insect cells using baculovirus expression vectors. Baculovirus vectors which are available for the expression of proteins in cultured insect cells (for example Sf9 cells) comprise the pAc series (Smith 1983, *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow 1989, *Virology* 170:31-39).

The abovementioned vectors are only a small overview of vectors to be used in accordance with the present invention. Further vectors are known to the skilled worker and are described, for example, in: *Cloning Vectors* (Ed., Pouwels, P.H., et al., Elsevier, Amsterdam-New York-Oxford, 1985, ISBN 0 444 904018). For further suitable expression systems for prokaryotic and eukaryotic cells see the chapters 16 and 17 of Sambrook, loc cit.

It follows from the above that, preferably, said vector is an expression vector. More preferably, the said polynucleotide of the present invention is under the control of a seed-specific promoter in the vector of the present invention. A preferred seed-specific promoter as meant herein is selected from the group consisting of Conlinin 1, Conlinin 2, napin, LuFad3, USP, LeB4, Arc, Fae, ACP, LuPXR, and SBP. For details, see, e.g., US 2003-0159174.

The polynucleotide of the present invention can be expressed in single-cell plant cells (such as algae), see Falciatore 1999, *Marine Biotechnology* 1 (3):239-251 and the references cited therein, and plant cells from higher plants (for example Spermatophytes, such as arable crops) by using plant expression vectors. Examples of plant expression vectors comprise those which are described in detail in: Becker 1992, *Plant Mol. Biol.* 20:1195-1197; Bevan 1984, *Nucl. Acids Res.* 12:8711-8721; *Vectors for Gene Transfer in Higher Plants*; in: *Transgenic Plants*, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, p. 15-38. A plant expression cassette, preferably, comprises regulatory sequences which are capable of controlling the gene expression in plant cells and which are functionally linked so that each sequence can fulfill its function, such as transcriptional termination, for example polyadenylation

signals. Preferred polyadenylation signals are those which are derived from *Agrobacterium tumefaciens* T-DNA, such as the gene 3 of the Ti plasmid pTiACH5, which is known as octopine synthase (Gielen 1984, EMBO J. 3, 835) or functional equivalents of these, but all other terminators which are functionally active in plants are also suitable. Since plant gene expression is very often not limited to transcriptional levels, a plant expression cassette preferably comprises other functionally linked sequences such as translation enhancers, for example the overdrive sequence, which comprises the 5' -untranslated tobacco mosaic virus leader sequence, which increases the protein/RNA ratio (Gallie 1987, Nucl. Acids Research 15:8693-8711). As described above, plant gene expression must be functionally linked to a suitable promoter which performs the expression of the gene in a timely, cell-specific or tissue-specific manner. Promoters which can be used are constitutive promoters (Benfey 1989, EMBO J. 8:2195-2202) such as those which are derived from plant viruses such as 35S CAMV (Frank 1980, Cell 21:285-294), 19S CaMV (see US 5,352,605 and WO 84/02913) or plant promoters such as the promoter of the Rubisco small subunit, which is described in US 4,962,028. Other preferred sequences for the use in functional linkage in plant gene expression cassettes are targeting sequences which are required for targeting the gene product into its relevant cell compartment (for a review, see Kermode 1996, Crit. Rev. Plant Sci. 15, 4: 285-423 and references cited therein), for example into the vacuole, the nucleus, all types of plastids, such as amyloplasts, chloroplasts, chromoplasts, the extracellular space, the mitochondria, the endoplasmic reticulum, oil bodies, peroxisomes and other compartments of plant cells. As described above, plant gene expression can also be facilitated via a chemically inducible promoter (for a review, see Gatz 1997, Annu. Rev. Plant Physiol. Plant Mol. Biol., 48:89-108). Chemically inducible promoters are particularly suitable if it is desired that genes are expressed in a time-specific manner. Examples of such promoters are a salicylic-acid-inducible promoter (WO 95/19443), a tetracyclin-inducible promoter (Gatz 1992, Plant J. 2, 397-404) and an ethanol-inducible promoter. Promoters which respond to biotic or abiotic stress conditions are also suitable promoters, for example the pathogen-induced PRP1-gene promoter (Ward 1993, Plant Mol. Biol. 22:361-366), the heat-inducible hsp80 promoter from tomato (US 5,187,267), the cold-inducible alpha-amylase promoter from potato (WO 96/12814) or the wound-inducible pinII promoter (EP 0 375 091 A). The promoters which are especially preferred are those which bring about the expression of genes in tissues and organs in which fatty acid, lipid and oil biosynthesis takes place, in seed cells such as the cells of endosperm and of the developing embryo. Suitable promoters are the napin gene promoter from oilseed rape (US 5,608,152), the USP promoter from *Vicia faba* (Baeumlein 1991, Mol. Gen. Genet. 225 (3):459-67), 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 legumin B4 promoter (LeB4; Baeumlein 1992, Plant Journal, 2 (2):233-9), and promoters which bring about the seed-specific expression in monocotyledonous plants such as maize, barley, wheat, rye, rice and the like. Suitable promoters to be taken into consideration are the lpt2 or lpt1 gene promoter from barley (WO 95/15389 and WO 95/23230) or those which are described in WO 99/16890 (promoters from the barley hordein gene, the rice glutelin gene, the rice oryzin gene, the rice prolamin gene, the wheat gliadin gene, wheat glutelin gene, the maize zein gene, the oat

glutelin gene, the sorghum kasirin gene, the rye secalin gene). Likewise, especially suitable are promoters which bring about the plastid-specific expression since plastids are the compartment in which the precursors and some end products of lipid biosynthesis are synthesized. Suitable promoters such as the viral RNA-polymerase promoter, are described in WO 95/16783 and WO 97/06250, and the clpP promoter from Arabidopsis, described in WO 99/46394.

Moreover, the present invention relates to a host cell comprising the polynucleotide or the vector of the present invention. The term "host cell" is also meant as "host cell culture".

Preferably, said host cell is a plant cell or plant cell culture and, more preferably, a plant cell obtained from an oilseed crop. More preferably, said oilseed crop is selected from the group consisting of flax (*Linum* sp.), rapeseed (*Brassica* sp.), soybean (*Glycine* sp.), sunflower (*Helianthus* sp.), cotton (*Gossypium* sp.), corn (*Zea mays*), olive (*Olea* sp.), safflower (*Carthamus* sp.), cocoa (*Theobroma cacao*), peanut (*Arachis* sp.), hemp, camelina, crambe, oil palm, coconuts, groundnuts, sesame seed, castor bean, lesquerella, tallow tree, sheanuts, tungnuts, kapok fruit, poppy seed, jojoba seeds and perilla.

Also preferably, said host cell is a microorganism. More preferably, said microorganism is a bacterium, a fungus or algae. More preferably, it is selected from the group consisting of *Candida*, *Cryptococcus*, *Lipomyces*, *Rhodospiridium*, *Yarrowia* and *Schizochytrium*.

Moreover, a host cell host cell culture according to the present invention may also be an animal cell. Preferably, said animal host cell is a host cell of a fish or a cell line obtained therefrom. More preferably, the fish host cell is from herring, salmon, sardine, redfish, eel, carp, trout, halibut, mackerel, zander or tuna.

Generally, the controlling steps in the production of LCPUFAs, i.e., the long chain unsaturated fatty acid biosynthetic pathway, are catalyzed by membrane-associated fatty acid desaturases and elongases. Plants and most other eukaryotic organisms have specialized desaturase and elongase systems for the introduction of double bonds and the extension of fatty acids beyond C18 atoms. The elongase reactions have several important features in common with the fatty acid synthase complex (FAS). However, the elongase complex is different from the FAS complex as the complex is localized in the cytosol and membrane bound, ACP is not involved and the elongase 3-keto-acyl-CoA-synthase catalyzes the condensation of malonyl-CoA with an acyl primer. The elongase complex consists of four components with different catalytic functions, the keto-acyl-synthase (condensation reaction of malonyl-CoA to acyl-CoA, creation of a 2 C atom longer keto-acyl-CoA fatty acid), the keto-acyl-reductase (reduction of the 3-keto group to a 3-hydroxy-group), the dehydratase (dehydration results in a 3-enoyl-acyl-CoA fatty acid) and the enoyl-CoA-reductase (reduction of the double bond at position 3, release from the complex). For the production of LCPUFAs including ARA, EPA and/or DHA the elongation reactions, beside the desaturation reactions, are essential. Higher plants do not have the necessary enzyme set to produce LCPUFAs (4 or more double bonds, 20 or more C atoms).

Therefore the catalytic activities have to be conferred to the plants or plant cells. The polynucleotides of the present invention catalyze the desaturation and elongation activities necessary for the formation of ARA, EPA and/or DHA. By delivering the novel desaturases and elongases increased levels of PUFAs and LCPUFAs are produced.

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However, person skilled in the art knows that dependent on the host cell, further, enzymatic activities may be conferred to the host cells, e.g., by recombinant technologies. Accordingly, the present invention, preferably, envisages a host cell which in addition to the polynucleotide of the present invention comprises polynucleotides encoding such desaturases and/or elongases as required depending on the selected host cell. Preferred desaturases and/or elongases which shall be present in the host cell are at least one enzyme selected from the group consisting of: Δ -4-desaturase, Δ -5-desaturase, Δ -5-elongase, Δ -6-desaturase, Δ 12-desaturase, Δ 15-desaturase, ω 3-desaturase and Δ -6-elongase. Especially preferred are the bifunctional d12d15-Desaturases d12d15Des(Ac) from *Acanthamoeba castellanii* (WO2007042510), d12d15Des(Cp) from *Claviceps purpurea* (WO2008006202) and d12d15Des(Lg)1 from *Lottia gigantea* (WO2009016202), the d12-Desaturases d12Des(Co) from *Calendula officinalis* (WO200185968), d12Des(Lb) from *Laccaria bicolor* (WO2009016202), d12Des(Mb) from *Monosiga brevicollis* (WO2009016202), d12Des(Mg) from *Mycosphaerella graminicola* (WO2009016202), d12Des(Nh) from *Nectria haematococca* (WO2009016202), d12Des(Ol) from *Ostreococcus lucimarinus* (WO2008040787), d12Des(Pb) from *Phycomyces blakesleeanus* (WO2009016202), d12Des(Ps) from *Phytophthora sojae* (WO2006100241) and d12Des(Tp) from *Thalassiosira pseudonana* (WO2006069710), the d15-Desaturases d15Des(Hr) from *Helobdella robusta* (WO2009016202), d15Des(Mc) from *Microcoleus chthonoplastes* (WO2009016202), d15Des(Mf) from *Mycosphaerella fijiensis* (WO2009016202), d15Des(Mg) from *Mycosphaerella graminicola* (WO2009016202) and d15Des(Nh)2 from *Nectria haematococca* (WO2009016202), the d4-Desaturases d4Des(Eg) from *Euglena gracilis* (WO2004090123), d4Des(Tc) from *Thraustochytrium* sp. (WO2002026946) and d4Des(Tp) from *Thalassiosira pseudonana* (WO2006069710), the d5-Desaturases d5Des(Ol)2 from *Ostreococcus lucimarinus* (WO2008040787), d5Des(Pp) from *Physcomitrella patens* (WO2004057001), d5Des(Pt) from *Phaeodactylum tricornutum* (WO2002057465), d5Des(Tc) from *Thraustochytrium* sp. (WO2002026946), d5Des(Tp) from *Thalassiosira pseudonana* (WO2006069710) and the d6-Desaturases d6Des(Cp) from *Ceratodon purpureus* (WO2000075341), d6Des(Ol) from *Ostreococcus lucimarinus* (WO2008040787), d6Des(Ot) from *Ostreococcus tauri* (WO2006069710), d6Des(Pf) from *Primula farinosa* (WO2003072784), d6Des(Pir)_BO from *Pythium irregulare* (WO2002026946), d6Des(Pir) from *Pythium irregulare* (WO2002026946), d6Des(Plu) from *Primula luteola* (WO2003072784), d6Des(Pp) from *Physcomitrella patens* (WO200102591), d6Des(Pt) from *Phaeodactylum tricornutum* (WO2002057465), d6Des(Pv) from *Primula vialii* (WO2003072784) and d6Des(Tp) from *Thalassiosira pseudonana* (WO2006069710), the d8-Desaturases d8Des(Ac) from *Acanthamoeba castellanii* (EP1790731), d8Des(Eg) from *Euglena gracilis* (WO200034439) and d8Des(Pm) from *Perkinsus marinus* (WO2007093776), the ω 3-Desaturases ω 3Des(Pi) from *Phytophthora infestans* (WO2005083053), ω 3Des(Pir) from *Pythium irregulare*

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(WO2008022963), o3Des(Pir)2 from *Pythium irregulare* (WO2008022963) and o3Des(Ps) from *Phytophthora sojae* (WO2006100241), the bifunctional d5d6-elongases d5d6Elo(Om)2 from *Oncorhynchus mykiss* (WO2005012316), d5d6Elo(Ta) from *Thraustochytrium aureum* (WO2005012316) and d5d6Elo(Tc) from *Thraustochytrium* sp. (WO2005012316), the d5-elongases d5Elo(At) from *Arabidopsis thaliana* (WO2005012316), d5Elo(At)2 from *Arabidopsis thaliana* (WO2005012316), d5Elo(Ci) from *Ciona intestinalis* (WO2005012316), d5Elo(OI) from *Ostreococcus lucimarinus* (WO2008040787), d5Elo(Ot) from *Ostreococcus tauri* (WO2005012316), d5Elo(Tp) from *Thalassiosira pseudonana* (WO2005012316) and d5Elo(XI) from *Xenopus laevis* (WO2005012316), the d6-elongases d6Elo(OI) from *Ostreococcus lucimarinus* (WO2008040787), d6Elo(Ot) from *Ostreococcus tauri* (WO2005012316), d6Elo(Pi) from *Phytophthora infestans* (WO2003064638), d6Elo(Pir) from *Pythium irregulare* (WO2009016208), d6Elo(Pp) from *Physcomitrella patens* (WO2001059128), d6Elo(Ps) from *Phytophthora sojae* (WO2006100241), d6Elo(Ps)2 from *Phytophthora sojae* (WO2006100241), d6Elo(Ps)3 from *Phytophthora sojae* (WO2006100241), d6Elo(Pt) from *Phaeodactylum tricornutum* (WO2005012316), d6Elo(Tc) from *Thraustochytrium* sp. (WO2005012316) and d6Elo(Tp) from *Thalassiosira pseudonana* (WO2005012316), the d9-elongases d9Elo(Ig) from *Isochrysis galbana* (WO2002077213), d9Elo(Pm) from *Perkinsus marinus* (WO2007093776) and d9Elo(Ro) from *Rhizopus oryzae* (WO2009016208). Particularly, if the manufacture of ARA is envisaged in higher plants, the enzymes recited in Table 3, below (i.e. additionally a d6-desaturase, d6-elongase, d5-elongase, d5-desaturase, d12-desaturase, and d6-elongase) or enzymes having essentially the same activity may be combined in a host cell. If the manufacture of EPA is envisaged in higher plants, the enzymes having additionally a d6-desaturase, d6-elongase, d5-desaturase, d12-desaturase, d6-elongase, omega 3-desaturase and d15-desaturase, or enzymes having essentially the same activity may be combined in a host cell. If the manufacture of DHA is envisaged in higher plants, the enzymes having additionally a d6-desaturase, d6-elongase, d5-desaturase, d12-desaturase, d6-elongase, omega 3-desaturase, d15-desaturase, d5-elongase, and d4-desaturase activity, or enzymes having essentially the same activity may be combined in a host cell.

The present invention also relates to a cell, preferably a host cell as specified above or a cell of a non-human organism specified elsewhere herein, said cell comprising a polynucleotide which is obtained from the polynucleotide of the present invention by a point mutation, a truncation, an inversion, a deletion, an addition, a substitution and homologous recombination. How to carry out such modifications to a polynucleotide is well known to the skilled artisan and has been described elsewhere in this specification in detail.

The present invention furthermore pertains to a method for the manufacture of a polypeptide encoded by a polynucleotide of any the present invention comprising

- a) cultivating the host cell of the invention under conditions which allow for the production of the said polypeptide; and
- b) obtaining the polypeptide from the host cell of step a).

Suitable conditions which allow for expression of the polynucleotide of the invention comprised by the host cell depend on the host cell as well as the expression control sequence used for governing expression of the said polynucleotide. These conditions and how to select them are very well known to those skilled in the art. The expressed polypeptide may be obtained, for example, by all conventional purification techniques including affinity chromatography, size exclusion chromatography, high pressure liquid chromatography (HPLC) and precipitation techniques including antibody precipitation. It is to be understood that the method may – although preferred – not necessarily yield an essentially pure preparation of the polypeptide. It is to be understood that depending on the host cell which is used for the aforementioned method, the polypeptides produced thereby may become posttranslationally modified or processed otherwise.

The present invention also encompasses a polypeptide encoded by the polynucleotide of the present invention or which is obtainable by the aforementioned method.

The term “ polypeptide” as used herein encompasses essentially purified polypeptides or polypeptide preparations comprising other proteins in addition. Further, the term also relates to the fusion proteins or polypeptide fragments being at least partially encoded by the polynucleotide of the present invention referred to above. Moreover, it includes chemically modified polypeptides. Such modifications may be artificial modifications or naturally occurring modifications such as phosphorylation, glycosylation, myristylation and the like (Review in Mann 2003, Nat. Biotechnol. 21, 255– 261, review with focus on plants in Huber 2004, Curr. Opin. Plant Biol. 7, 318-322). Currently, more than 300 posttranslational modifications are known (see full ABFRC Delta mass list at <http://www.abrf.org/index.cfm/dm.home>). The polypeptides of the present invention shall exhibit the desaturase or elongase activity referred to above.

Moreover, the present invention contemplates a non-human transgenic organism comprising the polynucleotide or the vector of the present invention.

