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ABSTRACT

The present invention relates to methods of producing industrial products from plant lipids, particularly from vegetative parts of plants. In particular, the present invention provides oil products such as biofuel, and processes for producing these products, as well as plants having an increased level medium chain fatty acids such as lauric acid and myristic acid. In one particular embodiment, the present invention relates to combinations of modifications in a fatty acid thioesterase and one or more acyltransferases. In an embodiment, the present invention relates to a process for extracting lipids. In another embodiment, the lipid is converted to one or more hydrocarbon products in harvested plant vegetative parts to produce alkyl esters of the fatty acids which are suitable for use as a renewable biofuel.

PLANTS PRODUCING MODIFIED LEVELS OF MEDIUM CHAIN FATTY ACIDS

This is a divisional application of Australian Patent Application 2018201932, the entire contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to methods of producing industrial products from plant lipids, particularly from vegetative parts of plants. In particular, the present invention provides oil products such as biofuel, and processes for producing these products, as well as plants having an increased level medium chain fatty acids such as lauric acid and myristic acid. In one particular embodiment, the present invention relates to combinations of modifications in a fatty acid thioesterase and one or more acyltransferases. In an embodiment, the present invention relates to a process for extracting lipids. In another embodiment, the lipid is converted to one or more hydrocarbon products in harvested plant vegetative parts to produce alkyl esters of the fatty acids which are suitable for use as a renewable biofuel.

BACKGROUND OF THE INVENTION

Over recent years the global production of vegetable oils has experienced constant growth, with over 179 million metric tons (MMT) being produced in 2015 (OECD/FAO, 2015), with the four major oil production crops being oil palm, soybean, canola and sunflower. An important component of global oil consumption is medium-chain fatty acids (MCFA), here defined as fatty acids in the range of 6-14 carbons in length. As well as their application within the food industry MCFAs are an ideal source for biodiesel and also for a wide range of oleochemical feedstocks including pharmaceuticals, personal care products, lubricants and detergents (Arkcoll, 1988; Basiron and Weng, 2004). Currently, the predominant crop sources of MCFA-enriched oils are coconut palm and oil palm (both palm oil and palm kernel oil) (Arkcoll, 1988). The production of these crops is limited to tropical and subtropical climates. The development of new crops that can produce MCFA-enriched oils in temperate climates has been proposed (Dehesh, 2001; Eccleston et al., 1996; Reynolds et al., 2015; Tjellstrom et al., 2013; Voelker et al., 1992; Wiberg et al., 2000) as a way to meet the growing global demand for MCFA in oleochemical production, pharmaceutical applications, and personal care products.

Many studies have investigated the modification of seed oils to contain increased MCFA content, predominantly focused on the engineering of lauric acid (C12:0) (Eccleston and Ohlrogge, 1998; Knutzon et al., 1999; Voelker et al., 1992). In oilseeds the engineered pathway begins with the overexpression of a specialised

thioesterase (FATB) that prematurely truncates the standard fatty acid elongation cycle within the plastid allowing export into the cytoplasm. The MCFA in the cytoplasm is available for incorporation into triacylglycerols (TAG) via the endogenous oilseed pathways which can occur via the acyl-CoA dependent reactions of the Kennedy pathway (glycerol-3-phosphate acyltransferase (GPAT), lysophosphatidic acid acyltransferase (LPAAT) and diacylglycerol acyltransferase (DGAT). Previous studies have investigated the incorporation of MCFA into seed oils following the coordinated over-expression of FATB and LPAAT, achieving up to 67% of laurate (C12:0) in seed oil (Knutzon et al., 1999). More recently, transcriptomic analyses have enabled the identification of new FATB and LPAAT genes from many *Cuphea* species, which have been used to both modify the fatty acid profiles and improve the incorporation of MCFA into the TAG, respectively, of transgenic *C amelina sativa* seeds (Kim et al., 2015a; Kim et al., 2015b).

Evidence, although, has found that endogenous TAG synthesis pathways in developing oilseeds are not ideal for incorporating MCFA into TAG (Wiberg et al., 1997; Wiberg et al., 2000), and that newly-synthesised MCFA becomes incorporated into membrane bound lipids, impeding lipid flux, agronomic performance and can even result in cell death through chlorosis (Bates et al., 2014; Voelker et al., 1996). Therefore it would seem that although MCFA can be produced in plant cells there is a poor pathway for incorporation into seed TAG. It has also been recognised that the accumulation of unusual fatty acids in PC appears to be a bottleneck for their enriched incorporation into TAG (Bates and Browse, 2011; Reynolds et al., 2015). In the example of engineering ricinoleic acid into oilseeds it has been demonstrated that the endogenous pathways need to be removed in conjunction with the ectopic expression of the specialised pathway counterpart (Adhikari et al., 2016; Bates and Browse, 2011; Burgal et al., 2008; Chen et al., 2016; van Erp et al., 2011; van Erp et al., 2015).

Recent work has demonstrated that engineering high oil levels in plant biomass is a realistic proposition (Vanhercke et al., 2014a; Vanhercke et al., 2013; Vanhercke et al., 2014b) with the accumulation of levels of TAG in *Nicotiana tabacum* leaves of up to 15% being attained by the coordinated transgenic expression of genes normally involved in oil production in seeds (Vanhercke et al., 2014a). Such approaches have uncovered a synergism involving an increase in the production of fatty acids in the plastid (WRINKLED1 (WRI1)), improving the assembly of fatty acids into leaf oils (DGAT) and slowing the catabolism of these oils (OLEOSIN, OLE1 (Winichayakul et al., 2013)); and sugar-dependent-1, SDP1 (Fan et al., 2014; Kelly et al., 2013a and b; Kim et al., 2014b; Vanhercke, 2014a). Although the production of TAG in biomass

offers a new source of common vegetable oils, these new expression platforms could also be adapted to produce high levels of novel fatty acids, such as MCFA (Reynolds et al., 2015; Wood, 2014).

The inventors first steps in this direction involved the overexpression of thioesterases from *Umbellularia californica*, *Cinnamomum camphora* and *Cocos nucifera* which resulted in the production of MCFA in leaf tissues (Reynolds et al., 2015). However, these metabolic pathways also resulted in high levels of MCFA in PC resulting in severe chlorosis and cell death (Bates et al., 2014; Wiberg et al., 2000), similar to conclusions drawn from oilseed engineering. The incorporation of MCFA into the membrane lipids of vegetative tissues is therefore particularly problematic.

The inventors have improved the MCFA metabolic pathway by combining a series of gene ensembles with three different DGAT1 genes isolated from *Elaeis guineensis* (African oil palm). A functional GPAT9 from *C. nucifera* was identified that was included in the metabolic pathway for improving the incorporation of MCFA into seed oils. An improvement in MCFA utilisation was demonstrated in vegetative plant cells such as leaf cells, which resulted in more efficient sequestering of MCFA in TAG while also effectively limiting the accumulation of MCFA in membrane lipids.

SUMMARY OF THE INVENTION

In a first aspect, the present invention provides a process for producing extracted plant lipid, comprising the steps of:

a) obtaining one or more plant parts comprising lipid, preferably vegetative plant parts, the lipid comprising a total fatty acid content which comprises fatty acids in an esterified form, the fatty acids comprising a level of total, or new, medium chain fatty acids (MCFA) that is at least 25% of the total fatty acid content on a weight basis, and

b) extracting lipid from the plant part(s),

thereby producing the extracted plant lipid.

In an embodiment, the plant part comprises one or more exogenous polynucleotides which encode polypeptides having fatty acid thioesterase (TE) activity, and either glycerol-3-phosphate acyltransferase (GPAT) activity, preferably GPAT9 activity, or diacylglycerol acyltransferase (DGAT) activity, preferably DGAT1 activity, or both GPAT and DGAT,

wherein the exogenous polynucleotide is operably linked to a promoter which is capable of directing expression of the polynucleotide in a cell of the plant part.

In a further embodiment, the plant part further comprises one or more or all of:

i. an exogenous polynucleotide which encodes a second polypeptide having glycerol-3-phosphate acyltransferase (GPAT) activity, preferably GPAT9 activity, or diacylglycerol acyltransferase (DGAT) activity, preferably DGAT1 activity;

ii. an exogenous polynucleotide which encodes a third polypeptide having 1-acyl-glycerol-3-phosphate acyltransferase (LPAAT) activity;

iii. an exogenous polynucleotide which encodes a transcription factor polypeptide that increases the expression of one or more glycolytic and/or fatty acid biosynthetic genes in a cell of the plant part compared to a corresponding cell lacking the exogenous polynucleotide;

iv. an exogenous polynucleotide which encodes a polypeptide which increases the export of fatty acids out of plastids of a cell in the plant part when compared to a corresponding cell lacking the exogenous polynucleotide; and

v. an exogenous polynucleotide which encodes an oil body coating (OBC) polypeptide,

wherein each exogenous polynucleotide is operably linked to a promoter which is capable of directing expression of the polynucleotide in a cell of the plant part.

In an embodiment, the OBC polypeptide is an oleosin, such as a polyoleosin or a caleosin, or a lipid droplet associated protein (LDAP).

In an embodiment, the transcription factor polypeptide is selected from the group consisting of Wrinkled 1 (WRI1), Leafy Cotyledon 1 (LEC1), LEC1-like, Leafy Cotyledon 2 (LEC2), BABY BOOM (BBM), FUS3, ABI3, ABI4, ABI5, Dof4 and Dof11, preferable WRI1, or the group consisting of MYB73, bZIP53, AGL15, MYB115, MYB118, TANMEI, WUS, GFR2a1, GFR2a2 and PHR1.

In an embodiment, the polypeptide which increases the export of fatty acids out of plastids of the cell is a fatty acid thioesterase such as a FATA polypeptide or a FATB polypeptide, a fatty acid transporter such as an ABCA9 polypeptide or a long-chain acyl-CoA synthetase (LACS), preferably a FATB polypeptide.

In an embodiment, the fatty acid thioesterase is capable of hydrolysing a substrate which is an acyl carrier protein (ACP) esterified to a medium chain fatty acid and/or a C16:0, preferably wherein the MCFA is a C10, C12 and/or C14.

In an embodiment, the plant part further comprises one or more or all of:

i. a genetic modification which down-regulates endogenous production and/or activity of a polypeptide involved in the catabolism of triacylglycerols (TAG) in a cell of the plant part when compared to a corresponding cell lacking the genetic modification;

ii. a genetic modification which down-regulates endogenous production and/or activity of a polypeptide involved in importing fatty acids into plastids of a cell in the plant part when compared to a corresponding cell lacking the genetic modification; and

iii. a genetic modification which down-regulates endogenous production and/or activity of a polypeptide involved in diacylglycerol (DAG) production in the plastid when compared to a corresponding cell in the plant part lacking the genetic modification.

In an embodiment, the polypeptide involved in the catabolism of triacylglycerols (TAG) in the plant, or part thereof, is an SDP1 lipase, a Cgi58 polypeptide, an acyl-CoA oxidase such as ACX1 or ACX2, or a polypeptide involved in β-oxidation of fatty acids in the plant or part thereof such as a PXA1 peroxisomal ATP-binding cassette transporter, preferably an SDP1 lipase.

In an embodiment, the polypeptide involved in importing fatty acids into plastids of the cell is a fatty acid transporter, or subunit thereof, preferably a TGD polypeptide.

In an embodiment, the polypeptide involved in diacylglycerol (DAG) production in the plastid is a plastidial GPAT, a plastidial LPAAT or a plastidial PAP.

In another embodiment, the plant part further comprises one or both of:

i. an exogenous polynucleotide which encodes a second transcription factor polypeptide that increases the expression of one or more glycolytic and/or fatty acid biosynthetic genes in the cell; and

ii. a second genetic modification which down-regulates endogenous production and/or activity of a polypeptide involved in importing fatty acids into plastids of the cell when compared to a corresponding cell lacking the second genetic modification.

In a preferred embodiment, the presence of a) a genetic modification which down-regulates endogenous production and/or activity of a polypeptide involved in the catabolism of triacylglycerols (TAG) in a cell of the plant part when compared to a corresponding cell lacking the genetic modification, b) an exogenous polynucleotide which encodes a polypeptide which increases the export of fatty acids out of plastids of a cell in the plant part when compared to a corresponding cell lacking the exogenous polynucleotide, or c) an exogenous polynucleotide which encodes a second transcription factor polypeptide that increases the expression of one or more glycolytic and/or fatty acid biosynthetic genes in the cell, together with an exogenous polynucleotide which encodes a WRI1 polypeptide and an exogenous polynucleotide

which encodes a polypeptide having DGAT1 activity, increases the total non-polar lipid content of the plant part, preferably a vegetative plant part such as a leaf or stem, relative to a corresponding plant part comprising the exogenous polynucleotides encoding the WRI1 and DGAT1 polypeptides but lacking each of the other exogenous polynucleotide and genetic modifications. Most preferably, at least the promoter that directs expression of the exogenous polynucleotide which encodes the transcription factor is a promoter other than a constitutive promoter. Alternatively for *Sorghum* or *Zea mays*, the promoter is preferably a constitutive promoter such as, for example a ubiquitin gene promoter.

In an embodiment, the addition of one or more of the exogenous polynucleotides or genetic modifications, preferably the exogenous polynucleotide encoding an OBC or a fatty acyl thioesterase or the genetic modification which down-regulates endogenous production and/or activity of a polypeptide involved in the catabolism of triacylglycerols (TAG) in the plant or part thereof, more preferably the exogenous polynucleotide which encodes a FATA thioesterase or an LDAP or which decreases expression of an endogenous TAG lipase such as a SDP1 TAG lipase in the plant or part thereof, results in a synergistic increase in the total non-polar lipid content of the plant or part thereof when added to the pair of transgenes WRI1 and DGAT, particularly before the plant flowers and even more particularly in the stems and/or roots of the plant.

In a preferred embodiment, the increase in the TAG content of a stem or root is at least 2-fold, more preferably at least 3-fold, relative to a corresponding plant part transformed with genes encoding WRI1 and DGAT1 but lacking the FATA thioesterase, LDAP and the genetic modification which down-regulates endogenous production and/or activity of a polypeptide involved in the catabolism of triacylglycerols (TAG) in the plant part. Most preferably, at least the promoter that directs expression of the exogenous polynucleotide which encodes the transcription factor is a promoter other than a constitutive promoter. Alternatively for *Sorghum* or *Zea mays*, the promoter is preferably a constitutive promoter such as, for example a ubiquitin gene promoter.

In an embodiment, each genetic modification is, independently, a mutation of an endogenous gene which partially or completely inactivates the gene, such as a point mutation, an insertion, or a deletion, or an exogenous polynucleotide encoding an RNA molecule which inhibits expression of the endogenous gene, wherein the exogenous polynucleotide is operably linked to a promoter which is capable of directing expression of the polynucleotide in the plant, or part thereof. The point mutation may

be a premature stop codon, a splice-site mutation, a frame-shift mutation or an amino acid substitution mutation that reduces activity of the gene or the encoded polypeptide. The deletion may be of one or more nucleotides within a transcribed exon or promoter of the gene, or extend across or into more than one exon, or extend to deletion of the entire gene. Preferably the deletion is introduced by use of ZF, TALEN or CRISPR technologies. In an alternate embodiment, one or more or all of the genetic modifications is an exogenous polynucleotide encoding an RNA molecule which inhibits expression of the endogenous gene, wherein the exogenous polynucleotide is operably linked to a promoter which is capable of directing expression of the polynucleotide in the plant, or part thereof. Examples of exogenous polynucleotide which reduces expression of an endogenous gene are selected from the group consisting of an antisense polynucleotide, a sense polynucleotide, a microRNA, a polynucleotide which encodes a polypeptide which binds the endogenous enzyme, a double stranded RNA molecule and a processed RNA molecule derived therefrom. In an embodiment, the plant or part thereof comprises genetic modifications which are an introduced mutation in an endogenous gene and an exogenous polynucleotide encoding an RNA molecule which reduces expression of another endogenous gene. In an alternate embodiment, all of the genetic modifications that provide for the increased TTQ and or TAG levels are mutations of endogenous genes.

In an embodiment, the activity of PDCT or CPT in a cell in the plant part is increased relative to a wild-type plant part. Alternatively, the activity of PDCT or CPT is decreased, for example by mutation in the endogenous gene encoding the enzyme or by downregulation of the gene through an RNA molecule which reduces its expression.

In an embodiment, when present, the two transcription factors are WRI1 and LEC2, or WRI1 and LEC1.

In the above embodiments, the plant part preferably comprises an exogenous polynucleotide which encodes a DGAT and a genetic modification which down-regulates production of an endogenous SDP1 lipase. More preferably, the plant part does not comprise an exogenous polynucleotide encoding a PDAT, and/or is a plant part other than a *Nicotiana benthamiana* or part thereof, and/or the WRI1 is a WRI1 other than *Arabidopsis thaliana* WRI1 and/or is a plant part other than a *Brassica napus* or part thereof. In an embodiment, at least one of the exogenous polynucleotides in the plant part is expressed from a promoter which is not a constitutive promoter such as, for example, a promoter which is expressed preferentially in green tissues or stems of the plant or that is up-regulated after commencement of flowering or during senescence.

In an embodiment, the plant part comprises an increased level or activity of polypeptides which are:

i. a GPAT, a LPAAT, and a WRI1 polypeptide;

ii. a GPAT, a LPAAT, a DGAT and a WRI1 polypeptide;

iii. a GPAT9, a LPAAT, and a WRI1 polypeptide;

iv. a GPAT9, a LPAAT, a DGAT, and a WRI1 polypeptide;

v. a GPAT, a LPAAT, a DGAT1, and a WRI1 polypeptide;

vi. a GPAT9, a LPAAT, a DGAT1, and a WRI1 polypeptide;

vii. a GPAT, a LPAAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

viii. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

ix. a GPAT9, a LPAAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

x. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xi. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xii. a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xiii. a GPAT, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xiv. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xv. a GPAT9, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xvi. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xvii. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xviii. a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xix. a GPAT, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xx. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxi. a GPAT9, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxii. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxiii. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxiv. a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxv. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxvi. a GPAT9, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxvii. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as

an oleosin, preferably a caleosin or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxviii. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin, or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase; or a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin, or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase.

In an embodiment, the one or more or all of the polypeptides are encoded by one or more exogenous polynucleotides in the plant parts.

In a second aspect, the present invention provides a process for producing extracted plant lipid, comprising the steps of:

a) obtaining one or more plant parts comprising lipid, preferably vegetative plant parts, the lipid comprising a total fatty acid content which comprises fatty acids in an esterified form, the fatty acids comprising an increased level of medium chain fatty acids (MCFA) relative to a corresponding wild-type plant part, wherein the plant part comprises an increased level or activity of polypeptides which are:

i. a GPAT, a LPAAT, and a WRI1 polypeptide;
ii. a GPAT, a LPAAT, a DGAT and a WRI1 polypeptide;
iii. a GPAT9, a LPAAT, and a WRI1 polypeptide;
iv. a GPAT9, a LPAAT, a DGAT, and a WRI1 polypeptide;
v. a GPAT, a LPAAT, a DGAT1, and a WRI1 polypeptide;
vi. a GPAT9, a LPAAT, a DGAT1, and a WRI1 polypeptide;
vii. a GPAT, a LPAAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;
viii. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;
ix. a GPAT9, a LPAAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;
x. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xi. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xii. a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xiii. a GPAT, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xiv. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xv. a GPAT9, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xvi. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xvii. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xviii. a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xix. a GPAT, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xx. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxi. a GPAT9, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxii. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxiii. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxiv. a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxv. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxvi. a GPAT9, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxvii. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxviii. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin, or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase; or

xxix. a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin, or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase,

and

b) extracting lipid from the plant part(s),

thereby producing the extracted plant lipid.

In an embodiment, the one or more or all of the polypeptides are encoded by one or more exogenous polynucleotides in the plant parts.

In an embodiment, the level of total, or new, MCFA is increased relative to a corresponding wild-type plant part, preferably the level is at least 25% of the total fatty acid content on a weight basis.

In an embodiment of the first and second aspects, the one or more or all of the encoded GPAT, LPAAT and DGAT have a preference for utilising medium chain fatty acid substrates. GPAT, LPAAT and DGAT each use an acyl-CoA substrate, with a second substrate that is G3P, LPA or DAG, respectively.

In an embodiment of the first and second aspects, the extracted lipid has one or more or all of the following features:

i. the level of medium chain fatty acids in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid, is at least about 30%, at least about 35%, at least about 40%, at least about 50%, at least about 55%, or between about 25% and about 55%, between about 25% and about 50%, between about 30% and about 50%, between about 35% and about 50%, between about 25% and about 40%, or between about 30% and about 40%;

ii. the level of lauric acid (C12:0) in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid, is, or is increased by, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 50%, at least or about 55%, or between about 15% and about 55%, between about 20% and about 50%, between about 30% and about 50%, between about 35% and about 50%, between about 15% and about 25%, or between about 20% and about 30%;

iii. the level of myristic acid (C14:0) in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid, is, or is increased by, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, or between about 25% and about 45%, between about 20% and about 50%, between about 30% and about 50%, between about 35% and about 50%, between about 30% and about 40%, between about 15% and about 25%, or between about 20% and about 30%;

iv. the level of palmitic acid (C16:0) in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid, is, or is increased by, between about 2% and about 18%, or between about 2% and

about 16%, or between about 2% and about 15%, or between about 15% and about 50%;

v. the level of lauric acid (C12:0) in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid is, or is increased by, at least about 25%, at least about 30%, at least about 40%, at least about 45%, or at least about 50%, and the level of myristic acid (C14:0) in the total fatty acid content of the extracted lipid and/or in the total fatty acid content of the TAG of the extracted lipid is, or is increased by, at least about 1%, at least about 2%, at least about 5%, or at least about 10%, or between about 1% and about 10%, or between about 2% and 10%, or between about 2% and about 6%, or less than about 10%, or less than about 8% or less than about 5%, or less than about 2%;

vi. the level of myristic acid (C14:0) in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid is, or is increased by, at least about 20%, at least about 25%, at least about 30%, or at least about 40%, and the level of lauric acid (C12:0) in the total fatty acid content of the extracted lipid and/or in the total fatty acid content of the TAG of the extracted lipid is, or is increased by, at least about 1%, at least about 2%, at least about 5%, or at least about 10%, or between about 1% and about 10%, or between about 2% and about 10%, or between about 2% and about 6%, or less than about 10%, or less than about 8% or less than about 5%, or less than about 2%;

vii. the level of myristic acid (C14:0) in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid is, or is increased by, at least about 20%, at least about 25%, at least about 30%, and the level of palmitic acid (C16:0) in the total fatty acid content of the extracted lipid and/or in the total fatty acid content of the TAG of the extracted lipid is, or is increased by, at least about 2%, at least about 3%, at least about 4%, or at least about 5%.

viii. the ratio of lauric acid (C12:0):myristic acid (C14:0) in the fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid, is increased, or is about 1:4, about 1:5, about 1:10, about 1:15, about 1:20, about 1:25, or about 4:1, about 5:1, about 10:1, about 15:1, about 20:1, about 30:1, about 40:1, or about 45:1;

ix. the ratio of lauric acid (C12:0):palmitic acid (C16:0) in the fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the

extracted lipid, is increased, or is about 1:2, about 1:3, about 1:4, about 1:5, about 1:10, about 1:15, about 10:1, about 20:1, about 30:1, about 40:1, or about 45:1;

x. the ratio of myristic acid (C14:0):palmitic acid (C16:0) in the fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid, is increased, or is about 1:2, about 1:3, about 1:4, about 1:5, about 1:10, about 1:15, about 10:1, about 20:1, about 30:1, or about 40:1;

xi. the level of oleic acid in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid, is decreased, or is less than about 10%, less than about 8%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, less than about 1%;

xii. the level of linoleic acid (LA) in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid, is increased or decreased, or is less than about 20%, less than about 15%, less than about 10%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1%;

xiii. the level of α-linolenic acid (ALA) in the total fatty acid content of the extracted lipid, or in the total fatty acid content of the TAG of the extracted lipid, is decreased or is less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 10%, less than about 8%, less than about 6%, less than about 5%, less than about 2%, or less than about 1%;

xiv. the level of total unsaturated fatty acids in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid, is decreased, or is less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 15%, less than about 10%, less than about 8%, less than about 6%, less than about 5%, less than about 2%, or less than about 1%;

xv. the level of total monounsaturated fatty acids in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid, is decreased, or is less than about 10%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1%;

xvi. the level of total polyunsaturated fatty acids in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid, is less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 15%, less than about 10%, less than about 8%,

less than about 6%, less than about 5%, less than about 2%, or less than about 1%;

xvii. the triacylglycerol (TAG) content of the extracted lipid is at least about 80%, at least about 85%, at least about 90%, or least about 95%, and about 98%, or between about 95% and about 98%, by weight of the extracted lipid;

xviii. the TAG content of the extracted lipid comprises, or is increased in a level of, one or more or all of the TAG species 36:0, 38:0, 40:0 and 42:0;

xix. the extracted lipid comprises tri-laurin (tri-C12:0) and/or tri-myristin (tri-C14:0); and

xx. the phosphocholine (PC) content of the extracted lipid comprises one or both of the PC species 28:0 and 30:0,

wherein any increase or decrease is relative to a corresponding wild-type plant part.

In an embodiment, the plant part comprises one or more of the features defined with respect to the first aspect.

In an embodiment of the first and second aspects, the plant part has one or more or all of the following features:

a) an increased soluble protein content relative to a corresponding wild-type plant part,

b) an increased nitrogen content in plant part relative to a corresponding wild-type plant part,

f) decreased carbon:nitrogen ratio relative to a corresponding wild-type plant part,

g) increased photosynthetic gene expression relative to a corresponding wild-type plant part,

h) increased photosynthetic capacity relative to a corresponding wild-type plant part,

i) decreased total dietary fibre (TDF) content relative to a corresponding wild-type plant part,

j) increased carbon content relative to a corresponding wild-type plant part, and

k) increased energy content relative to a corresponding wild-type plant part,

wherein each exogenous polynucleotide is operably linked to a promoter which is capable of directing expression of the polynucleotide in the cell.

In an embodiment, the plant part, preferably a *Sorghum sp*. or *Zea mays* plant part, further comprises:

l) an increased TTQ relative to a corresponding wild-type plant part,

wherein each exogenous polynucleotide is operably linked to a promoter which is capable of directing expression of the polynucleotide in the plant, or part thereof.

In an embodiment, the plant part is derived from an ancestor plant, for example, as described herein.

In an embodiment, the plant part has one or more or all of:

i) the plant part has an increased soluble protein relative to the corresponding wild-type plant part of at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 100%, between about 10% and about 200%, between about 50% and about 150%, or between about 50% and about 125%,

ii) the plant part has an increased nitrogen content relative to the corresponding wild-type plant part of at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 100%, between about 10% and about 200%, between about 50% and about 150% or between about 50% and about 125%,

iii) the plant part is a leaf which has an increased soluble protein content relative to a corresponding wild-type leaf of at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 100%, between about 10% and about 200%, between about 50% and about 150%, or between about 50% and about 125%,

iv) the plant part is a leaf which has an increased nitrogen content relative to a corresponding wild-type leaf of at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 100%, between about 10% and about 200%, between about 50% and about 150%, or between about 50% and about 125%,

v) the plant part has a decreased carbon:nitrogen content relative to the corresponding wild-type plant or part thereof of at least about 10%, at least about 25%, at least about 40%, between about 10% and about 50%, or between about 25% and about 50%,

vi) expression of one or more genes involved in photosynthesis is increased in the plant part relative to the corresponding wild-type plant part,

vii) the plant part has an increased carbon content relative to the corresponding wild-type plant part of at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 100%, at least about 125%, at least about 150%, between about 10% and about 300%, between about 50% and about 250%, or between about 100% and about 200%,

viii) the plant part has an increased energy content in the plant part relative to the corresponding wild-type plant part of at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 100%, at least about 125%, at least about

150%, at least about 200%, at least about 250%, between about 10% and about 400%, between about 50% and about 300%, or between about 200% and about 300%,

ix) the plant part has a decreased starch content relative to the corresponding wild-type plant part of at least about 2 fold, at least about 5 fold, at least about 10 fold, at least about 15 fold, at least about 20 fold, at least about 25 fold, between about 5 fold and about 35 fold, between about 10 fold and about 30 fold, or between about 20 fold and about 30 fold,

x) the plant part has a decreased TDF content relative to the corresponding wild-type plant part of at least about 10%, at least about 30%, at least about 50%, between about 10% and about 70%, or between about 30% and about 65%, and

xi) the plant part has a soluble sugar content relative to the corresponding wild-type plant part which is about 0.5 fold to 2 fold.

In an embodiment of the first and second aspects, plant part has one or more or all of;

i) the plant part comprises a total non-polar lipid content of at least about 8%, at least about 10%, at least about 11%, at least about 12%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, between 8% and 75%, between 10% and 75%, between 11% and 75%, between about 15% and 75%, between about 20% and 75%, between about 30% and 75%, between about 40% and 75%, between about 50% and 75%, between about 60% and 75%, or between about 25% and 50% (w/w dry weight), preferably before flowering,

ii) the plant part is a vegetative part that comprises a TAG content of at least about 8%, at least about 10%, at least about 11%, at least about 12%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, between 8% and 75%, between 10% and 75%, between 11% and 75%, between about 15% and 75%, between about 20% and 75%, between about 30% and 75%, between about 40% and 75%, between about 50% and 75%, between about 60% and 75%, or between about 25% and 50% (w/w dry weight), preferably before flowering,

iii) one or more or all of the promoters are selected from a tissue-specific promoter such as a leaf and/or stem specific promoter, a developmentally regulated promoter such as a senescence-specific promoter such as a SAG12 promoter, an inducible promoter, or a circadian-rhythm regulated promoter,

iv) the plant part is one member of a population or collection of at least about 1,500, at least about 3,000 or at least about 5,000 such plant parts, preferably vegetative plant parts.

In a further embodiment, the plant part is:

i) a 16:3 plant part, and which comprises one or more or all of the following:

a) an exogenous polynucleotide which encodes a polypeptide which increases the export of fatty acids out of plastids of the plant when compared to a corresponding plant lacking the exogenous polynucleotide,

b) a first genetic modification which down-regulates endogenous production and/or activity of a polypeptide involved in importing fatty acids into plastids of the plant when compared to a corresponding plant lacking the first genetic modification, and

c) a second genetic modification which down-regulates endogenous production and/or activity of a polypeptide involved in diacylglycerol (DAG) production in the plastid when compared to a corresponding plant lacking the second genetic modification,

wherein the exogenous polynucleotide is operably linked to a promoter which is capable of directing expression of the polynucleotide in the plant part, or

ii) a 18:3 plant part.

In an embodiment, the plant part has one or more or all of:

i) the plant part, preferably a vegetative plant part which has an increased synthesis of total fatty acids relative to a corresponding plant part lacking the exogenous polynucleotide(s) and/or genetic modification(s),

ii) the plant part, preferably a vegetative plant part which has an increased expression and/or activity of a fatty acyl acyltransferase which catalyses the synthesis of TAG, DAG or MAG, preferably TAG, relative to a corresponding plant part lacking the exogenous polynucleotide(s) and/or genetic modification(s),

iii) the plant part, preferably a vegetative plant part which has a decreased production of lysophosphatidic acid (LPA) from acyl-ACP and G3P in its plastids relative to a corresponding plant part lacking the exogenous polynucleotide(s) and/or genetic modification(s),

iv) the plant part, preferably a vegetative plant part which has an altered ratio of C16:3 to C18:3 fatty acids in its total fatty acid content and/or its galactolipid content relative to a corresponding part lacking the exogenous polynucleotide(s) and/or genetic modification(s), preferably a decreased ratio,

v) one or more or all of the promoters are selected from promoter other than a constitutive promoter, preferably a tissue-specific promoter such as a leaf and/or stem specific promoter, a developmentally regulated promoter such as a senescense-specific promoter such as a SAG12 promoter, an inducible promoter, or a circadian-rhythm regulated promoter, preferably wherein at least one of the promoters operably linked to an exogenous polynucleotide which encodes a transcription factor polypeptide is a promoter other than a constitutive promoter,

vi) the plant part, preferably a vegetative plant part, comprises a total fatty acid content whose oleic acid level and/or palmitic acid level is increased by at least 2% relative to a corresponding plant, or part thereof, lacking the exogenous polynucleotide(s) and/or genetic modification(s), and/or whose α-linolenic acid (ALA) level and /or linoleic acid level is decreased by at least 2% relative to a corresponding plant part lacking the exogenous polynucleotide(s) and/or genetic modification(s),

vii) non-polar lipid in the plant part, preferably a vegetative plant part, comprises a modified level of total sterols, preferably free (non-esterified) sterols, steroyl esters, steroyl glycosides, relative to the non-polar lipid in a corresponding plant, or part thereof, lacking the exogenous polynucleotide(s) and/or genetic modification(s),

viii) non-polar lipid in the plant part comprises waxes and/or wax esters,

ix) the plant part comprises an exogenous polynucleotide encoding a silencing suppressor, wherein the exogenous polynucleotide is operably linked to a promoter which is capable of directing expression of the polynucleotide in the plant,

x) the level of one or more non-polar lipid(s) and/or the total non-polar lipid content of the plant or part thereof, preferably a vegetative plant part, is at least 2% greater on a weight basis than in a corresponding plant or part, respectively, which comprises exogenous polynucleotides encoding an *Arabidposis thaliana* WRI1 and an *Arabidopsis thaliana* DGAT1 (SEQ ID NO:1),

xi) a total polyunsaturated fatty acid (PUFA) content which is decreased relative to the total PUFA content of a corresponding plant lacking the exogenous polynucleotide(s) and/or genetic modification(s),

xii) if the plant part is a seed, the seed germinates at a rate substantially the same as for a corresponding wild-type seed or when sown in soil produces a plant whose seed germinate at a rate substantially the same as for corresponding wild-type seed, and

xiii) the plant is an algal plant such as from diatoms (bacillariophytes), green algae (chlorophytes), blue-green algae (cyanophytes), golden-brown algae (chrysophytes), haptophytes, brown algae or heterokont algae.

In an embodiment, the plant part comprises a first exogenous polynucleotide encoding a WRI1, a second exogenous polynucleotide encoding a DGAT or a PDAT, preferably a DGAT1, a third exogenous polynucleotide encoding an RNA which reduces expression of a gene encoding an SDP1 polypeptide, and a fourth exogenous polynucleotide encoding an oleosin. In preferred embodiments, the plant part has one or more or all of the following features:

i) a total lipid content of at least 8%, at least 10%, at least 12%, at least 14%, or at least 15.5% (% weight),

ii) at least a 3 fold, at least a 5 fold, at least a 7 fold, at least an 8 fold, or least a 10 fold, at higher total lipid content in the plant part relative to a corresponding the plant part lacking the exogenous polynucleotides and/or genetic modifications,

iii) a total TAG content of at least 5%, at least 6%, at least 6.5% or at least 7% (% weight of dry weight or seed weight),

iv) at least a 40 fold, at least a 50 fold, at least a 60 fold, or at least 70 fold, at least 100 fold, or at least a 120-fold higher total TAG content relative to a corresponding the plant part lacking the exogenous polynucleotides and/or genetic modifications,

v) palmitic acid comprises at least 20%, at least 25%, at least 30% or at least 33% (% weight) of the fatty acids in TAG,

vi) at least a 1.5 fold higher level of palmitic acid in TAG relative to a corresponding the plant part lacking the exogenous polynucleotides and/or genetic modifications,

vii) linoleic acid comprises at least 22%, at least 25%, at least 30% or at least 34% (% weight) of the fatty acids in TAG, and

viii) α-linolenic acid comprises less than 20%, less than 15%, less than 11% or less than 8% (% weight) of the fatty acids in TAG.

ix) at least a 5 fold, or at least an 8 fold, lower level of α-linolenic acid in TAG relative to a corresponding the plant or part thereof lacking the exogenous polynucleotides and/or genetic modifications, In the above embodiments, a preferred plant part is a leaf piece having a surface area of at least 1cm^2 or a stem piece having a length of at least 1cm.

In an embodiment of the above aspects, the plant part has been treated so it is no longer able to be propagated or give rise to a living plant, i.e. it is dead (for example a brown leaf or stem). For example, the plant part has been dried and/or ground. In another embodiment, the plant part is alive (for example, a green leaf or stem).

In an embodiment, the part is a seed, fruit, or a vegetative part such as an aerial plant part or a green part such as a leaf or stem.

In the above embodiments, it is preferred that the plant part is a vegetative plant part which is growing in soil or which was grown in soil and the plant part was subsequently harvested, and wherein the vegetative part comprises at least 8% TAG on a weight basis (% dry weight) such as for example between 8% and 75% or between 8% and 30%. More preferably, the TAG content is at least 10%, such as for example between 10% and 75% or between 10% and 30%. Preferably, these TAG levels are present in the vegetative parts prior to or at flowering of the plant or prior to seed setting stage of plant development. In these embodiments, it is preferred that the ratio of the TAG content in the leaves to the TAG content in the stems of the plant is between 1:1 and 10:1, and/or the ratio is increased relative to a corresponding vegetative part comprising the first and second exogenous polynucleotides and lacking the first genetic modification. Preferably, the vegetative plant part has an increased soluble protein content relative to the corresponding wild-type vegetative plant part of at least about 100%, or between about 50% and about 125%. Preferably, the vegetative plant part has an increased nitorgen content relative to the corresponding wild-type vegetative part of at least about 100%, or between about 50% and about 125%. Preferably, the vegetative plant part has an decreased carbon:nitrogen content relative to the corresponding wild-type vegetative part of at least about 40%, or between about 25% and about 50%. Preferably, the vegetative plant part has a decreased TDF content in the part or at least a part of the transgenic plant relative to the corresponding wild-type vegetative plant part of at least about 30%, or between about 30% and about 65%.

In an embodiment, the plant part, preferably a leaf, a grain, a stem, a root or an endosperm is from a monocotyledonous plant, which has a total fatty acid content or TAG content which is increased at least 5-fold on a weight basis when compared to a corresponding non-transgenic monocotyledonous plant. Alternatively, the transgenic monocotyledonous plant has endosperm comprising a TAG content which is at least 2.0%, preferably at least 3%, more preferably at least 4% or at least 5%, on a weight basis. In an embodiment, the endosperm has a TAG content of at least 2% which is increased at least 5-fold relative to a corresponding non-transgenic endosperm. Preferably, the plant is fully male and female fertile, its pollen is essentially 100% viable, and its grain has a germination rate which is between 70% and 100% relative to corresponding wild-type grain. In an embodiment, the transgenic plant is a progeny plant at least two generations derived from an initial transgenic wheat plant, and is preferably homozygous for the transgenes. In embodiments, the monocotyledonous

plant, or part thereof, preferably a leaf, stem, grain or endosperm, is further characterised by one or more features as defined in the context of a plant or part thereof of the invention. In embodiments, the monocotyledonous plant, or part thereof, preferably a leaf, a grain, stem or an endosperm of the invention preferably has an increased level of monounsaturated fatty acids (MUFA) and/or a lower level of polyunsaturated fatty acids (PUFA) in both the total fatty acid content and in the TAG fraction of the total fatty acid content, such as for example an increased level of oleic acid and a reduced level of LA (18:2), when compared to a corresponding plant or part thereof lacking the genetic modifications and/or exogenous polynucleotide(s). Preferably, the linoleic acid (LA, 18:2) level in the total fatty acid content of the grain or endosperm of the monocotyledonous plant is reduced by at least 5% and/or the level of oleic acid in the total fatty acid content is increased by at least 5% relative to a corresponding wild-type plant or part thereof, preferably at least 10% or more preferably at least 15%, when compared to a corresponding plant or part thereof lacking the genetic modifications and/or exogenous polynucleotide(s).

In an embodiment of the first and second aspects, the extracted lipid is in the form of an oil, wherein at least about 90%, or least about 95%, at least about 98%, or between about 95% and about 98%, by weight of the oil is the lipid.

In an embodiment of the first and second aspects, the plant part is a vegetative plant part such as a plant leaf or stem, or the plant part is a seed or a fruit.

In an embodiment of the first and second aspects the plant part is from a species selected from a group consisting of a *Acrocomia aculeata* (macauba palm), *Arabidopsis thaliana*, *Aracinis hypogaea* (peanut), *Astrocaryum murumuru* (murumuru), *Astrocaryum vulgare* (tucumã), *Attalea geraensis* (Indaiá-rateiro), *Attalea humilis* (American oil palm), *Attalea oleifera* (andaiá), *Attalea phalerata* (uricuri), *Attalea speciosa* (babassu), *Avena sativa* (oats), *Beta vulgaris* (sugar beet), *Brassica sp*. such as *Brassica carinata*, *Brassica juncea*, *Brassica napobrassica*, *Brassica napus* (canola), *Camelina sativa* (false flax), *Cannabis sativa* (hemp), *Carthamus tinctorius* (safflower), *Caryocar brasiliense* (pequi), *Cocos nucifera* (Coconut), *Crambe abyssinica* (Abyssinian kale), *Cucumis melo* (melon), *Elaeis guineensis* (African palm), *Glycine max* (soybean), *Gossypium hirsutum* (cotton), *Helianthus sp.* such as *Helianthus annuus* (sunflower), *Hordeum vulgare* (barley), *Jatropha curcas* (physic nut), *Joannesia princeps* (arara nut-tree), *Lemna sp*. (duckweed) such as *Lemna aequinoctialis*, *Lemna disperma*, *Lemna ecuadoriensis*, *Lemna gibba* (swollen duckweed), *Lemna japonica*, *Lemna minor*, *Lemna minuta*, *Lemna obscura*, *Lemna paucicostata*, *Lemna perpusilla*, *Lemna tenera*, *Lemna trisulca*, *Lemna turionifera*,

Lemna valdiviana, *Lemna yungensis*, *Licania rigida* (oiticica), *Linum usitatissimum* (flax), *Lupinus angustifolius* (lupin), *Mauritia flexuosa* (buriti palm), *Maximiliana maripa* (inaja palm), *Miscanthus sp.* such as *Miscanthus x giganteus* and *Miscanthus sinensis*, *Nicotiana sp.* (tabacco) such as *Nicotiana tabacum* or *Nicotiana benthamiana*, *Oenocarpus bacaba* (bacaba-do-azeite), *Oenocarpus bataua* (patauã), *Oenocarpus distichus* (bacaba-de-leque), *Oryza sp.* (rice) such as *Oryza sativa* and *Oryza glaberrima*, *Panicum virgatum* (switchgrass), *Paraqueiba paraensis* (mari), *Persea amencana* (avocado), *Pongamia pinnata* (Indian beech), *Populus trichocarpa*, *Ricinus communis* (castor), *Saccharum sp.* (sugarcane), *Sesamum indicum* (sesame), *Solanum tuberosum* (potato), *Sorghum sp.* such as *Sorghum bicolor*, *Sorghum vulgare, Theobroma grandifor um* (cupuassu), *Trifolium sp.*, *Trithrinax brasiliensis* (Brazilian needle palm), *Triticum sp.* (wheat) such as *Triticum aestivum* and *Zea mays* (corn). For example, the plant part is from a monocotyledonous plant, preferably a plant from the family Poaceae, more preferably a *Sorghum* sp., a *Zea mays*, *Miscanthus sp.* such as *Miscanthus x giganteus* and *Miscanthus sinensis*, and/or a *Panicum virgatum* (switchgrass) plant.

