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(54) Title: PRODUCTION OF OMEGA-3 LONG CHAIN POLYUNSATURATED FATTY ACIDS

(57) Abstract: A recombinant camelina plant or cell comprising one or more polynucleotides encoding a A6-desaturase, a A6-elongase and a A5-desaturase operably linked with one or more regulatory sequences.

## Production of omega-3 long chain polyunsaturated fatty acids

### 5 Field of the Invention

The present invention relates to a recombinant oilseed plant or cell for producing omega-3 long chain polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The invention further relates to the oil produced  
10 by said recombinant oilseed plant or cell.

### Background to the Invention

Two main families of poly-unsaturated fatty acids are the omega-3 fatty acids, exemplified by EPA, and the omega-6 fatty acids, exemplified by arachidonic acid  
15 (Figure 1).

The starting material for the omega-6 metabolic pathway is the fatty acid linoleic acid while the omega-3 pathway proceeds via linolenic acid. Linolenic acid is formed by  
20 the activity of an omega-3 desaturase (Tocher et al. 1998, Prog. Lipid Res. 37, 73-117 ; Domergue et al. 2002, Eur. J. Biochem. 269, 4105-4113).

Omega-3 highly unsaturated fatty acids are recognized as being important dietary compounds for preventing arteriosclerosis and coronary heart disease, for alleviating  
25 inflammatory conditions and for retarding the growth of tumor cells. These beneficial effects are a result both of omega-3 highly unsaturated fatty acids causing competitive inhibition of compounds produced from omega-6 fatty acids, and from beneficial compounds produced directly from the omega-3 highly unsaturated fatty acids themselves (Simopoulos et al. (1986) Health Effects of Polyunsaturated Fatty  
30 Acids in Seafoods, Academic Press, New York).

Omega-3 long chain polyunsaturated fatty acids are crucial to brain development and normal brain functioning (Neuringer, et al., (1988) *Annu Rev Nutr* 8, 517-541) with DHA particularly important to brain functioning due to its influence on neural membrane properties, which modulate cell signalling (Uauy, R., and Dangour, A. D.  
35 (2006) *Nutr Rev* 64, S24-33; discussion S72-91). DHA concentration in the brain

decreases with age in humans, something that has been postulated to be consequential to the age-related deterioration in central nervous system functions (Soderberg et al. (1991) *Lipids* 26, 421-425). Evidence from animal studies supports this, with animals fed a low omega-3 long chain polyunsaturated fatty acid diet showing cognitive deficits (Suzuki et al. (1998) *Mech Ageing Dev* 101, 119-128) that are ameliorated by DHA supplementation (Moriguchi, T., and Salem, N., Jr. (2003) *J Neurochem* 87, 297-309; Chung, et al. (2008) *J Nutr* 138, 1165-1171).

In humans, it has been consistently reported that a higher intake of fish is related to reduced cognitive decline (van Gelder et al. (2007) *Am J Clin Nutr* 85, 1142-1147; Kalmijn et al. (1997) *Ann Neurol* 42, 776-782; Morris et al. (2005) *Arch Neurol* 62, 1849-1853; Kalmijn et al. (1997) *Am J Epidemiol* 145, 33-41) and incidence of dementia (Kalmijn et al. (1997) *Ann Neurol* 42, 776-782), and associated with better cognitive performance (Morris et al., (2005) *Arch Neurol* 62, 1849-1853; Kalmijn et al. (2004) *Neurology* 62, 275-280; Nurk et al. (2007) *Am J Clin Nutr* 86, 1470-1478; Dangour et al. (2009) *J Nutr Health Aging* 13, 198-202). Significant positive relationships between cognitive outcomes and dietary intake levels of omega-3 long chain polyunsaturated fatty acids have also been established (van Gelder et al. (2007) *Am J Clin Nutr* 85, 1142-1147; Morris et al. (2003) *Arch Neurol* 60, 940-946; Kalmijn et al. (2004) *Neurology* 62, 275-280). These positive relationships are further strengthened if one considers the plasma or erythrocyte level/status of omega-3 long chain polyunsaturated fatty acids. Here, DHA/EPA levels have been associated with better cognitive function in normal older adult (Whalley et al. (2004) *Am J Clin Nutr* 80, 1650-1657; Whalley et al. (2008) *Am J Clin Nutr* 87, 449-454), better cognitive outcomes over time (Whalley et al. (2008) *Am J Clin Nutr* 87, 449-454), and reduced risk of cognitive decline (Heude et al. (2003) *Am J Clin Nutr* 77, 803-808) and a lower risk of developing dementia (Schaefer et al. (2006) *Arch Neurol* 63, 1545-1550).

Studies in cognitively healthy populations have failed to show efficacy in improving cognition (Dangour et al. (2010) *Am J Clin Nutr* 91, 1725-1732; van de Rest et al. (2008) *Neurology* 71, 430-438). However, omega-3 long chain polyunsaturated fatty acid supplementation has been shown to be efficacious in cognitively impaired individuals (primarily mild cognitively impaired; MCI), where interventions had a beneficial effect on cognitive outcomes (Chiu et al. (2008) *Prog*

*Neuropsychopharmacol Biol Psychiatry* 32, 1538-1544; Freund-Levi et al. (2006) *Arch Neurol* 63, 1402-1408; Yurko-Mauro et al. (2010) *Alzheimers Dement* 6, 456-464). Furthermore, they have been shown to be beneficial in at risk AD individuals carrying the apolipoprotein E (ApoE)  $\epsilon$ 4 allele, with these individuals showing an improvement in sustained attention after 26 weeks intervention with both low (226 mg EPA, 176 mg DHA) and high (1093 mg EPA, 847 mg DHA) doses of fish oil.

Bacteria and yeast are not known to synthesize omega-3 highly unsaturated fatty acids and only a few fungi are known which can produce minor and trace amounts of omega-3 highly unsaturated fatty acids (Weete (1980) *Lipid Biochemistry of Fungi and Other Organism*. Plenum Press, New York; Wassef, M. (1977) "Fungal lipids." *Adv. Lipid Res.*).

Currently the primary dietary source of omega-3 highly unsaturated fatty acids is from certain fish oils which can contain up to 20-30% of these fatty acids in their triacylglycerides. Consequently large quantities of fish oil are processed and encapsulated each year for sale as a dietary supplement.

However, fish stocks may undergo natural fluctuations or may be depleted by overfishing. Furthermore, fish oils, can accumulate environmental pollutants and may contain high levels of fat-soluble vitamins that are found naturally in fish oils. When ingested, these vitamins are stored and metabolized in fat in the human body rather than excreted in urine. Additionally, fish oils have an unpleasant taste and odour when they undergo oxidation, and as such cannot be added to processed foods as a food additive, without impairing the taste of the food product. Moreover, the refining of pure omega-3 highly unsaturated fatty acids from crude extracts of fish oils is an involved and expensive process resulting in very high prices for pure forms of these fatty acids.

The primary natural source of omega-3 unsaturated fatty acids in fish oil is in fact marine unicellular microbes such as algae and diatoms, at the base of the aquatic foodweb. These highly unsaturated fatty acids are important components of photosynthetic membranes. Omega-3 highly unsaturated fatty acids accumulate in the food chain and are eventually incorporated into fish oils.

Owing to the positive characteristics of omega-3 polyunsaturated fatty acids, genes have been identified which are involved in the biosynthesis of these fatty acids in a variety of organisms.

- 5 Linoleic acid (LA, 18:2<sup>Δ<sup>9,12</sup></sup>) is converted to α-linolenic acid (ALA, 18:3<sup>Δ<sup>9,12,15</sup></sup>) the first of the omega-3 fatty acids, by the action of a Δ15 desaturase. Subsequently, ALA is converted to stearidonic acid (SDA, 18:4<sup>Δ<sup>6,9,12,15</sup></sup>) by the activity of a Δ6 desaturase; SDA is converted to eicosatetraenoic acid (ETA, 20:4<sup>Δ<sup>8,11,14,17</sup></sup>) by the activity of an elongase; and ETA is converted to eicosapentaenoic acid (EPA, 20:5<sup>Δ<sup>5,8,11,14,17</sup></sup>) by
- 10 the activity of a Δ5 desaturase. Alternatively, ETA and EPA can be produced from di-homo γ-linolenic acid (DGLA, 20:3<sup>Δ<sup>8,11,14</sup></sup>) and arachidonic acid (ARA, 20:4<sup>Δ<sup>5,8,11,14</sup></sup>) respectively, by the activity of a Δ17 desaturase. EPA can be further converted to DHA by the activity of an elongase and a Δ4 desaturase (see Figure 1).
- 15 While higher plants comprise polyunsaturated fatty acids such as linoleic acid and linolenic acid, long-chain polyunsaturated fatty acids such as DHA and EPA are not found at all in the seed oil of such plants, or only in miniscule, nutritionally-irrelevant amounts. The production of long-chain polyunsaturated fatty acids, in particular
- 20 omega-3 fatty acids, in higher plants would be advantageous since large amounts of high-quality long-chain polyunsaturated fatty acids (and associated triacylglycerides) for the food industry, animal nutrition and pharmaceutical purposes might be obtained economically.

25 Transgenic linseed oil plants have been shown to result in the accumulation of high levels of Δ6 desaturated C<sub>18</sub> fatty acids. However, only very low levels of C<sub>20</sub> polyunsaturated fatty acids have been obtained. The synthesis and accumulation of omega-3 LC-PUFAs such as EPA and DHA in the seeds of transgenic plants has previously reported in the literature but with limited success and unpredictable results.

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Abbadi et al. (Plant Cell. 2004 Oct;16(10):2734-48. Epub 2004 Sep 17) described attempts to produce EPA in the seeds of transgenic linseed, using a three-gene construct containing a Δ6-desaturase (D6D) from *Phaeodactylum tricornutum* (AY082393), A6-elongase (D6E) from *Physcomitrella patens* (AF428243) and Δ5-

desaturase (D5D) from *Phaeodactylum tricornutum* (AY082392). Linseed was chosen as a host species for the seed-specific expression of these genes on account of the very high levels of endogenous substrate (ALA) for prospective conversion to EPA. However, despite the presence of almost 50% ALA in the seeds of developing  
5 linseed, less than 1% EPA (0.8% of total fatty acids) was generated. In addition, very high levels of the undesired biosynthetic intermediate the omega-6 fatty acid  $\gamma$ -linolenic acid (GLA) were reported (16.8% of total fatty acids). This simultaneous accumulation of high levels of GLA and low synthesis of EPA was ascribed by Abbadi et al. (Plant Cell. 2004 Oct;16(10):2734-48. Epub 2004 Sep 17) to the  
10 phospholipid-dependent substrate-requirements of the D6D.

Similar results were also reported by Wu et al. (Nat Biotechnol, 2005, 23:1013-7) who described the seed-specific expression of a 3 gene construct (D6D from *Pythium irregulare*, CAJ30866; D6E from *Physcomitrella patens*; D5D from *Thraustochytrium*,  
15 AX467713) in *Brassica juncea*, yielding 0.8% EPA but 27.7% of the undesirable omega-6 GLA. More complex gene constructs were also reported by Wu et al. in which they attempted to boost the accumulation of EPA in transgenic *B. juncea*. A four gene construct comprising the same D6D, D6E, D5D activities and additionally the FAD2 A12-desaturase from *Calendula officinalis* (AF343065) resulted in a small  
20 increase in EPA to 1.2% but also a concomitant increase in GLA to 29.4%. A five gene construct, comprising D6D, D6E, D5D, FAD2 and a second  $\Delta\delta$ -elongase D6E#2 from *Thraustochytrium* (AX214454) had equally marginal impact on the fatty acid composition of the seeds of transgenic *B. juncea*, yielding 1.4% EPA and 28.6% GLA. A six gene construct, comprising the same D6D, D6E, D5D, FAD2, D6E#2 and  
25 a  $\omega$ 3-desaturase  $\omega$ 3D from *Phytophthora infestans* (CS160901), yielded the best levels of EPA at 8.1% - however, the levels of GLA remained high at 27.1%. In a further iteration, Wu et al. (Nat Biotechnol, 2005, 23:1013-7) also attempted to engineer the accumulation of both EPA and DHA, through the seed-specific expression of nine genes (D6D, D6E, D5D, FAD2, D6E#2,  $\omega$ 3D, and additionally a  
30 A5-elongase (D5E) from fish (*Oncorhynchus mykiss*; CS020097), a A4-desaturase (D4D) from *Thraustochytrium* (AF489589), and an acyltransferase also from the same organism). This yielded *B. juncea* seeds containing on average 8.1% EPA and 0.2% DHA. Again, GLA levels remained markedly higher (27.3%). Wu et al. reported

a maximal level of EPA observed in transgenic *B. juncea* as 15% and a maximal DHA level of 1.5% (based on individual plants for their nine gene construct).

5 Similar experiments were carried out in the model oilseed species *Arabidopsis thaliana*: Robert et al. (Functional Plant Biol, 2005, 32: 473-479) reported the low level accumulation of EPA (3.2% of total fatty acids) in the seeds of *Arabidopsis* on the expression of two genes, a functional D6D/D5D from zebrafish (*Danio rerio*, AF309556) and a D6E from the nematode *Caenorhabditis elegans* (Z68749). Interestingly, this construct also showed significantly reduced accumulation of GLA, a fact that Robert et al. attributed to the acyl-CoA-dependent substrate requirement of the D6D/D5D. Further transformation of this EPA-accumulating *Arabidopsis* line with genes for DHA synthesis (D4D and D5E from *Pavlova salina*, AY926605, AY926606) resulted in a mean level of 0.3% DHA, again with basal levels of the unwanted co-product GLA (0.3%).

15 Very similar results were reported by Hoffmann et al. (J Biol Chem, 2008, 283:22352-62) who postulated that the use of an "acyl-CoA-dependent" pathway in transgenic plants would decrease the build-up of biosynthetic intermediates such as GLA whilst simultaneously increase the accumulation of EPA. However, the seed-specific expression in *Arabidopsis* of acyl-CoA-dependent D6D and D5D activities from *Mantoniella squamata* (AM949597, AM949596) (in conjunction with the previously described D6E from *P. patens*) yielded barely detectable levels of EPA (<0.1% of total seed fatty acids and < 0.05% GLA. Analogous data have been reported by Ruiz-Lopez et al. (Transgenic Res. 2012 (doi:10.1007/s1 1248-012-9596-0)) who expressed a number of different gene combinations in *Arabidopsis*. Notably, a six gene construct comprising a D6D from *Pythium irregulare*, (CAJ30866); D6E from *Physcomitrella patens* (AF428243); D5D from *Thraustochytrium*, (AX467713); a bifunctional D12/15 desaturase from *Acanthamoeba castellanii*, EF017656; w3D from *Phytophthora infestans* (CS160901) and a second D6E from *Thalassiosira pseudonana*, (AY591337) yielded 2.5% EPA of total seed fatty acids with the concomitant accumulation of 13.3% GLA. In contrast, a four gene construct that contained an acyl-CoA-dependent D6D from *Ostreococcus tauri* (AY746357), D6E from *Thalassiosira pseudonana* (AY591337), D5D from *Thraustochytrium*, (AX467713) and FAD2 from *Phytophthora sojae* (CS423998) generated low levels of both EPA (2% of total fatty acids) and GLA (1.0%).

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More recently, Cheng et al. (Transgenic Res, 2010, 19:221-9) reported the accumulation of EPA in transgenic *Brassica carinata*. For example, the seed-specific expression of 3 genes (D6D from *Pythium irregulare*, CAJ30866; D6E from  
5 *Thalassiosira pseudonana*, AY591337; D5D from *Thraustochytrium*, AX467713) resulted in a mean level of 2.3% EPA, with high level co-accumulation of GLA (17.6%). A four gene construct (D6D, D6E, D5D and w3D from *Claviceps purpurea*, EF536898) resulted in 4.2% EPA and 11.8% GLA, whilst a five gene construct (D6D, D6E, D5D, w3D and an additional w3-desaturase from *Pythium irregulare*,  
10 (FB753541)) yielded 9.7% EPA and 11.1% GLA. Such levels are very similar to that observed with five and six gene constructs in *B. juncea* (Wu et al. 2005, Nat Biotechnol, 2005, 23:1013-7). Cheng et al. introduced a different 5 gene construct (D6D from *Pythium irregulare*, CAJ30866; D6E from *Thraustochytrium*, HC476134; D5D from *Thraustochytrium*, AX467713; FAD2 from *Calendula officinalis*, AF343065  
15 and w3D from *Pythium irregulare*, FB753541) into two different cultivars of *B. carinata*, differing in their accumulation of the C22 monounsaturated fatty acid erucic acid. Expression of this construct in conventional high erucic acid *B. carinata* resulted again in a mean accumulation of 9.3% EPA and 18.2% GLA. Expression in the zero-erucic acid genotype yielded an increase in EPA though this genotype also  
20 resulted in the co-accumulation of high levels of GLA (26.9%).

The present invention addresses the need for systems that produce commercially useful levels of omega-3 highly unsaturated fatty acids in the seeds of terrestrial plants.

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### Summary of the Invention

*Camelina sativa* is a genus within the flowering plant family *Brassicaceae*. Camelina is a short season crop, and has gained notoriety for its ability to withstand water  
30 shortages in early stages of development. In recent years, there has been increasing interest in the use of camelina oil as a biofuel and bio-lubricant, mainly in view of this crop's low nitrogen requirements.

The present invention relates to the surprising finding that camelina can be  
35 transformed with desaturase and elongase enzymes to produce omega-3 fatty acids.



Indeed, following the introduction of these enzymes into camelina, it is not only possible to generate omega-3 fatty acids, but it is possible to create novel oil compositions.

5 According to a first aspect of the present invention there is provided a recombinant camelina plant or cell comprising one or more polynucleotides encoding a  $\Delta 6$ -desaturase, a  $\Delta \delta$ -elongase and a A5-desaturase operably linked with one or more regulatory sequences.

10 Thus, there is provided a camelina plant or cell transformed with genes encoding a  $\Delta 6$ -desaturase, a A6-elongase and a  $\Delta \delta$ -desaturase.

The  $\Delta \delta$ -desaturase,  $\Delta \delta$ -elongase and  $\Delta \delta$ -desaturase enzymes can be encoded by a single or separate polynucleotide(s). What is important is that the recombinant  
15 camelina plant or host according to the first aspect of the invention comprises polynucleotide sequences for all three enzymes.

In a preferred embodiment of the first aspect of the invention, the recombinant camelina plant or cell is produced by transforming a camelina plant or cell with a  
20 polynucleotide encoding a  $\Delta 6$ -desaturase, a  $\Delta 6$ -elongase and a A5-desaturase operably linked with one or more regulatory sequences.

Alternatively, the recombinant camelina plant or cell may be produced by transforming a camelina plant or cell with separate polynucleotides each encoding a  
25  $\Delta \beta$ -desaturase and/or a  $\Delta \beta$ -elongase and/or a A5-desaturase.

The recombinant camelina plant or cell of this aspect of the invention may further comprise one or more polynucleotides encoding a A12-desaturase and/or a  $\omega 3$  desaturase operably linked with one or more regulatory sequences. Thus, there is  
30 provided a recombinant camelina plant or cell comprising one or more polynucleotides encoding a  $\Delta 6$ -desaturase, a  $\Delta 6$ -elongase, a A5-desaturase, a  $\Delta 12$ -desaturase and  $\omega 3$  desaturase operably linked to one or more regulatory elements. In a preferred embodiment, the recombinant camelina plant or cell is produced by transforming a camelina plant or cell with a polynucleotide encoding a  $\Delta 6$ -

desaturase, a  $\Delta 6$ -elongase, a  $\Delta 5$ -desaturase, a  $\Delta 12$ -desaturase and a  $\omega 3$  desaturase operably linked with one or more regulatory sequences.

5 According to a second aspect of the present invention there is provided a method for producing eicosapentaenoic acid (EPA) comprising growing a plant or cell according to the first aspect of the invention under conditions wherein said desaturase and elongase enzymes are expressed and EPA is produced in said plant or cell.

10 According to third aspect of the present invention there is provided a method for producing a plant seed oil comprising growing a recombinant camelina plant or cell of the first aspect of the invention whereby said desaturase and elongase enzymes are expressed and oil is produced in said plant or cell.

15 According to fourth aspect of the present invention there is provided a plant seed oil produced by the recombinant camelina plant or cell of the first aspect of the present invention.

20 According to a fifth aspect of the present invention there is provided a plant seed oil wherein EPA constitutes at least 5%, at least 10%, at least 20%, at least 25% or at least 30% (mol %) of the total of the total amount of fatty acid present in said oil. Said oil may be produced by a recombinant camelina plant or cell of the first aspect of the present invention.

25 In one embodiment, the EPA constitutes at least 15, 20, 25 or 30% (mol %) of the total fatty acid content of said oil, and the  $\gamma$ -linolenic (GLA) constitutes less than 10% (mol %) of the total fatty acid content of said oil.

In one embodiment, the EPA constitutes 20% to 35%, preferably 20 to 31% (mol %) of the total fatty acid content of said oil.

30 The GLA may constitute less than 7% (mol %) of the total fatty acid content of said oil. In one embodiment, the GLA constitutes 1% to 6% (mol %) of the total fatty acid content of said oil.

The ratio of the molar percentages of EPA to  $\gamma$ -linolenic (GLA) may be, for example, about 3:1 to about 22:1, preferably about 5:1 to about 20:1, preferably about 8:1 to about 20:1.

5 According to a sixth aspect of the present invention there is provided a recombinant camelina plant or cell comprising one or more polynucleotides encoding a  $\Delta 6$ -desaturase, a  $\Delta 6$ -elongase, a  $\Delta 5$ -desaturase, a  $\Delta 5$ -elongase and a A4-desaturase operably linked with one or more regulatory sequences.

10 Thus, there is provided a camelina plant or cell transformed with genes encoding a  $\Delta\beta$ -desaturase, a  $\Delta 6$ -elongase, a  $\Delta 5$ -desaturase, a  $\Delta 5$ -elongase and a  $\Delta 4$ -desaturase.

The A6-desaturase,  $\Delta\beta$ -elongase,  $\Delta 5$ -desaturase,  $\Delta 5$ -elongase and A4-desaturase  
15 can be encoded by a single or separate polynucleotide(s). What is essential is that the recombinant camelina plant or cell according to the sixth aspect of the invention comprises polynucleotide sequences for all five enzymes.

Preferably, the recombinant camelina plant or cell according to this aspect of the  
20 invention is produced by transforming a camelina plant or cell with a polynucleotide encoding a A6-desaturase, a A6-elongase, a A5-desaturase, a  $\Delta 5$ -elongase and a A4-desaturase operably linked with one or more regulatory sequences.

Alternatively, the recombinant camelina plant or cell may be produced by  
25 transforming a camelina plant or cell with separate polynucleotides each encoding a  $\Delta 6$ -desaturase, and/or  $\Delta\delta$ -elongase, and/or  $\Delta 5$ -desaturase, and/or  $\Delta\delta$ -elongase and/or a A4-desaturase.

The recombinant camelina plant or cell of this aspect of the invention may further  
30 comprise one or more polynucleotides encoding a  $\Delta 12$ -desaturase and/or a  $\omega 3$  desaturase operably linked with one or more regulatory sequences. Thus, there is provided a recombinant camelina plant or cell comprising one or more polynucleotides encoding a  $\Delta 6$ -desaturase, a  $\Delta 6$ -elongase, a A5-desaturase, a  $\Delta 12$ -desaturase, a  $\Delta\delta$ -elongase, a A4-desaturase and a  $\omega 3$  desaturase operably linked to  
35 one or more regulatory elements. In a preferred embodiment, the recombinant

camelina plant or cell is produced by transforming a camelina plant or cell with a polynucleotide encoding a A6-desaturase, a  $\Delta 6$ -elongase, a  $\Delta 5$ -desaturase, a  $\Delta 5$ -elongase, a A4-desaturase, a A12-desaturase and a  $\omega 3$  desaturase operably linked with one or more regulatory sequences.

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According to a seventh aspect of the present invention there is provided a method for producing docosahexaenoic acid (DHA) and/or EPA comprising growing a plant or cell according to the sixth aspect of the invention under conditions wherein said desaturase and elongase enzymes are expressed and DHA and/or EPA is produced

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in said plant or cell.

According to eighth aspect of the present invention there is provided a method for producing a plant seed oil comprising growing a recombinant camelina plant or cell of the sixth aspect of the invention whereby said desaturase and elongase enzymes are expressed and oil is produced in said plant or cell.

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According to a ninth aspect of the present invention there is provided a plant seed oil produced by the recombinant camelina plant or cell of the sixth aspect of the present invention.

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According to a tenth aspect of the present invention there is provided a plant seed oil wherein DHA constitutes at least 1%, preferably at least 3%, more preferably at least 5%, still more preferably at least 7%, still more preferably at least 10%, still more preferably at least 13% or still more preferably at least 15% (mol %) of the total amount of fatty acid present in said oil. Said oil may be produced by a recombinant camelina plant or cell according to the sixth aspect of the present invention.

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Preferably, according to this aspect of the invention the  $\gamma$ -linolenic (GLA) constitutes less than 5%, more preferably less than 4.5%, still more preferably less than 4%, still more preferably less than 3.5%, still more preferably less than 3%, still more preferably less than 2.5%, still more preferably less than 2% (mol %) of the total fatty acid content of said oil.

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In one embodiment the DHA constitutes 5% to 20% (mol %) of the total fatty acid content of said oil.

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In another embodiment the DHA constitutes 5% to 20% (mol %) of the total fatty acid content of said oil.

- 5 In another embodiment the DHA constitutes 10% to 20% (mol %) of the total fatty acid content of said oil.

In another embodiment the DHA constitutes 10 to 15% (mol %) of the total fatty acid content of said oil.

10

In another embodiment the DHA constitutes 10 to 13.7% (mol %) of the total fatty acid content of said oil.

- 15 Preferably the combined percentage of DHA and EPA is at least 20% of the total fatty acid content of said oil.

In one embodiment the combined percentage of DHA and EPA is 20 to 30% of the total fatty acid content of said oil.

- 20 In one embodiment the combined percentage of DHA and EPA is 21 to 27% of the total fatty acid content of said oil.

In one embodiment the DHA constitutes 4% to 10%, preferably 4% to 8%, preferably 5% to 7.5% (mol %) of the total fatty acid content of said oil.

25

In one embodiment the GLA constitutes 0% to 4.5% (mol %) of the total fatty acid content of said oil.

- 30 In one embodiment the GLA constitutes 0.5% to 4.5% (mol %) of the total fatty acid content of said oil.

In another embodiment the GLA constitutes 1.0% to 4.5% (mol %) of the total fatty acid content of said oil.

In another embodiment the GLA constitutes 1.5% to 4.5% (mol %) of the total fatty acid content of said oil.

5 In another embodiment the GLA constitutes 0% to 3.5% (mol %) of the total fatty acid content of said oil.

In another embodiment the GLA constitutes 0.5% to 3.5% (mol %) of the total fatty acid content of said oil.

10 In another embodiment the GLA constitutes 1.0% to 3.5% (mol %) of the total fatty acid content of said oil.

In another embodiment the GLA constitutes 1.5% to 3.5% (mol %) of the total fatty acid content of said oil.

15 In one embodiment the GLA constitutes 1.5% to 3.2% (mol %) of the total fatty acid content of said oil.

20 The ratio of the molar percentages of EPA to DHA may be, for example, about 0.8:1 to about 1.4:1, preferably about 1:1 to about 1:1.3.