Preferably, the non-human transgenic organism is a plant or a plant part. Preferred plants to be used for introducing the polynucleotide or the vector of the invention are plants which are capable of synthesizing fatty acids, such as all dicotyledonous or monocotyledonous plants, algae or mosses. It is to be understood that host cells derived from a plant may also be used for producing a plant according to the present invention. Preferred plant parts are seeds from the plants. Preferred plants are selected from the group of the plant families Adelotheceaceae, Anacardiaceae, Asteraceae, Apiaceae, Betulaceae, Boraginaceae, Brassicaceae, Bromeliaceae, Caricaceae, Cannabaceae, Convolvulaceae, Chenopodiaceae, Crypthecodiniaceae, Cucurbitaceae, Ditrichaceae, Elaeagnaceae, Ericaceae, Euphorbiaceae, Fabaceae, Geraniaceae, Gramineae, Juglandaceae, Lauraceae, Leguminosae, Linaceae, Prasinophyceae or vegetable plants or ornamentals such as Tagetes. Examples which may be mentioned are the following plants selected from the group consisting of: Adelotheceaceae such as the genera Physcomitrella, such as the genus and species Physcomitrella patens,

Anacardiaceae such as the genera Pistacia, Mangifera, Anacardium, for example the genus and species Pistacia vera [pistachio], Mangifera indica [mango] or Anacardium occidentale [cashew], Asteraceae, such as the genera Calendula, Carthamus, Centaurea, Cichorium, Cynara, Helianthus, Lactuca, Locusta, Tagetes, Valeriana, for example the genus and species

5 Calendula officinalis [common marigold], Carthamus tinctorius [safflower], Centaurea cyanus [cornflower], Cichorium intybus [chicory], Cynara scolymus [artichoke], Helianthus annuus [sunflower], Lactuca sativa, Lactuca crisper, Lactuca esculenta, Lactuca scariola L. ssp. sativa, Lactuca scariola L. var. integrata, Lactuca scariola L. var. integrifolia, Lactuca sativa subsp. romana, Locusta communis, Valeriana locusta [salad vegetables], Tagetes lucida, Tagetes

10 erecta or Tagetes tenuifolia [african or french marigold], Apiaceae, such as the genus Daucus, for example the genus and species Daucus carota [carrot], Betulaceae, such as the genus Corylus, for example the genera and species Corylus avellana or Corylus columnata [hazelnut], Boraginaceae, such as the genus Borago, for example the genus and species Borago officinalis [borage], Brassicaceae, such as the genera Brassica, Melanosinapis, Sinapis, Arabidopsis, for

15 example the genera and species Brassica napus, Brassica rapa ssp. [oilseed rape], Sinapis arvensis Brassica juncea, Brassica juncea var. juncea, Brassica juncea var. crispifolia, Brassica juncea var. foliosa, Brassica nigra, Brassica sinapioides, Melanosinapis communis [mustard], Brassica oleracea [fodder beet] or Arabidopsis thaliana, Bromeliaceae, such as the genera Ananas, Bromelia (pineapple), for example the genera and species Ananas comosus, Ananas

20 ananas or Bromelia comosa [pineapple], Caricaceae, such as the genus Carica, such as the genus and species Carica papaya [papaya], Cannabaceae, such as the genus Cannabis, such as the genus and species Cannabis sativa [hemp], Convolvulaceae, such as the genera Ipomoea, Convolvulus, for example the genera and species Ipomoea batatas, Ipomoea pandurata, Convolvulus batatas, Convolvulus tiliaceus, Ipomoea fastigiata, Ipomoea tiliacea,

25 Ipomoea triloba or Convolvulus panduratus [sweet potato, batate], Chenopodiaceae, such as the genus Beta, such as the genera and species Beta vulgaris, Beta vulgaris var. altissima, Beta vulgaris var. Vulgaris, Beta maritima, Beta vulgaris var. perennis, Beta vulgaris var. conditiva or Beta vulgaris var. esculenta [sugarbeet], Cryptocodiaceae, such as the genus Cryptocodium, for example the genus and species Cryptocodium cohnii, Cucurbitaceae,

30 such as the genus Cucurbita, for example the genera and species Cucurbita maxima, Cucurbita mixta, Cucurbita pepo or Cucurbita moschata [pumpkin/squash], Cymbellaceae such as the genera Amphora, Cymbella, Okedenia, Phaeodactylum, Reimeria, for example the genus and species Phaeodactylum tricornutum, Ditrichaceae such as the genera Ditrichaceae, Astomiopsis, Ceratodon, Chrysoblastella, Ditrichum, Distichum, Eccremidium, Lophidium, Philibertiella, Pleuridium, Saelania, Trichodon, Skottsbergia, for example the genera and

35 species Ceratodon antarcticus, Ceratodon columbiae, Ceratodon heterophyllus, Ceratodon purpureus, Ceratodon purpureus, Ceratodon purpureus ssp. convolutus, Ceratodon, purpureus spp. stenocarpus, Ceratodon purpureus var. rotundifolius, Ceratodon ratodon, Ceratodon stenocarpus, Chrysoblastella chilensis, Ditrichum ambiguum, Ditrichum brevisetum, Ditrichum crispatisimum, Ditrichum difficile, Ditrichum falcifolium, Ditrichum flexicaule, Ditrichum

40 giganteum, Ditrichum heteromallum, Ditrichum lineare, Ditrichum lineare, Ditrichum montanum, Ditrichum montanum, Ditrichum pallidum, Ditrichum punctulatum, Ditrichum pusillum, Ditrichum

pusillum var. tortile, Ditrichum rhynchostegium, Ditrichum schimperi, Ditrichum tortile, Distichium capillaceum, Distichium hagenii, Distichium inclinatum, Distichium macounii, Eccremidium floridanum, Eccremidium whiteleggei, Lophidion strictus, Pleuridium acuminatum, Pleuridium alternifolium, Pleuridium holdridgei, Pleuridium mexicanum, Pleuridium ravenellii, Pleuridium subulatum, Saelania glaucescens, Trichodon borealis, Trichodon cylindricus or Trichodon cylindricus var. oblongus, Elaeagnaceae such as the genus Elaeagnus, for example the genus and species Olea europaea [olive], Ericaceae such as the genus Kalmia, for example the genera and species Kalmia latifolia, Kalmia angustifolia, Kalmia microphylla, Kalmia polifolia, Kalmia occidentalis, Cistus chamaerhodendros or Kalmia lucida [mountain laurel], Euphorbiaceae such as the genera Manihot, Janipha, Jatropha, Ricinus, for example the genera and species Manihot utilissima, Janipha manihot, Jatropha manihot, Manihot aipil, Manihot dulcis, Manihot manihot, Manihot melanobasis, Manihot esculenta [manihot] or Ricinus communis [castor-oil plant], Fabaceae such as the genera Pisum, Albizia, Cathormion, Feuillea, Inga, Pithecolobium, Acacia, Mimosa, Medicago, Glycine, Dolichos, Phaseolus, Soja, for example the genera and species Pisum sativum, Pisum arvense, Pisum humile [pea], Albizia berteriana, Albizia julibrissin, Albizia lebbeck, Acacia berteriana, Acacia littoralis, Albizia berteriana, Albizzia berteriana, Cathormion berteriana, Feuillea berteriana, Inga fragrans, Pithecellobium berterianum, Pithecellobium fragrans, Pithecolobium berterianum, Pseudalbizzia berteriana, Acacia julibrissin, Acacia nemu, Albizia nemu, Feuillea julibrissin, Mimosa julibrissin, Mimosa speciosa, Sericanrda julibrissin, Acacia lebbeck, Acacia macrophylla, Albizia lebbek, Feuillea lebbeck, Mimosa lebbeck, Mimosa speciosa [silk tree], Medicago sativa, Medicago falcata, Medicago varia [alfalfa], Glycine max Dolichos soja, Glycine gracilis, Glycine hispida, Phaseolus max, Soja hispida or Soja max [soybean], Funariaceae such as the genera Aphanorrhagma, Entosthodon, Funaria, Physcomitrella, Physcomitrium, for example the genera and species Aphanorrhagma serratum, Entosthodon attenuatus, Entosthodon bolanderi, Entosthodon bonplandii, Entosthodon californicus, Entosthodon drummondii, Entosthodon jamesonii, Entosthodon leibergii, Entosthodon neoscoticus, Entosthodon rubrissetus, Entosthodon spathulifolius, Entosthodon tucsoni, Funaria americana, Funaria bolanderi, Funaria calcarea, Funaria californica, Funaria calvescens, Funaria convoluta, Funaria flavicans, Funaria groutiana, Funaria hygrometrica, Funaria hygrometrica var. arctica, Funaria hygrometrica var. calvescens, Funaria hygrometrica var. convoluta, Funaria hygrometrica var. muralis, Funaria hygrometrica var. utahensis, Funaria microstoma, Funaria microstoma var. obtusifolia, Funaria muhlenbergii, Funaria orcuttii, Funaria plano-convexa, Funaria polaris, Funaria ravenellii, Funaria rubrisseta, Funaria serrata, Funaria sonora, Funaria sublimbatus, Funaria tucsoni, Physcomitrella californica, Physcomitrella patens, Physcomitrella readeri, Physcomitrium australe, Physcomitrium californicum, Physcomitrium collenchymatum, Physcomitrium coloradense, Physcomitrium cupuliferum, Physcomitrium drummondii, Physcomitrium eurytostomum, Physcomitrium flexifolium, Physcomitrium hookeri, Physcomitrium hookeri var. serratum, Physcomitrium immersum, Physcomitrium kellermanii, Physcomitrium megalocarpum, Physcomitrium pyriforme, Physcomitrium pyriforme var. serratum, Physcomitrium rufipes, Physcomitrium sandbergii, Physcomitrium subsphaericum, Physcomitrium washingtoniense, Geraniaceae, such as the genera Pelargonium, Cocos, Oleum, for example the genera and

species *Cocos nucifera*, *Pelargonium grossularioides* or *Oleum cocois* [coconut], Gramineae, such as the genus *Saccharum*, for example the genus and species *Saccharum officinarum*, Juglandaceae, such as the genera *Juglans*, *Wallia*, for example the genera and species *Juglans regia*, *Juglans ailanthifolia*, *Juglans sieboldiana*, *Juglans cinerea*, *Wallia cinerea*, *Juglans bixbyi*,
5 *Juglans californica*, *Juglans hindsii*, *Juglans intermedia*, *Juglans jamaicensis*, *Juglans major*, *Juglans microcarpa*, *Juglans nigra* or *Wallia nigra* [walnut], Lauraceae, such as the genera *Persea*, *Laurus*, for example the genera and species *Laurus nobilis* [bay], *Persea americana*, *Persea gratissima* or *Persea persea* [avocado], Leguminosae, such as the genus *Arachis*, for example the genus and species *Arachis hypogaea* [peanut], Linaceae, such as the genera
10 *Linum*, *Adenolinum*, for example the genera and species *Linum usitatissimum*, *Linum humile*, *Linum austriacum*, *Linum bienne*, *Linum angustifolium*, *Linum catharticum*, *Linum flavum*, *Linum grandiflorum*, *Adenolinum grandiflorum*, *Linum lewisii*, *Linum narbonense*, *Linum perenne*, *Linum perenne* var. *lewisii*, *Linum pratense* or *Linum trigynum* [linseed], Lythraeae, such as the genus *Punica*, for example the genus and species *Punica granatum* [pomegranate], Malvaceae,
15 such as the genus *Gossypium*, for example the genera and species *Gossypium hirsutum*, *Gossypium arboreum*, *Gossypium barbadense*, *Gossypium herbaceum* or *Gossypium thurberi* [cotton], Marchantiaceae, such as the genus *Marchantia*, for example the genera and species *Marchantia berteroana*, *Marchantia foliacea*, *Marchantia macropora*, Musaceae, such as the genus *Musa*, for example the genera and species *Musa nana*, *Musa acuminata*, *Musa paradisiaca*, *Musa* spp. [banana], Onagraceae, such as the genera *Camissonia*, *Oenothera*, for example the genera and species *Oenothera biennis* or *Camissonia brevipes* [evening primrose],
20 Palmae, such as the genus *Elaeis*, for example the genus and species *Elaeis guineensis* [oil palm], Papaveraceae, such as the genus *Papaver*, for example the genera and species *Papaver orientale*, *Papaver rhoeas*, *Papaver dubium* [poppy], Pedaliaceae, such as the genus
25 *Sesamum*, for example the genus and species *Sesamum indicum* [sesame], Piperaceae, such as the genera *Piper*, *Artanthe*, *Peperomia*, *Steffensia*, for example the genera and species *Piper aduncum*, *Piper amalago*, *Piper angustifolium*, *Piper auritum*, *Piper betel*, *Piper cubeba*, *Piper longum*, *Piper nigrum*, *Piper retrofractum*, *Artanthe adunca*, *Artanthe elongata*, *Peperomia elongata*, *Piper elongatum*, *Steffensia elongata* [cayenne pepper], Poaceae, such as the genera
30 *Hordeum*, *Secale*, *Avena*, *Sorghum*, *Andropogon*, *Holcus*, *Panicum*, *Oryza*, *Zea* (maize), *Triticum*, for example the genera and species *Hordeum vulgare*, *Hordeum jubatum*, *Hordeum murinum*, *Hordeum secalinum*, *Hordeum distichon*, *Hordeum aegiceras*, *Hordeum hexastichon*, *Hordeum hexastichum*, *Hordeum irregulare*, *Hordeum sativum*, *Hordeum secalinum* [barley], *Secale cereale* [rye], *Avena sativa*, *Avena fatua*, *Avena byzantina*, *Avena fatua* var. *sativa*,
35 *Avena hybrida* [oats], *Sorghum bicolor*, *Sorghum halepense*, *Sorghum saccharatum*, *Sorghum vulgare*, *Andropogon drummondii*, *Holcus bicolor*, *Holcus sorghum*, *Sorghum aethiopicum*, *Sorghum arundinaceum*, *Sorghum caffrorum*, *Sorghum cernuum*, *Sorghum dochna*, *Sorghum drummondii*, *Sorghum durra*, *Sorghum guineense*, *Sorghum lanceolatum*, *Sorghum nervosum*, *Sorghum saccharatum*, *Sorghum subglabrescens*, *Sorghum verticilliflorum*, *Sorghum vulgare*,
40 *Holcus halepensis*, *Sorghum miliaceum*, *Panicum militaceum* [millet], *Oryza sativa*, *Oryza latifolia* [rice], *Zea mays* [maize], *Triticum aestivum*, *Triticum durum*, *Triticum turgidum*, *Triticum hybernum*, *Triticum macha*, *Triticum sativum* or *Triticum vulgare* [wheat], Porphyridiaceae, such

as the genera Chrootheca, Flintiella, Petrovanella, Porphyridium, Rhodella, Rhodosorus, Vanhoeffenia, for example the genus and species Porphyridium cruentum, Proteaceae, such as the genus Macadamia, for example the genus and species Macadamia intergrifolia [macadamia], Prasinophyceae such as the genera Nephroselmis, Prasinococcus, Scherffelia, 5 Tetraselmis, Mantoniella, Ostreococcus, for example the genera and species Nephroselmis olivacea, Prasinococcus capsulatus, Scherffelia dubia, Tetraselmis chui, Tetraselmis suecica, Mantoniella squamata, Ostreococcus tauri, Rubiaceae such as the genus Cofea, for example the genera and species Cofea spp., Coffea arabica, Coffea canephora or Coffea liberica [coffee], Scrophulariaceae such as the genus Verbascum, for example the genera and species 10 Verbascum blattaria, Verbascum chaixii, Verbascum densiflorum, Verbascum lagurus, Verbascum longifolium, Verbascum lychnitis, Verbascum nigrum, Verbascum olympicum, Verbascum phlomoides, Verbascum phoenicum, Verbascum pulverulentum or Verbascum thapsus [mullein], Solanaceae such as the genera Capsicum, Nicotiana, Solanum, Lycopersicon, for example the genera and species Capsicum annuum, Capsicum annuum var. 15 glabriusculum, Capsicum frutescens [pepper], Capsicum annuum [paprika], Nicotiana tabacum, Nicotiana alata, Nicotiana attenuata, Nicotiana glauca, Nicotiana langsdorffii, Nicotiana obtusifolia, Nicotiana quadrivalvis, Nicotiana repanda, Nicotiana rustica, Nicotiana sylvestris [tobacco], Solanum tuberosum [potato], Solanum melongena [eggplant], Lycopersicon esculentum, Lycopersicon lycopersicum, Lycopersicon pyriforme, Solanum integrifolium or 20 Solanum lycopersicum [tomato], Sterculiaceae, such as the genus Theobroma, for example the genus and species Theobroma cacao [cacao] or Theaceae, such as the genus Camellia, for example the genus and species Camellia sinensis [tea]. In particular preferred plants to be used as transgenic plants in accordance with the present invention are oil fruit crops which comprise large amounts of lipid compounds, such as peanut, oilseed rape, canola, sunflower, safflower, 25 poppy, mustard, hemp, castor-oil plant, olive, sesame, Calendula, Punica, evening primrose, mullein, thistle, wild roses, hazelnut, almond, macadamia, avocado, bay, pumpkin/squash, linseed, soybean, pistachios, borage, trees (oil palm, coconut, walnut) or crops such as maize, wheat, rye, oats, triticale, rice, barley, cotton, cassava, pepper, Tagetes, Solanaceae plants such as potato, tobacco, eggplant and tomato, Vicia species, pea, alfalfa or bushy plants 30 (coffee, cacao, tea), Salix species, and perennial grasses and fodder crops. Preferred plants according to the invention are oil crop plants such as peanut, oilseed rape, canola, sunflower, safflower, poppy, mustard, hemp, castor-oil plant, olive, Calendula, Punica, evening primrose, pumpkin/squash, linseed, soybean, borage, trees (oil palm, coconut). Especially preferred are sunflower, safflower, tobacco, mullein, sesame, cotton, pumpkin/squash, poppy, evening 35 primrose, walnut, linseed, hemp, thistle or safflower. Very especially preferred plants are plants such as safflower, sunflower, poppy, evening primrose, walnut, linseed, or hemp.