In an embodiment of the first and second aspects, the one or more or all of the promoters are expressed at a higher level in a vegetative plant part relative to seed of a plant.

In another aspect, the present invention provides extracted plant lipid produced by the process of both the first and second aspects, preferably comprising plant leaf lipid.

In another aspect, the present invention provides extracted plant lipid, comprising fatty acids in an esterified form, wherein the level of medium chain fatty acids in the total fatty acid content of the lipid in the vegetative plant part is at least about 25%. In an embodiment, the lipid has one or more of the features defined above in relation to the first or second aspects.

In another aspect, the present invention provides a cell comprising an increased level or activity of polypeptides which are:

i. a GPAT, a LPAAT, and a WRI1 polypeptide;
ii. a GPAT, a LPAAT, a DGAT and a WRI1 polypeptide;
iii. a GPAT9, a LPAAT, and a WRI1 polypeptide;
iv. a GPAT9, a LPAAT, a DGAT, and a WRI1 polypeptide;
v. a GPAT, a LPAAT, a DGAT1, and a WRI1 polypeptide;
vi. a GPAT9, a LPAAT, a DGAT1, and a WRI1 polypeptide;

vii. a GPAT, a LPAAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

viii. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

ix. a GPAT9, a LPAAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

x. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xi. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xii. a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xiii. a GPAT, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xiv. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xv. a GPAT9, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xvi. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xvii. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xviii. a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xix. a GPAT, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xx. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which

reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxi. a GPAT9, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxii. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxiii. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxiv. a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxv. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxvi. a GPAT9, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxvii. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xxviii. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin, or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase; or

xxix. a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin, or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase.

In an embodiment, the one or more or all of the polypeptides are encoded by one or more exogenous polynucleotides in the plant parts.

In an embodiment, the level of total, or new, MCFA is increased relative to a corresponding wild-type plant part, preferably at least 25% of the total fatty acid content on a weight basis.

In an embodiment, the one or more or all of the encoded GPAT, LPAAT and/or DGAT have a preference for utilising medium chain fatty acid substrates.

In an embodiment, the cell further comprises one or more or all of:

i. a genetic modification which down-regulates endogenous production and/or activity of a polypeptide involved in the catabolism of triacylglycerols (TAG) in the cell when compared to a corresponding cell lacking the genetic modification;

ii. a genetic modification which down-regulates endogenous production and/or activity of a polypeptide involved in importing fatty acids into plastids of the cell when compared to a corresponding cell lacking the genetic modification; and

iii. a genetic modification which down-regulates endogenous production and/or activity of a polypeptide involved in diacylglycerol (DAG) production in the plastid when compared to a corresponding cell lacking the genetic modification.

In an embodiment, the genetic modification is a mutation of an endogenous gene which partially or completely inactivates the gene, such as a point mutation, an insertion, or a deletion, or the genetic modification is an exogenous polynucleotide encoding an RNA molecule which inhibits expression of the endogenous gene, wherein the exogenous polynucleotide is operably linked to a promoter which is capable of directing expression of the polynucleotide in the cell.

In an embodiment, the one or more or all of the promoters are expressed at a higher level in a vegetative plant part relative to seed of a plant.

In an embodiment, the cell has one or more or all of the following features:

i. the level of medium chain fatty acids in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is at least about 30%, at least about 35%, at least about 40%, at least about 50%, at least about

55%, or between about 25% and about 55%, between about 25% and about 50%, between about 30% and about 50%, between about 35% and about 50%, between about 25% and about 40%, or between about 30% and about 40%;

ii. the level of lauric acid (C12:0) in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is, or is increased by, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 50%, at least or about 55%, or between about 15% and about 55%, between about 20% and about 50%, between about 30% and about 50%, between about 35% and about 50%, between about 15% and about 25%, or between about 20% and about 30%;

iii. the level of myristic acid (C14:0) in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is, or is increased by, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, or between about 25% and about 45%, between about 20% and about 50%, between about 30% and about 50%, between about 35% and about 50%, between about 30% and about 40%, between about 15% and about 25%, or between about 20% and about 30%;

iv. the level of palmitic acid (C16:0) in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is, or is increased by, between about 2% and about 18%, or between about 2% and about 16%, or between about 2% and about 15%, or between about 15% and about 50%;

v. the level of lauric acid (C12:0) in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell is, or is increased by, at least about 25%, at least about 30%, at least about 40%, at least about 45%, or at least about 50%, and the level of myristic acid (C14:0) in the total fatty acid content of the cell and/or in the total fatty acid content of the TAG of the cell is, or is increased by, at least about 1%, at least about 2%, at least about 5%, or at least about 10%, or between about 1% and about 10%, or between about 2% and 10%, or between about 2% and about 6%, or less than about 10%, or less than about 8% or less than about 5%, or less than about 2%;

vi. the level of myristic acid (C14:0) in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell is, or is increased by, at least about 20%, at least about 25%, at least about 30%, or at least about 40%, and the level of lauric acid (C12:0) in the total fatty acid content of the cell and/or in the total fatty acid content of the TAG of the cell is, or is increased by, at least about 1%, at least about 2%, at least about 5%, or at least

about 10%, or between about 1% and about 10%, or between about 2% and about 10%, or between about 2% and about 6%, or less than about 10%, or less than about 8% or less than about 5%, or less than about 2%;

vii. the level of myristic acid (C14:0) in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell is, or is increased by, at least about 20%, at least about 25%, at least about 30%, and the level of palmitic acid (C16:0) in the total fatty acid content of the cell and/or in the total fatty acid content of the TAG of the cell is, or is increased by, at least about 2%, at least about 3%, at least about 4%, or at least about 5%.

viii. the ratio of lauric acid (C12:0):myristic acid (C14:0) in the fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is increased, or is about 1:4, about 1:5, about 1:10, about 1:15, about 1:20, about 1:25, or about 4:1, about 5:1, about 10:1, about 15:1, about 20:1, about 30:1, about 40:1, or about 45:1;

ix. the ratio of lauric acid (C12:0):palmitic acid (C16:0) in the fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is increased, or is about 1:2, about 1:3, about 1:4, about 1:5, about 1:10, about 1:15, about 10:1, about 20:1, about 30:1, about 40:1, or about 45:1;

x. the ratio of myristic acid (C14:0):palmitic acid (C16:0) in the fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is increased, or is about 1:2, about 1:3, about 1:4, about 1:5, about 1:10, about 1:15, about 10:1, about 20:1, about 30:1, or about 40:1;

xi. the level of oleic acid in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is decreased, or is less than about 10%, less than about 8%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, less than about 1%;

xii. the level of linoleic acid (LA) in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is increased or decreased, or is less than about 20%, less than about 15%, less than about 10%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1%;

xiii. the level of α-linolenic acid (ALA) in the total fatty acid content of the cell, or in the total fatty acid content of the TAG of the cell, is decreased or is less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 10%, less than about 8%, less than about 6%, less than about 5%, less than about 2%, or less than about 1%;

xiv. the level of total unsaturated fatty acids in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is decreased, or is less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 15%, less than about 10%, less than about 8%, less than about 6%, less than about 5%, less than about 2%, or less than about 1%;

xv. the level of total monounsaturated fatty acids in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is decreased, or is less than about 10%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1%;

xvi. the level of total polyunsaturated fatty acids in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 15%, less than about 10%, less than about 8%, less than about 6%, less than about 5%, less than about 2%, or less than about 1%;

xvii. the triacylglycerol (TAG) content of the cell is at least about 80%, at least about 85%, at least about 90%, or least about 95%, and about 98%, or between about 95% and about 98%, by weight of the cell;

xviii. the TAG content of the cell comprises, or is increased in a level of, one or more or all of the TAG species 36:0, 38:0, 40:0 and 42:0;

xix. the cell comprises tri-laurin (tri-C12:0) and/or tri-myristin (tri-C14:0);

xx. the phosphocholine (PC) content of the cell comprises one or both of the PC species 28:0 and 30:0,

xxi. the cell has a reduced level of medium chain fatty acids, preferably C14:0, in membrane lipids relative to a corresponding cell;

xxii. the cell has less chlorosis relative to a corresponding cell which comprises the exogenous polynucleotide encoding the thioesterase but lacks the exogenous polynucleotide encoding the DGAT; and

xxiii. the cell is in a vegetative plant part and the part has an alleviated chlorosis phenotype relative to a corresponding vegetative plant part,

wherein any increase or decrease is relative to a corresponding wild-type cell.

In another aspect, the present invention provides a plant or a part thereof comprising the cell of the invention, or which is transgenic for one or more exogenous polynucleotides defined above.

In an embodiment, before the plant flowers, a vegetative part of the plant comprises a total non-polar lipid content of at least about 8%, at least about 10%, about 11%, between 8% and 15%, or between 9% and 12% (w/w dry weight).

In an embodiment, the plant is a monocotyledonous plant, or part thereof preferably a leaf, a grain, a stem, a root or an endosperm, which has a total fatty acid content or TAG content which is increased at least 5-fold on a weight basis when compared to a corresponding non-transgenic monocotyledonous plant, or part thereof. Alternatively, the transgenic monocotyledonous plant has endosperm comprising a TAG content which is at least 2.0%, preferably at least 3%, more preferably at least 4% or at least 5%, on a weight basis, or part of the plant, preferably a leaf, a stem, a root, a grain or an endosperm. In an embodiment, the endosperm has a TAG content of at least 2% which is increased at least 5-fold relative to a corresponding non-transgenic endosperm. Preferably, the plant is fully male and female fertile, its pollen is essentially 100% viable, and its grain has a germination rate which is between 70% and 100% relative to corresponding wild-type grain. In an embodiment, the transgenic plant is a progeny plant at least two generations derived from an initial transgenic wheat plant, and is preferably homozygous for the transgenes. In embodiments, the monocotyledonous plant, or part thereof, preferably a leaf, stem, grain or endosperm, is further characterised by one or more features as defined in the context of a plant or part thereof of the invention. In embodiments, the monocotyledonous plant, or part thereof preferably a leaf, a grain, stem or an endosperm of the invention preferably has an increased level of monounsaturated fatty acids (MUFA) and/or a lower level of polyunsaturated fatty acids (PUFA) in both the total fatty acid content and in the TAG fraction of the total fatty acid content, such as for example an increased level of oleic acid and a reduced level of LA (18:2), when compared to a corresponding plant or part thereof lacking the genetic modifications and/or exogenous polynucleotide(s). Preferably, the linoleic acid (LA, 18:2) level in the total fatty acid content of the grain or endosperm of the the monocotyledonous plant is reduced by at least 5% and/or the level of oleic acid in the total fatty acid content is increased by at least 5% relative to a corresponding wild-type plant or part thereof, preferably at least 10% or more preferably at least 15%, when compared to a corresponding plant or part thereof lacking the genetic modifications and/or exogenous polynucleotide(s).

In an embodiment, the plant, or part thereof, is a member of a population or collection of at least about 1,500, at least about 3,000 or at least about 5,000 such plants or parts.

In an embodiment, the TFA content, the the TAG content, the total non-polar lipid content, or the one or more non-polar lipids, and/or the level of the oleic acid or a PUFA in the plant or part thereof is determinable by analysis by using gas

chromatography of fatty acid methyl esters obtained from the plant or vegetative part thereof.

In a further embodiment, wherein the plant part is a leaf and the total non-polar lipid content of the leaf is determinable by analysis using Nuclear Magnetic Resonance (NMR).

In each of the above embodiments, it is preferred that the plant is a transgenic progeny plant at least two generations derived from an initial transgenic plant, and is preferably homozygous for the transgenes.

In an embodiment, the plant or the part thereof is phenotypically normal, in that it is not significantly reduced in its ability to grow and reproduce when compared to an unmodified plant or part thereof. In an embodiment, the biomass, growth rate, germination rate, storage organ size, seed size and/or the number of viable seeds produced is not less than 70%, not less than 80% or not less than 90% of that of a corresponding wild-type plant when grown under identical conditions. In an embodiment, the plant is male and female fertile to the same extent as a corresponding wild-type plant and its pollen (if produced) is as viable as the pollen of the corresponding wild-type plant, preferably at least about 75%, or at least about 90%, or close to 100% viable. In an embodiment, the plant produces seed which has a germination rate of at least about 75% or at least about 90% relative to the germination rate of corresponding seed of a wild-type plant, where the plant species produces seed. In an embodiment, the plant of the invention has a plant height which is at least about 75%, or at least about 90% relative to the height of the corresponding wild-type plant grown under the same conditions. A combination of each of these features is envisaged. In an alternative embodiment, the plant of the invention has a plant height which is between 60% and 90% relative to the height of the corresponding wild-type plant grown under the same conditions. In an embodiment, the plant or part thereof of the invention, preferably a plant leaf, does not exhibit increased necrosis, i.e. the extent of necrosis, if present, is the same as that exhibited by a corresponding wild-type plant or part thereof grown under the same conditions and at the same stage of plant development. This feature applies in particular to the plant or part thereof comprising an exogenous polynucleotide which encodes a fatty acid thioesterase such as a FATB thioesterase.

In another aspect, the present invention provides a population of at least about 1,500, at least about 3,000 or at least about 5,000 plants, each being a plant of the invention, growing in a field.

In an embodiment, the exogenous polynucleotides are inserted at the same chromosomal location in the genome of each of the plants, preferably in the nuclear genome of each of the plants.

In another aspect, the present invention provides a population of at least about 1000 plants , each being a plant according to the invention, growing in a field, or a collection of at least about 1000 plant parts, each being a plant part according to the invention, wherein the plant parts have been harvested from plants growing in a field.

Also provided is a storage bin comprising a collection of plants or plant parts of the invention.

In another aspect, the present invention provides an extract of a plant or a part thereof of the invention. The extract preferably has a different fatty acid composition relative to a corresponding wild-type extract.

In an embodiment, the extract is lacking at least 50% or at least 90% of the chlorophyll and/or soluble sugars of the plant or part thereof.

In a further aspect, the present invention provides a process for selecting a plant or a part thereof with a desired phenotype, the process comprising

i) obtaining a plurality of candidate plants, or parts thereof, which each comprise

a) a first exogenous polynucleotide which encodes a transcription factor polypeptide that increases the expression of one or more glycolytic and/or fatty acid biosynthetic genes in a plant or part thereof, and

b) a second exogenous polynucleotide which encodes a polypeptide involved in the biosynthesis of one or more non-polar lipids,

wherein each exogenous polynucleotide is operably linked to a promoter which is capable of directing expression of the polynucleotide in the plant, or part thereof,

ii) analysing lipid in the plurality of parts, or at least a part of each plant in the plurality of candidate plants, from step i),

iii) analysing the plurality of parts, or at least a part of each plant in the plurality of candidate plants, from step i) for one or more or all of;

a) soluble protein content,

b) nitrogen content,

c) carbon:nitrogen ratio,

d) photosynthetic gene expression,

e) photosynthetic capacity,

f) total dietary fibre (TDF) content,

g) carbon content, and

h) energy content, and

iv) selecting a plant or part thereof which comprises an increased triacylglycerol (TAG) content in the part or at least a part of the plant relative to a corresponding wild-type plant or part thereof and a desired phenotype selected from one or more or all of the following;

A) an increased soluble protein content in the part or at least a part of the plant relative to a corresponding wild-type plant or part thereof,

B) an increased nitrogen content in the part or at least a part of the plant relative to a corresponding wild-type plant or part thereof,

C) decreased carbon:nitrogen ratio in the part or at least a part of the plant relative to a corresponding wild-type plant or part thereof,

D) increased photosynthetic gene expression in the part or at least a part of the plant relative to a corresponding wild-type plant or part thereof,

E) increased photosynthetic capacity in the part or at least a part of the plant relative to a corresponding wild-type plant or part thereof,

F) decreased total dietary fibre (TDF) content in the part or at least a part of the plant relative to a corresponding wild-type plant or part thereof,

G) increased carbon content in the part or at least a part of the plant relative to a corresponding wild-type plant or part thereof, and

H) increased energy content in the part or at least a part of the plant relative to a corresponding wild-type plant or part thereof.

In an embodiment, the process further comprises a step of obtaining seed or a progeny plant from the transgenic plant, wherein the seed or progeny plant comprises the exogenous polynucleotides.

In an embodiment, the increased triacylglycerol (TAG) content is determined by analysing one or more of the total fatty acid content, TAG content, fatty acid composition, by any means, which might or might not involve first extracting the lipid.

In yet another embodiment, the selected plant or part thereof has one or more of the features as defined herein.

In another aspect, the present invention provides seed of, or obtained from, a plant according to the invention.

In another aspect, the present invention provides a process for obtaining a cell according to the invention, the process comprising the steps of:

i) introducing into a cell at least one exogenous polynucleotide and/or at least one genetic modification as defined above to produce a cell as defined above,

ii) expressing the exogenous polynucleotide(s) in the cell or a progeny cell thereof,

iii) analysing the lipid content of the cell or progeny cell, and

iv) selecting a cell according to the invention.

In another aspect, the present invention provides a method of producing a plant which has integrated into its genome a set of exogenous polynucleotides and/or genetic modifications as defined above, the method comprising the steps of:

i) crossing two parental plants, wherein one plant comprises at least one of the exogenous polynucleotides and/or at least one genetic modifications as defined in any one of claims 24 to 31; and the other plant comprises at least one of the exogenous polynucleotides and/or at least one genetic modifications as defined in any one of claims 24 to 31, and wherein between them the two parental plants comprise a set of exogenous polynucleotides and/or genetic modifications as defined in any one of claims 24 to 31,

ii) screening one or more progeny plants from the cross for the presence or absence of the set of exogenous polynucleotides and/or genetic modifications as defined in any one of claims 24 to 31, and

iii) selecting a progeny plant which comprise the set of exogenous polynucleotides and/or genetic modifications as defined in any one of claims 24 to 31,

thereby producing the plant.

In another aspect, the present invention provides a process for producing an industrial product, the process comprising the steps of:

i) obtaining a cell of the invention, a plant or a part thereof of the invention, or seed the invention, and

ii) either

a) converting at least some of the lipid in the cell, plant or part thereof, or seed, of step i) to the industrial product by applying heat, chemical, or enzymatic means, or any combination thereof, to the lipid *in situ* in the cell, or plant or vegetative part thereof, or seed, or

b) physically processing the cell, plant or part thereof, or seed, of step i), and subsequently or simultaneously converting at least some of the lipid in the processed cell, plant or part thereof, or seed, to the industrial product by applying heat, chemical, or enzymatic means, or any combination thereof, to the lipid in the processed cell, plant or part thereof, or seed, and

iii) recovering the industrial product,

thereby producing the industrial product.

In an embodiment, the step of physically processing the the cell, plant or part thereof, or seed, of step i), comprises one or more of rolling, pressing, crushing or grinding the plant or part thereof, or seed.

In an embodiment, the invention further comprises steps of:

(a) extracting at least some of the non-polar lipid content of the cell, or plant or part thereof, or seed, as non-polar lipid, and

(b) recovering the extracted non-polar lipid,

wherein steps (a) and (b) are performed prior to the step of converting at least some of the lipid in the cell, plant or part thereof, or seed, to the industrial product.

In another aspect, the present invention provides a process for producing extracted lipid, the process comprising the steps of:

i) obtaining a plant cell of the invention, or a plant or a part thereof of the invention, or seed of the invention,

ii) extracting lipid from the cell, or plant or part thereof, or seed, and

iii) recovering the extracted lipid,

thereby producing the extracted lipid.

In an embodiment, a process of extraction comprises one or more of drying, rolling, pressing crushing or grinding the plant or part thereof, or seed, and/or purifying the extracted lipid or seedoil.

In an embodiment, the process uses an organic solvent in the extraction process to extract the oil.

In an embodiment, the process comprises recovering the extracted lipid by collecting it in a container and/or one or more of degumming, deodorising, decolourising, drying, fractionating the extracted lipid, removing wax esters from the extracted lipid, or analysing the fatty acid composition of the extracted lipid.

In an embodiment, the volume of the extracted lipid or oil is at least 1 litre.

In a further embodiment, one or more or all of the following features apply:

(i) the extracted lipid or oil comprises triacylglycerols, wherein the triacylglycerols comprise at least 90%, preferably at least 95% or at least 96%, of the extracted lipid or oil,

(ii) the extracted lipid or oil comprises free sterols, steroyl esters, steroyl glycosides, waxes or wax esters, or any combination thereof, and

(iii) the total sterol content and/or composition in the extracted lipid or oil is significantly different to the sterol content and/or composition in the extracted lipid or oil produced from a corresponding plant or part thereof, or seed.

In an embodiment, the process further comprises converting the extracted lipid to an industrial product.

In an embodiment, the industrial product is a hydrocarbon product such as fatty acid esters, preferably fatty acid methyl esters and/or a fatty acid ethyl esters, an alkane such as methane, ethane or a longer-chain alkane, a mixture of longer chain alkanes, an alkene, a biofuel, carbon monoxide and/or hydrogen gas, a bioalcohol such as ethanol, propanol, or butanol, biochar, or a combination of carbon monoxide, hydrogen and biochar.

In a further embodiment, the plant part is an aerial plant part or a green plant part, preferably a vegetative plant part such as a plant leaf or stem.

In yet a further embodiment, the process further comprises a step of harvesting the plant or part thereof such as a vegetative plant part, tuber or beet, or seed, preferably with a mechanical harvester.

In another embodiment, the level of a lipid in the plant or part thereof, or seed and/or in the extracted lipid or oil is determinable by analysis by using gas chromatography of fatty acid methyl esters prepared from the extracted lipid or oil.

In yet another embodiment, the process further comprises harvesting the part from a plant.

In an embodiment, the plant part is a vegetative plant part which comprises a total non-polar lipid content of at least about 18%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, between 18% and 75%, between about 20% and 75%, between about 30% and 75%, between about 40% and 75%, between about 50% and 75%, between about 60% and 75%, or between about 25% and 50% (w/w dry weight).

In a further embodiment, the plant part is a vegetative plant part which comprises a total TAG content of at least about 18%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, between 18% and 75%, between about 20% and 75%, between about 30% and 75%, between about 40% and 75%, between about 50% and 75%, between about 60% and 75%, or between about 25% and 50% (w/w dry weight).

In another embodiment, the plant part is a vegetative plant part which comprises a total non-polar lipid content of at least about 11%, at least about 12%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least

about 60%, at least about 65%, at least about 70%, between 8% and 75%, between 10% and 75%, between 11% and 75%, between about 15% and 75%, between about 20% and 75%, between about 30% and 75%, between about 40% and 75%, between about 50% and 75%, between about 60% and 75%, or between about 25% and 50% (w/w dry weight), and wherein the vegetative plant part is from a 16:3 plant.

In yet another embodiment, the plant part is a vegetative plant part which comprises a total TAG content of at least about 11%, at least about 12%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, between 8% and 75%, between 10% and 75%, between 11% and 75%, between about 15% and 75%, between about 20% and 75%, between about 30% and 75%, between about 40% and 75%, between about 50% and 75%, between about 60% and 75%, or between about 25% and 50% (w/w dry weight), and wherein the vegetative plant part is from a 16:3 plant.

In an embodiment, the vegetative plant parts have a TAG/TFA Quotient (TTQ) of between 0.01 and 0.6. In an embodiment, the vegetative plant parts have a TTQ of between 0.01 and 0.55, or between 0.01 and 0.5, or about 0.1, or about 0.2 or about 0.3, or about 0.4 or about 0.5. Preferably, the TTQ is between 0.60 and 0.84, which corresponds to a TAG:TFA ratio of between 1.5:1 and 5:1, or between 0.84 and 0.95 which corresponds to a TAG:TFA ratio of between 5:1 and 20:1.

In an embodiment, the vegetative plant parts comprise an average TFA content of about 6%, or about 8%, or about 9% or about 10% (w/w dry weight).

In an embodiment, the TFA content of the vegetative plant parts comprises a palmitic acid content which is increased by at least 2% or at least 3% relative to the palmitic acid content of a corresponding wild-type vegetative plant part.

In an embodiment, the TFA content of the vegetative plant parts comprises a α-linoleic acid (ALA) content which is decreased by at least 2% or at least 3% relative to the ALA content of a corresponding wild-type vegetative plant part.

In an embodiment, one or more or all of the following features apply:

(i) the vegetative plant parts are leaves and/or stems or parts thereof which comprise one or more of an increased carbon content, an increased energy content, an increased soluble protein content, a reduced starch content, a reduced total dietary fibre (TDF) content and an increased nitrogen content, each on a weight basis relative to a corresponding wild-type leaf or stem or parts thereof from a wild-type *Sorghum* sp. or *Zea mays* plant at the same stage of growth,

(ii) the TFA content of the vegetative plant parts is at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 11%, at least about 12%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, between about 6% and about 20%, between 8% and 75%, between 10% and 75%, between 11% and 75%, between about 15% and 75%, between about 20% and 75%, between about 30% and 75%, between about 40% and 75%, between about 50% and 75%, between about 60% and 75%, or between about 25% and 50% (w/w dry weight) TFA,

(iii) the fatty acids esterified in the form of TAG in the vegetative plant parts is at least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 11%, at least about 12%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, between about 6% and about 20%, between 8% and 75%, between 10% and 75%, between 11% and 75%, between about 15% and 75%, between about 20% and 75%, between about 30% and 75%, between about 40% and 75%, between about 50% and 75%, between about 60% and 75%, or between about 25% and 50% (w/w dry weight),

(iv) the vegetative plant parts comprise an increased content of a WRI1 polypeptide, an increased content of a DGAT polypeptide, and a decreased content of a SDP1 polypeptide, each relative to a corresponding wild-type vegetative plant part,

(v) the vegetative plant parts comprise an increased content of a WRI1 polypeptide, an increased content of a DGAT polypeptide, and an increased content of a LEC2 polypeptide, each relative to a corresponding wild-type vegetative plant part,

(vi) the vegetative plant parts comprise an increased content of a PDAT or DGAT polypeptide, a decreased content of a TGD polypeptide, and a decreased content of a SDP1 polypeptide, each relative to a corresponding wild-type vegetative plant part, and

(vii) the vegetative plant parts comprise a decreased content of a TAG lipase such as a SDP1 TAG lipase, a decreased content of a TGD polypeptide such as a TGD5 polypeptide, and optionally a decreased content of a TST polypeptide such as a TST1 polypeptide, each decrease being relative to a corresponding wild-type vegetative plant part.

In an embodiment, one or more or all of the following features apply:

(i) the vegetative plant parts are harvested from the plant between the time of first flowering of the plant and first maturity of seed,

(ii) the *Sorghum* sp. plant is a *Sorghum bicolor* plant,

(iii) the vegetative plant parts include leaves and/or stems or parts thereof,

(iv) the vegetative plant parts comprise an average total fatty acid content of about 8% or about 10% (w/w dry weight),

In another aspect, the present invention provides a process for producing seed, the process comprising:

i) growing a plant according to the invention, and

ii) harvesting seed from the plant.

In an embodiment, the above process comprises growing a population of at least about 1,500, at least about 3,000 or at least about 5,000 plants, each being a plant of the invention, and harvesting seed from the population of plants.

In another aspect, the present invention provides recovered or extracted lipid obtainable from a cell according to the invention, a plant or a part thereof of the invention, seed of the invention, or obtainable by the process of the invention.

In another aspect, the present invention provides an industrial product produced by the process according to the invention, which is a hydrocarbon product such as fatty acid esters, preferably fatty acid methyl esters and/or a fatty acid ethyl esters, an alkane such as methane, ethane or a longer-chain alkane, a mixture of longer chain alkanes, an alkene, a biofuel, carbon monoxide and/or hydrogen gas, a bioalcohol such as ethanol, propanol, or butanol, biochar, or a combination of carbon monoxide, hydrogen and biochar. In an embodiment the industrial product comprises MCFA, preferably an increased level of MCFA relative to a corresponding industrial product produced from a wild-type plant or part thereof.

In a further aspect, the present invention provides for the use of a transgenic plant or part thereof of the invention, seed of the invention, extract of the invention or the recovered or extracted lipid or soluble protein of the invention for the manufacture of an industrial product.

Examples of industrial products of the invention include, but are not limited to, a hydrocarbon product such as fatty acid esters, preferably fatty acid methyl esters and/or a fatty acid ethyl esters, an alkane such as methane, ethane or a longer-chain alkane, a mixture of longer chain alkanes, an alkene, a biofuel, carbon monoxide and/or hydrogen gas, a bioalcohol such as ethanol, propanol, or butanol, biochar, or a combination of carbon monoxide, hydrogen and biochar.

In another aspect, the present invention provides use of a cell according to the invention, a plant or part thereof of the invention, seed of the invention, or the lipid of the invention, for the manufacture of an industrial product.

In another aspect, the present invention provides a process for producing fuel, the process comprising:

i) reacting the lipid of the invention with an alcohol, optionally, in the presence of a catalyst, to produce alkyl esters, and

ii) optionally, blending the alkyl esters with petroleum based fuel.

In another aspect, the present invention provides a process for producing a synthetic diesel fuel, the process comprising:

i) converting the lipid in a cell of the invention, or a plant or a part thereof of the invention, or seed of the invention, to a bio-oil by a process comprising pyrolysis or hydrothermal processing or to a syngas by gasification, and

ii) converting the bio-oil to synthetic diesel fuel by a process comprising fractionation, preferably selecting hydrocarbon compounds which condense between about 150ºC to about 200ºC or between about 200ºC to about 300ºC, or converting the syngas to a biofuel using a metal catalyst or a microbial catalyst.

In another aspect, the present invention provides a process for producing a biofuel, the process comprising converting the lipid in a cell of the invention, a plant or a part thereof of the invention, or seed of the invention, to bio-oil by pyrolysis, a bioalcohol by fermentation, or a biogas by gasification or anaerobic digestion.

In another aspect, the present invention provides a process for producing a feedstuff, the process comprising admixing a plant cell of the invention, a plant or a part thereof of the invention, seed of the invention, or the lipid of any one of claims the invention, or an extract or portion thereof, with at least one other food ingredient.

In another aspect, the present invention provides feedstuffs, cosmetics or chemicals comprising a plant cell of the invention, a plant or a part thereof of the invention, seed of the invention, or the lipid of the invention, or an extract or portion thereof.

In an embodiment, the feedstuff is silage, pellets or hay.

In another aspect, the present invention provides a process for feeding an animal, the process comprising providing to the animal a plant or a part thereof of the invention, seed of the invention, or the lipid of the invention.

In an embodiment, the animal ingests an increased amount of MCFA, nitrogen, protein, carbon and/or energy potential relative to when the animal ingests the same

amount on a dry weight basis of a corresponding wild-type plant or part thereof, seed or extract or feedstuff produced from the corresponding wild-type plant or part thereof.

Any embodiment herein shall be taken to apply *mutatis mutandis* to any other embodiment unless specifically stated otherwise.

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended for the purpose of exemplification only. Functionally-equivalent products, compositions and methods are clearly within the scope of the invention, as described herein.

Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps or group of compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, compositions of matter, groups of steps or group of compositions of matter.

The invention is hereinafter described by way of the following non-limiting Examples and with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWINGS

Figure 1. A representation of lipid synthesis in eukaryotic cells, showing export of some of the fatty acids synthesized in the plastids to the Endoplasmic Reticulum (ER) via the Plastid Associated Membrane (PLAM), and import of some of the fatty acids into the plastid from the ER for eukaryotic galactolipid synthesis. Abbreviations:

Acetyl-CoA and Malonyl-CoA: acetyl-coenzyme A and malonyl-coenzymeA;
ACCase: Acetyl-CoA carboxylase;
FAS: fatty acid synthase complex;
16:0-ACP, 18:0-ACP and 18:1-ACP: C16:0-acyl carrier protein (ACP), C18:0-acyl carrier protein, C18:1-acyl carrier protein;
KAS II: ketoacyl-ACP synthase II (EC 2.3.1.41);
PLPAAT: plastidial LPAAT;
PGPAT: plastidial GPAT;
PAP: PA phosphorylase (EC 3.1.3.4);
G3P: glycerol-3-phosphate;
LPA: lysophosphatidic acid;
PA: phosphatidic acid;
DAG: diacylglycerol;
TAG: triacylglycerol;
Acyl-CoA and Acyl-PC: acyl-coenzyme A and acyl- phosphatidylcholine;

PC: phosphatidylcholine;
GPAT: glycerol-3-phosphate acyltransferase;
LPAAT: lysophosphatidic acid acyltransferase (EC 2.3.1.51);
LPCAT: acyl-CoA:lysophosphatidylcholine acyltransferase; or synonyms 1-acylglycerophosphocholine *O*-acyltransferase; acyl-CoA:1-acyl-*sn*-glycero-3-phosphocholine *O*-acyltransferase (EC 2.3.1.23);
CPT: CDP-choline:diacylglycerol cholinephosphotransferase; or synonyms 1-alkyl-2-acetylglycerol cholinephosphotransferase; alkylacylglycerol cholinephosphotransferase; cholinephosphotransferase; phosphorylcholine-glyceride transferase (EC 2.7.8.2);
PDCT: phosphatidylcholine:diacylglycerol cholinephosphotransferase;
PLC: phospholipase C (EC 3.1.4.3);
PLD: Phospholipase D; choline phosphatase; lecithinase D; lipophosphodiesterase II (EC 3.1.4.4);
PDAT: phospholipid:diacylglycerol acyltransferase; or synonym phospholipid:1,2-diacyl-*sn*-glycerol O-acyltransferase (EC 2.3.1.158);
FAD2: fatty acid Δ12-desaturase; FAD3, fatty acid Δ15-desaturase;
UDP-Gal: Uridine diphosphate galactose;
MGDS: monogalactosyldiacylglycerol synthase;
MGDG: monogalactosyldiacylglycerol; DGDG: digalactosyldiacylglycerol
FAD6, 7, 8: plastidial fatty acid Δ12-desaturase, plastidial ω3-desaturase, plastidial ω3-desaturase induced at low temperature, respectively.

Figure 2. Schematic diagram of vector pOIL122. Abbreviations: TER Agrtu-Nos, *Agrobacterium tumefaciens* nopaline synthase terminator; NPTII, neomycin phosphotransferase protein coding region; PRO CaMV35S-Ex2, Cauliflower Mosaic Virus 35S promoter with double enhancer region; Arath-DGAT1, *Arabidopsis thaliana* DGAT1 acyltransferase protein coding region; PRO Arath-Rubisco SSU, *A. thaliana* Rubisco small subunit promoter; Arath-FATA2, *A. thaliana* FATA2 thioesterase protein coding region; Arath-WRI, *A. thaliana* WRI1 transcription factor protein coding region; TER Glyma-Lectin, *Glycine max* lectin terminator; enTCUP2 promoter, *Nicotiana tabacum* cryptic constitutive promoter; attB1 and attB2, Gateway recombination sites; NB SDP1 fragment, *Nicotiana benthamiana* SDP1 region targeted for hpRNAi silencing; OCS terminator, *A. tumefaciens* octopine synthase terminator. Backbone features outside the T-DNA region are derived from pORE04 (Coutu et al., 2007).

Figure 3. TAG levels (% leaf dry weight) in *N. benthamiana* leaf tissue, infiltrated with genes encoding different WRI1 polypeptides either with (right hand bars) or without (left hand bars) co-expression of DGAT1 (n=3). All samples were infiltrated with the P19 construct as well.

Figure 4. Phylogenetic tree of LDAP polypeptides (Example 6).

Figure 5. Schematic representation of the genetic construct pJP3506 including the T-DNA region between the left and right borders. TAG, triacylglycerol; FFA, free fatty acids; DAG, diacylglycerol; Sesin-Oleosin, *Sesame indicum* oleosin protein coding region.

Figure 6. Triacylglycerol accumulation upon the expression of AtCaleosin 3 and SiOleosin L.

Figure 7. Total fatty acid methyl ester (FAME) profiles (weight %) illustrating the effect of WRI1+DGAT1-mediated high oil background on MCFA production in *Nicotiana benthamiana* leaf (n=4). Highest MCFA production was observed after the addition of Arath-WRI1.

Figure 8. TAG content in leaf samples of transformed tobacco plants at seed-setting stage of growth, transformed with the T-DNA from pOIL049, lines #23c and #32b. The controls (parent) samples were from plants transformed with the T-DNA from pJP3502. The upper line shows 18:2 percentage in the TAG and the lower line shows the 18:3 (ALA) percentage in the fatty acid content.

Figure 9. Leaf total FAME profiles (weight %) elucidating the effect of WRI1 on MCFA accumulation (n=4). Addition of Arath-WRI1 greatly increased the production of the relevant fatty acid (C12:0, C14:0 or C16:0) relative to the previous addition of Cocnu-LPAAT alone.

Figure 10. TFA levels (% weight), TAG levels, levels of MCFA (C16:0 and C14:0, % of total fatty acids) in TFA and MCFA in TAG (% of total fatty acid content in TAG) in plant cells after expression of combinations of three oil palm DGATs with FATB, LPAAT and WRI1. Numbers 1-10 are as listed in the text (Example 10).

Figure 11. Phylogenetic relationship of glycerol-3-phosphate acyltransferase (*GPAT*) genes from various species including the *Arabidopsis thaliana* (*AtGPAT9*) and *Cocos nucifera* (*CnGPAT9*) genes used in this study. The plant GPAT9 cluster is shaded in grey. BrGPAT3 = *Brassica rapa* glycerol-3-phosphate acyltransferase 3-like (Accession: XM_009105753); BnGPAT3 = *Brassica napus* glycerol-3-phosphate acyltransferase 3-like (Accession: XM_013896062); CsGPAT3 = *Camelina sativa* glycerol-3-phosphate acyltransferase 3 (Accession: XM_010458322); AtGPAT9 = *A. thaliana* glycerol-3-phosphate acyltransferase 9 (Accession: NM_125455); ThGPAT3 = *Tarenaya hassleriana* glycerol-3-phosphate acyltransferase 3-like (Accession: XM_010549847); RcGPAT3 = *Ricinus communis* glycerol-3-phosphate acyltransferase 3 (Accession: NM_001323761); JcGPAT3 = *Jatropha curcas* glycerol-3-phosphate acyltransferase 3 (Accession: NM_001308751); EgGPAT3 = *Elaeis guineensis* glycerol-3-phosphate acyltransferase 3-like (Accession: XM_010913693); CnGPAT9 = *C. nucifera* GPAT9 (Accession: KX235871); Mouse GPAT = Mus musculus 1-acylglycerol-3-phosphate O-acyltransferase 9 (Accession: NM_172715); LrGPAT = *Lilium regale* GPAT (Accession: JX524740); LpGPAT = *Lilium pensylvanicum* GPAT (Accession: JX524741); LlGPAT = *Lilium longiflorum* GPAT (Accession: JX524738); EgGPAT mRMA = *E. guineensis* mRNA for acylation enzyme (Accession: AJ272082); ChGPAT = *Corylus heterophylla* GPAT (Accession: JF428134); JcGPAT = *J. curcas* glycerol-3-phosphate acyltransferase, chloroplastic (Accession: NM_001305998); BnGPAT = *B. napus* glycerol-3-phosphate acyltransferase gene (Accession: KM243174); PsGPAT = *Pisum sativum* chloroplast mRNA for acyl-ACP:sn-glycerol-3-phosphate-acyltransferase (Accession: X59041); SlGPAT = *Solanum lycopersicum* glycerol-3-phosphate acyltransferase (Accession: NM_001306067); AtGPAT3 = *A. thaliana* putative sn-glycerol-3-phosphate 2-O-acyltransferase (Accession: NM_116426); AtGPAT2 = *A. thaliana* glycerol-3-phosphate sn-2-acyltransferase 2 (Accession: NM_100120); AtGPAT1 = *A. thaliana* sn-glycerol-3-phosphate 2-O-acyltransferase (Accession: NM_100531); AtGPAT7 = *A. thaliana* glycerol-3-phosphate acyltransferase 7 (Accession: NM_120691); AtGPAT5 = *A. thaliana* glycerol-3-phosphate acyltransferase 5 (Accession: NM_111976); QsGPAT = *Quercus suber* glycerol-3-phosphate acyltransferase (Accession: JN819185); EgGPAT5 = *E. guineensis* glycerol-3-phosphate acyltransferase 5 (Accession: XM_010923983); EgGPAT6 = *E. guineensis* glycerol-3-phosphate 2-O-acyltransferase 6 (Accession: XM_010924793); AtGPAT6 = *A. thaliana* bifunctional sn-glycerol-3-phosphate 2-O-acyltransferase/phosphatase (Accession: NM_129367); AtGPAT8 = *A.*

thaliana bifunctional sn-glycerol-3-phosphate 2-O-acyltransferase/phosphatase (Accession: NM_116264); AtGPAT4 = *A. thaliana* glycerol-3-phosphate sn-2-acyltransferase (Accession: NM_100043); GhGPAT = *Gossypium hirsutum* probable glycerol-3-phosphate acyltransferase 3 (Accession: XM_016838669); EgGPAT4 = *E. guineensis* glycerol-3-phosphate 2-O-acyltransferase 4-like (Accession: XM_010942191).

Figure 12. Testing the effect of GPAT9 genes from *Arabidopsis thaliana* (*AtGPAT9*) and from *Cocos nucifera* (*CnGPAT9*) expression on TAG content, determined by transient *Nicotiana benthamiana* leaf expression (n=4).

Figure 13. Fatty acid composition analysis of triacylglycerol (TAG), determined by the analysis of fatty acid methyl esters (FAME) via gas chromatography-flame ionisation detection (GC-FID) (n=3). Each thioesterase was infiltrated in combination with CnGPAT9 alone, CnGPAT9 + CnLPAAT or CnGPAT9 + CnLPAAT + EgDGAT1, with all treatments including expression of AtWRI1 (*Arabidopsis thaliana* WRINKLED1). CcTE = *Cinnamomum camphora* thioesterase; CnTE2 = Cocos nucifera thioesterase; UcTE = *Umbellularia californica* thioesterase; CnGPAT9 = C. nucifera glycerol-3-phosphate acyltransferase 9; CnLPAAT = *C. nucifera* lysophosphatidic acid acyltransferase; EgDGAT1 = *Elaeis guineensis* diacylglycerol acyltransferase.