In another embodiment the ratio of the molar percentages of the sum of (EPA + DHA) to GLA is about 20:1 to about 3:1, 5:1, 7:1 or 10:1.

25 In another embodiment the ratio of the molar percentages of the sum of (EPA + DHA) to GLA is about 17:1 to about 3:1, 5:1, 7:1 or 10:1.

In another embodiment the ratio of the molar percentages of the sum of (EPA + DHA) to GLA is about 16.4:1 to about 3:1, 5:1, 7:1 or 10:1.

30 In another embodiment the ratio of the molar percentages of the sum of (EPA + DHA) to GLA is about 8:1 to about 3:1.

35 According to an eleventh aspect of the present invention there is provided use of camelina in the manufacture of an omega-3 fatty acid, preferably EPA or DHA.

According to a twelfth aspect of the present invention there is provided a camelina seed comprising a phosphatidylcholine wherein the total number of carbon atoms of the fatty acid acyl groups of said phosphatidylcholine is 40. Preferably the seed is a seed of the plant of the first aspect of the invention.

5

According to a thirteenth aspect of the present invention there is provided a camelina seed comprising phosphatidylcholine, wherein the total number of carbon atoms:double bonds of the fatty acid acyl groups of said phosphatidylcholine species is selected from the group consisting of: 34:4, 34:0, 36:7, 38:1 1, 38:9, 38:8, 38:7, 10 38:6, 38:5, 40:1 1, 40:10, 40:9, 40:8, 40:7, 40:6, 40:5 and 40:4. Preferably the seed comprises all the phosphatidylcholine species 34:4, 34:0, 36:7, 38:1 1, 38:9, 38:8, 38:7, 38:6, 38:5, 40:1 1, 40:10, 40:9, 40:8, 40:7, 40:6, 40:5 and 40:4. Preferably the seed is a seed of the plant of the first aspect of the invention.

15

According to a fourteenth aspect of the present invention there is provided a camelina seed comprising one or more phosphatidylethanolamine species wherein the total number of carbon atoms:double bonds of the fatty acid acyl groups of said phosphatidylethanolamine species is selected from the group consisting of 34:4, 36:7, 38:8, 38:7, 38:6, 38:5, 40:10, 40:9, 40:8, 40:7, 40:6, 40:5. Preferably the seed 20 comprises all of the phosphatidylethanolamine species 34:4, 36:7, 38:8, 38:7, 38:6, 38:5, 40:10, 40:9, 40:8, 40:7, 40:6, 40:5. Preferably the seed is a seed of the plant of the first aspect of the invention.

25

According to a fifteenth aspect of the present invention there is provided a camelina seed oil comprising the phosphatidylcholine species 34:4, 34:0, 36:7, 38:1 1, 38:9, 38:8, 38:7, 38:6, 38:5, 40:1 1, 40:10, 40:9, 40:8, 40:7, 40:6, 40:5 and 40:4. Preferably the seed is a seed of the plant of the sixth aspect of the invention.

30

According to a sixteenth aspect of the present invention there is provided a camelina seed wherein the seed comprises one or more phosphatidylethanolamine species wherein the total number of carbon atoms:double bonds of the fatty acid acyl groups of said phosphatidylethanolamine species is selected from the group consisting of 34:4, 36:7, 38:8, 38:7, 38:6, 38:5, 40:10, 40:9, 40:8, 40:7, 40:6, 40:5. Preferably the camelina oil comprises all of the phosphatidylethanolamine species 34:4, 36:7, 38:8,

38:7, 38:6, 38:5, 40:10, 40:9, 40:8, 40:7, 40:6, 40:5. Preferably the seed is a seed of the plant of the sixth aspect of the invention.

5 According to a seventeenth aspect of the present invention there is provided a camelina seed or oil wherein said seed or oil comprises triglycerides wherein the number of carbon atom double bonds of said triglycerides is 58:8, 58:9 and 58:10. The seed or oil may be derived from the transgenic camelina plant of the invention.

10 In addition to the specific elongase and desaturase enzymes referred to herein, the recombinant camelina plant or cell defined herein may further encode other enzymes involved in polyunsaturated fatty acid synthesis, in particular enzymes involved in omega-3 polyunsaturated fatty acid synthesis. Alternatively, the recombinant camelina plant may only be transformed with the fatty acid synthesis enzymes referred to herein.

15

The recombinant camelina plant defined herein may be in the form of a seed.

The desaturase and elongase enzymes used in the present invention may be derived from, for example, algae, bacteria, mould or yeast.

20

In one embodiment, the A6-desaturase used in the present invention is derived from *Ostreococcus*, preferably OtD6 from *Ostreococcus tauri* (Domergue et al. Biochem. J. 389 (PT 2), 483-490 (2005)). In one embodiment, the A6-desaturase is encoded by a polynucleotide sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or  
25 100% identity to SEQ ID NO:1. In another embodiment, the A6-desaturase comprises an amino acid sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:2.

In another embodiment, the A6-desaturase used in the present invention is O809D6  
30 from *Ostreococcus* RCC809. In one embodiment, the A6-desaturase is encoded by a polynucleotide sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:19. In another embodiment, the A6-desaturase comprises an amino acid sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:20.

35



In one embodiment, the  $\Delta\delta$ -elongase used in the present invention is derived from *Physcomitrella*, and is preferably from *Physcomitrella patens*. Preferably the  $\Delta 6$ -elongase is PSE1 derived from *Physcomitrella patens* (Zank et al., Plant J. 31 (3), 255-268 (2002); AB238914). In one embodiment, the A6-elongase is encoded by a polynucleotide sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:3. In another embodiment, the A6-elongase comprises an amino acid sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:4.

10

In another embodiment, the  $\Delta 6$ -elongase used in the present invention is FcElo6, a  $\Delta 6$  fatty acid elongase from *Fragilariopsis cylindrus* CCMP 1102. In one embodiment, the  $\Delta\delta$ -elongase is encoded by a polynucleotide sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:21. In another embodiment, the  $\Delta\delta$ -elongase comprises an amino acid sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:22.

15

In another embodiment, the A6-elongase used in the present invention is CeElo6, a  $\Delta 6$  fatty acid elongase from *Caenorhabditis elegans* (Beaudoin et al., 2000, Proc Natl Acad Sci U S A. 2000 Jun 6;97(12):6421-6). In one embodiment, the A6-elongase is encoded by a polynucleotide sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:23. In another embodiment, the  $\Delta 6$ -elongase comprises an amino acid sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:24.

20

In one embodiment, the A5-desaturase used in the present invention is derived from *Thraustochytrium* sp. Preferably the  $\Delta 5$ -desaturase is Tc $\Delta 5$  derived from *Thraustochytrium* sp. (Qiu et al. J Biol Chem. 2001 Aug 24;276(34):31561-6; AF489588). In one embodiment, the  $\Delta 5$ -desaturase is encoded by a polynucleotide sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:5. In another embodiment, the  $\Delta 5$ -desaturase comprises an amino acid sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:6.

25

30

In another embodiment, the EMod5  $\Delta 5$ -desaturase from *E. huxleyi* (Sequence ID 9, 10) can be used. In one embodiment, the  $\Delta 5$ -desaturase is encoded by a polynucleotide sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:9. In another embodiment, the  $\Delta 5$ -desaturase  
5 comprises an amino acid sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO: 10.

In one embodiment, the A5-elongase used in the present invention is derived from *Ostreococcus*, preferably *Ostreococcus tauri*. Preferably the A5-elongase is OtElo5  
10 derived from *Ostreococcus tauri* (WO 20050 123 16-A2; CS020 123). In one embodiment, the A5-elongase is encoded by a polynucleotide sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:7. In another embodiment, the  $\Delta 5$ -elongase comprises an amino acid sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:8.

15

In one embodiment, the A4-desaturase is derived from *Thraustochytrium sp* (ATCC21685). In one embodiment, the A4-desaturase is encoded by a polynucleotide sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:17. In another embodiment, the A4-desaturase  
20 comprises an amino acid sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO: 18.

In another embodiment the A4-desaturase is EhD4 derived from *Emiliana huxleyi* (WO 20091 331 45-A1; HC086723; et al. Phytochemistry. 201 1 May; 72(7).594-600).

25 In one embodiment, the A4-desaturase is encoded by a polynucleotide sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID 15. In another embodiment, the A4-desaturase comprises an amino acid sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO: 16.

30 In another embodiment the A4-desaturase is TpDesk, a A4-desaturase from *Thalassiosira pseudonana* (Tonon et al, 2005 FEBS J. 2005 Jul;272(13):3401-12). In one embodiment, the A4-desaturase is encoded by a polynucleotide sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID 25. In another embodiment, the A4-desaturase comprises an amino acid sequence that has  
35 at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:26.

In one embodiment, the A12-desaturase used in the present invention is derived from Phytophthora, and is preferably PsA12 from *Phytophthora sojae* (WO 2006100241 A2; CS423998). In one embodiment, the A12-desaturase is encoded by a  
5 polynucleotide sequence that has at least 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:11. In another embodiment, the A12-desaturase comprises an amino acid sequence that has at least 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:12.

10 In a one embodiment, the  $\omega$ 3-desaturase used in the present invention is derived from *phytophthora*, preferably *phytophthora infestans*. Preferably the  $\omega$ 3-desaturase is  $\pi(\omega$ 3) derived from *phytophthora infestans* (JP 2007527716; DJ418322). In one embodiment, the  $\omega$ 3-desaturase is encoded by a polynucleotide sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:13. In  
15 another embodiment, the  $\omega$ 3-desaturase comprises an amino acid sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO: 14.

In another embodiment, the  $\omega$ 3-desaturase used in the present invention is Hpw-3, a  $\omega$ 3 desaturase gene from *Hyaloperonospora parasitica*. In one embodiment, the  $\omega$ 3-  
20 desaturase is encoded by a polynucleotide sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:27. In another embodiment, the  $\omega$ 3-desaturase comprises an amino acid sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:28.

25 Preferably the camelina referred to herein is *Camelina satrva*.

In one embodiment, the plant seed oil described herein comprises triglycerides wherein the number of carbon atoms:double bonds of said triglycerides is 58:8, 58:9 and 58:10.

30

#### Detailed description

Various preferred features and embodiments of the present invention will now be  
35 described by way of non-limiting examples.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA and immunology, which are within the capabilities of a person of ordinary skill in the art. Such techniques are explained in the literature. See, for example, J. Sambrook, E. F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning: A Laboratory Manual*, Second Edition, Books 1-3, Cold Spring Harbor Laboratory Press; Ausubel, F. M. et al. (1995 and periodic supplements; *Current Protocols in Molecular Biology*, ch. 9, 13, and 16, John Wiley & Sons, New York, N.Y.); B. Roe, J. Crabtree, and A. Kahn, 1996, *DNA Isolation and Sequencing: Essential Techniques*, John Wiley & Sons; J. M. Polak and James O'D. McGee, 1990, *In Situ Hybridization: Principles and Practice*; Oxford University Press; M. J. Gait (Editor), 1984, *Oligonucleotide Synthesis: A Practical Approach*, Irl Press; D. M. J. Lilley and J. E. Dahlberg, 1992, *Methods of Enzymology: DNA Structure Part A: Synthesis and Physical Analysis of DNA Methods in Enzymology*, Academic Press; and E. M. Shevach and W. Strober, 1992 and periodic supplements, *Current Protocols in Immunology*, John Wiley & Sons, New York, NY. Each of these general texts is herein incorporated by reference.

## 20 Camelina

*Camelina* is a genus within the flowering plant family Brassicaceae. *Camelina* is an emerging biofuel crop, in particular *Camelina sativa*. It is also known by other names such as German sesame, false-flax, gold-of-pleasure, and Siberian oilseed. Renewed interest in *C. sativa* as a biofuel feedstock is due in part to its drought tolerance and minimal requirements for supplemental nitrogen and other agricultural inputs (Gehring et al. (2006) *Genome* 49(12): 1555-63; Gugel and Falk (2006) *Canadian Journal of Plant Science* 86(4): 1047-1058).

30 Similar to other non-traditional, renewable oilseed feedstocks such as *Jatropha curcas* L. ("jatropha"), *C. sativa* grows on marginal land. Unlike *jatropha*, which is a tropical and subtropical shrub, *C. sativa* is native to Europe and is naturalized in North America, where it grows well in the northern United States and southern Canada.

In addition to its drought tolerance and broad distribution, several other aspects of *C. sativa* biology make it well suited for development as an oilseed crop. First, *C. sativa* is a member of the family *Brassicaceae*, and thus is a relative of both the genetic model organism *Arabidopsis thaliana* and the common oilseed crop *Brassica napus* (also known as canola). Second, the oil content of *C. sativa* seeds is comparable to that of *B. napus*, ranging from 30 to 40% (w/w) (Budin et al. (1995). Journal of the American Oil Chemists' Society 72(3): 309-315; Gugel and Falk (2006) Canadian journal of plant science 86(4): 1047-1058). Finally, the properties of *C. sativa* biodiesel are already well described and both seed oil and biodiesel from *C. sativa* were used as fuel in engine trials with promising results (Bernardo et al. (2003) Industrial Crops and Products 17(3): 191-197; Frohlich and Rice (2005). Industrial Crops and Products 21(1): 25-31).

#### 15 Oils, lipids and fatty acids

Polyunsaturated fatty acids can be classified into two major families (depending on the position (n) of the first double bond nearest the methyl end of the fatty acid carbon chain. Thus, the omega-6 fatty acids have the first unsaturated double bond six carbon atoms from the omega (methyl) end of the molecule and additionally have a total of two or more double bonds, with each subsequent unsaturation occurring 3 additional carbon atoms toward the carboxyl end of the molecule. In contrast, the omega-3 fatty acids have the first unsaturated double bond three carbon atoms away from the omega end of the molecule and additionally have a total of three or more double bonds, with each subsequent unsaturation occurring 3 additional carbon atoms toward the carboxyl end of the molecule.

Table 1 summarizes the common names of omega-3 fatty acids and the abbreviations that will be used throughout the specification:

30

Table 1

Common Name	Abbreviation	Shorthand notation
oleic acid	OA	18:1 <sup>Δ9</sup>

Linoleic acid	LA	18:2 <sup>Δ9,12</sup>
γ-Linolenic	GLA	18:3 <sup>Δ6,9,12</sup>
di-homo γ-linolenic acid	DGLA	20:3 <sup>Δ8,11,14</sup>
Arachidonic acid	ARA	20:4 <sup>Δ5,8,11,14</sup>
α-linolenic acid	ALA	18:3 <sup>Δ9,12,15</sup>
stearidonic acid	SDA	18:4 <sup>Δ6,9,12,15</sup>
eicosatetraenoic acid	ETA	20:4 <sup>Δ8,11,14,17</sup>
eicosapentaenoic acid	EPA	20:5 <sup>Δ5,8,11,14,17</sup>
docosapentaenoic acid	DPA	22:5 <sup>Δ7,10,13,16,19</sup>
docosahexaenoic acid	DHA	22:6 <sup>Δ4,7,10,13,16,19</sup>

- 5 The fatty acids produced by the processes of the present invention can be isolated from the camelina in the form of an oil, a lipid or a free fatty acid. One embodiment of the invention is therefore oils, lipids or fatty acids or fractions thereof which have been produced by the methods of the invention, especially preferably oil, lipid or a fatty acid composition comprising EPA or DHA and being derived from the transgenic camelina.
- 10 The term "oil", or "lipid" is understood as meaning a fatty acid mixture comprising unsaturated, preferably esterified, fatty acid(s). The oil or lipid is preferably high in omega-3 polyunsaturated or, advantageously, esterified fatty acid(s). In a particularly preferred embodiment the oil or lipid has a high ALA, ETA, EPA, DPA and/or DHA content, preferably a high EPA and/or DHA content.
- 15 For the analysis, the fatty acid content of the seed can, for example, be determined by gas chromatography after converting the fatty acids into the methyl esters by transesterification of lipids such as triacylglycerides and/or phospholipids.
- 20 The omega-3 polyunsaturated acids produced in the method of the present invention, for example EPA and DHA, may be in the form of fatty acid derivatives, for example sphingolipids, phosphoglycerides, lipids, glycolipids, phospholipids, monoacylglycerol, diacylglycerol, triacylglycerol or other fatty acid esters.

5 The omega-3 and other polyunsaturated fatty acids which are present can be liberated for example via treatment with alkali, for example aqueous KOH or NaOH, or acid hydrolysis, advantageously in the presence of an alcohol such as methanol or ethanol, or via enzymatic cleavage, and isolated via, for example, phase separation and subsequent acidification via, for example, H<sub>2</sub>SO<sub>4</sub>. The fatty acids can also be liberated directly without the above-described processing step.

10 If further purification is necessary, standard methods can be employed. Such methods may include extraction, treatment with urea, fractional crystallization, HPLC, fractional distillation, silica gel chromatography, high-speed centrifugation or distillation, or combinations of these techniques. Protection of reactive groups, such as the acid or alkenyl groups, may be done at any step through known techniques (e.g., alkylation, iodination, use of butylated hydroxytoluene (BHT)). Methods used include methylation of the fatty acids to produce methyl esters. Similarly, protecting groups may be removed at any step. Desirably, purification of fractions containing, for example, ALA, STA, ETA, EPA, DPA and DHA may be accomplished by treatment with urea and/or fractional distillation.

20 The present invention encompasses the use of the oil, lipid, the fatty acids and/or the fatty acid composition in feedstuffs, foodstuffs, cosmetics or pharmaceuticals. The oils, lipids, fatty acids or fatty acid mixtures according to the invention can be used in the manner with which the skilled worker is familiar for mixing with other oils, lipids, fatty acids or fatty acid mixtures of animal origin, such as, for example, fish oils. Thus, the invention also provides feedstuffs, foodstuffs, cosmetics or pharmacologicals which comprise the oils, lipids, fatty acids or fatty acid mixtures of the present invention.

#### Total fatty acid content

30 The term "total fatty acids content" herein refers to the sum of all cellular fatty acids that can be derivitized to fatty acid methyl esters by the base transesterification method in a given sample (as known in the art, for example as described in Sayanova et al., (1997) Proc Natl Acad Sci U S A. 1997 Apr 15;94(8):421 1-6; Sayanova et al., (2003) FEBS Lett. 2003 May 8;542(1-3): 100-4).

35

Polyunsaturated fatty acid biosynthetic genes

Microorganisms, including algae, bacteria, moulds and yeasts, can synthesize polyunsaturated fatty acids and omega fatty acids in the ordinary course of cellular metabolism. Particularly well-studied are fungi including *Schizochytrium aggregatum*, species of the genus *Thraustochytrium* and *Mortierella aipina*. Additionally, many dinoflagellates (Dinophyceae) naturally produce high concentrations of polyunsaturated fatty acids. As such, a variety of genes involved in oil production have been identified through genetic means and the DNA sequences of some of these genes are publicly available. Non-limiting examples are shown below:

	Accession No.	Description
5		
10		
15	AY131238	Argania spinosa $\Delta 6$ desaturase
	Y055118	Echium pitardii var. pitardii $\Delta 6$ desaturase
	AY055117	Echium gentianoides $\Delta 6$ desaturase
	AF296076	Mucor rouxii, $\Delta 6$ desaturase
	AF007561	Borago officinalis $\Delta 6$ desaturase
20	L11421	Synechocystis sp. $\Delta 6$ desaturase
	NM_031344	Rattus norvegicus $\Delta 6$ fatty acid desaturase
	AF465283,	Mortierella aipina $\Delta 6$ fatty acid desaturase
	AF465282	Mortierella isabellina $\Delta 6$ fatty acid desaturase
	AF419296	Pythium irregulare $\Delta 6$ fatty acid desaturase
25	AB052086	Mucor circinelloides D6d mRNA for $\Delta 6$ fatty acid desaturase
	AJ250735	Ceratodon purpureus mRNA for $\Delta 6$ fatty acid desaturase
	AF126799	Homo sapiens $\Delta 6$ fatty acid desaturase
	AF126798	Mus musculus $\Delta 6$ fatty acid desaturase
	AF199596,	Homo sapiens $\Delta 5$ desaturase
30	AF320509	Rattus norvegicus liver $\Delta 5$ desaturase
	AB072976	Mus musculus D5D mRNA for $\Delta 5$ desaturase
	AF489588	Thraustochytrium sp. ATCC21685 $\Delta 5$ fatty acid desaturase
	AJ510244	Phytophthora megasperma mRNA for $\Delta 5$ fatty acid desaturase
	AF419297	Pythium irregulare $\Delta 5$ fatty acid desaturase
35	AF07879	Caenorhabditis elegans $\Delta 5$ fatty acid desaturase



	AF067654	Mortierella alpina $\Delta 5$ fatty acid desaturase
	AB022097	Dictyostelium discoideum mRNA for $\Delta 5$ fatty acid desaturase
	AF489589.1	Thraustochytrium sp. ATCC21685 $\Delta 4$ fatty acid desaturase
	AY332747	Pavlova lutheri $\Delta 4$ fatty acid desaturase (desl) mRNA
5	AAG36933	Emericella nidulans oleate $\Delta 12$ desaturase
	AF1 10509,	Mortierella alpina $\Delta 12$ fatty acid desaturase mRNA
	AAL13300	Mortierella alpina $\Delta 12$ fatty acid desaturase
	AF417244	Mortierella alpina ATCC 16266 $\Delta 12$ fatty acid desaturase
	AF161219	Mucor rouxii $\Delta 12$ desaturase mRNA
10	X86736 S	piruline platensis $\Delta 12$ desaturase
	AF240777	Caenorhabditis elegans $\Delta 12$ desaturase
	AB007640	Chlamydomonas reinhardtii $\Delta 12$ desaturase
	AB075526	Chlorella vulgaris $\Delta 12$ desaturase
	AP002063	Arabidopsis thaliana microsomal $\Delta 12$ desaturase
15	NP_441622,	Synechocystis sp. PCC 6803 $\Delta 15$ desaturase
	AAL36934	Perilla frutescens $\Delta 15$ desaturase

Additionally, the patent literature provides many additional DNA sequences of genes (and/or details concerning several of the genes above and their methods of isolation) involved in polyunsaturated fatty acid production. See, for example: U.S. Pat. No. 5,968,809 ( $\Delta 6$  desaturases); U.S. Pat. No. 5,972,664 and U.S. Pat. No. 6,075,183 ( $\Delta 5$  desaturases); WO 91/13972 and U.S. Pat. No. 5,057,419 ( $\Delta 9$  desaturases); WO 93/1 1245 ( $\Delta 15$  desaturases); WO 94/1 1516, U.S. Pat. No. 5,443,974 and WO 03/099216 ( $\Delta 12$  desaturases); U.S. 2003/0196217 A1 ( $\Delta 17$  desaturase); WO 02/090493 ( $\Delta 4$  desaturases); and WO 00/12720 and U.S. 2002/01 39974A1 (elongases).

The term "desaturase" refers to a polypeptide component of a multi-enzyme complex that can desaturate, i.e., introduce a double bond in one or more fatty acids to produce a mono- or polyunsaturated fatty acid or precursor of interest. Some desaturases have activity on two or more substrates. It may be desirable to empirically determine the specificity of a fatty acid desaturase by transforming a suitable host with the gene for the fatty acid desaturase and determining its effect on the fatty acid profile of the host.

In the context of the present invention a  $\omega$ 3 desaturase catalyzes the conversion of LA to ALA (WO 2008022963-A 30 28-FEB-2008; FB753570)

5 In the context of the present invention a  $\Delta$ 6 desaturases catalyzes the conversion of ALA to SDA and also LA to GLA. A6-Desaturases are described in WO 93/06712, US 5,614, 393, US 5614393, WO 96/21022, W00021557 and WO 99/271 11 and their application to production in transgenic organisms is also described, e. g. in WO 9846763, WO 9846764 and WO 9846765. In one embodiment, the A6-desaturase used in the present invention is derived from *Ostreococcus*, preferably OtD6 from  
10 *Ostreococcus tauri* (Domergue et al. Biochem. J. 389 (PT 2), 483-490 (2005); AY746357). In one embodiment, the A6-desaturase is encoded by a polynucleotide sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:1. In another embodiment, the  $\Delta$ 6-desaturase comprises an amino acid sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to  
15 SEQ ID NO:2.

In the context of the present invention a  $\Delta$ 5 desaturase catalyzes the conversion of ETA to EPA. In one embodiment, the  $\Delta$ 5-desaturase used in the present invention is derived from *Thraustochytrium* sp. Preferably the  $\Delta$ 5-desaturase is To $\Delta$ 5 derived  
20 from *Thraustochytrium* sp. (Qiu et al. J Biol Chem. 2001 Aug 24;276(34):31561-6; AF489588). In one embodiment, the  $\Delta$ 5-desaturase is encoded by a polynucleotide sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:5. In another embodiment, the  $\Delta$ 5-desaturase comprises an amino acid sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to  
25 SEQ ID NO:6.

In the context of the present invention a  $\Delta$ 12 desaturases catalyzes the conversion OA to LA. In one embodiment, the A12-desaturase used in the present invention is P $\epsilon$  $\Delta$  12 derived from Phytophthora, preferably *Phytophthora sojae* (WO 2006100241  
30 A2; CS423998). In one embodiment, the A12-desaturase is encoded by a polynucleotide sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:11. In another embodiment, the  $\Delta$ 12-desaturase comprises an amino acid sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO: 12.

In the context of the present invention a  $\Delta 4$  desaturase catalyzes the conversion of DPA to DHA. In one embodiment, the A4-desaturase is derived from *Thraustochytrium* sp (ATCC21685). In one embodiment, the A4-desaturase is encoded by a polynucleotide sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO: 17. In another embodiment, the  $\Delta 4$ -  
5 desaturase comprises an amino acid sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO: 18.

In another embodiment the A4-desaturase is EhD4 derived from *Emiliana huxleyi*  
10 (Sayanova et al. *Phytochemistry*. 2011 May; 72(7):594-600). In one embodiment, the A4-desaturase is encoded by a polynucleotide sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID 15. In another embodiment, the A4-desaturase comprises an amino acid sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO: 16.

15

The term "eiongase" refers to a polypeptide that can elongate a fatty acid carbon chain to produce an acid two carbons longer than the fatty acid substrate that the eiongase acts upon.

20 Examples of reactions catalyzed by eiongase systems are the conversion of GLA to DGLA, SDA to ETA, ARA to DTA and EPA to DPA. In general, the substrate selectivity of eiongases is somewhat broad but segregated by both chain length and the degree and type of unsaturation.

25 For example, a C14/16 eiongase will utilize a C14 substrate (e.g., myristic acid), a C16/18 eiongase will utilize a C16 substrate (e.g., palmitate), a C18/20 eiongase will utilize a C18 substrate (e.g., GLA, SDA, LA, ALA) and a C20/22 eiongase (also referred to as a  $\Delta 5$  eiongase) will utilize a C20 substrate (e.g., ARA, EPA).

30 Since some eiongases have broad specificity, a single enzyme may be capable of catalyzing several eiongase reactions (e.g., thereby acting as both a C16/18 eiongase and a C18/20 eiongase). It may be desirable to empirically determine the specificity of a fatty acid eiongase by transforming a suitable host with the gene for the fatty acid eiongase and determining its effect on the fatty acid profile of the host.