Preferred mosses are Physcomitrella or Ceratodon. Preferred algae are Isochrysis, Mantoniella, Ostreococcus or Cryptothecodinium, and algae/diatoms such as Phaeodactylum or 40 Thraustochytrium. More preferably, said algae or mosses are selected from the group consisting of: Emiliana, Shewanella, Physcomitrella, Thraustochytrium, Fusarium, Phytophthora, Ceratodon, Isochrysis, Aleurita, Muscarioides, Mortierella, Phaeodactylum, Cryptothecodinium,

specifically from the genera and species *Thallasiosira pseudonona*, *Euglena gracilis*, *Physcomitrella patens*, *Phytophthora infestans*, *Fusarium gramineum*, *Cryptocodium cohnii*, *Ceratodon purpureus*, *Isochrysis galbana*, *Aleurita farinosa*, *Thraustochytrium* sp., *Muscarioides viallii*, *Mortierella alpina*, *Phaeodactylum tricornutum* or *Caenorhabditis elegans* or especially advantageously *Phytophthora infestans*, *Thallasiosira pseudonona* and *Cryptocodium cohnii*.

Transgenic plants may be obtained by transformation techniques as elsewhere in this specification. Preferably, transgenic plants can be obtained by T-DNA-mediated transformation. Such vector systems are, as a rule, characterized in that they contain at least the *vir* genes, which are required for the *Agrobacterium*-mediated transformation, and the sequences which delimit the T-DNA (T-DNA border). Suitable vectors are described elsewhere in the specification in detail.

Also encompassed are transgenic non-human animals comprising the vector or polynucleotide of the present invention. Preferred non-human transgenic animals envisaged by the present invention are fish, such as herring, salmon, sardine, redfish, eel, carp, trout, halibut, mackerel, zander or tuna.

However, it will be understood that dependent on the non-human transgenic organism specified above, further, enzymatic activities may be conferred to the said organism, e.g., by recombinant technologies. Accordingly, the present invention, preferably, envisages a non-human transgenic organism specified above which in addition to the polynucleotide of the present invention comprises polynucleotides encoding such desaturases and/or elongases as required depending on the selected host cell. Preferred desaturases and/or elongases which shall be present in the organism are at least one enzyme selected from the group of desaturases and/or elongases or the combinations specifically recited elsewhere in this specification (see above and Tables 3, 4 and 5).

Furthermore, the present invention encompasses a method for the manufacture of polyunsaturated fatty acids comprising:

- a) cultivating the host cell of the invention under conditions which allow for the production of polyunsaturated fatty acids in said host cell;
- b) obtaining said polyunsaturated fatty acids from the said host cell.

The term "polyunsaturated fatty acids (PUFA)" as used herein refers to fatty acids comprising at least two, preferably, three, four, five or six, double bonds. Moreover, it is to be understood that such fatty acids comprise, preferably from 18 to 24 carbon atoms in the fatty acid chain. More preferably, the term relates to long chain PUFA (LCPUFA) having from 20 to 24 carbon atoms in the fatty acid chain. Preferred unsaturated fatty acids in the sense of the present invention are selected from the group consisting of DGLA 20:3 (8,11,14), ARA 20:4 (5,8,11,14), iARA 20:4(8,11,14,17), EPA 20:5 (5,8,11,14,17), DPA 22:5 (4,7,10,13,16), DHA 22:6 (4,7,10,13,16,19), 20:4 (8,11,14,17), more preferably, arachidonic acid (ARA) 20:4 (5,8,11,14),

eicosapentaenoic acid (EPA) 20:5 (5,8,11,14,17), and docosahexaenoic acid (DHA) 22:6 (4,7,10,13,16,19). Thus, it will be understood that most preferably, the methods provided by the present invention pertaining to the manufacture of ARA, EPA or DHA. Moreover, also encompassed are the intermediates of LCPUFA which occur during synthesis. Such intermediates are, preferably, formed from substrates by the desaturase or elongase activity of the polypeptides of the present invention. Preferably, substrates encompass LA 18:2 (9,12), ALA 18:3(9,12,15), Eicosadienoic acid 20:2 (11,14), Eicosatrienoic acid 20:3 (11,14,17)), DGLA 20:3 (8,11,14), ARA 20:4 (5,8,11,14), eicosatetraenoic acid 20:4 (8,11,14,17), Eicosapentaenoic acid 20:5 (5,8,11,14,17), Docosahexapentanoic acid 22:5 (7,10,13,16,19).

The term “cultivating” as used herein refers maintaining and growing the host cells under culture conditions which allow the cells to produce the said polyunsaturated fatty acid, i.e. the PUFA and/or LCPUFA referred to above. This implies that the polynucleotide of the present invention is expressed in the host cell so that the desaturase and/or elongase activity is present. Suitable culture conditions for cultivating the host cell are described in more detail below.

The term “obtaining” as used herein encompasses the provision of the cell culture including the host cells and the culture medium as well as the provision of purified or partially purified preparations thereof comprising the polyunsaturated fatty acids, preferably, ARA, EPA, DHA, in free or in -CoA bound form, as membrane phospholipids or as triacylglyceride esters. More preferably, the PUFA and LCPUFA are to be obtained as triglyceride esters, e.g., in form of an oil. More details on purification techniques can be found elsewhere herein below.

The host cells to be used in the method of the invention are grown or cultured in the manner with which the skilled worker is familiar, depending on the host organism. Usually, host cells are grown in a liquid medium comprising a carbon source, usually in the form of sugars, a nitrogen source, usually in the form of organic nitrogen sources such as yeast extract or salts such as ammonium sulfate, trace elements such as salts of iron, manganese and magnesium and, if appropriate, vitamins, at temperatures of between 0°C and 100°C, preferably between 10°C and 60°C under oxygen or anaerobic atmosphere dependent on the type of organism. The pH of the liquid medium can either be kept constant, that is to say regulated during the culturing period, or not. The cultures can be grown batchwise, semibatchwise or continuously. Nutrients can be provided at the beginning of the fermentation or administered semicontinuously or continuously: The produced PUFA or LCPUFA can be isolated from the host cells as described above by processes known to the skilled worker, e.g., by extraction, distillation, crystallization, if appropriate precipitation with salt, and/or chromatography. It might be required to disrupt the host cells prior to purification. To this end, the host cells can be disrupted beforehand. The culture medium to be used must suitably meet the requirements of the host cells in question. Descriptions of culture media for various microorganisms which can be used as host cells according to the present invention can be found in the textbook "Manual of Methods for General Bacteriology" of the American Society for Bacteriology (Washington D.C., USA, 1981). Culture media can also be obtained from various commercial suppliers. All media components are

sterilized, either by heat or by filter sterilization. All media components may be present at the start of the cultivation or added continuously or batchwise, as desired. If the polynucleotide or vector of the invention which has been introduced in the host cell further comprises an expressible selection marker, such as an antibiotic resistance gene, it might be necessary to add a selection agent to the culture, such as an antibiotic in order to maintain the stability of the introduced polynucleotide. The culture is continued until formation of the desired product is at a maximum. This is normally achieved within 10 to 160 hours. The fermentation broths can be used directly or can be processed further. The biomass may, according to requirement, be removed completely or partially from the fermentation broth by separation methods such as, for example, centrifugation, filtration, decanting or a combination of these methods or be left completely in said broth. The fatty acid preparations obtained by the method of the invention, e.g., oils, comprising the desired PUFA or LCPUFA as triglyceride esters are also suitable as starting material for the chemical synthesis of further products of interest. For example, they can be used in combination with one another or alone for the preparation of pharmaceutical or cosmetic compositions, foodstuffs, or animal feeds. Chemically pure triglycerides comprising the desired PUFA or LCPUFA can also be manufactured by the methods described above. To this end, the fatty acid preparations are further purified by extraction, distillation, crystallization, chromatography or combinations of these methods. In order to release the fatty acid moieties from the triglycerides, hydrolysis may be also required. The said chemically pure triglycerides or free fatty acids are, in particular, suitable for applications in the food industry or for cosmetic and pharmacological compositions.

Moreover, the present invention relates to a method for the manufacture of poly-unsaturated fatty acids comprising:

- a) cultivating the non-human transgenic organism of the invention under conditions which allow for the production of poly-unsaturated fatty acids in said non-human transgenic organism; and
- b) obtaining said poly-unsaturated fatty acids from the said non-human transgenic organism.

Further, it follows from the above that a method for the manufacture of an oil, lipid or fatty acid composition is also envisaged by the present invention comprising the steps of any one of the aforementioned methods and the further step of formulating PUFA or LCPUFA as oil, lipid or fatty acid composition. Preferably, said oil, lipid or fatty acid composition is to be used for feed, foodstuffs, cosmetics or medicaments. Accordingly, the formulation of the PUFA or LCPUFA shall be carried out according to the GMP standards for the individual envisaged products. For example, an oil may be obtained from plant seeds by an oil mill. However, for product safety reasons, sterilization may be required under the applicable GMP standard. Similar standards will apply for lipid or fatty acid compositions to be applied in cosmetic or pharmaceutical compositions. All these measures for formulating oil, lipid or fatty acid compositions as products are comprised by the aforementioned manufacture.

The term "oil" refers to a fatty acid mixture comprising unsaturated and/or saturated fatty acids which are esterified to triglycerides. Preferably, the triglycerides in the oil of the invention comprise PUFA or LCPUFA as referred to above. The amount of esterified PUFA and/or LCPUFA is, preferably, approximately 30%, a content of 50% is more preferred, a content of 60%, 70%, 80% or more is even more preferred. The oil may further comprise free fatty acids, preferably, the PUFA and LCPUFA referred to above. For the analysis, the fatty acid content can be, e.g., determined by GC analysis after converting the fatty acids into the methyl esters by transesterification. The content of the various fatty acids in the oil or fat can vary, in particular depending on the source. The oil, however, shall have a non-naturally occurring composition with respect to the PUFA and/or LCPUFA composition and content. It will be understood that such a unique oil composition and the unique esterification pattern of PUFA and LCPUFA in the triglycerides of the oil shall only be obtainable by applying the methods of the present invention specified above. Moreover, the oil of the invention may comprise other molecular species as well. Specifically, it may comprise minor impurities of the polynucleotide or vector of the invention. Such impurities, however, can be detected only by highly sensitive techniques such as PCR.

Another embodiment is the use of the polynucleotide comprising NEENA or the recombinant vector comprising the polynucleotide with NEENA as defined above for enhancing expression of at least one enzyme of the polyunsaturated fatty acid biosynthetic pathway as defined in plants or parts thereof, in a more preferably embodiment the polynucleotide comprising NEENA or the recombinant vector comprising the polynucleotide with NEENA as defined above for enhancing expression of at least one enzyme of the polyunsaturated fatty acid biosynthetic pathway is used in plant seeds.

Another preferred embodiment is the use of a host cell or a host cell culture or of a non-human transgenic organism, transgenic plant, plant parts or plant seeds derived from the transgenic non-human organism or plant as described above for the production of foodstuffs, animal feeds, seeds, pharmaceuticals or fine chemicals.

DEFINITIONS

Abbreviations: NEENA – nucleic acid expression enhancing nucleic acid, GFP – green
5 fluorescence protein, GUS – beta-Glucuronidase, BAP – 6-benzylaminopurine; MS -
Murashige and Skoog medium; Kan: Kanamycin sulfate; GA3 - Gibberellic acid; microl:
Microliter.

It is to be understood that this invention is not limited to the particular methodology or protocols.

10 It is also to be understood that the terminology used herein is for the purpose of describing
particular embodiments only, and is not intended to limit the scope of the present invention
which will be limited only by the appended claims. It must be noted that as used herein and in
the appended claims, the singular forms "a," "and," and "the" include plural reference unless the
15 context clearly dictates otherwise. Thus, for example, reference to "a vector" is a reference to
one or more vectors and includes equivalents thereof known to those skilled in the art, and so
forth. The term "about" is used herein to mean approximately, roughly, around, or in the region
of. When the term "about" is used in conjunction with a numerical range, it modifies that range
by extending the boundaries above and below the numerical values set forth. In general, the
20 term "about" is used herein to modify a numerical value above and below the stated value by a
variance of 20 percent, preferably 10 percent up or down (higher or lower). As used herein, the
word "or" means any one member of a particular list and also includes any combination of
members of that list. The words "comprise," "comprising," "include," "including," and "includes"
when used in this specification and in the following claims are intended to specify the presence
25 of one or more stated features, integers, components, or steps, but they do not preclude the
presence or addition of one or more other features, integers, components, steps, or groups
thereof. For clarity, certain terms used in the specification are defined and used as follows:

Antiparallel: "Antiparallel" refers herein to two nucleotide sequences paired through hydrogen
bonds between complementary base residues with phosphodiester bonds running in the 5'-3'
30 direction in one nucleotide sequence and in the 3'-5' direction in the other nucleotide sequence.

Antisense: The term "antisense" refers to a nucleotide sequence that is inverted relative to its
normal orientation for transcription or function and so expresses an RNA transcript that is
complementary to a target gene mRNA molecule expressed within the host cell (e.g., it can
35 hybridize to the target gene mRNA molecule or single stranded genomic DNA through Watson-
Crick base pairing) or that is complementary to a target DNA molecule such as, for example
genomic DNA present in the host cell.

Coding region: As used herein the term "coding region" when used in reference to a structural
40 gene refers to the nucleotide sequences which encode the amino acids found in the nascent
polypeptide as a result of translation of a mRNA molecule. The coding region is bounded, in
eukaryotes, on the 5'-side by the nucleotide triplet "ATG" which encodes the initiator methionine

and on the 3'-side by one of the three triplets which specify stop codons (i.e., TAA, TAG, TGA). In addition to containing introns, genomic forms of a gene may also include sequences located on both the 5'- and 3'-end of the sequences which are present on the RNA transcript. These sequences are referred to as "flanking" sequences or regions (these flanking sequences are located 5' or 3' to the non-translated sequences present on the mRNA transcript). The 5'-flanking region may contain regulatory sequences such as promoters and enhancers which control or influence the transcription of the gene. The 3'-flanking region may contain sequences which direct the termination of transcription, post-transcriptional cleavage and polyadenylation.

Complementary: "Complementary" or "complementarity" refers to two nucleotide sequences which comprise antiparallel nucleotide sequences capable of pairing with one another (by the base-pairing rules) upon formation of hydrogen bonds between the complementary base residues in the antiparallel nucleotide sequences. For example, the sequence 5'-AGT-3' is complementary to the sequence 5'-ACT-3'. Complementarity can be "partial" or "total." "Partial" complementarity is where one or more nucleic acid bases are not matched according to the base pairing rules. "Total" or "complete" complementarity between nucleic acid molecules is where each and every nucleic acid base is matched with another base under the base pairing rules. The degree of complementarity between nucleic acid molecule strands has significant effects on the efficiency and strength of hybridization between nucleic acid molecule strands. A "complement" of a nucleic acid sequence as used herein refers to a nucleotide sequence whose nucleic acid molecules show total complementarity to the nucleic acid molecules of the nucleic acid sequence.

Double-stranded RNA: A "double-stranded RNA" molecule or "dsRNA" molecule comprises a sense RNA fragment of a nucleotide sequence and an antisense RNA fragment of the nucleotide sequence, which both comprise nucleotide sequences complementary to one another, thereby allowing the sense and antisense RNA fragments to pair and form a double-stranded RNA molecule.

Endogenous: An "endogenous" nucleotide sequence refers to a nucleotide sequence, which is present in the genome of the untransformed plant cell.

Enhanced expression: "enhance" or "increase" the expression of a nucleic acid molecule in a plant cell are used equivalently herein and mean that the level of expression of the nucleic acid molecule in a plant, part of a plant or plant cell after applying a method of the present invention is higher than its expression in the plant, part of the plant or plant cell before applying the method, or compared to a reference plant lacking a recombinant nucleic acid molecule of the invention. For example, the reference plant is comprising the same construct which is only lacking the respective NEENA. The term "enhanced" or "increased" as used herein are synonymous and means herein higher, preferably significantly higher expression of the nucleic acid molecule to be expressed. As used herein, an "enhancement" or "increase" of the level of an agent such as a protein, mRNA or RNA means that the level is increased relative to

a substantially identical plant, part of a plant or plant cell grown under substantially identical conditions, lacking a recombinant nucleic acid molecule of the invention, for example lacking the NEENA molecule, the recombinant construct or recombinant vector of the invention. As used herein, "enhancement" or "increase" of the level of an agent, such as for example a preRNA, mRNA, rRNA, tRNA, snoRNA, snRNA expressed by the target gene and/or of the protein product encoded by it, means that the level is increased 50% or more, for example 100% or more, preferably 200% or more, more preferably 5 fold or more, even more preferably 10 fold or more, most preferably 20 fold or more for example 50 fold relative to a cell or organism lacking a recombinant nucleic acid molecule of the invention. The enhancement or increase can be determined by methods with which the skilled worker is familiar. Thus, the enhancement or increase of the nucleic acid or protein quantity can be determined for example by an immunological detection of the protein. Moreover, techniques such as protein assay, fluorescence, Northern hybridization, nuclease protection assay, reverse transcription (quantitative RT-PCR), ELISA (enzyme-linked immunosorbent assay), Western blotting, radioimmunoassay (RIA) or other immunoassays and fluorescence-activated cell analysis (FACS) can be employed to measure a specific protein or RNA in a plant or plant cell. Depending on the type of the induced protein product, its activity or the effect on the phenotype of the organism or the cell may also be determined. Methods for determining the protein quantity are known to the skilled worker. Examples, which may be mentioned, are: the micro-Biuret method (Goa J (1953) Scand J Clin Lab Invest 5:218-222), the Folin-Ciocalteu method (Lowry OH et al. (1951) J Biol Chem 193:265-275) or measuring the absorption of CBB G-250 (Bradford MM (1976) Analyt Biochem 72:248-254). As one example for quantifying the activity of a protein, the detection of luciferase activity is described in the Examples below.