KEY TO THE SEQUENCE LISTING

SEQ ID NO:1 *Arabidopsis thaliana* DGAT1 polypeptide (CAB44774.1)

SEQ ID NO:2 YFP tripeptide – conserved DGAT2 and/or MGAT1/2 sequence motif

SEQ ID NO:3 HPHG tetrapeptide – conserved DGAT2 and/or MGAT1/2 sequence motif

SEQ ID NO:4 EPHS tetrapeptide – conserved plant DGAT2 sequence motif

SEQ ID NO:5 RXGFX(K/R)XAXXXGXXX(L/V)VPXXXFG(E/Q) – long conserved sequence motif of DGAT2 which is part of the putative glycerol phospholipid domain

SEQ ID NO:6 FLXLXXXN – conserved sequence motif of mouse DGAT2 and MGAT1/2 which is a putative neutral lipid binding domain

SEQ ID NO:7 Conserved GPAT amino acid sequence GDLVICPEGTTCREP

SEQ ID NO:8 Conserved GPAT/phosphatase amino acid sequence (Motif I)

SEQ ID NO:9 Conserved GPAT/phosphatase amino acid sequence (Motif III)

SEQ ID NO:10 Sorbi-WRL1

SEQ ID NO:11 Lupan-WRL1

SEQ ID NO:12 Ricco-WRL1

SEQ ID NO:13 *Lupin angustifolius* WRI1 polypeptide

SEQ ID NO:14 WRI1 motif (R G V T/S R H R W T G R)

SEQ ID NO:15 WRI1 motif (F/Y E A H L W D K)

SEQ ID NO:16 WRI1 motif (D L A A L K Y W G)

SEQ ID NO:17 WRI1 motif (S X G F S/A R G X)

SEQ ID NO:18 WRI1 motif (H H H/Q N G R/K W E A R I G R/K V)

SEQ ID NO:19 WRI1 motif (Q E E A A A X Y D)

SEQ ID NO:20 pJP3502 TDNA (inserted into genome) sequence

SEQ ID NO:21 pJP3507 vector sequence

SEQ ID NO:22 Linker sequence

SEQ ID NO:23 Partial *Nicotiana benthamiana* CGI-58 sequence selected for hpRNAi silencing (pTV46)

SEQ ID NO:24 Partial *N. tabacum* AGPase sequence selected for hpRNAi silencing (pTV35)

SEQ ID NO:25 GXSXG lipase motif

SEQ ID NO:26 HX(4)D acyltransferase motif

SEQ ID NO:27 VX(3)HGF probable lipid binding motif

SEQ ID NO:28 *Arabidopsis thaliana* BBM polypeptide (NP_197245.2)

SEQ ID NO:29 Inducible *Aspergilus niger* alcA promoter

SEQ ID NO:30 AlcR inducer that activates the AlcA promotor in the presence of ethanol

SEQ ID NO:31 *Arabidopsis thaliana* LEC1; (AAC39488)

SEQ ID NO:32 *Zea mays* LEC1 (AAK95562)

SEQ ID NO:33 *Arabidopsis thaliana* LEC1-like (AAN15924)

SEQ ID NO:34 *Arabidopsis thaliana* FUS3 (AAC35247)

SEQ ID NO:35 *Brassica napus* FUS3

SEQ ID NO:36 *Medicago truncatula* FUS3

SEQ ID NO:37 *Arabidopsis thaliana* SDP1 cDNA sequence, Accession No. NM_120486, 3275nt

SEQ ID NO:38 *Sorghum bicolor* SDP1 cDNA XM_002458486; 2724nt

SEQ ID NO:39 *Nicotiana benthamiana* SDP1 cDNA, Nbv5tr6404201SEQ ID NO:40 *Nicotiana benthamiana* SDP1 cDNA region targeted for hpRNAi silencing

SEQ ID NO:41 Promoter of *Arabidopsis thaliana SDP1* gene, 1.5kb

SEQ ID NO:42 Nucleotide sequence of the complement of the pSSU-Oleosin gene in the T-DNA of pJP3502. In order (complementary sequences): *Glycine max* Lectin terminator 348nt, 3' exon 255nt, UBQ10 intron 304nt, 5' exon 213nt, SSU promoter 1751nt

SEQ ID NO:43 *Arabidopsis thaliana* FATA1

SEQ ID NO:44 *Arabidopsis thaliana* FATA2

SEQ ID NO:45 *Arabidopsis thaliana* FATB

SEQ ID NO:46 *Arabidopsis thaliana* WRI3

SEQ ID NO:47 *Arabidopsis thaliana* WRI4

SEQ ID NO:48 *Avena sativa* WRI1

SEQ ID NO:49 *Sorghum bicolor* WRI1

SEQ ID NO:50 *Zea mays* WRI1

SEQ ID NO:51 *Triadica sebifera WRI1*

SEQ ID NO:52 *S. tuberosum* Patatin B33 promoter sequence

SEQ ID NO:53 *Z. mays* SEE1 promoter region (1970nt from Accession number AJ494982)

SEQ ID NO:54 *A. littoralis* AlSAP promoter sequence, Accession No DQ885219

SEQ ID NO:55 *A. rhizogenes* ArRolC promoter sequence, Accession No. DQ160187

SEQ ID NO:56 *Elaeis guineensis* (oil palm) DGAT1

SEQ ID NO:57 *G. max* MYB73, Accession No. ABH02868

SEQ ID NO:58 *A. thaliana* bZIP53, Accession No. AAM14360

SEQ ID NO:59 *A. thaliana* AGL15, Accession No NP_196883

SEQ ID NO:60 *A. thaliana* MYB118, Accession No. AAS58517

SEQ ID NO:61 *A. thaliana* MYB115, Accession No. AAS10103

SEQ ID NO:62 *A. thaliana* TANMEI, Accession No. BAE44475

SEQ ID NO:63 *A. thaliana* WUS, Accession No. NP_565429

SEQ ID NO:64 *B. napus* GFR2a1, Accession No. AFB74090

SEQ ID NO:65 *B. napus* GFR2a2, Accession No. AFB74089

SEQ ID NO:66 *A. thaliana* PHR1, Accession No. AAN72198

SEQ ID NO:67 *Sapium sebiferum* LDAP-1 nucleotide sequence

SEQ ID NO:68 *Sapium sebiferum* LDAP-1 amino acid sequence

SEQ ID NO:69 *Sapium sebiferum* LDAP-2 nucleotide sequence

SEQ ID NO:70 *Sapium sebiferum* LDAP-2 amino acid sequence

SEQ ID NO:71 *Sapium sebiferum* LDAP-3 nucleotide sequence

SEQ ID NO:72 *Sapium sebiferum* LDAP-3 amino acid sequence

SEQ ID NO:73 *S. bicolor* SDP1 (accession number XM_002463620)

SEQ ID NO:74 *T. aestivum* SDP1 nucleotide sequence (Accession number AK334547)
SEQ ID NO:75 *S. bicolor* SDP1 hpRNAi fragment.
SEQ ID NO's 76 to 81 Oligonucleotide primer sequence
SEQ ID NO:82 *Saccharum* hybrid DIRIGENT (DIR16) promoter sequence
SEQ ID NO:83 *Saccharum* hybrid O-Methyl transferase (OMT) promoter sequence
SEQ ID NO:84 Sequence of the A1 promoter allele of the *Saccharum* hybrid *R1MYB1* gene
SEQ ID NO:85 *Saccharum* hybrid Loading Stem Gene 5 (LSG5) promoter sequence
SEQ ID NO:86 Amino acid sequence of *Sesamum indicum* oleosinL polypeptide (Accession No. AF091840)
SEQ ID NO:87 Amino acid sequence of *Cinnamomum camphora* 14:0-ACP thioesterase (Accession No. Q39473.1)
SEQ ID NO:88 Amino acid sequence of *Cocos nucifera* acyl-ACP thioesterase FatB1 (Accession No. AEM72519.1)
SEQ ID NO:89 Amino acid sequence of *Cocos nucifera* acyl-ACP thioesterase FatB2 (Accession No. AEM72520.1)
SEQ ID NO:90 Amino acid sequence of *Cocos nucifera* acyl-ACP thioesterase FatB3 (Accession No. AEM72521.1)
SEQ ID NO:91 Amino acid sequence of *Cuphea lanceolata* acyl-(ACP) thioesterase type B (Accession No. CAB60830.1)
SEQ ID NO:92 Amino acid sequence of *Cuphea viscosissima* FatB1 (Accession No. AEM72522.1)
SEQ ID NO:93 Amino acid sequence of and *Umbellularia californica* 12:0-ACP thioesterase (Accession No. Q41635.1)
SEQ ID NO:94 Amino acid sequence of *C. nucifera* LPAAT (Accession No. Q42670.1)
SEQ ID NO:95 Amino acid sequence of *A. thaliana* plastidial LPAAT1 (Accession No. AEE85783.1)
SEQ ID NO:96 Codon optimised nucleotide sequence of *Elaeis guineensis* DGAT1
SEQ ID NO:97 Amino acid sequence of *Cocos nucifera* GPAT9
SEQ ID NO:98 Amino acid sequence of *Arabidopsis thaliana* GPAT9
SEQ ID NO:99 Amino acid sequence of *Elaeis guineensis* GPAT9
SEQ ID NO:100 Amino acid sequence of *Phoenix dactylifera* GPAT9
SEQ ID NO:101 Amino acid sequence of *Musa acuminata* GPAT9
SEQ ID NO:102 Amino acid sequence of *Ananas comosus* GPAT9
SEQ ID NO:103 Amino acid sequence of *Asparagus officinalis* GPAT9

SEQ ID NO:104 Amino acid sequence of *Oryza brachyantha* GPAT9
SEQ ID NO:105 Amino acid sequence of *Oryza sativa* GPAT9
SEQ ID NO:106 Amino acid sequence of *Nelumbo nucifera* GPAT9
SEQ ID NO:107 Amino acid sequence of *Vitis vinifera* GPAT9
SEQ ID NO:108 Amino acid sequence of *Nicotiana tomentosiformis* GPAT9
SEQ ID NO:109 Amino acid sequence of *Jatropha curcas* GPAT9
SEQ ID NO:110 Amino acid sequence of *Glycine max* GPAT9
SEQ ID NO:111 Amino acid sequence of *Sesamum indicum* GPAT9
SEQ ID NO:112 Amino acid sequence of *Brachypodium distachyon* GPAT9
SEQ ID NO:113 Amino acid sequence of *Setaria italica* GPAT9
SEQ ID NO:114 Amino acid sequence of *Cicer arietinum* GPAT9
SEQ ID NO:115 Amino acid sequence of *Zea mays* GPAT9
SEQ ID NO:116 Amino acid sequence of *Gossypium hirsutum* GPAT9
SEQ ID NO:117 Amino acid sequence of *Eucalyptus grandis* GPAT9
SEQ ID NO:118 Amino acid sequence of *Cucumis sativus* GPAT9
SEQ ID NO:119 Amino acid sequence of *Gossypium arboreum* GPAT9
SEQ ID NO:120 Nucleotide sequence of *Cocos nucifera* GPAT9
SEQ ID NO:121 Nucleotide sequence of *Arabidopsis thaliana* GPAT9
SEQ ID NO:122 Nucleotide sequence of *Elaeis guineensis* GPAT9
SEQ ID NO:123 Nucleotide sequence of *Phoenix dactylifera* GPAT9
SEQ ID NO:124 Nucleotide sequence of *Musa acuminata* GPAT9
SEQ ID NO:125 Nucleotide sequence of *Ananas comosus* GPAT9
SEQ ID NO:126 Nucleotide sequence of *Asparagus officinalis* GPAT9
SEQ ID NO:127 Nucleotide sequence of *Oryza brachyantha* GPAT9
SEQ ID NO:128 Nucleotide sequence of *Oryza sativa* GPAT9
SEQ ID NO:129 Nucleotide sequence of *Nelumbo nucifera* GPAT9
SEQ ID NO:130 Nucleotide sequence of *Vitis vinifera* GPAT9
SEQ ID NO:131 Nucleotide sequence of *Nicotiana tomentosiformis* GPAT9
SEQ ID NO:132 Nucleotide sequence of *Jatropha curcas* GPAT9
SEQ ID NO:133 Nucleotide sequence of *Glycine max* GPAT9
SEQ ID NO:134 Nucleotide sequence of *Sesamum indicum* GPAT9
SEQ ID NO:135 Nucleotide sequence of *Brachypodium distachyon* GPAT9
SEQ ID NO:136 Nucleotide sequence of *Setaria italica* GPAT9
SEQ ID NO:137 Nucleotide sequence of *Cicer arietinum* GPAT9
SEQ ID NO:138 Nucleotide sequence of *Zea mays* GPAT9
SEQ ID NO:139 Nucleotide sequence of *Gossypium hirsutum* GPAT9

SEQ ID NO:140 Nucleotide sequence of *Eucalyptus grandis* GPAT9
SEQ ID NO:141 Nucleotide sequence of *Cucumis sativus* GPAT9
SEQ ID NO:142 Nucleotide sequence of *Gossypium arboreum* GPAT9
SEQ ID NO:143 Amino acid sequence of *E. guineensis* NF-YB1
SEQ ID NO:144 Amino acid sequence of *E. guineensis* ZFP1
SEQ ID NO:145 Amino acid sequence of *A. thaliana* NF-YB2
SEQ ID NO:146 Amino acid sequence of *A. thaliana* NF-YB3
SEQ ID NO:147 Amino acid sequence of *A. thaliana* ZFP2
SEQ ID NO:148 Amino acid sequence of *E. guineensis* ABI5
SEQ ID NO:149 Amino acid sequence of *E. guineensis* NF-YC2
SEQ ID NO:150 Amino acid sequence of *E. guineensis* NF-YA3
SEQ ID NO:151 Amino acid sequence of *G. max* DOF4
SEQ ID NO:152 Amino acid sequence of *G. max* ZF351

DETAILED DESCRIPTION OF THE INVENTION

General Techniques

Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (e.g., in cell culture, molecular genetics, plant biology, cell biology, protein chemistry, lipid and fatty acid chemistry, animal nutrition, biofeul production, and biochemistry).

Unless otherwise indicated, the recombinant protein, cell culture, and immunological techniques utilized in the present invention are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), DNA Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996), F.M. Ausubel et al. (editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present), Ed Harlow and David Lane (editors) Antibodies: A Laboratory Manual, Cold Spring Harbour Laboratory, (1988), and J.E. Coligan et al. (editors) Current Protocols in Immunology, John Wiley & Sons (including all updates until present).

Selected Definitions

The term "exogenous" in the context of a polynucleotide or polypeptide refers to the polynucleotide or polypeptide when present in a cell or a plant or part thereof which does not naturally comprise the polynucleotide or polypeptide. Such a cell is referred to herein as a “recombinant cell” or a “transgenic cell” and a plant comprising the cell as a “transgenic plant”. In an embodiment, the exogenous polynucleotide or polypeptide is from a different genus to the cell of the plant or part thereof comprising the exogenous polynucleotide or polypeptide. In another embodiment, the exogenous polynucleotide or polypeptide is from a different species. In one embodiment, the exogenous polynucleotide or polypeptide expressed in the plant cell is from a different species or genus. The exogenous polynucleotide or polypeptide may be non-naturally occurring, such as for example, a synthetic DNA molecule which has been produced by recombinant DNA methods. The DNA molecule may, preferably, include a protein coding region which has been codon-optimised for expression in the plant cell, thereby producing a polypeptide which has the same amino acid sequence as a naturally occurring polypeptide, even though the nucleotide sequence of the protein coding region is non-naturally occurring. The exogenous polynucleotide may encode, or the exogenous polypeptide may be, for example: a diacylglycerol acyltransferase (DGAT) such as a DGAT1 or a DGAT2, a Wrinkled 1 (WRI1) transcription factor, on OBC such as an Oleosin or preferably an LDAP, a fatty acid thioesterase such as a FATA or FATB polypeptide, or a silencing suppressor polypeptide. In an embodiment, a cell of the invention is a recombinant cell.

As used herein, the term “triacylglycerol (TAG) content” or variations thereof refers to the amount of TAG in the cell, plant or part thereof. TAG content can be calculated using techniques known in the art such as the sum of glycerol and fatty acyl moieties using a relation: % TAG by weight = 100x ((41x total mol FAME/3)+(total g FAME- (15x total mol FAME)))/g, where 41 and 15 are molecular weights of glycerol moiety and methyl group, respectively (where FAME is fatty acid methyl esters) (see Examples such as Example 1).

As used herein, the term “total fatty acid (TFA) content” or variations thereof refers to the total amount of fatty acids in the cell, plant or part thereof on a weight basis, as a percentage of the weight of the cell, plant or part thereof. Unless otherwise specified, the weight of the cell, plant or part thereof is the dry weight of the cell, plant or part thereof. TFA content is measured as described in Example 1 herein. The method involves conversion of the fatty acids in the sample to FAME and measurement of the amount of FAME by GC, using addition of a known amount of a distinctive fatty acid

standard such as C17:0 as a quantitation standard in the GC. TFA therefore represents the weight of just the fatty acids, not the weight of the fatty acids and their linked moieties in the plant lipid.

As used herein, the“TAG/TFA Quotient” or “TTQ” parameter is calculated as the level of TAG (%) divided by the level of TFA (%), each as a percentage of the dry weight of the plant material. For example, a TAG level of 6% comprised in a TFA level of 10% yields a TTQ of 0.6. The TAG and TFA levels are measured as described herein. It is understood that, in this context, the TFA level refers to the weight of the total fatty acid content and the TAG level refers to the weight of TAG, including the glycerol moiety of TAG.

As used herein, the term “soluble protein content” or variations thereof refers to the amount of soluble protein in the plant or part thereof. Soluble protein content can be calculated using techniques known in the art. For instance, fresh tissue can be ground, chlorophyll and soluble sugars extracted by heating to 80°C in 50-80% (v/v) ethanol in 2.5 mM HEPES buffer at pH 7.5, centriguation, washing pellet in distilled water, resuspending the pellet 0.1 M NaOH and heating to 95°C for 30 min, and then the Bradford assay (Bradford, 1976) is used determined soluble protein content. Alternatively, fresh tissue can be ground in buffer containing 100 mM Tris-HCl pH 8.0 and 10 mM $MgCl_2$.

As used herein, the term “nitrogen content” or variations thereof refers to the amount of nitrogen in the plant or part thereof. Nitrogen content can be calculated using techniques known in the art. For example, freeze-dried tissue can be analysed using a Europa 20-20 isotope ratio mass spectrometer with an ANCA preparation system, comprising a combustion and reduction tube operating at 1000°C and 600°C, respectively, to determine nitrogen content.

As used herein, the term “carbon content” or variations thereof refers to the amount of carbon in the plant or part thereof. Carbon content can be calculated using techniques known in the art. For example, organic carbon levels can be deteremined using the method described by Shaw (1959), or as described in Example 1 of WO 2016/004473.

As used herein, the term “carbon:nitrogen ratio” or variations thereof refers to the relative amount of carbon in the cell, plant or part thereof when compared to the amount of nitrogen in the cell, plant or part thereof. Carbon and nitrogen contents can be calculated as described above and representated as a ratio.

As used herein, the term “photosynthetic gene expression” or variations thereof refers to one or more genes expressing proteins involved in photosynthetic pathways in

the plant ot part thereof. Examples of photosynthetic genes which may be upregulated in plants or parts thereof of the invention include, but are not limited to, one or more of the genes listed in Table 10 of WO 2016/004473.

As used herein, the term “photosynthetic capacity” or variations thereof refers to the ability of the plant or part thereof to photosynthesize (convert light energy to chemical energy). Photosynthetic capacity (A_{max}) is a measure of the maximum rate at which leaves are able to fix carbon during photosynthesis. It is typically measured as the amount of carbon dioxide that is fixed per metre squared per second, for example as μmol m^{-2} sec^{-1}. Photosynthetic capacity can be calculated using techniques known in the art.

As used herein, the term “total dietary fibre (TDF) content” or variations thereof refers to the amount of fiber (including soluble and insoluble fibre) in the cell, plant or part thereof. As the skilled person would understand, dietary fiber includes non-starch polysaccharides such as arabinoxylans, cellulose, and many other plant components such as resistant starch, resistant dextrins, inulin, lignin, chitins, pectins, β-glucans, and oligosaccharides. TDF can be calculated using techniques known in the art. For example, using the Prosky method (Prosky et al. 1985), the McCleary method (McCleary et al., 2007) or the rapid integrated total dietary fiber method (McCleary et al., 2015).

As used herein, the term “energy content” or variations thereof refers to the amount of food energy in the plant or part thereof. More specifically, the amount of chemical energy that animals (including humans) derive from their food. Energy content can be calculated using techniques known in the art. For example, energy content can be deteremined based on heats of combustion in a bomb calorimeter and corrections that take into consideration the efficiency of digestion and absorption and the production of urea and other substances in the urine. As another example, energy content can be calculated as described in Example 1 of WO 2016/004473.

As used herein, the term "extracted lipid" refers to a composition extracted from a cell, plant or part thereof of the invention, such as a transgenic cell, plant or part thereof of the invention, which comprises at least 60% (w/w) lipid.

As used herein, the term "non-polar lipid" refers to fatty acids and derivatives thereof which are soluble in organic solvents but insoluble in water. The fatty acids may be free fatty acids and/or in an esterified form. Examples of esterified forms of non-polar lipid include, but are not limited to, triacylglycerol (TAG), diacylyglycerol (DAG), monoacylglycerol (MAG). Non-polar lipids also include sterols, sterol esters and wax esters. Non-polar lipids are also known as "neutral lipids". Non-polar lipid is

typically a liquid at room temperature. In an embodiment, at least 50%, more preferably at least 70%, more preferably at least 80%, more preferably at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99% of the fatty acids in non-polar lipid of the invention are present as TAG. The non-polar lipid may be further purified or treated, for example by hydrolysis with a strong base to release the free fatty acid, or by fractionation, distillation, or the like. Non-polar lipid may be present in or obtained from plant parts such as seed, leaves, tubers, beets or fruit. Non-polar lipid of the invention may form part of "seedoil" if it is obtained from seed.

The free and esterified sterol (for example, sitosterol, campesterol, stigmasterol, brassicasterol, Δ5-avenasterol, sitostanol, campestanol, and cholesterol) concentrations in the extracted lipid may be as described in Phillips et al. (2002). Sterols in plant oils are present as free alcohols, esters with fatty acids (esterified sterols), glycosides and acylated glycosides of sterols. Sterol concentrations in naturally occurring vegetable oils (seedoils) ranges up to a maximum of about 1100mg/100g. Hydrogenated palm oil has one of the lowest concentrations of naturally occurring vegetable oils at about 60mg/100g. The recovered or extracted seedoils of the invention preferably have between about 100 and about 1000mg total sterol/100g of oil. For use as food or feed, it is preferred that sterols are present primarily as free or esterified forms rather than glycosylated forms. In the seedoils of the present invention, preferably at least 50% of the sterols in the oils are present as esterified sterols, except for soybean seedoil which has about 25% of the sterols esterified. The canola seedoil and rapeseed oil of the invention preferably have between about 500 and about 800 mg total sterol/100g, with sitosterol the main sterol and campesterol the next most abundant. The corn seedoil of the invention preferably has between about 600 and about 800 mg total sterol/100g, with sitosterol the main sterol. The soybean seedoil of the invention preferably has between about 150 and about 350 mg total sterol/100g, with sitosterol the main sterol and stigmasterol the next most abundant, and with more free sterol than esterified sterol. The cottonseed oil of the invention preferably has between about 200 and about 350 mg total sterol/100g, with sitosterol the main sterol. The coconut oil and palm oil of the invention preferably have between about 50 and about 100mg total sterol/100g, with sitosterol the main sterol. The safflower seedoil of the invention preferably has between about 150 and about 250mg total sterol/100g, with sitosterol the main sterol. The peanut seedoil of the invention preferably has between about 100 and about 200mg

total sterol/100g, with sitosterol the main sterol. The sesame seedoil of the invention preferably has between about 400 and about 600mg total sterol/100g, with sitosterol the main sterol. The sunflower seedoil of the invention preferably has between about 200 and 400mg total sterol/100g, with sitosterol the main sterol. Oils obtained from vegetative plant parts according to the invention preferably have less than 200mg total sterol/100g, more preferably less than 100mg total sterol/100g, and most preferably less than 50mg total sterols/100g, with the majority of the sterols being free sterols. In an embodiment, the lipid or oil is from a vegetative plant part which comprises one or more or all of sitosterol, campesterol, stigmasterol and cholesterol. In an embodiment, the lipid or oil is from a vegetative plant part and has more galactosylglycerides than phosphoglycerides. In an embodiment, the lipid or oil is from a seed and has more phosphoglycerides than galactosylglycerides. Further guidance regarding sterols and other lipids components of plant cells can be found in Gunstone et al. (2007) The Lipid Handbook, Third Edition, CRC Press.

As used herein, the term "vegetative oil" refers to a composition obtained from vegetative parts of a plant which comprises at least 60% (w/w) lipid, or obtainable from the vegetative parts if the oil is still present in the vegetative part. That is, vegetative oil of the invention includes oil which is present in the vegetative plant part, as well as oil which has been extracted from the vegetative part (extracted oil). The vegetative oil is preferably extracted vegetative oil. Vegetative oil is typically a liquid at room temperature. The fatty acids are typically in an esterified form such as for example, TAG, DAG, acyl-CoA, galactolipid or phospholipid. The fatty acids may be free fatty acids and/or in an esterified form. In an embodiment, at least 50%, more preferably at least 70%, more preferably at least 80%, more preferably at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99% of the fatty acids in vegetative oil of the invention can be found as TAG. In an embodiment, vegetative oil of the invention is "substantially purified" or "purified" oil that has been separated from one or more other lipids, nucleic acids, polypeptides, or other contaminating molecules with which it is associated in the vegetative plant part or in a crude extract. It is preferred that the substantially purified vegetative oil is at least 60% free, more preferably at least 75% free, and more preferably, at least 90% free from other components with which it is associated in the vegetative plant part or extract. Vegetative oil of the invention may further comprise non-fatty acid molecules such as, but not limited to, sterols. In an embodiment, the vegetative oil is canola oil

(*Brassica sp*. such as *Brassica carinata*, *Brassica juncea*, *Brassica napobrassica*, *Brassica napus*) mustard oil (*Brassica juncea*), other Brassica oil (e.g., *Brassica napobrassica, Brassica camelina)*, sunflower oil (*Helianthus sp.* such as *Helianthus annuus*), linseed oil (*Linum usitatissimum*), soybean oil (*Glycine max*), safflower oil (*Carthamus tinctorius*), corn oil (*Zea mays*), tobacco oil (*Nicotiana sp.* such as *Nicotiana tabacum* or *Nicotiana benthamiana*), peanut oil (*Arachis hypogaea*), palm oil (*Elaeis guineensis)*, cotton oil (*Gossypium hirsutum*), coconut oil (*Cocos nucifera*), avocado oil (*Persea americana*), olive oil (*Olea europaea*), cashew oil (*Anacardium occidentale*), macadamia oil (*Macadamia intergrifolia*), almond oil (*Prunus amygdalus*), oat oil (*Avena sativa),* rice oil (*Oryza sp.* such as *Oryza sativa* and *Oryza glaberrima*), *Arabidopsis* oil (*Arabidopsis thaliana*), *Aracinis hypogaea* (peanut), *Beta vulgaris* oil (sugar beet), *Camelina sativa* oil (false flax), *Crambe abyssinica* oil (Abyssinian kale), *Cucumis melo* oil (melon), *Hordeum vulgare* oil (barley), *Jatropha curcas* oil (physic nut), *Joannesia princeps* oil (arara nut-tree), *Licania rigida* oil (oiticica), *Lupinus angustifolius* oil (lupin), *Miscanthus sp.* oil such as *Miscanthus x giganteus* oil and *Miscanthus sinensis* oil, *Panicum virgatum* (switchgrass) oil, *Pongamia pinnata* oil (Indian beech), *Populus trichocarpa* oil, *Ricinus communis* oil (castor), *Saccharum sp.* oil (sugarcane), *Sesamum indicum* oil (sesame), *Solanum tuberosum* oil (potato), *Sorghum sp.* oil such as *Sorghum bicolor* oil, *Sorghum vulgare* oil*, Theobroma grandiforum* oil (cupuassu), *Trifolium sp.* oil, and *Triticum sp.* oil (wheat) such as *Triticum aestivum*. oil Vegetative oil may be extracted from vegetative plant parts by any method known in the art, such as for extracting seedoils. This typically involves extraction with nonpolar solvents such as diethyl ether, petroleum ether, chloroform/methanol or butanol mixtures, generally associated with first crushing of the seeds. Lipids associated with the starch or other polysaccharides may be extracted with water-saturated butanol. The seedoil may be "de-gummed" by methods known in the art to remove polar lipids such as phospholipids or treated in other ways to remove contaminants or improve purity, stability, or colour. The TAGs and other esters in the vegetative oil may be hydrolysed to release free fatty acids, or the oil hydrogenated, treated chemically, or enzymatically as known in the art. As used herein, the term “seedoil” has an analogous meaning except that it refers to a lipid composition obtained from seeds of plants of the invention.

As used herein, the term "fatty acid" refers to a carboxylic acid with an aliphatic tail of at least 6 carbon atoms in length, either saturated or unsaturated. Preferred fatty acids have a carbon-carbon bonded chain of at least 12 carbons in length, more preferably fatty acids having have a carbon-carbon bonded chain of 12 and/or 14

carbons in length . Most naturally occurring fatty acids have an even number of carbon atoms because their biosynthesis involves acetate which has two carbon atoms. The fatty acids may be in a free state (non-esterified) or in an esterified form such as part of a TAG, DAG, MAG, acyl-CoA (thio-ester) bound, acyl-ACP bound, or other covalently bound form. When covalently bound in an esterified form, the fatty acid is referred to herein as an "acyl" group. The fatty acid may be esterified as a phospholipid such as a phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidylinositol (PI), or diphosphatidylglycerol. Saturated fatty acids do not contain any double bonds or other functional groups along the chain. The term "saturated" refers to hydrogen, in that all carbons (apart from the carboxylic acid [-COOH] group) contain as many hydrogens as possible. In other words, the omega (ω) end contains 3 hydrogens (CH_3-) and each carbon within the chain contains 2 hydrogens (-CH_2-). Unsaturated fatty acids are of similar form to saturated fatty acids, except that one or more alkene functional groups exist along the chain, with each alkene substituting a singly-bonded "-CH_2-CH_2-" part of the chain with a doubly-bonded "-CH=CH-" portion (that is, a carbon double bonded to another carbon). The two next carbon atoms in the chain that are bound to either side of the double bond can occur in a *cis* or *trans* configuration.

As used herein, a fatty acid with a “medium chain length”, also referred to as “MCFA”, comprises an acyl chain of 6 to 14 carbons. The acyl chain may be modified (for example it may comprise one or more double bonds, a hydroxyl group, an expoxy group, etc) or preferably is a saturated MCFA. This terms at least includes one or more or all of caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), and myristic acid (C14:0). In an embodiment, the medium chain length fatty acids are lauric acid and/or myristic acid, or capric, lauric and myristic.

As used herein, “new medium chain fatty acids” or “new medium chain fatty acid content” or the like refers to the difference between the total MCFA content of the extracted lipid, oil, recombinant cell, plant or plant part, or seed, of the invention as the context determines, expressed as a percentage of the total fatty acid content, and the total MCFA content of a corresponding wild-type extracted lipid, oil, recombinant cell, plant or plant part, or seed, obtained from a wild-type plant. That is, the new MCFA refers to the increased MCFA of the product of the invention relative to the corresponding wild-type product. These new medium chain fatty acids are the fatty acids that are produced in the cells, plants and plant parts, or seeds, of the invention by the expression of the genetic constructs (exogenous polynucleotides) introduced into the cells, and include (if present) lauric acid and/or myristic acid. Exemplary total

medium chain fatty acid contents and new medium chain fatty acid contents are determined by conversion of fatty acids in a sample to FAME and analysis by GC, as described in Example 1.

As used herein, “new medium chain fatty acids in the total fatty acid content of the TAG of the extracted lipid” or the like refers to the difference of the total MCFA content esterified in the form of triacylglycerols in the extracted lipid, oil, recombinant cell, plant or plant part, or seed, as the context determines, expressed as a percentage of the total fatty acid content esterified in TAG, and the total MCFA content esterified in the form of triacylglycerols in a corresponding wild-type extracted lipid, oil, recombinant cell, plant or plant part, or seed, obtained from a wild-type plant.

As used herein, the terms "monounsaturated fatty acid" or "MUFA" refer to a fatty acid which comprises at least 12 carbon atoms in its carbon chain and only one alkene group (carbon-carbon double bond), which may be in an esterified or non-esterified (free) form. As used herein, the terms "polyunsaturated fatty acid" or "PUFA" refer to a fatty acid which comprises at least 12 carbon atoms in its carbon chain and at least two alkene groups (carbon-carbon double bonds), which may be in an esterified or non-esterified form.

"Monoacylglyceride" or "MAG" is glyceride in which the glycerol is esterified with one fatty acid. As used herein, MAG comprises a hydroxyl group at an *sn*-1/3 (also referred to herein as *sn*-1 MAG or 1-MAG or 1/3-MAG) or *sn*-2 position (also referred to herein as 2-MAG), and therefore MAG does not include phosphorylated molecules such as PA or PC. MAG is thus a component of neutral lipids in a plant or part thereof.

"Diacylglyceride" or "DAG" is glyceride in which the glycerol is esterified with two fatty acids which may be the same or, preferably, different. As used herein, DAG comprises a hydroxyl group at a *sn*-1,3 or *sn*-2 position, and therefore DAG does not include phosphorylated molecules such as PA or PC. DAG is thus a component of neutral lipids in a plant or part thereof. In the Kennedy pathway of DAG synthesis (Figure 1), the precursor *sn*-glycerol-3-phosphate (G3P) is esterified to two acyl groups, each coming from a fatty acid coenzyme A ester, in a first reaction catalysed by a glycerol-3-phosphate acyltransferase (GPAT) at position *sn*-1 to form LysoPA, followed by a second acylation at position *sn*-2 catalysed by a lysophosphatidic acid acyltransferase (LPAAT) to form phosphatidic acid (PA). This intermediate is then de-phosphorylated by PAP to form DAG. DAG may also be formed from TAG by removal of an acyl group by a lipase, or from PC essentially by removal of a choline headgroup by any of the enzymes PDCT, PLC or PLD (Figure 1).

"Triacylglyceride" or "TAG" is a glyceride in which the glycerol is esterified with three fatty acids which may be the same (e.g. as in tri-olein) or, more commonly, different. In the Kennedy pathway of TAG synthesis, DAG is formed as described above, and then a third acyl group is esterified to the glycerol backbone by the activity of DGAT. Alternative pathways for formation of TAG include one catalysed by the enzyme PDAT (Figure 1) and the MGAT pathway described herein.

As used herein, the term "wild-type" or variations thereof refers to a cell, plant or part thereof such as a cell, vegetative plant part, seed, tuber or beet, that has not been genetically modified, such as cells, plants or parts thereof that do not comprise the one or more exogenous polynucleotides, according to this invention.

The term "corresponding" refers to a cell, plant or part thereof such as a cell, vegetative plant part, seed, tuber or beet, that has the same or similar genetic background as a cell, plant or part thereof such as a vegetative plant part, seed, tuber or beet of the invention but which has not been modified as described herein (for example, a vegetative plant part or seed which lacks the defined exogenous polynucleotide(s)). In a preferred embodiment, the corresponding plant or part thereof such as a vegetative plant part is at the same developmental stage as the plant or part thereof such as a vegetative plant part of the invention. For example, if the plant is a flowering plant, then preferably the corresponding plant is also flowering. A corresponding cell, plant or part thereof such as a vegetative plant part, can be used as a control to compare levels of nucleic acid or protein expression, or the extent and nature of trait modification, for example MCFA and/or TAG content, with the cell, plant or part thereof such as a vegetative plant part of the invention which is modified as described herein. A person skilled in the art is readily able to determine an appropriate "corresponding" cell, plant or part thereof such as a vegetative plant part for such a comparison.

As used herein, "compared with" or “relative to” refers to comparing levels of, for example, MCFA or triacylglycerol (TAG) content, one or more or all of soluble protein content, nitrogen content, carbon:nitrogen ratio, photosynthetic gene expression, photosynthetic capacity, total dietary fibre (TDF) content, carbon content, and energy content, or non-polar lipid content or composition, total non-polar lipid content, total fatty acid content or other parameter of the cell, plant or part thereof comprising the one or more exogenous polynucleotides, genetic modifications or exogenous polypeptides with a cell, plant or part thereof such as a vegetative plant part lacking the one or more exogenous polynucelotides, genetic modifications or polypeptides.

As used herein, “synergism”, “synergistic”, “acting synergistically” and related terms are each a comparative term that means that the effect of a combination of elements present in a plant or part thereof of the invention, for example a combination of elements A and B, is greater than the sum of the effects of the elements separately in corresponding plants or parts thereof, for example the sum of the effect of A and the effect of B. Where more than two elements are present in the plant or part thereof, for example elements A, B and C, it means that the effect of the combination of all of the elements is greater than the sum of the effects of the individual effects of the elements. In a preferred embodiment, it means that the effect of the combination of elements A, B and C is greater than the sum of the effect of elements A and B combined and the effect of element C. In such a case, it can be said that element C acts synergistically with elements A and B. As would be understood, the effects are measured in corresponding cells, plants or parts thereof, for example grown under the same conditions and at the same stage of biological development.

As used herein, "germinate at a rate substantially the same as for a corresponding wild-type plant" or similar phrases refers to seed of a plant of the invention being relatively able to germinate when compared to seed of a wild-type plant lacking the defined exogenous polynucleotide(s) and genetic modifications. Germination may be measured *in vitro* on tissue culture medium or in soil as occurs in the field. In one embodiment, the number of seeds which germinate, for instance when grown under optimal greenhouse conditions for the plant species, is at least 75%, more preferably at least 90%, when compared to corresponding wild-type seed. In another embodiment, the seeds which germinate, for instance when grown under optimal glasshouse conditions for the plant species, produce seedlings which grow at a rate which, on average, is at least 75%, more preferably at least 90%, when compared to corresponding wild-type plants. This is referred to as “seedling vigour”. In an embodiment, the rate of initial root growth and shoot growth of seedlings of the invention is essentially the same compared to a corresponding wild-type seedling grown under the same conditions. In an embodiment, the leaf biomass (dry weight) of the plants of the invention is at least 80%, preferably at least 90%, of the leaf biomass relative to a corresponding wild-type plant grown under the same conditions, preferably in the field. In an embodiment, the height of the plants of the invention is at least 70%, preferably at least 80%, more preferably at least 90%, of the plant height relative to a corresponding wild-type plant grown under the same conditions, preferably in the field and preferably at maturity.

As used herein, the term "an exogenous polynucleotide which down-regulates the production and/or activity of an endogenous polypeptide" or variations thereof, refers to a polynucleotide that encodes an RNA molecule, herein termed a “silencing RNA molecule” or variations thereof (for example, encoding an amiRNA or hpRNAi), that down-regulates the production and/or activity, or itself down-regulates the production and/or activity (for example, is an amiRNA or hpRNA which can be delivered directly to, for example, the plant or part thereof) of an endogenous polypeptide. This includes where the initial RNA transcript produced by expression of the exogenous polynucleotide is processed in the cell to form the actual silencing RNA molecule. The endogenous polypeptides whose production or activity are downregulated include, for example, SDP1 TAG lipase, plastidial GPAT, plastidial LPAAT, TGD polypeptide such as TGD5, TST such as TST1 or TST2, AGPase, PDCT, CPT or Δ12 fatty acid desturase (FAD2), or a combination of two or more thereof. Typically, the RNA molecule decreases the expression of an endogenous gene encoding the polypeptide. The extent of down-regulation is typically less than 100%, for example the production or activity is reduced by between 25% and 95% relative to the wild-type. The optimal level of remaining production or activity can be routinely determined.

As used herein, the term "on a weight basis" refers to the weight of a substance (for example, TAG, DAG, fatty acid, protein, nitrogen, carbon) as a percentage of the weight of the composition comprising the substance (for example, seed, leaf dry weight). For example, if a transgenic seed has 25 μg total fatty acid per 120 μg seed weight; the percentage of total fatty acid on a weight basis is 20.8%.

As used herein, the term "on a relative basis" refers to a parameter such as the amount of a substance in a composition comprising the substance in comparison with the parameter for a corresponding composition, as a percentage. For example, a reduction from 3 units to 2 units is a reduction of 33% on a relative basis.

As used herein, “plastids” are organelles in plants, including algae, which are the site of manufacture of carbon-based compounds from photosynthesis including sugars, starch and fatty acids. Plastids include chloroplasts which contain chlorophyll and carry out photosynthesis, etioplasts which are the predecessors of chloroplasts, as well as specialised plastids such as chromoplasts which are coloured plastids for synthesis and storage of pigments, gerontoplasts which control the dismantling of the photosynthetic apparatus during senescence, amyloplasts for starch synthesis and storage, elaioplasts for storage of lipids, and proteinoplasts for storing and modifying proteins.

As used herein, the term "biofuel" refers to any type of fuel, typically as used to power machinery such as automobiles, planes, boats, trucks or petroleum powered motors, whose energy is derived from biological carbon fixation. Biofuels include fuels derived from biomass conversion, as well as solid biomass, liquid fuels and biogases. Examples of biofuels include bioalcohols, biodiesel, synthetic diesel, vegetable oil, bioethers, biogas, syngas, solid biofuels, algae-derived fuel, biohydrogen, biomethanol, 2,5-Dimethylfuran (DMF), biodimethyl ether (bioDME), Fischer-Tropsch diesel, biohydrogen diesel, mixed alcohols and wood diesel.

As used herein, the term "bioalcohol" refers to biologically produced alcohols, for example, ethanol, propanol and butanol. Bioalcohols are produced by the action of microorganisms and/or enzymes through the fermentation of sugars, hemicellulose or cellulose.

As used herein, the term "biodiesel" refers to a composition comprising fatty acid methyl- or ethyl- esters derived from lipids by transesterification, the lipids being from living cells not fossil fuels.

As used herein, the term "synthetic diesel" refers to a form of diesel fuel which is derived from renewable feedstock rather than the fossil feedstock used in most diesel fuels.

As used herein, the term "vegetable oil" includes a pure plant oil (or straight vegetable oil) or a waste vegetable oil (by product of other industries), including oil produced in either a vegetative plant part or in seed. Vegetable oil includes vegetative oil and seedoil, as defined herein.

As used herein, the term "biogas" refers to methane or a flammable mixture of methane and other gases produced by anaerobic digestion of organic material by anaerobes.

As used herein, the term "syngas" refers to a gas mixture that contains varying amounts of carbon monoxide and hydrogen and possibly other hydrocarbons, produced by partial combustion of biomass. Syngas may be converted into methanol in the presence of catalyst (usually copper-based), with subsequent methanol dehydration in the presence of a different catalyst (for example, silica-alumina).

As used herein, the term "biochar" refers to charcoal made from biomass, for example, by pyrolysis of the biomass.

As used herein, the term "feedstock" refers to a material, for example, biomass or a conversion product thereof (for example, syngas) when used to produce a product, for example, a biofuel such as biodiesel or a synthetic diesel.

As used herein, the term "industrial product" refers to a hydrocarbon product which is predominantly made of carbon and hydrogen such as, for example, fatty acid methyl- and/or ethyl-esters or alkanes such as methane, mixtures of longer chain alkanes which are typically liquids at ambient temperatures, a biofuel, carbon monoxide and/or hydrogen, or a bioalcohol such as ethanol, propanol, or butanol, or biochar. The term "industrial product" is intended to include intermediary products that can be converted to other industrial products, for example, syngas is itself considered to be an industrial product which can be used to synthesize a hydrocarbon product which is also considered to be an industrial product. The term industrial product as used herein includes both pure forms of the above compounds, or more commonly a mixture of various compounds and components, for example the hydrocarbon product may contain a range of carbon chain lengths, as well understood in the art.

As used herein, “progeny” means the immediate and all subsequent generations of offspring produced from a parent, for example a second, third or later generation offspring.

As used herein, the term “ancestor” refers to any earlier generation of the plant comprising the first and second exogenous polynucleotides. The ancestor may be the parent plant, grandparent plant, great grandparent plant and so on.

As used herein, the term “selecting a plant” means actively selecting the plant on the basis that it has the desired phenotype, such as increased MCFA when compared to the corresponding wild-type plant.

As used herein, phrases such as “comprise a TFA content of about 5% (w/w dry weight)”, or “comprise a total TAG content of about 6% (w/w dry weight)”, or similary structured phrases, mean that more than the defined level may be present. For instance, the phrase “comprise a TFA content of about 5% (w/w dry weight)” can be used interchangeably with “comprises at least about 5% TFA (w/w dry weight)”. Extending this example further, a vegetative plant part which comprise a TFA content of about 5% (w/w dry weight) may have a 6%, or 7.5% or higher TFA content.