35

In the context of the present invention a  $\Delta 6$  elongase catalyzes the conversion of SDA to ETA. In one embodiment, the  $\Delta 6$ -elongase used in the present invention is derived from *Physcomitrella*, and is preferably from *Physcomitrella patens*. Preferably the  $\Delta 6$ -elongase is PSE1 derived from *Physcomitrella patens* (Zank, et al., Plant J. 31  
5 (3), 255-268 (2002); AB238914). In one embodiment, the  $\Delta 6$ -elongase is encoded by a polynucleotide sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:3. In another embodiment, the  $\Delta 6$ -elongase comprises an amino acid sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:4.

10

In the context of the present invention a  $\Delta 5$  elongase catalyzes the conversion of EPA to DPA. In one embodiment, the  $\Delta 5$ -elongase used in the present invention is derived from *Ostreococcus*, preferably *Ostreococcus tauri*. Preferably the  $\Delta 5$ -elongase is OtElo5 derived from *Ostreococcus tauri* (WO 200501 231 6-A2;  
15 CS020123). In one embodiment, the  $\Delta 5$ -elongase is encoded by a polynucleotide sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:7. In another embodiment, the  $\Delta 5$ -elongase comprises an amino acid sequence that has at least 50, 60, 70, 80, 85, 90, 95, 97, 99% or 100% identity to SEQ ID NO:8.

20

Although the particular source of a polyunsaturated fatty acid desaturase or elongase is not critical in the invention herein, it will be obvious to one of skill in the art that heterologous genes will be expressed with variable efficiencies in an alternate host. Furthermore, it may be desirable to modify the expression of particular  
25 polyunsaturated fatty acid biosynthetic pathway enzymes to achieve optimal conversion efficiency of each, according to the specific polyunsaturated fatty acid product composition of interest. A variety of genetic engineering techniques are available to optimize expression of a particular enzyme. Two such techniques include codon optimization and gene mutation, as described below. Genes produced by e.g.,  
30 either of these two methods, having desaturase and/or elongase activity(s) would be useful in the invention herein for synthesis of omega-3 polyunsaturated fatty acids.

Sequence Homology or Sequence identity

"Sequence Homology or Sequence identity" is used herein interchangeably. The terms "identical" or percent "identity" in the context of two or more nucleic acid or protein sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection. If two sequences which are to be compared with each other differ in length, sequence identity preferably relates to the percentage of the nucleotide residues of the shorter sequence which are identical with the nucleotide residues of the longer sequence.

5

10 Sequence identity can be determined conventionally with the use of computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive Madison, WI 5371 1). Bestfit utilizes the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2 (1981), 482-489, in order

15 to find the segment having the highest sequence identity between two sequences. When using Bestfit or another sequence alignment program to determine whether a particular sequence has for instance 95% identity with a reference sequence of the present invention, the parameters are preferably so adjusted that the percentage of identity is calculated over the entire length of the reference sequence and that

20 homology gaps of up to 5% of the total number of the nucleotides in the reference sequence are permitted. When using Bestfit, the so-called optional parameters are preferably left at their preset ("default") values. The deviations appearing in the comparison between a given sequence and the above-described sequences of the invention may be caused for instance by addition, deletion, substitution, insertion or

25 recombination. Such a sequence comparison can preferably also be carried out with the program "fasta20u66" (version 2.0u66, September 1998 by William R. Pearson and the University of Virginia; see also W.R. Pearson (1990), *Methods in Enzymology* 183, 63-98, appended examples and <http://workbench.sdsc.edu/>). For this purpose, the "default" parameter settings may be used.

30

Preferably, reference to a sequence which has a percent identity to any one of the SEQ ID NOs as detailed herein refers to a sequence which has the stated percent identity over the entire length of the SEQ ID NO referred to.

Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions.

#### Hybridization

5

Hybridization is the binding of complementary strands of DNA, DNA/RNA, or RNA.

10

Polynucleotides that hybridize to the polynucleotide sequences provided herein may also be used in the invention. Particularly preferred are polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization occurring only if there is at least 90%, 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1 x SSC at about 65°C.

15

The polynucleotides of the invention, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate other genes that have a high identity, particularly high sequence identity.

20

#### Codon-optimization

25

Codon degeneracy refers to the nature in the genetic code permitting variation of the nucleotide sequence without affecting the amino acid sequence of an encoded polypeptide. The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. As will be appreciated by one skilled in the art, it is frequently useful to modify a portion of the codons encoding a particular polypeptide that is to be expressed in a foreign host, such that the modified polypeptide uses codons that are preferred by the alternate host. Use of host-preferred codons can substantially enhance the expression of the foreign gene encoding the polypeptide.

30

In general, host-preferred codons can be determined within a particular host species of interest by examining codon usage in proteins (preferably those expressed in the largest amount) and determining which codons are used with highest frequency.

5 Then, the coding sequence for a polypeptide of interest having desaturase or elongase activity can be synthesized in whole or in part using the codons preferred in the host species. All (or portions) of the DNA also can be synthesized to remove any destabilizing sequences or regions of secondary structure that would be present in the transcribed mRNA. All (or portions) of the DNA also can be synthesized to alter  
10 the base composition to one more preferable in the desired host cell.

In the present invention, it may be desirable to modify a portion of the codons encoding the polypeptide having the relevant activity e.g., desaturase or elongase activity, to enhance the expression of the gene in camelina.

15

#### Gene Mutation

Methods for synthesizing sequences and bringing sequences together are well established in the literature. For example, *in vitro* mutagenesis and selection, site-  
20 directed mutagenesis, error prone PGR (Melnikov et al., Nucleic Acids Research, 27(4): 1056-1 062 (Feb. 15, 1999)), "gene shuffling" or other means can be employed to obtain mutations of naturally occurring desaturase or elongase genes. This would permit production of a polypeptide having desaturase or elongase activity, respectively, *in vivo* with more desirable physical and kinetic parameters for function  
25 in the host cell such as a longer half-life or a higher rate of production of a desired PUFA.

If desired, the regions of a polypeptide of interest (i.e., a desaturase or an elongase) important for enzymatic activity can be determined through routine mutagenesis,  
30 expression of the resulting mutant polypeptides and determination of their activities. Mutants may include deletions, insertions and point mutations, or combinations thereof. A typical functional analysis begins with deletion mutagenesis to determine the N- and C-terminal limits of the protein necessary for function, and then internal deletions, insertions or point mutants are made to further determine regions  
35 necessary for function. Other techniques such as cassette mutagenesis or total

synthesis also can be used. Deletion mutagenesis is accomplished, for example, by using exonucleases to sequentially remove the 5' or 3' coding regions. Kits are available for such techniques. After deletion, the coding region is completed by ligating oligonucleotides containing start or stop codons to the deleted coding region after the 5' or 3' deletion, respectively. Alternatively, oligonucleotides encoding start or stop codons are inserted into the coding region by a variety of methods including site-directed mutagenesis, mutagenic PCR or by ligation onto DNA digested at existing restriction sites. Internal deletions can similarly be made through a variety of methods including the use of existing restriction sites in the DNA, by use of mutagenic primers via site-directed mutagenesis or mutagenic PCR. Insertions are made through methods such as linker-scanning mutagenesis, site-directed mutagenesis or mutagenic PCR, while point mutations are made through techniques such as site-directed mutagenesis or mutagenic PCR.

#### 15 Transformation

The term "transgenic" or "recombinant" is preferably understood as meaning the expression of the nucleic acids encoding the enzymes involved in omega-3 fatty acid synthesis referred to herein at an unnatural locus in the genome, i.e. preferably, heterologous expression of the nucleic acids takes place. Thus, the genes introduced in to the camelina according to the present invention are preferably derived from a different organism.

The polynucleotides encoding the enzymes (e.g., desaturase and elongase enzymes) may be introduced into expression cassettes and/or vectors. In principal, the expression cassettes can be used directly for introduction into the camelina. However, preferably the nucleic acids are cloned into expression cassettes, which are then used for transforming camelina with the aid of vectors such as *Agrobacterium*.

30

After their introduction into the camelina plant cell or plant, the polynucleotides used in the present invention can either be present on a separate plasmid or, advantageously, integrated into the genome of the host cell.



As used in the present context, the term "vector" refers to a nucleic acid molecule which is capable of transporting another nucleic acid to which it is bound. One type of vector is a "plasmid", a circular double-stranded DNA loop into which additional DNA segments can be ligated. A further type of vector is a viral vector, it being possible for additional DNA segments to be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they have been introduced. Other vectors are advantageously integrated into the genome of a host cell when they are introduced into the host cell, and thus replicate together with the host genome. Moreover, certain vectors can govern the expression of genes with which they are in operable linkage. These vectors are referred to in the present context as "expression vectors". Usually, expression vectors which are suitable for DNA recombination techniques take the form of plasmids.

The recombinant expression vectors used in the present invention are suitable for expressing nucleic acids in a camelina host cell. The recombinant expression vectors/polynucleotides preferably comprise one or more regulatory sequences, which regulatory sequence(s) is/are operably linked with the nucleic acid sequence to be expressed.

The term "operably linked" refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

The term "regulatory sequence" is intended to comprise promoters, enhancers and other expression control elements such as polyadenylation signals. These regulatory sequences are described, for example, in Goeddel: Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990), or see: Gruber and Crosby, in: Methods in Plant Molecular Biology and Biotechnology, CRC Press, Boca Raton, Florida, Ed.: Glick and Thompson, Chapter 7, 89-108, including the references cited therein.

Examples of plant expression vectors comprise those which are described in detail in: Becker, D., Kemper, E., Schell, J., and Masterson, R. (1992) "New plant binary vectors with selectable markers located proximal to the left border", *Plant Mol. Biol.* 20:1 195-1 197; Bevan, M.W. (1984) "Binary *Agrobacterium* vectors for plant transformation", *Nucl. Acids Res.* 12:8711-8721; and *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.

The polynucleotide/vector preferably comprises regulatory sequences which are capable of governing the expression of genes in plant cells and which are linked operably so that each sequence can fulfill its function, such as transcriptional termination, for example polyadenylation signals. Examples of polyadenylation signals are those which are derived from *Agrobacterium tumefaciens* T-DNA, such as gene 3 of the Ti plasmid pTiACH5 (Gielen et al., *EMBO J.* 3 (1984) 835 et seq.), which is known as octopine synthase, or functional equivalents thereof, but other terminator sequences which are functionally active in plants are also suitable.

Since plant gene expression is very often not limited to the transcriptional level, a plant expression cassette or vector preferably comprises other sequences which are linked operably, such as translation enhancers.

Plant gene expression is preferably linked operably with a suitable promoter which triggers gene expression with the correct timing or in a cell- or tissue- specific manner. Examples of promoters are constitutive promoters (Benfey et al., *EMBO J.* 8 (1989) 2195-2202), such as those which are derived from plant viruses, such as 35S CaMV (Franck et al., *Cell* 21 (1980) 285-294), 19S CaMV (see also US 5352605 and WO 84/02913), or plant promoters, such as the promoter of the Rubisco subunit, which is described in US 4,962,028. Other sequences for use in operable linkage in plant gene expression cassettes are targeting sequences, which are required for steering the gene product into its corresponding cell compartment (see a review in Kermode, *Grit. Rev. Plant Sci.* 15, 4 (1996) 285-423 and references cited therein), for example into the vacuole, into the nucleus, all types of plastids, such as amyloplasts, chloroplasts, chromoplasts, the extracellular space, the mitochondria, the endoplasmid reticulum, elaioplasts, peroxisomes and other compartments of plant cells.

Plant gene expression can also be achieved via a chemically inducible promoter (see review in Gatz 1997, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 48:89-108). Chemically inducible promoters are particularly suitable when it is desired that the gene expression takes place in a time-specific manner. Examples of such promoters are a salicylic acid-inducible promoter (WO 95/19443), a tetracyclin-inducible promoter (Gatz et al. (1992) *Plant J.* 2, 397-404) and an ethanol-inducible promoter.

Promoters which respond to biotic or abiotic stress conditions are also suitable, for example the pathogen-induced PRP1 gene promoter (Ward et al., *Plant. Mol. Biol.* 22 (1993) 361-366), the heat-inducible tomato hsp80 promoter (US 5,187,267), the chill-inducible potato alpha-amylase promoter (WO 96/12814), the wound-inducible pinll promoter (EP-A-0 375 091) and the cis-jasmone-responsive promoter (Matthes MC, Bruce TJ, Ton J, Verrier PJ, Pickett JA, Napier JA. The transcriptome of cis-jasmone-induced resistance in *Arabidopsis thaliana* and its role in indirect defence. *Planta.* 2010 Oct;232(5): 1163-80).

Especially preferred are those promoters which bring about the gene expression in tissues and organs in which the biosynthesis of fatty acids, lipids and oils takes place, in seed cells, such as cells of the endosperm and of the developing embryo. Examples of such promoters are the oilseed rape napin promoter (US 5,608,152), the *Vicia faba* USP promoter (Baeumlein et al., *Mol Gen Genet.* 1991 , 225 (3):459-67), the *Arabidopsis oleosin* promoter (WO 98/45461), the *Phaseolus vulgaris* phaseolin promoter (US 5,504,200), the Brassica Bce4 promoter (WO 91/13980) or the legumine B4 promoter (LeB4; Baeumlein et al., 1992, *Plant Journal*, 2 (2):233-9). It is also envisaged that a mesocarp-specific promoter could direct the synthesis of the omega-3 trait in oil palm and similar crops.

Other promoters are those which bring about a plastid-specific expression, since plastids constitute the compartment in which the precursors and some end products of lipid biosynthesis are synthesized. Examples of 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.

To ensure the stable integration of the biosynthesis genes into the transgenic plant over a plurality of generations, it is usually necessary for each of the nucleic acids which encodes a protein of interest to be expressed under the control of a separate promoter, preferably a promoter which differs from the other promoters, since  
5 repeating sequence motifs can lead to instability of the T-DNA, or to recombination events. However, it is also possible to insert a plurality of nucleic acid sequences behind a promoter and, if appropriate, before a terminator sequence. Here, the insertion site, or the sequence, of the inserted nucleic acids in the expression cassette is not of critical importance, that is to say a nucleic acid sequence can be  
10 inserted at the first or last position in the cassette without its expression being substantially influenced thereby.

Preferably, each gene introduced into the camelina plant or cell is under the control of a specific promoter.

15

Vector DNA can be introduced into 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 methods known in the prior art for the introduction of foreign nucleic acid (for example DNA) into a host cell, including calcium phosphate or calcium  
20 chloride coprecipitation, DEAE-dextran-mediated transfection, lipofection, natural competence, 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  
25 ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) and other laboratory textbooks such as Methods in Molecular Biology, 1995, Vol. 44, Agrobacterium protocols, Ed.: Gartland and Davey, Humana Press, Totowa, New Jersey.

30 Transformation systems for camelina are known in the art. For example, US 2009/0151023 describe a method which involves:

- (a) Providing sterilized *Camelina sativa* seeds collected from a plants grown in controlled conditions;

- (b) Germinating the seeds on agar in sterilized conditions and growing in vitro seedlings;
- (c) Obtaining explants from the in vitro grown seedlings;
- (d) Inoculating the explants with *Agrobacterium tumefaciens* strain containing at least one recombinant DNA construct;
- (e) Cocultivating the explant with the *Agrobacterium* strain;
- (f) Transferring the explants to a callus forming medium, said medium being supplemented with hormones and containing 2% sucrose;
- (g) Transferring the explants to a shoot regeneration medium, said medium being supplemented with hormones and containing 2-6% sucrose;
- (h) Transferring the shoots to a root elongation medium, said medium being supplemented with hormones and containing 1-4%; and
- (i) Transferring the regenerated shoots into soil and growing them to transgenic *Cameiina sativa* plants.

15

The methods for transforming cameiina disclosed in US 2009/0151028 and US 2009/0151023 are incorporated herein by reference.

20

Transgenic plants which comprise the polyunsaturated fatty acids synthesized in the process according to the invention can advantageously be marketed directly without there being any need for the oils, lipids or fatty acids synthesized to be isolated.

25

Plants for the process according to the invention are listed as meaning intact plants and all plant parts, plant organs or plant parts such as leaf, stem, seeds, root, tubers, anthers, fibers, root hairs, stalks, embryos, calli, cotyledons, petioles, harvested material, plant tissue, reproductive tissue and cell cultures which are derived from the actual transgenic plant and/or can be used for bringing about the transgenic plant. In this context, the seed comprises all parts of the seed such as the seed coats, epidermal cells, seed cells, endosperm or embryonic tissue.

30

The compounds produced in the process according to the invention can also be isolated from the organisms, advantageously plants, in the form of their oils, fats, lipids and/or free fatty acids. This can be done via pressing or extraction of the cameiina plant parts, preferably the plant seeds. In this context, the oils, fats, lipids and/or free fatty acids can be obtained by what is known as cold-beating or cold-

35

pressing without applying heat. To allow for greater ease of disruption of the plant parts, specifically the seeds, they are previously comminuted, steamed or roasted. The seeds which have been pretreated in this manner can subsequently be pressed or extracted with solvents such as warm hexane. Thereafter, the resulting products are processed further, i.e. refined. In this process, substances such as the plant mucilages and suspended matter are first removed. What is known as desliming can be effected enzymatically or, for example, chemico-physically by addition of acid such as phosphoric acid. Thereafter, the free fatty acids are removed by treatment with a base, for example sodium hydroxide solution. The resulting product is washed thoroughly with water to remove the alkali remaining in the product and then dried. To remove the pigment remaining in the product, the products are subjected to bleaching, for example using filler's earth or active charcoal. At the end, the product is deodorized, for example using steam.

#### 15 Growing

In the case of plant (including plant tissue or plant organs) or plant cells, "growing" is understood as meaning, for example, the cultivation on or in a nutrient medium, or of the intact plant on or in a substrate, for example in a hydroponic culture, potting compost or on arable land.

Further preferred features and embodiments of the present invention will now be described by way of non-limiting example and with reference to the accompanying drawings in which:

25

Figure 1 is a schematic showing the biosynthesis pathway for long chain polyunsaturated fatty acids.

Figure 2 shows a schematic of the vector constructs used for *Camelina* transformation.

30

Figure 3 shows neutral loss survey of total seed triglycerols (TAG) from wild type and *Camelina* lines transformed with the five gene construct BC.

Figure 4 shows the ESI-MS analysis of wild type and Camelina lines transformed with the five gene construct BC.

5 Figure 5 shows acyl composition as determined by precursor ion scanning of phosphatidyl choline for the wild type and Camelina lines transformed with the five gene construct BC

Figure 6 shows the distribution of acyl chains within phosphatidylcholine of wild type and Camelina lines transformed with the five gene construct BC.

10

Figure 7 shows the distribution of acyl chains within phosphatidylethanolamine of wild type and Camelina lines transformed with the five gene construct BC.

15 Figure 8 shows the distribution of acyl chains within phosphatidic acid and phosphoinositol of wild type and Camelina lines transformed with the five gene construct BC.

20 Figure 9 shows the distribution of acyl chains within phosphatidylserine acid and phosphatidylglycerol of wild type and Camelina lines transformed with the five gene construct BC.

Figures 10 and 11 show the acyl-CoA pool of Arabidopsis and transgenic Camelina seeds harvested at mid-stage of seed development.

## 25 **Example 1 - Materials and Methods**

### *Fatty-acid analysis*

30 Fatty acids were extracted and methylated as described Sayanova et al., (1997) Proc Natl Acad Sci U S A. 1997 Apr 15;94(8):421 1-6 and Sayanova et al., (2003) FEBS Lett. 2003 May 8;542(1-3):100-4. Methyl ester derivatives of total fatty acids extracted were analysed by GC and GC-MS. Data presented as representative numbers derived from replicated analysis.

### *Acyl-CoA profiling*

Twenty-milligrams of developing (15 days after flowering) seed material were collected, frozen in liquid nitrogen and extracted after Larson and Graham 2001  
5 (Larson TR, Graham IA. (2001), Plant J. 2001 Jan;25(1):1 15-25), for reverse-phase LC with either quantitative analysis of fluorescent acyl-etheno-CoA derivatives or with  
electrospray ionization tandem mass spectrometry (multi reaction monitoring) in  
positive ion mode For the analysis of etheno-CoA derivatives HPLC (Agilent 1200 LC  
10 system; Phenomenex LUNA 150 - 2 mm C18(2) column) was performed using the methodology and gradient conditions described previously (Larson and Graham  
2001); whilst LC-MS/MS +MRM analysis followed the methods described by Haynes  
et al. 2008 (Agilent 1200 LC system ;Gemini C18 column, 2 mm inner diameter, 150  
mm with 5 mm particles). For the purpose of identification and calibration, standard  
15 acyl-CoA esters with acyl chain lengths from C14 to C20 were purchased from Sigma  
as free acids or lithium salts.

### *Lipid Profiling*

The molecular species of TAGs and PLs were analysed by electrospray ionisation  
20 triple quadrupole mass spectrometry (API 4000 QTRAP; Applied Biosystems). The  
molecular species of polar lipid were defined by the presence of a head-group  
fragment and the mass/charge of the intact lipid ion formed by ESI (Welti *et al.*, 2002,  
J Biol Chem. 2002 Aug 30;277(35):31 994-2002. Devaiah *et al.*, 2006,  
Phytochemistry. 2006 Sep;67(17):1907-24. with modifications described by Xiao et  
25 al. 2010; Plant Cell. 2010 May;22(5): 1463-82.). Such tandem ESI-MS/MS precursor  
and product ion scanning, based on head group fragment, do not determine the  
individual fatty acyl species. Instead, polar lipids are identified at the level of class,  
total acyl carbons, and total number of acyl carbon-carbon double bonds. Polar lipids  
were quantified in comparison with a series of polar lipid internal standards.  
30 Triacylglycerols (TAGs) measured after Krank et al. (2007, Methods Enzymol.  
2007;432:1-20) were defined by the presence of one acyl fragment and the  
mass/charge of the ion formed from the intact lipid (neutral loss profiling). This allows  
identification of one TAG acyl species and the total acyl carbons and total number of  
acyl double bonds in the other two chains. The procedure does not allow  
35 identification of the other two fatty acids individually nor the positions (sn-1 , sn-2, or



sn-3) that individual acyl chains occupy on the glycerol. TAGs were quantified in a manner similar to the polar lipids, including background subtraction, smoothing, integration, isotope deconvolution and comparison of sample peaks with those of the internal standard (using LipidView, Applied Biosystems). However, whereas polar lipids within a class exhibit similar mass spectral response factors, the mass spectral responses of various TAG species are variable, owing to differential ionization of individual molecular TAG species. In the data shown herein, no response corrections were applied to the data. The data were normalized to the internal standards tri15:0 and tri19:0.

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### **Example 2 - Production of EPA in transgenic Camelina**

We were interested in engineering the accumulation of bona fide omega-3 LC-PUFAs normally associated with fish oils such as eicosapentaenoic acid (EPA; 20:5  $\Delta^{5,8,11,14,17}$ ) and docosahexaenoic acid (DHA; 22:6  $\Delta^{4,7,10,13,16,19}$ ). To that end, a modular reconstruction of their biosynthetic pathway (Fig 1) was undertaken in transgenic Camelina. The heterologous biosynthetic activities were all placed under the regulatory control of common seed-specific promoters. In addition, given the variation in codon-usage observed between angiosperms and marine algae, a number of genes were resynthesised with codon-optimisation for expression in Cruciferae.

20

#### *Constructs design*

Four constructs containing from 3- to 7- gene cassettes were built using the Gateway® recombination system (Invitrogen). Respective genes were inserted as *NcoI/PacI* fragments into the promoter/terminator cassettes and then moved into pENTRY vectors (Fig 2). As shown, the simplest (MC) construct contained a three expression cassettes, comprising 1) a seed specific promoter (the sucrose binding protein SBP1800 promoter), *Oi* $\Delta$ 6, *Ostreococcus tauri*  $\Delta$ 6-desaturase gene (Domergue et al. Biochem. J. 389 (PT 2), 483-490 (2005); AY746357) and *CatpA*, terminator; 2) a seed specific promoter (USP1 promoter (Baumlein et al. 1991 Mol Gen Genet. 1991 Mar;225(3):459-67), PSE1, a  $\Delta$ 6 fatty acid elongase from *Physcomitrella patens* (Zank et al., Plant J. 31 (3), 255-268 (2002); AB238914) and CaMV35S terminator; 3) a seed specific promoter (Cnl, a conlininI promoter (Truksa 2003; Plant Physiol Biochem 41:141-147), TcA5, a  $\Delta$ 5-desaturase from

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*Thraustochytrium* sp. (Qiu et al. J Biol Chem. 2001 Aug 24;276(34):31561-6) and OCS, a terminator region of OCS, octoplin synthase gene of *A. tumefaciens*.

5 The BC construct contained five- gene cassettes including the same 3 gene cassettes as in the MC described above plus two additional gene cassettes consisting of PsA12, a  $\Delta$ 12-desaturase gene from *Phytophthora sojae* (see above) and P $\omega$ 3, a  $\omega$ 3 desaturase gene from *Phytophthora infestans* (Wu et al., 2005 Nat Biotechnol. 2005 Aug;23(8):1013-7) flanked by Np, a BnNapin promoter and E9 terminator regions.

10

To build DHA-1 construct we combined BC construct with additional two-gene cassettes, containing OtElo5, an *Ostreococcus tauri*  $\Delta$ 5 fatty acid elongase (Meyer et al., J Lipid Res. 2004 Oct;45(10): 1899-909) and EhA4, a A4-desaturase from *Emiliania huxleyi* (Sayanova et al. 2011 Phytochemistry. 2011 May;72(7):594-600) flanked by napin promoters and OCS terminators.

15

#### *Synthesis of EPA in transgenic Camelina*

In a first iteration, the simplest 3-gene construct (MC) was introduced into transgenic Camelina using standard floral infiltration technique to infect inflorescences with  
20 *Agrobacterium tumefaciens* strains carrying binary transformation vectors. Table 2 exemplifies the accumulation of non-native omega-3 long chain polyunsaturated fatty acids such as eicosapentaenoic acid (EPA). Total fatty acid composition of seeds from wild-type and transgenic plants of *C.sativa* lines expressing MC construct are shown below, confirming the presence of EPA in transgenics in the range 12.9-  
25 17.3% of total seed fatty acids. Note the complete absence of this fatty acid from the wildtype non-transgenic control.

30

Table 2

	16:0	18:0	18:1	LA	GLA	ALA	SDA	20:1	DHGLA	ARA	ETA	EPA	Others
Wt	7.0	3.1	15.1	21.2	0.0	29.6	0.0	14.1	0.0	0.0	0.0	0.0	9.9
Line2	9.3	5.0	4.7	24.7	1.8	12.2	1.8	7.8	0.6	2.4	2.0	16.8	10.9
Line3	9.3	4.9	6.4	25.6	2.1	13.4	2.0	8.3	0.7	2.0	1.8	12.9	10.6
Line	9.2	5.6	4.1	21.3	1.4	13.5	1.3	6.2	1.5	2.2	5.1	17.3	11.3

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In a second iteration of engineering *Camelina* with the capacity to accumulate high levels of EPA, we transformed *C. Sativa* with the 5-gene construct BC, again by floral infiltration. As shown in Table 3 below, the total fatty acid composition of T2 seeds from transgenic plants of *C.sativa* expressing BC construct contains very high levels of EPA (in the range 20.0 - 30.7%). Moreover, as shown in Table 4 below, it was also possible to obtain EPA at a level of 30.7% EPA. This fatty acid is totally absent from WT controls.