Expression: "Expression" refers to the biosynthesis of a gene product, preferably to the transcription and/or translation of a nucleotide sequence, for example an endogenous gene or a heterologous gene, in a cell. For example, in the case of a structural gene, expression involves transcription of the structural gene into mRNA and - optionally - the subsequent translation of mRNA into one or more polypeptides. In other cases, expression may refer only to the transcription of the DNA harboring an RNA molecule.

Expression construct: "Expression construct" as used herein mean a DNA sequence capable of directing expression of a particular nucleotide sequence in an appropriate part of a plant or plant cell, comprising a promoter functional in said part of a plant or plant cell into which it will be introduced, operatively linked to the nucleotide sequence of interest which is - optionally - operatively linked to termination signals. If translation is required, it also typically comprises sequences required for proper translation of the nucleotide sequence. The coding region may code for a protein of interest but may also code for a functional RNA of interest, for example RNAa, siRNA, snoRNA, snRNA, microRNA, ta-siRNA or any other noncoding regulatory RNA, in the sense or antisense direction. The expression construct comprising the nucleotide sequence of interest may be chimeric, meaning that one or more of its components is heterologous with respect to one or more of its other components. The expression construct

may also be one, which is naturally occurring but has been obtained in a recombinant form useful for heterologous expression. Typically, however, the expression construct is heterologous with respect to the host, i.e., the particular DNA sequence of the expression construct does not occur naturally in the host cell and must have been introduced into the host cell or an ancestor of the host cell by a transformation event. The expression of the nucleotide sequence in the expression construct may be under the control of a seed-specific promoter or of an inducible promoter, which initiates transcription only when the host cell is exposed to some particular external stimulus. In the case of a plant, the promoter can also be specific to a particular tissue or organ or stage of development.

Foreign: The term "foreign" refers to any nucleic acid molecule (e.g., gene sequence) which is introduced into the genome of a cell by experimental manipulations and may include sequences found in that cell so long as the introduced sequence contains some modification (e.g., a point mutation, the presence of a selectable marker gene, etc.) and is therefore distinct relative to the naturally-occurring sequence.

Functional linkage: The term "functional linkage" or "functionally linked" is to be understood as meaning, for example, the sequential arrangement of a regulatory element (e.g. a promoter) with a nucleic acid sequence to be expressed and, if appropriate, further regulatory elements (such as e.g., a terminator or a NEENA) in such a way that each of the regulatory elements can fulfill its intended function to allow, modify, facilitate or otherwise influence expression of said nucleic acid sequence. As a synonym the wording " operable linkage" or " operably linked" may be used. The expression may result depending on the arrangement of the nucleic acid sequences in relation to sense or antisense RNA. To this end, direct linkage in the chemical sense is not necessarily required. Genetic control sequences such as, for example, enhancer sequences, can also exert their function on the target sequence from positions which are further away, or indeed from other DNA molecules. Preferred arrangements are those in which the nucleic acid sequence to be expressed recombinantly is positioned behind the sequence acting as promoter, so that the two sequences are linked covalently to each other. The distance between the promoter sequence and the nucleic acid sequence to be expressed recombinantly is preferably less than 200 base pairs, especially preferably less than 100 base pairs, very especially preferably less than 50 base pairs. In a preferred embodiment, the nucleic acid sequence to be transcribed is located behind the promoter in such a way that the transcription start is identical with the desired beginning of the chimeric RNA of the invention. Functional linkage, and an expression construct, can be generated by means of customary recombination and cloning techniques as described (e.g., in Maniatis T, Fritsch EF and Sambrook J (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor (NY); Silhavy et al. (1984) *Experiments with Gene Fusions*, Cold Spring Harbor Laboratory, Cold Spring Harbor (NY); Ausubel et al. (1987) *Current Protocols in Molecular Biology*, Greene Publishing Assoc. and Wiley Interscience; Gelvin et al. (Eds) (1990) *Plant Molecular Biology Manual*; Kluwer Academic Publisher, Dordrecht, The Netherlands). However, further sequences, which, for example, act as a linker with specific cleavage sites for restriction

enzymes, or as a signal peptide, may also be positioned between the two sequences. The insertion of sequences may also lead to the expression of fusion proteins. Preferably, the expression construct, consisting of a linkage of a regulatory region for example a promoter and nucleic acid sequence to be expressed, can exist in a vector-integrated form and be inserted into a plant genome, for example by transformation.

Gene: The term "gene" refers to a region operably joined to appropriate regulatory sequences capable of regulating the expression of the gene product (e.g., a polypeptide or a functional RNA) in some manner. A gene includes untranslated regulatory regions of DNA (e.g., promoters, enhancers, repressors, etc.) preceding (up-stream) and following (downstream) the coding region (open reading frame, ORF) as well as, where applicable, intervening sequences (i.e., introns) between individual coding regions (i.e., exons). The term "structural gene" as used herein is intended to mean a DNA sequence that is transcribed into mRNA which is then translated into a sequence of amino acids characteristic of a specific polypeptide.

Genome and genomic DNA: The terms "genome" or "genomic DNA" is referring to the heritable genetic information of a host organism. Said genomic DNA comprises the DNA of the nucleus (also referred to as chromosomal DNA) but also the DNA of the plastids (e.g., chloroplasts) and other cellular organelles (e.g., mitochondria). Preferably the terms genome or genomic DNA is referring to the chromosomal DNA of the nucleus.

Heterologous: The term "heterologous" with respect to a nucleic acid molecule or DNA refers to a nucleic acid molecule which is operably linked to, or is manipulated to become operably linked to, a second nucleic acid molecule to which it is not operably linked in nature, or to which it is operably linked at a different location in nature. A heterologous expression construct comprising a nucleic acid molecule and one or more regulatory nucleic acid molecule (such as a promoter or a transcription termination signal) linked thereto for example is a constructs originating by experimental manipulations in which either a) said nucleic acid molecule, or b) said regulatory nucleic acid molecule or c) both (i.e. (a) and (b)) is not located in its natural (native) genetic environment or has been modified by experimental manipulations, an example of a modification being a substitution, addition, deletion, inversion or insertion of one or more nucleotide residues. Natural genetic environment refers to the natural chromosomal locus in the organism of origin, or to the presence in a genomic library. In the case of a genomic library, the natural genetic environment of the sequence of the nucleic acid molecule is preferably retained, at least in part. The environment flanks the nucleic acid sequence at least at one side and has a sequence of at least 50 bp, preferably at least 500 bp, especially preferably at least 1,000 bp, very especially preferably at least 5,000 bp, in length. A naturally occurring expression construct - for example the naturally occurring combination of a promoter with the corresponding gene - becomes a transgenic expression construct when it is modified by non-natural, synthetic "artificial" methods such as, for example, mutagenization. Such methods have been described (US 5,565,350; WO 00/15815). For example a protein encoding nucleic acid molecule operably linked to a promoter, which is not the native promoter of this molecule, is

considered to be heterologous with respect to the promoter. Preferably, heterologous DNA is not endogenous to or not naturally associated with the cell into which it is introduced, but has been obtained from another cell or has been synthesized. Heterologous DNA also includes an endogenous DNA sequence, which contains some modification, non-naturally occurring, multiple copies of an endogenous DNA sequence, or a DNA sequence which is not naturally associated with another DNA sequence physically linked thereto. Generally, although not necessarily, heterologous DNA encodes RNA or proteins that are not normally produced by the cell into which it is expressed.

High expression seed-specific promoter: A "high expression seed-specific promoter" as used herein means a promoter causing seed-specific or seed-preferential expression in a plant or part thereof wherein the accumulation or rate of synthesis of RNA or stability of RNA derived from the nucleic acid molecule under the control of the respective promoter is higher, preferably significantly higher than the expression caused by the promoter lacking the NEENA of the invention. Preferably the amount of RNA and/or the rate of RNA synthesis and/or stability of RNA is increased 50% or more, for example 100% or more, preferably 200% or more, more preferably 5 fold or more, even more preferably 10 fold or more, most preferably 20 fold or more for example 50 fold relative to a seed-specific or a seed-preferential promoter lacking a NEENA of the invention.

Hybridization: The term "hybridization" as used herein includes "any process by which a strand of nucleic acid molecule joins with a complementary strand through base pairing." (J. Coombs (1994) Dictionary of Biotechnology, Stockton Press, New York). Hybridization and the strength of hybridization (i.e., the strength of the association between the nucleic acid molecules) is impacted by such factors as the degree of complementarity between the nucleic acid molecules, stringency of the conditions involved, the T_m of the formed hybrid, and the G:C ratio within the nucleic acid molecules. As used herein, the term " T_m " is used in reference to the "melting temperature." The melting temperature is the temperature at which a population of double-stranded nucleic acid molecules becomes half dissociated into single strands. The equation for calculating the T_m of nucleic acid molecules is well known in the art. As indicated by standard references, a simple estimate of the T_m value may be calculated by the equation: $T_m = 81.5 + 0.41(\% \text{ G+C})$, when a nucleic acid molecule is in aqueous solution at 1 M NaCl [see e.g., Anderson and Young, Quantitative Filter Hybridization, in Nucleic Acid Hybridization (1985)]. Other references include more sophisticated computations, which take structural as well as sequence characteristics into account for the calculation of T_m . Stringent conditions, are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6.

"Identity" : "Identity" when used in respect to the comparison of two or more nucleic acid or amino acid molecules means that the sequences of said molecules share a certain degree of sequence similarity, the sequences being partially identical.

To determine the percentage identity (homology is herein used interchangeably) of two amino acid sequences or of two nucleic acid molecules, the sequences are written one underneath the other for an optimal comparison (for example gaps may be inserted into the sequence of a protein or of a nucleic acid in order to generate an optimal alignment with the other protein or the other nucleic acid).

The amino acid residues or nucleic acid molecules at the corresponding amino acid positions or nucleotide positions are then compared. If a position in one sequence is occupied by the same amino acid residue or the same nucleic acid molecule as the corresponding position in the other sequence, the molecules are homologous at this position (i.e. amino acid or nucleic acid "homology" as used in the present context corresponds to amino acid or nucleic acid "identity". The percentage homology between the two sequences is a function of the number of identical positions shared by the sequences (i.e. % homology = number of identical positions/total number of positions x 100). The terms "homology" and "identity" are thus to be considered as synonyms.

For the determination of the percentage identity of two or more amino acids or of two or more nucleotide sequences several computer software programs have been developed. The identity of two or more sequences can be calculated with for example the software fasta, which presently has been used in the version fasta 3 (W. R. Pearson and D. J. Lipman, PNAS 85, 2444(1988); W. R. Pearson, Methods in Enzymology 183, 63 (1990); W. R. Pearson and D. J. Lipman, PNAS 85, 2444 (1988); W. R. Pearson, Enzymology 183, 63 (1990)). Another useful program for the calculation of identities of different sequences is the standard blast program, which is included in the Biomax pedant software (Biomax, Munich, Federal Republic of Germany). This leads unfortunately sometimes to suboptimal results since blast does not always include complete sequences of the subject and the query. Nevertheless as this program is very efficient it can be used for the comparison of a huge number of sequences. The following settings are typically used for such a comparisons of sequences:

Intron: refers to sections of DNA (intervening sequences) within a gene that do not encode part of the protein that the gene produces, and that is spliced out of the mRNA that is transcribed from the gene before it is exported from the cell nucleus. Intron sequence refers to the nucleic acid sequence of an intron. Thus, introns are those regions of DNA sequences that are transcribed along with the coding sequence (exons) but are removed during the formation of mature mRNA. Introns can be positioned within the actual coding region or in either the 5' or 3' untranslated leaders of the pre-mRNA (unspliced mRNA). Introns in the primary transcript are excised and the coding sequences are simultaneously and precisely ligated to form the mature mRNA. The junctions of introns and exons form the splice site. The sequence of an intron begins with GU and ends with AG. Furthermore, in plants, two examples of AU-AC introns have been described: the fourteenth intron of the RecA-like protein gene and the seventh intron of the G5 gene from Arabidopsis thaliana are AT-AC introns. Pre-mRNAs containing introns have three short sequences that are – beside other sequences- essential for the intron to be

accurately spliced. These sequences are the 5' splice-site, the 3' splice-site, and the branchpoint. mRNA splicing is the removal of intervening sequences (introns) present in primary mRNA transcripts and joining or ligation of exon sequences. This is also known as cis-splicing which joins two exons on the same RNA with the removal of the intervening sequence (intron).

5 The functional elements of an intron is comprising sequences that are recognized and bound by the specific protein components of the spliceosome (e.g. splicing consensus sequences at the ends of introns). The interaction of the functional elements with the spliceosome results in the removal of the intron sequence from the premature mRNA and the rejoining of the exon sequences. Introns have three short sequences that are essential -although not sufficient- for
10 the intron to be accurately spliced. These sequences are the 5' splice site, the 3' splice site and the branch point. The branchpoint sequence is important in splicing and splice-site selection in plants. The branchpoint sequence is usually located 10-60 nucleotides upstream of the 3' splice site.

15 Isolated: The term "isolated" as used herein means that a material has been removed by the hand of man and exists apart from its original, native environment and is therefore not a product of nature. An isolated material or molecule (such as a DNA molecule or enzyme) may exist in a purified form or may exist in a non-native environment such as, for example, in a transgenic host cell. For example, a naturally occurring polynucleotide or polypeptide present in a living
20 plant is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides can be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and would be isolated in that such a vector or composition is not part of its original environment. Preferably, the term "isolated" when used in relation to a nucleic acid molecule, as in "an
25 isolated nucleic acid sequence" refers to a nucleic acid sequence that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in its natural source. Isolated nucleic acid molecule is nucleic acid molecule present in a form or setting that is different from that in which it is found in nature. In contrast, non-isolated nucleic acid molecules are nucleic acid molecules such as DNA and RNA, which are
30 found in the state they exist in nature. For example, a given DNA sequence (e.g., a gene) is found on the host cell chromosome in proximity to neighboring genes; RNA sequences, such as a specific mRNA sequence encoding a specific protein, are found in the cell as a mixture with numerous other mRNAs, which encode a multitude of proteins. However, an isolated nucleic acid sequence comprising for example SEQ ID NO: 1 includes, by way of example, such nucleic
35 acid sequences in cells which ordinarily contain SEQ ID NO:1 where the nucleic acid sequence is in a chromosomal or extrachromosomal location different from that of natural cells, or is otherwise flanked by a different nucleic acid sequence than that found in nature. The isolated nucleic acid sequence may be present in single-stranded or double-stranded form. When an isolated nucleic acid sequence is to be utilized to express a protein, the nucleic acid sequence
40 will contain at a minimum at least a portion of the sense or coding strand (i.e., the nucleic acid sequence may be single-stranded). Alternatively, it may contain both the sense and anti-sense strands (i.e., the nucleic acid sequence may be double-stranded).

Minimal Promoter: promoter elements, particularly a TATA element, that are inactive or that have greatly reduced promoter activity in the absence of upstream activation. In the presence of a suitable transcription factor, the minimal promoter functions to permit transcription.

NEENA: see " Nucleic acid expression enhancing nucleic acid" .

Nucleic acid expression enhancing nucleic acid (NEENA): The term " nucleic acid expression enhancing nucleic acid" refers to a sequence and/or a nucleic acid molecule of a specific sequence having the intrinsic property to enhance expression of a nucleic acid under the control of a promoter to which the NEENA is functionally linked. Unlike promoter sequences, the NEENA as such is not able to drive expression. In order to fulfill the function of enhancing expression of a nucleic acid molecule functionally linked to the NEENA, the NEENA itself has to be functionally linked to a promoter. In distinction to enhancer sequences known in the art, the NEENA is acting in cis but not in trans and has to be located close to the transcription start site of the nucleic acid to be expressed.

Nucleic acids and nucleotides: The terms "Nucleic Acids" and "Nucleotides" refer to naturally occurring or synthetic or artificial nucleic acid or nucleotides. The terms " nucleic acids" and "nucleotides" comprise deoxyribonucleotides or ribonucleotides or any nucleotide analogue and polymers or hybrids thereof in either single- or double-stranded, sense or antisense form. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. The term "nucleic acid" is used inter-changeably herein with "gene", "cDNA", "mRNA", "oligonucleotide," and "polynucleotide". Nucleotide analogues include nucleotides having modifications in the chemical structure of the base, sugar and/or phosphate, including, but not limited to, 5-position pyrimidine modifications, 8-position purine modifications, modifications at cytosine exocyclic amines, substitution of 5-bromo-uracil, and the like; and 2'-position sugar modifications, including but not limited to, sugar-modified ribonucleotides in which the 2'-OH is replaced by a group selected from H, OR, R, halo, SH, SR, NH₂, NHR, NR₂, or CN. Short hairpin RNAs (shRNAs) also can comprise non-natural elements such as non-natural bases, e.g., inosine and xanthine, non-natural sugars, e.g., 2'-methoxy ribose, or non-natural phosphodiester linkages, e.g., methylphosphonates, phosphorothioates and peptides.

Nucleic acid sequence: The phrase "nucleic acid sequence" refers to a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5'- to the 3'-end. It includes chromosomal DNA, self-replicating plasmids, infectious polymers of DNA or RNA and DNA or RNA that performs a primarily structural role. "Nucleic acid sequence" also refers to a consecutive list of abbreviations, letters, characters or words, which represent nucleotides. In one embodiment, a nucleic acid can be a "probe" which is a relatively short nucleic acid, usually less than 100 nucleotides in length. Often a nucleic acid probe is from about 50 nucleotides in

length to about 10 nucleotides in length. A "target region" of a nucleic acid is a portion of a nucleic acid that is identified to be of interest.