As used herein, unless the context indicates otherwise, the term “increased content” when used in reference to a polypeptide, or similar pharses including refrence to specific polypeptide, refers to either an exogenous polypeptide or an endogenous polypeptide. For example, a vegetative plant part of the invention may comprise an increased content of a WRI1 polypeptide, am increased GPAT9 content, an increased LPAAT content, an increased content of a DGAT polypeptide, and a decreased content of a SDP1 polypeptide, each relative to a corresponding wild-type vegetative plant part, wherein each of the WRI1 and DGAT polypeptides is independently either an

exogenous polypeptide or an endogenous polypeptide. As another example, a vegetative plant part of the invention may comprise an increased content of a WRI1 polypeptide, an increased content of a DGAT polypeptide, and an increased content of a LEC2 polypeptide, each relative to a corresponding wild-type vegetative plant part, wherein each of the WRI1, DGAT and LEC2 polypeptides is independently either an exogenous polypeptide or an endogenous polypeptide. As a further example, a vegetative plant part of the invention may comprise an increased content of a PDAT or DGAT polypeptide, a decreased content of a TGD polypeptide, and a decreased content of a SDP1 polypeptide, each relative to a corresponding wild-type vegetative plant part wherein the PDAT or DGAT is either an exogenous polypeptide or an endogenous polypeptide, and so on. An exogenous polypepetide may be the result of expression of a transgene encoding the polypeptide in the cell or plant or part thereof of the invention. The endogenous polypeptide may be the result of increased expression of an endogenous gene, such as inducing overexpression and/or providing increased levels of a transcription factor(s) for the gene.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

The term "and/or", e.g., "X and/or Y" shall be understood to mean either "X and Y" or "X or Y" and shall be taken to provide explicit support for both meanings or for either meaning.

As used herein, the term about, unless stated to the contrary, refers to +/- 10%, more preferably +/- 5%, more preferably +/- 2%, more preferably +/- 1%, even more preferably +/- 0.5%, of the designated value.

Production of Plants with Modified Traits

The present invention is based on the finding that plant traits, such as MFCA content and TAG content, in plants or parts thereof can be increased by a combination of two or more modifications selected from those designated herein as: (A). Push, (B). Pull, (C). Protect, (D). Package, (E). Plastidial Export, (F). Plastidial Import and (G). Prokaryotic Pathway.

Plants or parts thereof such as a vegetative plant parts of the invention therefore have a number of combinations of exogenous polynucleotides and/or genetic modifications each of which provide for one of the modifications. These exogenous polynucleotides and/or genetic modifications include:

(A) an exogenous polynucleotide which encodes a transcription factor polypeptide that increases the expression of one or more glycolytic and/or fatty acid biosynthetic genes in the plant or part thereof such as a vegetative plant part, providing the "Push" modification,

(B) an exogenous polynucleotide which encodes a polypeptide involved in the biosynthesis of one or more non-polar lipids in the plant or part thereof such as a vegetative plant part, providing the "Pull" modification,

(C) a genetic modification which down-regulates endogenous production and/or activity of a polypeptide involved in the catabolism of triacylglycerols (TAG) in the plant or part thereof such as a vegetative plant part when compared to a corresponding plant or part thereof such as a vegetative plant part lacking the genetic modification, providing the "Protect" modification,

(D) an exogenous polynucleotide which encodes an oil body coating (OBC) polypeptide such as a lipid droplet associated polypeptide (LDAP), providing the "Package" modification,

(E) an exogenous polynucleotide which encodes a polypeptide which increases the export of fatty acids out of plastids of the plant or part thereof such as a vegetative plant part, when compared to a corresponding plant or part thereof such as a vegetative plant part lacking the exogenous polynucleotide, providing the "Plastidial Export" modification,

(F) a genetic modification which down-regulates endogenous production and/or activity of a polypeptide involved in importing fatty acids into plastids of the plant or part thereof such as a vegetative plant part when compared to a corresponding plant or part thereof such as a vegetative plant part lacking the genetic modification, providing the "Plastidial Import" modification, and

G) a genetic modification which down-regulates endogenous production and/or activity of a polypeptide involved in diacylglycerol (DAG) production in the plastid of the plant or part thereof such as a vegetative plant part when compared to a corresponding plant or part thereof such as a vegetative plant part lacking the genetic modification, providing the "prokaryotic Pathway" modification.

Preferred combinations (also referred to herein as sets) of exogenous polynucleotides and/or genetic modifications of the invention are;

1) A, B and optionally one of C, D, E, F or G;

2) A, C and optionally one of D, E, F or G;

3) A, D and optionally one of E, F or G;

4) A, E and optionally F or G;

5) A, F and optionally G;

6) A and G;

7) A, B, C and optionally one of D, E, F or G;

8) A, B, D and optionally one of E, F or G;

9) A, B, E and optionally F or G;

10) A, B, F and optionally G;

11) A, B, C, D and optionally one of E, F or G;

12) A, B, C, E and optionally F or G;

13) A, B, C, F and optionally G;

14) A, B, D, E and optionally F or G;

15) A, B, D, F and optionally G;

16) A, B, E, F and optionally G;

17) A, C, D and optionally one of E, F or G;

18) A, C, E and optionally F or G;

19) A, C, F and optionally G;

20) A, C, D, E and optionally F or G;

21) A, C, D, F and optionally G;

22) A, C, E, F and optionally a fifth modification G;

23) A, D, E and optionally F or G;

24) A, D, F and optionally G;

25) A, D, E, F and optionally G;

26) A, E, F and optionally G;

27) Six of A, B, C, D, E, F and G omitting one of A, B, C, D, E, F or G, and

28) Any one of 1-26 above where there are two or more exogenous polynucleotides encoding two or more different transcription factor polypeptides that increases the expression of one or more glycolytic and/or fatty acid biosynthetic genes in the plant or part thereof, for example one exogenous polynucleotide encoding WRI1 and another exogenous polynucleotide encoding LEC2.

In each of the above preferred combinations there may be at least two different exogenous polynucleotides which encode at least two different transcription factor polypeptides that increases the expression of one or more glycolytic and/or fatty acid biosynthetic genes in theplant or part thereof such as a vegetative plant part.

These modifications are described more fully as follows:

A. The “Push” modification is characterised by an increased synthesis of total fatty acids in the plastids of the plant or part thereof. In an embodiment, this occurs by the increased expression and/or activity of a transcription factor which regulates fatty

acid synthesis in the plastids. In one embodiment, this can be achieved by expressing in a transgenic plant or part thereof an exogenous polynucleotide which encodes a transcription factor polypeptide that increases the expression of one or more glycolytic and/or fatty acid biosynthetic genes in the plant or part thereof. In an embodiment, the increased fatty acid synthesis is not caused by the provision to the plant or part thereof of an altered ACCase whose activity is less inhibited by fatty acids, relative to the endogenous ACCase in the plant or part thereof. In an embodiment, the plant or part thereof comprises an exogenous polynucleotide which encodes the transcription factor, preferably under the control of a promoter other than a constitutive promoter. The transcription factor may be selected from the group consisting of WRI1, LEC1, LEC1-like, LEC2, BBM, FUS3, ABI3, ABI4, ABI5, Dof4, Dof11 or the group consisting of MYB73, bZIP53, AGL15, MYB115, MYB118, TANMEI, WUS, GFR2a1, GFR2a2 and PHR1, and is preferably WRI1, LEC1 or LEC2, or WRI1 alone. In a further embodiment, the increased synthesis of total fatty acids is relative to a corresponding wild-type plant or part thereof. In an embodiment, there are two or more exogenous polynucleotides encoding two or more different transcription factor polypeptides. The "Push" modification may also be achieved by increased expression of polypeptides which modulate activity of WRI1, such as MED15 or 14-3-3 polypeptides.

B. The "Pull" modification is characterised by increased expression and/or activity in the plant or part thereof of a fatty acyl acyltransferase which catalyses the synthesis of TAG, DAG or MAG in the plant or part thereof, such as a DGAT, PDAT, LPAAT, GPAT or MGAT, preferably a DGAT or a PDAT. In one embodiment, this can be achieved by expressing in a transgenic plant or part thereof an exogenous polynucleotide which encodes a polypeptide involved in the biosynthesis of one or more non-polar lipids. In an embodiment, the acyltransferase is a membrane-bound acyltransferase that uses an acyl-CoA substrate as the acyl donor in the case of DGAT, LPAAT, GPAT or MGAT, or an acyl group from PC as the acyl donor in the case of PDAT. The Pull modification can be relative to a corresponding wild-type plant or part thereof or, preferably, relative to a corresponding plant or part thereof which has the Push modification. In an embodiment, the plant or part thereof comprises an exogenous polynucleotide which encodes the fatty acyl acyltransferase. The "Pull" modification can also be achieved by increased expression of a PDCT, CPT or phospholipase C or D polypeptide which increases the production of DAG from PC.

In a preferred embodiment, the cell comprises an exogenous polynucleotide(s) encoding one or more or all of a GPAT, LPAAT and/or DGAT which have a preference for utilising medium chain fatty acid substrates, particularly for lauric acid

and/or myristic acid. Such GPAT, LPAAT and/or DGAT having a preference for utilising medium chain fatty acid substrates include those described herein, as well as those which can be isolated from plants which naturally produce high levels of medium chain fatty acids, such as but not limited to, *Elaeis guineensis*, *Cocus nucifera*, *Attalea dubia*, *Orbignya phalerata*, *Astrocaryum murumuru*, *Bactris gasipaes*, *Pycnanthus angolensis*, *Cuphea wrightii*, *Attalea colenda*, *Laurus nobilis*, *Umbellularia californica*, *Qualea grandiflora* and *Actinodaphne hookeri*. The skilled person would appreciate that the sequences provided herein which readily be used to screen sequence databases to identify orthologous genes and proteins from the above species.

C. The "Protect" modification is characterised by a reduction in the catabolism of triacylglycerols (TAG) in the plant or part thereof. In an embodiment, this can be achieved through a genetic modification in the plant or part thereof which down-regulates endogenous production and/or activity of a polypeptide involved in the catabolism of triacylglycerols (TAG) in the plant or part thereof when compared to a corresponding plant or part thereof lacking the genetic modification. In an embodiment, the plant or part thereof has a reduced expression and/or activity of an endogenous TAG lipase in the plant or part thereof, preferably an SDP1 lipase, a Cgi58 polypeptide, an acyl-CoA oxidase such as the ACX1 or ACX2, or a polypeptide involved in β-oxidation of fatty acids in the plant or part thereof such as a PXA1 peroxisomal ATP-binding cassette transporter. This may occur by expression in the plant or part thereof of an exogenous polynucleotide which encodes an RNA molecule which reduces the expression of, for example, an endogenous gene encoding the TAG lipase such as the SDP1 lipase, acyl-CoA oxidase or the polypeptide involved in β-oxidation of fatty acids in the plant or part thereof, or by a mutation in an endogenous gene encoding, for example, the TAG lipase, acyl-CoA oxidase or polypeptide involved in β-oxidation of fatty acids. In an embodiment, the reduced expression and/or activity is relative to a corresponding wild-type plant or part thereof or relative to a corresponding plant or part thereof which has the Push modification.

D. The "Package" modification is characterised by an increased expression and/or accumulation of an oil body coating (OBC) polypeptide. In an embodiment, this can be achieved by expressing in a transgenic plant or part thereof an exogenous polynucleotide which encodes an oil body coating (OBC) polypeptide. The OBC polypeptide may be an oleosin, such as for example a polyoleosin, a caoleosin or a steroleosin, or preferably an LDAP. In an embodiment, the level of oleosin that is accumulated in the plant or part thereof is at least 2-fold higher relative to the corresponding plant or part thereof comprising the oleosin gene from the T-DNA of

pJP3502. In an embodiment, the increased expression or accumulation of the OBC polypeptide is not caused solely by the Push modification. In an embodiment, the expression and/or accumulation is relative to a corresponding wild-type plant or part thereof or, preferably, relative to a corresponding plant or part thereof which has the Push modification.

E. The "Plastidial Export" modification is characterised by an increased rate of export of total fatty acids out of the plastids of the plant or part thereof. In one embodiment, this can be achieved by expressing in a plant or part thereof an exogenous polynucleotide which encodes a polypeptide which increases the export of fatty acids out of plastids of the plant or part thereof when compared to a corresponding plant or part thereof lacking the exogenous polynucleotide. In an embodiment, this occurs by the increased expression and/or activity of a fatty acid thioesterase (TE), a fatty acid transporter polypeptide such as an ABCA9 polypeptide, or a long-chain acyl-CoA synthetase (LACS). In an embodiment, the plant or part thereof comprises an exogenous polynucleotide which encodes the TE, fatty acid transporter polypeptide or LACS. The TE may be a FATB polypeptide or preferably a FATA polypeptide. In an embodiment, the TE is preferably a TE which has a preference for hydrolysing MCFA, or MCFA and C16:0 substrates. In an embodiment, the Plastidial Export modification is relative to a corresponding wild-type plant or part thereof or, preferably, relative to a corresponding plant or part thereof which has the Push modification.

F. The "Plastidial Import" modification is characterised by a reduced rate of import of fatty acids into the plastids of the plant or part thereof from outside of the plastids. In an embodiment, this can be achieved through a genetic modification in the plant or part thereof which down-regulates endogenous production and/or activity of a polypeptide involved in importing fatty acids into plastids of the plant or part thereof when compared to a corresponding plant or part thereof lacking the genetic modification. For example, this may occur by expression in the plant or part thereof of an exogenous polynucleotide which encodes an RNA molecule which reduces the expression of an endogenous gene encoding an transporter polypeptide such as a TGD polypeptide, for example a TGD1, TGD2, TGD3, TGD4 or preferably a TGD5 polypeptide, or by a mutation in an endogenous gene encoding the TGD polypeptide. In an embodiment, the reduced rate of import is relative to a corresponding wild-type plant or part thereof or relative to a corresponding plant or part thereof which has the Push modification.

G. The "Prokaryotic Pathway" modification is characterised by a decreased amount of DAG or rate of production of DAG in the plastids of the plant or part

thereof. In an embodiment, this can be achieved through a genetic modification in the plant or part thereof which down-regulates endogenous production and/or activity of a polypeptide involved in diacylglycerol (DAG) production in the plastid when compared to a corresponding plant or part thereof lacking the genetic modification. In an embodiment, the decreased amount or rate of production of DAG occurs by a decreased production of LPA from acyl-ACP and G3P in the plastids. The decreased amount or rate of production of DAG may occur by expression in the plant or part thereof of an exogenous polynucleotide which encodes an RNA molecule which reduces the expression of an endogenous gene encoding a plastidial GPAT, plastidial LPAAT or a plastidial PAP, preferably a plastidial GPAT, or by a mutation in an endogenous gene encoding the plastidial polypeptide. In an embodiment, the decreased amount or rate of production of DAG is relative to a corresponding wild-type plant or part thereof or, preferably, relative to a corresponding plant or part thereof which has the Push modification.

The Push modification is highly desirable in the invention, and the Pull modification is preferred. The Protect and Package modifications may be complementary i.e. one of the two may be sufficient. The plant or part thereof may comprise one, two or all three of the Plastidial Export, Plastidial Import and Prokaryotic Pathway modifications. In an embodiment, at least one of the exogenous polynucleotides in the plant or part thereof, preferably at least the exogenous polynucleotide encoding the transcription factor which regulates fatty acid synthesis in the plastids, is expressed under the control of (H) a promoter other than a constitutive promoter such as, for example, a developmentally related promoter, a promoter that is preferentially active in photosynthetic cells, a tissue-specific promoter, a promoter which has been modified by reducing its expression level relative to a corresponding native promoter, or is preferably a senesence-specific promoter. More preferably, at least the exogenous polynucleotide encoding the transcription factor which regulates fatty acid synthesis in the plastids is expressed under the control of a promoter other than a constitutive promoter and the exogenous polynucleotide which encodes an RNA molecule which down-regulates endogenous production and/or activity of a polypeptide involved in the catabolism of triacylglycerols is also expressed under the control of a promoter other than a constitutive promoter, which promoters may be the same or different. Alternatively in monocotyledonous plants, the exogenous polynucleotide encoding the transcription factor which regulates fatty acid synthesis in the plastids is expressed under the control of a constitutive promoter such as, for example, a ubiquitin gene promoter or an actin gene promoter.

Plants produce some, but not all, of their membrane lipids such as MGDG in plastids by the so-called prokaryotic pathway (Figure 1). In plants, there is also a eukaryotic pathway for synthesis of galactolipids and glycerolipids which synthesizes FA first of all in the plastid and then assembles the FA into glycerolipids in the ER. MGDG synthesised by the eukaryotic pathway contains C18:3 (ALA) fatty acid esterified at the *sn*-2 position of MGDG. The DAG backbone including the ALA for the MGDG synthesis by this pathway is assembled in the ER and then imported into the plastid. In contrast, the MGDG synthesized by the prokaryotic pathway contains C16:3 fatty acid esterified at the *sn*-2 position of MGDG. The ratio of the contribution of the prokaryotic pathway relative to the eukaryotic pathway in producing MGDG (16:3) vs MGDG (18:3) is a characteristic and distinctive feature of different plant species (Mongrand et al. 1998). This distinctive fatty acid composition of MGDG allows all higher plants (angiosperms) to be classified as either so-called 16:3 or 18:3 plants. 16:3 species, exemplified by *Arabidopsis* and *Brassica napus*, generally have both of the prokaryotic and eukaryotic pathways of MGDG synthesis operating, whereas the 18:3 species exemplified by *Sorghum bicolor*, *Zea mays*, *Nicotiana tabacum*, *Pisum sativum* and *Glycine max* generally have only (or almost entirely) the eukaryotic pathway of MGDG synthesis, providing little or no C16:3 fatty acid accumulation in the vegetative tissues.

As used herein, a “16:3 plant” or “16:3 species” is one which has more than 2% C16:3 fatty acid in the total fatty acid content of its photosynthetic tissues. As used herein, a “18:3 plant” or “18:3 species” is one which has less than 2% C16:3 fatty acid in the total fatty acid content of its photosynthetic tissues. As described herein, a plant can be converted from being a 16:3 plant to an 18:3 plant by suitable genetic modifications. The proportion of flux between the prokaryote and eukaryote pathways is not conserved across different plant species or tissues. In 16:3 species up to 40% of flux in leaves occurs via the prokaryotic pathway (Browse et al., 1986), while in 18:3 species, such as pea and soybean, about 90% of FAs which are synthesized in the plastid are exported out of the plastid to the ER to supply the source of FA for the eukaryotic pathway (Ohlrogge and Browse, 1995; Somerville et al., 2000).

Therefore different amounts of 18:3 and 16:3 fatty acids are found within the glycolipids of different plant species. This is used to distinguish between 18:3 plants whose fatty acids with 3 double bonds are almost entirely C18 fatty acids and the 16:3 plants that contain both C_{16}- and C_{18}-fatty acids having 3 double bonds. In chloroplasts of 18:3 plants, enzymic activities catalyzing the conversion of phosphatidate to diacylglycerol and of diacylglycerol to monogalactosyl diacylglycerol (MGD) are

significantly less active than in 16:3 chloroplasts. In leaves of 18:3 plants, chloroplasts synthesize stearoyl-ACP2 in the stroma, introduce the first double bond into the saturated hydrocarbon chain, and then hydrolyze the thioester by thioesterases (Figure 1). Released oleate is exported across chloroplast envelopes into membranes of the cell, probably the endoplasmic reticulum, where it is incorporated into PC. PC-linked oleoyl groups are desaturated in these membranes and subsequently move back into the chloroplast. The MGD-linked acyl groups are substrates for the introduction of the third double bond to yield MGD with two linolenoyl residues. This galactolipid is characteristic of 18:3 plants such as Asteraceae and Fabaceae, for example. In photosynthetically active cells of 16:3 plants which are represented, for example, by members of Apiaceae and Brassicaceae, two pathways operate in parallel to provide thylakoids with MGD.

In one embodiment, the plant or part thereof such as a vegetative plant part of the invention produces higher levels of non-polar lipids such as TAG, or MFCA content, preferably both, than a corresponding plant or part thereof such as a vegetative plant part which lacks the genetic modifications or exogenous polynucleotides. In one example, plants of the invention produce seeds, leaves, or have leaf portions of at least 1cm^2 in surface area, stems and/or tubers having an increased non-polar lipid content such as TAG or MCFA content, preferably both, when compared to corresponding seeds, leaves, leaf portions of at least 1cm^2 in surface area, stems or tubers.

Preferably, the plant or part thereof such as a vegetative plant part of the invention is transformed with one or more exogenous polynucleotides such as chimeric DNAs. In the case of multiple chimeric DNAs, these are preferably covalently linked on one DNA molecule such as, for example, a single T-DNA molecule, and preferably integrated at a single locus in the host cell genome, preferably the host nuclear genome. Alternatively, the chimeric DNAs are on two or more DNA molecules which may be unlinked in the host genome, or the DNA molecule(s) is not integrated into the host genome, such as occurs in transient expression experiments. The plant or part thereof such as a vegetative plant part is preferably homozygous for the one DNA molecule inserted into its genome.

Transcription Factors

Various transcription factors are involved in plant cells in the synthesis of fatty acids and lipids incorporating the fatty acids such as TAG, and therefore can be manipulated for the Push modification. A preferred transcription factor is WRI1. As used herein, the term "Wrinkled 1" or "WRI1" or "WRL1" refers to a transcription

factor of the AP2/ERWEBP class which regulates the expression of several enzymes involved in glycolysis and *de novo* fatty acid biosynthesis. WRI1 has two plant-specific (AP2/EREB) DNA-binding domains. WRI1 in at least *Arabidopsis* also regulates the breakdown of sucrose via glycolysis thereby regulating the supply of precursors for fatty acid biosynthesis. In other words, it controls the carbon flow from the photosynthate to storage lipids. *wri1* mutants in at least *Arabidopsis* have a wrinkled seed phenotype, due to a defect in the incorporation of sucrose and glucose into TAGs.

Examples of genes which are transcribed by WRI1 include, but are not limited to, one or more, preferably all, of genes encoding pyruvate kinase (At5g52920, At3g22960), pyruvate dehydrogenase (PDH) E1alpha subunit (At1g01090), acetyl-CoA carboxylase (ACCase), BCCP2 subunit (At5g15530), enoyl-ACP reductase (At2g05990; EAR), phosphoglycerate mutase (At1g22170), cytosolic fructokinase, and cytosolic phosphoglycerate mutase, sucrose synthase (SuSy) (see, for example, Liu et al., 2010; Baud et al., 2007; Ruuska et al., 2002).

WRI1 contains the conserved domain AP2 (cd00018). AP2 is a DNA-binding domain found in transcription regulators in plants such as APETALA2 and EREBP (ethylene responsive element binding protein). In EREBPs the domain specifically binds to the 11bp GCC box of the ethylene response element (ERE), a promotor element essential for ethylene responsiveness. EREBPs and the C-repeat binding factor CBF1, which is involved in stress response, contain a single copy of the AP2 domain. APETALA2-like proteins, which play a role in plant development contain two copies.

Other sequence motifs which may be found in WRI1 and its functional homologs include:

1. R G V T/S R H R W T G R (SEQ ID NO:14).
2. F/Y E A H L W D K (SEQ ID NO:15).
3. D L A A L K Y W G (SEQ ID NO:16).
4. S X G F S/A R G X (SEQ ID NO:17).
5. H H H/Q N G R/K W E A R I G R/K V (SEQ ID NO:18).
6. Q E E A A A X Y D (SEQ ID NO:19).

As used herein, the term "Wrinkled 1" or "WRI1" also includes "Wrinkled 1-like" or "WRI1-like" proteins. Examples of WRI1 proteins include Accession Nos: A8MS57 (*Arabidopsis thaliana*), Q6X5Y6, (*Arabidopsis thaliana*), XP_002876251.1 (*Arabidopsis lyrata subsp. Lyrata*), ABD16282.1 (*Brassica napus*), ADO16346.1 (*Brassica napus*), XP_003530370.1 (*Glycine max*), AEO22131.1 (*Jatropha curcas*), XP_002525305.1 (*Ricinus communis*), XP_002316459.1 (*Populus trichocarpa*),

CBI29147.3 (*Vitis vinifera*), XP_003578997.1 (*Brachypodium distachyon*), BAJ86627.1 (*Hordeum vulgare* subsp. *vulgare*), EAY79792.1 (*Oryza sativa*), XP_002450194.1 (*Sorghum bicolor*), ACG32367.1 (*Zea mays*), XP_003561189.1 (*Brachypodium distachyon*), ABL85061.1 (*Brachypodium sylvaticum*), BAD68417.1 (*Oryza sativa*), XP_002437819.1 (*Sorghum bicolor*), XP_002441444.1 (*Sorghum bicolor*), XP_003530686.1 (*Glycine max*), XP_003553203.1 (*Glycine max*), XP_002315794.1 (*Populus trichocarpa*), XP_002270149.1 (*Vitis vinifera*), XP_003533548.1 (*Glycine max*), XP_003551723.1 (*Glycine max*), XP_003621117.1 (*Medicago truncatula*), XP_002323836.1 (*Populus trichocarpa*), XP_002517474.1 (*Ricinus communis*), CAN79925.1 (*Vitis vinifera*), XP_003572236.1 (*Brachypodium distachyon*), BAD10030.1 (*Oryza sativa*), XP_002444429.1 (*Sorghum bicolor*), NP_001170359.1 (*Zea mays*), XP_002889265.1 (*Arabidopsis lyrata subsp. lyrata*), AAF68121.1 (*Arabidopsis thaliana*), NP_178088.2 (*Arabidopsis thaliana*), XP_002890145.1 (*Arabidopsis lyrata subsp. lyrata*), BAJ33872.1 (*Thellungiella halophila*), NP_563990.1 (*Arabidopsis thaliana*), XP_003530350.1 (*Glycine max*), XP_003578142.1 (*Brachypodium distachyon*), EAZ09147.1 (*Oryza sativa*), XP_002460236.1 (*Sorghum bicolor*), NP_001146338.1 (*Zea mays*), XP_003519167.1 (*Glycine max*), XP_003550676.1 (*Glycine max*), XP_003610261.1 (*Medicago truncatula*), XP_003524030.1 (*Glycine max*), XP_003525949.1 (*Glycine max*), XP_002325111.1 (*Populus trichocarpa*), CBI36586.3 (*Vitis vinifera*), XP_002273046.2 (*Vitis vinifera)*, XP_002303866.1 (*Populus trichocarpa*), and CBI25261.3 (*Vitis vinifera*). Further examples include Sorbi-WRL1 (SEQ ID NO:10), Lupan-WRL1 (SEQ ID NO:11), Ricco-WRL1 (SEQ ID NO:12), and *Lupin angustifolius* WRI1 (SEQ ID NO:13). A preferred WRI1 is a maize WRI1 or a sorghum WRI1. In an embodiment, an exogenous polynucleotide of the invention which encodes a WRI1 which comprises one or more of the following:

i) nucleotides encoding a polypeptide comprising amino acids whose sequence is set forth in any of the above mentioned accessions, or a biologically active fragment thereof, or a polypeptide whose amino acid sequence is at least 30% identical to that set forth in any of the above mentioned accessions,

ii) nucleotides whose sequence is at least 30% identical to i), and

iii) a polynucleotide which hybridizes to one or both of i) or ii) under stringent conditions.

More recently, a subset of WRI1-like transcription factors have been re-classified as WRI2, WRI3 or WRI4 transcription factors, which are characterised by preferential expression in stems and/or roots of plants rather than in developing seeds

(To et al., 2012). Despite their re-classification, these are included in the definition of "WRI1" herein. Preferred WRI1-like transcription factors are those which can complement the function of a *wri1* mutation in a plant, particularly the function in developing seed of the plant such as in an *A. thaliana wri1* mutant. The function of a WRI1-like polypeptide can also be assayed in the *N. benthamiana* transient assays as described herein.

The WRI1 transcription factor may be endogenous to the plant or cell, or exogenous to the plant or cell, for example expressed from an exogenous polynucleotide. The WRI1 transcription factor may be a naturally occurring WRI1 polypeptide or a variant thereof, provided it retains transcription factor activity. The level or activity of an endogenous WRI1 polypeptide may also be increased by increased expression of a MED15 polypeptide (Kim et al., 2016), for example polypeptides whose amino acid sequences are provided in Accession No: NM_101446.4 or NM_001321633.1, or of a 14-3-3 polypeptide (Ma et al., 2016), for example Accession Nos: AY079350, AY079350, XM_002445734.1, XM_002445734.1, NM_001203346, NM_001203346, XM_002445734.1, or XM_002445734.1. MED15 polypeptide is thought to assist in directing WRI1 to its target promoters and expression of WRI1 expression itself, while 14-3-3 polypeptides are thought to interact with WRI1 polypeptide to increase the WRI1 effect.

As used herein, a "LEAFY COTYLEDON" or "LEC" polypeptide means a transcription factor which is a LEC1, LEC1-like, LEC2, ABI3 or FUS3 transcription factor which exhibits broad control on seed maturation and fatty acid synthesis. LEC2, FUS3 and ABI3 are related polypeptides that each contain a B3 DNA-binding domain of 120 amino acids (Yamasaki et al., 2004) that is only found in plant proteins. They can be distinguished by phylogenetic analysis to determine relatedness in amino acid sequence to the members of the *A. thaliana* polypeptides having the Accession Nos as follows: LEC2, Accession No. AAL12004.1; FUS3 (also known as FUSCA3), Accession No. AAC35247. LEC1 belongs to a different class of polypeptides and is homologous to a HAP3 polypeptide of the CBF binding factor class (Lee et al., 2003). The LEC1, LEC2 and FUS3 genes are required in early embryogenesis to maintain embryonic cell fate and to specify cotyledon identity and in later in initiation and maintenance of embryo maturation (Santos-Mendoza et al., 2008). They also induce expression of genes encoding seed storage proteins by binding to RY motifs present in the promoters, and oleosin genes. They can also be distinguished by their expression patterns in seed development or by their ability to complement the corresponding mutation in *A. thaliana*.

As used herein, the term "Leafy Cotyledon 1" or "LEC1" refers to a NF-YB-type transcription factor which participates in zygotic development and in somatic embryogenesis. The endogenous gene is expressed specifically in seed in both the embryo and endosperm. LEC1 activates the gene encoding WRI1 as well as a large class of fatty acid synthesis genes. Ectopic expression of LEC2 also causes rapid activation of auxin-responsive genes and may cause formation of somatic embryos. Examples of LEC1 polypeptides include proteins from *Arabidopsis thaliana* (AAC39488, SEQ ID NO:31), *Medicago truncatula* (AFK49653) and *Brassica napus* (ADF81045), *A. lyrata* (XP_002862657), *R. communis* (XP_002522740), *G. max* (XP_006582823), *A. hypogaea* (ADC33213), *Z. mays* (AAK95562, SEQ ID NO:32). In an embodiment, an exogenous polynucleotide of the invention which encodes a LEC1 which comprises one or more of the following:

i) nucleotides encoding a polypeptide comprising amino acids whose sequence is set forth in any of the above mentioned accessions, or a biologically active fragment thereof, or a polypeptide whose amino acid sequence is at least 30% identical to that set forth in any of the above mentioned accessions,

ii) nucleotides whose sequence is at least 30% identical to i), and

iii) a polynucleotide which hybridizes to one or both of i) or ii) under stringent conditions.

LEC1-like (L1L) is closely related to LEC1 but has a different pattern of gene expression, being expressed earlier during embryogenesis (Kwong et al., 2003). Examples of LEC1-like polypeptides include proteins from *Arabidopsis thaliana* (AAN15924, SEQ ID NO:33), *Brassica napus* (AHI94922), and *Phaseolus coccineus* LEC1-like (AAN01148).

As used herein, the term "Leafy Cotyledon 2" or "LEC2" refers to a B3 domain transcription factor which participates in zygotic development and in somatic embryogenesis and which activates expression of a gene encoding WRI1. Its ectopic expression facilitates the embryogenesis from vegetative plant tissues (Alemanno et al., 2008). Examples of LEC2 polypeptides include proteins from *Arabidopsis thaliana* (Accession No. NP_564304.1), *Medicago truncatula* (Accession No. CAA42938.1) and *Brassica napus* (Accession No. ADO16343.1). In an embodiment, an exogenous polynucleotide of the invention which encodes a LEC2 which comprises one or more of the following:

i) nucleotides encoding a polypeptide comprising amino acids whose sequence is set forth in any of the above mentioned accessions, or a biologically active fragment

thereof, or a polypeptide whose amino acid sequence is at least 30% identical to that set forth in any of the above mentioned accessions,

ii) nucleotides whose sequence is at least 30% identical to i), and

iii) a polynucleotide which hybridizes to one or both of i) or ii) under stringent conditions.

As used herein, the term "FUS3" refers to a B3 domain transcription factor which participates in zygotic development and in somatic embryogenesis and is detected mainly in the protodermal tissue of the embryo (Gazzarrini et al., 2004). Examples of FUS3 polypeptides include proteins from *Arabidopsis thaliana* (AAC35247, SEQ ID NO:34), *Brassica napus* (XP_006293066.1, SEQ ID NO:35) and *Medicago truncatula* (XP_003624470, SEQ ID NO:36). Over-expression of any of LEC1, L1L, LEC2, FUS3 and ABI3 from an exogenous polynucleotide is preferably controlled by a developmentally regulated promoter such as a senescence specific promoter, an inducible promoter, or a promoter which has been engineered for providing a reduced level of expression relative to a native promoter, particularly in plants other than *Arabidopsis thaliana* and *B. napus* cv. Westar, in order to avoid developmental abnormalities in plant development that are commonly associated with over-expression of these transcription factors (Mu et al., 2008). In an embodiment, an exogenous polynucleotide of the invention which encodes a FUS3 which comprises one or more of the following:

i) nucleotides encoding a polypeptide comprising amino acids whose sequence is set forth in any of the above mentioned accessions, or a biologically active fragment thereof, or a polypeptide whose amino acid sequence is at least 30% identical to that set forth in any of the above mentioned accessions,

ii) nucleotides whose sequence is at least 30% identical to i), and

iii) a polynucleotide which hybridizes to one or both of i) or ii) under stringent conditions.

As used herein, the term "BABY BOOM" or "BBM" refers an AP2/ERF transcription factor that induces regeneration under culture conditions that normally do not support regeneration in wild-type plants. Ectopic expression of *Brassica napus BBM* (*BnBBM*) genes in *B. napus* and *Arabidopsis* induces spontaneous somatic embryogenesis and organogenesis from seedlings grown on hormone-free basal medium (Boutilier et al., 2002). In tobacco, ectopic *BBM* expression is sufficient to induce adventitious shoot and root regeneration on basal medium, but exogenous cytokinin is required for somatic embryo (SE) formation (Srinivasan et al., 2007). Examples of BBM polypeptides include proteins from *Arabidopsis thaliana* (Accession

No. NP_197245.2, SEQ ID NO:28), maize (US 7579529), *Sorghum bicolor* (Accession No. XP_002458927) and *Medicago truncatula* (Accession No. AAW82334.1). In an embodiment, an exogenous polynucleotide of the invention which encodes a BBM which comprises one or more of the following:

i) nucleotides encoding a polypeptide comprising amino acids whose sequence is set forth in any of the above mentioned accessions, or a biologically active fragment thereof, or a polypeptide whose amino acid sequence is at least 30% identical to that set forth in any of the above mentioned accessions,

ii) nucleotides whose sequence is at least 30% identical to i), and

iii) a polynucleotide which hybridizes to one or both of i) or ii) under stringent conditions.

An ABI3 polypeptide (*A. thaliana* Accession No. NP_189108) is related to the maize VP1 protein, is expressed at low levels in vegetative tissues and affects plastid development. An ABI4 polypeptide (*A. thaliana* Accession NP_181551) belongs to a family of transcription factors that contain a plant-specific AP2 domain (Finkelstein et al., 1998) and acts downstream of ABI3. ABI5 (*A. thaliana* Accession No. NP_565840) is a transcription factor of the bZIP family which affects ABA sensitivity and controls the expression of some LEA genes in seeds. It binds to an ABA-responsive element.

Each of the following transcription factors was selected on the basis that they functioned in embryogenesis in plants. Accession numbers are provided in Table 8. Homologs of each can be readily identified in many other plant species and tested as described in Example 4.

MYB73 is a transcription factor that has been identified in soybean, involved in stress responses.

bZIP53 is a transcription factor in the bZIP protein family, identified in *Arabidopsis*.

AGL15 (Agamous-like 15) is a MADS box transcription factor which is natively expressed during embryogenesis. AGL15 is also natively expressed in leaf primordia, shoot apical meristems and young floral buds, suggesting that AGL15 may also have a function during post-germinative development. AGL15 has a role in embryogenesis and gibberellic acid catabolism. It targets B3 domain transcription factors that are key regulators of embryogenesis.

MYB115 and MYB118 are transcription factors in the MYB family from *Arabidopsis* involved in embryogenesis.

TANMEI also known as EMB2757 encodes a WD repeat protein required for embryo development in *Arabidopsis*.

WUS, also known as Wuschel, is a homeobox gene that controls the stem cell pool in embryos. It is expressed in the stem cell organizing center of meristems and is required to keep the stem cells in an undifferentiated state. The transcription factor binds to a TAAT element core motif.

GFR2a1 and GFR2a2 are transcription factors at least from soybean.

Fatty Acyl Acyltransferases

As used herein, the term "fatty acyl acyltransferase" refers to a protein which is capable of transferring an acyl group from acyl-CoA, PC or acyl-ACP, preferably acyl-CoA or PC, onto a substrate to form TAG, DAG or MAG. These acyltransferases include DGAT, PDAT, MGAT, GPAT and LPAAT.

As used herein, the term "diacylglycerol acyltransferase" (DGAT) refers to a protein which transfers a fatty acyl group from acyl-CoA to a DAG substrate to produce TAG. Thus, the term "diacylglycerol acyltransferase activity" refers to the transfer of an acyl group from acyl-CoA to DAG to produce TAG. A DGAT may also have MGAT function but predominantly functions as a DGAT, i.e., it has greater catalytic activity as a DGAT than as a MGAT when the enzyme activity is expressed in units of nmoles product/min/mg protein (see for example, Yen et al., 2005). The activity of DGAT may be rate-limiting in TAG synthesis in seeds (Ichihara et al., 1988). DGAT uses an acyl-CoA substrate as the acyl donor and transfers it to the *sn*-3 position of DAG to form TAG. The enzyme functions in its native state in the endoplasmic reticulum (ER) of the cell.

There are three known types of DGAT, referred to as DGAT1, DGAT2 and DGAT3, respectively. DGAT1 polypeptides are membrane proteins that typically have 10 transmembrane domains, DGAT2 polypeptides are also membrane proteins but typically have 2 transmembrane domains, whilst DGAT3 polypeptides typically have none and are thought to be soluble in the cytoplasm, not integrated into membranes. Plant DGAT1 polypeptides typically have about 510-550 amino acid residues while DGAT2 polypeptides typically have about 310-330 residues. DGAT1 is the main enzyme responsible for producing TAG from DAG in most developing plant seeds, whereas DGAT2s from plant species such as tung tree (*Vernicia fordii*) and castor bean (*Ricinus communis*) that produce high amounts of unusual fatty acids appear to have important roles in the accumulation of the unusual fatty acids in TAG. Over-expression of AtDGAT1 in tobacco leaves resulted in a 6-7 fold increased TAG content (Bouvier-Nave et al., 2000).

Examples of DGAT1 polypeptides include DGAT1 proteins from *Aspergillus fumigatus* (XP_755172.1), *Arabidopsis thaliana* (CAB44774.1; SEQ ID NO:1), *Ricinus communis* (AAR11479.1), *Vernicia fordii* (ABC94472.1), *Vernonia galamensis* (ABV21945.1 and ABV21946.1), *Euonymus alatus* (AAV31083.1), *Caenorhabditis elegans* (AAF82410.1), *Rattus norvegicus* (NP_445889.1), *Homo sapiens* (NP_036211.2), as well as variants and/or mutants thereof. In an embodiment, an exogenous polynucleotide of the invention which encodes a DGAT1 which comprises one or more of the following:

i) nucleotides encoding a polypeptide comprising amino acids whose sequence is set forth in any of the above mentioned accessions, or a biologically active fragment thereof, or a polypeptide whose amino acid sequence is at least 30% identical to that set forth in any of the above mentioned accessions,

ii) nucleotides whose sequence is at least 30% identical to i), and

iii) a polynucleotide which hybridizes to one or both of i) or ii) under stringent conditions.

Examples of DGAT2 polypeptides include proteins encoded by DGAT2 genes from *Arabidopsis thaliana* (NP_566952.1), *Ricinus communis* (AAY16324.1), *Vernicia fordii* (ABC94474.1), *Mortierella ramanniana* (AAK84179.1), *Homo sapiens* (Q96PD7.2) (Q58HT5.1), *Bos taurus* (Q70VZ8.1), *Mus musculus* (AAK84175.1), as well as variants and/or mutants thereof. DGAT1 and DGAT2 amino acid sequences show little homology. Expression in leaves of an exogenous DGAT2 was twice as effective as a DGAT1 in increasing oil content (TAG). Further, *A. thaliana* DGAT2 had a greater preference for linoleoyl-CoA and linolenoyl-CoA as acyl donors relative to oleoyl-CoA, compared to DGAT1. This substrate preference can be used to distinguish the two DGAT classes in addition to their amino acid sequences. In an embodiment, an exogenous polynucleotide of the invention which encodes a DGAT2 which comprises one or more of the following:

i) nucleotides encoding a polypeptide comprising amino acids whose sequence is set forth in any of the above mentioned accessions, or a biologically active fragment thereof, or a polypeptide whose amino acid sequence is at least 30% identical to that set forth in any of the above mentioned accessions,

ii) nucleotides whose sequence is at least 30% identical to i), and

iii) a polynucleotide which hybridizes to one or both of i) or ii) under stringent conditions.

Examples of DGAT3 polypeptides include proteins encoded by DGAT3 genes from peanut (*Arachis hypogaea*, Saha, et al., 2006), as well as variants and/or mutants

thereof. A DGAT has little or no detectable MGAT activity, for example, less than 300 pmol/min/mg protein, preferably less than 200 pmol/min/mg protein, more preferably less than 100 pmol/min/mg protein.

In a particularly preferred embodiment, the DGAT has a preference for medium chain fatty acids. For instance, the DGAT comprises one or more of the following:

i) nucleotides encoding a polypeptide comprising amino acids whose sequence is set forth in SEQ ID NO:56, or a biologically active fragment thereof, or a polypeptide whose amino acid sequence is at least 30% identical to SEQ ID NO:56,

ii) nucleotides whose sequence is at least 30% identical to i), and

iii) a polynucleotide which hybridizes to one or both of i) or ii) under stringent conditions.

As used herein, the term “phospholipid:diacylglycerol acyltransferase” (PDAT; EC 2.3.1.158) or its synonym “phospholipid:1,2-diacyl-*sn*-glycerol O-acyltransferase” means an acyltransferase that transfers an acyl group from a phospholipid, typically PC, to the sn-3 position of DAG to form TAG. This reaction is different to DGAT and uses phospholipids as the acyl-donors. Increased expression of PDAT such as PDAT1, which may be exogenous or endogenous to the cell or plant of the invention, increases the production of TAG from PC. There are several forms of PDAT in plant cells including PDAT1, PDAT2 or PDAT3 (Ghosal et al., 2007). Sequences of exemplary PDAT coding regions and polypeptides are provided in Accession Nos: XM_002462417.1, (*Sorghum*), NM_001147943 (*Zea mays*), (Dahlqvist et al., 2000; Fan et al., 2013a and b; Fan et al., 2014) although any PDAT encoding gene can be used. The PDAT may be exogenous or endogenous to the plant or part thereof.