15 Table3

																EPA (MOL%)	
	16:0	18:0	18:1	18:2	GLA	ALA	SDA	20:1	DHGLA	ARA	20:3n3	ETA	EPA	Others	Sum	Average	SD
162a	9.8	7.6	4.6	18.3	2.4	10.8	1.5	6.5	0.5	2.0	1.6	2.4	25.0	7.0	100.0		
162b	8.6	7.1	5.0	18.7	3.6	11.4	2.3	7.4	0.5	1.8	1.4	2.2	22.8	7.1	100.0		
162c	11.3	7.0	4.9	20.4	4.0	9.4	2.1	5.3	0.6	1.9	1.3	2.0	24.0	5.8	100.0	23.9	1.1
26a	9.9	6.2	4.0	19.1	1.9	10.3	1.1	6.4	0.8	2.1	1.6	3.5	26.0	7.0	100.0		
26b	8.9	5.6	4.5	18.6	1.2	14.9	0.8	7.7	1.4	2.0	1.5	4.7	21.6	6.6	100.0		
26c	9.2	5.8	4.8	18.6	1.6	14.9	1.1	7.3	1.2	1.9	1.5	4.2	21.6	6.3	100.0	23.1	2.6
169a	10.3	5.4	4.8	19.8	1.0	15.0	0.7	6.4	1.3	1.8	1.6	4.6	21.3	6.0	100.0		
169b	11.3	6.7	4.0	18.0	1.2	14.4	0.8	5.8	1.1	1.6	1.6	4.5	22.4	6.5	100.0		
169c	11.3	6.5	4.7	19.9	2.2	13.5	1.3	6.5	1.3	1.9	1.4	4.1	18.9	6.5	100.0	20.9	1.8
158a	8.5	8.0	5.3	20.2	3.5	10.7	2.0	7.2	0.6	1.9	1.3	2.5	21.3	7.0	100.0		
158b	10.4	9.0	5.0	19.5	5.9	8.1	3.1	6.4	0.6	1.8	1.2	1.8	19.0	8.2	100.0		
158c	8.9	8.5	5.2	19.8	3.6	9.8	1.9	6.6	0.5	2.1	1.3	2.3	21.6	7.8	100.0	20.6	1.4
216a	7.6	8.1	5.3	21.5	2.1	11.2	1.1	8.1	0.6	1.9	1.5	2.5	19.8	8.5	100.0		
216b	7.8	7.7	5.3	21.2	2.4	10.9	1.2	7.9	0.6	2.0	1.6	2.4	20.7	8.5	100.0		
216c	7.4	7.8	5.2	21.3	1.8	11.2	1.0	8.1	0.6	2.0	1.6	2.6	20.8	8.7	100.0	20.4	0.5
29a	9.2	6.3	4.9	21.2	1.5	13.3	0.7	7.0	0.9	2.1	1.7	3.3	21.2	6.6	100.0		
29b	8.8	6.8	5.7	20.8	1.7	15.5	1.0	7.7	1.1	2.0	1.3	3.5	17.9	6.3	100.0		
29c	8.6	6.2	5.1	20.1	1.5	14.5	0.8	8.0	0.8	2.0	1.5	3.2	21.1	6.5	100.0	20.1	1.9
105a	8.6	8.9	5.3	20.3	2.3	10.1	1.2	7.4	0.5	2.1	1.6	2.0	21.0	8.5	100.0		
105b	8.9	9.8	5.4	20.3	3.2	9.8	1.7	7.2	0.5	1.9	1.4	1.8	19.2	8.9	100.0		
105c	9.2	10.1	5.5	20.8	2.2	8.8	1.0	7.0	0.6	2.3	1.5	1.9	19.8	9.3	100.0	20.0	1.0

Table 4

		MOL%																		
		16:0	18:0	18:1	18:2	GLA	ALA	SDA	20:1	DHGLA	ARA	20:3n3	ETA	EPA	Others	Sum				
T3 seeds	LineBBC_3_26 1seed/vial	26_10	10.4	6.3	4.0	14.2	2.1	8.2	1.6	6.5	0.3	1.4	2.1	2.9	30.7	9.2	100.0	NVR1	36	
T3 seeds	LineBBC_3_162 1seed/vial	162_13	11.3	7.3	4.1	19.4	2.6	7.6	1.2	6.2	0.3	1.6	1.5	2.1	26.1	8.6	100.0	NVR1	16	
T3 seeds	LineBBC_3_26 1seed/vial	26_15	9.9	7.7	4.2	17.1	3.0	9.3	1.4	6.8	0.3	1.6	1.6	2.8	25.8	8.4	100.0	NVR2	6	
T3 seeds	LineBBC_3_26 1seed/vial	26_19	9.3	6.1	4.2	15.8	1.3	13.8	1.0	7.7	0.5	1.4	1.9	3.9	24.9	8.0	100.0	NVR2	11	
T3 seeds	LineBBC_3_169 1seed/vial	169_16	10.3	7.2	4.2	20.7	1.9	7.8	0.9	6.0	0.5	1.7	1.8	2.9	24.9	9.3	100.0	NVR2	32	
T3 seeds	LineBBC_3_162 1seed/vial	162_20	8.9	7.0	5.0	16.6	2.1	12.2	1.3	8.1	0.4	1.7	1.6	2.7	24.6	7.9	100.0	NVR1	24	

5

#### Targeted lipidomic analysis of transgenic *Camelina* accumulating EPA.

To provide further and more detailed characterisation of the *Camelina* plants accumulating high levels of EPA in the seed oil, detailed analysis was carried out using tandem mass spectrometry as detailed below. As shown in Fig 3, neutral loss surveys of the total seed triacylglycerols (TAG) from either WT or two high EPA lines (162, 26 - cf Table 3) confirmed the presence of EPA in TAGs from lines 162 and 26 and confirmed the complete absence of this fatty acid in WT seed oil. To further define the composition of the TAGs present in the high EPA lines, ESI-MS was used to identify their molecular composition, compared with WT. As shown in Fig 4, several novel TAG species are clearly present in lines 162 and 26 which are not present in WT, notably 58:8, 58:9 and 58:10. Given that the predominant TAG species in WT

are 54:5-8, this upward shift represents the accumulation of longer chain fatty acids containing additional double bonds - i.e. EPA is accumulating at 1 (or possibly 2) positions on the glycerol backbone of TAG.

- As a corollary to the analysis of neutral lipids in these *Camelina* lines, we also analysed the acyl composition of phospholipids. Using precursor ion scanning, the acyl composition of phosphatidylcholine (PC, the major phospholipid present in plant seeds) was determined for WT and lines 162 and 26 (Fig 5). Again major differences were identified between the WT and the high EPA transgenics, including the identification of a series of C38 and C40 lipids were essentially absent from WT.
- 10 This alteration to the composition of phospholipids resulting from the transgenic synthesis and accumulation of EPA was further investigated by more detailed profiling of individual phospholipid classes (Figs 6-9). As seen in Fig 6, this analysis confirmed the presence of a suite of novel PC species, arising from the incorporation of EPA into this phospholipid. It is also clear that a number of endogenous PC
- 15 species are reduced as a consequence of this accumulation, most notably the reduction in C36 PC species containing 1-4 double bonds. A very similar profile was observed for phosphatidylethanolamine (PE) (Fig 8), which also showed the accumulation of novel C38 and C40 polyunsaturated species, with a concomitant reduction in the levels of C36 PE species. We profiled the other, more minor,
- 20 phospholipid species (phosphatidic acid [PA], phosphoinositol [PI], phosphatidylserine [PS] and phosphatidyl glycerol [PG]) and observed some more pronounced perturbations. For example, overall levels of all PA species were increased in the transgenic lines, albeit from a very low baseline (Fig 8). Conversely many C34 and C36 PI species were decreased in the high EPA transgenics, though these lines did
- 25 also contain some novel C38 PUFA-containing species (Fig 8). Interestingly, PS, which normally accumulates di + monounsaturated C20+ fatty acids was reduced in the transgenic lines, as were C34/36 PG species (Fig 9). No novel C38/40 PS species could be detected in our transgenic lines, whereas novel C38 PG PUFA-containing species were observed (Fig 9).
- 30 Acyl-CoA profiling was also used to define the composition of this key metabolic hub. As can be seen in Figs 10 & 11, the acyl-CoA pool of transgenic *Camelina* seeds harvested at mid-stage of seed development revealed the presence of significant levels of EPA-CoA.

### **Example 3 - Production of DHA in transgenic Camelina**

Having successfully engineered the significant accumulation of EPA in transgenic  
5 Camelina seeds, we next attempted to direct the synthesis of DHA. Since DHA is a  
metabolite of EPA (Fig 1), having sufficient levels of EPA are a prerequisite for such  
manipulations. Using the construct detailed in Fig 2, we generated transgenic  
Camelina plants engineered to accumulate both EPA and DHA.

10 Since genotyping of the T2 generation indicated that this material was not  
homozygous for the transgene, we decided to carry out half-seed analysis, in which a  
portion of the seed is subject to destructive FAMES analysis, but the residual portion  
containing the embryo is retained and can be used to regenerate a plant. As shown  
below in Table 5, the single (half) seed analysis indeed confirmed the presence of  
15 transgene nulls (samples 9-11) as would be expected from a non-homozygous  
population. However, FAMES analysis of total seed lipids did indeed confirm the  
presence of EPA and DHA, the later up to levels greater than 13% of total fatty acids.  
The best line showing combined levels of EPA and DHA (C20+ omega-3 LC-PUFAs)  
was at 26.3 % of total seed fatty acids. Importantly, this line contained only very low  
20 levels of the omega-6 fatty acids ARA, GLA and DHGLA and the omega-3  
biosynthetic intermediates SDA, ETA and DPA. Thus this novel Camelina oil  
represents a new and valuable terrestrial source of C20+ omega-3 LC-PUFAs  
normally found in aquatic environments.

25 Table 5. Total fatty acid composition of T2 seeds from transgenic plants of C.sativa  
best lines expressing DHA-1 construct. (Half seed analysis)

**Half seed analysis on T2 seeds**

MOL%	16:0	18:0	18:1	18:2	GLA	ALA	SDA	20:0	20:1	20:2	DHGLA	ARA	20:3n3	ETA	EPA	22:0	22:1	DPA	DHA	Others			
BBC OE3	1	15.0	7.5	7.2	23.4	1.6	6.4	0.9	3.5	6.7	1.4	1.3	1.5	0.9	1.9	5.3	0.5	1.0	1.3	4.4	8.4	NVX1	7
	2	12.1	8.0	6.4	20.7	3.2	11.2	2.4	3.6	6.0	1.1	1.0	1.5	0.8	2.6	5.6	0.6	0.7	1.7	4.2	6.8	NVX1	56
	3	15.7	7.5	5.4	18.5	2.5	10.4	2.0	4.0	6.7	1.3	1.0	1.3	0.9	2.6	5.2	0.6	0.8	1.4	4.1	8.1	NVX1	9
	4	14.8	4.9	7.5	18.8	1.6	13.2	1.4	1.8	7.4	1.5	1.0	1.2	1.0	2.7	4.9	0.4	0.9	1.5	4.7	8.6	NVX1	10
	5	11.1	4.8	6.5	23.3	1.9	14.6	1.3	2.5	8.5	1.5	1.4	1.1	1.1	3.3	5.2	0.4	0.9	1.3	4.3	4.6	NVX1	57
	6	11.5	4.4	8.6	23.1	2.3	13.4	1.7	2.1	8.5	1.4	1.7	1.5	0.8	3.0	4.8	0.3	0.8	1.1	3.8	5.3	NVX1	13
	7	11.3	5.0	6.8	23.4	2.1	13.7	1.5	2.5	8.2	1.3	1.3	1.5	1.0	2.8	5.8	0.4	1.0	1.3	5.0	4.2	NVX1	14
	8	13.3	4.8	5.7	19.4	2.1	12.3	1.9	1.9	7.6	1.5	0.9	1.5	1.2	2.3	7.2	0.4	1.0	1.5	7.3	6.5	NVX1	15
	9	9.8	3.8	9.3	23.5	0.2	27.6	0.2	2.7	11.6	2.4	0.1	0.0	1.1	0.3	0.4	0.5	3.4	0.0	0.3	3.1	NVX1	58
	10	12.6	4.9	9.7	28.0	0.0	21.4	0.0	2.9	9.2	2.3	0.0	0.0	0.6	0.0	0.0	0.5	2.5	0.0	0.0	5.5	NVX1	17
	11	11.9	3.9	8.6	23.4	0.0	26.9	0.0	2.8	10.6	2.4	0.0	0.0	1.0	0.0	0.0	0.5	3.5	0.0	0.0	4.4	NVX1	19
	12	15.1	4.8	6.7	21.6	1.7	13.4	1.3	2.0	7.7	1.3	1.3	1.4	1.0	2.7	5.1	0.4	0.9	1.3	4.5	5.7	NVX1	20
	13	13.1	5.4	6.9	24.8	2.1	11.0	1.2	2.7	8.1	1.2	1.4	1.4	0.9	2.5	5.6	0.4	1.0	1.1	4.4	4.8	NVX1	21
	14	12.0	4.9	5.5	17.2	3.2	13.8	3.2	2.2	7.4	1.0	0.7	1.4	1.2	2.3	8.8	0.4	0.7	2.2	7.5	4.6	NVX1	22
	15	10.7	8.0	6.1	21.5	1.9	15.3	1.9	4.0	7.6	1.1	0.8	1.2	1.0	2.5	4.8	0.6	1.0	1.8	5.2	3.0	NVX1	59
	16	12.1	5.7	6.4	18.1	2.3	15.3	2.2	2.7	6.9	1.0	0.7	1.5	1.2	2.6	6.9	0.5	0.7	2.2	7.2	3.9	NVX1	25
	17	10.8	5.4	7.5	22.5	1.7	16.4	1.4	3.2	7.8	1.3	1.2	1.4	1.1	3.0	5.2	0.4	0.7	1.6	4.4	2.8	NVX1	26
	18	14.0	5.0	6.5	23.2	1.8	9.4	1.2	2.5	7.3	1.3	1.2	1.7	1.1	2.2	7.0	0.5	1.1	1.4	6.1	5.4	NVX1	27
	19	12.6	4.7	6.5	21.6	1.8	14.4	1.5	2.0	7.5	1.3	1.0	1.4	1.1	2.6	5.5	0.4	1.0	1.6	5.7	5.6	NVX1	28
	20	15.2	6.0	6.8	23.8	1.5	7.8	0.8	3.2	7.3	1.3	1.1	1.5	1.0	2.4	5.8	0.5	1.2	1.6	5.3	5.9	NVX1	29

Line	16:0	18:0	18:1	18:2	GLA	ALA	SDA	20:0	20:1	20:2	DHGLA	ARA	20:3n3	ETA	EPA	22:0	22:1	DPA	DHA	Others
OE_33_2	15.9	5.2	5.8	16.6	1.6	7.4	1.4	0.8	2.7	1.0	0.4	1.2	1.5	2.7	12.6	0.0	0.0	5.0	13.7	4.6
OE_33_24	13.2	4.2	5.3	15.7	2.6	9.2	2.0	1.1	4.1	1.0	0.6	2.1	1.7	3.2	13.0	0.2	0.6	3.8	12.7	3.7
OE_33_66	14.0	4.1	6.0	15.3	3.5	9.4	2.9	0.9	3.5	0.9	0.5	1.7	1.4	2.3	12.9	0.0	0.7	3.5	12.6	3.8
OE_33_11	15.4	5.2	6.2	13.2	4.4	7.5	3.0	1.2	4.2	0.7	0.2	1.6	1.0	1.7	13.7	0.3	0.5	3.9	11.7	4.3
OE_33_5	14.5	5.0	5.8	15.4	3.1	10.1	2.3	1.1	3.7	0.9	0.5	1.8	1.1	2.8	12.5	0.3	0.4	3.6	11.5	3.8
OE_33_86	13.3	4.2	6.0	17.6	3.4	10.4	2.7	1.1	4.0	1.1	0.5	1.9	1.4	2.2	12.5	0.0	0.6	3.0	10.7	3.5
OE_33_91	11.8	3.9	5.3	16.6	2.4	12.9	2.3	1.0	4.1	1.3	0.6	2.4	1.4	3.1	13.0	0.0	0.5	3.6	10.6	3.3
OE_33_27	12.6	4.5	5.9	17.1	2.5	12.0	2.2	1.2	4.3	0.0	0.8	2.3	1.3	3.1	12.1	0.2	0.4	3.5	10.3	3.7
OE_33_97	11.9	4.0	6.4	17.6	3.1	10.7	2.4	1.1	4.2	1.1	0.6	2.4	1.3	2.9	12.9	0.0	0.5	3.0	10.3	3.5
OE_33_13	13.3	4.9	5.7	16.9	2.3	11.2	1.9	1.2	4.1	1.2	0.8	2.1	1.2	3.2	11.5	0.3	0.4	4.0	10.2	3.8
OE_33_3	13.8	4.5	6.0	18.0	2.2	11.9	2.0	1.0	4.0	1.3	0.8	2.2	1.3	3.0	11.1	0.3	0.4	3.5	10.0	2.8
OE_33_90	11.4	4.0	5.4	16.9	2.5	13.2	2.6	1.2	4.5	1.3	0.7	2.2	1.3	3.2	12.8	0.0	0.4	3.5	10.0	3.2
OE_33_31	10.6	4.2	5.6	16.3	2.7	13.3	2.3	1.2	4.4	1.2	0.7	2.3	1.4	3.3	13.0	0.2	0.4	3.3	9.8	3.7
OE_33_4	15.7	4.4	5.0	16.7	1.9	10.5	1.9	1.1	3.9	1.5	1.0	2.8	1.4	3.0	11.4	0.0	0.4	5.1	9.8	2.7
OE_33_92	10.8	4.2	5.3	16.4	3.1	14.0	2.6	1.1	4.0	1.0	0.7	2.3	1.1	3.2	13.6	0.0	0.3	2.9	9.7	3.8
OE_33_15	12.1	4.8	5.8	16.3	2.4	13.1	2.1	1.3	4.9	1.0	0.7	2.0	1.2	3.0	12.0	0.3	0.5	3.3	9.7	3.6
OE_33_34	10.9	4.1	5.9	18.2	2.8	12.5	2.5	1.1	4.2	1.2	0.8	2.6	1.3	3.2	12.5	0.2	0.4	3.1	9.6	3.0
OE_33_19	10.2	4.5	6.3	13.4	4.0	12.8	3.3	1.4	5.5	0.8	0.2	1.8	1.3	1.9	14.3	0.3	0.6	2.7	9.6	5.1
OE_33_74	11.8	4.0	6.0	19.9	3.2	11.1	2.3	1.1	4.2	1.2	0.6	2.4	1.3	2.7	12.2	0.0	0.5	2.7	9.3	3.4
OE_33_44	11.7	4.5	6.0	17.1	2.3	12.4	2.1	1.3	4.5	1.4	0.7	2.2	1.5	3.2	12.0	0.3	0.5	3.1	9.3	4.0
OE_33_63	10.7	4.4	5.9	17.1	3.2	12.0	2.4	1.2	4.4	1.2	0.8	2.6	1.2	3.1	13.0	0.2	0.4	2.9	9.2	4.0
OE_33_23	12.2	4.3	6.2	19.0	2.5	12.6	1.9	1.1	4.4	0.0	0.9	2.5	1.3	3.2	11.2	0.2	0.4	3.0	9.2	3.9
OE_33_64	11.1	4.4	6.2	18.5	2.8	10.9	2.0	1.2	4.4	1.3	0.8	2.5	1.3	3.2	12.5	0.3	0.5	3.1	9.2	3.9
OE_33_77	10.9	4.2	6.5	16.5	4.3	11.6	3.3	1.1	4.6	0.9	0.4	2.4	1.1	2.0	13.3	0.2	0.5	2.7	9.2	4.4
OE_33_7	15.1	5.0	5.8	16.4	2.3	11.7	1.9	1.2	4.5	1.2	0.6	1.8	1.3	2.9	11.5	0.3	0.4	3.5	9.1	3.5
OE_33_55	10.3	4.8	5.9	16.6	2.8	12.7	2.4	1.5	5.4	1.1	0.6	2.3	1.3	2.9	12.7	0.3	0.6	3.0	9.0	4.0
OE_33_59	11.1	3.9	5.7	17.2	2.9	14.5	2.6	1.2	5.1	1.1	0.6	1.9	1.5	3.1	11.2	0.2	0.7	3.2	9.0	3.4
OE_33_95	10.9	4.6	6.2	18.2	2.8	11.9	2.1	1.3	4.4	1.2	0.7	2.6	1.2	2.9	12.5	0.3	0.5	2.7	9.0	4.0

5 To further examine the feasibility of producing EPA and DHA in transgenic Camelina seeds, we evaluated additional activities for this capacity - 4 examples are shown below.

Example 4 - EPA-B4.3

To the original MC construct (Fig 2; comprising 1) a seed specific promoter (the sucrose binding protein SBP1800 promoter), OtA6, *Ostreococcus tauri*  $\Delta 6$ -desaturase gene (Domergue et al. *Biochem. J.* 389 (PT 2), 483-490 (2005); AY746357) and CatpA, terminator; 2) a seed specific promoter (USP1 promoter (Baumlein et al. 1991 *Mol Gen Genet.* 1991 Mar;225(3):459-67), PSE1, a  $\Delta 6$  fatty acid elongase from *Physcomitrella patens* (Zank.et al., *Plant J.* 31 (3), 255-268 (2002); AB238914) and CaMV35S terminator; 3) a seed specific promoter (Cnl, a conlininl promoter (Truksa 2003; *Plant Physiol Biochem* 41:141-147), TcA5, a  $\Delta 5$ -desaturase from *Thraustochytrium* sp. (Qiu et al. *J Biol Chem.* 2001 Aug 24;276(34):31561-6) and OCS, a terminator region of OCS, octopin synthase gene of *A. tumefaciens*) we added Hpw-3, a  $\omega 3$  desaturase gene from *Hyaloperonospora parasitica* behind the Cnl promoter and in front of OCS, a terminator region of OCS, octopin synthase gene of *A. tumefaciens*

#### 15 Example s - EPA-B5.1

We varied the genes present in the original BC construct (Fig 2), such that the PsA12, a A12-desaturase gene from *Phytophthora sojae* and Pi $\omega 3$ , a  $\omega 3$  desaturase gene from *Phytophthora infestans* flanked by Np, a BnNapin promoter and E9 terminator regions were retained, but the activities were replaced with: 1) O809d6, a D6-desaturase from *Ostreococcus* RCC809, flanked by the Cnl conlininl seed-specific promoter and OCS, a terminator region of OCS, octopin synthase gene of *A. tumefaciens* 2) FcElo6, a  $\Delta 6$  fatty acid elongase from *Fragilariopsis cylindrus* CCMP 1102, flanked by the Cnl conlininl seed-specific promoter and OCS, a terminator region of OCS, octopin synthase gene of *A. tumefaciens* and 3) EmiD5, a  $\Delta 5$ -desaturase from *Emiliana huxleyi* (Sayanova et al., 2011, *Phytochemistry* 72: 594-600) flanked by the Cnl conlininl seed-specific promoter and OCS, a terminator region of OCS, octopin synthase gene of *A. tumefaciens*

#### 30 Example 6 - EPA-B5.2

We varied the genes present in the original BC construct (Fig 2), such that the **PSA12**, a A12-desaturase gene from *Phytophthora sojae* and Pi $\omega 3$ , a  $\omega 3$  desaturase gene from *Phytophthora infestans* flanked by Np, a BnNapin promoter and E9 terminator regions were retained, but the activities were replaced with: 1) O809d6, a



D6-desaturase from *Ostreococcus* RCC809, flanked by the Cnl conlinin1 seed-specific promoter and OCS, a terminator region of OCS, octopin synthase gene of *A. tumefaciens* 2) CeElo6, a  $\Delta 6$  fatty acid elongase from *Caenorhabditis elegans* (Beaudoin et al., 2000, *Proc Natl Acad Sci U S A.* 2000 Jun 6;97(12):6421-6) flanked  
5 by the Cnl conlinin1 seed-specific promoter and OCS, a terminator region of OCS, octopin synthase gene of *A. tumefaciens* 3) EmiD5, a A5-desaturase from *Emiliana huxleyi* (Sayanova et al., 2011, *Phytochemistry* 72: 594-600) flanked by the Cnl conlinin1 seed-specific promoter and OCS, a terminator region of OCS, octopin synthase gene of *A. tumefaciens*

10

#### Example 4 - DHA-B7.2

To the original DHA-1 construct (Fig 2), the EhD4 D4-desaturase from *Emiliana huxleyi* (Sayanova et al, 2011) was replaced by TpDesk, a D4-desaturase from  
15 *Thalassiosira pseudonana* (Tonon et al, 2005 *FEBS J.* 2005 Jul;272(13):3401-12), under the same regulatory elements (Cni1, OCS).

Half-seeds of primary T1 transgenic lines were analysed by GC-FID as described earlier (Example 1 - Fatty acid analysis) and examples of the fatty acid profiles  
20 observed are shown in Table 6 below. These data indicate that the capacity of *Camelina* to produce EPA and DHA is not limited to the gene sets initially described.

25

30

Table 6

line	I6	I6:1b	I6:1d	I6:2	I6:3	18:0	18:1a	18:1b	b18:2	18:2	GLA	bALA	ALA	SDA	20:0	20:1	20:1a	20:1c	20:2	DHGLA	ARA	20:3n3	20:4n3	EPA	22:0	22:1	22:2	DPA	DHA	24:0
DHA-B7.2_1	5	2	1	0	0	4	6	1	1	15	5	0	19	5	3	11	0	2	1	2	0	2	5	1	1	3	4	1	0	1
DHA-B7.2_2	5	2	4	1	2	7	3	0	0	15	3	0	13	4	5	12	0	3	1	2	0	1	3	3	0	4	5	2	2	1
DHA-B7.2_3	5	2	3	0	1	5	3	0	1	12	6	0	17	9	4	15	0	2	1	1	0	2	2	0	0	5	4	0	0	2
DHA-B7.2_4	5	2	1	0	0	6	5	0	1	15	2	0	19	2	4	10	0	2	2	1	1	2	3	6	0	3	3	9	6	0
WILDTYPE	5	4	2	0	1	4	5	0	0	14	1	0	27	0	4	17	0	0	5	0	0	2	0	0	0	7	3	0	0	2
EPA-B4.3_1	5	2	1	0	0	4	15	0	1	8	0	0	21	1	3	13	0	1	2	1	2	2	2	11	0	3	2	1	0	1
EPA-B4.3_2	4	0	0	0	0	3	14	0	0	14	0	0	35	0	2	19	0	0	2	0	0	2	0	0	0	0	4	0	0	1
EPA-B4.3_3	5	1	1	0	0	2	10	0	0	14	0	0	38	0	2	17	0	0	2	0	0	2	0	0	0	5	0	0	0	2
EPA-B5.1_1	6	1	0	0	0	4	9	0	0	20	2	0	30	5	3	14	0	0	1	0	0	1	0	1	0	3	0	0	0	0
EPA-B5.1_2	7	1	0	0	0	5	8	1	0	25	3	0	22	5	4	12	0	0	1	0	0	1	0	2	1	2	0	0	0	0
EPA-B5.1_3	6	1	0	0	0	5	10	1	0	24	2	0	27	4	3	12	0	0	1	0	0	1	0	1	1	1	0	0	0	0
EPA-B5.1_4	6	1	0	0	0	4	12	1	0	27	3	0	18	5	3	14	0	0	1	0	0	1	1	2	0	2	0	0	0	0
EPA-B5.1_5	5	1	0	0	0	3	12	1	0	23	2	0	25	5	3	14	0	0	1	0	0	1	0	1	0	2	0	0	0	0
EPA-B5.1_6	6	1	0	1	0	3	11	2	1	26	4	0	18	5	2	11	0	0	1	0	1	1	1	4	0	2	0	0	0	0
EPA-B5.1_7	7	1	0	1	0	4	7	1	0	24	0	0	29	1	4	15	0	0	2	0	0	2	0	0	1	3	0	0	0	0
EPA-B5.1_8	7	1	0	1	0	6	8	2	0	32	4	0	17	4	3	10	0	0	0	0	0	1	0	3	0	1	0	0	0	0
EPA-B5.2_1	7	2	0	0	0	5	7	1	0	27	2	0	16	6	0	4	11	0	1	1	1	1	1	7	0	2	0	0	0	1
EPA-B5.2_2	6	1	0	0	0	4	13	0	0	22	1	0	27	4	0	3	14	0	1	0	0	1	0	1	0	2	0	0	0	1
EPA-B5.2_3	7	2	0	0	0	4	6	0	0	22	2	0	22	6	0	5	15	0	1	0	0	1	0	4	1	3	0	0	0	1
EPA-B5.2_4	7	2	0	0	0	5	8	0	0	24	1	0	27	4	0	3	11	0	1	0	0	1	1	3	0	1	0	0	0	1
EPA-B5.2_5	5	1	0	0	0	3	15	0	0	24	1	0	24	4	0	2	14	0	1	0	0	1	1	3	0	2	0	0	0	0
EPA-B5.2_6	6	1	0	0	0	3	12	0	0	24	0	0	29	3	0	2	13	0	1	0	0	1	1	1	0	1	0	0	0	1
EPA-B5.2_7	7	2	0	0	0	5	6	0	0	19	2	0	22	4	0	6	16	0	2	0	1	1	1	2	2	4	0	0	0	1
EPA-B5.2_8	7	2	0	0	0	4	7	0	0	23	1	0	28	4	0	4	12	0	1	0	0	1	1	2	1	3	0	0	0	1

5

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the present invention. Although the present invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in biochemistry and biotechnology or related fields are intended to be within the scope of the following claims.