5 Oligonucleotide: The term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics thereof, as well as oligonucleotides having non-naturally-occurring portions which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target and increased stability in the presence of nucleases. An oligonucleotide preferably includes two or more
10 nucleomonomers covalently coupled to each other by linkages (e.g., phosphodiester) or substitute linkages.

Plant: is generally understood as meaning any eukaryotic single-or multi-celled organism or a cell, tissue, organ, part or propagation material (such as seeds or fruit) of same which is capable
15 of photosynthesis. Included for the purpose of the invention are all genera and species of higher and lower plants of the Plant Kingdom. Annual, perennial, monocotyledonous and dicotyledonous plants are preferred. The term includes the mature plants, seed, shoots and seedlings and their derived parts, propagation material (such as seeds or microspores), plant organs, tissue, protoplasts, callus and other cultures, for example cell cultures, and any other
20 type of plant cell grouping to give functional or structural units. Mature plants refer to plants at any desired developmental stage beyond that of the seedling. Seedling refers to a young immature plant at an early developmental stage. Annual, biennial, monocotyledonous and dicotyledonous plants are preferred host organisms for the generation of transgenic plants. The expression of genes is furthermore advantageous in all ornamental plants, useful or ornamental
25 trees, flowers, cut flowers, shrubs or lawns. Plants which may be mentioned by way of example but not by limitation are angiosperms, bryophytes such as, for example, Hepaticae (liverworts) and Musci (mosses); Pteridophytes such as ferns, horsetail and club mosses; gymnosperms such as conifers, cycads, ginkgo and Gnetatae; algae such as Chlorophyceae, Phaeophyceae, Rhodophyceae, Myxophyceae, Xanthophyceae, Bacillariophyceae (diatoms),
30 and Euglenophyceae. Preferred are plants which are used for food or feed purpose such as the families of the Leguminosae such as pea, alfalfa and soya; Gramineae such as rice, maize, wheat, barley, sorghum, millet, rye, triticale, or oats; the family of the Umbelliferae, especially the genus *Daucus*, very especially the species *carota* (carrot) and *Apium*, very especially the species *Graveolens dulce* (celery) and many others; the family of the Solanaceae, especially
35 the genus *Lycopersicon*, very especially the species *esculentum* (tomato) and the genus *Solanum*, very especially the species *tuberosum* (potato) and *melongena* (egg plant), and many others (such as tobacco); and the genus *Capsicum*, very especially the species *annuum* (peppers) and many others; the family of the Leguminosae, especially the genus *Glycine*, very especially the species *max* (soybean), alfalfa, pea, lucerne, beans or peanut and many others;
40 and the family of the Cruciferae (Brassicaceae), especially the genus *Brassica*, very especially the species *napus* (oil seed rape), *campestris* (beet), *oleracea* cv *Tastie* (cabbage), *oleracea* cv *Snowball Y* (cauliflower) and *oleracea* cv *Emperor* (broccoli); and of the genus *Arabidopsis*, very

especially the species thaliana and many others; the family of the Compositae, especially the genus Lactuca, very especially the species sativa (lettuce) and many others; the family of the Asteraceae such as sunflower, Tagetes, lettuce or Calendula and many other; the family of the Cucurbitaceae such as melon, pumpkin/squash or zucchini, and linseed. Further preferred are
5 cotton, sugar cane, hemp, flax, chillies, and the various tree, nut and wine species.

Polypeptide: The terms "polypeptide", "peptide", "oligopeptide", "polypeptide", "gene product", "expression product" and "protein" are used interchangeably herein to refer to a polymer or oligomer of consecutive amino acid residues.

10 Primary transcript: The term "primary transcript" as used herein refers to a premature RNA transcript of a gene. A "primary transcript" for example still comprises introns and/or is not yet comprising a polyA tail or a cap structure and/or is missing other modifications necessary for its correct function as transcript such as for example trimming or editing.

15 Promoter: The terms "promoter", or "promoter sequence" are equivalents and as used herein, refer to a DNA sequence which when ligated to a nucleotide sequence of interest is capable of controlling the transcription of the nucleotide sequence of interest into RNA. Such promoters can for example be found in the following public databases

20 <http://www.grassius.org/grasspromdb.html>,

<http://mendel.cs.rhul.ac.uk/mendel.php?topic=plantprom>, [http://ppdb.gene.nagoya-u.ac.jp/cgi-](http://ppdb.gene.nagoya-u.ac.jp/cgi-bin/index.cgi)
bin/index.cgi. Promoters listed there may be addressed with the methods of the invention and

are herewith included by reference. A promoter is located 5' (i.e., upstream), proximal to the transcriptional start site of a nucleotide sequence of interest whose transcription into mRNA it controls, and provides a site for specific binding by RNA polymerase and other transcription factors for initiation of transcription. Said promoter comprises for example the at least 10 kb, for example 5 kb or 2 kb proximal to the transcription start site. It may also comprise the at least 1500 bp proximal to the transcriptional start site, preferably the at least 1000 bp, more preferably the at least 500 bp, even more preferably the at least 400 bp, the at least 300 bp, the
25 at least 200 bp or the at least 100 bp. In a further preferred embodiment, the promoter comprises the at least 50 bp proximal to the transcription start site, for example, at least 25 bp.

The promoter does not comprise exon and/or intron regions or 5' untranslated regions. The promoter may for example be heterologous or homologous to the respective plant. A polynucleotide sequence is "heterologous to" an organism or a second polynucleotide sequence
35 if it originates from a foreign species, or, if from the same species, is modified from its original form. For example, a promoter operably linked to a heterologous coding sequence refers to a coding sequence from a species different from that from which the promoter was derived, or, if from the same species, a coding sequence which is not naturally associated with the promoter (e.g. a genetically engineered coding sequence or an allele from a different ecotype or variety).

40 Suitable promoters can be derived from genes of the host cells where expression should occur or from pathogens for this host cells (e.g., plants or plant pathogens like plant viruses). A plant specific promoter is a promoter suitable for regulating expression in a plant. It may be derived

from a plant but also from plant pathogens or it might be a synthetic promoter designed by man. If a promoter is an inducible promoter, then the rate of transcription increases in response to an inducing agent. Also, the promoter may be regulated in a tissue-specific or tissue preferred manner such that it is only or predominantly active in transcribing the associated coding region in a specific tissue type(s) such as leaves, roots or meristem. The term "tissue specific" as it applies to a promoter refers to a promoter that is capable of directing selective expression of a nucleotide sequence of interest to a specific type of tissue (e.g., petals) in the relative absence of expression of the same nucleotide sequence of interest in a different type of tissue (e.g., roots). Tissue specificity of a promoter may be evaluated by, for example, operably linking a reporter gene to the promoter sequence to generate a reporter construct, introducing the reporter construct into the genome of a plant such that the reporter construct is integrated into every tissue of the resulting transgenic plant, and detecting the expression of the reporter gene (e.g., detecting mRNA, protein, or the activity of a protein encoded by the reporter gene) in different tissues of the transgenic plant. The detection of a greater level of expression of the reporter gene in one or more tissues relative to the level of expression of the reporter gene in other tissues shows that the promoter is specific for the tissues in which greater levels of expression are detected. The term "cell type specific" as applied to a promoter refers to a promoter, which is capable of directing selective expression of a nucleotide sequence of interest in a specific type of cell in the relative absence of expression of the same nucleotide sequence of interest in a different type of cell within the same tissue. The term "cell type specific" when applied to a promoter also means a promoter capable of promoting selective expression of a nucleotide sequence of interest in a region within a single tissue. Cell type specificity of a promoter may be assessed using methods well known in the art, e.g., GUS activity staining, GFP protein or immunohistochemical staining. The term "constitutive" when made in reference to a promoter or the expression derived from a promoter means that the promoter is capable of directing transcription of an operably linked nucleic acid molecule in the absence of a stimulus (e.g., heat shock, chemicals, light, etc.) in the majority of plant tissues and cells throughout substantially the entire lifespan of a plant or part of a plant. Typically, constitutive promoters are capable of directing expression of a transgene in substantially any cell and any tissue.

Promoter specificity: The term "specificity" when referring to a promoter means the pattern of expression conferred by the respective promoter. The specificity describes the tissues and/or developmental status of a plant or part thereof, in which the promoter is conferring expression of the nucleic acid molecule under the control of the respective promoter. Specificity of a promoter may also comprise the environmental conditions, under which the promoter may be activated or down-regulated such as induction or repression by biological or environmental stresses such as cold, drought, wounding or infection.

Purified: As used herein, the term "purified" refers to molecules, either nucleic or amino acid sequences that are removed from their natural environment, isolated or separated. "Substantially purified" molecules are at least 60% free, preferably at least 75% free, and more

preferably at least 90% free from other components with which they are naturally associated. A purified nucleic acid sequence may be an isolated nucleic acid sequence.

Recombinant: The term "recombinant" with respect to nucleic acid molecules refers to nucleic acid molecules produced by recombinant DNA techniques. Recombinant nucleic acid molecules may also comprise molecules, which as such does not exist in nature but are modified, changed, mutated or otherwise manipulated by man. Preferably, a "recombinant nucleic acid molecule" is a non-naturally occurring nucleic acid molecule that differs in sequence from a naturally occurring nucleic acid molecule by at least one nucleic acid. A "recombinant nucleic acid molecule" may also comprise a "recombinant construct" which comprises, preferably operably linked, a sequence of nucleic acid molecules not naturally occurring in that order. Preferred methods for producing said recombinant nucleic acid molecule may comprise cloning techniques, directed or non-directed mutagenesis, synthesis or recombination techniques.

"Seed-specific promoter" in the context of this invention means a promoter which is regulating transcription of a nucleic acid molecule under control of the respective promoter in seeds wherein the transcription in any tissue or cell of the seeds contribute to more than 90%, preferably more than 95%, more preferably more than 99% of the entire quantity of the RNA transcribed from said nucleic acid sequence in the entire plant during any of its developmental stage. The term "seed-specific expression" and "seed-specific NEENA" are to be understood accordingly. Hence a "seed-specific NEENA" enhances the transcription of a seed-specific or seed-preferential promoter in a way, that the transcription in seeds derived from said promoter functionally linked to a respective NEENA contribute to more than 90%, preferably more than 95%, more preferably more than 99% of the entire quantity of the RNA transcribed from the respective promoter functionally linked to a NEENA in the entire plant during any of its developmental stage.

"Seed-preferential promoter" in the context of this invention means a promoter which is regulating transcription of a nucleic acid molecule under control of the respective promoter in seeds wherein the transcription in any tissue or cell of the seeds contribute to more than 50%, preferably more than 70%, more preferably more than 80% of the entire quantity of the RNA transcribed from said nucleic acid sequence in the entire plant during any of its developmental stage. The term "seed-preferential expression" and "seed-preferential NEENA" are to be understood accordingly. Hence a "seed-preferential NEENA" enhances the transcription of a seed-specific or seed-preferential promoter in a way, that the transcription in seeds derived from said promoter functionally linked to a respective NEENA contribute to more than 50%, preferably more than 70%, more preferably more than 80% of the entire quantity of the RNA transcribed from the respective promoter functionally linked to a NEENA in the entire plant during any of its developmental stage.

Sense: The term "sense" is understood to mean a nucleic acid molecule having a sequence which is complementary or identical to a target sequence, for example a sequence which binds

to a protein transcription factor and which is involved in the expression of a given gene. According to a preferred embodiment, the nucleic acid molecule comprises a gene of interest and elements allowing the expression of the said gene of interest.

- 5 Significant increase or decrease: An increase or decrease, for example in enzymatic activity or in gene expression, that is larger than the margin of error inherent in the measurement technique, preferably an increase or decrease by about 2-fold or greater of the activity of the control enzyme or expression in the control cell, more preferably an increase or decrease by about 5-fold or greater, and most preferably an increase or decrease by about 10-fold or greater.

Substantially complementary: In its broadest sense, the term "substantially complementary", when used herein with respect to a nucleotide sequence in relation to a reference or target nucleotide sequence, means a nucleotide sequence having a percentage of identity between the substantially complementary nucleotide sequence and the exact complementary sequence of said reference or target nucleotide sequence of at least 60%, more desirably at least 70%, more desirably at least 80% or 85%, preferably at least 90%, more preferably at least 93%, still more preferably at least 95% or 96%, yet still more preferably at least 97% or 98%, yet still more preferably at least 99% or most preferably 100% (the later being equivalent to the term "identical" in this context). Preferably identity is assessed over a length of at least 19 nucleotides, preferably at least 50 nucleotides, more preferably the entire length of the nucleic acid sequence to said reference sequence (if not specified otherwise below). Sequence comparisons are carried out using default GAP analysis with the University of Wisconsin GCG, SEQWEB application of GAP, based on the algorithm of Needleman and Wunsch (Needleman and Wunsch (1970) J Mol. Biol. 48: 443-453; as defined above). A nucleotide sequence "substantially complementary" to a reference nucleotide sequence hybridizes to the reference nucleotide sequence under low stringency conditions, preferably medium stringency conditions, most preferably high stringency conditions (as defined above).

- 30 Transgene: The term "transgene" as used herein refers to any nucleic acid sequence, which is introduced into the genome of a cell by experimental manipulations. A transgene may be an "endogenous DNA sequence," or a "heterologous DNA sequence" (i.e., "foreign DNA"). The term "endogenous DNA sequence" refers to a nucleotide sequence, which is naturally found in the cell into which it is introduced so long as it does not contain some modification (e.g., a point mutation, the presence of a selectable marker gene, etc.) relative to the naturally-occurring sequence.

Transgenic: The term transgenic when referring to an organism means transformed, preferably stably transformed, with a recombinant DNA molecule that preferably comprises a suitable promoter operatively linked to a DNA sequence of interest.

Vector: As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid molecule to which it has been linked. One type of vector is a genomic integrated vector, or "integrated vector", which can become integrated into the chromosomal DNA of the host cell. Another type of vector is an episomal vector, i.e., a nucleic acid molecule capable of extra-chromosomal replication. Vectors capable of directing the expression of genes to which they are operatively linked are referred to herein as "expression vectors". In the present specification, "plasmid" and "vector" are used interchangeably unless otherwise clear from the context. Expression vectors designed to produce RNAs as described herein in vitro or in vivo may contain sequences recognized by any RNA polymerase, including mitochondrial RNA polymerase, RNA pol I, RNA pol II, and RNA pol III. These vectors can be used to transcribe the desired RNA molecule in the cell according to this invention. A plant transformation vector is to be understood as a vector suitable in the process of plant transformation.

Wild-type: The term "wild-type", "natural" or "natural origin" means with respect to an organism, polypeptide, or nucleic acid sequence, that said organism is naturally occurring or available in at least one naturally occurring organism which is not changed, mutated, or otherwise manipulated by man.

The contents of all references cited throughout this application are herewith incorporated by reference in general and with respect to their specific disclosure content referred to above.

FIGURES

Figure 1: Schematic figure of the different enzymatic activities leading to the production of ARA, EPA and DHA.

Figure 2 Strategy employed for stepwise buildup of plant expression plasmids of the invention. A detailed description is given in example 4. Abbreviations: Nco I, Pac I, Kas I, Sfo I, Fse I, Sbf I, Xma I, Not I indicate restriction endonucleases used for cloning; attLx and attRx—where x are numbers from 1 to 4—designate attachment sites for site specific recombination of the Multisite Gateway™ System (Invitrogen); pENTR_A, pENTR_B, pENTR_C are Multisite Gateway™ System-Entry-vectors; Kan (Kanamycin) and Strep (Streptomycin) designate antibiotic selection markers used for cloning; ori-origin of replication.

Figure 3 Orientation and combination of the functional elements (promoter, NEENA, gene, terminator) of the plant expression vecotrs VC-LJB913-1qcz (SEQ-ID 38), VC-LJB1327-1qcz (SEQ-ID 39), VC-LJB2003-1qcz (SEQ-ID 40) and VC-LJB2197-1qcz (SEQ-ID 146).

EXAMPLES

Example 1: General cloning methods

Cloning methods as e.g. use of restriction endonucleases to cut double stranded DNA at specific sites, agarose gel electrophoreses, purification of DNA fragments, transfer of nucleic acids onto nitrocellulose and nylon membranes, joining of DNA-fragments, transformation of E.coli cells and culture of bacteria were performed as described in Sambrook et al. (1989) (Cold Spring Harbor Laboratory Press: ISBN 0-87965-309-6). Polymerase chain reaction was performed using Phusion™ High-Fidelity DNA Polymerase (NEB, Frankfurt, Germany) according to the manufactures instructions. In general, primers used in PCR were designed such, that at least 20 nucleotides of the 3' end of the primer anneal perfectly with the template to amplify. Restriction sites were added by attaching the corresponding nucleotides of the recognition sites to the 5' end of the primer. Fusion PCR, for example described by K. Heckman and L. R. Pease, Nature Protocols (2007) 2, 924– 932 was used as an alternative method to join two fragments of interest, e.g. a promoter to a gene or a gene to a terminator.

Example 2: Sequence Analysis of recombinant DNA

Sequencing of recombinant DNA-molecules was performed using a laser-fluorescence DNA sequencer (Applied Biosystems Inc, USA) employing the sanger method (Sanger et al. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467). Expression constructs harboring fragments obtained by polymerase chain reactions (PCR) were subjected to sequencing to confirm the correctness of expression cassettes consisting of promoter, nucleic acid molecule to be expressed and terminator to avoid mutations that might result from handling of the DNA during cloning, e.g. due to incorrect primers, mutations from exposure to UV-light or errors of polymerases.