As used herein, the term "monoacylglycerol acyltransferase" or "MGAT" refers to a protein which transfers a fatty acyl group from acyl-CoA to a MAG substrate, for example *sn*-2 MAG, to produce DAG. Thus, the term "monoacylglycerol acyltransferase activity" at least refers to the transfer of an acyl group from acyl-CoA to MAG to produce DAG. The term "MGAT" as used herein includes enzymes that act on *sn*-1/3 MAG and/or *sn*-2 MAG substrates to form *sn*-1,3 DAG and/or *sn*-1,2/2,3-DAG, respectively. In a preferred embodiment, the MGAT has a preference for *sn*-2 MAG substrate relative to *sn*-1 MAG, or substantially uses only *sn*-2 MAG as substrate. As used herein, MGAT does not include enzymes which transfer an acyl group preferentially to LysoPA relative to MAG, such enzymes are known as LPAATs. That is, a MGAT preferentially uses non-phosphorylated monoacyl substrates, even though they may also have low catalytic activity on LysoPA. A preferred MGAT does not have detectable activity in acylating LysoPA. A MGAT may also have DGAT

function but predominantly functions as a MGAT, i.e., it has greater catalytic activity as a MGAT than as a DGAT when the enzyme activity is expressed in units of nmoles product/min/mg protein (also see Yen et al., 2002). There are three known classes of MGAT, referred to as, MGAT1, MGAT2 and MGAT3, respectively. Examples of MGAT1, MGAT2 and MGAT3 polypeptides are described in WO2013/096993.

As used herein, an "MGAT pathway" refers to an anabolic pathway, different to the Kennedy pathway for the formation of TAG, in which DAG is formed by the acylation of either *sn*-1 MAG or preferably *sn*-2 MAG, catalysed by MGAT. The DAG may subsequently be used to form TAG or other lipids. WO2012/000026 demonstrated firstly that plant leaf tissue can synthesise MAG from G-3-P such that the MAG is accessible to an exogenous MGAT expressed in the leaf tissue, secondly MGAT from various sources can function in plant tissues, requiring a successful interaction with other plant factors involved in lipid synthesis and thirdly the DAG produced by the exogenous MGAT activity is accessible to a plant DGAT, or an exogenous DGAT, to produce TAG. MGAT and DGAT activity can be assayed by introducing constructs encoding the enzymes (or candidate enzymes) into *Saccharomyces cerevisiae* strain H1246 and demonstrating TAG accumulation.

Some of the motifs that have been shown to be important for catalytic activity in some DGAT2s are also conserved in MGAT acyltransferases. Of particular interest is a putative neutral lipid-binding domain with the concensus sequence FLXLXXXN (SEQ ID NO:6) where each X is independently any amino acid other than proline, and N is any nonpolar amino acid, located within the N-terminal transmembrane region followed by a putative glycerol/phospholipid acyltransferase domain. The FLXLXXXN motif (SEQ ID NO:6) is found in the mouse DGAT2 (amino acids 81-88) and MGAT1/2 but not in yeast or plant DGAT2s. It is important for activity of the mouse DGAT2. Other DGAT2 and/or MGAT1/2 sequence motifs include:

1. A highly conserved YFP tripeptide (SEQ ID NO:2) in most DGAT2 polypeptides and also in MGAT1 and MGAT2, for example, present as amino acids 139-141 in mouse DGAT2. Mutating this motif within the yeast DGAT2 with non-conservative substitutions rendered the enzyme non-functional.

2. HPHG tetrapeptide (SEQ ID NO:3), highly conserved in MGATs as well as in DGAT2 sequences from animals and fungi, for example, present as amino acids 161-164 in mouse DGAT2, and important for catalytic activity at least in yeast and mouse DGAT2. Plant DGAT2 acyltransferases have a EPHS (SEQ ID NO:4) conserved sequence instead, so conservative changes to the first and fourth amino acids can be tolerated.

3. A longer conserved motif which is part of the putative glycerol phospholipid domain. An example of this motif is RXGFX(K/R)XAXXXGXXX(L/V)VPXXXFG(E/Q) (SEQ ID NO:5), which is present as amino acids 304-327 in mouse DGAT2. This motif is less conserved in amino acid sequence than the others, as would be expected from its length, but homologs can be recognised by motif searching. The spacing may vary between the more conserved amino acids, i.e., there may be additional X amino acids within the motif, or less X amino acids compared to the sequence above.

One important component in glycerolipid synthesis from fatty acids esterified to ACP or CoA is the enzyme *sn*-glycerol-3-phosphate acyltransferase (GPAT), which is another of the polypeptides involved in the biosynthesis of non-polar lipids. This enzyme is involved in different metabolic pathways and physiological functions. It catalyses the following reaction: G3P + fatty acyl-ACP or -CoA → LPA + free-ACP or -CoA. The GPAT-catalyzed reaction occurs in three distinct plant subcellular compartments: plastid, endoplasmic reticulum (ER) and mitochondria. These reactions are catalyzed by three different types of GPAT enzymes, a soluble form localized in plastidial stroma which uses acyl-ACP as its natural acyl substrate (PGPAT in Figure 1), and two membrane-bound forms localized in the ER and mitochondria which use acyl-CoA and acyl-ACP as natural acyl donors, respectively (Chen et al., 2011).

As used herein, the term "glycerol-3-phosphate acyltransferase" (GPAT; EC 2.3.1.15) and its synonym “glycerol-3-phosphate *O*-acyltransferase” refer to a protein which acylates glycerol-3-phosphate (G-3-P) to form LysoPA and/or MAG, the latter product forming if the GPAT also has phosphatase activity on LysoPA. The acyl group that is transferred is from acyl-CoA if the GPAT is an ER-type GPAT (an “acyl-CoA:*sn*-glycerol-3-phosphate 1-O-acyltransferase” also referred to as “microsomal GPAT”) or from acyl-ACP if the GPAT is a plastidial-type GPAT (PGPAT). Thus, the term "glycerol-3-phosphate acyltransferase activity" refers to the acylation of G-3-P to form LysoPA and/or MAG. The term "GPAT" encompasses enzymes that acylate G-3-P to form *sn*-1 LPA and/or *sn*-2 LPA, preferably *sn*-2 LPA. Preferably, the GPAT which may be over-expressed in the Pull modification is a membrane bound GPAT that functions in the ER of the cell, more preferably a GPAT9, and the plastidial GPAT that is down-regulated in the Prokaryotic Pathway modification is a soluble GPAT (“plastidial GPAT”). In a preferred embodiment, the GPAT has phosphatase activity. In a most preferred embodiment, the GPAT is a *sn*-2 GPAT having phosphatase activity which produces *sn*-2 MAG.

As used herein, the term "*sn*-1 glycerol-3-phosphate acyltransferase" (*sn*-1 GPAT) refers to a protein which acylates *sn*-glycerol-3-phosphate (G-3-P) to preferentially form 1-acyl-*sn*-glycerol-3-phosphate (*sn*-1 LPA). Thus, the term "*sn*-1 glycerol-3-phosphate acyltransferase activity" refers to the acylation of *sn*-glycerol-3-phosphate to form 1-acyl-*sn*-glycerol-3-phosphate (*sn*-1 LPA).

As used herein, the term "*sn*-2 glycerol-3-phosphate acyltransferase" (*sn*-2 GPAT) refers to a protein which acylates *sn*-glycerol-3-phosphate (G-3-P) to preferentially form 2-acyl-*sn*-glycerol-3-phosphate (*sn*-2 LPA). Thus, the term "*sn*-2 glycerol-3-phosphate acyltransferase activity" refers to the acylation of *sn*-glycerol-3-phosphate to form 2-acyl-*sn*-glycerol-3-phosphate (*sn*-2 LPA).

The GPAT family is large and all known members contain two conserved domains, a plsC acyltransferase domain (PF01553) and a HAD-like hydrolase (PF12710) superfamily domain and variants thereof. In addition to this, at least in *Arabidopsis thaliana*, GPATs in the subclasses GPAT4-GPAT8 all contain a N-terminal region homologous to a phosphoserine phosphatase domain (PF00702), and GPATs which produce MAG as a product can be identified by the presence of such a homologous region. Some GPATs expressed endogenously in leaf tissue comprise the conserved amino acid sequence GDLVICPEGTTCREP (SEQ ID NO:7). GPAT4 and GPAT6 both contain conserved residues that are known to be critical to phosphatase activity, specifically conserved amino acids in Motif I (DXDX[T/V][L/V]; SEQ ID NO:8) and Motif III (K-[G/S][D/S]XXX[D/N]; SEQ ID NO:9) located at the N-terminus (Yang et al., 2010).

Homologues of *Arabidopsis* GPAT4 (Accession No. NP_171667.1) and GPAT6 (NP_181346.1) include AAF02784.1 (*Arabidopsis thaliana*), AAL32544.1 (*Arabidopsis thaliana*), AAP03413.1 (*Oryza sativa*), ABK25381.1 (*Picea sitchensis*), ACN34546.1 (*Zea Mays*), BAF00762.1 (*Arabidopsis thaliana*), BAH00933.1 (*Oryza sativa*), EAY84189.1 (*Oryza sativa*), EAY98245.1 (*Oryza sativa*), EAZ21484.1 (*Oryza sativa*), EEC71826.1 (*Oryza sativa*), EEC76137.1 (*Oryza sativa*), EEE59882.1 (*Oryza sativa*), EFJ08963.1 (*Selaginella moellendorffii*), EFJ11200.1 (*Selaginella moellendorffii*), NP_001044839.1 (*Oryza sativa*), NP_001045668.1 (*Oryza sativa*), NP_001147442.1 (*Zea mays*), NP_001149307.1 (*Zea mays*), NP_001168351.1 (*Zea mays*), AFH02724.1 (*Brassica napus*) NP_191950.2 (*Arabidopsis thaliana*), XP_001765001.1 (*Physcomitrella patens*), XP_001769671.1 (*Physcomitrella patens*), (*Vitis vinifera*), XP_002275348.1 (*Vitis vinifera*), XP_002276032.1 (*Vitis vinifera*), XP_002279091.1 (*Vitis vinifera*), XP_002309124.1 (*Populus trichocarpa*), XP_002309276.1 (*Populus trichocarpa*), XP_002322752.1 (*Populus trichocarpa*),

XP_002323563.1 (*Populus trichocarpa*), XP_002439887.1 (*Sorghum bicolor*), XP_002458786.1 (*Sorghum bicolor*), XP_002463916.1 (*Sorghum bicolor*), XP_002464630.1 (*Sorghum bicolor*), XP_002511873.1 (*Ricinus communis*), XP_002517438.1 (*Ricinus communis*), XP_002520171.1 (*Ricinus communis*), ACT32032.1 (*Vernicia fordii*), NP_001051189.1 (*Oryza sativa*), AFH02725.1 (*Brassica napus*), XP_002320138.1 (*Populus trichocarpa*), XP_002451377.1 (*Sorghum bicolor*), XP_002531350.1 (*Ricinus communis*), and XP_002889361.1 (*Arabidopsis lyrata*).

In an embodiment, an exogenous polynucleotide of the invention which encodes a GPAT which comprises one or more of the following:

i) nucleotides encoding a polypeptide comprising amino acids whose sequence is set forth in any of the above mentioned accessions, or a biologically active fragment thereof, or a polypeptide whose amino acid sequence is at least 30% identical to that set forth in any of the above mentioned accessions,

ii) nucleotides whose sequence is at least 30% identical to i), and

iii) a polynucleotide which hybridizes to one or both of i) or ii) under stringent conditions.

In a particularly preferred embodiment, the GPAT, preferablty a GPAT9, has a preference for utilising medium chain fatty acid substrates. For instance, the GPAT9 comprises one or more of the following:

i) nucleotides encoding a polypeptide comprising amino acids whose sequence is set forth in any of SEQ ID NO:97 to 119, preferably SEQ ID NO:97, or a biologically active fragment thereof, or a polypeptide whose amino acid sequence is at least 30% identical to any one of SEQ ID NO:97 to 119, preferably at least 30% identical to SEQ ID NO:97,

ii) nucleotides whose sequence is at least 30% identical to i), and

iii) a polynucleotide which hybridizes to one or both of i) or ii) under stringent conditions.

The soluble plastidial GPATs (PGPAT, also known as ATS1 in *Arabidopsis thaliana*) have been purified and genes encoding them cloned from several plant species such as pea (*Pisum sativum*, Accession number: P30706.1), spinach (*Spinacia oleracea*, Accession number: Q43869.1), squash (*Cucurbita moschate*, Accession number: P10349.1), cucumber (*Cucumis sativus*, Accession number: Q39639.1) and *Arabidopsis thaliana* (Accession number: Q43307.2). The soluble plastidial GPAT is the first committed step for what is known as the prokaryotic pathway of glycerolipid synthesis and is operative only in the plastid (Figure 1). The so-called prokaryotic

pathway is located exclusively in plant plastids and assembles DAG for the synthesis of galactolipids (MGDG and DGMG) which contain C16:3 fatty acids esterified at the *sn*-2 position of the glycerol backbone.

Conserved motifs and/or residues can be used as a sequence-based diagnostic for the identification of GPAT enzymes. Alternatively, a more stringent function-based assay could be utilised. Such an assay involves, for example, feeding labelled glycerol-3-phosphate to cells or microsomes and quantifying the levels of labelled products by thin-layer chromatography or a similar technique. GPAT activity results in the production of labelled LPA whilst GPAT/phosphatase activity results in the production of labelled MAG.

As used herein, the term "lysophosphatidic acid acyltransferase" (LPAAT; EC 2.3.1.51) and its synonyms “1-acyl-glycerol-3-phosphate acyltransferase”, “acyl-CoA:1-acyl-*sn*-glycerol-3-phosphate 2-O-acyltransferase” and “1-acylglycerol-3-phosphate *O*-acyltransferase” refer to a protein which acylates lysophosphatidic acid (LPA) to form phosphatidic acid (PA). The acyl group that is transferred is from acyl-CoA if the LPAAT is an ER-type LPAAT or from acyl-ACP if the LPAAT is a plastidial-type LPAAT (PLPAAT). Thus, the term "lysophosphatidic acid acyltransferase activity" refers to the acylation of LPA to form PA.

In a particularly preferred embodiment, the LPAAT has a preference for medium chain fatty acids. For instance, the LPAAT comprises one or more of the following:

i) nucleotides encoding a polypeptide comprising amino acids whose sequence is set forth in SEQ ID NO:94, or a biologically active fragment thereof, or a polypeptide whose amino acid sequence is at least 30% identical to SEQ ID NO:94,

ii) nucleotides whose sequence is at least 30% identical to i), and

iii) a polynucleotide which hybridizes to one or both of i) or ii) under stringent conditions.

Oil Body Coating Polypeptides

TAGs are accumulated in plant tissues as subcellular spherical lipid droplets (LDs, also called oil bodies or lipid bodies) of approximately 0.5-2 μm in diameter. In seeds, each LD has a matrix of TAGs surrounded by a layer of phospholipids (PLs) and structural proteins termed oleosins (Chapman and Ohlrogge, 2012; Hsieh and Huang, 2004; Murphy, 2012). The small size of LDs provides a large surface area per unit TAG, which would facilitate lipase binding and lipolysis during germination (Huang and Huang, 2016). Recent proteomics and homology based studies have led to the

identification of several new protein components involved in the formation, maintenance, and/or turnover of LDs (Pyc et al., 2017).

Regarding protein structural organization, oleosin comprises an N-terminal domain, a central hydrophobic domain, and a C-terminal domain (Hsiao and Tzen, 2011). Oleosin-H is distinguished from the other isoform oleosin-L by an extra 18-residue segment in its C-terminal domain (Tai et al., 2002). Ubiquitin is a highly-conserved regulatory protein that attaches to lysine ε-amino groups of target proteins by its C-terminal glycine residue (Hsiao and Tzen, 2011). Protein ubiquitination is integral to many biological pathways such as proteasomal degradation, stress responses, hormone biosynthesis and signaling, morphogenesis, chromatin structure, self-incompatibility, and battling pathogens (Sorokin et al., 2009). Some studies suggested that oleosin might be involved in storage lipid degradation after germination (Poxleitner et al., 2006). It has been noticed that protein ubiquitination is involved not only in the ubiquitin/26S proteasome pathway, but also in various biological functions possibly associated with different ubiquitin linkages (Weissman, 2001). Ectopic expression of several LD proteins, such as the plant oleosins and SEIPINs as well as the human perilipins, was shown to modulate LD morphology and accumulation in yeast (*S. cerevisiae*) (Cai et al., 2015). Lipid reserves are metabolized via the successive events of lipolysis, fatty acid (FA) transport to glyoxysomes, activation of acyl-CoA derivatives, β-oxidation, glyoxylate cycle, partial tricarboxylic acid cycle, and gluconeogenesis (Deruyffelaere et al., 2015).

In an embodiment, the oil body coating polypeptide is non-allergenic, or not known to be allergenic, such as to humans.

As used herein, the term "Oleosin" refers to an amphipathic protein present in the membrane of oil bodies in the storage tissues of seeds (see, for example, Huang, 1996; Tai et al., 2002; Lin et al., 2005; Capuano et al., 2007; Lui et al., 2009; Shimada and Hara-Nishimura, 2010) and artificially produced variants (see for example WO2011/053169 and WO2011/127118).

Oleosins are of low M_r (15-26,000), corresponding to about 140-230 amino acid residues, which allows them to become tightly packed on the surface of oil bodies. Within each seed species, there are usually two or more oleosins of different M_r. Each oleosin molecule contains a relatively hydrophilic, variable N-terminal domain (for example, about 48 amino acid residues), a central totally hydrophobic domain (for example, of about 70-80 amino acid residues) which is particularly rich in aliphatic amino acids such as alanine, glycine, leucine, isoleucine and valine, and an amphipathic α-helical domain of about 30-40 amino acid residues at or near the C-

terminus. The central hydrophobic domain typically contains a proline knot motif of about 12 residues at its center. Generally, the central stretch of hydrophobic residues is inserted into the lipid core and the amphiphatic N-terminal and/or amphiphatic C-terminal are located at the surface of the oil bodies, with positively charged residues embedded in a phospholipid monolayer and the negatively charged ones exposed to the exterior.

As used herein, the term "Oleosin" encompasses polyoleosins which have multiple oleosin polypeptides fused together in a head-to-tail fashion as a single polypeptide (WO2007/045019), for example 2x, 4x or 6x oleosin peptides, and caleosins which bind calcium and which are a minor protein component of the proteins that coat oil bodies in seeds (Froissard et al., 2009), and steroleosins which bind sterols (WO2011/053169). However, generally a large proportion (at least 80%) of the oleosins of oil bodies will not be caleosins and/or steroleosins. The term “oleosin” also encompasses oleosin polypeptides which have been modified artificially, such oleosins which have one or more amino acid residues of the native oleosins artificially replaced with cysteine residues, as described in WO2011/053169. Typically, 4-8 residues are substituted artificially, preferably 6 residues, but as many as between 2 and 14 residues can be substituted. Preferably, both of the amphipathic N-terminal and C-terminal domains comprise cysteine substitutions. The modification increases the cross-linking ability of the oleosins and increases the thermal stability and/or the stability of the proteins against degradation by proteases.

A substantial number of oleosin protein sequences, and nucleotide sequences encoding therefor, are known from a large number of different plant species. Examples include, but are not limited to, oleosins from sesame, *Arabidposis*, canola, corn, rice, peanut, castor, soybean, flax, grape, cabbage, cotton, sunflower, sorghum and barley. Examples of oleosins (with their Accession Nos) include *Brassica napus* oleosin (CAA57545.1), *Brassica napus* oleosin S1-1 (ACG69504.1), *Brassica napus* oleosin S2-1 (ACG69503.1), *Brassica napus* oleosin S3-1 (ACG69513.1), *Brassica napus* oleosin S4-1 (ACG69507.1), *Brassica napus* oleosin S5-1 (ACG69511.1), *Arachis hypogaea* oleosin 1 (AAZ20276.1), *Arachis hypogaea* oleosin 2 (AAU21500.1), *Arachis hypogaea* oleosin 3 (AAU21501.1), *Arachis hypogaea* oleosin 5 (ABC96763.1), *Ricinus communis* oleosin 1 (EEF40948.1), *Ricinus communis* oleosin 2 (EEF51616.1), *Glycine max* oleosin isoform a (P29530.2), *Glycine max* oleosin isoform b (P29531.1), *Linum usitatissimum* oleosin low molecular weight isoform (ABB01622.1), *Linum usitatissimum* oleosin high molecular weight isoform (ABB01624.1), *Helianthus annuus* oleosin (CAA44224.1), *Zea mays* oleosin

(NP_001105338.1), *Brassica napus* steroleosin (ABM30178.1), *Brassica napus* steroleosin SLO1-1 (ACG69522.1), *Brassica napus* steroleosin SLO2-1 (ACG69525.1), *Sesamum indicum* steroleosin (AAL13315.1), *Sesame indicum* OleosinL (Tai et al., 2002; Accession number AF091840; SEQ ID NO:86), *Ficus pumila var. awkeotsang* oleosin L-isoform (Accession No. ABQ57397.1), *Cucumis sativus* oleosinL (Accession No. XP_004146901.1), *Linum usitatissimum* oleosinL (Accession No. ABB01618.1), *Glycine max* oleosinL (Accession No. XP_003556321.2), *Ananas comosus* oleosinL (Accession No. OAY72596.1), *Setaria italica* oleosinL (Accession No. XP_004956407.1), *Fragaria vesca subsp. vesca* oleosinL (Accession No. XP_004307777.1), *Brassica napus* oleosinL (Accession No. CDY03377.1), *Solanum lycopersicum* oleosinL (Accession No. XP_004240765.1), *Sesame indicum* OleosinH1 (Tai et al., 2002; Accession number AF302807), *Vanilla planifolia* leaf OleosinU1 (Huang and Huang, 2016; Accession number SRX648194), *Persea americana* mesocarp OleosinM lipid droplet associated protein (Huang and Huang, 2016; Accession number SRX627420), *Arachis hypogaea* Oleosin 3 (Parthibane et al., 2012a and b; Accession number AY722696), *A. thaliana* Caleosin3 (Shen et al., 2014; Laibach et al., 2015; Accession number AK317039), *A. thaliana* steroleosin (Accession number AT081653), *Zea mays* steroleosin (NP_001152614.1), *Brassica napus* caleosin CLO-1 (ACG69529.1), *Brassica napus* caleosin CLO-3 (ACG69527.1), *Sesamum indicum* caleosin (AAF13743.1), *Zea mays* caleosin (NP_001151906.1), *Glycine max* caleosin (AAB71227). Other lipid encapsulation polypeptides that are functionally equivalent are plastoglobulins and MLDP polypeptides (WO2011/127118). In an embodiment, an exogenous polynucleotide of the invention which encodes a oleosin (such as an OleosinL) or steroleosin which comprises one or more of the following:

i) nucleotides encoding a polypeptide comprising amino acids whose sequence is set forth in any of the above mentioned accessions, or a biologically active fragment thereof, or a polypeptide whose amino acid sequence is at least 30% identical to that set forth in any of the above mentioned accessions,

ii) nucleotides whose sequence is at least 30% identical to i), and

iii) a polynucleotide which hybridizes to one or both of i) or ii) under stringent conditions.

In an embodiment, the oleosin is oleosinL or an ortholog thereof. OleosinL lacks the about 18 amino acid H-form insertion towards the C-terminus of other oleosins (see, for example, Tai et al., 2002). Thus, OleosinL's can readily be distinguished from other oleosins based on protein alignment.

As used herein, a "lipid droplet associated protein" or "LDAP" means a polypeptide which is associated with lipid droplets in plants in tissues or organs other than seeds, anthers and pollen, such as fruit tissues including pericarp and mesocarp. LDAPs may be associated with oil bodies in seeds, anthers or pollen as well as in the tissues or organs other than seeds, anthers and pollen. They are distinct from oleosins which are polypeptides associated with the surface of lipid droplets in seed tissues, anthers and pollen. LDAPs as used herein include LDAP polypeptides that are produced naturally in plant tissues as well as amino acid sequence variants that are produced artificially. The function of such variants can be tested as exemplified in Example 6.

Horn et al. (2013) identified two LDAP genes which are expressed in avocado pericarp. The encoded avocado LDAP1 and LDAP2 polypeptides were 62% identical in amino acid sequence and had homology to polypeptide encoded by *Arabidopsis* At3g05500 and a rubber tree SRPP-like protein. Gidda et al. (2013) identified three LDAP genes that were expressed in oil palm (*Elaeis guineensis*) mesocarp but not in kernels and concluded that LDAP genes were plant specific and conserved amongst all plant species. LDAP polypeptides may contain additional domains (Gidda et al., (2013). Genes encoding LDAPs are generally up-regulated in non-seed tissues with abundant lipid and can be identified thereby, but are thought to be expressed in all non-seed cells that produce oil including for transient storage. Horn et al. (2013) shows a phylogenetic tree of SRPP-like proteins in plants. Exemplary LDAP polypeptides are described in Example 6 and Example 9 herein, such as *Rhodococcus opacus* TadA lipid droplet associated protein (MacEachran et al., 2010; Accession number HM625859), *Nannochloropsis oceanica* LSDP oil body protein (Vieler et al., 2012; Accession number JQ268559) and *Trichoderma reesei* HFBI hydrophobin (Linder et al., 2005; Accession number Z68124). Homologs of LDAPs in other plant species can be readily identified by those skilled in the art. In an embodiment, an exogenous polynucleotide of the invention which encodes an LDAP which comprises one or more of the following:

i) nucleotides encoding a polypeptide comprising amino acids whose sequence is set forth in any of the above mentioned accessions, or a biologically active fragment thereof, or a polypeptide whose amino acid sequence is at least 30% identical to that set forth in any of the above mentioned accessions,

ii) nucleotides whose sequence is at least 30% identical to i), and

iii) a polynucleotide which hybridizes to one or both of i) or ii) under stringent conditions.

As used herein, the term a "polypeptide involved in starch biosynthesis" refers to any polypeptide, the downregulation of which in a plant cell below normal (wild-type) levels results in a reduction in the level of starch synthesis and a decrease in the levels of starch. This reduces the flow of carbon from sugars into starch. An example of such a polypeptide is AGPase.

As used herein, the term "ADP-glucose phosphorylase" or "AGPase" refers to an enzyme which regulates starch biosynthesis, catalysing conversion of glucose-1-phosphate and ATP to ADP-glucose which serves as the building block for starch polymers. The active form of the AGPase enzyme consists of 2 large and 2 small subunits.

The AGPase enzyme in plants exists primarily as a tetramer which consists of 2 large and 2 small subunits. Although these subunits differ in their catalytic and regulatory roles depending on the species (Kuhn et al., 2009), in plants the small subunit generally displays catalytic activity. The molecular weight of the small subunit is approximately 50-55 kDa. Sequences of exemplary AGPase small subunit polypeptides are provided in Accession Nos: XM_002462095.1 (*Sorghum*) and XM_008666513.1 (*Zea mays*) (Sanjaya et al., 2011; Zale et al., 2016). The molecular weight of the large subunit is approximately 55-60 kDa. The plant enzyme is strongly activated by 3-phosphoglycerate (PGA), a product of carbon dioxide fixation; in the absence of PGA, the enzyme exhibits only about 3% of its activity. Plant AGPase is also strongly inhibited by inorganic phosphate (Pi). In contrast, bacterial and algal AGPase exist as homotetramers of 50kDa. The algal enzyme, like its plant counterpart, is activated by PGA and inhibited by Pi, whereas the bacterial enzyme is activated by fructose-1, 6-bisphosphate (FBP) and inhibited by AMP and Pi.

TAG Lipases and Beta-Oxidation

As used herein, the term "polypeptide involved in the degradation of lipid and/or which reduces lipid content" refers to any polypeptide which catabolises lipid, the downregulation of which in a plant cell below normal (wild-type) levels results an increase in the level of oil, such as fatty acids and/or TAGs, in a cell of a transgenic plant or part thereof such as a vegetative part, tuber, beet or a seed. Examples of such polypeptides include, but are not limited to, lipases, or a lipase such as a CGi58 (Comparative Gene identifier-58-Like) polypeptide, a SUGAR-DEPENDENT1 (SDP1) triacylglycerol lipase (see, for example, Kelly et al., 2011) and a lipase described in WO 2009/027335.

As used herein, the term “TAG lipase” (EC.3.1.1.3) refers to a protein which hydrolyzes TAG into one or more fatty acids and any one of DAG, MAG or glycerol. Thus, the term “TAG lipase activity” refers to the hydrolysis of TAG into glycerol and fatty acids.

As used herein, the term “CGi58” refers to a soluble acyl-CoA-dependent lysophosphatidic acid acyltransferase encoded by the At4g24160 gene in *Arabidopsis thaliana* and its homologs in other plants and "Ict1p" in yeast and its homologs. The plant gene such as that from *Arabidopsis* gene locus At4g24160 is expressed as two alternative transcripts: a longer full-length isoform (At4g24160.1) and a smaller isoform (At4g24160.2) missing a portion of the 3′ end (see James et al., 2010; Ghosh et al., 2009; US 201000221400). Both mRNAs code for a protein that is homologous to the human CGI-58 protein and other orthologous members of this α/β hydrolase family (ABHD). In an embodiment, the CGI58 (At4g24160) protein contains three motifs that are conserved across plant species: a GXSXG lipase motif (SEQ ID NO:25), a HX(4)D acyltransferase motif (SEQ ID NO:26), and VX(3)HGF, a probable lipid binding motif (SEQ ID NO:27). The human CGI-58 protein has lysophosphatidic acid acyltransferase (LPAAT) activity but not lipase activity. In contrast, the plant and yeast proteins possess a canonical lipase sequence motif GXSXG (SEQ ID NO:25), that is absent from vertebrate (humans, mice, and zebrafish) proteins, and have lipase and phospholipase activity (Ghosh et al., 2009). Although the plant and yeast CGI58 proteins appear to possess detectable amounts of TAG lipase and phospholipase A activities in addition to LPAAT activity, the human protein does not.

Disruption of the homologous *CGI-58* gene in *Arabidopsis thaliana* results in the accumulation of neutral lipid droplets in mature leaves. Mass spectroscopy of isolated lipid droplets from *cgi-58* loss-of-function mutants showed they contain triacylglycerols with common leaf-specific fatty acids. Leaves of mature *cgi-58* plants exhibit a marked increase in absolute triacylglycerol levels, more than 10-fold higher than in wild-type plants. Lipid levels in the oil-storing seeds of *cgi-58* loss-of-function plants were unchanged, and unlike mutations in β-oxidation, the *cgi-58* seeds germinated and grew normally, requiring no rescue with sucrose (James et al., 2010).

Examples of nucleotides encoding CGi58 polypeptides include those from *Arabidopsis thaliana* (NM_118548.1 encoding NP_194147.2), *Brachypodium distachyon* (XP_003578450.1), *Glycine max* (XM_003523590.1 encoding XP_003523638.1), *Zea mays* (NM_001155541.1 encoding NP_001149013.1), *Sorghum bicolor* (XM_002460493.1 encoding XP_002460538.1), *Ricinus communis* (XM_002510439.1 encoding XP_002510485.1), *Medicago truncatula*

(XM_003603685.1 encoding XP_003603733.1), and *Oryza sativa* (encoding EAZ09782.1). In an embodiment, a genetic modification of the invention down-regulates endogenous production of CGi58, wherein CGi58 is encoded by one or more of the following:

i) nucleotides comprising a sequence set forth a the above mentioned accessions,

ii) nucleotides whose sequence is at least 30% identical to i), and

iii) a polynucleotide which hybridizes to one or both of i) or ii) under stringent conditions.

Other lipases which have lipase activity on TAG include SUGAR-DEPENDENT1 triacylglycerol lipase (SDP1, see for example Eastmond et al., 2006; Kelly et al., 2011) and SDP1-like polypeptides found in plant species as well as yeast (TGL4 polypeptide) and animal cells, which are involved in storage TAG breakdown. The SDP1 and SDP1-like polypeptides appear to be responsible for initiating TAG breakdown in seeds following germination (Eastmond et al., 2006). Plants that are mutant in *SDP1*, in the absence of exogenous WRI1 and DGAT1, exhibit increased levels of PUFA in their TAG. As used herein, "SDP1 polypeptides" include SDP1 polypeptides, SDP1-like polypeptides and their homologs in plant species. SDP1 and SDP1-like polypeptides in plants are 800-910 amino acid residues in length and have a patatin-like acylhydrolase domain that can associate with oil body surfaces and hydrolyse TAG in preference to DAG or MAG. SDP1 is thought to have a preference for hydrolysing the acyl group at the *sn*-2 position of TAG. *Arabidopsis* contains at least three genes encoding SDP1 lipases, namely *SDP1* (Accession No. NP_196024, nucleotide sequence SEQ ID NO:37 and homologs in other species), *SDP1L* (Accession No. NM_202720 and homologs in other species, Kelly et al., 2011) and *ATGLL* (At1g33270) (Eastmond et al, 2006). Of particular interest for reducing gene activity are *SDP1* genes which are expressed in vegetative tissues in plants, such as in leaves, stems and roots. Levels of non-polar lipids in vegetative plant parts can therefore be increased by reducing the activity of SDP1 polypeptides in the plant parts, for example by either mutation of an endogenous gene encoding a SDP1 polypeptide or introduction of an exogenous gene which encodes a silencing RNA molecule which reduces the expression of an endogenous *SDP1* gene. Such a reduction is of particular benefit in tuber crops such as sugarbeet and potato, and in "high sucrose" plants such as sweet sorghum, sugarcane and and sugarbeet.

Genes encoding SDP1 homologues (including SDP1-like homologues) in a plant species of choice can be identified readily by homology to known *SDP1* gene sequences. Known SDP1 nucleotide or amino acid sequences include Accession Nos.:

in *Brassica napus,* GN078290, GN078281, GN078283; *Capsella rubella,* XP_006287072; *Theobroma cacao,* XP_007028574.1; *Populus trichocarpa,* XP_002308909; *Prunus persica,* XP_007203312; *Prunus mume,* XP_008240737; *Malus domestica,* XP_008373034; *Ricinus communis,* XP_002530081; *Medicago truncatula,* XP_003591425; *Solanum lycopersicum,* XP_004249208; *Phaseolus vulgaris,* XP_007162133; *Glycine max,* XP_003554141; *Solanum tuberosum,* XP_006351284; *Glycine max,* XP_003521151; *Cicer arietinum,* XP_004493431; *Cucumis sativus,* XP_004142709; *Cucumis melo,* XP_008457586; *Jatropha curcas,* KDP26217; *Vitis vinifera,* CBI30074; *Oryza sativa,* Japonica Group BAB61223; *Oryza sativa,* Indica Group EAY75912; *Oryza sativa,* Japonica Group NP_001044325; *Sorghum bicolor,* XP_002458531 (SEQ ID NO:38); *Brachypodium distachyon,* XP_003567139; *Zea mays,* AFW85009; *Hordeum vulgare,* BAK03290; *Aegilops tauschii,* EMT32802; *Sorghum bicolor,* XP_002463665; *Zea mays,* NP_001168677; *Hordeum vulgare*, BAK01155; *Aegilops tauschii,* EMT02623; *Triticum urartu,* EMS67257; *Physcomitrella patens,* XP_001758169. Preferred SDP1 sequences for use in genetic constructs for inhibiting expression of the endogenous genes are from cDNAs corresponding to the genes which are expressed most highly in the plant cells, vegetative plant parts or the seeds, whichever is to be modified. Nucleotide sequences which are highly conserved between cDNAs corresponding to all of the *SDP1* genes in a plant species are preferred if it is desired to reduce the activity of all members of a gene family in that species. In an embodiment, a genetic modification of the invention down-regulates endogenous production of SDP1, wherein SDP1 is encoded by one or more of the following:

i) nucleotides comprising a sequence set forth a the above mentioned accessions,

ii) nucleotides whose sequence is at least 30% identical to i), and

iii) a polynucleotide which hybridizes to one or both of i) or ii) under stringent conditions.

As shown in the Examples, reduction of the expression and/or activity of SDP1 TAG lipase in plant leaves greatly increased the TAG content, both in terms of the amount of TAG that accumulated and the earlier timing of accumulation during plant development, in the context of co-expression of the transcription factor WRI1 and a fatty acyl acyltransferase. In particular, the increase was observed in plants prior to flowering, and was up to about 70% on a weight basis (% dry weight) at the onset of senescence. The increase was relative to the TAG levels observed in corresponding plant leaves transformed with exogenous polynucleotides encoding the WRI1 and fatty

acyl acyltransferase but lacking the modification that reduced SDP1 expression and/or activity.

Reducing the expression of other TAG catabolism genes in plant parts can also increase TAG content, such as the *ACX* genes encoding acyl-CoA oxidases such as the *Acx1* (At4g16760 and homologs in other plant species) or *Acx2* (At5g65110 and homologs in other plant species) genes. Another polypeptide involved in lipid catabolism is PXA1 which is a peroxisomal ATP-binding cassette transporter that is requires for fatty acid import for β-oxidation (Zolman et al. 2001).

Export of Fatty Acids from Plastids

As used herein, the term "polypeptide which increases the export of fatty acids out of plastids of the cell" refers to any polypeptide which aids in fatty acids being transferred from within plastids of plant cells in a plant or part thereof to outside the plastid, which may be any other part of the cell such as for example the endoplasmic reticulum (ER). Examples of such polypeptides include, but are not limited to, a C16 or C18 fatty acid thioesterase such as a FATA polypeptide or a FATB polypeptide, a C6 to C14 fatty acid thioesterase (which is also a FATB polypeptide), a fatty acid transporter such as an ABCA9 polypeptide or a long-chain acyl-CoA synthetase (LACS).

As used herein, the term “fatty acid thioesterase” or “FAT” or “acyl-ACP thioesterase” refers to an enzyme which catalyses the hydrolysis of the thioester bond between an acyl moiety and acyl carrier protein (ACP) in acyl-ACP and the release of a free fatty acid. Such enzymes typically function in the plastids of an organism which is synthesizing *de novo* fatty acids. As used herein, the term “C16 or C18 fatty acid thioesterase” refers to an enzyme which catalyses the hydrolysis of the thioester bond between a C16 and/or C18 acyl moiety and ACP in acyl-ACP and the release of free C16 or C18 fatty acid in the plastid. The free fatty acid is then re-esterified to CoA in the plastid envelope as it is transported out of the plastid. The substrate specificity of the fatty acid thioesterase (FAT) enzyme in the plastid is involved in determining the spectrum of chain length and degree of saturation of the fatty acids exported from the plastid. FAT enzymes can be classified into two classes based on their substrate specificity and nucleotide sequences, FATA and FATB (EC 3.1.2.14) (Jones et al., 1995). FATA polypeptides prefer oleoyl-ACP as substrate, while FATB polypeptides show higher activity towards saturated acyl-ACPs of different chain lengths such as acting on palmitoyl-ACP to produce free palmitic acid. Examples of FATA polypeptides useful for the invention include, but are not limited to, those from

Arabidopsis thaliana (NP_189147), *Arachis hypogaea* (GU324446), *Helianthus annuus* (AAL79361), *Carthamus tinctorius* (AAA33020), *Morus notabilis* (XP_010104178.1), *Brassica napus* (CDX77369.1), *Ricinus communis* (XP_002532744.1) and *Camelina sativa* (AFQ60946.1). Examples of FATB polypeptides useful for the invention include, but are not limited to, those from *Zea mays* (AIL28766), *Brassica napus* (ABH11710), *Helianthus annuus* (AAX19387), *Arabidopsis thaliana* (AEE28300), *Umbellularia californica* (AAC49001), *Arachis hypogaea* (AFR54500), *Ricinus communis* (EEF47013) and *Brachypodium sylvaticum* (ABL85052.1). In an embodiment, an exogenous polynucleotide of the invention which encodes a thioesterase which comprises one or more of the following:

i) nucleotides encoding a polypeptide comprising amino acids whose sequence is set forth in any of the above mentioned accessions, or a biologically active fragment thereof, or a polypeptide whose amino acid sequence is at least 30% identical to that set forth in any of the above mentioned accessions,

ii) nucleotides whose sequence is at least 30% identical to i), and

iii) a polynucleotide which hybridizes to one or both of i) or ii) under stringent conditions.

A subclass of FATB polypeptides are fatty acid thioesterases which have hydrolysis activity on a C6C14 saturated acyl moiety linked by a thioester bond to ACP. Such enzymes are also referred to as medium chain fatty acid (MCFA) thioesterases or MC-FAT enzymes. Such enzymes may also have thioesterase activity on C16-ACP, indeed they may have greater thioesterase activity on a C16 acyl-ACP substrate than on a MCFA-ACP substrate, nevertheless they are considered herein to be an MCFA thioesterase if they produce at least 0.5% MCFA in the total fatty acid content when expressed exogenously in a plant cell. Examples of MCFA thioesterases are given in Example 10 herein. In a particularly preferred embodiment, the thioesterase has a preference for hydrolysing medium chain fatty acid substartes. For instance, the thioesterease comprises one or more of the following:

i) nucleotides encoding a polypeptide comprising amino acids whose sequence is set forth in any one of SEQ ID NOs 87 to 93, or a biologically active fragment thereof, or a polypeptide whose amino acid sequence is at least 30% identical to any one or more of both of SEQ ID NOs 87 to 93,

ii) nucleotides whose sequence is at least 30% identical to i), and

iii) a polynucleotide which hybridizes to one or both of i) or ii) under stringent conditions.

More particularly preferred embodiment, the thioesterease is a C12:0-ACP thioestersae which comprises one or more of the following:

i) nucleotides encoding a polypeptide comprising amino acids whose sequence is set forth in SEQ ID NO:93, or a biologically active fragment thereof, or a polypeptide whose amino acid sequence is at least 30% identical to SEQ ID NO:93,

ii) nucleotides whose sequence is at least 30% identical to i), and

iii) a polynucleotide which hybridizes to one or both of i) or ii) under stringent conditions.

As used herein, the term "fatty acid transporter" relates to a polypeptide present in the plastid membrane which is involved in actively transferring fatty acids from a plastid to outside the plastid. Examples of ABCA9 (ABC transporter A family member 9) polypeptides useful for the invention include, but are not limited to, those from *Arabidopsis thaliana* (Q9FLT5), *Capsella rubella* (XP_006279962.1), *Arabis alpine* (KFK27923.1), *Camelina sativa* (XP_010457652.1), *Brassica napus* (CDY23040.1) and *Brassica rapa* (XP_009136512.1).