15

## SEQUENCES

SEQ ID NO: 1 (Codon-optimised OtD6  $\Delta$ 6-desaturase from *Ostreococcus tauri*)

5           1   ATGTGTGTTGAGACCGAGAACAACGATGGAATCCCTACTGTGGAGATCGCTTTCGATGGA  
           61   GAGAGAGAAAGAGCTGAGGCTAACGTGAAAGTTGTCTGCTGAGAAGATGGAACCTGCTGCT  
          121   TTGGCTAAGACCTTCGCTAGAAGATACGTGGTTATCGAGGGAGTTGAGTACGATGTGACC  
          181   GATTTCAAACATCCTGGAGGAACCGTGATTTTCTACGCTCTCTCTAACACTGGAGCTGAT  
          241   GCTACTGAGGCTTCAAGGAGTTCACCACAGATCTAGAAAGGCTAGGAAGGCTTTGGCT  
 10       301   GCTTTGCCTTCTAGACCTGCTAAGACCGCTAAAAGTGGATGATGCTGAGATGCTCCAGGAT  
          361   TTCGCTAAGTGGAGAAAGGAGTTGGAGAGGGACGGATTCTTCAAGCCTTCTCCTGCTCAT  
          421   GTTGCTTACAGATTGCTGAGTTGGCTGCTATGTACGCTTTGGGAACCTACTTGATGTAC  
          481   GCTAGATACGTTGTGTCCTCTGTGTTGGTTTACGCTTGCTTCTTCGGAGCTAGATGTGGA  
          541   TGGGTCAACATGAGGGAGGACATTCCTTCTTGACCGGAAACATCTGGTGGGATAAGAGA  
 15       601   ATCCAAGCTTTCACTGCTGGATTTCGGATTGGCTGGATCTGGAGATATGTGGAACCTCCATG  
          661   CACAAACAGCACCATGCTACTCCTCAAAAAGTGGGCACGATATGGATTTGGATAACCACT  
          721   CCTGCTGTTGCTTTCTTCAACACCGCTGTGGAGGATAATAGACCTAGGGGATTCTCTAAG  
          781   TACTGGCTCAGATTGCAAGCTTGGACCTTCATTCCTGTGACTTCTGGATTGGTGTGCTC  
          841   TTCTGGATGTTCTTCCATCCTTCTAAGGCTTTGAAGGGAGGAAAGTACGAGGAGCTT  
 20       901   GTGTGGATGTTGGCTGCTCATGTGATTAGAACCTGGACCATTAAGGCTGTTACTGGATTTC  
          961   ACCGCTATGCAATCCTACGGACTCTTCTTGGCTACTTCTTGGGTTTCCGGATGCTACTTG  
          1021   TTCGCTCACTTCTTACTTCTCACACCCATTTGGATGTTGTTTCCTGATGAGCATTTG  
          1081   TCTTGGGTTAGGTACGCTGTGGATCACACCATTGATATCGATCCTTCTCAGGGATGGGTT  
          1141   AACTGGTTGATGGGATACTTGAAGTCCAAAGTGATTTCATCACCTTCTCCCTTCTATGCCT  
 25       1201   CAATTGAGACAACCTGAGGTGTCCAGAAGATTGCTTGCCTTTCGCTAAGAAGTGGAAACCTC  
          1261   AACTACAAGGTGATGACTTATGCTGGAGCTTGGAAAGGCTACTTTGGGAAACCTCGATAAT  
          1321   GTGGGAAAGCACTACTACGTGCACGGACAACATTCGGAAAGACCGCTTGA  
  
 30

SEQ ID NO: 2 (OtD6  $\Delta$ 6-desaturase from *Ostreococcus tauri*)

35   MCVETENNDGIPTVEIAFDGERERAEANVKLSAEKMEPAALAKTFARRYVVIEGVEYDVT  
      DFKHPGGTVIFYALSNTGADATEAFKEFHHSRKRKALALPSRPAKTAKVDDAEMLQD  
      FAKWRKELERDGFKPSPAHVAYRFAELAAMYALGTYLMYARYVVS SVLVYACFFGARGC  
      WVQHEGGHSSLTGNIIWWDKRIQAF TAGFGLAGSGDMWNSMHNKHHATPQKVRHDMDLDTT  
      PAVAFFNTAVEDNRPRGFSKYWLRLQAWTFIPVTSGLVLLFWMFFLHPSKALKGGKYEEL  
 40   VWMLAAHVIRTWTIKAVTGFTAMQSYGLFLATSWVSGCYLFAHFSTSHTHLDVVPAD EHL  
      SWVRYAVDHTIDIDPSQGWVNWLMGYLNCQVIHHLFSPMPQFRQPEVSRRFVAFKKNWL  
      NYKVMTYAGAWKATLGNLDNVGKHYYVHGQHS GKTA\*

**SEQ ID NO: 3** (Codon-optimised PSE1  $\Delta 6$ -elongase from *Physcomitrella patens*)

1 ATGGAAGTTGTTGAGAGGTTCTACGGAGAGTTGGATGGAAAGGTTTCCCAAGGAGTGAAC  
 5 61 GCTTTGTTGGGATCTTTTCGGAGTTGAGTTGACTGATACCCCAACTACTAAGGGATTGCCA  
 121 CTCGTTGATTCTCCAACCTCCAATTGTGTTGGGAGTGTCTGTTTACTTGACCATCGTGATC  
 181 GGAGGATTGCTTTGGATCAAGGCTAGAGATCTCAAGCCAAGAGCTTCTGAGCCATTCTTG  
 241 TTGCAAGCTTTGGTGTGGTGCACAACCTGTTCTGCTTCGCTTTGTCTCTTTACATGTGC  
 301 GTGGGTATCGCTTACCAAGCTATCACCTGGAGATATTCCTTGTGGGGAAACGCTTATAAC  
 361 CCAAAGCACAAAGGAGATGGCTATCCTCGTTTACCTCTTCTACATGTCCAAGTACGTGGAG  
 10 421 TTCATGGATAACCGTGATCATGATCCTCAAGAGATCCACCAGACAGATTTCTTTCCCTCCAC  
 481 GTGTACCACCATCTTCTATCTCCCTTATCTGGTGGGCTATTGCTCATCATGCTCCAGGA  
 541 GGAGAGGCTTATTGGAGTGCTGCTCTCAACTCTGGAGTGCATGTGTTGATGTACGCTTAC  
 601 TACTTCTTGGCTGCTTGCTTGAGATCTTCCCAAAGCTCAAGAACAAGTACCTCTTCTGG  
 661 GGAAGATACCTCACCCAATTCCAGATGTTCCAGTTCATGCTCAACTTGGTGCAAGCTTAC  
 15 721 TACGATATGAAAACCAACGCTCCATATCCACAATGGCTCATCAAGATCCTCTTCTACTAC  
 781 ATGATCTCCCTCTTGTTCCTCTTCGGAACTTCTACGTGCAAAAGTACATCAAGCCATCC  
 841 GATGGAAAGCAAAGGGAGCTAAGACCGAGTGA

20

**SEQ ID NO: 4** (PSE1  $\Delta 6$ -elongase from *Physcomitrella patens*)

MEVVERFYGELDGKVSQGVNALLGSFGVELTDTPTTKGLPLVDSPTPIVLGVSVYLTIVIGLLWIKAR  
 25 DLKPRASEPFLLQALVLVHNLFCFALSLYMCGIAYQAITWRYSLWGNAYNPKHKEMAILVYLFYMSKY  
 VEFMDTVIMILKRSTRQISFLHVYHHSSI SLIWWAIAHHPGGEAYWSAALNSGVHVLMYAYYFLAACL  
 RSSPKLKNKYLFWGRYLTQFQMFQFMLNLVQAYYDMKTNAPYPQWLIKILFYMI SLLFLFGNFYVQKY  
 IKPSDGKQKGAKTE\*

**SEQ ID NO: 5** (Codon-optimised Tc $\Delta$ 5-desaturase from *Thraustochytrium* sp.)

1 ATGGGAAAAGGATCTGAGGGAAGATCTGCTGCTAGAGAGATGACTGCTGAGGCTAACGGA  
 5 61 GATAAGAGAAAGACCATCCTCATTGAGGGAGTGTGTACGATGCTACCAACTCAAACAC  
 121 CCAGGAGGTTCCATTATTAACTTCCTCACCGAGGGAGAAGCTGGAGTTGATGCTACCCAA  
 181 GCTTACAGAGAGTTCATCAGAGATCCGGAAAGGCTGATAAGTACCTCAAGTCCCTCCCA  
 241 AAGTTGGATGCTTCTAAGGTGGAGTCTAGGTTCTCTGCTAAGGAGCAGGCTAGAAGGGAC  
 301 GCTATGACCAGGGATTACGCTGCTTTCAGAGAGGAGTTGGTTGCTGAGGGATACTTCGAT  
 10 361 CCATCTATCCCACACATGATCTACAGAGTGGTGGAGATTGTGGCTTTGTTTCGCTTTGTCT  
 421 TTCTGGTTGATGTCTAAGGCTTCTCCAACCTCTTTGGTTTTGGGAGTGGTGTGAACGGA  
 481 ATCGCTCAAGGAAGATGCGGATGGGTTATGCATGAGATGGGACACGGATCTTTCAGTGA  
 541 GTTATCTGGCTCGATGATAGGATGTGCGAGTTCCTTCTACGGAGTTGGATGTGGAATGTCT  
 601 GGACACTACTGGAAGAACCAGCATTCTAAGCACCATGCTGCTCCAAACAGATTGGAGCAC  
 661 GATGTGGATTTGAACACCTTGCCACTCGTTGCTTTCAACGAGAGAGTTGTGAGGAAGGTT  
 15 721 AAGCCAGGATCTTTGTTGGCTTTGTGGCTCAGAGTTCAGGCTTATTTGTTTCGCTCCAGTG  
 781 TCTTGCTTGTGATCGGATTGGGATGGACCTTGTACTTGCACCCAAGATATATGCTCAGG  
 841 ACCAAGAGACATATGGAGTTTGTGTGGATCTTCGCTAGATATATCGGATGGTTCTCCTTG  
 901 ATGGGAGCTTTGGGATATTCTCCTGGAACCTTCTGTGGGAATGTACCTCTGCTCTTTCGGA  
 961 CTTGGATGCATCTACATCTTCCCTCCAATTGCTGTGTCTCATAACCCATTTGCCAGTTACC  
 20 1021 AACCCAGAGGATCAATTGCATTGGCTTGAGTACGCTGCTGATCATAACCGTGAACATCTCT  
 1081 ACCAAGTCTTGGTTGGTTACCTGGTGGATGTCTAACCTCAACTTCCAAATCGAGCATCAT  
 1141 TTGTTCCCAACCGCTCCACAATTCAAGTTCAAGGAGATCTCTCCAAGAGTTGAGGCTCTC  
 1201 TTCAAGAGACATAACCTCCCTTACTACGATTTGCCATACACCTCTGCTGTTTCTACTACC  
 25 1261 TTCGCTAACCTCTACTCTGTTGGACATTCTGTTGGAGCTGATACCAAGAAGCAGGATTGA

**SEQ ID NO: 6** (Tc $\Delta$ 5-desaturase from *Thraustochytrium* sp.)

30 MGKGSEGRSAAREMTAEANGDKRKTILIEGVLYDATNFKHPGGSIIINFLTEGEAGVDATQAYREFHQRS  
 GKADKYLKSLPKLDASKVESRFSAKEQARRDAMTRDYAAFREELVAEGYFDPSIPHMIYRVVEIVALFA  
 LSFWLMSKASPTSLVLGVVMNGIAQGRCGWVMHEMHHGHSFTGVIWLDLDRMCEFFYGVGCGMSGHYWKNQ  
 HSKHHAAPNRLEHDVDLNTLPLVAFNERVVRKVKPGSLALWLRVQAYLFAPVSCLLIGLWTLYLHPR  
 YMLRTRKHMEFVWI FARYIGWFSLMGALGYS PGT SVGMYLCSFGLGCIYIFLQFAVSHTHLPVTNPEDQ  
 35 LHWLEYAADHTVNI STKSWLV TWMSNLNFQIEHHLFPTAPQFRFKEISPRVEALFKRHNLPYYDLPYT  
 SAVSTTFANLYSVGHSVGADTKKQD\*

**SEQ ID NO: 7** (Codon-optimised OtELo5  $\Delta 5$ -elongase from *Ostreococcus tauri*)

1 ATGTCTGCTTCTGGAGCTTTGTTGCCTGCTATTGCTTTTCGCTGCTTACGCTTACGCTACC  
 5 61 TACGCTTATGCTTTTCGAGTGGTCTCATGCTAACGGAATCGATAACGTGGATGCTAGAGAG  
 121 TGGATTGGAGCTTTGTCTTTGAGACTCCCTGCAATTGCTACCACCATGTACCTCTTGTTT  
 181 TGCCTTGTGGGACCTAGATTGATGGCTAAGAGGGAGGCTTTTGTATCCTAAGGGATTCATG  
 241 CTCGCTTACAACGCTTACCAAACCGCTTTCAACGTTGTGGTGTGCGGAATGTTTCGCTAGA  
 301 GAGATCTCTGGATTGGGACAACCTGTTTGGGGATCTACTATGCCTTGGAGCGATAGGAAG  
 361 TCCTTCAAGATTTTGTGGGAGTGTGGCTCCATTACAACAATAAGTACCTCGAGTTGTTG  
 10 421 GATACTGTGTTTCATGGTGGCTAGGAAAAGACCAAGCAGCTCTCTTCTTGCATGTGTAC  
 481 CATCATGCTTTGTTGATTTGGGCTTGGTGGCTTGTGTCATCTCATGGCTACCAACGAT  
 541 TGCATCGATGCTTATTTTCGGAGCTGCTTGCAACTCTTTCATCCACATCGTGATGTACTCC  
 601 TACTACCTCATGTCTGCTTTGGGAATTAGATGCCCTTGGAGAGATATATCACCCAGGCT  
 661 CAGATGTTGCAATTCGTGATCGTGTTCGCTCATGCTGTTTTTCGTGCTCAGACAAAAGCAC  
 15 721 TGCCCTGTTACTTTGCCTTGGGCACAAATGTTTCGTGATGACAAATATGTTGGTGTCTTTC  
 781 GGAACTTCTACCTCAAGGCTTACTCTAACAAGTCTAGGGGAGATGGAGCTTCTTCTGTT  
 841 AAGCCTGCTGAGACTACTAGAGCACCTTCTGTGAGAAGAACCAGGTCCAGGAAGATCGAT  
 901 TGA  
 20

**SEQ ID NO: 8** (OtELo5  $\Delta 5$ -elongase from *Ostreococcus tauri*)

25 MSASGALLPAIAFAAYAYATYAYAFEWSHANGIDNVDAREWIGALSLRLPAIATTTMYLLFCLVGPRLMA  
 KREAFDPKGFMLAYNAYQTAFNVVVLGMFAREISGLGQPVWGSTMPSDRKSFKILLGVWLHYNNKYLE  
 LLDTVFMVARKKTKQLSFLHVYHHALLIWAWWLVCHLMATNDCIDAYFGAACNSFIHIVMYSYYLMSAL  
 GIRCPWKRYITQAQMLQFVIVFAHAVFVLRQKHCPVTL PWAQMFVMTNMLVLFNGFYLKAYSNKSRGDG  
 ASSVKPAETTRAPSVRRTRSRKID\*  
 30

**SEQ ID NO: 9** (Codon-optimised EMoD5  $\Delta 5$ -desaturase from *Emiliana huxleyi*)

1 ATGTCATTGGCTGCTAAAGATGCAGCCTCGGCCACTCATCCGTCTTGGACCCTAAGTAT  
 61 CACGGAGCTACAAATAAGTCAAGAACTGATGCAGCAGACCTTACAGTTAGTTCTATCGAC  
 5 121 ACTTCTAAGGAGATGATCATAAGGGGTCGTGTGTATGATGTCTCTGATTTTATTTAAAGG  
 181 CACCCGGGAGGAAGCATTATTAAGCTCTCCTTAGGTTCTGATGCAACAGACGCTTATAAC  
 241 AACTTCCATATTAGGTCTAAAAAAGCGGATAAAAATGTTGAGAGCTTTGCCAAGTAGGCCA  
 301 GTAGCGGATGGATTTCGCTAGAGACGCTTTGTCTGCAGACTTCGAGGCCCTGAGAGCCCAA  
 361 CTCGAGGCCGAAGGTTACTTCGAACCGAATCTGTGGCATGTAGCTTATCGAGTTGCGGAA  
 10 421 GTCGTTGCTATGTACTGGGCGGGTATTAGACTTATCTGGGCGGGTTATTGGTTTTTATAGGA  
 481 GCCATTGTAGCAGGAATAGCTCAGGGGAGATGCGGTTGGCTTCAGCATGAGGGTGGTCAT  
 541 TATTTCGCTCACAGGTAATATTAAGCTTGCAGACATGCAAAATGATTATCTATGGATTA  
 601 GGTTCGGAATGTCCGGTTGTTATTGGAGAAACCAACATAACAAGCACCATGCGACACCG  
 661 CAAAAGTTGGGTGCAGATCCAGACCTTCAAACAATGCCTCTGGTTGCGTTCATGGACTC  
 15 721 ATCGGTGCTAAGGCTAGGGGAGCAGGAAAGTCGTGGCTAGCATGGCAAGCTCCACTTTTC  
 781 TTTGGAGGCGTTATCACAACCTGGTATCTTTTGGTTGGCAGTTCGTCCAACATCCAAAG  
 841 CACGATTGAGAGTAGGAAACCAACTCGAATTAGGCTATATGGCTTTACGATATGCTTTA  
 901 TGGTATGCAGCATTCGGTCATCTTGGGCTTGGTGGTGCTTTTCAGATTGTACGCTTTTTAT  
 961 GTGGCAGTCGGAGGTACATATATCTTACGAACCTTTGCGGTGTCTCACACACATAAGGAT  
 20 1021 GTTGTTCACACAGATAAGCATATTTCTTGGACCTTGTATTCTGCAAACCATACCACTAAT  
 1081 CAATCTAACACACCTCTAGTCAATTTGGTGGATGGCCTATCTGAATTTTCAAATTGAACAT  
 1141 CACCTTTTCCCTAGCATGCCACAATATAACCATCCTAAAATCTGCGGAAGAGTGAAACAA  
 1201 TTGTTTGAAAAACATGGCGTAGAGTACGATGTCAGAACTTACGCGAAGTCAATGCGTGAT  
 1261 ACATACGTGAATCTCTTGGCTGTGGGAAATGCATCTCATTCCCTTCATCAGAGAAACGAG  
 25 1321 GGATTAACGACTAGGGAGTCTGCGGCTGTTAGAGTTACAGGTCATTGA

**SEQ ID NO: 10** (EMoD5  $\Delta 5$ -desaturase from *Emiliana huxleyi*)

30 1 MSLAAKDAASAHSSVLDPKYHGATNKSRTDAADLTVSSIDTSKEMIIRGRVYDVSDFIKR  
 61 HPGGSI IKLSLGS DATDAYNNFHIRSKKADKMLRALPSRPVADGFARDALSADFEALRAQ  
 121 LEAEGYFEPNLWHVAYRVAEVVAMYWAGIRLIWAGYWFLGAI VAGIAQGRGWLQHEGGH  
 35 181 YSLTGNIKLDRHMQMI IYGLGCGMSGCYWRNQH NKHHATPQKLGADPDLQTMPLVAFHGL  
 241 IGAKARGAGKSWLAWQAPLFFGGVITTLV SFGWQFVQHPKHALRVGNQLELG YMALRYAL  
 301 WYAAFHGLGLGGA FRLYAFYVAVGGTYIFTNFAVSH THKD VVPHDKHISWTLYSANHTTN  
 361 QSNTPLVNWWW MAYLNFQIEHHLFPSMPQYNHPKICGRVKQLFEKHGVEYDVRTYAKSMRD  
 421 TYVNLLAVGNASHSLHQ RNEGLTTRESAAVRVTGH\*

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**SEQ ID NO: 11** (Codon-optimised Ps $\Delta$ 12-desaturase from *Phytophthora sojae*)

1 ATGGCTATTTTGAACCCTGAGGCTGATTCTGCTGCTAACCTCGCTACTGATTCTGAGGCT  
 5 61 AAGCAAAGACAATTGGCTGAGGCTGGATACACTCATGTTGAGGGTGCTCCTGCTCCTTTG  
 121 CCTTTGGAGTTGCCTCATTTCCTCTCAGAGATCTCAGAGCTGCTATTCCTAAGCACTGC  
 181 TTCGAGAGATCTTTCGTGACCTCCACCTACTACATGATCAAGAACGTGTTGACTTGCGCT  
 241 GCTTTGTTCTACGCTGCTACCTTCATTGATAGAGCTGGAGCTGCTGCTTATGTTTTGTGG  
 301 CCTGTGTACTGGTTCTTCCAGGGATCTTACTTGACTGGAGTGTGGGTTATCGCTCATGAG  
 361 TGTGGACATCAGGCTTATTGCTCTTCTGAGGTGGTGAACAACCTTGATTGGACTCGTGTTG  
 10 421 CATTCTGCTTTGTTGGTGCCTTACCCTCTTGGAGAATCTCTCACAGAAAGCACCATTCC  
 481 AACACTGGATCTTGCAGAACGATGAGGTTTTCGTTCCTGTGACCAGATCTGTGTTGGCT  
 541 TCTTCTTGAACGAGACCTTGGAGGATTCTCCTCTCTACCAACTCTACCGTATCGTGTAC  
 601 ATGTTGGTGTGTTGGATGGATGCCTGGATACCTCTTCTTCAACGCTACTGGACCTACTAAG  
 661 TACTGGGGAAAGTCTAGGTCTCACTTCAACCCTTACTCCGCTATCTATGCTGATAGGGAG  
 15 721 AGATGGATGATCGTGTCTCCGATATTTTTCTTGGTGGCTATGTTGGCTGTTTTGGCTGCT  
 781 TTGGTGCACACTTCTCCTTCAACACCATGGTGAAGTTCTACGTGGTGCCTTACTTCATT  
 841 GTGAACGCTTACTTGGTGTGATTACCTACCTCCAACACACCGATACCTACATCCCTCAT  
 901 TTCAGAGAGGGAGAGTGGAATTGGTTGAGAGGAGCTTTGTGCACTGTGGATAGATCATT  
 961 GGTCCATTCTCGATTCTGTGGTGCATAGAATCGTGGATACCCATGTTTGCCACCACATC  
 20 1021 TTCTCCAAGATGCCTTTCTATCATTGCGAGGAGGCTACCAACGCTATTAAGCCTCTCCTC  
 1081 GGAAAGTTCTACTTGAAGGATACCACTCCTGTTCTGTTGCTCTCTGGAGATCTTACACC  
 1141 CATTGCAAGTTCGTTGAGGATGATGGAAAGGTGGTGTCTACAAGAACAAGCTCTAG

**25 SEQ ID NO: 12** (Ps $\Delta$ 12-desaturase from *Phytophthora sojae*)

MAILNPEADSAANLATDSEAKQRQLAEAGYTHVEGAPAPLPLELPHFSLRDLRAAI PKHCFERSFVTST  
 YYMIKNVLTCAALFYAATFIDRAGAAAYVLWPVYWFQGSYLTGVVWVIAHECGHQAYCSSEVVNNLIGL  
 30 VLHSALLVPYHSWRI SHRKHSNTGSCENDEVFVPVTRSVLASSWNETLEDSPLYQLYRIVYMLVVGWM  
 PGYLFFNATGPTKYWGKSRSHFNPSAIYADRERWMIVLSDFLVAMLAVLAALVHTFSFNTMVKFYV  
 PYFIVNAYLVLITYLQHTDTYI PHFREGEWNWLRGALCTVDRSFGPFLDSVVHRIVDTHVCHHIFSKMP  
 FYHCEEATNAIKPLLKGFYLDKTT PVPVALWRSYTHCKFVEDDGKVVIFYKNKL\*