Example 3: Identification of Nucleic Acid Expression Enhancing Nucleic Acids (NEENA) from genes with seed preferred expression

3.1 Identification of NEENA molecules from A. thaliana genes

Using publicly available genomic DNA sequences (e.g. <http://www.ncbi.nlm.nih.gov/genomes/PLANTS/PlantList.html>) and transcript expression data (e.g. <http://www.weigelworld.org/resources/microarray/AtGenExpress/>), a set of 19 NEENA candidates deriving from Arabidopsis thaliana transcripts with seed preferred expression was selected for detailed analyses. The candidates were named as follows:

Table 1: seed specific NEENA candidates (NEENAss).

NEENA name	Locus	Annotation	SEQ ID No
NEENAss1	At1g62290	aspartyl protease family protein	6
NEENAss2	At1g65090	expressed protein	7
NEENAss15	At2g27040	PAZ domain-containing protein	9
NEENAss18	At1g01170	ozone-responsive stress-related protein, putative	10
NEENAss14	At5g63190	MA3 domain-containing protein	8
NEENAss4	At5g07830	glycosyl hydrolase family 79 N-terminal domain-containing protein similar to beta-glucuronidase AtGUS2	11
NEENAss13	At2g04520	eukaryotic translation initiation factor 1A, putative / eIF-1A	12
NEENAss3	At5g60760	2-phosphoglycerate kinase-related	13
NEENAss5	At1g11170	expressed protein contains Pfam profile PF05212	14
NEENAss11	At4g37050	PLA V/PLP4 (Patatin-like protein 4)	15
NEENAss8	At1g56170	HAP5B (Heme activator protein (yeast) homolog 5B)	16
NEENAss16	At1g54100	aldehyde dehydrogenase, putative / antiquitin	17
NEENAss9	At3g12670	CTP synthase, putative / UTP--ammonia ligase, putative	18
NEENAss20	At4g04460	aspartyl protease family protein	19
NEENAss10	At1g04120	ATMRP5 (Arabidopsis thaliana multidrug resistance-associated protein 5)	20
NEENAss6	At2g41070	basic leucine zipper transcription factor (BZIP12)	21
NEENAss12	At1g05450	protease inhibitor/seed storage/lipid transfer protein (LTP)-related	22
NEENAss7	At4g03050	2-oxoglutarate-dependent dioxygenase, putative (AOP3)	23
NEENAss17	At3g12490	cysteine protease inhibitor, putative / cystatin	24

3.2 Isolation of the NEENA candidates

Genomic DNA was extracted from *A. thaliana* green tissue using the Qiagen DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Genomic DNA fragments containing NEENA molecules were isolated by conventional polymerase chain reaction (PCR). Primers were designed on the basis of the *A. thaliana* genome sequence with a multitude of NEENA candidates. The reaction comprised 19 sets of primers (Table 2) and followed the protocol outlined by Phusion High Fidelity DNA Polymerase (Cat No F-540L, New England Biolabs, Ipswich, MA, USA). The isolated DNA was used as template DNA in a PCR amplification using the following primers:

Table 2: Primer sequences for isolation of NEENAs

Primer name	Sequence	SEQ ID No	PCR yielding SEQ ID No
NEENAss1_for	tggtgcttaaacactctggtgagt	42	6
NEENAss1_rev	tttgacctacaaaatcaaagcagtca	43	
NEENAss2_for	agttcttgcttcgaagttgc	44	7
NEENAss2_rev	tactacgtactgtttcaattct	45	
NEENAss3_for	atttcacacgcgtttctatcatttc	46	13
NEENAss3_rev	ttatctctctctaaaaataaaaaacgaatc	47	
NEENAss4_for	gtccagaattttctccattga	48	11
NEENAss4_rev	tcttcactatccaaagctctca	49	
NEENAss5_for	gtctactttcattacagtgactctg	50	14
NEENAss5_rev	ttatatatttacctgcaacacaattcaa	51	
NEENAss6_for	cactcgaatactgcatgcaa	52	21
NEENAss6_rev	ttatgtagcctttacacagaaaacaa	53	
NEENAss7_for	aacaactatggcctgaggggt	54	23
NEENAss7_rev	ttatcttactgttttaaccaaaaaataaaat	55	
NEENAss8_for	atcttagggtttcgagatctca	56	16
NEENAss8_rev	tgctaagctatctctgttaataataaaattg	57	
NEENAss9_for	atttttgttggtgaaaggtaga	58	18
NEENAss9_rev	ttacgtttttgtctctgcttcttct	59	
NEENAss10_for	tctgggaaatatcgattttgatct	60	20
NEENAss10_rev	tctcaccacatcccaaagctc	61	
NEENAss11_for	gcacaatcttagcttaccttgaa	62	15
NEENAss11_rev	ttatttaatccacaagccttgccctc	63	
NEENAss12_for	tgtcggagaagtgggcg	64	22
NEENAss12_rev	agaagtgggcgacg	65	
NEENAss13_for	tagcttaatctcagattcgaatcgt	66	12
NEENAss13_rev	tagtatctacataccaatcatacaaatg	67	
NEENAss14_for	ttcacgatttgaatttga	68	8

NEENAss14_rev	tctacaacattaaaacgaccatta	69	
NEENAss15_for	agggtttcgttttgtttca	70	9
NEENAss15_rev	ttatctcctgctcaaagaaacca	71	
NEENAss16_for	agaagctcatttctcgatac	72	17
NEENAss16_rev	tctctgcgcaaaaattcacc	73	
NEENAss17_for	tctaaaaatacagggcacc	74	24
NEENAss17_rev	ttactcttcggtgcagaagccta	75	
NEENAss18_for	actgtttaagcttcactgtct	76	10
NEENAss18_rev	tttcttctaaagctgaaagt	77	
NEENAss20_for	ttaagcttttaagaatcttactcaca	78	19
NEENAss20_rev	ttaaattttacctgtcatcaaaaacaaca	79	

Amplification during the PCR was carried out with the following composition (50 microl):

3,00 microl A. thaliana genomic DNA
 5 10,00 microl 5x Phusion HF Buffer
 4,00 microl dNTP (2,5 mM)
 2,50 microl for Primer (10 microM)
 2,50 microl rev Primer (10 microM)
 0,50 microl Phusion HF DNA Polymerase (2U/microl)

10 A touch-down approach was employed for the PCR with the following parameters: 98,0°C for 30 sec (1 cycle), 98,0°C for 30 sec, 56,0°C for 30 sec and 72,0°C for 60 sec (4 cycles), 4 additional cycles each for 54,0°C, 51,0°C and 49,0°C annealing temperature, followed by 20 cycles with 98,0°C for 30 sec, 46,0°C for 30 sec and 72,0°C for 60 sec (4 cycles) and 72,0°C for 5 min. The
 15 amplification products was loaded on a 2 % (w/v) agarose gel and separated at 80V. The PCR products were excised from the gel and purified with the Qiagen Gel Extraction Kit (Qiagen, Hilden, Germany). The purified PCR prudcts were cloned into the pCR2.1 TOPO (Invitrogen) vector according to the manufacturer' s manual and subsequently sequenced. These plasmids served as source for further cloning steps or as template for further PCR, e.g. fusion PCR for
 20 fusion with promoters as described in example 4.

Example 4: Assembly of genes required for PUFA synthesis within a T-plasmid

25 For synthesis of LC-PUFA in Brassica napus seeds, the set of genes encoding the proteins of the metabolic LC-PUFA pathway (table 3) was combined with expression elements (promoter, terminators, NEENAs, table 5) and transfered into binary t-plasmids that were used for agrobacteria mediated transformation of plants as described in example 5. To this end, the general cloning strategy depicted in figure 2 was employed: Genes listed in table 3 were PCR-amplified using Phusion™ High-Fidelity DNA Polymerase (NEB, Frankfurt, Germany) according
 30 to the manufactures instructions from cDNA using primer introducing a Nco I and/or Asc I

restriction site at the 5' terminus, and a Pac I restriction site at the 3' terminus (figure 2A). Promoter-terminator modules or promoter-NEENA-terminator modules were created by joining the corresponding expression elements listed in table 2 using fusion PCR as described in example 1 and cloning the PCR-product into the TOPO-vector pCR2.1 (Invitrogen) according to the manufactures instructions (figure 2B). As a non limiting example, primer combinations are listed in table 6 were used to create fusions of promoter-NEENAs harbored by the plasmid VC-LJB2003-1qcz (SEQ-ID 40) and VC-LJB2197-1qcz (SEQ-ID 146) containing the required set of pathway genes to synthesize arachidonic acid in seeds of rapeseed. While joining terminator sequences to promoter sequences or promoter-NEENA sequences using fusion PCR, primers were designed such, that recognition sequences for the restriction endonucleases depicted in figure 2 were added to either side of the modules, and the recognition sites for the restriction endonucleases Nco I, Asc I and Pac I were introduced between promoter and terminator or between NEENA and terminator (see figure 2B). To obtain the final expression modules, PCR-amplified genes were cloned between promoter and terminator or NEENA and terminator via Nco I and/or Pac I restriction sites (figure 2C). Employing the custom multiple cloning site (MCS) SEQ-ID 41, up to three of those expression modules were combined as desired to expression cassettes harbored by either one of pENTR/A, pENTR/B or pENTR/C (figure 2D). Deviating from the strategy depicted in figure 2, some elements or joined elements were synthesized by a service provider or cloned using blunt-end ligation. Finally, the Multisite Gateway™ System (Invitrogen) was used to combine three expression cassette harbored by pENTR/A, pENTR/B and pENTR/C (figure 2E) to obtain the final binary pSUN T-plasmids VC-LJB913-1qcz (SEQ-ID 38), VC-LJB1327-1qcz (SEQ-ID 39) and VC-LJB2003-1qcz (SEQ-ID 40) and VC-LJB2197-1qcz (SEQ-ID 146).. The orientation and combination of the functional elements (promoter, NEENA, gene, terminator) is depicted in figure 3A, 3B, 3C and 3D. An overview of binary vectors and their usage is given by Hellens et al, Trends in Plant Science (2000) 5: 446– 451.

Table 3: Genes used for synthesis of 20:4n-6 (ARA) in rapeseed.

Gene	Source Organism	Activity	SEQ-ID
d12Des(Ps_GA)	Phytophthora sojae	Δ 12-Desaturase	95
d6Des(Ot_febit)	Ostreococcus tauri	Δ 6-Desaturase	96
d6Des(Ot_GA2)	Ostreococcus tauri	Δ 6-Desaturase	97
d6Des(Pir_GAI)	Pythium irregulare	Δ 6-Desaturase	98
d6Elo(Pp_GA2)	Physcomitrella patens	Δ 6-Elongase	99
d6Elo(Tp_GA2)	Thalassiosira pseudonana	Δ 6-Elongase	100
d5Des(Tc_GA2)	Thraustochytrium ssp.	Δ 5-Desaturase	101

Table 4: Genes used in addition to genes listed in table 1 for synthesis of 22:6n-3 (DHA) in rapeseed.

Gene	Source Organism	Activity	SEQ-ID
d5Elo(Ot_GA3)	Ostreococcus tauri	Δ 5-Elongase	102
d4Des(Tc_GA3)	Traustochytrium ssp.	Δ 4-Desaturase	103

5 Table 5: Expression elements used for synthesis of 20:4n-6 (ARA) or 22:6n-3 (DHA) in rapeseed

Element	Source Organism	Function	SEQ-ID
p-VfSBP-NEENAss1	Vicia faba; Arabidopsis	Promotor+NEENA	1
p-BnNapin-NEENAss2	Brassica napus; Arabidopsis	Promotor+NEENA	2
p-LuCnl-NEENAss14	Linum usitatissimum; Arabidopsis	Promotor+NEENA	3
p-LuPxr-NEENAss15	Linum usitatissimum; Arabidopsis	Promotor+NEENA	4
p-VfUSP-NEENAss18	Vicia faba; Arabidopsis	Promotor+NEENA	5
p-VfSBP-NEENAss2	<i>Vicia faba</i> , Arabidopsis	Promoter+NEENA	147
p-LuPxr-NEENAss1	<i>Linum usitatissimum</i> , Arabidopsis	Promoter+NEENA	148
p-BnNapin-NEENAss14	<i>Brassica napus</i> , Arabidopsis	Promoter+NEENA	149
NEENAss1	Arabidopsis	NEENA from locus At1g62290 (aspartyl protease family protein)	6
NEENAss2	Arabidopsis	NEENA from locus At1g65090 (expressed protein)	7
NEENAss14	Arabidopsis	NEENA from locus At5g63190 (MA3 domain-containing protein)	8
NEENAss15	Arabidopsis	NEENA from locus At2g27040 (PAZ domain-containing protein)	9
NEENAss18	Arabidopsis	NEENA from locus At1g01170 (ozone-responsive stress-related protein, putative)	10
NEENAss4	Arabidopsis	NEENA from locus At5g07830 (glycosyl hydrolase family 79 N- terminal domain-containing)	11

		protein similar to beta-glucuronidase AtGUS2)	
NEENAss13	Arabidopsis	NEENA from locus At2g04520 (eukaryotic translation initiation factor 1A, putative / eIF-1A)	12
NEENAss3	Arabidopsis	NEENA from locus At5g60760 (2-phosphoglycerate kinase-related)	13
NEENAss5	Arabidopsis	NEENA from locus At1g11170 (expressed protein contains Pfam profile PF05212)	14
NEENAss11	Arabidopsis	NEENA from locus At4g37050 (PLA V/PLP4 (Patatin-like protein 4))	15
NEENAss8	Arabidopsis	NEENA from locus At1g56170 (HAP5B (Heme activator protein (yeast) homolog 5B))	16
NEENAss16	Arabidopsis	NEENA from locus At1g54100 (aldehyde dehydrogenase, putative / antiquitin)	17
NEENAss9	Arabidopsis	NEENA from locus At3g12670 (CTP synthase, putative / UTP--ammonia ligase, putative)	18
NEENAss20	Arabidopsis	NEENA from locus At4g04460 (aspartyl protease family protein)	19
NEENAss10	Arabidopsis	NEENA from locus At1g04120 (ATMRP5 (Arabidopsis thaliana multidrug resistance-associated protein 5))	20
NEENAss6	Arabidopsis	NEENA from locus At2g41070 (basic leucine zipper transcription factor (BZIP12))	21
NEENAss12	Arabidopsis	NEENA from locus At1g05450 (protease inhibitor/seed storage/lipid transfer protein (LTP)-related)	22
NEENAss7	Arabidopsis	NEENA from locus At4g03050 (2-oxoglutarate-dependent dioxygenase, putative (AOP3))	23
NEENAss17	Arabidopsis	NEENA from locus At3g12490 (cysteine protease inhibitor, putative / cystatin)	24

p-BnNapin	Brassica napus	Promotor	25
p-LuCnI	Linum usitatissimum	Promotor	26
p-LuPXR	Linum usitatissimum	Promotor	27
p-VfSBP	Vicia faba	Promotor	28
p-VfUSP	Vicia faba	Promotor	29
p-VfLeB4	Vicia faba	Promotor	30
t-AtPXR	Arabidopsis	Terminator	31
t-CaMV35S	CaMV	Terminator	32
t-E9	Pisum sativum	Terminator	33
t-AgrOCS	Agrobacterium tumefaciens	Terminator	34
t-PvArc	Phaseolus vulgaris	Terminator	35
t-StCat	Solanum tuberosum	Terminator	36
t-VfLeB3	Vicia faba	Terminator	37

5

Table 6: Primers used for creation of fusions between promotor and NEENA elements using fusion PCR as described in example 1.