As used herein, the term "acyl-CoA synthetase" or "ACS" (EC 6.2.1.3) means a polypeptide which is a member of a ligase family that catalyzes the formation of fatty acyl-CoA by a two-step process proceeding through an adenylated intermediate, using a non-esterified fatty acid, CoA and ATP as substrates to produce an acyl-CoA ester, AMP and pyrophosphate as products. As used herein, the term "long-chain acyl-CoA synthetase" (LACS) is an ACS that has activity on at least a C18 free fatty acid substrate although it may have broader activity on any of C14-C20 free fatty acids. The endogenous plastidial LACS enzymes are localised in the outer membrane of the plastid and function with fatty acid thioesterase for the export of fatty acids from the plastid (Schnurr et al., 2002). In *Arabidopsis*, there are at least nine identified LACS genes (Shockey et al., 2002). Preferred LACS polypeptides are of the LACS9 subclass, which in *Arabidopsis* is the major plastidial LACS. Examples of LACS polypeptides useful for the invention include, but are not limited to, those from *Arabidopsis thaliana* (Q9CAP8), *Camelina sativa* (XP_010416710.1), *Capsella rubella* (XP_006301059.1), *Brassica napus* (CDX79212.1), *Brassica rapa* (XP_009104618.1), *Gossypium raimondii* (XP_012450538.1) and *Vitis Vinifera* (XP_002285853.1). Homologs of the above mentioned polypeptides in other species can readily be identified by those skilled in the art.

Polypeptides Involved in Diacylglycerol (DAG) Production

Levels of non-polar lipids in, for example, vegetative plant parts can also be increased by reducing the activity of polypeptides involved in diacylglycerol (DAG) production in the plastid in the plant parts, for example by either mutation of an endogenous gene encoding such a polypeptide or introduction of an exogenous gene which encodes a silencing RNA molecule which reduces the expression of a target gene involved in diacylglycerol (DAG) production in the plastid.

As used herein, the term "polypeptide involved in diacylglycerol (DAG) production in the plastid" refers to any polypeptide in the plastid of plant cells in a plant or part thereof that is directly involved in the synthesis of diacylglycerol. Examples of such polypeptides include, but are not limited to, a plastidial GPAT, a plastidial LPAAT or a plastidial PAP.

GPATs are described elsewhere in the present document. Examples of plastidial GPAT polypeptides which can be targeted for down-regulation in the invention include, but are not limited to, those from *Arabidopsis thaliana* (BAA00575), *Capsella rubella* (XP_006306544.1), *Camelina sativa* (010499766.1), *Brassica napus* (CDY43010.1), *Brassica rapa* (XP_009145198.1), *Helianthus annuus* (ADV16382.1) and *Citrus unshiu* (BAB79529.1). Homologs in other species can readily be identified by those skilled in the art.

LPAATs are described elsewhere in the present document. As the skilled person would appreciate, plastidial LPAATs to be targeted for down-regulation for reducing DAG synthesis in the plastid are not endogenous LPAATs which function outside of the plastid such as those in the ER, for example being useful for producing TAG comprising medium chain length fatty acids. Examples of plastidial LPAAT polypeptides which can be targeted for down-regulation in the invention include, but are not limited to, those from *Brassica napus* (ABQ42862), *Brassica rapa* (XP_009137939.1), *Arabidopsis thaliana* (NP_194787.2), *Camelina sativa* (XP_010432969.1), *Glycine max* (XP_006592638.1) and *Solanum tuberosum* (XP_006343651.1). Homologs in other species of the above mentioned polypeptides can readily be identified by those skilled in the art.

As used herein, the term "phosphatidic acid phosphatase" (PAP) (EC 3.1.3.4) refers to a protein which hydrolyses the phosphate group on 3-*sn*-phosphatidate to produce 1,2-diacyl-sn-glycerol (DAG) and phosphate. Examples of plastidial PAP polypeptides which can be targeted for down-regulation in the invention include, but are not limited to, those from *Arabidopsis thaliana* (Q6NLA5), *Capsella rubella* (XP_006288605.1), *Camelina sativa* (XP_010452170.1), *Brassica napus* (CDY10405.1), *Brassica rapa* (XP_009122733.1), *Glycine max* (XP_003542504.1)

and *Solanum tuberosum* (XP_006361792.1). Homologs in other species of the above mentioned polypeptides can readily be identified by those skilled in the art.

Another enzyme that results in DAG production, but in the ER rather than the plastid, is PDCT. As used herein, the term "phosphatidylcholine:diacylglycerol cholinephosphotransferase" (PDCT; EC 2.7.8.2) means an cholinephosphotransferase that transfers a phospho-choline headgroup from a phospholipid, typically PC, to produce DAG, or the reverse reaction to produce PC from DAG. Thus, the two substrates of the forward reaction are cytidine monophosphate (CMP) and phosphatidylcholine and the two products are CDP-choline and DAG. PDCT belongs to the phosphatidic acid phosphatase-related protein family and typically possesses lipid phosphatase/phosphotransferase (LPT) domains. In *Arabidopsis thaliana*, PDCT is encoded by the *ROD1* (At3g15820) and *ROD2* (At3g15830) genes (Lu et al., 2009). Homologous genes are readily identified in other plant species (Guan et al., 2015). Sequences of exemplary PDCT coding regions and polypeptides are provided in, Accession Nos XM_002437214 and EU973573.1), although any PDCT encoding gene can be used. In an embodiment, the PDCT is other than *A. thaliana* PDCT (Lu et al., 2009). Increased expression of PDCT, which may be exogenous or endogenous to the cell or plant of the invention and which is preferably expressed from an exogenous polynucleotide, increases the flow of esterified acyl groups from PC to DAG and thereby increases the TTQ in the total fatty acid content and the level of TAG in vegetative plant parts or cells of the invention. Alternatively, decreasing the level of PDCT activity in the cell or plant by mutation in the gene or by a silencing RNA molecule reduces the production of PC from DAG, the reverse PDCT reaction.

Import of Fatty Acids into Plastids

Levels of non-polar lipids in vegetative plant parts can also be increased by reducing the activity of TGD polypeptides in the plant parts, for example by either mutation of an endogenous gene encoding a TGD polypeptide or introduction of an exogenous gene which encodes a silencing RNA molecule which reduces the expression of an endogenous *TGD* gene. As used herein, a "Trigalactosyldiacylglycerol (TGD) polypeptide" is one which is involved in the ER to chloroplast lipid trafficking (Xu et al., 2010; Fan et al., 2015) and involved in forming a protein complex which has permease function for lipids. Four such polypeptides are known to form or be associated with a TGD permease, namely TGD-1 (Accession No. At1g19800 and homologs in other species), TGD-2 (Accession No At2g20320 and homologs in other species), TGD-3 (Accession No. NM-105215 and homologs in other species) and

TGD-4 (At3g06960 and homologs in other species) (US 20120237949). TGD5 is also involved in ER to choroplast lipid trafficking, and down-regulation of TGD5 is associated with increased oil production (US2015/337017; Fan et al., 2015). Sequences of exemplary TGD5 polypeptides are provided in Accession Nos XM_002442154 and EU972796.1). TGD-1, -2 and -3 polypeptides are thought to be components of an ATP-Binding Cassette (ABC) transporter associated with the inner envelope membrane of the chloroplast. TGD-2 and TGD-4 polypeptides bind to phosphatidic acid whereas TGD-3 polypetide functions as an ATPase in the chloroplast stroma. As used herein, an "endogenous TGD gene" is a gene which encodes a TGD polypeptide in a plant. Mutations in *TGD-1* gene in *A. thaliana* caused accumulation of triacylglycerols, oligogalactolipids and phosphatidic acid (PA) (Xu et al., 2005). Mutations in *TGD* genes or *SDP1* genes, or indeed in any desired gene in a plant, can be introduced in a site-specific manner by artificial zinc finger nuclease (ZFN), TAL effector (TALEN) or CRISPR technologies (using a Cas9 type nuclease) as known in the art. Preferred exogenous genes encoding silencing RNAs are those encoding a double-stranded RNA molecule such as a hairpin RNA or an artificial microRNA precursor.

Sucrose Metabolism

The TAG levels and/or the TTQ of the total fatty content in the cells, plants and plant parts of the invention can also be increased by modifying sucrose metabolism, particularly in the stems of plants such as sugarcane, *Sorghum* and *Zea mays*. In an embodiment, this is achieved by increasing expression of a sucrose metabolism polypeptide such as invertase or sucrose synthase, or of a sucrose transport polypeptide such as SUS1, SUS4, SUT2, SUT4, or SWEET. The effect of these polypeptides is to increase the supply of sucrose and its monosaccharide components in the cytosol of the cells and/or to decrease the transfer and/or storage of sucrose in the vacuoles of the cells, particularly in stem cells. Sequences of examples of these polypeptides are provided in SEQ ID NOs:274-292 of WO 2016/004473. Invertase such as bCIN, INV2 or INV3 acts to convert sucrose into hexoses which can be exported from the vacuoles into the cytoplasm (McKinley et al., 2016). Increased expression of SUS1 or SUS4 breaks down cytosolic sucrose into hexoses for glycolysis and *de novo* fatty acid synthesis rather than transfer of the sucrose into vacuoles, such as in stem parenchyma cells (McKinley et al., 2016). Increased expression of sugar transport polypeptides such as tonoplast sucrose exporter, for example SUT2 or SUT4, or SWEET polypeptide releases vacuolar sucrose for cytosolic glycolysis and increases *de novo*

fatty acid biosynthesis (Bihmidine et al., 2016; Qazi et al., 2012; Schneider et al., 2012; Hedrich et al., 2015; Klemens et al., 2013).

The TAG levels and/or the TTQ of the total fatty content in the cells, plants and plant parts of the invention can also be increased by reducing the level of TST polypeptides such as TST1 or TST2, particularly in the stems of plants such as sugarcane, *Sorghum* and *Zea mays*. TST polypeptide can be decreased by mutation of the endogenous genes encoding the polypeptide, or by introduction of an exogenous polynucleotide that encodes a silencing RNA molecule. Sequences of exemplary TST cDNAs and polypeptides are provided as SEQ ID NOs:266-273 of WO 2016/004473.

Fatty Acid Modifying Enzymes

As used herein, the term "FAD2" refers to a membrane bound delta-12 fatty acid desturase that desaturates oleic acid (C18:1 $^{\Delta 9}$) to produce linoleic acid (C18:2$^{\Delta 9,12}$).

As used herein, the term "epoxygenase" or "fatty acid epoxygenase" refers to an enzyme that introduces an epoxy group into a fatty acid resulting in the production of an epoxy fatty acid. In preferred embodiment, the epoxy group is introduced at the 12th carbon on a fatty acid chain, in which case the epoxygenase is a Δ12-epoxygenase, especially of a C16 or C18 fatty acid chain. The epoxygenase may be a Δ9-epoxygenase, a Δ15 epoxygenase, or act at a different position in the acyl chain as known in the art. The epoxygenase may be of the P450 class. Preferred epoxygenases are of the mono-oxygenase class as described in WO98/46762. Numerous epoxygenases or presumed epoxygenases have been cloned and are known in the art. Further examples of expoxygenases include proteins comprising an amino acid sequence provided in SEQ ID NO:21 of WO 2009/129582, polypeptides encoded by genes from *Crepis paleastina* (CAA76156, Lee et al., 1998), *Stokesia laevis* (AAR23815) (monooxygenase type), *Euphorbia lagascae* (AAL62063) (P450 type), human CYP2J2 (arachidonic acid epoxygenase, U37143); human CYPIA1 (arachidonic acid epoxygenase, K03191), as well as variants and/or mutants thereof.

As used herein, the term, "hydroxylase" or "fatty acid hydroxylase" refers to an enzyme that introduces a hydroxyl group into a fatty acid resulting in the production of a hydroxylated fatty acid. In a preferred embodiment, the hydroxyl group is introduced at the 2nd, 12th and/or 17th carbon on a C18 fatty acid chain. Preferably, the hydroxyl group is introduced at the 12th carbon, in which case the hydroxylase is a Δ12-hydroxylase. In another preferred embodiment, the hydroxyl group is introduced at the 15th carbon on a C16 fatty acid chain. Hydroxylases may also have enzyme activity as a fatty acid desaturase. Examples of genes encoding Δ12-hydroxylases include those

from *Ricinus communis* (AAC9010, van de Loo 1995); *Physaria lindheimeri*, (ABQ01458, Dauk et al., 2007); *Lesquerella fendleri*, (AAC32755, Broun et al., 1998); *Daucus carota*, (AAK30206); fatty acid hydroxylases which hydroxylate the terminus of fatty acids, for example: *A, thaliana* CYP86A1 (P48422, fatty acid ω-hydroxylase); *Vicia sativa* CYP94A1 (P98188, fatty acid ω-hydroxylase); mouse CYP2E1 (X62595, lauric acid ω-1 hydroxylase); rat CYP4A1 (M57718, fatty acid ω-hydroxylase), as well as as variants and/or mutants thereof.

As used herein, the term "conjugase" or "fatty acid conjugase" refers to an enzyme capable of forming a conjugated bond in the acyl chain of a fatty acid. Examples of conjugases include those encoded by genes from *Calendula officinalis* (AF343064, Qiu et al., 2001); *Vernicia fordii* (AAN87574, Dyer et al., 2002); *Punica granatum* (AY178446, Iwabuchi et al., 2003) and *Trichosanthes kirilowii* (AY178444, Iwabuchi et al., 2003); as well as as variants and/or mutants thereof.

As used herein, the term "acetylenase" or "fatty acid acetylenase" refers to an enzyme that introduces a triple bond into a fatty acid resulting in the production of an acetylenic fatty acid. In a preferred embodiment, the triple bond is introduced at the 2nd, 6th, 12th and/or 17th carbon on a C18 fatty acid chain. Examples acetylenases include those from *Helianthus annuus* (AA038032, ABC59684), as well as as variants and/or mutants thereof.

Examples of such fatty acid modifying genes include proteins according to the following Accession Numbers which are grouped by putative function, and homologues from other species: Δ12-acetylenases ABC00769, CAA76158, AAO38036, AAO38032; Δ12 conjugases AAG42259, AAG42260, AAN87574; Δ12-desaturases P46313, ABS18716, AAS57577, AAL61825, AAF04093, AAF04094; Δ12 epoxygenases XP_001840127, CAA76156, AAR23815; Δ12-hydroxylases ACF37070, AAC32755, ABQ01458, AAC49010; and Δ12 P450 enzymes such as AF406732.

Silencing Suppressors

In an embodiment, a transgenic plant or part thereof of the invention may comprise a silencing suppressor.

As used herein, a “silencing suppressor” enhances transgene expression in a plant or part thereof of the invention. For example, the presence of the silencing suppressor results in higher levels of a polypeptide(s) produced an exogenous polynucleotide(s) in a plant or part thereof of the invention when compared to a corresponding plant or part thereof lacking the silencing suppressor. In an embodiment, the silencing suppressor preferentially binds a dsRNA molecule which is

21 base pairs in length relative to a dsRNA molecule of a different length. This is a feature of at least the p19 type of silencing suppressor, namely for p19 and its functional orthologs. In another embodiment, the silencing suppressor preferentially binds to a double-stranded RNA molecule which has overhanging 5' ends relative to a corresponding double-stranded RNA molecule having blunt ends. This is a feature of the V2 type of silencing suppressor, namely for V2 and its functional orthologs. In an embodiment, the dsRNA molecule, or a processed RNA product thereof, comprises at least 19 consecutive nucleotides, preferably whose length is 19-24 nucleotides with 19-24 consecutive basepairs in the case of a double-stranded hairpin RNA molecule or processed RNA product, more preferably consisting of 20, 21, 22, 23 or 24 nucleotides in length, and preferably comprising a methylated nucleotide, which is at least 95% identical to the complement of the region of the target RNA, and wherein the region of the target RNA is i) within a 5' untranslated region of the target RNA, ii) within a 5' half of the target RNA, iii) within a protein-encoding open-reading frame of the target RNA, iv) within a 3' half of the target RNA, or v) within a 3' untranslated region of the target RNA.

Further details regarding silencing suppressors are well known in the art and described in WO 2013/096992 and WO 2013/096993.

Polynucleotides

The terms "polynucleotide", and "nucleic acid" are used interchangeably. They refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. A polynucleotide of the invention may be of genomic, cDNA, semisynthetic, or synthetic origin, double-stranded or single-stranded and by virtue of its origin or manipulation: (1) is not associated with all or a portion of a polynucleotide with which it is associated in nature, (2) is linked to a polynucleotide other than that to which it is linked in nature, or (3) does not occur in nature. The following are non-limiting examples of polynucleotides: coding or non-coding regions of a gene or gene fragment, exons, introns, messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), ribozymes, cDNA, recombinant polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, chimeric DNA of any sequence, nucleic acid probes, and primers. For *in vitro* use, a polynucleotide may comprise modified nucleotides such as by conjugation with a labeling component.

As used herein, an "isolated polynucleotide" refers to a polynucleotide which has been separated from the polynucleotide sequences with which it is associated or linked in its native state, or a non-naturally occurring polynucleotide.

As used herein, the term "gene" is to be taken in its broadest context and includes the deoxyribonucleotide sequences comprising the transcribed region and, if translated, the protein coding region, of a structural gene and including sequences located adjacent to the coding region on both the 5' and 3' ends for a distance of at least about 2 kb on either end and which are involved in expression of the gene. In this regard, the gene includes control signals such as promoters, enhancers, termination and/or polyadenylation signals that are naturally associated with a given gene, or heterologous control signals, in which case, the gene is referred to as a "chimeric gene". The sequences which are located 5' of the protein coding region and which are present on the mRNA are referred to as 5' non-translated sequences. The sequences which are located 3' or downstream of the protein coding region and which are present on the mRNA are referred to as 3' non-translated sequences. The term "gene" encompasses both cDNA and genomic forms of a gene. A genomic form or clone of a gene contains the coding region which may be interrupted with non-coding sequences termed "introns", "intervening regions", or "intervening sequences." Introns are segments of a gene which are transcribed into nuclear RNA (nRNA). Introns may contain regulatory elements such as enhancers. Introns are removed or "spliced out" from the nuclear or primary transcript; introns are therefore absent in the mRNA transcript. A gene which contains at least one intron may be subject to variable splicing, resulting in alternative mRNAs from a single transcribed gene and therefore polypeptide variants. A gene in its native state, or a chimeric gene may lack introns. The mRNA functions during translation to specify the sequence or order of amino acids in a nascent polypeptide. The term "gene" includes a synthetic or fusion molecule encoding all or part of the proteins of the invention described herein and a complementary nucleotide sequence to any one of the above.

As used herein, "chimeric DNA" refers to any DNA molecule that is not naturally found in nature; also referred to herein as a "DNA construct" or “genetic construct”. Typically, a chimeric DNA comprises regulatory and transcribed or protein coding sequences that are not naturally found together in nature. Accordingly, chimeric DNA may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. The open reading frame may or may not be linked to its natural upstream and downstream

regulatory elements. The open reading frame may be incorporated into, for example, the plant genome, in a non-natural location, or in a replicon or vector where it is not naturally found such as a bacterial plasmid or a viral vector. The term "chimeric DNA" is not limited to DNA molecules which are replicable in a host, but includes DNA capable of being ligated into a replicon by, for example, specific adaptor sequences.

A "transgene" is a gene that has been introduced into the genome by a transformation procedure. The term includes a gene in a progeny plant or part thereof such as a vegetative plant part which was introducing into the genome of a progenitor cell thereof. Such progeny cells etc may be at least a 3rd or 4th generation progeny from the progenitor cell which was the primary transformed cell, or of the progenitor transgenic plant (referred to herein as a T0 plant). Progeny may be produced by sexual reproduction or vegetatively such as, for example, from tubers in potatoes or ratoons in sugarcane. The term "genetically modified", “genetic modification” and variations thereof, is a broader term that includes introducing a gene into a cell by transformation or transduction, mutating a gene in a cell and genetically altering or modulating the regulation of a gene in a cell, or the progeny of any cell modified as described above.

A "genomic region" as used herein refers to a position within the genome where a transgene, or group of transgenes (also referred to herein as a cluster), have been inserted into a cell, or predecessor thereof. Such regions only comprise nucleotides that have been incorporated by the intervention of man such as by methods described herein.

A "recombinant polynucleotide" of the invention refers to a nucleic acid molecule which has been constructed or modified by artificial recombinant methods. The recombinant polynucleotide may be present in a cell of a plant or part thereof in an altered amount or expressed at an altered rate (e.g., in the case of mRNA) compared to its native state. In one embodiment, the polynucleotide is introduced into a cell that does not naturally comprise the polynucleotide. Typically an exogenous DNA is used as a template for transcription of mRNA which is then translated into a continuous sequence of amino acid residues coding for a polypeptide of the invention within the transformed cell. In another embodiment, the polynucleotide is endogenous to the plant or part thereof and its expression is altered by recombinant means, for example, an exogenous control sequence is introduced upstream of an endogenous gene of interest to enable the transformed plant or part thereof to express the polypeptide encoded by the gene, or a deletion is created in a gene of interest by ZFN, Talen or CRISPR methods.

A recombinant polynucleotide of the invention includes polynucleotides which have not been separated from other components of the cell-based or cell-free expression system, in which it is present, and polynucleotides produced in said cell-based or cell-free systems which are subsequently purified away from at least some other components. The polynucleotide can be a contiguous stretch of nucleotides or comprise two or more contiguous stretches of nucleotides from different sources (naturally occurring and/or synthetic) joined to form a single polynucleotide. Typically, such chimeric polynucleotides comprise at least an open reading frame encoding a polypeptide of the invention operably linked to a promoter suitable of driving transcription of the open reading frame in a cell of interest.

With regard to the defined polynucleotides, it will be appreciated that % identity figures higher than those provided above will encompass preferred embodiments. Thus, where applicable, in light of the minimum % identity figures, it is preferred that the polynucleotide comprises a polynucleotide sequence which is at least 60%, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99%, more preferably at least 99.1%, more preferably at least 99.2%, more preferably at least 99.3%, more preferably at least 99.4%, more preferably at least 99.5%, more preferably at least 99.6%, more preferably at least 99.7%, more preferably at least 99.8%, and even more preferably at least 99.9% identical to the relevant nominated SEQ ID NO.

A polynucleotide of, or useful for, the present invention may selectively hybridise, under stringent conditions, to a polynucleotide defined herein. As used herein, stringent conditions are those that: (1) employ during hybridisation a denaturing agent such as formamide, for example, 50% (v/v) formamide with 0.1% (w/v) bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42ºC; or (2) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 g/ml), 0.1% SDS and 10% dextran sulfate at 42ºC in 0.2 x SSC and 0.1% SDS, and/or (3) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0.1% SDS at 50ºC.

Polynucleotides of the invention may possess, when compared to naturally occurring molecules, one or more mutations which are deletions, insertions, or substitutions of nucleotide residues. Polynucleotides which have mutations relative to a reference sequence can be either naturally occurring (that is to say, isolated from a natural source) or synthetic (for example, by performing site-directed mutagenesis or DNA shuffling on the nucleic acid as described above).

Polynucleotides for Reducing Expression of Genes

RNA Interference

RNA interference (RNAi) is particularly useful for specifically reducing the expression of a gene, which results in reduced production of a particular protein if the gene encodes a protein. Although not wishing to be limited by theory, Waterhouse et al. (1998) have provided a model for the mechanism by which dsRNA (duplex RNA) can be used to reduce protein production. This technology relies on the presence of dsRNA molecules that contain a sequence that is essentially identical to the mRNA of the gene of interest or part thereof. Conveniently, the dsRNA can be produced from a single promoter in a recombinant vector or host cell, where the sense and anti-sense sequences are flanked by an unrelated sequence which enables the sense and anti-sense sequences to hybridize to form the dsRNA molecule with the unrelated sequence forming a loop structure. The design and production of suitable dsRNA molecules is well within the capacity of a person skilled in the art, particularly considering Waterhouse et al. (1998), Smith et al. (2000), WO 99/32619, WO 99/53050, WO 99/49029, and WO 01/34815.

In one example, a DNA is introduced that directs the synthesis of an at least partly double stranded RNA product(s) with homology to the target gene to be inactivated such as, for example, a *SDP1*, *TGD*, plastidial *GPAT*, plastidial *LPAAT*, plastidial *PAP*, *AGPase* gene. The DNA therefore comprises both sense and antisense sequences that, when transcribed into RNA, can hybridize to form the double stranded RNA region. In one embodiment of the invention, the sense and antisense sequences are separated by a spacer region that comprises an intron which, when transcribed into RNA, is spliced out. This arrangement has been shown to result in a higher efficiency of gene silencing (Smith et al., 2000). The double stranded region may comprise one or two RNA molecules, transcribed from either one DNA region or two. The presence of the double stranded molecule is thought to trigger a response from an endogenous system that destroys both the double stranded RNA and also the homologous RNA

transcript from the target gene, efficiently reducing or eliminating the activity of the target gene.

The length of the sense and antisense sequences that hybridize should each be at least 19 contiguous nucleotides, preferably at least 50 contiguous nucleotides, more preferably at least 100 or at least 200 contiguous nucleotides. Generally, a sequence of 100-1000 nucleotides corresponding to a region of the target gene mRNA is used. The full-length sequence corresponding to the entire gene transcript may be used. The degree of identity of the sense sequence to the targeted transcript (and therefore also the identity of the antisense sequence to the complement of the target transcript) should be at least 85%, at least 90%, or 95-100%. The RNA molecule may of course comprise unrelated sequences which may function to stabilize the molecule. The RNA molecule may be expressed under the control of a RNA polymerase II or RNA polymerase III promoter. Examples of the latter include tRNA or snRNA promoters.

Preferred small interfering RNA ("siRNA") molecules comprise a nucleotide sequence that is identical to about 19-25 contiguous nucleotides of the target mRNA. Preferably, the siRNA sequence commences with the dinucleotide AA, comprises a GC-content of about 30-70% (preferably, 30-60%, more preferably 40-60% and more preferably about 45%-55%), and does not have a high percentage identity to any nucleotide sequence other than the target in the genome of the organism in which it is to be introduced, for example, as determined by standard BLAST search.

microRNA

MicroRNAs (abbreviated miRNAs) are generally 19–25 nucleotides (commonly about 20-24 nucleotides in plants) non-coding RNA molecules that are derived from larger precursors that form imperfect stem-loop structures. miRNAs bind to complementary sequences on target messenger RNA transcripts (mRNAs), usually resulting in translational repression or target degradation and gene silencing. Artificial miRNAs (amiRNAs) can be designed based on natural miRNAs for reducing the expression of any gene of interest, as well known in the art.

In plant cells, miRNA precursor molecules are believed to be largely processed in the nucleus. The pri-miRNA (containing one or more local double-stranded or "hairpin" regions as well as the usual 5' "cap" and polyadenylated tail of an mRNA) is processed to a shorter miRNA precursor molecule that also includes a stem-loop or fold-back structure and is termed the "pre-miRNA". In plants, the pre-miRNAs are cleaved by distinct DICER-like (DCL) enzymes, yielding miRNA:miRNA* duplexes. Prior to transport out of the nucleus, these duplexes are methylated.

In the cytoplasm, the miRNA strand from the miRNA:miRNA duplex is selectively incorporated into an active RNA-induced silencing complex (RISC) for target recognition.The RISC- complexes contain a particular subset of Argonaute proteins that exert sequence-specific gene repression (see, for example, Millar and Waterhouse, 2005; Pasquinelli et al., 2005; Almeida and Allshire, 2005).

Cosuppression

Genes can suppress the expression of related endogenous genes and/or transgenes already present in the genome, a phenomenon termed homology-dependent gene silencing. Most of the instances of homologydependent gene silencing fall into two classes - those that function at the level of transcription of the transgene, and those that operate post-transcriptionally.

Post-transcriptional homology-dependent gene silencing (i.e., cosuppression) describes the loss of expression of a transgene and related endogenous or viral genes in transgenic plants. Cosuppression often, but not always, occurs when transgene transcripts are abundant, and it is generally thought to be triggered at the level of mRNA processing, localization, and/or degradation. Severa1 models exist to explain how cosuppression works (see in Taylor, 1997).

Cosuppression involves introducing an extra copy of a gene or a fragment thereof into a plant in the sense orientation with respect to a promoter for its expression. The size of the sense fragment, its correspondence to target gene regions, and its degree of sequence identity to the target gene can be determined by those skilled in the art. In some instances, the additional copy of the gene sequence interferes with the expression of the target plant gene. Reference is made to WO 97/20936 and EP 0465572 for methods of implementing co-suppression approaches.

Recombinant Vectors

One embodiment of the present invention includes a recombinant vector, which comprises at least one polynucleotide defined herein and is capable of delivering the polynucleotide into a host cell. Recombinant vectors include expression vectors. Recombinant vectors contain heterologous polynucleotide sequences, that is, polynucleotide sequences that are not naturally found adjacent to a polynucleotide defined herein, that preferably, are derived from a different species. The vector can be either RNA or DNA, and typically is a viral vector, derived from a virus, or a plasmid. Plasmid vectors typically include additional nucleic acid sequences that provide for easy selection, amplification, and transformation of the expression cassette in

prokaryotic cells, e.g., pUC-derived vectors, pGEM-derived vectors or binary vectors containing one or more T-DNA regions. Additional nucleic acid sequences include origins of replication to provide for autonomous replication of the vector, selectable marker genes, preferably encoding antibiotic or herbicide resistance, unique multiple cloning sites providing for multiple sites to insert nucleic acid sequences or genes encoded in the nucleic acid construct, and sequences that enhance transformation of prokaryotic and eukaryotic (especially plant) cells.

"Operably linked" as used herein, refers to a functional relationship between two or more nucleic acid (e.g., DNA) segments. Typically, it refers to the functional relationship of a transcriptional regulatory element (promoter) to a transcribed sequence. For example, a promoter is operably linked to a coding sequence of a polynucleotide defined herein, if it stimulates or modulates the transcription of the coding sequence in an appropriate cell. Generally, promoter transcriptional regulatory elements that are operably linked to a transcribed sequence are physically contiguous to the transcribed sequence, i.e., they are *cis*-acting. However, some transcriptional regulatory elements such as enhancers need not be physically contiguous or located in close proximity to the coding sequences whose transcription they enhance.

When there are multiple promoters present, each promoter may independently be the same or different.

Recombinant vectors may also contain one or more signal peptide sequences to enable an expressed polypeptide defined herein to be retained in the endoplasmic reticulum (ER) in the cell, or transfer into a plastid, and/or contain fusion sequences which lead to the expression of nucleic acid molecules as fusion proteins. Examples of suitable signal segments include any signal segment capable of directing the secretion or localisation of a polypeptide defined herein.

To facilitate identification of transformants, the recombinant vector desirably comprises a selectable or screenable marker gene. By "marker gene" is meant a gene that imparts a distinct phenotype to cells expressing the marker gene and thus, allows such transformed cells to be distinguished from cells that do not have the marker. A selectable marker gene confers a trait for which one can "select" based on resistance to a selective agent (e.g., a herbicide, antibiotic). A screenable marker gene (or reporter gene) confers a trait that one can identify through observation or testing, that is, by "screening" (e.g., β-glucuronidase, luciferase, GFP or other enzyme activity not present in untransformed cells). Exemplary selectable markers for selection of plant transformants include, but are not limited to, a *hyg* gene which encodes hygromycin B resistance; a neomycin phosphotransferase (*nptII*) gene conferring resistance to

kanamycin, paromomycin; a glutathione-S-transferase gene from rat liver conferring resistance to glutathione derived herbicides as for example, described in EP 256223; a glutamine synthetase gene conferring, upon overexpression, resistance to glutamine synthetase inhibitors such as phosphinothricin as for example, described in WO 87/05327; an acetyltransferase gene from *Streptomyces viridochromogenes* conferring resistance to the selective agent phosphinothricin as for example, described in EP 275957; a gene encoding a 5-enolshikimate-3-phosphate synthase (EPSPS) conferring tolerance to N-phosphonomethylglycine as for example, described by Hinchee et al. (1988); a *bar* gene conferring resistance against bialaphos as for example, described in WO91/02071; a nitrilase gene such as *bxn* from *Klebsiella ozaenae* which confers resistance to bromoxynil (Stalker et al., 1988); a dihydrofolate reductase (DHFR) gene conferring resistance to methotrexate (Thillet et al., 1988); a mutant acetolactate synthase gene (ALS) which confers resistance to imidazolinone, sulfonylurea, or other ALS-inhibiting chemicals (EP 154,204); a mutated anthranilate synthase gene that confers resistance to 5-methyl tryptophan; or a dalapon dehalogenase gene that confers resistance to the herbicide.

Preferably, the recombinant vector is stably incorporated into the genome of the cell such as the plant cell. Accordingly, the recombinant vector may comprise appropriate elements which allow the vector to be incorporated into the genome, or into a chromosome of the cell.

Expression Vector

As used herein, an "expression vector" is a DNA vector that is capable of transforming a host cell and of effecting expression of one or more specified polynucleotides. Expression vectors of the present invention contain regulatory sequences such as transcription control sequences, translation control sequences, origins of replication, and other regulatory sequences that are compatible with the host cell and that control the expression of polynucleotides of the present invention. In particular, expression vectors of the present invention include transcription control sequences. Transcription control sequences are sequences which control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which control transcription initiation such as promoter, enhancer, operator and repressor sequences. The choice of the regulatory sequences used depends on the target organism such as a plant and/or target organ or tissue of interest. Such regulatory sequences may be obtained from any eukaryotic organism such as plants or plant viruses, or may be chemically synthesized. A number of vectors

suitable for stable transfection of plant cells or for the establishment of transgenic plants have been described in for example, Pouwels et al., Cloning Vectors: A Laboratory Manual, 1985, supp. 1987, Weissbach and Weissbach, Methods for Plant Molecular Biology, Academic Press, 1989, and Gelvin et al., Plant Molecular Biology Manual, Kluwer Academic Publishers, 1990. Typically, plant expression vectors include for example, one or more cloned plant genes under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant expression vectors also can contain a promoter regulatory region (e.g., a regulatory region controlling inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, a ribosome binding site, a transcription termination site, and/or a polyadenylation signal.

A number of constitutive promoters that are active in plant cells have been described. Suitable promoters for constitutive expression in plants include, but are not limited to, the cauliflower mosaic virus (CaMV) 35S promoter, the Figwort mosaic virus (FMV) 35S, the light-inducible promoter from the small subunit (SSU) of the ribulose-1,5-bis-phosphate carboxylase, the rice cytosolic triosephosphate isomerase promoter, the adenine phosphoribosyltransferase promoter of *Arabidopsis*, the rice actin 1 gene promoter, the mannopine synthase and octopine synthase promoters, the Adh promoter, the sucrose synthase promoter, the R gene complex promoter, and the chlorophyll α/β binding protein gene promoter. These promoters have been used to create DNA vectors that have been expressed in plants, see for example, WO 84/02913. All of these promoters have been used to create various types of plant-expressible recombinant DNA vectors.

For the purpose of expression in source tissues of the plant such as the leaf, seed, root or stem, it is preferred that the promoters utilized in the present invention have relatively high expression in these specific tissues. For this purpose, one may choose from a number of promoters for genes with tissue- or cell-specific, or -enhanced expression. Examples of such promoters reported in the literature include, the chloroplast glutamine synthetase GS2 promoter from pea, the chloroplast fructose-1,6-biphosphatase promoter from wheat, the nuclear photosynthetic ST-LS1 promoter from potato, the serine/threonine kinase promoter and the glucoamylase (CHS) promoter from *Arabidopsis thaliana*. Also reported to be active in photosynthetically active tissues are the ribulose-1,5-bisphosphate carboxylase promoter from eastern larch (*Larix laricina*), the promoter for the Cab gene, Cab6, from pine, the promoter for the Cab-1 gene from wheat, the promoter for the Cab-1 gene from spinach, the promoter for the Cab 1R gene from rice, the pyruvate, orthophosphate dikinase (PPDK) promoter

from *Zea mays*, the promoter for the tobacco Lhcb1*2 gene, the *Arabidopsis thaliana* Suc2 sucrose-H^{30} symporter promoter, and the promoter for the thylakoid membrane protein genes from spinach (PsaD, PsaF, PsaE, PC, FNR, AtpC, AtpD, Cab, RbcS). Other promoters for the chlorophyll α/β-binding proteins may also be utilized in the present invention such as the promoters for LhcB gene and PsbP gene from white mustard (*Sinapis alba*).

A variety of plant gene promoters that are regulated in response to environmental, hormonal, chemical, and/or developmental signals, also can be used for expression of RNA-binding protein genes in plant cells, including promoters regulated by (1) heat, (2) light (e.g., pea RbcS-3A promoter, maize RbcS promoter), (3) hormones such as abscisic acid, (4) wounding (e.g., WunI), or (5) chemicals such as methyl jasmonate, salicylic acid, steroid hormones, alcohol, Safeners (WO 97/06269), or it may also be advantageous to employ (6) organ-specific promoters.

As used herein, the term "plant storage organ specific promoter" refers to a promoter that preferentially, when compared to other plant tissues, directs gene transcription in a storage organ of a plant. For the purpose of expression in sink tissues of the plant such as the tuber of the potato plant, the fruit of tomato, or the seed of soybean, canola, cotton, *Zea mays*, wheat, rice, and barley, it is preferred that the promoters utilized in the present invention have relatively high expression in these specific tissues. The promoter for *β*-conglycinin or other seed-specific promoters such as the napin, zein, linin and phaseolin promoters, can be used. Root specific promoters may also be used. An example of such a promoter is the promoter for the acid chitinase gene. Expression in root tissue could also be accomplished by utilizing the root specific subdomains of the CaMV 35S promoter that have been identified.

In a particularly preferred embodiment, the promoter directs expression in tissues and organs in which lipid biosynthesis takes place. Such promoters may act in seed development at a suitable time for modifying lipid composition in seeds. Preferred promoters for seed-specific expression include: 1) promoters from genes encoding enzymes involved in lipid biosynthesis and accumulation in seeds such as desaturases and elongases, 2) promoters from genes encoding seed storage proteins, and 3) promoters from genes encoding enzymes involved in carbohydrate biosynthesis and accumulation in seeds. Seed specific promoters which are suitable are, the oilseed rape napin gene promoter (US 5,608,152), the *Vicia faba* USP promoter (Baumlein et al., 1991), 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 legumin B4 promoter (Baumlein et al., 1992), and promoters which lead to the

seed-specific expression in monocots such as maize, barley, wheat, rye, rice and the like. Notable promoters which are suitable are the barley lpt2 or lpt1 gene promoter (WO 95/15389 and WO 95/23230), or the promoters described in WO 99/16890 (promoters from the barley hordein gene, the rice glutelin gene, the rice oryzin gene, the rice prolamin gene, the wheat gliadin gene, the wheat glutelin gene, the maize zein gene, the oat glutelin gene, the sorghum kasirin gene, the rye secalin gene). Other promoters include those described by Broun et al. (1998), Potenza et al. (2004), US 20070192902 and US 20030159173. In an embodiment, the seed specific promoter is preferentially expressed in defined parts of the seed such as the cotyledon(s) or the endosperm. Examples of cotyledon specific promoters include, but are not limited to, the FP1 promoter (Ellerstrom et al., 1996), the pea legumin promoter (Perrin et al., 2000), and the bean phytohemagglutnin promoter (Perrin et al., 2000). Examples of endosperm specific promoters include, but are not limited to, the maize zein-1 promoter (Chikwamba et al., 2003), the rice glutelin-1 promoter (Yang et al., 2003), the barley D-hordein promoter (Horvath et al., 2000) and wheat HMW glutenin promoters (Alvarez et al., 2000). In a further embodiment, the seed specific promoter is not expressed, or is only expressed at a low level, in the embryo and/or after the seed germinates.

In another embodiment, the plant storage organ specific promoter is a fruit specific promoter. Examples include, but are not limited to, the tomato polygalacturonase, E8 and Pds promoters, as well as the apple ACC oxidase promoter (for review, see Potenza et al., 2004). In a preferred embodiment, the promoter preferentially directs expression in the edible parts of the fruit, for example the pith of the fruit, relative to the skin of the fruit or the seeds within the fruit.

In an embodiment, the inducible promoter is the *Aspergillus nidulans* alc system. Examples of inducible expression systems which can be used instead of the *Aspergillus nidulans* alc system are described in a review by Padidam (2003) and Corrado and Karali (2009). In another embodiment, the inducible promoter is a safener inducible promoter such as, for example, the maize *ln2-1* or *ln2-2* promoter (Hershey and Stoner, 1991), the safener inducible promoter is the maize GST-27 promoter (Jepson et al., 1994), or the soybean GH2/4 promoter (Ulmasov et al., 1995).

In another embodiment, the inducible promoter is a senescence inducible promoter such as, for example, senescence-inducible promoter SAG (senescence associated gene) 12 and SAG 13 from *Arabidopsis* (Gan, 1995; Gan and Amasino, 1995) and LSC54 from *Brassica napus* (Buchanan-Wollaston, 1994). Such promoters

show increased expression at about the onset of senescence of plant tissues, in particular the leaves.

For expression in vegetative tissue leaf-specific promoters, such as the ribulose biphosphate carboxylase (RBCS) promoters, can be used. For example, the tomato RBCS1, RBCS2 and RBCS3A genes are expressed in leaves and light grown seedlings (Meier et al., 1997). A ribulose bisphosphate carboxylase promoters expressed almost exclusively in mesophyll cells in leaf blades and leaf sheaths at high levels, described by Matsuoka et al. (1994), can be used. Another leaf-specific promoter is the light harvesting chlorophyll a/b binding protein gene promoter (see, Shiina et al., 1997). The *Arabidopsis thaliana* myb-related gene promoter (Atmyb5) described by Li et al. (1996), is leaf-specific. The Atmyb5 promoter is expressed in developing leaf trichomes, stipules, and epidermal cells on the margins of young rosette and cauline leaves, and in immature seeds. A leaf promoter identified in maize by Busk et al. (1997), can also be used.

In some instances, for example when LEC2 or BBM is recombinantly expressed, it may be desirable that the transgene is not expressed at high levels. An example of a promoter which can be used in such circumstances is a truncated napin A promoter which retains the seed-specific expression pattern but with a reduced expression level (Tan et al., 2011).

The 5' non-translated leader sequence can be derived from the promoter selected to express the heterologous gene sequence of the polynucleotide of the present invention, or may be heterologous with respect to the coding region of the enzyme to be produced, and can be specifically modified if desired so as to increase translation of mRNA. For a review of optimizing expression of transgenes, see Koziel et al. (1996). The 5' non-translated regions can also be obtained from plant viral RNAs (Tobacco mosaic virus, Tobacco etch virus, Maize dwarf mosaic virus, Alfalfa mosaic virus, among others) from suitable eukaryotic genes, plant genes (wheat and maize chlorophyll a/b binding protein gene leader), or from a synthetic gene sequence. The present invention is not limited to constructs wherein the non-translated region is derived from the 5' non-translated sequence that accompanies the promoter sequence. The leader sequence could also be derived from an unrelated promoter or coding sequence. Leader sequences useful in context of the present invention comprise the maize Hsp70 leader (US 5,362,865 and US 5,859,347), and the TMV omega element.

The termination of transcription is accomplished by a 3' non-translated DNA sequence operably linked in the expression vector to the polynucleotide of interest. The 3' non-translated region of a recombinant DNA molecule contains a

polyadenylation signal that functions in plants to cause the addition of adenylate nucleotides to the 3' end of the RNA. The 3' non-translated region can be obtained from various genes that are expressed in plant cells. The nopaline synthase 3' untranslated region, the 3' untranslated region from pea small subunit Rubisco gene, the 3' untranslated region from soybean 7S seed storage protein gene are commonly used in this capacity. The 3' transcribed, non-translated regions containing the polyadenylate signal of *Agrobacterium* tumor-inducing (Ti) plasmid genes are also suitable.

Recombinant DNA technologies can be used to improve expression of a transformed polynucleotide by manipulating, for example, the efficiency with which the resultant transcripts are translated by codon optimisation according to the host cell species or the deletion of sequences that destabilize transcripts, and the efficiency of post-translational modifications.