**SEQ ID NO: 13** (Codon-optimised pi(w3)-desaturase from *Phytophthora infestans*)

1 ATGGCTACAAAGGAGGCTTACGTTTTCCCAACTCTCACCGAGATCAAGAGATCTCTCCCA  
 61 AAGGATTGCTTCGAGGCTTCTGTGCCTTTGTCTCTCTACTACACTGTGAGATGCTTGGTT  
 5 121 ATTGCTGTGGCTTTGACCTTCGGATTGAACTACGCTAGAGCTTTGCCAGAGGTTGAGTCT  
 181 TTCTGGGCTTTGGATGCTGCTTTGTGCACTGGATATATCCTCCTCCAGGGAATTGTGTT  
 241 TGGGGATTCTTCACTGTTGGACACGATGCTGGACACGGAGCTTCTCTAGATACCACCTC  
 301 TTGAACTTCGTTGTGGGAACCTTCATGCACTCTCTCATCTTGACCCCATTCGAGTCTTGG  
 361 AAGTTGACCCACAGACACCACCACAAGAACACCGGAAACATCGATAGAGATGAGGTGTT  
 10 421 TACCCACAGAGAAAAGGCTGATGATCACCCATTGTCCAGGAACTTGATCTTGGCTTTGGGA  
 481 GCTGCTTGGCTTGGCTTATTTGGTGGAGGGATTCCCACCAAGAAAGGTGAACCACTTCAAC  
 541 CCATTCGAGCCACTTTTTGTGAGACAAGTGTCCGCTGTGGTTATCTCTTTGCTCGCTCAC  
 601 TTCTTCGTTGCTGGACTCTCTATCTACTTGTCTCTCCAGTTGGGACTTAAGACCATGGCT  
 661 ATCTACTACTACGGACCAGTTTTTCGTGTTCCGATCTATGTTGGTGATTACCACCTTCTTG  
 15 721 CACCACAACGATGAGGAGACTCCATGGTATGCTGATCTGAGTGGACTTACGTGAAGGGA  
 781 AACTTGTCCCTCTGTGGATAGATCTTACGGTGTCTCATCGATAACCTCTCCACAACATC  
 841 GGAACTCACCAGATCCACCACCTCTTCCCAATTATCCACACTACAAGCTCAAGAAGGCT  
 901 ACTGCTGCTTTCCACCAAGCTTTCCAGAGCTTGTGAGAAAAGTCCGATGAGCCAATCATC  
 961 AAGGCTTTCTTCAGAGTGGGAAGGTTGTATGCTAACTACGGAGTGGTTGATCAAGAGGCT  
 20 1021 AAGCTCTTCACTTTGAAGGAGGCTAAGGCTGCTACTGAAGCTGCTGCTAAGACCAAGTCT  
 1081 ACCTGA

**SEQ ID NO: 14** (pi(w3)-desaturase from *Phytophthora infestans*)

25 1 MATKEAYVFPTLTEIKRSLPKDCFEASVPLSLYYTVRCLVIAVALTFGLNYARALPEVES  
 61 FWALDAALCTGYILLQGI VFWGFFTVGH DAGHGAF SRYHLLN FVVGTFM HSLILTPFESW  
 121 KLTHRHHHKNTGNIDRDEVFYPQRKADHPLSRNLILALGAAWLAYLVEGFPPRKVNHFN  
 181 PFEPLFVRQVS AVVISLLAHFFVAGLSIYLSLQLGLKTMAYYYGPFVVFVGSMLVITTF  
 30 241 HHNDEETPWYADSEWTYVKG NLSVDRSYGALIDNLSHNI GTHQIHHLFPPIIPHYKLKKA  
 301 TAAFHQAFPELV RKSDEPIIKAFFRVGRLYANYGVVDQEAKLFTLKEAKAATEAAAKTKS  
 361 T\*

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5 SEQ ID NO: 15 (Codon-optimized EhD4  $\Delta$ 4-desaturase from *Emiliana huxleyi*)

1 ATGGGGGGTGCAGGCGCTTCGGAAGCAGAGAGGCCAAAGTGGACAACCTATCCACGGAAGA  
 61 CACGTTGATGTGTCAAAGTTTACACACCCTGGAGGTAATATCATTGAATTGTTCTATGGC  
 10 121 ATGGATAGTACATCCGCTTTCGAGCAATTTACGGACATCATAAGGGGGCATGGAAGATG  
 181 CTCAAGGCTCTTCCTACCAAGGAGGTTGACCCAGCTGACGTCCACAGCAACCTCAAGAA  
 241 CATGTCGCGGAGATGACCAGACTTATGACATCCTGGAGAGAAAGGGGTTTATTCAAGCCT  
 301 CGTCCGGTTGCATCTGGCATATATGGACTTGCAGTAGTTGCTGCTATAGTTGCATGCATT  
 361 GCATGTGCTCCGCACGCACCGGTTCTGTGGGGATTGGTTTAGGGTCTTGTGGGCCCAA  
 421 TGCGGTTTCTTGCAGCATATGGGGGACATAGGGAGTGGGGGTCAGGTATTCTTTCTTG  
 15 481 CTCCAACACTTCTTTGAGGGTTTACTAAAGGGAGGATCAGCTAGCTGGTGGAGGAACAGA  
 541 CATAATAAGCATCATGCGAAAACCAATGTTCTTGGAGAGGATGGTGCCTTCGAACTACT  
 601 CCATTCTTTGCGTGGGACCCGACTCTCGCTAAAAGGTGCCGATTGGTCTCTGAAGACA  
 661 CAAGCTTTCACCTTCCCTCCAGCACTAGGAGCCTATGTTTTCGTTTTCGCTTTCACAATT  
 721 AGAAAATACGCTGTGGTGAAAAAATCTGGCACGAACTTGCTCTAATGATTGCTCATTAC  
 20 781 GCAATGTTTCTACTATGCCCTGCAGTTGGCTGGAGCCAGTTTGGGTTCTGGACTTGCATTT  
 841 TACTGCACAGGTTACGCATGGCAGGGAATCTACCTCGGATTCTTCTTCGGTTTGGCCAC  
 901 TTTGCAGTCGAGAGAGTACCAAGCACAGCGACATGGCTCGAAAGCTCAATGATAGGTTCA  
 961 TGGCAGGGAATCTACCTCGGATTCTTCTTCGGTTTGGCCACTTTGCAGTCGAGAGAGTA  
 1021 CCAAGCACAGCGACATGGCTCGAAAGCTCAATGATAGGTACGGTAGACTGGGGAGGTTCA  
 25 1081 TCTGCTTTTTGTGGTTATGTTTCTGGTTTCTTGAATATCAAATTGAACATCACATGGCC  
 1141 CCTCAAATGCCTATGGAAAATCTGAGACAGATCAGGGCAGATTGTAAGGCTAGTGCTGAG  
 1201 AAACCTCGGCTTGCCATATAGAGAGTTGTCATTTCGAGGTGCTGTCAAACCTCATGATGGTA  
 1261 GGTCTCTGGAGGACTGGAAGAGACGAATTACAGCTCCGAAGTGATCGAAGAAAGTACTCA  
 1321 AGAACCCAGGCTTACATGGCGGCTGCTTCAGCTGTTGTTGAAAATCTGAAGGCAGATTAA

30 SEQ ID NO: 16 (EhD4  $\Delta$ 4-desaturase from *Emiliana huxleyi*)

1 MGGAGASEAERPKWTTIHGRHVDVSKFRHPGGNIIELFYGMDSSTSAFEQFHGHKGAWKM  
 61 LKALPTKEVDPADVPQQPQEHVAEMTRLMTSWRERGLFKPRPVASGIYGLAVVAIIVACI  
 35 121 ACAPHAPVLSGIGLGSWAQCGFLQHMGGHREWGVRYSFLLQHFFEGLLKGSASWWRNR  
 181 HNKHHAKTNVLGEDGDLRTPFFAWDPTLAKKVPDWSLKTQAFTFLPALGAYVVFVFAFTI  
 241 RKYAVVKKLWHELALMIAHYAMFYALQLAGASLGSLAFYCTGYAWQGIYLGFFFGLSH  
 301 FAVERVPSTATWLESSMIGSWQGIYLGFFFGLSHFAVERVPSTATWLESSMIGTVDWGS  
 361 SAFCGYVSGFLNIQIEHHMAPQMPMENLRQIRADCKASAELGLPYRELSFAGAVKLMV  
 40 421 GLWRTGRDELQLRSDRRKYSRTQAYMAAASAVVENLKAD\*

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5 **SEQ ID NO: 17** (Codon-optimized  $\Delta 4$ -desaturase from *Thraustochytrium* sp., ATCC21685)

1 ATGACTGTTGGATACGATGAGGAGATCCCATTCGAGCAAGTTAGGGCTCATAACAAGCCA  
 61 GATGATGCTTGGTGTGCTATTCATGGACACGTTACGATGTTACCAAGTTCGCTTCTGTT  
 121 CATCCAGGAGGAGATATTATCTTGCTCGCTGCTGGAAAGGAAGCTACTGTGCTCTACGAG  
 10 181 ACCTACCATGTTAGAGGAGTGTCTGATGCTGTGCTCAGAAAAGTACAGAAATCGGAAAAGTTG  
 241 CCAGATGGACAAGGAGGAGCTAACGAGAAGSAGAAGAGAACCTTGTCTGGATTGTCCTCT  
 301 GCTTCTTACTACACCTGGAACCTCCGATTTCTACAGAGTGATGAGGGAGAGAGTTGTGGCT  
 361 AGATTGAAGGAGAGAGGAAAGGCTAGAAGAGGAGGATACGAGTTGTGGATCAAGGCTTTC  
 421 TTGCTCCTTGTGGATTCTGGTCTCTCTTTACTGGATGTGCACCCTCGATCCATCTTTC  
 15 481 GGAGCTATCTTGGCTGCTATGCTTTGGGAGTGTTGCTGCTTTTGTGGAACTGCATC  
 541 CAACATGATGGAAACCATGGAGCTTTCGCTCAATCTAGATGGGTAAACAAGGTGGCAGGA  
 601 TGGACTTTGGATATGATCGGAGCTTCTGGAATGACTTGGGAGTCCAACATGTGTTGGGA  
 661 CATCACCCATACACTAACTTGATCGAGGAGGAGAACGGATTGCAAAAAGGTGCCGAAAG  
 721 AAGATGGATAACCAAGTTGGCTGATCAAGAGTCTGATCCAGATGTGTTCTCCACCTACCCA  
 20 781 ATGATGAGATTGCATCCATGGCATCAGAAGAGATGGTATCACAGGTTCCAGCATATCTAC  
 841 GGACCATTCATCTTCGATTTCATGACCATCAACAAGGTGGTACTCAAGATGTTGGAGTG  
 901 GTGTTGAGAAAAGAGGCTCTTCCAAATCGATGCTGAGTGAGATATGCTTCCCCAATGTAC  
 961 GTTGCTAGGTTCTGGATCATGAAGGCTTTGACCGTGTGTACATGGTTGCTCTCCCATGT  
 1021 TATATGCAAGGACCATGGCATGGATTGAAGCTCTTCGCTATCGCTCATTTCACTTGCAGGA  
 25 1081 GAGGTTTTGGCTACCATGTTTCATCGTGAACCAATTCATCGAGGGAGTGCTTACGCTTCT  
 1141 AAGGATGCTGTTAAGGGAACTATGGCTCCACCAAAGACTATGCATGGAGTGACCCCAATG  
 1201 AACAACTAGAAAAGGAGGTTGAGGCTGAGGCTTCTAAGTCTGGAGCTGTGGTTAAGTCT  
 1261 GTGCCATTGGATGATTGGGCTGCTGTTCAATGCCAAACCTCTGTGAACTGGTCTGTTGGA  
 1321 TCTTGTTCTGGAACCATTTCTCTGGAGGACTCAACCATCAAATCGAGCATCATCTCTTC  
 30 1381 CCAGGATTGCTCACGAGACCTACTACCACATCCAAGATGTGGTTCAATCTACCTGTGCT  
 1441 GAGTACGGAGTCCATACCAACATGAGCCATCTTTGTGGACTGCTTACTGGAAGATGCTC  
 1501 GAACATTTGAGACAATTGGGAAACGAGGAGACTCACGAGTCTTGGCAAAGAGCTGCTTGA

35 **SEQ ID NO: 18** ( $\Delta 4$ -desaturase from *Thraustochytrium* sp., ATCC21685)

1 MTVGYDEEIPFEQVRAHNKPDDAWCAIHGHVYDVTKFASVHPGGDIIILLAGKEATVLYE  
 61 TYHVRGVSDAVLRKYRIGKLPDQGGANEKEKRTL SGLSSASYTWN SDFYRVMRERVVA  
 121 RLKERGKARRGGYELWIKAFLLL VGFWS SLYWMCTLDPSFGAIIAAMSLGVFAAFVGTCTI  
 40 181 QHDGNHGAFQSRWVNKVAGWTLDMIGASGMTWEEFQHV LGHHPYTNLIEEENGLQKVS GK  
 241 KMDTKLADQESDPDVFSTYPMMRLHPWHQKRWYHRFQHIYGPPIFGFMTINKVVTQDVGV  
 301 VLRKRLFQIDAECRYASPMYVARFWIMKALTVLYMVALPCYMQGPWHGLKLF AIAHF TCG  
 361 EVLATMFIVNHIIEGVS YASKDAVKGTMAPPKTMHGVT PMNNTRKEVEAEASKSGAVVKS  
 421 VPLDDWAAVQCQTSVNWSVGSWFWNHFSGGLNHQIEHHLFPGLSHETYYHIQDVVQSTCA  
 45 481 EYGVPYQHEPSLWTAYWKMLEHLRQLGNEETHESWQRAA\*

**SEQ ID NO: 19 (codon optimised O809D6  $\Delta$ 6-desaturase from *Ostreococcus* RCC809)**

5 ATGGGAAAGGGAGCAAGGAACCCAGGAGCAAGGGCATGGAAGTCAACATTGGAGCCTCACGCAGTGGCA  
 AAGTCATTCGATAGGAGATGGGTTAAGGTGGATGGAGTTGAATACGATGTGACTGATTTCAAGCATCCT  
 GGAGGTAGTGTATATACTACATGCTTTCTAACACAGGTGCTGATGCAACCGAAGCTTTTAAGGAGTTC  
 CATTACAGGAGTAAGAAAGCTAGGAAAGCACTTGCATGCAATGCCTCAAAGAGAACCAGAGGATGCTTCA  
 CCAGTTGAAGATGCAAACATGCTCAAGGATTCGCTAAGTGGAGAAAGGATCTCGAAAGGGAGGGATTT  
 10 TTCAAACCTTCTCCAGCTCATGTGGCATAATAGATTTGCTGAGCTTGCATGCAATGTTTCGCTCTCGGTACA  
 GCATTAATGTACGCTAGATGGCAGCAACTTCTGTTTTCTGACAGCTTGTTCCTTGGAGCAAGATGC  
 GGTTGGGTTCAACATGAGGGAGGTCACTCTTCATTGACTGGATCAATCTGGTGGGATAAGAGAATACAG  
 GCTTTTACAGCAGGATTCGGTCTCGCTAGTTCTGGTGATATGTGGAATTTAATGCATAACAAGCATCAC  
 GCAACCCCTCAAAAAGTTAGGCACGATATGGATTTGGATACTACACCAGCTGTTGCATTTTTCAATACT  
 15 CCTGTGACTTCAGGACTCGTGCTTTTGGCTTGGATGTATCTCTTACATCCAAGACACATTGCAAGAAGG  
 AAGAATTACGAAGAGGCTGCATGGATCGTTGCTGCACATGTGATAAGGACATCAGTTATTTAAAGCTGTG  
 ACAGGATATAGTTGGATAACCTGTTACGGTCTCTTTTTTAAGTACCATGTGGGTTTTCTGGATGCTATCTT  
 TTTGCTCATTTCTCAACCAGTCATACTCACCTTGATGTTGTGCCTTCAGATAAGCATTTGAGTTGGGTT  
 20 AGATATGCTGTGGATCACACTATTGATATCGATCCATCTAAATCAGTTGTGAATTTGGCTTATGGGTTAC  
 TTGAACTGTGAGTTATCCATCACTTGTTCCTGATATGCCACAATTCAGACAGCCAGAAGTTTCTAGA  
 AGGTTTGTGTCATTGCTAAGAAATGGAATCTCAACTACAAGGTTATGTCTTATTACGGAGCTTGAAA  
 GCAACATTCCGTAACCTTAACGAAGTTGGAAAGCACTACTATATTCAGGGTTCTCAAATCACAAAAAAG  
 ACCGTGTAA

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**SEQ ID NO: 20 (O809D6  $\Delta$ 6-desaturase from *Ostreococcus* RCC809)**

30 1 MGKGARNPGARAWKSTLEPHAVAKSFDRRWVKVDGVEYDVTDFKHPGGSVIYYMLSNTGA  
 61 DATEAFKEFHYSKARKALALPQREPEDASPVEDANMLKDFAKWRKDLEREGFFKPS  
 121 AHVAYRFAELAAMFALGTALMYARWHATSVFVTACFFGARCGWVQHEGGHSSLTGSIWWD  
 181 KRIQAFTAGFLASSGDMWNLHMKHHATPQKVRHDMDLDTTPAVAFFNTAVEENRPRKF  
 241 SKLWLRVQAWTFVPVTSGLVLLAWMYLLHPRHIARRKNYEEAAWIVAHVIRTSVIKAVT  
 35 301 GYSWITCYGLFLSTMWVSGCYLFAHFSTSHTHLDVVP SDKHLSWVRYAVDHTIDIDPSKS  
 361 VVNWLMGYLNCQVIHHLFPDMPQFRQPEVSRRFVSVFAKKWNLNYKVMSYYGAWKATFGNL  
 421 NEVGKHYIIGSQITKKTV-

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**SEQ ID NO: 21 (codon-optimised FcELO6  $\Delta$ 6-Elongase from *Fragilariopsis cylindrus* CCMP 1102)**

5 ATGGATGAATACAAGGCAACTTTAGAGAGTGTGGGAGATGCTATAATACAATGGGCAGATCCTGAGAGT  
 CAATTTACTGGTTTTACAAAGGGATGGTTTCTTACAGATTTACCTCAGCTTTCAGTATAGCACTTGTT  
 TACGTGTTGTTTCGTTATTATCGGTAGTCAAGTTATGAAGGTGCTTCCTGCTATTGATCCTTACCCAATA  
 AAGTTTTTCTACAATGTTTCTCAGATCATGTTGTGTGCATACATGACTATAGAAGCTTGCCTTTTGGCA  
 10 TATAGAAACGGATACACAATCATGCCTTGTGTTGGTTATAATAGGGATGATCCAGCTATAGGAAACCTC  
 TTATGGCTCTTTTACGTTTCAAAGTGTGGGATTTCTGGGATACCATCTTCATTGTTCTTGGTAAGAAA  
 TGGAGACAACCTCAGTTTCTTACATGTGTATCATCACACTACAATCTTTCTCTTCTACTGGTTAAATGCT  
 AACGTTTTCTATGATGGAGATATATACCTTACAATCGCATTGAATGGTTTCATACATACTGTGATGTAC  
 ACATACTACTTTATCTGTATGCACACCAAGGATAAGAAAACTGGAAAAGTCTTTGCCTATATGGTGGGAAG  
 15 TCTTCACTCACACTTTTGCAATTATTTAGTTTCATCACCATGATGTCACAGGGACTCTATTTAATAATT  
 TTCGGTTGCGAGAGTTTGTCTATAAGGGTTACCGCTACTTACGTTGTGTACATACTTTCTTTGTTTTTC  
 CTCTTCGCTCAATTTTTTCGTGGCATCTTACATGCAGCCAAAGAAATCAAAAACCTGCTTGA

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**SEQ ID NO: 22 (FcELO6  $\Delta$ 6-Elongase from *Fragilariopsis cylindrus* CCMP 1102)**

25 1 MDEYKATLESVGDALIQWADPESQFTGFTKGWFLTDFTSAFSAFIALVYVLFVVIIGSQVMKV  
 61 LPAIDPYPIKFFYNVSQIMLCAYMTIEACLLAYRNGYTIMPCVGYNRDDPAIGNLLWLFY  
 121 VSKVWDFWDTIFIVLGKKWRQLSEFLHVVHHTTIFLFYWLNANVFYDGDIIYLTIALNGFIH  
 181 TVMYTYFFICMHTKDKKTGKSLPIWWKSSLTLLQLFQFITMMSQGLYLIIFGCESLSIRV  
 241 TATYVVYIILSLFFLEAQFFVASYMOPKKSSTA-

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**SEQ ID NO: 23 (codon-optimised CeELO6  $\Delta$ 6-elongase from *Caenorhabditis elegans*)**

5  
 ATGGCTCAGCACCCACTCGTTCAGAGGTTACTTGATGTTAAATTCGATACAAAGAGGTTTCGTGGCAATA  
 GCAACTCATGGTCCTAAAAATTTCCCTGATGCTGAAGGAAGAAAGTTTTTCGCAGATCATTTTCGATGTT  
 ACTATTTCAAGCTAGTATACTCTACATGGTTGTGGTTTTTGGTACTAAATGGTTCATGAGAAACAGGCAA  
 CCTTTCCAGTTAACAATCCCACTTAACATATGGAACCTTCATTTTGGCTGCATTCTCAATCGCTGGAGCA  
 10  
 GTGAAGATGACCCCTGAGTTTTTCGGAACATTGCTAACAAGGGTATTGTGGCATCATACTGTAAGGTT  
 TTCGATTTACCAAAGGAGAAAACGGTTACTGGGTTTTGGCTTTTCATGGCTAGTAAGCTTTTTGAGTTG  
 GTGGATACTATCTTCCTTGTGTTTTGAGAAAAAGGCCACTCATGTTCCCTCCATTGGTACCATCACATCCTC  
 ACAATGATATACGCTTGGTACTCTCACCTCTTACCCCAGGATTCAACAGATACGGTATTTACTTGAAC  
 TTTGTGGTTCACGCATTCATGTACTCTTATTACTTCCTCAGATCAATGAAGATCAGGGTTCCAGGATTT  
 15  
 ATTGCTCAAGCAATCACAAGTTTACAAATAGTGCAGTTCATTATCTCTTGTGCTGTTCTTGACATTTG  
 GGTTATCTCATGCACTTTACCAATGCTAACTGCGATTTTGAACCTTCTGTGTTCAAATTGGCTGTTTTT  
 ATGGATACTACATACCTCGCACTCTTCGTGAATTTCTTTCTTCAGTCATATGTTCTCAGGGGTGGTAAG  
 GATAAGTACAAAGCTGTTCCAAAGAAAAAGAATAACTGA

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**SEQ ID NO: 24 (CeELO6  $\Delta$ 6-elongase from *Caenorhabditis elegans*)**

25  
 1 MAQHPLVQRLLDVKFDTKR FVAIATHGPKNF PDAEGRKFFADHFDVTIQASILYMVVVF  
 61 TKWFMRNRQPFQLTIPLNIWNFILAAFSIAGAVKMTPEFFGTIANKGIVASYCKVDFDFTK  
 121 GENGYVWVWLFMASKL FELVDTI FLVLRKRPLMFLHWHYHHIL TMIYAWYSHPLTPGFENRYG  
 181 IYLN FVVHAFMYSYYFLRSMKIRVPGFIAQAITS LQIVQFI IISCAVLAHLGLYLMHFTNAN  
 30 241 CDFEPSVFKLAVFMDTTYLALFVNFFLQSYVLRGGKDKYKAVPKKNN-

**SEQ ID NO: 25 (codon-optimised TpDesK  $\Delta$ 4-desaturase from *Thalassiosira pseudonana*)**

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ATGGGTAATGGTAATCTTCCAGCATCTACAGCACAACTCAAGTCAACAAGTAAACCTCAACAGCAACAC  
 GAGCACAGAACAATCAGTAAATCTGAATTGGCACAACATAAACACTCCTAAGTCTGCTTGGTGTGCAGTT  
 CATTCAACTCCTGCTACAGATCCAAGTCACTCTAATAACAAACAGCATGCACACCTTGTTTTGGATATT  
 ACAGATTTTCGCTTCTAGACATCCAGGAGGAGATTTGATTTCTTTTGGCTTCAGGAAAAGATGCAAGTGTG  
 CTCTTCGAGACCTACCACCCTAGGGGAGTTCCAACTTCATTAATTCAAAAGCTTCAGATCGGTGTTATG  
 GAAGAGGAAGCTTTTAGAGATAGTTTCTACTCTTGGACAGATTCTGATTTCTACACCGTTCTTAAGAGA  
 AGGGTTGTGAAAAGATTAGAGGAAAAGGGGACTTGATAGAAGGGGTTCAAAAAGAGATTTGGATCAAGGCT  
 TTATTTCTCTTAGTTGGATTCTGGTACTGTCTTTACAAGATGTACACTACATCAGATATAGATCAATAC  
 GGAATAGCTATTGCATATAGTATCGGAATGGGTACTTTTGTCTGCATTTCATCGGTACATGCATACAACAT  
 GATGGAAACCACGGTGTCTTTCGCACAGAACAAGCTTTTGAACAAGTTGGCTGGATGGACACTCGATATG  
 ATCGGTGCTTCTGCATTACCTGGGAATTGCAGCATATGCTCGGTTCATCACCCCTTACACTAATGTTCTT  
 GATGGAGTGGAGGAAGAGAGAAAAGAAAAGGGGAGAGGATGTGGCTTTTGAAGAGAAGGATCAAGAGTCA  
 GATCCAGATGTTTTCTCTTCATTCCTCTCATGAGAATGCATCCACATCACACCCTAGTTGGTACCAT  
 AAATATCAGCACCTTTATGCTCCTCCACTCTTTGCATTAATGACCCTTGCTAAGGTGTTTCAACAGGAT  
 TTCGAAGTTGCAACATCTGGAAGATTGTACCATATTGATGCTAACGTTAGATATGGTTCAGTTTGGAAAT  
 GTGATGAGATTCTGGGCTATGAAAGTTATCACAATGGGATACATGATGGGTTTGCCTATTTACTTTCAT  
 GGAGTTCTCAGGGGAGTGGGTCTTTTCGTTATCGGACACCTTGCATGTGGTGAACCTTAGCTACTATG  
 TTCATAGTTAACCATGTGATTTGAGGGAGTGAAGTTATGGTACAAAAGATCTTGTGGAGGTTGCATCTCAC  
 GGAGATGAAAAGAAAATTGTGAAGCCTACAACCGTTTTAGGTGATACCCCAATGGAGAAAACATAGAGAA  
 GAGGCTCTCAAGTCAACAGTAACAACAACAAGAAAAGGGGAGAAAAGAACTCAGTTCTTAGTGTGCCA  
 TTTAATGATTGGGCTGCAGTGCAATGCCAGACTTCTGTAACTGGTCTCCTGGTTCATGGTTTTTGGAAAT  
 CATTTTCAGTGGAGGTTTTGTCTCACCAAATCGAGCATCACCTCTTCCCAAGTATATGTCATACTAACTAC  
 TGCCACATTCGAAGATGTTGTGGAATCTACATGTGCTGAGTACGGTGTGCCATATCAGTCTGAATCAAAC  
 TTGTTTCGTTGCATACGGAAAGATGATCTCACATTTGAAGTTCTTCGGTAAGGCTAAGTGCGAGTGA

**35 SEQ ID NO: 26 (TpDesK  $\Delta$ 4-desaturase from *Thalassiosira pseudonana*)**

40  
 45

1 MGNGLPASTAQLKSTSKPQQQHEHRTISKSELAQHNTPKSAWCAVHSTPATDPSHSNNK  
 61 QHAHLVLDITDFASRHPGGDLILLASGKDASVLFETYHPRGVPTSLIQKLQIGVMEEEF  
 121 RDSFYSWTDSDFYTVLKRRVVERLEERGLDRRGSKEIWIKALFLLVGFWYCLYKMYTTSD  
 181 IDQYGIAYSIGMGTFAAFI GTCIQH DGNHGAF AQNKLLNKL AGWTLDMIGASAF TWEL  
 241 QHMLGHHPYTNVLDGVEEERKERGEDVALEEKDQESDPDVFSSFPLMRMHPHHTTSWYHK  
 301 YQHLYAPPLFALMTLAKVFOQDFEVATSGRLYHIDANVRYGSVWNVMRFRWAMKVIITMGYM