Promoter/NEENA cassette	Primer pair 1. PCR Promoter	Primer pair 1. PCR NEENA	Primer pair 2. PCR Promotor-NEENA
p-VfSBP-NEENAss1	Forw: tcgacggcccggactgta tccaac (SEQ-ID No:80) Rev: actcaccagagtgtttaag caccagttcagcttgatcg ctctattaat (SEQ-ID No:81)	Forw: attaatagagcgatcaag ctgaactgggtgcttaaaca ctctgggtgagt (SEQ-ID No:82) Rev: tttgacctacaaaatcaaa gcagtca (SEQ-ID No:43)	Forw: tcgacggcccggactgta tccaac (SEQ-ID No:80) Rev: tttgacctacaaaatcaaa gcagtca (SEQ-ID No:43)
p-BnNapin-NEENAss2	Forw: taaggatgacctaccatt cttga (SEQ-ID No:83) Rev: gcaacttcgaaagcaaa gaactgttttaatctgtttg tattga (SEQ-ID No:84)	Forw: tcaatacaaaacaagatta aaaacaagttcttgccttc gaagttgc (SEQ-ID No:85) Rev: tactacgtactgtttcaatt ct (SEQ-ID No:45)	Forw: taaggatgacctaccatt cttga (SEQ-ID No:83) Rev: tactacgtactgtttcaatt ct (SEQ-ID No:45)

Promoter/NEENA cassette	Primer pair 1. PCR Promoter	Primer pair 1. PCR NEENA	Primer pair 2. PCR Promotor-NEENA
p-LuCnl-NEENAss14	Forw: ttagcagatatttggtgtctaat (SEQ-ID No:86) Rev: tcaaattccaaatcgtgaaattttgggtggtgattggtctt (SEQ-ID No:87)	Forw: aaagaaccaatcaccacaaaaaatttcacgatttgaatttga (SEQ-ID No:88) Rev: tctacaacattaaaacgaccatta (SEQ-ID No:69)	Forw: ttagcagatatttggtgtctaat (SEQ-ID No:86) Rev: tctacaacattaaaacgaccatta (SEQ-ID No:69)
p-LuPxr-NEENAss15	Forw: cacgggcaggacataggactact (SEQ-ID No:89) Rev: tgaaacaaaaacgaaaccctgatttatgataaaaatgtcggttt (SEQ-ID No:90)	Forw: aaaccgacattttatcataatcagggtttcgttttgtttca (SEQ-ID No:91) Rev: ttatctcctgctcaaagaaacca (SEQ-ID No:71)	Forw: cacgggcaggacataggactact (SEQ-ID No:89) Rev: ttatctcctgctcaaagaaacca (SEQ-ID No:71)
p-VfUSP-NEENAss18	Forw: ctgcagcaaatttacacattgcc (SEQ-ID No:92) Rev: agacagtgaagcttaaacagtactggctatgaagaattataatc (SEQ-ID No:93)	Forw: gattataatttctcatagccagtactgtttaagcttactgtct (SEQ-ID No:94) Rev: tttcttctaaagctgaaagt (SEQ-ID No:77)	Forw: ctgcagcaaatttacacattgcc (SEQ-ID No:92) Rev: tttcttctaaagctgaaagt (SEQ-ID No:77)
p-VfSBP-NEENAss2	Forw: Tcgacggcccgactgtatccaac (SEQ-ID No:80) Rev: Gcaacttcgaaagcaagaactgttcagcttgatcgctctattaat (SEQ-ID No:150)	Forw: Attaatagagcgatcaagctgaacagttcttgctttcgaaagtgc (SEQ-ID No:151) Rev: Tactacgtactgtttcaattct (SEQ-ID No:45)	Forw: Tcgacggcccgactgtatccaac (SEQ-ID No:80) Rev: Tactacgtactgtttcaattct (SEQ-ID No:45)

Promoter/NEENA cassette	Primer pair 1. PCR Promoter	Primer pair 1. PCR NEENA	Primer pair 2. PCR Promotor-NEENA
p-LuPxr-NEENAss1	Forw: Cacgggcaggacatagggactact (SEQ-ID No:89) Rev: Actcaccagagtgtttaagcaccagatttatgataaaaatgtcgggtt (SEQ_ID No:152)	Forw: aaaccgacattttatcataaatctggtgcttaaacactctggtgagt (SEQ-ID No:153) Rev: tggtgcttaaacactctggtgagt (SEQ-ID No:42)	Forw: Cacgggcaggacatagggactact (SEQ-ID No:89) Rev: tggtgcttaaacactctggtgagt (SEQ-ID No:42)
p-BnNapin-NEENAss14	Forw: taaggatgacctaccattcttga (SEQ-ID No:83) Rev: tcaaattccaaatcgtgaatgttttaatctgtgttga (SEQ_ID No:154)	Forw: tcaatacaaacaagattaaaaacatttcacgatttgaattga (SEQ-ID No:155) Rev: tctacaacattaaaacgaccatta (SEQ_ID No:69)	Forw: taaggatgacctaccattcttga (SEQ-ID No:83) Rev: tctacaacattaaaacgaccatta (SEQ_ID No:69)

Binary T-plasmids harboring functional expression modules for synthesis of docosaehaenoic acid (DHA) in rapeseed can be obtained in a similar manner. To this end, in addition to the functional modules (promotor-gene-terminator and/or promotor-NEENA-gene-terminator) described for synthesis ARA, constructs also contain functional modules required for the expression of the genes listed in table 4. Promoters used in those expression modules can be SEQ-ID No. 25, 26, 27, 28, 29 and/or 30, NEENAs can be any or none of SEQ-ID No. 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, and(or 24,, and terminators can be SEQ-ID No. 31, 32, 33, 34, 35, 36 and 37.

Example 5: Production of transgenic plants

- a) Generation of transgenic rape seed plants (amended protocol according to Moloney et al. 1992, Plant Cell Reports, 8:238-242)

For the generation of transgenic rapeseed plants, the binary vectors described in example 3 were transformed into *Agrobacterium tumefaciens* C58C1:pGV2260 (Deblaere et al. 1984, Nucl. Acids. Res. 13: 4777-4788). For the transformation of rapeseed plants (cv. Kumily,) a 1:50 dilution of an overnight culture of positive transformed acrobacteria colonies grown in Murashige-Skoog Medium (Murashige and Skoog 1962 Physiol. Plant. 15, 473) supplemented by 3% saccharose (3MS-Medium) was used. Petioles or Hypocotyledones of sterial rapeseed plants were incubated in a petri dish with a 1:50 acrobacterial dilution for 5-10 minutes. This was followed by a tree day co-incubation in darkness at 25°C on 3MS-Medium with 0.8% bacto-Agar. After three days the culture was put on to 16 hours light/8 hours darkness weekly on MS-

medium containing 500mg/l Claforan (Cefotaxime-Natrium), 100 nM Imazetapyr, 20 mikrom Benzylaminopurin (BAP) and 1,6g/l Glucose. Growing sprouts were transferred to MS-Medium containing 2% saccharose, 250mg/l Claforan and 0.8%Bacto-Agar. Even after three weeks no root formation was observed, a growth hormone 2-Indolbutyl acid was added to the medium for enhancing root formation.

Regenerated sprouts have been obtained on 2MS-Medium with Imazetapyr and Claforan and were transferred to the green house for sprouting. After flowering, the mature seeds were harvested and analysed for expression of the Desaturase gene via lipid analysis as described in Qui et al. 2001, J. Biol. Chem. 276, 31561-31566.

b) Production of transgenic flax plants

The production of transgenic flax plants can be carried out according to the method of Bell et al., 1999, In Vitro Cell. Dev. Biol. Plant 35(6):456-465 using particle bombardment. Acrobacterial transformation could be carried out according to Mlynarova et al. (1994), Plant Cell Report 13: 282-285.

Example 6: Lipid extraction and lipid analysis of plant oils

The results of genetic modifications in plants or on the production of a desired molecule, e.g. a certain fatty acid, can be determined by growing the plant under suitable conditions, e.g. as described below, and analysing the growth media and/or the cellular components for enhanced production of the desired molecule, e.g. lipids or a certain fatty acid. Lipids can be extracted as described in the standard literature including Ullman, Encyclopedia of Industrial Chemistry, Bd. A2, S. 89-90 und S. 443-613, VCH: Weinheim (1985); Fallon, A., et al., (1987) "Applications of HPLC in Biochemistry" in: Laboratory Techniques in Biochemistry and Molecular Biology, Bd. 17; Rehm et al. (1993) Biotechnology, Bd. 3, Kapitel III: "Product recovery and purification", S. 469-714, VCH: Weinheim; Belter, P.A., et al. (1988) Bioseparations: downstream processing for Biotechnology, John Wiley and Sons; Kennedy, J.F., und Cabral, J.M.S. (1992) Recovery processes for biological Materials, John Wiley and Sons; Shaeiwitz, J.A., und Henry, J.D. (1988) Biochemical Separations, in: Ullmann's Encyclopedia of Industrial Chemistry, Bd. B3; Kapitel 11, S. 1-27, VCH: Weinheim; und Dechow, F.J. (1989) Separation and purification techniques in biotechnology, Noyes Publications.

Alternatively, extraction will be carried out as described in Cahoon et al. (1999) Proc. Natl. Acad. Sci. USA 96 (22):12935-12940, und Browse et al. (1986) Analytic Biochemistry 152:141-145. Quantitative and qualitative analysis of lipids or fatty acids are described in Christie, William W., Advances in Lipid Methodology, Ayr/Scotland: Oily Press (Oily Press Lipid Library; 2); Christie, William W., Gas Chromatography and Lipids. A Practical Guide - Ayr, Scotland: Oily Press, 1989, Repr. 1992, IX, 307 S. (Oily Press Lipid Library; 1); "Progress in Lipid Research, Oxford: Pergamon Press, 1 (1952) - 16 (1977) u.d.T.: Progress in the Chemistry of Fats and Other Lipids CODEN.

The binary T-plasmids described in example 4 were transformed into rapeseed (*Brassica napus*) as described in example 5. After selection of transgenic plants using PCR, plants were grown until development of mature seeds (Day/night cycle: 16h at 200mE and 21°C, 8h at darkness and 19°C). Fatty acids from harvested seeds were extracted and analysed using gas chromatography. Based on the analysed lipids, the effect of the NEENAs on expression of desaturases and elongases can be determined since the lipid pattern of successfully transformed plant seeds will differ from the pattern of control plant seeds, e.g. of plants expressing a set of desaturases and elongases without the enhancing effect of NEENAs.

Table 7 shows results of single seed measurements of the five best performing transgenic lines obtained for each binary T-plasmid. Table 8 shows the nomenclature for the fatty acids listed in the header of table 3.

Surprisingly, transgenic plants obtained from transformations with construct VC-LJB1327-1qcz (SEQ-ID 39) VC-LJB2003-1qcz (SEQ-ID 40) and VC-LJB2197-1qcz (SEQ-ID 146) showed a much higher ARA to GLA ratio compared to plants transformed with VC-LJB913-1qcz (SEQ-ID 38) and was highest for plants transformed with VC-LJB2003-1qcz (ARA:GLA ratio of up to 53.3). Such a ratio is beneficial if GLA is not desired. Even more surprising was that plants of constructs VC-LJB2003-1qcz and VC-LJB2197-1qcz (incorporating NEENAs) reached higher ARA levels than VC-LJB913-1qcz and VC-LJB1327-1qcz (maximal for VC-LJB913-1qcz: 25.6 %; VC-LJB1327-1qcz: 22 %, VC-LJB2003-1qcz: 28.7 % and for VC-LJB2197-1qcz: 33.1 %), despite removal of the expression module expressing the enzyme d6Des(Pir_GAI) compared to VC-LJB913-1qcz transformed plants.

Table 7: Gaschromatographical analysis of the fatty acid composition of seedoil from transgenic rapeseed plants.

Sample name	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-7	18:2 n-7	18:2 n-6	18:3 n-6	18:3 n-3	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-6	20:3 n-3	20:4 n-6 (ARA)	20:4 n-3	20:5 n-3	22:0	Ratio ARA:GLA	Ratio ARA:DGLA	Ratio LA:ALA	Ratio ARA:EPA
LJB2197_169_37	2,9	0,0	0,0	2,0	17,9	0,9	25,1	1,2	3,8	0,0	0,5	0,7	2,5	3,0	0,0	33,1	0,5	5,9	0,0	27,0	11,2	6,7	5,6
LJB2197_169_5	3,2	0,0	0,0	2,0	16,3	1,2	25,8	2,1	4,5	0,2	0,6	0,6	1,5	2,8	0,0	32,1	0,5	6,7	0,0	15,2	11,5	5,8	4,8
LJB2197_169_11	3,0	0,0	0,0	2,1	17,5	0,9	27,6	1,6	4,1	0,0	0,6	0,6	1,5	3,3	0,0	31,2	0,5	5,5	0,0	19,9	9,4	6,7	5,7
LJB2197_169_51	3,1	0,0	0,0	2,2	17,5	0,9	27,6	1,6	4,1	0,0	0,6	0,6	1,5	3,3	0,0	31,1	0,5	5,5	0,0	19,8	9,3	6,7	5,7
LJB2197_169_9	3,4	0,0	0,0	2,2	18,3	1,9	25,9	3,1	4,0	0,2	0,6	0,6	1,1	2,0	0,0	30,2	0,4	5,7	0,3	9,9	14,9	6,5	5,3
LJB2197_169_22	3,2	0,0	0,0	2,4	17,7	0,7	28,5	1,7	3,6	0,2	0,7	0,8	0,8	3,8	0,0	29,8	0,6	5,3	0,4	18,0	7,9	8,0	5,7
LJB2197_169_36	3,5	0,0	0,0	2,3	17,1	0,6	29,5	1,5	3,8	0,2	0,7	0,8	1,0	3,2	0,0	29,5	0,5	5,5	0,4	19,8	9,3	7,8	5,4
LJB2197_169_40	3,4	0,0	0,0	2,4	18,2	1,2	28,6	2,8	3,4	0,2	0,7	0,7	0,8	2,6	0,0	29,2	0,4	5,0	0,3	10,2	11,4	8,4	5,8
LJB2197_169_42	3,5	0,0	0,0	2,1	18,4	0,7	27,2	1,5	4,3	0,2	0,6	0,8	1,0	3,3	0,0	29,1	0,6	6,6	0,3	19,1	8,9	6,4	4,4
LJB2197_169_26	3,7	0,0	0,0	2,3	19,3	1,3	27,4	2,7	4,0	0,3	0,7	0,7	0,6	2,1	0,0	29,0	0,4	5,5	0,0	10,6	13,5	6,9	5,3
LJB2197_169_61	3,7	0,0	0,0	2,7	20,1	1,1	28,5	2,2	3,2	0,2	0,8	0,9	1,1	3,0	0,0	27,4	0,4	4,6	0,3	12,5	9,3	9,0	6,0
LJB2197_169_14	3,3	0,0	0,0	1,7	18,0	0,7	30,9	1,5	4,9	0,2	0,6	0,7	1,1	3,1	0,0	26,8	0,6	5,7	0,4	18,4	8,7	6,4	4,7
LJB2197_169_16	3,3	0,0	0,0	2,2	20,3	0,7	28,3	1,2	4,5	0,1	0,7	0,8	0,8	3,0	0,0	26,6	0,6	6,3	0,4	21,5	8,8	6,2	4,2
LJB2197_169_65	3,7	0,1	0,0	2,7	20,1	1,2	29,1	2,3	3,8	0,2	0,7	0,7	0,8	1,8	0,0	26,6	0,3	5,5	0,3	11,5	14,4	7,7	4,8
LJB2197_169_7	3,1	0,0	0,0	2,3	18,6	0,5	30,1	1,1	4,1	0,1	0,7	0,9	1,8	4,1	0,0	26,5	0,7	5,1	0,4	24,6	6,5	7,4	5,2
LJB2197_169_34	3,3	0,0	0,0	2,5	20,9	0,8	28,8	1,3	4,2	0,2	0,8	0,8	0,8	2,9	0,0	26,5	0,5	5,4	0,4	19,8	9,2	6,9	4,9
LJB2197_169_47	3,2	0,0	0,0	2,1	18,0	0,9	33,4	1,7	3,9	0,1	0,7	0,7	0,7	2,7	0,0	26,3	0,4	4,6	0,4	15,2	9,8	8,7	5,7
LJB2197_169_24	3,2	0,0	0,0	2,2	18,6	0,5	30,0	1,2	4,4	0,1	0,7	0,8	1,5	3,9	0,0	26,2	0,7	5,6	0,3	22,7	6,8	6,7	4,7
LJB2197_169_31	3,2	0,0	0,0	1,8	19,4	0,6	31,1	1,4	4,2	0,1	0,6	0,8	1,3	3,5	0,0	26,0	0,6	5,0	0,4	18,6	7,5	7,4	5,2
LJB2197_169_73	3,7	0,0	0,0	2,9	18,7	0,8	31,2	1,7	4,5	0,0	0,8	0,7	0,7	2,4	0,0	26,0	0,4	5,4	0,0	15,1	10,8	6,9	4,8
LJB2197_169_21	3,3	0,0	0,0	2,4	19,5	0,7	30,5	1,3	4,2	0,1	0,7	0,8	0,9	3,6	0,0	25,9	0,6	5,0	0,4	19,3	7,2	7,2	5,2
LJB2197_169_29	3,2	0,0	0,0	2,2	19,6	0,6	29,7	1,3	4,0	0,1	0,7	0,9	1,5	4,0	0,0	25,8	0,7	5,1	0,4	19,4	6,5	7,4	5,0
LJB2003_110_11	3,0	0,0	0,0	2,6	15,7	0,2	32,1	0,6	2,8	0,0	0,7	1,0	4,8	3,0	0,3	28,7	0,3	3,7	0,3	48,9	9,7	11,4	7,8
LJB2003_110_17	3,3	0,0	0,0	2,7	16,5	0,1	31,4	0,5	2,7	0,0	0,7	1,1	5,6	2,5	0,4	28,1	0,3	3,6	0,3	53,3	11,2	11,5	7,7
LJB2003_110_16	3,3	0,1	0,0	3,2	17,4	0,2	32,6	0,5	2,6	0,0	0,8	1,1	5,1	2,7	0,4	26,1	0,3	3,2	0,3	50,1	9,6	12,6	8,1
LJB2003_8_54	3,5	0,1	0,0	2,5	19,4	0,8	36,2	2,3	3,3	0,2	0,8	0,7	0,4	2,0	0,0	24,2	0,2	3,0	0,4	10,5	12,2	11,0	8,1
LJB2003_8_7	3,3	0,1	0,0	2,8	19,4	0,8	35,1	2,3	3,7	0,2	0,8	0,8	0,5	1,8	0,0	24,0	0,3	3,7	0,4	10,2	13,6	9,5	6,5
LJB2003_53_11	2,7	0,0	0,0	2,1	14,0	0,3	38,0	0,7	5,3	0,0	0,7	0,7	1,6	4,3	0,0	23,9	0,7	4,7	0,4	33,1	5,6	7,2	5,1