Transfer Nucleic Acids

Transfer nucleic acids can be used to deliver an exogenous polynucleotide to a cell and comprise one, preferably two, border sequences and one or more polynucleotides of interest. The transfer nucleic acid may or may not encode a selectable marker. Preferably, the transfer nucleic acid forms part of a binary vector in a bacterium, where the binary vector further comprises elements which allow replication of the vector in the bacterium, selection, or maintenance of bacterial cells containing the binary vector. Upon transfer to a eukaryotic cell, the transfer nucleic acid component of the binary vector is capable of integration into the genome of the eukaryotic cell or, for transient expression experiments, merely of expression in the cell.

As used herein, the term "extrachromosomal transfer nucleic acid" refers to a nucleic acid molecule that is capable of being transferred from a bacterium such as *Agrobacterium sp.*, to a plant cell such as a plant leaf cell. An extrachromosomal transfer nucleic acid is a genetic element that is well-known as an element capable of being transferred, with the subsequent integration of a nucleotide sequence contained within its borders into the genome of the recipient cell. In this respect, a transfer nucleic acid is flanked, typically, by two "border" sequences, although in some instances a single border at one end can be used and the second end of the transferred nucleic acid is generated randomly in the transfer process. A polynucleotide of interest is typically positioned between the left border-like sequence and the right border-like sequence of a transfer nucleic acid. The polynucleotide contained within the transfer nucleic acid may be operably linked to a variety of different promoter and terminator

regulatory elements that facilitate its expression, that is, transcription and/or translation of the polynucleotide. Transfer DNAs (T-DNAs) from *Agrobacterium sp.* such as *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes*, and man made variants/mutants thereof are probably the best characterized examples of transfer nucleic acids. Another example is P-DNA ("plant-DNA") which comprises T-DNA border-like sequences from plants.

As used herein, "T-DNA" refers to a T-DNA of an *Agrobacterium tumefaciens* Ti plasmid or from an *Agrobacterium rhizogenes* Ri plasmid, or variants thereof which function for transfer of DNA into plant cells. The T-DNA may comprise an entire T-DNA including both right and left border sequences, but need only comprise the minimal sequences required in *cis* for transfer, that is, the right T-DNA border sequence. The T-DNAs of the invention have inserted into them, anywhere between the right and left border sequences (if present), the polynucleotide of interest. The sequences encoding factors required in *trans* for transfer of the T-DNA into a plant cell such as vir genes, may be inserted into the T-DNA, or may be present on the same replicon as the T-DNA, or preferably are in *trans* on a compatible replicon in the *Agrobacterium* host. Such "binary vector systems" are well known in the art. As used herein, "P-DNA" refers to a transfer nucleic acid isolated from a plant genome, or man made variants/mutants thereof, and comprises at each end, or at only one end, a T-DNA border-like sequence.

As used herein, a "border" sequence of a transfer nucleic acid can be isolated from a selected organism such as a plant or bacterium, or be a man made variant/mutant thereof. The border sequence promotes and facilitates the transfer of the polynucleotide to which it is linked and may facilitate its integration in the recipient cell genome. In an embodiment, a border-sequence is between 10-80 bp in length. Border sequences from T-DNA from *Agrobacterium sp.* are well known in the art and include those described in Lacroix et al. (2008).

Whilst traditionally only *Agrobacterium sp.* have been used to transfer genes to plants cells, there are now a large number of systems which have been identified/developed which act in a similar manner to *Agrobacterium sp.* Several non-*Agrobacterium* species have recently been genetically modified to be competent for gene transfer (Chung et al., 2006; Broothaerts et al., 2005). These include *Rhizobium* sp. NGR234, *Sinorhizobium meliloti* and *Mezorhizobium loti*.

As used herein, the terms "transfection", "transformation" and variations thereof are generally used interchangeably. "Transfected" or "transformed" cells may have

been manipulated to introduce the polynucleotide(s) of interest, or may be progeny cells derived therefrom.

Plants

The invention also provides a plant or part thereof comprising two or more exogenous polynucleotides and/or genetic modifications as described herein. The term "plant" when used as a noun refers to whole plants, whilst the term "part thereof" refers to plant organs (e.g., leaves, stems, roots, flowers, fruit), single cells (e.g., pollen), seed, seed parts such as an embryo, endosperm, scutellum or seed coat, plant tissue such as vascular tissue, plant cells and progeny of the same. As used herein, plant parts comprise plant cells.

As used herein, the terms “in a plant” and “in the plant” in the context of a modification to the plant means that the modification has occurred in at least one part of the plant, including where the modification has occurred throughout the plant, and does not exclude where the modification occurs in only one or more but not all parts of the plant. For example, a tissue-specific promoter is said to be expressed “in a plant”, even though it might be expressed only in certain parts of the plant. Analogously, “a transcription factor polypeptide that increases the expression of one or more glycolytic and/or fatty acid biosynthetic genes in the plant” means that the increased expression occurs in at least a part of the plant.

As used herein, the term "plant" is used in it broadest sense, including any organism in the Kingdom Plantae. It also includes red and brown algae as well as green algae. It includes, but is not limited to, any species of flowering plant, grass, crop or cereal (e.g., oilseed, maize, soybean), fodder or forage, fruit or vegetable plant, herb plant, woody plant or tree. It is not meant to limit a plant to any particular structure. It also refers to a unicellular plant (e.g., microalga). The term "part thereof" in reference to a plant refers to a plant cell and progeny of same, a plurality of plant cells, a structure that is present at any stage of a plant's development, or a plant tissue. Such structures include, but are not limited to, leaves, stems, flowers, fruits, nuts, roots, seed, seed coat, embryos. The term "plant tissue" includes differentiated and undifferentiated tissues of plants including those present in leaves, stems, flowers, fruits, nuts, roots, seed, for example, embryonic tissue, endosperm, dermal tissue (e.g., epidermis, periderm), vascular tissue (e.g., xylem, phloem), or ground tissue (comprising parenchyma, collenchyma, and/or sclerenchyma cells), as well as cells in culture (e.g., single cells, protoplasts, callus, embryos, etc.). Plant tissue may be *in planta*, in organ culture, tissue culture, or cell culture.

As used herein, the term "vegetative tissue" or "vegetative plant part" is any plant tissue, organ or part other than organs for sexual reproduction of plants. The organs for sexual reproduction of plants are specifically seed bearing organs, flowers, pollen, fruits and seeds. Vegetative tissues and parts include at least plant leaves, stems (including bolts and tillers but excluding the heads), tubers and roots, but excludes flowers, pollen, seed including the seed coat, embryo and endosperm, fruit including mesocarp tissue, seed-bearing pods and seed-bearing heads. In one embodiment, the vegetative part of the plant is an aerial plant part. In another or further embodiment, the vegetative plant part is a green part such as a leaf or stem.

A "transgenic plant" or variations thereof refers to a plant that contains a transgene not found in a wild-type plant of the same species, variety or cultivar. Transgenic plants as defined in the context of the present invention include plants and their progeny which have been genetically modified using recombinant techniques to cause production of at least one polypeptide defined herein in the desired plant or part thereof. Transgenic plant parts has a corresponding meaning. The plant and plant parts of the invention may comprise genetic modifications, for example gene mutations, and be considered as “non-transgenic” provided they lack transgenes.

The terms "seed" and "grain" are used interchangeably herein. "Grain" refers to mature grain such as harvested grain or grain which is still on a plant but ready for harvesting, but can also refer to grain after imbibition or germination, according to the context. Mature grain commonly has a moisture content of less than about 18%. In a preferrd embodiment, the moisture content of the grain is at a level which is generally regarded as safe for storage, preferably between 5% and 15%, between 6% and 8%, between 8% and 10%, or between 10% and 15%. "Developing seed" as used herein refers to a seed prior to maturity, typically found in the reproductive structures of the plant after fertilisation or anthesis, but can also refer to such seeds prior to maturity which are isolated from a plant. Mature seed commonly has a moisture content of less than about 12%.

As used herein, the term "plant storage organ" refers to a part of a plant specialized to store energy in the form of for example, proteins, carbohydrates, lipid. Examples of plant storage organs are seed, fruit, tuberous roots, and tubers. A preferred plant storage organ of the invention is seed.

As used herein, the term "phenotypically normal" refers to a genetically modified plant or part thereof, for example a plant such as a tragsenic plant, or a storage organ such as a seed, tuber or fruit of the invention not having a significantly reduced ability to grow and reproduce when compared to an unmodified plant or part

thereof. Preferably, the biomass, growth rate, germination rate, storage organ size, seed size and/or the number of viable seeds produced is not less than 90% of that of a plant lacking said genetic modifications or exogenous polynucleotides when grown under identical conditions. This term does not encompass features of the plant which may be different to the wild-type plant but which do not effect the usefulness of the plant for commercial purposes such as, for example, a ballerina phenotype of seedling leaves. In an embodiment, the genetically modified plant or part thereof which is phenotypically normal comprises a recombinant polynucleotide encoding a silencing suppressor operably linked to a plant storage organ specific promoter and has an ability to grow or reproduce which is essentially the same as a corresponding plant or part thereof not comprising said polynucleotide.

Plants go through a series of growing stages from sowing of a seed, germination and emergence of a seedling, through to flowering, seed setting, physiological maturity and ultimately senescence. These stages are well known and readily defined, for example for *Sorghum* plants as follows. Taking the day the seedling first emerges above the soil as day 0, the vegetative stage of growth is defined herein as from 10 days to initiation of flowering at about 60-70 days, and physiogical maturity is reached at about 100 days, depending on the environmental conditions. The vegetative stage includes the boot leaf stage from about 45 days until the first flowering. The boot leaf is the last leaf formed on the plant, from which the panicle (head) emerges. The “boot leaf stage” is defined as from when the boot leaf has fully emerged to initiation of flowering.

As used herein, the term "commencement of flowering" or “initiation of flowering” with respect to a plant refers to the time that the first flower on the plant opens, or the time of onset of anthesis.

As used herein, the term "seed set" with respect to a seed-bearing plant refers to the time when the first seed of the plant reaches maturity. This is typically observable by the colour of the seed or its moisture content, well known in the art.

As used herein, the term “mature” as it relates to a plant leaf means that it has reached full size but has not begun to show signs of ageing or death such as yellowing and/or sensensce. The skilled person can readily determine whether a leaf of a particular plant can be considered as mature.

As used herein, the term "senescence” with respect to a whole plant refers to the final stage of plant development which follows the completion of growth, usually after the plant reaches maximum aerial biomass or height. Senescence begins when the plant aerial biomass reaches its maximum and begins to decline in amount and generally

ends with death of most of the plant tissues. It is during this stage that the plant mobilises and recycles cellular components from leaves and other parts which accumulated during growth to other parts to complete its reproductive development. Senescence is a complex, regulated process which involves new or increased gene expression of some genes. Often, some plant parts are senescing while other parts of the same plant continue to grow. Therefore, with respect to a plant leaf or other green organ, the term "senescence" as used herein refers to the time when the amount of chlorophyll in the leaf or organ begins to decrease. Senescence is typically associated with dessication of the leaf or organ, mostly in the last stage of senescence. Senescence is usually observable by the change in colour of the leaf from green towards yellow and eventually to brown when fully dessicated. It is believed that cellular senescence underlies plant and organ senescence.

Plants provided by or contemplated for use in the practice of the present invention include both monocotyledons and dicotyledons. In preferred embodiments, the plants of the present invention are crop plants (for example, cereals and pulses, maize, wheat, potatoes, rice, sorghum, millet, cassava, barley) or legumes such as soybean, beans or peas. The plants may be grown for production of edible roots, tubers, leaves, stems, flowers or fruit. The plants may be vegetable plants whose vegetative parts are used as food. The plants of the invention may be: *Acrocomia aculeata* (macauba palm), *Arabidopsis thaliana*, *Aracinis hypogaea* (peanut), *Astrocaryum murumuru* (murumuru), *Astrocaryum vulgare* (tucumã), *Attalea geraensis* (Indaiá-rateiro), *Attalea humilis* (American oil palm), *Attalea oleifera* (andaiá), *Attalea phalerata* (uricuri), *Attalea speciosa* (babassu), *Avena sativa* (oats), *Beta vulgaris* (sugar beet), *Brassica sp.* such as *Brassica carinata*, *Brassica juncea*, *Brassica napobrassica*, *Brassica napus* (canola), *Camelina sativa* (false flax), *Cannabis sativa* (hemp), *Carthamus tinctorius* (safflower), *Caryocar brasiliense* (pequi), *Cocos nucifera* (Coconut), *Crambe abyssinica* (Abyssinian kale), *Cucumis melo* (melon), *Elaeis guineensis* (African palm), *Glycine max* (soybean), *Gossypium hirsutum* (cotton), *Helianthus sp.* such as *Helianthus annuus* (sunflower), *Hordeum vulgare* (barley), *Jatropha curcas* (physic nut), *Joannesia princeps* (arara nut-tree), *Lemna sp.* (duckweed) such as *Lemna aequinoctialis*, *Lemna disperma*, *Lemna ecuadoriensis*, *Lemna gibba* (swollen duckweed), *Lemna japonica*, *Lemna minor*, *Lemna minuta*, *Lemna obscura*, *Lemna paucicostata*, *Lemna perpusilla*, *Lemna tenera*, *Lemna trisulca*, *Lemna turionifera*, *Lemna valdiviana*, *Lemna yungensis*, *Licania rigida* (oiticica), *Linum usitatissimum* (flax), *Lupinus angustifolius* (lupin), *Mauritia flexuosa* (buriti palm), *Maximiliana maripa* (inaja palm), *Miscanthus sp.* such as *Miscanthus x*

giganteus and *Miscanthus sinensis*, *Nicotiana sp.* (tabacco) such as *Nicotiana tabacum* or *Nicotiana benthamiana*, *Oenocarpus bacaba* (bacaba-do-azeite), *Oenocarpus bataua* (patauã), *Oenocarpus distichus* (bacaba-de-leque), *Oryza sp.* (rice) such as *Oryza sativa* and *Oryza glaberrima*, *Panicum virgatum* (switchgrass), *Paraqueiba paraensis* (mari), *Persea amencana* (avocado), *Pongamia pinnata* (Indian beech), *Populus trichocarpa*, *Ricinus communis* (castor), *Saccharum sp.* (sugarcane), *Sesamum indicum* (sesame), *Solanum tuberosum* (potato), *Sorghum sp.* such as *Sorghum bicolor*, *Sorghum vulgare, Theobroma grandiforum* (cupuassu), *Trifolium sp.*, *Trithrinax brasiliensis* (Brazilian needle palm), *Triticum sp.* (wheat) such as *Triticum aestivum*, *Zea mays* (corn), alfalfa (*Medicago sativa*), rye (*Secale cerale*), sweet potato (*Lopmoea batatus*), cassava (*Manihot esculenta*), coffee (*Cofea* spp.), pineapple (*Anana comosus*), citris tree (*Citrus* spp.), cocoa (*Theobroma cacao*), tea (*Camellia senensis*), banana (*Musa* spp.), avocado (*Persea americana*), fig (*Ficus casica*), guava (*Psidium guajava*), mango (*Mangifer indica*), olive (*Olea europaea*), papaya (*Carica papaya*), cashew (*Anacardium occidentale*), macadamia (*Macadamia intergrifolia*) and almond (*Prunus amygdalus*).

In an embodiment, the plant is not a *Nicotiana sp*.

Other preferred plants include C4 grasses such as, in addition to those mentioned above, *Andropogon gerardi*, *Bouteloua curtipendula*, *B. gracilis*, *Buchloe dactyloides, Schizachyrium scoparium, Sorghastrum nutans*, *Sporobolus cryptandrus*; C3 grasses such as *Elymus canadensis*, the legumes *Lespedeza capitata* and *Petalostemum villosum*, the forb *Aster azureus*; and woody plants such as *Quercus ellipsoidalis* and *Q. macrocarpa*. Other preferred plants include C3 grasses.

In a preferred embodiment, the plant is an angiosperm.

In an embodiment, the plant is an oilseed plant, preferably an oilseed crop plant. As used herein, an "oilseed plant" is a plant species used for the commercial production of lipid from the seeds of the plant. The oilseed plant may be, for example, oil-seed rape (such as canola), maize, sunflower, safflower, soybean, sorghum, flax (linseed) or sugar beet. Furthermore, the oilseed plant may be other *Brassicas*, cotton, peanut, poppy, rutabaga, mustard, castor bean, sesame, safflower, *Jatropha curcas* or nut producing plants. The plant may produce high levels of lipid in its fruit such as olive, oil palm or coconut. Horticultural plants to which the present invention may be applied are lettuce, endive, or vegetable Brassicas including cabbage, broccoli, or cauliflower. The present invention may be applied in tobacco, cucurbits, carrot, strawberry, tomato, or pepper.

In a preferred embodiment, the plant is a member of the family Fabaceae (or Leguminosae) such as alfalfa, clover, peas, lucerne, beans, lentils, lupins, mesquite, carob, soybeans, and peanuts, or a member of the family Poaceae such as corn, sorghum, wheat, barley and oats. In a particularly preferred embodiment, the plant is alfalfa, clover, corn or sorghum, each of which are particularly useful for forage or fodder for animals.

In a preferred embodiment, the transgenic plant is homozygous for each and every gene that has been introduced (transgene) so that its progeny do not segregate for the desired phenotype. The transgenic plant may also be heterozygous for the introduced transgene(s), preferably uniformly heterozygous for the transgene such as for example, in F1 progeny which have been grown from hybrid seed. Such plants may provide advantages such as hybrid vigour, well known in the art.

Transformation of plants

Transgenic plants can be produced using techniques known in the art, such as those generally described in Slater et al., Plant Biotechnology - The Genetic Manipulation of Plants, Oxford University Press (2003), and Christou and Klee, Handbook of Plant Biotechnology, John Wiley and Sons (2004).

As used herein, the terms "stably transforming", "stably transformed" and variations thereof refer to the integration of the polynucleotide into the genome of the cell such that they are transferred to progeny cells during cell division without the need for positively selecting for their presence. Stable transformants, or progeny thereof, can be identified by any means known in the art such as Southern blots on chromosomal DNA, or *in situ* hybridization of genomic DNA, enablimg their selection.

Agrobacterium-mediated transfer is a widely applicable system for introducing genes into plant cells because DNA can be introduced into cells in whole plant tissues, plant organs, or explants in tissue culture, for either transient expression, or for stable integration of the DNA in the plant cell genome. For example, floral-dip (*in planta*) methods may be used. The use of *Agrobacterium*-mediated plant integrating vectors to introduce DNA into plant cells is well known in the art. The region of DNA to be transferred is defined by the border sequences, and the intervening DNA (T-DNA) is usually inserted into the plant genome. It is the method of choice because of the facile and defined nature of the gene transfer.

Acceleration methods that may be used include for example, microprojectile bombardment and the like. One example of a method for delivering transforming nucleic acid molecules to plant cells is microprojectile bombardment. This method has

been reviewed by Yang et al., Particle Bombardment Technology for Gene Transfer, Oxford Press, Oxford, England (1994). Non-biological particles (microprojectiles) that may be coated with nucleic acids and delivered into cells, for example of immature embryos, by a propelling force. Exemplary particles include those comprised of tungsten, gold, platinum, and the like.

In another method, plastids can be stably transformed. Methods disclosed for plastid transformation in higher plants include particle gun delivery of DNA containing a selectable marker and targeting of the DNA to the plastid genome through homologous recombination (US 5,451,513, US 5,545,818, US 5,877,402, US 5,932479, and WO 99/05265). Other methods of cell transformation can also be used and include but are not limited to the introduction of DNA into plants by direct DNA transfer into pollen, by direct injection of DNA into reproductive organs of a plant, or by direct injection of DNA into the cells of immature embryos followed by the rehydration of desiccated embryos.

The regeneration, development, and cultivation of plants from single plant protoplast transformants or from various transformed explants is well known in the art (Weissbach et al., In: Methods for Plant Molecular Biology, Academic Press, San Diego, Calif., (1988)). This regeneration and growth process typically includes the steps of selection of transformed cells, culturing those individualized cells through the usual stages of embryonic development through the rooted plantlet stage. Transgenic embryos and seeds are similarly regenerated. The resulting transgenic rooted shoots are thereafter planted in an appropriate plant growth medium such as soil.

The development or regeneration of plants containing the foreign, exogenous gene is well known in the art. Preferably, the regenerated plants are self-pollinated to provide homozygous transgenic plants. Otherwise, pollen obtained from the regenerated plants is crossed to seed-grown plants of agronomically important lines. Conversely, pollen from plants of these important lines is used to pollinate regenerated plants. A transgenic plant of the present invention containing a desired polynucleotide is cultivated using methods well known to one skilled in the art.

To confirm the presence of the transgenes in transgenic cells and plants, a polymerase chain reaction (PCR) amplification or Southern blot analysis can be performed using methods known to those skilled in the art. Expression products of the transgenes can be detected in any of a variety of ways, depending upon the nature of the product, and include Northern blot hybridisation, Western blot and enzyme assay. Once transgenic plants have been obtained, they may be grown to produce plant tissues or parts having the desired phenotype. The plant tissue or plant parts, may be harvested,

and/or the seed collected. The seed may serve as a source for growing additional plants with tissues or parts having the desired characteristics. Preferably, the vegetative plant parts are harvested at a time when the yield of non-polar lipids are at their highest. In one embodiment, the vegetative plant parts are harvested about at the time of flowering, or after flowering has initiated. Preferably, the plant parts are harvested at about the time senescence begins, usually indicated by yellowing and drying of leaves.

Transgenic plants formed using *Agrobacterium* or other transformation methods typically contain a single genetic locus on one chromosome. Such transgenic plants can be referred to as being hemizygous for the added gene(s). More preferred is a transgenic plant that is homozygous for the added gene(s), that is, a transgenic plant that contains two added genes, one gene at the same locus on each chromosome of a chromosome pair. A homozygous transgenic plant can be obtained by self-fertilising a hemizygous transgenic plant, germinating some of the seed produced and analyzing the resulting plants for the gene of interest.

It is also to be understood that two different transgenic plants that contain two independently segregating exogenous genes or loci can also be crossed (mated) to produce offspring that contain both sets of genes or loci. Selfing of appropriate F1 progeny can produce plants that are homozygous for both of the exogenous genes or loci. Back-crossing to a parental plant and out-crossing with a non-transgenic plant are also contemplated, as is vegetative propagation. Similarly, a transgenic plant can be crossed with a second plant comprising a genetic modification such as a mutant gene and progeny containing both of the transgene and the genetic modification identified. Descriptions of other breeding methods that are commonly used for different traits and crops can be found in Fehr, In: Breeding Methods for Cultivar Development, Wilcox J. ed., American Society of Agronomy, Madison Wis. (1987).

TILLING

In one embodiment, TILLING (Targeting Induced Local Lesions IN Genomes) can be used to produce plants in which endogenous genes comprise a mutation, for example genes encoding an SDP1 or TGD polypeptide, TST, a plastidial GPAT, plastidial LPAAT, phosphatidic acid phosphatase (PAP), or a combination of two or more thereof. In a first step, introduced mutations such as novel single base pair changes are induced in a population of plants by treating seeds (or pollen) with a chemical mutagen, and then advancing plants to a generation where mutations will be stably inherited. DNA is extracted, and seeds are stored from all members of the population to create a resource that can be accessed repeatedly over time. For a

TILLING assay, heteroduplex methods using specific endonucleases can be used to detect single nucleotide polymorphisms (SNPs). Alternatively, Next Generation Sequencing of DNA from pools of mutagenised plants can be used to identify mutants in the gene of choice. Typically, a mutation frequency of one mutant per 1000 plants in the mutagenised population is achieved. Using this approach, many thousands of plants can be screened to identify any individual with a single base change as well as small insertions or deletions (1-30 bp) in any gene or specific region of the genome. TILLING is further described in Slade and Knauf (2005), and Henikoff et al. (2004).

In addition to allowing efficient detection of mutations, high-throughput TILLING technology is ideal for the detection of natural polymorphisms. Therefore, interrogating an unknown homologous DNA by heteroduplexing to a known sequence reveals the number and position of polymorphic sites. Both nucleotide changes and small insertions and deletions are identified, including at least some repeat number polymorphisms. This has been called Ecotilling (Comai et al., 2004).

Genome editing using site-specific nucleases

Genome editing uses engineered nucleases such as RNA guided DNA endonucleases or nucleases composed of sequence specific DNA binding domains fused to a non-specific DNA cleavage module. These engineered nucleases enable efficient and precise genetic modifications by inducing targeted DNA double stranded breaks that stimulate the cell's endogenous cellular DNA repair mechanisms to repair the induced break. Such mechanisms include, for example, error prone non-homologous end joining (NHEJ) and homology directed repair (HDR).

In the presence of donor plasmid with extended homology arms, HDR can lead to the introduction of single or multiple transgenes to correct or replace existing genes. In the absence of donor plasmid, NHEJ-mediated repair yields small insertion or deletion mutations of the target that cause gene disruption.

Engineered nucleases useful in the methods of the present invention include zinc finger nucleases (ZFNs), transcription activator-like (TAL) effector nucleases (TALEN) and CRISPR/Cas9 type nucleases, and related nucleases.

Typically nuclease encoded genes are delivered into cells by plasmid DNA, viral vectors or *in vitro* transcribed mRNA.

A zinc finger nuclease (ZFN) comprises a DNA-binding domain and a DNA-cleavage domain, wherein the DNA binding domain is comprised of at least one zinc finger and is operatively linked to a DNA-cleavage domain. The zinc finger DNA-

binding domain is at the N-terminus of the protein and the DNA-cleavage domain is located at the C-terminus of said protein.

A ZFN must have at least one zinc finger. In a preferred embodiment, a ZFN would have at least three zinc fingers in order to have sufficient specificity to be useful for targeted genetic recombination in a host cell or organism. Typically, a ZFN having more than three zinc fingers would have progressively greater specificity with each additional zinc finger.

The zinc finger domain can be derived from any class or type of zinc finger. In a particular embodiment, the zinc finger domain comprises the Cis_2His_2 type of zinc finger that is very generally represented, for example, by the zinc finger transcription factors TFIIIA or Sp1. In a preferred embodiment, the zinc finger domain comprises three Cis_2His_2 type zinc fingers. The DNA recognition and/or the binding specificity of a ZFN can be altered in order to accomplish targeted genetic recombination at any chosen site in cellular DNA. Such modification can be accomplished using known molecular biology and/or chemical synthesis techniques. (see, for example, Bibikova et al., 2002).

The ZFN DNA-cleavage domain is derived from a class of non-specific DNA cleavage domains, for example the DNA-cleavage domain of a Type II restriction enzyme such as FokI (Kim et al., 1996). Other useful endonucleases may include, for example, *Hha*I, *Hin*dIII, Nod, *Bbv*CI, *Eco*RI, *Bgl*I, and *Alw*I.

A transcription activator-like (TAL) effector nuclease (TALEN) comprises a TAL effector DNA binding domain and an endonuclease domain.

TAL effectors are proteins of plant pathogenic bacteria that are injected by the pathogen into the plant cell, where they travel to the nucleus and function as transcription factors to turn on specific plant genes. The primary amino acid sequence of a TAL effector dictates the nucleotide sequence to which it binds. Thus, target sites can be predicted for TAL effectors, and TAL effectors can be engineered and generated for the purpose of binding to particular nucleotide sequences.

Fused to the TAL effector-encoding nucleic acid sequences are sequences encoding a nuclease or a portion of a nuclease, typically a nonspecific cleavage domain from a type II restriction endonuclease such as *Fok*I (Kim et al., 1996). Other useful endonucleases may include, for example, *Hha*I, *Hin*dIII, *Nod*, *Bbv*CI, *Eco*RI, *Bgl*I, and *Alw*I. The fact that some endonucleases (e.g., *Fok*I) only function as dimers can be capitalized upon to enhance the target specificity of the TAL effector. For example, in some cases each *Fok*I monomer can be fused to a TAL effector sequence that recognizes a different DNA target sequence, and only when the two recognition sites

are in close proximity do the inactive monomers come together to create a functional enzyme. By requiring DNA binding to activate the nuclease, a highly site-specific restriction enzyme can be created.

A sequence-specific TALEN can recognize a particular sequence within a preselected target nucleotide sequence present in a cell. Thus, in some embodiments, a target nucleotide sequence can be scanned for nuclease recognition sites, and a particular nuclease can be selected based on the target sequence. In other cases, a TALEN can be engineered to target a particular cellular sequence.

Genome editing using programmable RNA-guided DNA endonucleases

Distinct from the site-specific nucleases described above, the clustered regulatory interspaced short palindromic repeats (CRISPR)/Cas system provides an alternative to ZFNs and TALENs for inducing targeted genetic alterations, via RNA-guided DNA cleavage.

CRISPR systems rely on CRISPR RNA (crRNA) and transactivating chimeric RNA (tracrRNA) for sequence-specific cleavage of DNA. Three types of CRISPR/Cas systems exist: in type II systems, Cas9 serves as an RNA-guided DNA endonuclease that cleaves DNA upon crRNA–tracrRNA target recognition. CRISPR RNA base pairs with tracrRNA to form a two-RNA structure that guides the Cas9 endonuclease to complementary DNA sites for cleavage.

The CRISPR system can be portable to plant cells by co-delivery of plasmids expressing the Cas endonuclease and the necessary crRNA components. The Cas endonuclease may be converted into a nickase to provide additional control over the mechanism of DNA repair (Cong et al., 2013).

CRISPRs are typically short partially palindromic sequences of 24-40bp containing inner and terminal inverted repeats of up to 11 bp. Although isolated elements have been detected, they are generally arranged in clusters (up to about 20 or more per genome) of repeated units spaced by unique intervening 20-58bp sequences. CRISPRs are generally homogenous within a given genome with most of them being identical. However, there are examples of heterogeneity in, for example, the Archaea (Mojica et al., 2000).

Feedstuffs

The present invention includes compositions which can be used as feedstuffs. For purposes of the present invention, "feedstuffs" include any food or preparation for animal (including human) consumption and which serves to nourish or build up tissues

or supply energy, and/or to maintain, restore or support adequate nutritional status or metabolic function. Feedstuffs of the invention include nutritional compositions for babies and/or young children.

As used herein, the term “animal” refers to any eukaryotic organism capable of ingesting plant derived material. In an embodiment, the animal is a ruminant animal (cattle, sheep, goats etc). Alternatively, the animal is a non-ruminant animal. In one embodiment, the animal is a mammal. In an embodiment, the animal is a human. In an embodiment, the animal is a livestock animal such, but not limited to, as cattle, goats, sheep, pigs, horses, poultry such as chickens and the like. In an embodiment, the cattle are diary cattle or beef cattle. In another embodiment, the animal is a fish, for instance fish bred using aquaculture including, but not limited to, salmon, trout, carp, bass, bream, turbot, sole, milkfish, grey mullet, grouper, flounder, sea bass, cod, haddock, Japanese flounder, catfish, char, whitefish, sturgeon, tench, roach, pike, pike-perch, yellowtail, tilapia, eel or tropical fish (such as the fresh, brackish, and salt water tropical fish). The animal may be a crustacean such as, but not limited to, krill, clams, shrimp (including prawns), crab, and lobster.

Feedstuffs of the invention may comprise for example, a plant or part thereof such as a vegetative plant part of the invention along with a suitable carrier(s). The term "carrier" is used in its broadest sense to encompass any component which may or may not have nutritional value. As the person skilled in the art will appreciate, the carrier must be suitable for use (or used in a sufficiently low concentration) in a feedstuff, such that it does not have deleterious effect on an organism which consumes the feedstuff. Feedstuffs may comprise plant parts which have been harvested and subsequently processed or treated, for example, by chopping, cutting, drying, pressing or pelleting the plant parts, into a form that is suitable for consumption by the animal, or altered by processes such as drying or fermentation to produce hay or silage.

The feedstuff of the present invention comprises a lipid and/or protein produced directly or indirectly by use of the methods, plants or parts thereof disclosed herein. The composition may either be in a solid or liquid form. Additionally, the composition may include edible macronutrients, vitamins, and/or minerals in amounts desired for a particular use. The amounts of these ingredients will vary depending on whether the composition is intended for use with normal individuals or for use with individuals having specialized needs such as individuals suffering from metabolic disorders and the like.

Examples of suitable carriers with nutritional value include, but are not limited to, macronutrients such as edible fats, carbohydrates and proteins. Examples of such

edible fats include, but are not limited to, coconut oil, borage oil, fungal oil, black current oil, soy oil, and mono- and di-glycerides. Examples of such carbohydrates include, but are not limited to, glucose, edible lactose, and hydrolyzed starch. Additionally, examples of proteins which may be utilized in the nutritional composition of the invention include, but are not limited to, soy proteins, electrodialysed whey, electrodialysed skim milk, milk whey, or the hydrolysates of these proteins.

With respect to vitamins and minerals, the following may be added to the feedstuff compositions of the present invention, calcium, phosphorus, potassium, sodium, chloride, magnesium, manganese, iron, copper, zinc, selenium, iodine, and vitamins A, E, D, C, and the B complex. Other such vitamins and minerals may also be added.

A feedstuff composition of the present invention may also be added to food even when supplementation of the diet is not required. For example, the composition may be added to food of any type, including, but not limited to, margarine, butter, cheeses, milk, yogurt, chocolate, candy, snacks, salad oils, cooking oils, cooking fats, meats, fish and beverages.

Additionally, material produced in accordance with the present invention may also be used as animal food supplements to alter an animal's tissue or milk fatty acid composition to one more desirable for human or animal consumption, or to reduce methane production in ruminant animals. Furthermore, feedstuffs of the invention can be used in aquaculture to increase the levels of fatty acids and nutrition in fish for human or animal consumption.

Preferred feedstuffs of the invention are the plants, seed and other plant parts such as leaves, fruits and stems which may be used directly as food or feed for humans or other animals. For example, animals may graze directly on such plants grown in the field, or be fed more measured amounts in controlled feeding. The invention includes the use of such plants and plant parts as feed for increasing the polyunsaturated fatty acid levels in humans and other animals.

For consumption by non-human animals the feedstuff may be in any suitable form for such as, but not limited to, silage, hay or pasture growing in a field. In an embodiment, the feedstuff for non-human consumption is a leguminous plant, or part thereof, which is a member of the family Fabaceae family (or Leguminosae) such as alfalfa, clover, peas, lucerne, beans, lentils, lupins, mesquite, carob, soybeans, and peanuts.

In embodiment, the animal is in a feedlot and/or a shed.

In an embodiment, the plant or fraction thereof comprises at least about 5%, at least about 10%, at least about 50%, at least about 75%, at least about 90% or all of the feedstuff.

Silage

As used herein, "silage" is a relatively high-moisture fodder which has been produced and stored in a process called ensilage and which is typically fed to cattle, sheep or other ruminants. During the storage time, carbohydrates, lipids and proteins in the plant material ferment, producing organic acids, or are broken down oxidatively, or both. The plant material upon harvest and the post-fermentation plant materials are both included in silage as the term is used herein. Silage is typically made from grass crops such as maize, sorghum, oats or other cereals, or from mixed pasture grasses and legumes such as alfalfa or clover, using the green, above-ground parts of the plants. Silage is made either by placing cut vegetation (usually the whole above-ground plant biomass which can include reproductive tissues) in a pit or silo or other means for storage, and compressing it down so as to leave as little air as possible with the plant material. Oxygen is excluded to some extent by covering it with a plastic sheet or by wrapping the plant material tightly within plastic film (baling) to reduce air inflow. Silage is made from plant material with a suitable moisture content, generally about 50% to 60% of the fresh weight, depending on the means of storage and the degree of compression used and the amount of water that will be lost in storage, but not exceeding 75%. For sorghum and corn, harvest begins when the whole-plant moisture is at a suitable level, ideally a few days before it is ripe. For pasture-type crops, the plants are mowed and allowed to wilt for a day or so until the moisture content drops to a suitable level. Ideally the crop is mowed when in full flower and deposited in the pit or silo on the day of its cutting. At harvesting, or after, the plant material is shredded or chopped by the harvester into pieces typically about 1-5 cm long. The plant material may be placed in large heaps on the ground and compressed to reduce the amount of air, then covered with plastic, or into a silo. Alternatively, the plant material may be baled in plastic wrapping to exclude air, which typically requires a lower moisture content of about 30-40%, but still too damp to be stored as dry hay.

The cut or chopped, stored plant material undergoes mostly anaerobic fermentation, which starts about 48 hours after the pit or silo is filled. The fermentation process converts sugars and other carbohydrates such as hemicellulose to organic acids, mostly acetic, propionic, lactic and butyric acids. Fermentation starts after the trapped oxygen is consumed and is essentially complete after about two weeks of storage, or

may continue for longer periods. When the plant material is closely packed, the supply of oxygen is limited and the fermentation results in the decomposition of the carbohydrates, some lipids and proteins in the material into the organic acids. This product is named sour silage. If, on the other hand, the fodder is more loosely packed, the main reaction is oxidation which proceeds more rapidly and the temperature rises. If the mass is compressed when the temperature is 60-75C, the reaction ceases and sweet silage results. Fermentation may be aided by inoculation with specific microorganisms such as lactic acid bacteria to speed fermentation or improve the resulting silage, e.g. with *Lactobacillus plantarum*.

Bulk silage is commonly fed to dairy cattle, while baled silage tends to be used for beef cattle, sheep and horses. The advantages of silage as animal feed are several. During fermentation, the silage bacteria act on the cellulose and other carbohydrates in the forage to produce the organic fatty acids, thereby lowering the pH. This inhibits competing bacteria that might cause spoilage and the organic acids thereby act as natural preservatives, improve digestibility and palatability. This preservative action is particularly important during winter in temperate regions, when green forage is unavailable.

Silage can be produced using techniques known in the art such as those described in CN 101940272 CN 103461658 CN 101946853, CN 101946853, CN 104381743, US3875304 and US 6224916. Pellets for animal feed can be produced using techniques known in the art such as those described in US 3035920, US3573924 and US 5871802.

Plant Biomass

An increase in the total lipid content of plant biomass equates to greater energy content, making its use as a feed or forage or in the production of biofuel more economical.

The main components of naturally occurring plant biomass are carbohydrates (approximately 75%, dry weight) and lignin (approximately 25%), which can vary with plant type. The carbohydrates are mainly cellulose or hemicellulose fibers, which impart strength to the plant structure, and lignin, which holds the fibers together. Plant biomass typically has a low energy density as a result of both its physical form and moisture content. This also makes it inconvenient and inefficient for storage and transport without some kind of pre-processing. There are a range of processes available to convert it into a more convenient form including: 1) physical pre-processing (for example, grinding) or 2) conversion by thermal (for example, combustion, gasification,

pyrolysis) or chemical (for example, anaerobic digestion, fermentation, composting, transesterification) processes. In this way, the biomass is converted into what can be described as a biomass fuel.

Combustion

Combustion is the process by which flammable materials are allowed to burn in the presence of air or oxygen with the release of heat. The basic process is oxidation. Combustion is the simplest method by which biomass can be used for energy, and has been used to provide heat. This heat can itself be used in a number of ways: 1) space heating, 2) water (or other fluid) heating for central or district heating or process heat, 3) steam raising for electricity generation or motive force. When the flammable fuel material is a form of biomass the oxidation is of predominantly the carbon (C) and hydrogen (H) in the cellulose, hemicellulose, lignin, and other molecules present to form carbon dioxide (CO_2) and water (H_2O). The plants of the invention provide improved fuel for combustion by virtue of the increased lipid content.

Gasification

Gasification is a partial oxidation process whereby a carbon source such as plant biomass, is broken down into carbon monoxide (CO) and hydrogen (H_2), plus carbon dioxide (CO_2) and possibly hydrocarbon molecules such as methane (CH_4). If the gasification takes place at a relatively low temperature, such as 700ºC to 1000ºC, the product gas will have a relatively high level of hydrocarbons compared to high temperature gasification. As a result it may be used directly, to be burned for heat or electricity generation via a steam turbine or, with suitable gas clean up, to run an internal combustion engine for electricity generation. The combustion chamber for a simple boiler may be close coupled with the gasifier, or the producer gas may be cleaned of longer chain hydrocarbons (tars), transported, stored and burned remotely. A gasification system may be closely integrated with a combined cycle gas turbine for electricity generation (IGCC - integrated gasification combined cycle). Higher temperature gasification (1200ºC to 1600ºC) leads to few hydrocarbons in the product gas, and a higher proportion of CO and H_2. This is known as synthesis gas (syngas or biosyngas) as it can be used to synthesize longer chain hydrocarbons using techniques such as Fischer-Tropsch (FT) synthesis. If the ratio of H_2 to CO is correct (2:1) FT synthesis can be used to convert syngas into high quality synthetic diesel biofuel which is compatible with conventional fossil diesel and diesel engines.

Pyrolysis

As used herein, the term "pyrolysis" means a process that uses slow heating in the absence of oxygen to produce gaseous, oil and char products from biomass. Pyrolysis is a thermal or thermo-chemical conversion of lipid-based, particularly triglyceride-based, materials. The products of pyrolysis include gas, liquid and a sold char, with the proportions of each depending upon the parameters of the process. Lower temperatures (around 400ºC) tend to produce more solid char (slow pyrolysis), whereas somewhat higher temperatures (around 500ºC) produce a much higher proportion of liquid (bio-oil), provided the vapour residence time is kept down to around 1s or less. Temperatures of about 275ºC to about 375ºC can be used to produce liquid bio-oil having a higher proportion of longer chain hydrocarbons. Pyrolysis involves direct thermal cracking of the lipids or a combination of thermal and catalytic cracking. At temperatures of about 400-500ºC, cracking occurs, producing short chain hydrocarbons such as alkanes, alkenes, alkadienes, aromatics, olefins and carboxylic acid, as well as carbon monoxide and carbon dioxide.

Four main catalyst types can be used including transition metal catalysts, molecular sieve type catalysts, activated alumina and sodium carbonate (Maher and Bressler, 2007). Examples are given in US 4102938. Alumina (Al_2O_3) activated by acid is an effective catalyst (US 5233109). Molecular sieve catalysts are porous, highly crystalline structures that exhibit size selectivity, so that molecules of only certain sizes can pass through. These include zeolite catalysts such as ZSM-5 or HZSM-5 which are crystalline materials comprising AlO_4 and SiO_4 and other silica-alumina catalysts. The activity and selectivity of these catalysts depends on the acidity, pore size and pore shape, and typically operate at 300-500ºC. Transition metal catalysts are described for example in US 4992605. Sodium carbonate catalyst has been used in the pyrolysis of oils (Dandik and Aksoy, 1998).

As used herein, “hydrothermal processing”, “HTP”, also referred to as “thermal depolymerisation” is a form of pyrolysis which reacts the plant-derived matter, specifically the carbon-containing material in the plant-derived matter, with hydrogen to produce a bio-oil product comprised predominantly of paraffinic hydrocarbons along with other gases and solids. A significant advantage of HTP is that the vegetative plant material does not need to be dried before forming the composition for the conversion reaction, although the vegetative plant material can be dried beforehand to aid in transport or storage of the biomass. The biomass can be used directly as harvested from the field. The reactor is any vessel which can withstand the high temperature and pressure used and is resistant to corrosion. The solvent used in the HTP includes water

or is entirely water, or may include some hydrocarbon compounds in the form of an oil. Generally, the solvent in HTP lacks added alcohols. The conversion reaction may occur in an oxidative, reductive or inert environment. “Oxidative” as used herein means in the presence of air, “reductive” means in the presence of a reducing agent, typically hydrogen gas or methane, for example 10-15% H_2 with the remainder of the gas being N_2, and “inert” means in the presence of an inert gas such as nitrogen or argon. The conversion reaction is preferably carried out under reductive conditions. The carbon-containing materials that are converted include cellulose, hemi-cellulose, lignin and proteins as well as lipids. The process uses a conversion temperature of between 270ºC and 400ºC and a pressure of between 70 and 350 bar, typically 300ºC to 350ºC and a pressure between 100-170bar. As a result of the process, organic vapours, pyrolysis gases and charcoal are produced. The organic vapours are condensed to produce the bio-oil. Recovery of the bio-oil may be achieved by cooling the reactor and reducing the pressure to atmospheric pressure, which allows bio-oil (organic) and water phases to develop and the bio-oil to be removed from the reactor.