**SEQ ID NO: 27 (codon-optimised Hpw-3, a w3-desaturase from *Hyaloperonospora parasitica*)**

5           1    ATGGCTACTAAACAATCAGTTGCTTTTCCTACTTTGACTGATCTTAAAAGATCTCTTCCT  
           61    TCTGAGTGTTTTGAATCTTCTTTGCCTCTTTCTCTTTACTATACACTTAGATCTTTGGTT  
 121       121    TTTGCTGGTTCTCTTGCTGTTTCTCTTTCTTACGCTCTTGCTCAACCTTTGGTTCAAAC  
 181       181    TTTTACCCTCTTAGAGTTGCTCTTATTGCTGGATACACTGTTTTTCAAGGAGTTATTTTC  
 241       241    TGGGGATTTTTCACTATTGGTCATGATGCTGGTCATGGTGCTTTTTCTAGATATCCTGTT  
 301       301    CTTAACTTCACTGTTGGAACACTTATGCATTCTCTTATTTTACTCCTTTTGAATCTTGG  
 10       361    AAGTTGACTCATAGACATCATCATAAAAACACTGGAAATATCGATAGAGATGAGATCTTC  
           421    TACCCTCAAAGAGAATCTGATGATCATCCTGTTTCTAGACATCTTACTTTCCTTGGG  
           481    GCTGCTTGGTTCGCTTACCTTGTGAGGGTTTTCCACCTAGAAAATTGAATCATTACAAT  
           541    CCTTTCGAGCCATTGTTGAGAGAAGAGTTTCTGCTGTTGTTATCTCTATCTTGGCTCAG  
           601    TTTTTCGTTGCAGGATTGTCTATTTACTTGTGTTTCCAGGTTGGAGTTCAGGCTGTTGCT  
 15       661    CTTTACTATTACGGTCCATCTTCGTTTTTGGTACTATGCTTGTIATTACTACTTTTTCTT  
           721    CATCATAACGATGAAGAGACTCCTTGGTACGGTGATGAGGATTGGTCTTACGTTAAGGGT  
           781    AACTTGTCTTCTGTTGATAGATCTTACGGTCCCTCTTATCGATAACTTGTCTCATAACATC  
           841    GGTACTCATCAAGTTCATCATCTTTCCCAATCATCCCTCATTACAAATTAAGCCTGCT  
 20       901    ACAGCTGCTTTCAGAAGAGCTTTCCACATCTTGTGTTAGAAAGTCTGATGAAAGAATTTG  
           961    CAGGCTTTTTACAGAATTGGTAGATTGTATGCTAAATATGGTGTGCTGATTCTTCTGCT  
 1021     1021    AAATTGTTTTACATTGAAGGAAGCTCAACTTACTTCTAAAGCTGCTTCTGATGCTAAAGCT  
 1081     1081    GCTTGA

25

**SEQ ID NO: 28 (Hpw-3, a w3-desaturase from *Hyaloperonospora parasitica*)**

          1    MATKQSVAFPTLTDLKRSLPSECFESSLPLSLYTLRSLVVFAGSLAVSLSYALAOPLVQN  
 61       61    FYPLRVALIAGYTVFQGVIFWGFFTIGHDAGHGAFSRYPVLNFTVGTLMHSLILTPFESW  
 30       121    KLTHRHHHKNTGNIDRDEIFYPQRESDDHPVSRHLTFTLGAAWFAYLVEGFPPRKLNHYN  
           181    PFEPLFERRVSAVVISILAQFFVAGLSIYLCFQVGVQAVALLYYGPIFVFGTMLVITTFLL  
           241    HHNDEETPWYGDEDWSYVKGNLSSVDRSYGPLIDNLSHNIGTHQVHHLFPIIPHYKLPKA  
           301    TAAFRRAFPHLVRKSDERILQAFYRIGRLYAKYGVADSSAKLFTLKEAQLTСКАASDAKA  
 361       361    A-

35



Claims:

- 5 1. A recombinant camelina plant or cell comprising one or more polynucleotides encoding a  $\Delta 6$ -desaturase, a  $\Delta 6$ -elongase and a  $\Delta 5$ -desaturase operably linked with one or more regulatory sequences.
- 10 2. A recombinant camelina plant or cell according to claim 1 which further comprises one or more polynucleotides encoding a  $\Delta 12$ -desaturase and/or a  $\omega 3$  desaturase operably linked with one or more regulatory sequences.
- 15 3. A recombinant camelina plant according to claim 1 or 2 wherein the plant is a seed.
- 20 4. A recombinant camelina plant or cell according to any one of claims 1 to 3 wherein the desaturase and elongase enzymes are independently derived from algae, bacteria, mould or yeast.
- 25 5. A recombinant camelina plant or cell according to any one of claims 1 to 3 wherein the  $\Delta 6$ -desaturase comprises an amino acid sequence having at least 50% identity to SEQ ID NO:2 or SEQ ID NO:20, the  $\Delta 6$ -elongase comprises an amino acid sequence having at least 50% identity to SEQ ID NO:4, SEQ ID NO: 22 or SEQ ID NO:24, and the  $\Delta 5$ -desaturase comprises an amino acid sequence having at least 50% identity to SEQ ID NO:6 or SEQ ID NO: 10.
- 30 6. A recombinant camelina plant or cell according to any one of claims 1 to 5 wherein the  $\Delta 6$ -desaturase is derived from *Ostreococcus tauri*, the  $\Delta 6$ -elongase is derived from *Physcomitrella patens* and the  $\Delta 5$ -desaturase is derived from *Thraustochytrium sp.*
- 35 7. A method for producing eicosapentaenoic acid (EPA) comprising growing a plant or cell according to any one of claims 1 or 6 under conditions wherein said desaturase and elongase enzymes are expressed and EPA is produced in said plant or cell.

- 5 8. A method for producing plant seed oil comprising growing a plant or cell according to any one of claims 1 or 6 under conditions wherein said desaturase and elongase enzymes are expressed and a plant seed oil is produced in said plant or cell.
9. A plant seed oil produced by the method of claim 8.
- 10 10. A plant seed oil according to claim 9 wherein EPA constitutes at least 5%, at least 10%, at least 15%, at least 20%, at least 25% or at least 30% (mol %) of the total fatty acid content of said oil.
- 15 11. A plant seed oil wherein the EPA constitutes at least 15% (mol %) of the total fatty acid content of said oil, and the  $\gamma$ -linolenic (GLA) constitutes less than 10% (mol %) of the total fatty acid content of said oil.
- 20 12. A plant seed oil according to claim 11 wherein the EPA constitutes at least 20% (mol %) of the total fatty acid content of said oil.
- 25 13. A plant seed oil according to claim 12 wherein the EPA constitutes 20% to 31% (mol %) of the total fatty acid content of said oil.
- 30 14. A plant seed oil according to any one of claims 11 to 13 wherein the GLA constitutes less than 7% (mol %) of the total fatty acid content of said oil.
- 35 15. A plant seed oil according to claim 14 wherein the GLA constitutes 1% to 6% (mol %) by weight of the total fatty acid content of said oil.
16. A plant seed oil according to any one of claims 11 to 15 wherein the ratio of the molar percentages of EPA to  $\gamma$ -linolenic (GLA) is about 3:1 to about 22:1, preferably about 5:1 to about 20:1.
17. A plant seed oil according to any one of claims 11 to 16 wherein the oil is produced by the method of claim 8.

18. A recombinant camelina plant or cell comprising one or more polynucleotides encoding a  $\Delta 6$ -desaturase, a  $\Delta 6$ -elongase, a  $\Delta \delta$ -desaturase, a  $\Delta 5$ -elongase and a A4-desaturase operably linked with one or more regulatory sequences.
- 5 19. A recombinant camelina plant or cell according to claim 18 which further comprises one or more polynucleotides encoding a A12-desaturase and/or a  $\omega 3$  desaturase operably linked with one or more regulatory sequences.
- 10 20. A recombinant camelina plant according to claim 18 or 19 wherein the plant is a seed.
21. A recombinant camelina plant or cell according to any one of claims 18 to 20 wherein the desaturase and elongase enzymes are independently derived from algae, bacteria, mould or yeast.
- 15 22. A recombinant camelina plant or cell according to any one of claims 18 to 21 wherein the  $\Delta \delta$ -desaturase comprises an amino acid sequence having at least 50% identity to SEQ ID NO:2 or SEQ ID NO:20, the A6-elongase comprises an amino acid sequence having at least 50% identity to SEQ ID NO:4, SEQ ID NO: 22 or SEQ ID NO:24, the  $\Delta 5$ -desaturase comprises an amino acid sequence having at least 50% identity to SEQ ID NO:6 or SEQ ID NO:10, the  $\Delta 5$ -elongase comprises an amino acid sequence having at least 50% identity to SEQ ID NO:8 and the A4-desaturase comprises an amino acid sequence having at least 50% identity to SEQ ID NO:16, SEQ ID NO: 20 25 18 or SEQ ID NO:26.
- 30 23. A recombinant camelina plant or cell according to any one of claims 18 to 22 wherein the  $\Delta 6$ -desaturase is derived from *Ostreococcus tauri*, the  $\Delta 6$ -elongase is derived from *Physcomitrella patens*, the  $\Delta 5$ -desaturase is derived from *Thraustochytrium sp.*, the  $\Delta 5$ -elongase is derived from *Ostreococcus tauri* and the A4-desaturase is derived from *Emiliana huxleyi* or *Thraustochytrium sp*

24. A method for producing docosahexaenoic acid (DHA) comprising growing a plant or cell according to any one of claims 18 to 23 under conditions wherein said desaturase and elongase enzymes are expressed and DHA is produced in said plant or cell.

5

25. A method for producing EPA comprising growing a plant or cell according to according to any one of claims 18 to 23 under conditions wherein said desaturase and elongase enzymes are expressed and EPA is produced in said plant.

10

26. A method for producing a camelina oil comprising growing a plant or cell according to any one of claims 18 to 25 wherein said desaturase and elongase enzymes are expressed and oil is produced in said plant or cell.

15

27. A plant seed oil produced by the method of claim 26.

28. A plant seed oil according to claim 27 wherein DHA constitutes at least 3%, at least 5%, at least 7%, at least 10%, or at least 13% (mol %) of the total amount of fatty acid present in said oil.

20

29. A plant seed oil wherein DHA constitutes at least 3%, at least 5%, at least 7%, at least 10%, or at least 13% (mol %) of the total fatty acid content of said oil and the  $\gamma$ -linolenic (GLA) constitutes less than 5% (mol %) of the total fatty acid content of said oil.

25

30. A plant seed oil according to claim 29 wherein the DHA constitutes at least 13% (mol %) of the total fatty acid content of said oil.

30

31. A plant seed oil according to claim 30 wherein the DHA constitutes 5% to 15%, 10% to 15% or 10% to 13.7% (mol %) of the total fatty acid content of said oil.

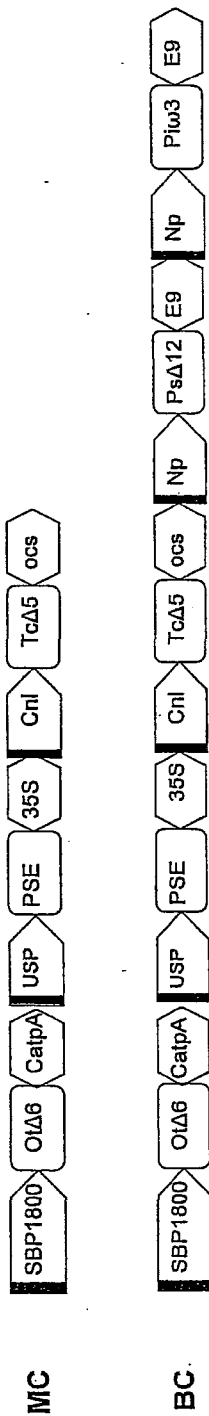
35

32. A plant seed oil according to any one of claims 29 to 31 wherein the GLA constitutes less than 3.5 % (mol %) of the total fatty acid content of said oil.

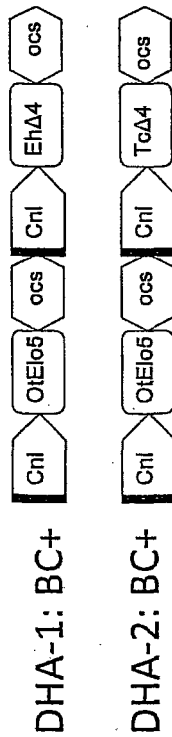
33. A plant seed oil according to any one of claims 29 to 31 wherein the GLA constitutes 0.5% to 5% (mol %) of the total fatty acid content of said oil.
- 5 34. A plant seed oil according to any one of claims 29 to 31 wherein the GLA constitutes 1.5% to 3.5% (mol %) of the total fatty acid content of said oil.
35. A plant seed oil according to any one of claims 29 to 34 wherein the ratio of the percentages by weight of EPA to DHA is about 0.8:1 to about 1.4:1.
- 10 36. A plant seed oil according to any one of claims 29 to 35 wherein the ratio of the molar percentages of the sum of (EPA + DHA) to GLA is about 20:1 to about 3:1, or about 17:1 to about 7:1.
- 15 37. A plant seed oil according to any one of claims 29 to 36 wherein the oil is produced by the method of claim 26.
38. Use of camelina in the manufacture of a long chain polyunsaturated acid.
- 20 39. Use according to claim 38 wherein the long chain polyunsaturated acid is an omega-3 long chain polyunsaturated acid
40. Use according to claim 38 wherein the long chain polyunsaturated acid is EPA.
- 25 41. Use according to claim 38 wherein the long chain polyunsaturated acid is acid DHA.
- 30 42. A feedstuff, food, cosmetic or pharmaceutical comprising the oil as defined in any one of claims 9 to 17 or 27 to 37.



Fig2 EPA-constructs



DHA-constructs



Simplified maps of the vector constructs used for Camelina transformation.

Cnl, conlinin 1promoter for the gene encoding the flax 2S storage protein conlinin; USP, promoter region of the unknown seed protein of *V.faba*; SBP1800; NP, napin; OtΔ6, a Δ6-desaturase from *O. taurii*; TcΔ5- a Δ5-desaturase from *Thraustochytrium* sp.; Pi ω3- an ω-3 desaturase from *P. infestans*; PsΔ12- a Δ12-desaturase from *P. sojae*; EhΔ4 – a Δ4-desaturases from *Emiliania huxleyi*; PSE1, a Δ6-elongase from *P. patens*, OtElo5- Δ5-elongase from *Ostreococcus tauri*; OCS, 35S, E9 and CatpA – represent terminators.

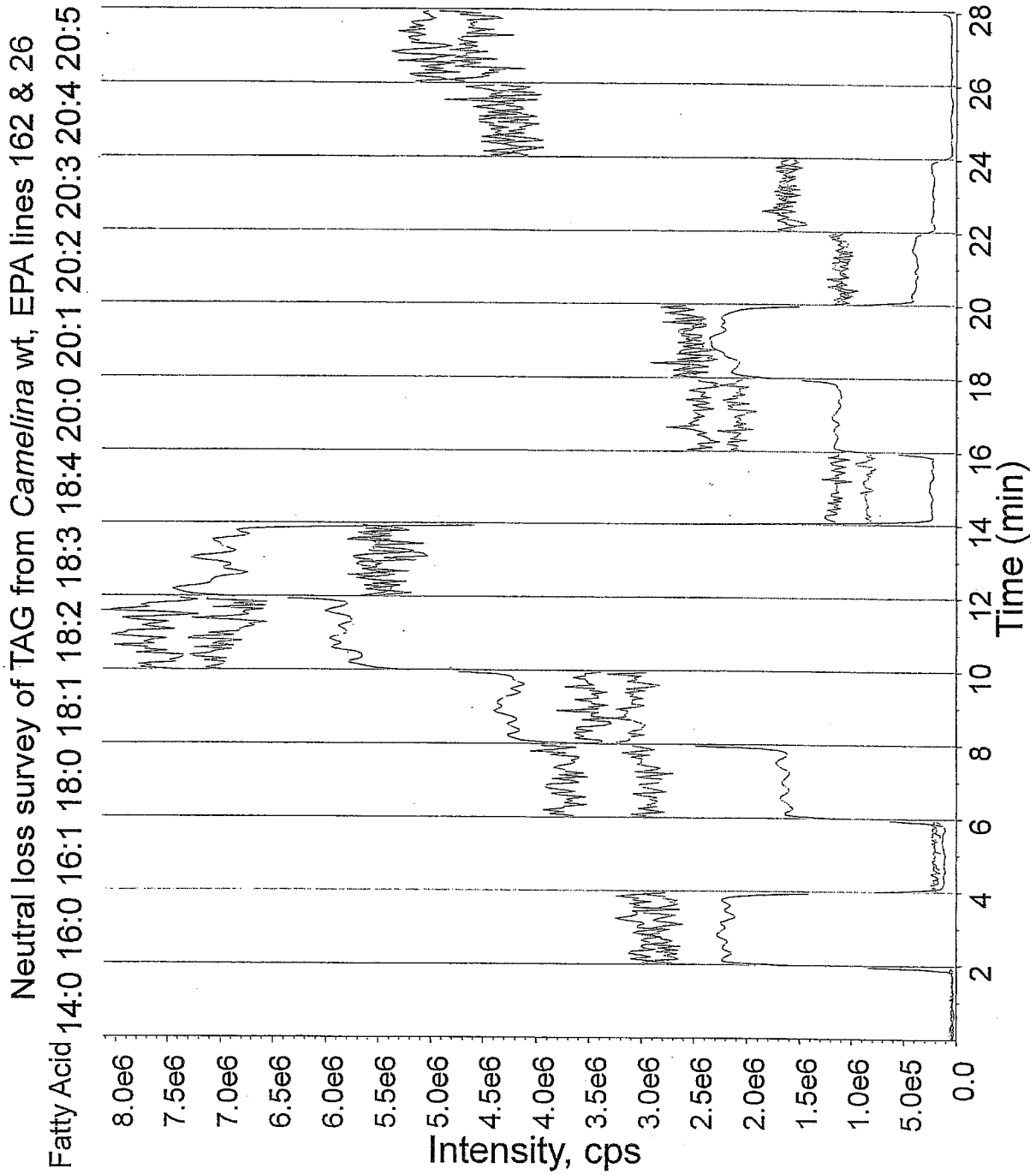
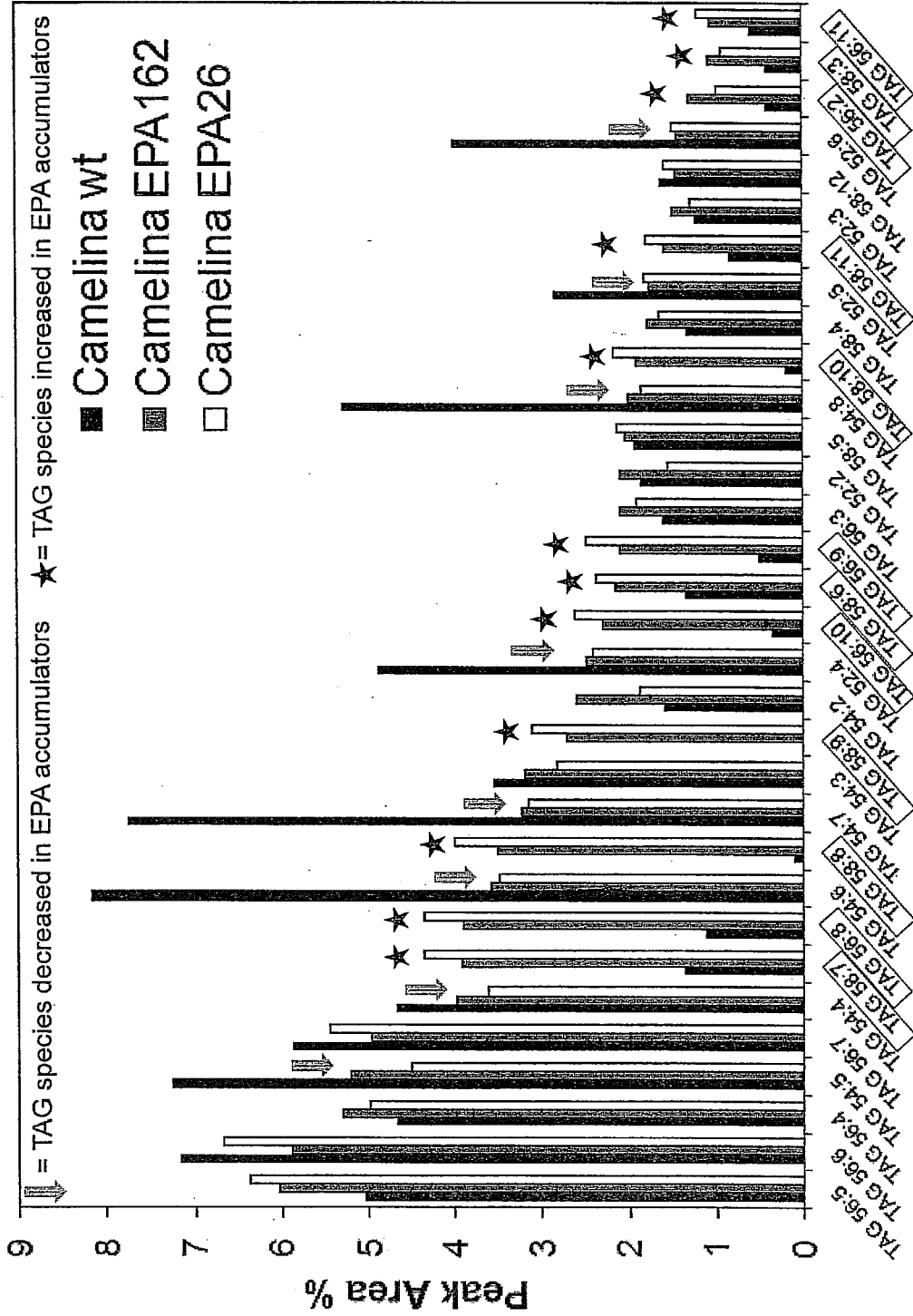


Fig 3



Triacylglycerol species (>1%) identified by ESI-MS

Fig 4



Sample MS spectra showing positive precursor ion scan for 184 (PC)