Sample name	16:0	16:1n-7	16:3n-3	18:0	18:1n-9	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:0	20:1n-9	20:2n-6	20:3n-6	20:3n-3	20:4n-6 (ARA)	20:4n-3	20:5n-3	22:0	Ratio ARA:GLA	Ratio ARA:DGLA	Ratio LA:ALA	Ratio ARA:EPA
LJB2003_110_49	3,9	0,0	0,0	3,4	18,6	0,3	34,9	0,5	2,5	0,0	0,8	1,1	4,4	2,4	0,5	23,6	0,2	2,7	0,3	45,1	9,9	13,9	8,7
LJB2003_53_37	3,2	0,0	0,0	3,4	16,0	0,4	36,4	1,0	3,5	0,0	0,8	0,7	1,3	5,2	0,0	23,5	0,7	3,4	0,4	23,6	4,5	10,3	6,8
LJB2003_8_49	3,4	0,1	0,0	2,7	20,0	0,9	37,1	2,1	3,4	0,1	0,8	0,7	0,5	1,7	0,0	22,8	0,2	2,9	0,4	11,1	13,1	11,0	7,9
LJB2003_8_23	3,6	0,1	0,0	3,0	20,7	1,0	33,6	2,9	4,0	0,3	0,8	0,8	0,5	1,4	0,0	22,8	0,2	3,8	0,4	7,8	16,5	8,4	6,1
LJB2003_8_42	3,6	0,1	0,0	2,6	20,7	0,8	35,3	2,4	3,8	0,2	0,7	0,7	0,5	1,6	0,0	22,6	0,2	3,7	0,4	9,6	14,1	9,3	6,0
LJB2003_8_57	3,7	0,1	0,0	3,0	20,8	1,0	36,0	2,3	3,1	0,2	0,9	0,7	0,4	1,8	0,0	22,6	0,2	2,8	0,5	9,8	12,6	11,8	8,0
LJB2003_53_34	2,8	0,0	0,0	2,6	16,4	0,3	39,3	0,8	4,2	0,0	0,7	0,7	1,7	4,1	0,0	22,5	0,5	3,1	0,4	28,5	5,5	9,4	7,2
LJB2003_54_13	3,7	0,1	0,0	2,3	17,9	0,5	39,5	1,3	4,0	0,1	0,8	0,7	0,8	1,9	0,0	22,4	0,2	3,2	0,5	17,4	11,9	9,8	6,9
LJB2003_8_58	3,7	0,1	0,0	2,5	23,6	0,9	34,1	2,1	3,5	0,2	0,7	0,8	0,3	1,4	0,0	22,3	0,2	3,4	0,3	10,7	16,3	9,9	6,5
LJB2003_8_62	3,7	0,1	0,0	2,5	21,3	0,8	35,6	2,2	3,9	0,2	0,7	0,7	0,4	1,4	0,0	22,2	0,2	3,6	0,4	10,2	15,4	9,1	6,3
LJB2003_110_25	3,2	0,0	0,0	3,2	20,1	0,2	34,7	0,4	2,9	0,0	0,9	1,1	3,9	3,2	0,4	22,2	0,4	3,0	0,3	49,8	7,0	12,1	7,5
LJB2003_54_17	3,4	0,2	0,0	2,4	18,2	0,4	39,5	1,1	4,2	0,1	0,8	0,7	1,0	2,1	0,0	21,9	0,3	3,4	0,4	20,5	10,7	9,5	6,5
LJB2003_8_19	3,6	0,1	0,0	3,1	20,6	0,9	36,9	2,5	3,1	0,2	0,8	0,8	0,4	1,5	0,0	21,9	0,2	2,9	0,4	8,7	14,6	11,7	7,6
LJB2003_53_23	3,4	0,2	0,0	2,3	18,0	0,2	37,0	0,7	4,3	0,1	0,6	0,8	2,0	3,6	0,2	21,9	0,6	3,8	0,4	31,0	6,1	8,5	5,7
LJB2003_8_68	3,5	0,1	0,0	2,8	20,8	0,8	36,4	2,2	3,7	0,2	0,8	0,7	0,4	1,7	0,0	21,8	0,2	3,4	0,4	9,9	13,1	9,9	6,4
LJB1327_305_31	3,7	0,1	0,0	2,8	21,0	1,3	35,3	3,4	3,1	0,2	0,9	0,7	0,2	1,5	0,9	22,0	0,1	2,2	0,5	6,4	15,1	11,4	9,8
LJB1327_305_48	4,3	0,1	0,0	2,2	19,5	0,9	37,5	1,9	4,2	0,1	0,7	0,6	0,2	2,1	0,7	21,6	0,2	2,8	0,5	11,5	10,4	8,9	7,8
LJB1327_305_32	4,0	0,0	0,0	2,1	18,8	0,7	39,2	1,8	4,2	0,1	0,8	0,6	0,2	1,7	1,2	21,4	0,2	2,5	0,6	11,7	12,9	9,3	8,7
LJB1327_458_92	3,3	0,0	0,0	2,0	17,8	0,9	39,1	2,1	4,6	0,2	0,8	0,7	0,2	2,0	1,4	21,2	0,2	3,1	0,6	10,2	10,4	8,6	6,9
LJB1327_305_38	4,0	0,1	0,0	2,7	19,7	1,0	36,9	2,9	4,1	0,3	0,9	0,6	0,2	1,7	0,6	20,9	0,2	2,8	0,6	7,2	12,6	9,0	7,5
LJB1327_305_43	4,0	0,1	0,0	2,6	19,2	1,0	37,2	2,2	4,0	0,1	0,9	0,6	0,2	3,1	0,8	20,8	0,3	2,4	0,6	9,5	6,8	9,3	8,7
LJB1327_305_45	3,9	0,1	0,0	2,7	19,9	0,9	37,3	2,4	4,3	0,2	0,9	0,6	0,2	1,7	0,9	20,7	0,2	2,7	0,6	8,6	12,0	8,7	7,7
LJB1327_305_30	3,9	0,1	0,0	2,7	22,0	1,0	36,4	2,5	3,5	0,2	0,9	0,7	0,2	1,6	0,6	20,6	0,2	2,5	0,5	8,3	13,1	10,3	8,3
LJB1327_305_35	4,2	0,1	0,0	2,7	20,3	1,0	38,0	2,2	3,7	0,2	0,9	0,6	0,2	1,5	0,8	20,6	0,2	2,3	0,5	9,3	14,1	10,4	9,0
LJB1327_305_37	4,3	0,0	0,0	3,0	19,7	1,1	38,8	2,6	3,2	0,2	1,1	0,6	0,2	1,4	0,7	20,5	0,1	1,9	0,6	7,9	14,9	12,2	10,9
LJB1327_305_47	4,1	0,1	0,0	2,4	20,4	1,1	38,3	2,4	3,8	0,2	0,8	0,6	0,2	1,4	0,7	20,4	0,1	2,2	0,5	8,5	14,4	10,0	9,3
LJB1327_305_34	3,9	0,0	0,0	2,8	18,9	1,0	39,0	2,6	3,6	0,2	1,1	0,6	0,1	1,7	1,2	20,4	0,2	2,1	0,7	8,0	11,7	10,7	9,9
LJB1327_305_44	4,1	0,1	0,0	2,9	20,3	0,9	38,0	2,3	4,3	0,2	0,9	0,6	0,2	1,4	0,7	19,9	0,1	2,5	0,6	8,5	14,2	8,9	8,0
LJB1327_458_94	4,0	0,0	0,0	2,1	18,8	0,8	37,1	2,2	5,9	0,3	0,8	0,6	0,2	2,2	1,5	19,3	0,3	3,5	0,5	8,7	8,9	6,3	5,5
LJB1327_305_50	4,0	0,0	0,0	2,4	19,6	0,8	40,3	2,2	4,2	0,2	0,9	0,6	0,2	1,5	0,7	19,2	0,2	2,4	0,6	8,8	12,6	9,7	8,0
LJB1327_305_42	3,7	0,1	0,0	2,5	23,6	1,1	37,0	2,4	3,8	0,2	0,8	0,7	0,2	1,1	0,7	19,2	0,2	2,4	0,5	8,1	16,8	9,7	8,0

Sample name	16:0	16:1n-7	16:3n-3	18:0	18:1n-9	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:0	20:1n-9	20:2n-6	20:3n-6	20:3n-3	20:4n-6 (ARA)	20:4n-3	20:5n-3	22:0	Ratio ARA:GLA	Ratio ARA:DGLA	Ratio LA:ALA	Ratio ARA:EPA
LJB1327_305_54	4,4	0,1	0,0	3,4	21,5	1,4	37,4	2,8	2,7	0,1	1,3	0,8	0,6	1,5	0,7	19,1	0,0	1,6	0,6	6,8	12,9	13,9	11,8
LJB1327_305_41	4,2	0,1	0,0	2,5	22,4	1,0	38,6	2,0	3,6	0,1	0,8	0,6	0,2	1,3	0,7	19,1	0,2	2,3	0,5	9,6	14,6	10,7	8,3
LJB1327_305_40	3,9	0,0	0,0	2,5	21,1	0,9	39,4	2,0	4,3	0,2	0,8	0,6	0,2	1,3	0,7	18,8	0,2	2,6	0,5	9,2	14,5	9,1	7,1
LJB1327_305_55	4,5	0,1	0,0	3,3	21,9	1,3	37,7	2,0	3,3	0,2	1,2	0,7	0,7	1,4	0,9	18,6	0,0	1,7	0,5	9,3	13,7	11,3	11,1
LJB1327_305_33	4,2	0,1	0,0	2,7	23,8	1,1	37,0	2,1	3,7	0,2	0,9	0,7	0,2	1,3	0,8	18,6	0,1	2,1	0,6	8,9	14,6	10,1	8,8
LJB913_64-13a	4,4	0,0	0,0	3,9	11,3	0,0	21,7	11,7	3,7	0,7	1,1	0,9	3,7	6,7	0,0	25,6	0,7	3,8	0,0	2,2	3,8	5,9	6,7
LJB913_64_9	3,8	0,0	0,2	2,7	9,8	0,0	21,8	12,7	4,3	0,8	0,9	1,0	4,5	8,7	0,6	23,6	1,0	3,8	0,0	1,8	2,7	5,1	6,1
LJB913_64_3	4,2	0,1	0,2	3,6	12,0	0,0	22,4	11,7	3,5	0,7	1,0	1,2	5,7	6,5	0,7	22,0	0,7	3,5	0,5	1,9	3,4	6,5	6,4
LJB913_64_20	3,5	0,2	0,1	3,3	14,1	0,1	25,9	8,7	3,0	0,5	0,9	1,2	5,0	7,4	0,5	21,2	0,8	3,2	0,4	2,4	2,9	8,5	6,6
LJB913_64_8	3,7	0,1	0,2	3,0	13,9	0,2	24,1	16,4	3,7	1,1	0,9	0,8	2,0	5,8	0,0	20,4	0,7	3,1	0,0	1,2	3,5	6,6	6,6
LJB913_91_5	3,5	0,1	0,1	2,8	15,7	0,1	27,1	9,0	4,9	0,5	0,8	0,9	3,1	6,0	0,4	20,3	0,7	3,5	0,4	2,2	3,4	5,5	5,8
LJB913_64_22	4,8	0,2	0,1	4,0	13,4	0,0	25,9	9,3	3,9	0,7	1,2	1,0	4,3	6,0	0,6	19,8	0,8	3,3	0,7	2,1	3,3	6,6	6,0
LJB913_64_23	4,5	0,1	0,1	3,9	13,4	0,0	25,0	9,9	3,9	0,7	1,1	1,1	5,2	6,1	0,7	19,5	0,8	3,4	0,5	2,0	3,2	6,4	5,8
LJB913_64-07a	4,2	0,0	0,0	4,5	13,7	0,0	25,9	7,3	4,4	0,0	1,3	1,4	7,6	6,2	1,0	19,4	0,0	3,1	0,0	2,7	3,1	5,9	6,3
LJB913_91_4	4,1	0,2	0,2	3,7	16,2	0,2	32,4	5,2	6,1	0,4	1,0	0,7	1,7	4,7	0,0	19,4	0,6	3,4	0,0	3,7	4,1	5,3	5,7
LJB913_64-05a	4,2	0,0	0,0	4,8	14,0	0,0	25,3	8,0	4,8	0,5	1,3	1,3	5,8	7,0	0,0	19,1	0,8	3,2	0,0	2,4	2,7	5,2	6,0
LJB913_64_10	3,9	0,0	0,1	4,6	15,0	0,0	27,3	6,0	4,5	0,3	1,2	1,3	6,2	6,4	0,7	18,9	0,6	2,5	0,5	3,2	3,0	6,0	7,6
LJB913_64_13	3,9	0,1	0,1	3,5	15,0	0,1	25,1	9,4	4,5	0,5	0,9	1,1	4,5	7,8	0,6	18,7	1,0	3,1	0,0	2,0	2,4	5,6	6,0
LJB913_91_14	3,6	0,0	0,2	3,5	17,0	0,1	26,6	9,3	5,1	0,5	0,9	0,9	2,8	6,5	0,4	18,7	0,7	3,1	0,0	2,0	2,9	5,2	6,0
LJB913_64-12a	4,8	0,0	0,0	4,8	13,9	0,0	24,1	7,1	5,0	0,0	1,4	1,3	7,7	6,5	1,2	18,5	0,0	3,5	0,0	2,6	2,8	4,8	5,2
LJB913_91_28	4,2	0,0	0,2	4,0	17,1	0,0	27,4	7,5	5,9	0,4	1,1	0,9	2,9	6,5	0,0	18,4	0,7	2,9	0,0	2,4	2,8	4,7	6,3
LJB913_91_20	3,2	0,1	0,1	3,0	18,7	0,1	28,9	7,2	4,7	0,3	0,7	0,9	2,5	7,1	0,3	18,3	0,8	2,9	0,0	2,5	2,6	6,1	6,3
LJB913_64_17	4,7	0,1	0,1	4,3	14,3	0,0	27,0	9,6	3,8	0,5	1,1	1,0	4,0	6,5	0,5	18,2	0,7	2,9	0,6	1,9	2,8	7,1	6,2
LJB913_91_18	3,6	0,0	0,1	4,0	17,8	0,1	31,0	6,1	4,6	0,3	1,0	0,9	2,5	5,6	0,3	18,2	0,6	2,8	0,5	3,0	3,2	6,8	6,6
LJB913_64_14	4,0	0,0	0,3	4,2	14,9	0,0	26,2	8,9	5,5	0,7	1,1	1,0	3,9	6,5	0,6	17,8	0,9	3,5	0,0	2,0	2,7	4,7	5,1
LJB913_91_3	3,8	0,1	0,2	3,1	18,1	0,0	29,7	6,5	6,8	0,5	0,9	0,8	1,9	5,3	0,0	17,7	0,8	3,7	0,0	2,7	3,3	4,4	4,8

Table 8: Used Nomenclature

Fatty acid	Nomenclature	
Oleic acid	18:1 Δ 9	18:1n-9
Linoleic acid	18:2 Δ 6,12	18:2n-6
α -Linolenic acid	18:3 Δ 9,12,15	α 18:3n-3
γ -Linolenic acid	18:3 Δ 6,9,12	γ 18:3n-6
Stearidonic acid	18:4 Δ 6,9,12,15	18:4n-3
Dihomo- γ -linolenic acid	20:3 Δ 8,11,14	20:3n-6
Eicosatrienoic acid	20:3 Δ 11,14,17	20:3n-3
iso-Arachidonic acid	20:4 Δ 8,11,14,17	20:4n-3
Arachidonic acid	20:4 Δ 5,8,11,14	20:4n-6
Eicosapentaenoic acid	20:5 Δ 5,8,11,14,17	20:5n-3

CLAIMS

1. A polynucleotide comprising:
 - a) at least one nucleic acid sequence encoding a polypeptide having desaturase or
5 elongase activity;
 - b) at least one seed-specific plant promoter operatively linked to the said nucleic acid sequence defined in a);
 - c) at least one terminator sequence operatively linked to the said
10 nucleic acid sequence and
 - d) one or more nucleic acid expression enhancing nucleic acid (NEENA)
molecule functionally linked to said promoter and which is/are heterologous to said
promoter and to said polypeptide defined in a).
2. The polynucleotide of claim 1 comprising:
 - e) at least one nucleic acid sequence encoding a polypeptide having beta-ketoacyl re-
15 ductase activity;
 - f) at least one nucleic acid sequence encoding a polypeptide having dehydratase ac-
tivity and/or
 - g) at least one nucleic acid sequence encoding a polypeptide having enoyl-CoA reduc-
20 tase activity,wherein the nucleic acid sequences defined in e) to g) are heterologous to said poly-
peptide having desaturase or elongase activity.
3. The polynucleotide of claim 1 or 2 comprising at least one nucleic acid sequence encod-
25 ing a polypeptide having acyltransferase activity, wherein the nucleic acid sequence is
heterologous to said polypeptide having desaturase, elongase, beta-ketoacyl reductase,
dehydratase or enoyl-CoA reductase activity.
4. A vector comprising the polynucleotide of claims 1 to 3.
5. A host cell comprising the polynucleotide of claims 1 to 3 or the vector of claim 4.
6. A method for the manufacture of a polypeptide encoded by a polynucleotide of claims 1
to 3 comprising
 - 35 a) cultivating the host cell of claim 5 under conditions which allow for the production of
the said polypeptide; and
 - b) obtaining the polypeptide from the host cell of step a).
7. A non-human transgenic organism comprising the polynucleotide of claims 1 to 3
40 or the vector of claim 4.
8. The non-human transgenic organism of claim 7, which is a plant or a plant part.

9. A method for the manufacture of polyunsaturated fatty acids comprising:
- a) cultivating the host cell of claim 5 under conditions which allow for the production of polyunsaturated fatty acids in said host cell; and
 - b) obtaining said polyunsaturated fatty acids from the said host cell.
- 5
10. A method for the manufacture of polyunsaturated fatty acids comprising:
- a) cultivating the non-human transgenic organism of claim 7 or 8 under conditions which allow for the production of polyunsaturated fatty acids in said non-human transgenic organism; and
 - 10 b) obtaining said polyunsaturated fatty acids from the said non-human transgenic organism.
11. The method of claim 9 or 10, wherein said poly-unsaturated fatty acid is arachidonic acid (ARA), eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA).
- 15
12. A method for the manufacture of an oil, lipid or fatty acid composition comprising the steps of the method of any one of claims 9 to 11 and the further step of formulating the polyunsaturated fatty acid as oil, lipid or fatty acid composition.
- 20
13. A use of the polynucleotide as defined in claims 1 to 3 or the recombinant vector comprising the polynucleotide as defined in claim 4 for enhancing expression of at least one enzyme of the polyunsaturated fatty acid biosynthetic pathway as defined in claims 1 to 3 in plants or parts thereof.
- 25
14. A use of a host cell or a host cell culture as claimed in claim 5 or a non-human transgenic organism, transgenic plant, plant parts or plant seeds derived from the transgenic non-human organism or plant as claimed in claim 7 or 8 for the production of foodstuffs, animal feeds, seeds, pharmaceuticals or fine chemicals.