Fig 5

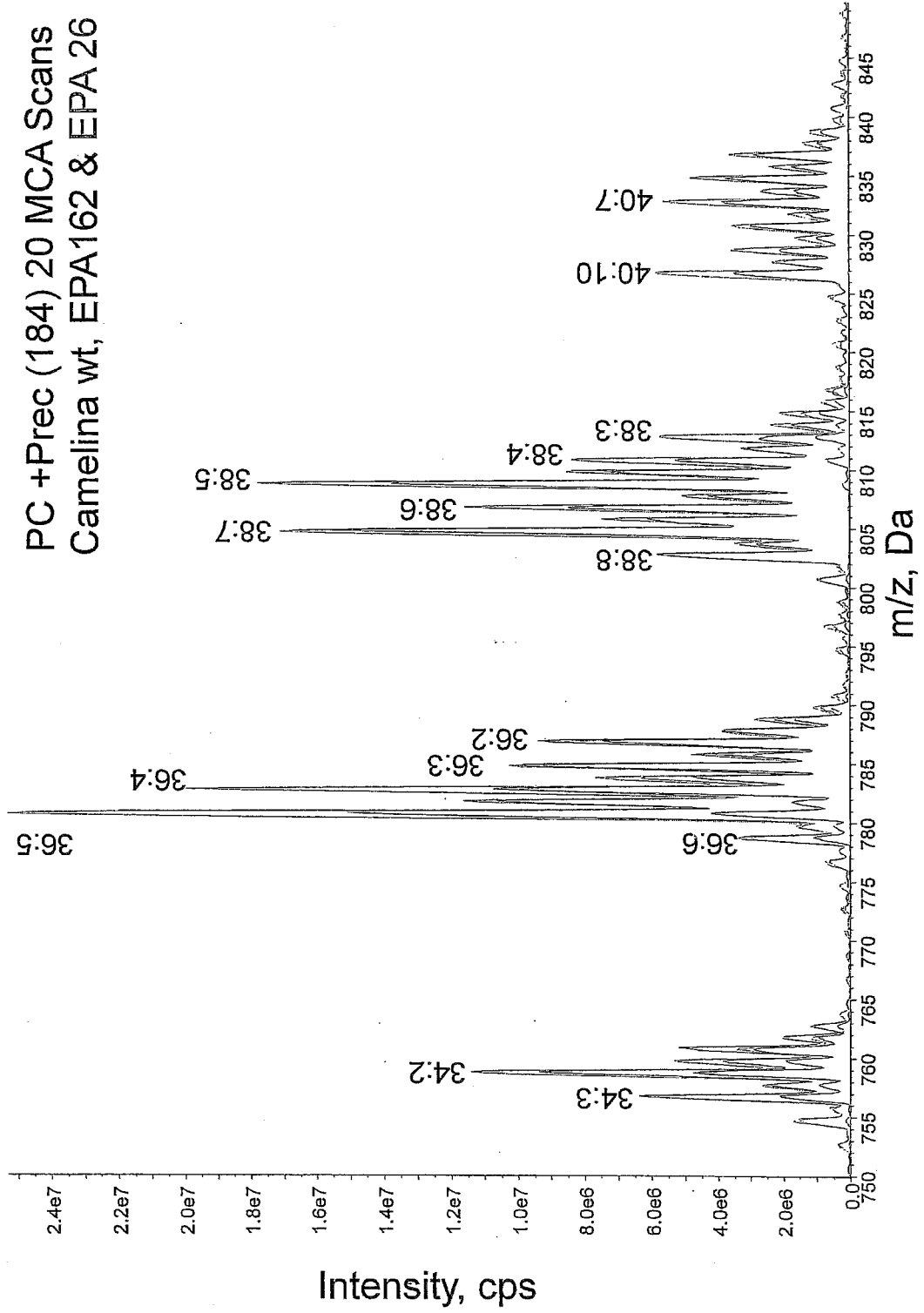
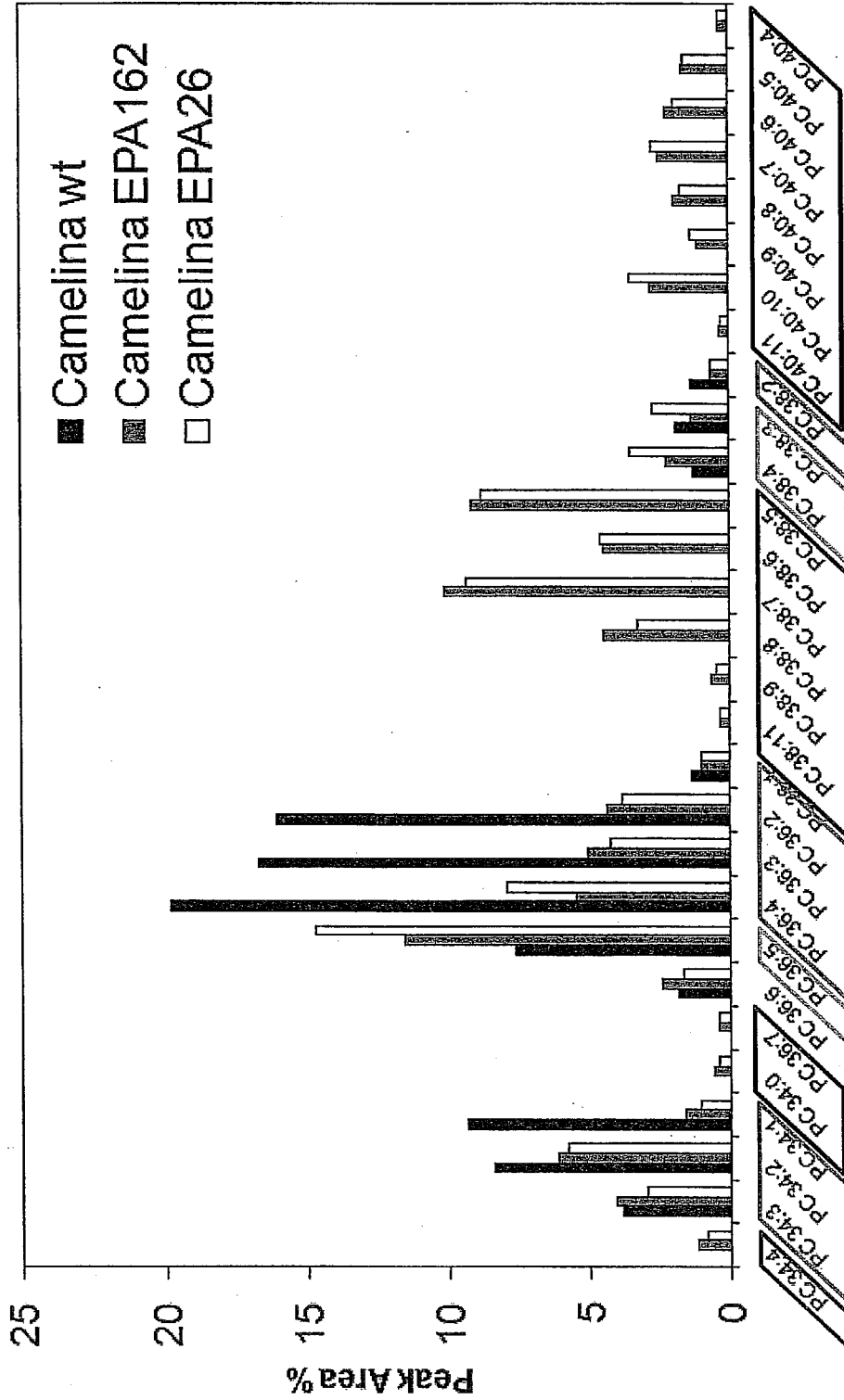
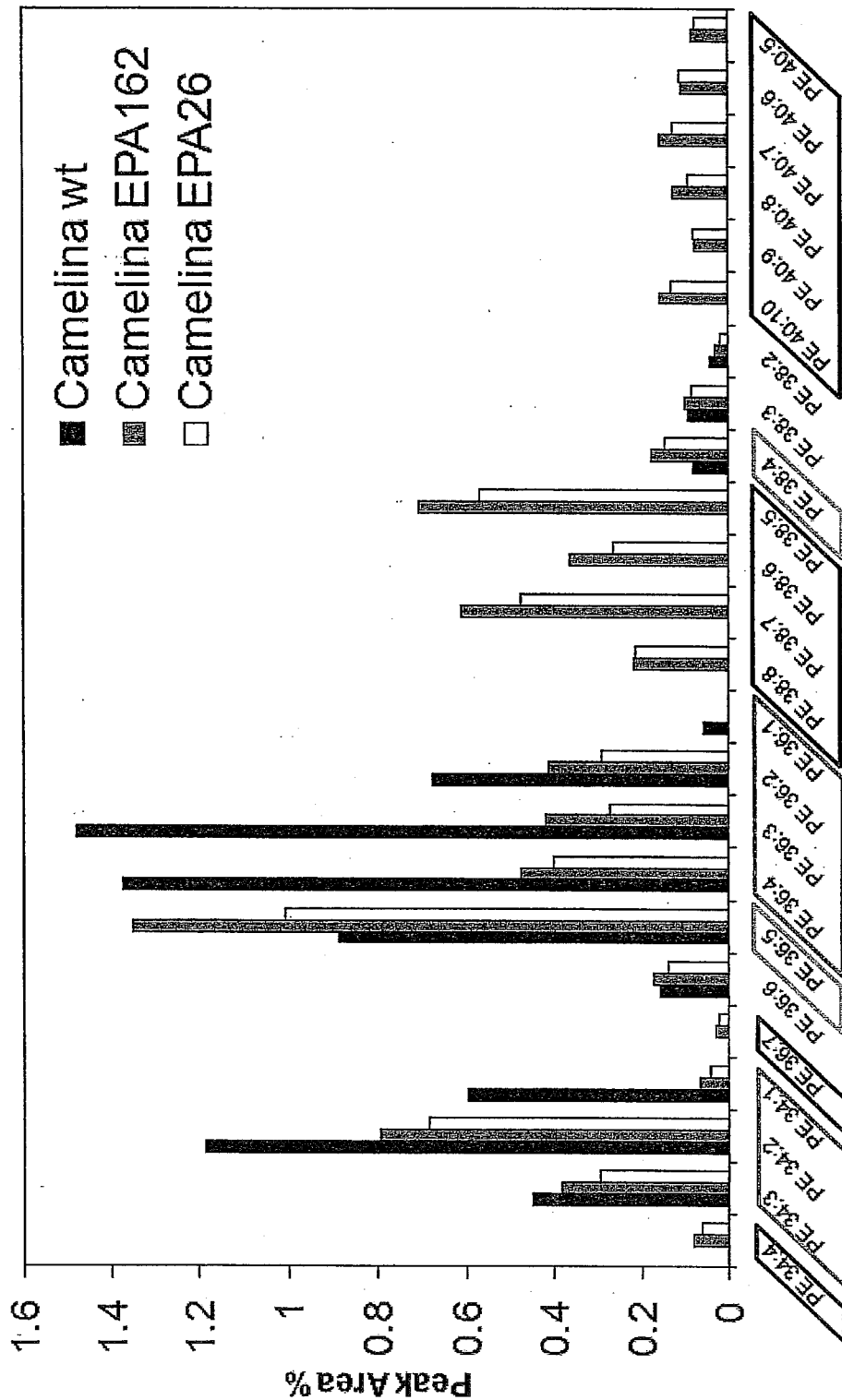


Fig 6 – Distribution of acyl chains within phospholipids of WT and EPA- accumulating Camelina: phosphatidylcholine (PC)



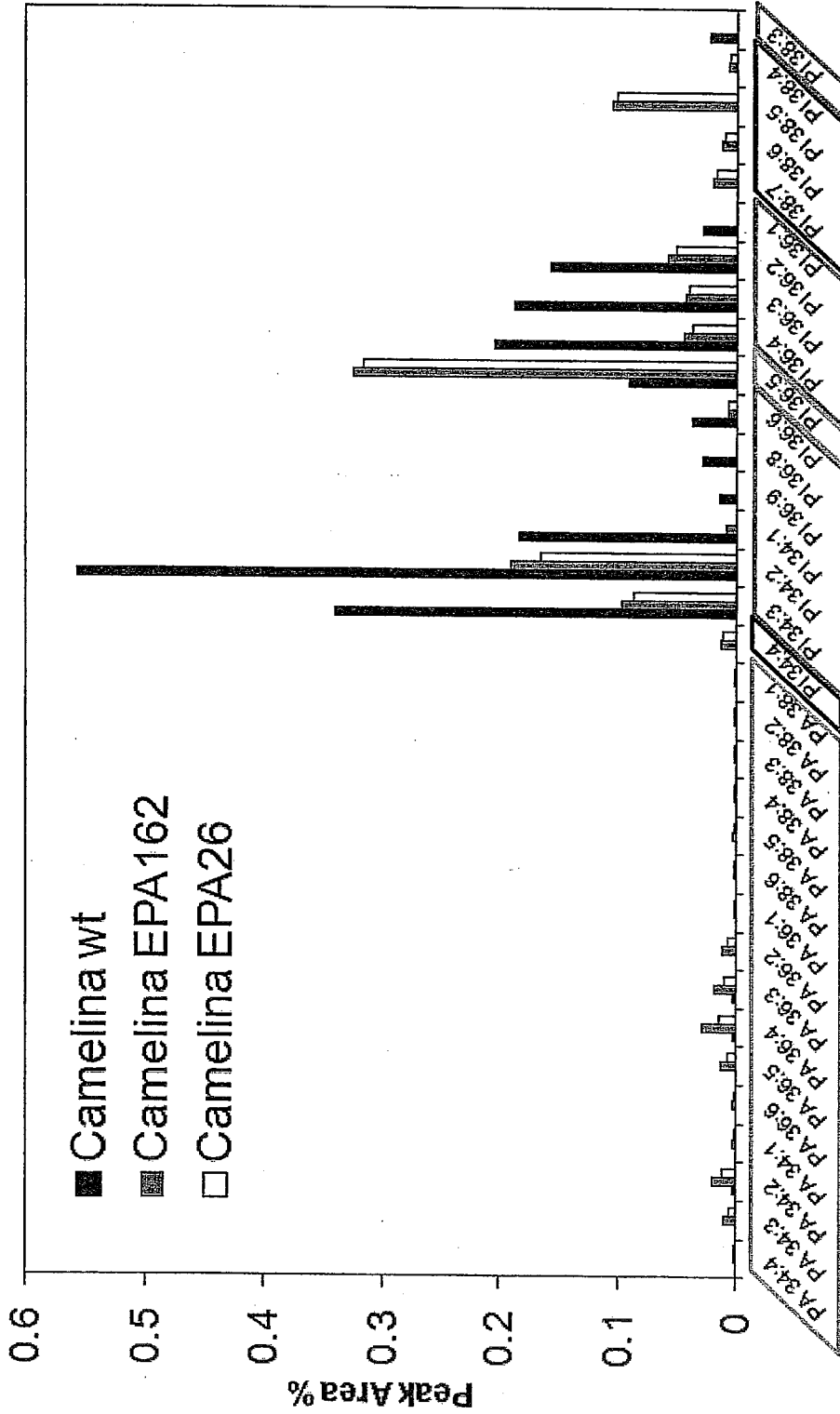
Novel species of PC present in transgenic lines indicated with red boxes [classification based on total chain length for sn-1 and sn-2 positions, and number of double bonds]. PC species significantly reduced in transgenics are boxed in blue; orange box = increased above WT levels

Fig 7 – Distribution of acyl chains within phospholipids of WT and EPA-accumulating Camelina: phosphatidylethanolamine (PE)



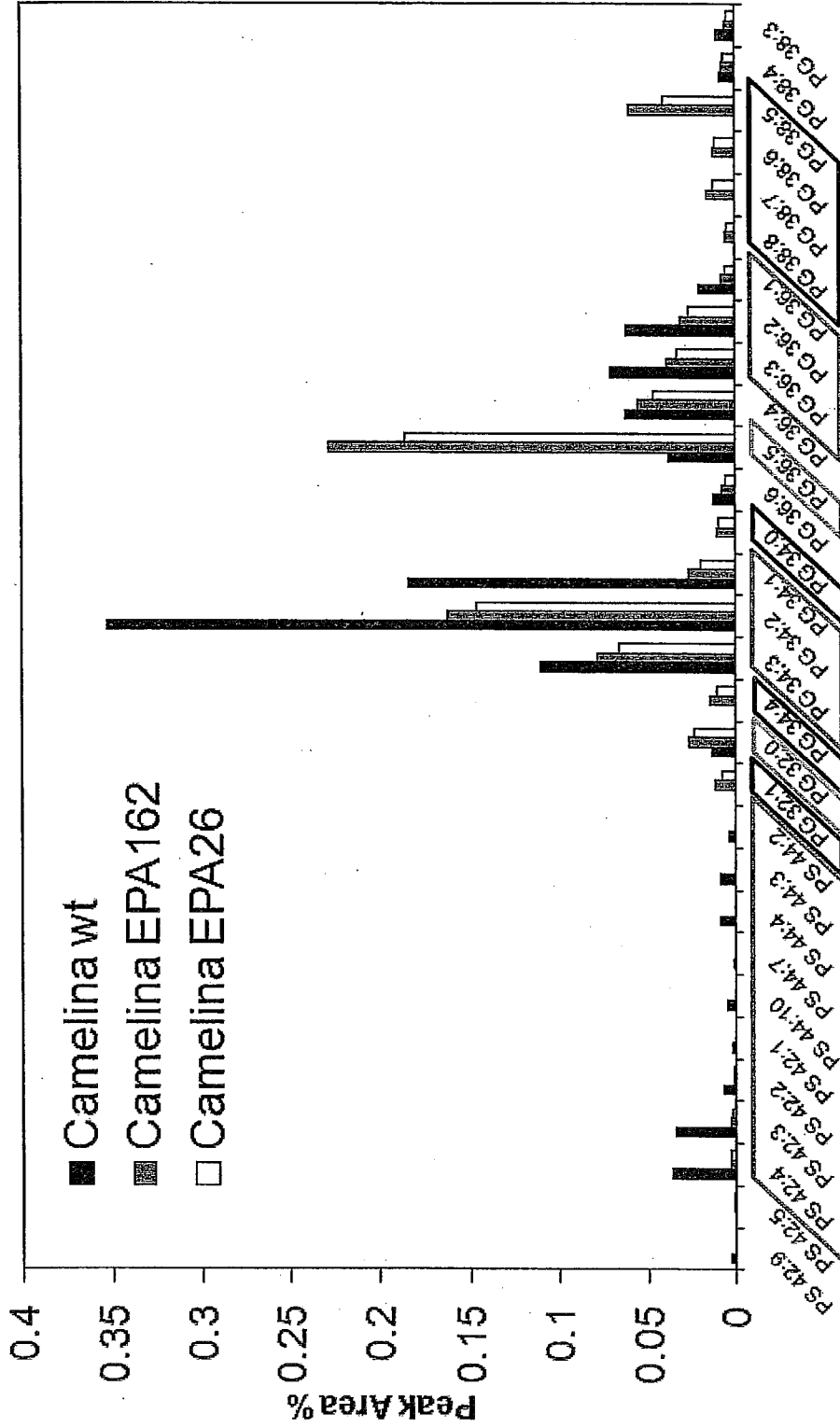
Novel species of PE present in transgenic lines indicated with red boxes [classification based on total chain length for sn-1 and sn-2 positions, and number of double bonds]. PE species significantly reduced in transgenics are boxed in blue; orange = increased above WT

Fig 8 – Distribution of acyl chains within phospholipids of WT and EPA- accumulating Camelina: phosphatidic acid (PA) and phosphoinositol (PI)



Novel species of PA or PI present in transgenic lines indicated with red boxes [classification based on total chain length for sn-1 and sn-2 positions, and number of double bonds]. PA/PI species significantly reduced in transgenics are boxed in blue; orange = increased above WT

Fig 9 – Distribution of acyl chains within phospholipids of WT and EPA-accumulating Camelina: phosphatidylserine (PS) and phosphatidylglycerol (PG)



Novel species of PS or PG present in transgenic lines indicated with red boxes [classification based on total chain length for sn-1 and sn-2 positions, and number of double bonds]. PS/PG species significantly reduced in transgenics are boxed in blue

Fig 10. Analysis of Acyl CoA (LC - MS MS QTRAP) from Mid Development Arabidopsis & Camelina Seed

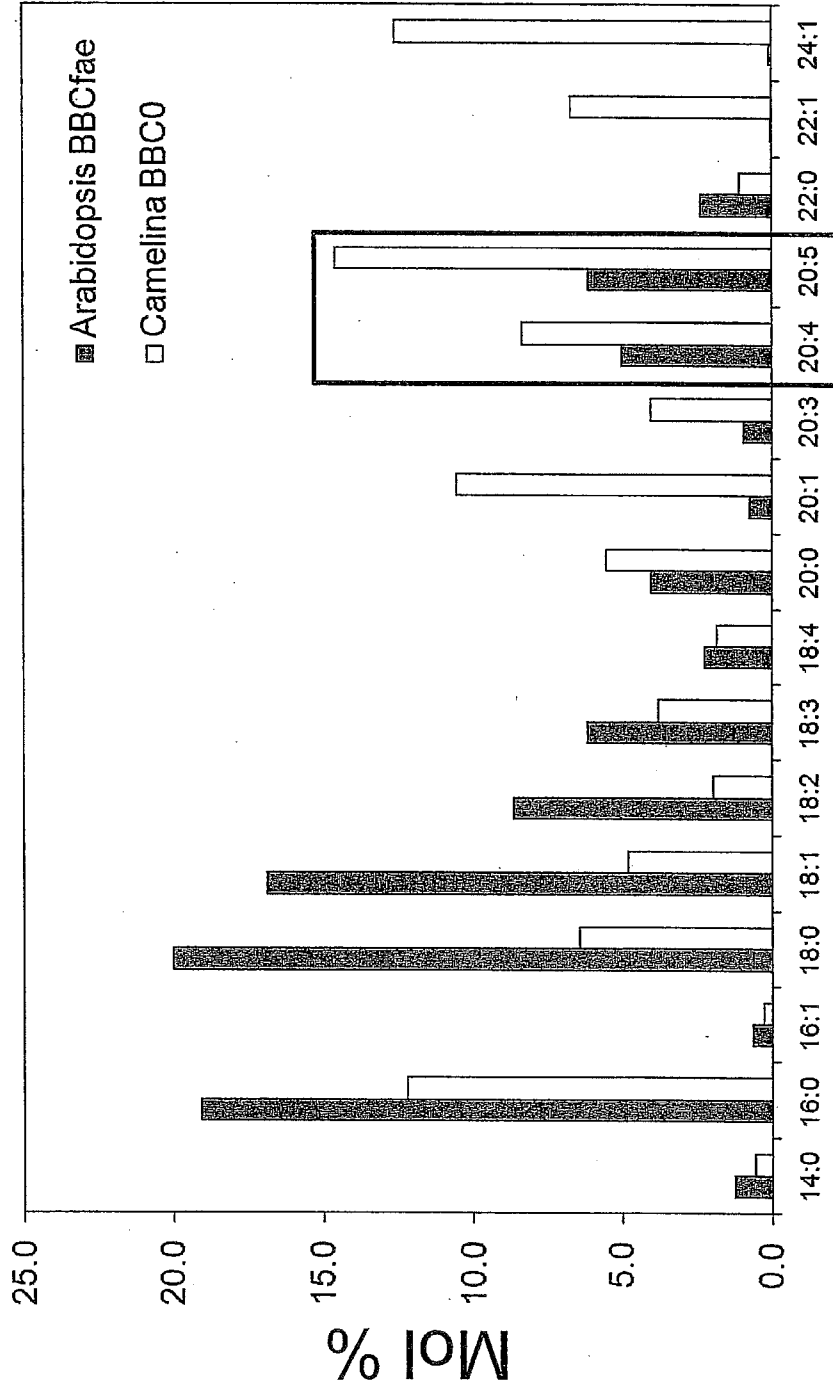
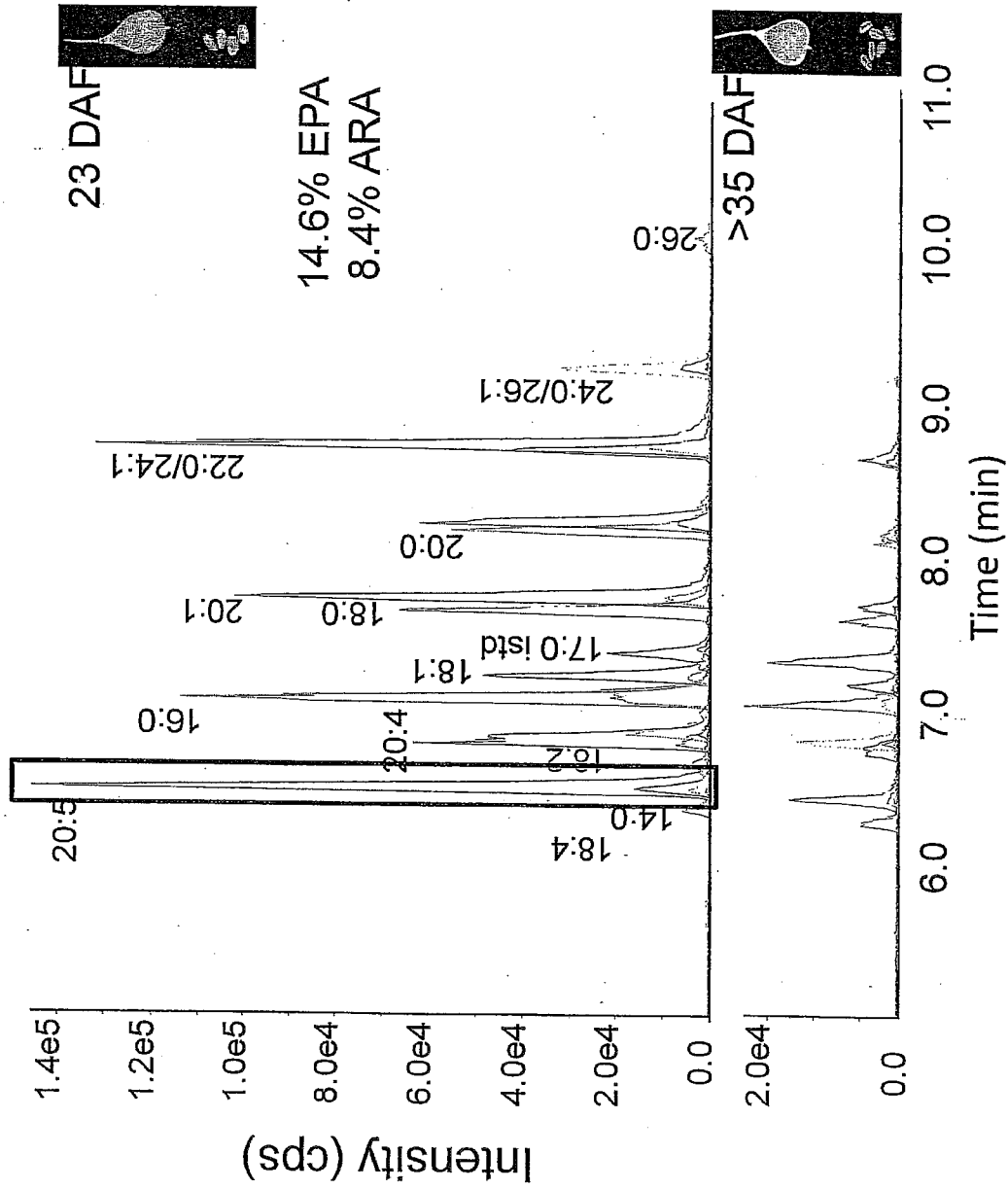


Fig 11: XIC of Acyl CoA MRM (26 pairs) *Camelina* Seed expressing MC (BBC0) construct





INTERNATIONAL SEARCH REPORT

International application No  
PCT/GB2013/050955

A. CLASSIFICATION OF SUBJECT MATTER  
INV. C12N15/82 C12N9/10 C12N9/02  
ADD.

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

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal , CHEM ABS Data, BIOSIS, Sequence Search , EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OLGA SAYANOVA ET AL: "The role of [Delta] 6-desaturase acyl-carrier specificity in the efficient synthesis of long-chain polyunsaturated fatty acids in transgenic plants" , PLANT BIOTECHNOLOGY JOURNAL, vol . 10, no. 2, 8 September 2011 (2011-09-08) , pages 195-206, XP055071442 , ISSN: 1467-7644, DOI : 10.1111/j.1467-7652.2011.00653.x	1,3-6, 18, 20-23 , 26,38,39
Y	the whole document	2, 7,8, 12, 13 , 19 , 24-26, 30,31 , 40,41
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Further documents are listed in the continuation of Box C.

See patent family annex.

\* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

18 September 2013

Date of mailing of the international search report

25/09/2013

Name and mailing address of the ISA/  
European Patent Office, P.B. 5818 Patentlaan 2  
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Fax: (+31-70) 340-3016

Authorized officer

Puonti -Kaerl as, J

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/GB2013/05Q955

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
  
2.  As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
  
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-10, 40(completely) ; 18-23, 25-27, 38, 39, 42(partially)

A recombinant camelina plant or cell expressing a delta 6-desaturase, a delta 6-elongase and/or a delta 5-desaturase and subject-matter relating thereto

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2. claims: 11-17, 29-36(completely) ; 37, 42(partially)

A plant seed oil wherein the EPA constitutes at least 15% (mol %) of the total fatty acid content of said oil, and the  $\gamma$ -linolenic (GLA) constitutes less than 10% (mol %) of the total fatty acid content of said oil, and subject-matter relating thereto

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3. claims: 24, 28, 41(completely) ; 18-23, 25-27, 37-39, 42(partially)

As invention group 1, but relating to plants further expressing a delta 5-elongase and/or a delta 4-desaturase

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## INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2013/050955

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/234006 AI (CI RPUS PETRA [DE] ET AL) 17 September 2009 (2009-09-17)	9-11 , 14-17
Y	the whole document	2, 7, 8, 12, 13 , 19 , 24-26, 30,31 , 40,41
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X	N. RUIZ-LOPEZ ET AL: "Metabolic engineering of the omega-3 long chain polyunsaturated fatty acid biosynthetic pathway into transgenic plants", JOURNAL OF EXPERIMENTAL BOTANY, vol . 63, no. 7, 1 April 2012 (2012-04-01) , pages 2397-2410, XP055071443 , ISSN: 0022-0957 , DOI : 10. 1093/jxb/err454	27-29 , 32-35 ,37
Y	the whole document	2, 7, 8, 19 , 24-26,41
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X	Wo 2011/006948 AI (BASF PLANT SCIENCE CO GMBH [DE] ; BAUER JOERG [DE] ; NAPIER JOHNATHAN A) 20 January 2011 (2011-01-20)	9, 10, 14, 15, 17 , 27-29 , 32-37 ,42
Y	the whole document	40,41
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A	OLGA SAYANOVA ET AL: "Transgenic oilseed crops as an alternative to fish oils", PROSTAGLANDINS LEUKOTRIENES AND ESSENTIAL FATTY ACIDS, vol . 85, no. 5, 2011 , pages 253-260, XP028309060, ISSN: 0952-3278, DOI : 10. 1016/J . PLEFA.2011 .04.013 [retrieved on 2011-05-07]	1-42
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A	VENEGAS-CALERON M ET AL: "An alternative to fish oils: Metabolic engineering of oil-seed crops to produce omega-3 long chain polyunsaturated fatty acids", PROGRESS IN LIPID RESEARCH, PERGAMON PRESS, PARIS, FR, vol . 49, no. 2, 1 April 2010 (2010-04-01) , pages 108-119 , XP026911073 , ISSN: 0163-7827 , DOI : 10. 1016/J . PLI PRES.2009 .10.001 [retrieved on 2009-10-24]	1-42
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## INTERNATIONAL SEARCH REPORT

International application No  
PCT/GB2013/050955

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHAOFU LU AND JINLING KANG: "Generati on of transgeni c plants of a potenti al oilseed crop Camelina sati va by Agrobacteri um-medi ated transformati on" , PLANT CELL REPORTS, SPRINGER VERLAG, DE, vol . 27, no. 2, 1 February 2008 (2008-02-01) , pages 273-278, XP002659396, ISSN: 0721-7714, DOI : 10. 1007/S00299-007-0454-0 [retri eved on 2007-09-27] -----	1-42
A	wo 2009/133145 AI (BASF PLANT SCIENCE GMBH [DE] ; BAUER JOERG [DE] ; NAPI ER JOHNATHAN A [GB] 5 November 2009 (2009-11-05) cited in the appl icati on -----	1-42

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2013/050955

Patent document cited in search report	Publication date	Patent family member(s)	Publication date	
US 2009234006	A1	17-09-2009	AR 058650 A1	20-02-2008
			AU 2006278035 A1	15-02-2007
			CA 2617714 A1	15-02-2007
			CN 101238216 A	06-08-2008
			DE 102005038036 A1	15-02-2007
			EP 1915451 A2	30-04-2008
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