The yield of the recovered bio-oil is calculated as a percentage of the dry weight of the input biomass on a dry weight basis. It is calculated according to the formula: weight of bio-oil x 100/dry weight of the vegetative plant parts. The weight of the bio-oil does not include the weight of any water or solids which may be present in a bio-oil mixture, which are readily removed by filtration or other known methods.

The bio-oil may then be separated into fractions by fractional distillation, with or without additional refining processes. Typically, the fractions that condense at these temperatures are termed: about 370ºC, fuel oil; about 300ºC, diesel oil; about 200ºC, kerosene; about 150ºC, gasoline (petrol). Heavier fractions may be cracked into lighter, more desirable fractions, well known in the art. Diesel fuel typically is comprised of C13-C22 hydrocarbon compounds.

Transesterification

"Transesterification" as used herein is the conversion of lipids, principally triacylglycerols, into fatty acid methyl esters or ethyl esters by reaction with short chain alcohols such as methanol or ethanol, in the presence of a catalyst such as alkali or acid. Methanol is used more commonly due to low cost and availability, but ethanol, propanol or butanol or mixtures of the alcohols can also be used. The catalysts may be homogeneous catalysts, heterogeneous catalysts or enzymatic catalysts. Homogeneous catalysts include ferric sulphate followed by KOH. Heterogeneous catalysts include

CaO, K_3PO_4, and WO_3/ZrO_2. Enzymatic catalysts include Novozyme 435 produced from *Candida antarctica*.

Transesterification can be carried out on extracted oil, or preferably directly *in situ* in the vegetative plant material. The vegetative plant parts may be dried and milled prior to being used to prepare the composition for the conversion reaction, but does not need to be. The advantage of direct conversion to fatty acid esters, preferably FAME, is that the conversion can use lower temperatures and pressures and still provide good yields of the product, for example, comprising at least 50% FAME by weight. The yield of recovered bio-oil by transesterification is calculated as for the HTP process.

Production of Non-Polar Lipids

Techniques that are routinely practiced in the art can be used to extract, process, purify and analyze the lipids such as the TAG produced by plants or parts thereof of the instant invention. Such techniques are described and explained throughout the literature in sources such as, Fereidoon Shahidi, Current Protocols in Food Analytical Chemistry, John Wiley & Sons, Inc. (2001) D1.1.1-D1.1.11, and Perez-Vich et al. (1998).

Production of oil from vegetative plant parts or seed

Typically, vegetative plant parts or plant seeds are cooked, pressed, and/or extracted to produce crude vegetative oil or seedoil, which is then degummed, refined, bleached, and deodorized. Generally, techniques for crushing seed are known in the art. For example, oilseeds can be tempered by spraying them with water to raise the moisture content to, for example, 8.5%, and flaked using a smooth roller with a gap setting of 0.23 to 0.27 mm. Depending on the type of seed, water may not be added prior to crushing. Application of heat deactivates enzymes, facilitates further cell rupturing, coalesces the lipid droplets, and agglomerates protein particles, all of which facilitate the extraction process. Vegetative plant parts can be similarly treated, depending on the moisture content.

In an embodiment, the majority of the vegetative oil or seedoil is released by passage through a screw press. Cakes (vegetative plant meal, seedmeal) expelled from the screw press may then be solvent extracted for example, with hexane, using a heat traced column, or not be solvent treated, in which case it may be more suitable as animal feed. Alternatively, crude vegetative oil or seedoil produced by the pressing operation can be passed through a settling tank with a slotted wire drainage top to remove the solids that are expressed with the vegetative oil or seedoil during the

pressing operation. The clarified vegetative oil or seedoil can be passed through a plate and frame filter to remove any remaining fine solid particles. Once the solvent is stripped from the crude oil, the pressed and extracted portions are combined and subjected to normal lipid processing procedures (i.e., degumming, caustic refining, bleaching, and deodorization).

Extraction of the lipid from vegetative plant parts of the invention uses analogous methods to those known in the art for seedoil extraction. One way is physical extraction, which often does not use solvent extraction. Expeller pressed extraction is a common type, as are the screw press and ram press extraction methods. Mechanical extraction is typically less efficient than solvent extraction where an organic solvent (e.g., hexane) is mixed with at least the plant biomass, preferably after the biomass is dried and ground. The solvent dissolves the lipid in the biomass, which solution is then separated from the biomass by mechanical action (e.g., with the pressing processes above). This separation step can also be performed by filtration (e.g., with a filter press or similar device) or centrifugation etc. The organic solvent can then be separated from the non-polar lipid (e.g., by distillation). This second separation step yields non-polar lipid from the plant and can yield a re-usable solvent if one employs conventional vapor recovery. In an embodiment, the oil and/or protein content of the plant part or seed is analysed by near-infrared reflectance spectroscopy as described in Hom et al. (2007) prior to extraction.

If the vegetative plant parts are not to be used immediately to extract the lipid it is preferably processed to ensure the lipid content is retained as much as possible (see, for example, Christie, 1993), such as by drying the vegetative plant parts.

Degumming

Degumming is an early step in the refining of oils and its primary purpose is the removal of most of the phospholipids from the oil, which may be present as approximately 1-2% of the total extracted lipid. Addition of ~2% of water, typically containing phosphoric acid, at 70–80°C to the crude oil results in the separation of most of the phospholipids accompanied by trace metals and pigments. The insoluble material that is removed is mainly a mixture of phospholipids and triacylglycerols and is also known as lecithin. Degumming can be performed by addition of concentrated phosphoric acid to the crude oil to convert non-hydratable phosphatides to a hydratable form, and to chelate minor metals that are present. Gum is separated from the oil by centrifugation. The oil can be refined by addition of a sufficient amount of a sodium hydroxide solution to titrate all of the fatty acids and removing the soaps thus formed.

Alkali refining

Alkali refining is one of the refining processes for treating crude oil, sometimes also referred to as neutralization. It usually follows degumming and precedes bleaching. Following degumming, the oil can treated by the addition of a sufficient amount of an alkali solution to titrate all of the fatty acids and phosphoric acids, and removing the soaps thus formed. Suitable alkaline materials include sodium hydroxide, potassium hydroxide, sodium carbonate, lithium hydroxide, calcium hydroxide, calcium carbonate and ammonium hydroxide. This process is typically carried out at room temperature and removes the free fatty acid fraction. Soap is removed by centrifugation or by extraction into a solvent for the soap, and the neutralised oil is washed with water. If required, any excess alkali in the oil may be neutralized with a suitable acid such as hydrochloric acid or sulphuric acid.

Bleaching

Bleaching is a refining process in which oils are heated at 90–120°C for 10–30 minutes in the presence of a bleaching earth (0.2–2.0%) and in the absence of oxygen by operating with nitrogen or steam or in a vacuum. This step in oil processing is designed to remove unwanted pigments (carotenoids, chlorophyll, gossypol etc), and the process also removes oxidation products, trace metals, sulphur compounds and traces of soap.

Deodorization

Deodorization is a treatment of oils and fats at a high temperature (200–260°C) and low pressure (0.1–1 mm Hg). This is typically achieved by introducing steam into the oil at a rate of about 0.1 ml/minute/100 ml of oil. Deodorization can be performed by heating the oil to 260ºC under vacuum, and slowly introducing steam into the oil at a rate of about 0.1 ml/minute/100 ml of oil. After about 30 minutes of sparging, the oil is allowed to cool under vacuum. The oil is typically transferred to a glass container and flushed with argon before being stored under refrigeration. If the amount of oil is limited, the oil can be placed under vacuum for example, in a Parr reactor and heated to 260ºC for the same length of time that it would have been deodorized. This treatment improves the colour of the oil and removes a majority of the volatile substances or odorous compounds including any remaining free fatty acids, monoacylglycerols and oxidation products.

Winterisation

Winterization is a process sometimes used in commercial production of oils for the separation of oils and fats into solid (stearin) and liquid (olein) fractions by crystallization at sub-ambient temperatures. It was applied originally to cottonseed oil to produce a solid-free product. It is typically used to decrease the saturated fatty acid content of oils.

Algae

Algae can produce 10 to 100 times as much mass as terrestrial plants in a year and can be cultured in open-ponds (such as raceway-type ponds and lakes) or in photobioreactors. The most common oil-producing algae can generally include the diatoms (bacillariophytes), green algae (chlorophytes), blue-green algae (cyanophytes), and golden-brown algae (chrysophytes). In addition a fifth group known as haptophytes may be used. Groups include brown algae and heterokonts. Specific non-limiting examples algae include the Classes: Chlorophyceae, Eustigmatophyceae, Prymnesiophyceae, Bacillariophyceae. Bacillariophytes capable of oil production include the genera Amphipleura, Amphora, Chaetoceros, Cyclotella, Cymbella, Fragilaria, Hantzschia, Navicula, Nitzschia, Phaeodactylum, and Thalassiosira. Specific non-limiting examples of chlorophytes capable of oil production include Ankistrodesmus, Botryococcus, Chlorella, Chlorococcum, Dunaliella, Monoraphidium, Oocystis, Scenedesmus, and Tetraselmis. In one aspect, the chlorophytes can be Chlorella or Dunaliella. Specific non-limiting examples of cyanophytes capable of oil production include Oscillatoria and Synechococcus. A specific example of chrysophytes capable of oil production includes Boekelovia. Specific non-limiting examples of haptophytes include Isochysis and Pleurochysis.

Specific algae useful in the present invention include, for example, *Chlamydomonas sp*. such as *Chlamydomonas reinhardtii*, *Dunaliella sp.* such as *Dunaliella salina, Dunaliella tertiolecta*, *D. acidophila, D. Lateralis. D.martima. D. parva, D. polmorpha, D. primolecta, D. pseudosalina, D. quartolecta. D. viridis, Haematococcus sp., Chlorella sp.* such as *Chlorella vulgaris, Chlorella sorokiniana* or *Chlorella protothecoides, Thraustochytrium sp., Schizochytrium sp., Volvox sp, Nannochloropsis sp., Botryococcus braunii* which can contain over 60wt% lipid, *Phaeodactylum tricornutum, Thalassiosira pseudonana, Isochrysis sp., Pavlova sp., Chlorococcum sp, Ellipsoidion sp., Neochloris sp., Scenedesmus sp*.

Algae of the invention can be harvested using microscreens, by centrifugation, by flocculation (using for example, chitosan, alum and ferric chloride) and by froth

flotation. Interrupting the carbon dioxide supply can cause algae to flocculate on its own, which is called "autoflocculation". In froth flotation, the cultivator aerates the water into a froth, and then skims the algae from the top. Ultrasound and other harvesting methods are currently under development.

Lipid may be extracted from the algae by mechanical crushing. When algal mass is dried it retains its lipid content, which can then be "pressed" out with an oil press. Osmotic shock may also be used to release cellular components such as lipid from algae, and ultrasonic extraction can accelerate extraction processes. Chemical solvents (for example, hexane, benzene, petroleum ether) are often used in the extraction of lipids from algae. Enzymatic extraction using enzymes to degrade the cell walls may also be used to extract lipids from algae. Supercritical CO_2 can also be used as a solvent. In this method, CO_2 is liquefied under pressure and heated to the point that it becomes supercritical (having properties of both a liquid and a gas), allowing it to act as a solvent.

Uses of Plant Lipids

The lipids produced by the methods described have a variety of uses. In some embodiments, the lipids are used as food oils. In other embodiments, the lipids are refined and used as lubricants or for other industrial uses such as the synthesis of plastics. In some preferred embodiments, the lipids are refined to produce biodiesel. Biodiesel can be made from oils derived from the plants, algae and fungi of the invention. Use of plant triacylglycerols for the production of biofuel is reviewed in Durrett et al. (2008). The resulting fuel is commonly referred to as biodiesel and has a dynamic viscosity range from 1.9 to 6.0 mm^2s^{-1} (ASTM D6751). Bioalcohol may produced from the fermentation of sugars or the biomass other than the lipid left over after lipid extraction. General methods for the production of biofuel can be found in, for example, Maher and Bressler (2007), Greenwell et al. (2010), Karmakar et al. (2010), Alonso et al. (2010), Liu et al. (2010). Gong and Jiang (2011), Endalew et al. (2011) and Semwal et al. (2011).

The present invention provides methods for increasing oil content in vegetative tissues. Plants of the present invention have increased energy content of leaves and/or stems such that the whole above-ground plant parts may be harvested and used to produce biofuel. Furthermore, the level of oleic acid is increased significantly while the polyunsaturated fatty acid alpha linolenic acid (ALA) was reduced. The plants, algae and fungi of the present invention thereby reduce the production costs of biofuel.

Biodiesel

The production of biodiesel, or alkyl esters, is well known. There are three basic routes to ester production from lipids: 1) Base catalysed transesterification of the lipid with alcohol; 2) Direct acid catalysed esterification of the lipid with methanol; and 3) Conversion of the lipid to fatty acids, and then to alkyl esters with acid catalysis. Any method for preparing fatty acid alkyl esters and glyceryl ethers (in which one, two or three of the hydroxy groups on glycerol are etherified) can be used. For example, fatty acids can be prepared, for example, by hydrolyzing or saponifying TAG with acid or base catalysts, respectively, or using an enzyme such as a lipase or an esterase. Fatty acid alkyl esters can be prepared by reacting a fatty acid with an alcohol in the presence of an acid catalyst. Fatty acid alkyl esters can also be prepared by reacting TAG with an alcohol in the presence of an acid or base catalyst. Glycerol ethers can be prepared, for example, by reacting glycerol with an alkyl halide in the presence of base, or with an olefin or alcohol in the presence of an acid catalyst. The alkyl esters can be directly blended with diesel fuel, or washed with water or other aqueous solutions to remove various impurities, including the catalysts, before blending.

Aviation Fuel

For improved performance of biofuels, thermal and catalytic chemical bond-breaking (cracking) technologies have been developed that enable converting bio-oils into bio-based alternatives to petroleum-derived diesel fuel and other fuels, such as jet fuel.

The use of medium chain fatty acid source, such produced by a cell of the invention, a plant or part thereof of the invention, a seed of of the invention, or a transgenic version of any one thereof, precludes the need for high-energy fatty acid chain cracking to achieve the shorter molecules needed for jet fuels and other fuels with low-temperature flow requirements. This method comprises cleaving one or more medium chain fatty acid groups from the glycerides to form glycerol and one or more free fatty acids. In addition, the method comprises separating the one or more medium chain fatty acids from the glycerol, and decarboxylating the one or more medium chain fatty acids to form one or more hydrocarbons for the production of the jet fuel.

Compositions

The present invention also encompasses compositions, particularly pharmaceutical compositions, comprising one or more plants, plant parts, lipids,

proteins, nitrogen containing molecules, or carbon containing molecules, produced using the methods of the invention.

A pharmaceutical composition may additionally comprise an active ingredient and a standard, well-known, non-toxic pharmaceutically-acceptable carrier, adjuvant or vehicle such as phosphate-buffered saline, water, ethanol, polyols, vegetable oils, a wetting agent, or an emulsion such as a water/oil emulsion. The composition may be in either a liquid or solid form. For example, the composition may be in the form of a tablet, capsule, ingestible liquid, powder, topical ointment or cream. Proper fluidity can be maintained for example, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. It may also be desirable to include isotonic agents for example, sugars, sodium chloride, and the like. Besides such inert diluents, the composition can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening agents, flavoring agents and perfuming agents.

A typical dosage of a particular fatty acid is from 0.1 mg to 20 g, taken from one to five times per day (up to 100 g daily) and is preferably in the range of from about 10 mg to about 1, 2, 5, or 10 g daily (taken in one or multiple doses). As known in the art, a minimum of about 300 mg/day of fatty acid, especially polyunsaturated fatty acid, is desirable. However, it will be appreciated that any amount of fatty acid will be beneficial to the subject.

Possible routes of administration of the pharmaceutical compositions of the present invention include for example, enteral and parenteral. For example, a liquid preparation may be administered orally. Additionally, a homogenous mixture can be completely dispersed in water, admixed under sterile conditions with physiologically acceptable diluents, preservatives, buffers or propellants to form a spray or inhalant.

The dosage of the composition to be administered to the subject may be determined by one of ordinary skill in the art and depends upon various factors such as weight, age, overall health, past history, immune status, etc., of the subject.

Additionally, the compositions of the present invention may be utilized for cosmetic purposes. The compositions may be added to pre-existing cosmetic compositions, such that a mixture is formed, or a fatty acid produced according to the invention may be used as the sole "active" ingredient in a cosmetic composition.

Polypeptides

The terms "polypeptide" and "protein" are generally used interchangeably herein.

A polypeptide or class of polypeptides may be defined by the extent of identity (% identity) of its amino acid sequence to a reference amino acid sequence, or by having a greater % identity to one reference amino acid sequence than to another. The % identity of a polypeptide to a reference amino acid sequence is typically determined by GAP analysis (Needleman and Wunsch, 1970; GCG program) with parameters of a gap creation penalty = 5, and a gap extension penalty = 0.3. The query sequence is at least 100 amino acids in length and the GAP analysis aligns the two sequences over a region of at least 100 amino acids. Even more preferably, the query sequence is at least 250 amino acids in length and the GAP analysis aligns the two sequences over a region of at least 250 amino acids. Even more preferably, the GAP analysis aligns two sequences over their entire length, and the extent of identity is determined over the full length of the reference sequence. The polypeptide or class of polypeptides may have the same enzymatic activity as, or a different activity than, or lack the activity of, the reference polypeptide. Preferably, the polypeptide has an enzymatic activity of at least 10% of the activity of the reference polypeptide.

As used herein a "biologically active fragment" is a portion of a polypeptide of the invention which maintains a defined activity of a full-length reference polypeptide for example, DGAT activity. Biologically active fragments as used herein exclude the full-length polypeptide. Biologically active fragments can be any size portion as long as they maintain the defined activity. Preferably, the biologically active fragment maintains at least 10% of the activity of the full length polypeptide.

With regard to a defined polypeptide or enzyme, it will be appreciated that % identity figures higher than those provided herein will encompass preferred embodiments. Thus, where applicable, in light of the minimum % identity figures, it is preferred that the polypeptide/enzyme comprises an amino acid sequence which is at least 60%, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99%, more preferably at least 99.1%, more preferably at least 99.2%, more preferably at least 99.3%, more preferably at least 99.4%, more preferably at least 99.5%, more preferably at least 99.6%, more preferably at least 99.7%, more preferably at least 99.8%, and even more preferably at least 99.9% identical to the relevant nominated SEQ ID NO.

Amino acid sequence mutants of the polypeptides defined herein can be prepared by introducing appropriate nucleotide changes into a nucleic acid defined herein, or by *in vitro* synthesis of the desired polypeptide. Such mutants include for example, deletions, insertions, or substitutions of residues within the amino acid sequence. A combination of deletions, insertions and substitutions can be made to arrive at the final construct, provided that the final polypeptide product possesses the desired characteristics.

Mutant (altered) polypeptides can be prepared using any technique known in the art, for example, using directed evolution or rathional design strategies (see below). Products derived from mutated/altered DNA can readily be screened using techniques described herein to determine if they possess transcription factor, fatty acid acyltransferase or OBC activities.

In designing amino acid sequence mutants, the location of the mutation site and the nature of the mutation will depend on characteristic(s) to be modified. The sites for mutation can be modified individually or in series for example, by (1) substituting first with conservative amino acid choices and then with more radical selections depending upon the results achieved, (2) deleting the target residue, or (3) inserting other residues adjacent to the located site.

Amino acid sequence deletions generally range from about 1 to 15 residues, more preferably about 1 to 10 residues and typically about 1 to 5 contiguous residues.

Substitution mutants have at least one amino acid residue in the polypeptide removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis to inactivate enzymes include sites identified as the active site(s). Other sites of interest are those in which particular residues obtained from various strains or species are identical. These positions may be important for biological activity. These sites, especially those falling within a sequence of at least three other identically conserved sites, are preferably substituted in a relatively conservative manner. Such conservative substitutions are shown in Table 1 under the heading of "exemplary substitutions".

Table 1. Exemplary substitutions.

Original Residue	Exemplary Substitutions
Ala (A)	val; leu; ile; gly

Arg (R)	lys
Asn (N)	gln; his
Asp (D)	glu
Cys (C)	ser
Gln (Q)	asn; his
Glu (E)	asp
Gly (G)	pro, ala
His (H)	asn; gln
Ile (I)	leu; val; ala
Leu (L)	ile; val; met; ala; phe
Lys (K)	arg
Met (M)	leu; phe
Phe (F)	leu; val; ala
Pro (P)	gly
Ser (S)	thr
Thr (T)	ser
Trp (W)	tyr
Tyr (Y)	trp; phe
Val (V)	ile; leu; met; phe, ala

In a preferred embodiment a mutant/variant polypeptide has only, or not more than, one or two or three or four conservative amino acid changes when compared to a naturally occurring polypeptide. Details of conservative amino acid changes are provided in Table 1. As the skilled person would be aware, such minor changes can reasonably be predicted not to alter the activity of the polypeptide when expressed in a transgenic plant or part thereof. Mutants with desired activity may be engineered using standard procedures in the art such as by performing random mutagenesis, targeted mutagenesis, or saturation mutagenesis on known genes of interest, or by subjecting different genes to DNA shuffling.

EXAMPLES

Example 1. General Materials and Methods

Expression of genes in plant cells in a transient expression system

Genes were expressed in plant cells using a transient expression system essentially as described by Voinnet et al. (2003) and Wood et al. (2009). Binary

vectors containing the coding region to be expressed by a strong constitutive e35S promoter containing a duplicated enhancer region were introduced into *Agrobacterium tumefaciens* strain AGL1. A chimeric binary vector, 35S:p19, for expression of the p19 viral silencing suppressor was separately introduced into AGL1, as described in WO2010/057246. A chimeric binary vector, 35S:V2, for expression of the V2 viral silencing suppressor was separately introduced into AGL1. The recombinant cells were grown to stationary phase at 28°C in LB broth supplemented with 50 mg/L kanamycin and 50 mg/L rifampicin. The bacteria were then pelleted by centrifugation at 5000 g for 5 min at room temperature before being resuspended to OD600 = 1.0 in an infiltration buffer containing 10 mM MES pH 5.7, 10 mM $MgCl_2$ and 100 uM acetosyringone. The cells were then incubated at 28°C with shaking for 3 hours after which the OD600 was measured and a volume of each culture, including the viral suppressor construct 35S:p19 or 35S:V2, required to reach a final concentration of OD600 = 0.125 added to a fresh tube. The final volume was made up with the above buffer. Leaves were then infiltrated with the culture mixture and the plants were typically grown for a further three to five days after infiltration before leaf discs were recovered for either purified cell lysate preparation or total lipid isolation.

Transformation of *Sorghum bicolor L.*

Plant Material

Sorghum plants of the inbred cultivar TX-430 (Miller, 1984) were grown in a plant growth chamber (Conviron, PGC-20 flex) at 28 ± 1˚C "day" temperature and 20 ± 1˚C "night" temperature, with a 16 hr photoperiod at a light intensity during the "day" of 900-1000 LUX. Panicles were covered with white translucent paper bags before flowering. Immature embryos were harvested from panicles 12–15 days after anthesis. Panicles were washed several times with water and developing seeds that were uniform in size were isolated and surface-sterilized using 20% commercial bleach mixed with 0.1% Tween-20 for 15-20 min. They were then washed with sterile distilled water 3 times each for 20 min, and blotted dry in a laminar flow hood. Immature embryos (IEs) ranging from 1.4 to 2.5 mm in length were aseptically isolated in the laminar flow hood and used as the starting tissue for preparation of green regenerative tissue.

Base Cultivation Media

Media used for plant transformation were based on MS (Murashige and Skoog, 1962), supplied by PhytoTechnology Laboratories (M519). The pH of the media was adjusted to 5.8 before sterilization at 121˚C for 15 min. Heat sensitive plant growth

regulators and other additives such as Geneticin (G418, Sigma) used as a selection agent, were filter sterilized (0.2 µm) and added to the media after sterilization when the media had cooled to about 55˚C. The optimized culture medium composition for the different stages of plant transformation from callus induction to plant regeneration from green tissue induced from immature embryos is presented in Table 2.

Cultivation Methods and Materials

The isolated IEs ranging from 1.4 to 2.5 mm in length were placed onto callus induction media-osmotic medium (CIM-osmotic medium, Table 2) with their scutellum facing upward. The CIM base medium was modified to improve callus quality and induction frequency from immature embryos, as well as callus regeneration media, by including α-Lipoic acid (1 to 5 mg/l), Melatonin (5 to 10 mg/l) and 2-Aminoidan-2-phosphonic acid HCl (1 to 2 mg/l) unless otherwise stated. For the development of green tissue, immature embryos were incubated under fluorescent light of approximately 45-50 µmol s^{-1} m^{-2} (16 h/day) in a tissue culture room at 24 ± 2˚C. After three days of culture, the root and shoot poles of the immature embryos were aseptically separated and re-inoculated on to the same CIM and maintained under the same conditions as described above. They were subcultured every two weeks onto the same CIM for 6 weeks and evaluated for callus quality, callus induction efficiency and transformation efficiency.

Table 2. Media used in DEC tissue induction and transformation of sorghum

Name of the medium	**Composition**	**Culture duration**
CIM-Osmotic Medium	MS medium powder with vitamins, 4.33 g/l; 2,4-D, 1 mg/l; BAP, 0.5 mg/l; L-proline, 0.7 g/l; L-Lipoic acid, 1 mg/l; peptone, 0.82 g/l; Myo-inositol, 150 mg/l; Copper sulfate, 0.8 mg/l; Manitol, 36.4 g/l; Sorbitol, 36.4 g/l; Agar, 8.5 g/l, pH 5.8	3-4 hrs before bombardment; o/n post bombardment
CIM- pre selection medium	MS medium powder with vitamins, 4.33 g/l; 2,4-D, 1 mg/l; BAP, 0.5 mg/l; L-proline, 0.7 g/l; L-Lipoic acid, 1 mg/l; peptone, 0.82 g/l; Myo-inosito,l 150 mg/l; Copper sulfate, 0.8 mg/l; Maltose, 30 g/l; L-cysteine, 50 mg/l; Ascorbic acid, 15 mg/l; Agar, 9 g/l, pH 5.8	3-4 days
CIM-callus induction medium/G25	MS medium powder with vitamins, 4.33 g/l; 2,4-D, 1 mg/l; BAP, 0.5 mg/l; L-proline, 0.7 g/l; L-Lipoic acid, 1 mg/l; peptone, 0.82 g/l; Myo-inositol, 150 mg/l; Copper sulfate, 0.8 mg/l; Maltose, 30 g/l; Geneticin, 25 mg/l; Agar, 9 g/l, pH 5.8	4 weeks

Name of the medium	Composition	Culture duration
SIM-shoot induction medium/G25	MS medium powder with vitamins, 4.33 g/l; BAP, 1.0 mg/l; 2,4-D, 0.5 mg/l; L-proline, 0.7 g/l; L-Lipoic acid, 1 mg/l; peptone, 0.82 g/l; Myo-inositol, 150 mg/l; Copper sulfate, 0.8 mg/l; Maltose, 30 g/l; Geneticin, 25 mg/l; Agar, 9 g/l, pH 5.8	2 weeks
SRM- shoot regeneration medium/G25	MS medium powder with vitamins, 4.33 g/l; BAP, 1.0 mg/l; TDZ, 0.5 mg/l; L-proline, 0.7 g/l; L-Lipoic acid, 1 mg/l; peptone, 0.82 g/l; Myo-inositol, 150 mg/l; Copper sulfate, 0.8 mg/l; Maltose, 30 g/l; Geneticin, 25 mg/l; Agar, 9 g/l, pH 5.8	2 weeks
SOG-shoot out growth medium/G30	MS medium powder with vitamins, 2.2 g/l; L-proline, 0.7 g/l; L-Lipoic acid, 1 mg/l; peptone, 0.82 g/l; Myo-inositol, 150 mg/l; Copper sulfate, 0.8 mg/l; Sucrose, 15 g/l; Geneticin, 30 mg/l; Agar, 9 g/l, pH 5.8	2 weeks
RIM-root induction medium/G15	MS medium powder with vitamins, 4.33 g/l; L-proline, 0.7 g/l; L-Lipoic acid, 1 mg/l; peptone, 0.82 g/l; Myo-inositol, 150 mg/l; Copper sulfate, 0.8 mg/l; sucrose, 15 g/l; IAA, 1 mg/l; IBA, 1 mg/l; NAA, 1 mg/l; PVP, 2 g/l; Geneticin, 15 mg/l; Agar 9 g/l, pH 5.8	4 weeks

Callus initiated from IEs in the first 3-4 weeks on CIM were mostly embryogenic and slowly differentiated into embryogenic callus with nodular structures which were coloured from pale to darker green. Embryogenic calli with green nodular structures were selected and maintained on the same medium (CIM) by subculturing every 2 weeks for up to 6 months or more, for use as explants for transformation. This type of tissue is termed herein as “differentiating embryogenic callus” tissue or “DEC” tissue, since this tissue forms nodular structures of differentiating cells which maintain embryogenic and organogenic potential, even though the tissues were really a mixture of callus cells, cells forming nodular structures and granular structures, and intermediate cells which the inventors understood were on the developmental pathway somewhere between callus (which is undifferentiated cells) and the nodular structures. Sometimes, the tissues included early stage (globular) somatic embryos.

Particle-bombardment of green regenerative DEC tissues

Plasmids containing a selectable marker gene encoding the neomycin phosphotransferase II (NptII) providing resistance to the antibiotic Geneticin, under the control of the pUbi promoter and terminated by the nos 3’ region, were made or

obtained for experiments to achieve stable transformation or for co-bombardment with other plasmids. Plasmid DNAs were isolated using a Zymopure™ Maxiprep kit (USA) according to the manufacturer's instructions. As a control vector for transformation, a genetic vector was obtained which contained *uidA* (GUS) and *bar* genes designed for expression in plant cells. The *uidA* gene was under the regulatory control of a maize polyubiquitin promoter (pUbi) and an *Agrobacterium tumefaciens* octopine synthase polyadenylation/terminator (*ocs* 3') sequence. The sequence between the promoter and the protein coding region included the 5' UTR and first intron of the *Ubi* gene. The *uidA* reporter gene also contained, within its protein coding region, an intron from a castor bean catalase gene which prevented translation of functional GUS protein in *Agrobacterium*, thereby reducing the background GUS gene expression in inoculated plant tissues. Therefore, any GUS expression would be due to expression of the *uidA* gene in the plant cells. The *bar* gene was also under the regulatory control of a pUbi promoter and terminated with an *Agrobacterium* nopaline synthase 3' regulatory sequence (*nos* 3'). The *uidA*/*bar* vector was initially used in experiments to detect transient gene expression in the sorghum DEC tissues.

Uniform healthy, green regenerative DEC tissues (4-5 mm in size), produced using methods described above and having been cultured for 6 weeks to 6 months from initiation, were used for microprojectile-mediated transformation (bombardment) with the plasmids. Approximately 15 uniform green DEC tissues (each 4-5 mm) were placed at the centre of a petri dish (90 mm diameter) containing CIM-osmotic medium (Table 2) and incubated in the dark for about 4 hrs prior to bombardment. Bombardment was performed with a PDS-1000 He device (Biorad, Hercules, CA) as described by Liu et al. (2014). Post bombardment, the tissues were kept on the same osmotic medium overnight and transferred to pre-selection medium the next morning

Green DEC tissues bombarded with the genetic vector plasmid having a selectable marker encoding NptII were transferred to CIM-PS medium for 3-4 days before any selection, with addition to the medium of two compounds as antioxidants, L-cysteine (50 mg/l) and ascorbic acid (15 mg/l) (Table 2). Without the addition of these antioxidants in pre-selection medium, many of the bombarded tissues turned brown, some quite dark brown in colour, and many lost any ability to grow further. After 3-4 days on pre-selection medium, some of the bombarded tissues were subjected to GUS staining and viewed under a microscope to count the distinctive blue (GUS positive) spots, to check that genes had been transferred and could be expressed. The inclusion of the two antioxidants in the pre-selection medium improved the efficiency of the transformation as shown by the transient expression of the GUS gene.

Selection and regeneration of transgenic plants with optimised conditions

Following bombardment and 3-4 days culture on pre-selection medium without selective agent (Geneticin), the bombarded tissues had increased in size from 4-5 mm to about 6-7 mm. These tissues were transferred to selective medium CIM/G25 containing 25 mg/l Geneticin (Table 2) and cultured for a further 4 weeks. When possible, the bombarded tissues were split into 2-6 pieces each, increasing the recovery of independent transformants. All of the tissues were cultured on the media as described in Table 2 and maintained in order to regenerate putative transgenic plants.

Plants were regenerated efficiently upon growth on these media. Each bombarded tissue and the shoots obtained from it were subcultured and maintained separately for calculation of the transformation efficiency. Positive transformation was confirmed by PCR on plant genomic DNA isolated from shoot samples, showing the presence of the selectable marker gene. The number of transformants was calculated per input DEC tissue. Transformation efficiencies of about 50% were obtained, expressed as independent transformants per input bombarded tissue.

Agrobacterium-*mediated transformation of green regenerative DEC tissues*

Uniform healthy, green regenerative DEC tissues (4-5 mm in size) produced using methods described in the foregoing examples and which have been cultured for 6 weeks to 6 months from initiation, are used for *Agrobacterium*-mediated transformation.

Genetic vectors having T-DNA regions containing the genes for transformation were designed and made for transformation of green regenerative DEC tissues using *Agrobacterium*-mediated transformation. A control binary vector contained *uidA* (GUS) and *bar* genes designed for expression in plant cells. The *uidA* gene was under the regulatory control of a maize polyubiquitin promoter (pUbi) and an *Agrobacterium tumefaciens* octopine synthase polyadenylation/terminator (*ocs* 3') sequence. The sequence between the promoter and the protein coding region included the 5' UTR and first intron of the *Ubi* gene. The *uidA* reporter gene also contained, within its protein coding region, an intron from a castor bean catalase gene which prevented translation of functional GUS protein in *Agrobacterium*, thereby reducing the background GUS gene expression in inoculated plant tissues. Therefore, any GUS expression was due to expression of the *uidA* gene in the plant cells. The *bar* gene was also under the regulatory control of a pUbi promoter and terminated with an *Agrobacterium* nopaline synthase 3' regulatory sequence (*nos* 3').

A suitable *Agrobacterium tumefaciens* strain was obtained *e.g.,* AGL1 as described in Lazo et al. (1991) and the genetic vector is introduced into the *Agrobacterium tumefaciens* strain by heat shock method.

Agrobacterium cultures harboring the genetic construct are grown in suitable medium *e.g.,* LB medium, and under appropriate conditions to produce an *Agrobacterium* inoculum, after which time the uniform healthy, green regenerative DEC tissues are infected with *Agrobacterium* inoculum. The infected DEC tissues are blotted on sterile filter paper to remove excess *Agrobacterium* and transferred to co-cultivation medium, optionally supplemented with antioxidants, and incubated in the dark at approximately 22-24ºC for 2-4 days. Following incubation, the DEC tissues are treated with an appropriate agent to kill the Agrobacterium, washed in sterile water, transferred to an appropriate medium and allowed to grow. After 4-6 weeks, shoots are excised and cultured on shoot elongation medium, after which time putative transgenic shoots are then detected using appropriate assays.

Brassica napus transformation

Brassica napus seeds were sterilized using chlorine gas as described by Kereszt et al. (2007) and germinated on tissue culture medium. Cotyledonary petioles with 2-4 mm stalk were isolated as described by Belide et al. (2013) and used as explants. *A. tumefaciens* AGL1 (Lazo et al., 1991) cultures containing the binary vector were prepared and cotyledonary petioles inoculated with the cultures as described by Belide et al. (2013). Infected cotyledonary petioles were cultured on MS medium supplemented with 1 mg/L TDZ + 0.1 mg/L NAA + 3 mg/L $AgNO_3$ + 250 mg/L cefotaxime, 50 mg/L timentin and 25 mg/L kanamycin and cultured for 4 weeks at 24°C with 16hr/8hr light-dark photoperiod with a biweekly subculture on to the same medium. Explants with green callus were transferred to shoot initiation medium (MS + 1 mg/L kinetin + 3 mg/L $AgNO_3$ + 250 mg/L cefotaxime + 50 mg/L timentin + 25 mg/L kanamycin) and cultured for another 2-3 weeks. Small shoots (~1 cm) were isolated from the resistant callus and transferred to shoot elongation medium (MS medium with 0.1 mg/L gibberelic acid + 3 mg/L $AgNO_3$ + 250 mg/L cefotaxime + 25 mg/L kanamycin) and cultured for another two weeks. Healthy shoots with one or two leaves were selected and transferred to rooting media (1/2 MS with 1 mg/L NAA + 20 mg/L ADS + 3 mg/L $AgNO_3$ + 250 mg/L cefotaxime) and cultured for 2-3 weeks. DNA was isolated from small leaves of resistant shoots using the plant DNA isolation kit (Bioline, Alexandria, NSW, Australia) as described by the manufacturer's protocol. The presence of T-DNA sequences was tested by PCR amplification on genomic DNA.

Positive, transgenic shoots with roots were transferred to pots containing seedling raising mix and grown in a glasshouse at 24ºC daytime/16ºC night-time (standard conditions).

Purified leaf lysate – enzyme assays

Nicotiana benthamiana leaf tissues previously infiltrated as described above were ground in a solution containing 0.1 M potassium phosphate buffer (pH 7.2) and 0.33 M sucrose using a glass homogenizer. Leaf homogenate was centrifuged at 20,000 g for 45 minutes at 4°C after which each supernatant was collected. Protein content in each supernatant was measured according to Bradford (1976) using a Wallac1420 multi-label counter and a Bio-Rad Protein Assay dye reagent (Bio-Rad Laboratories, Hercules, CA USA). Acyltransferase assays used 100 μg protein according to Cao et al. (2007) with some modifications. The reaction medium contained 100 mM Tris-HCl (pH 7.0), 5 mM $MgCl_2$, 1 mg/mL BSA (fatty acid-free), 200 mM sucrose, 40 mM cold oleoyl-CoA, 16.4 μM *sn*-2 monooleoylglycerol[^{14}C] (55mCi/mmol, American Radiochemicals, Saint Louis, MO USA) or 6.0 μM [^{14}C]glycerol-3-phosphate (G-3-P) disodium salt (150 mCi/mmol, American Radiochemicals). The assays were carried out for 7.5, 15, or 30 minutes.

Lipid analysis

Analysis of oil content in seeds

When seed oil content or total fatty acid composition was to be determined in small seeds such as *Arabidopsis* seeds, fatty acids in the seeds were directly methylated without crushing of seeds. Seeds were dried in a desiccator for 24 hours and approximately 4 mg of seed was transferred to a 2 ml glass vial containing a Teflon-lined screw cap. 0.05 mg triheptadecanoin (TAG with three C17:0 fatty acids) dissolved in 0.1 ml toluene was added to the vial as internal standard. Seed fatty acids were methylated by adding 0.7 ml of 1N methanolic HCl (Supelco) to the vial containing seed material. Crushing of the seeds was not necessary for complete methylation with small seeds such as *Arabidopsis* seeds. The mixture was vortexed briefly and incubated at 80°C for 2 hours. After cooling the mixtures to room temperature, 0.3 ml of 0.9% NaCl (w/v) and 0.1 ml hexane was added to the vial and mixed well for 10 minutes in a Heidolph Vibramax 110. The FAME were collected into a 0.3 ml glass insert and analysed by GC with a flame ionization detector (FID) as described below.

The peak area of individual FAME were first corrected on the basis of the peak area responses of a known amount of the same FAMEs present in a commercial standard GLC-411 (NU-CHEK PREP, INC., USA). GLC-411 contains equal amounts of 31 fatty acids (% by weight), ranging from C8:0 to C22:6. In case of fatty acids which were not present in the standard, the peak area responses of the most similar FAME was taken. For example, the peak area response of FAMEs of 16:1d9 was used for 16:1d7 and the FAME response of C22:6 was used for C22:5. The corrected areas were used to calculate the mass of each FAME in the sample by comparison to the internal standard mass. Oil is stored mainly in the form of TAG and its weight was calculated based on FAME weight. Total moles of glycerol was determined by calculating moles of each FAME and dividing total moles of FAMEs by three. TAG content was calculated as the sum of glycerol and fatty acyl moieties using a relation: % oil by weight = 100x ((41x total mol FAME/3)+(total g FAME- (15x total mol FAME)))/g seed, where 41 and 15 are molecular weights of glycerol moiety and methyl group, respectively.

Analysis of fatty acid content in larger *seeds*

To determine fatty acid composition in single seeds that were larger, such as canola and *Camelina* seeds, or Sorghum or corn seeds, direct methylation of fatty acids in the seed was performed as for *Arabidopsis* seeds except with breaking of the seed coats. This method extracted sufficient oil from the seed to allow fatty acid composition analysis. To determine the fatty acid composition of total extracted lipid from seeds, seeds were crushed and lipids extracted with $CHCl_3$/MeOH. Aliquots of the extracted lipid were methylated and analysed by GC. Pooled seed-total lipid content (seed oil content) of canola was determined by two extractions of lipid using $CHCl_3$/MeOH from a known weight of desiccated seeds after crushing, followed by methylation of aliquots of the lipids together with the 17:0 fatty acids as internal standard. In the case of larger seeds such as *Camelina*, the lipid from a known amount of seeds was methylated together with known amount of 17:0 fatty acids as for the *Arabidopsis* oil analysis and FAME were analysed by GC. For TAG quantitation, TAG was fractionated from the extracted lipid using TLC and directly methylated *in silica* using 17:0 TAG as an internal standard. These methods are described more fully as follows.

After harvest at plant maturity, seeds were desiccated by storing the seeds for 24 hours at room temperature in a desiccator containing silica gel as desiccant. Moisture content of the seeds was typically 6-8%. Total lipids were extracted from

known weights of the desiccated seeds by crushing the seeds using a mixture of chloroform and methanol (2/1 v/v) in an eppendorf tube using a Reicht tissue lyser (22 frequency/seconds for 3 minutes) and a metal ball. One volume of 0.1M KCl was added and the mixture shaken for 10 minutes. The lower non-polar phase was collected after centrifuging the mixture for 5 minutes at 3000 rpm. The remaining upper (aqueous) phase was washed with 2 volumes of chloroform by mixing for 10 minutes. The second non-polar phase was also collected and pooled with the first. The solvent was evaporated from the lipids in the extract under nitrogen flow and the total dried lipid was dissolved in a known volume of chloroform.

To measure the amount of lipid in the extracted material, a known amount of 17:0-TAG was added as internal standard and the lipids from the known amount of seeds incubated in 1 N methanolic-HCl (Supelco) for 2 hours at 80ºC. FAME thus made were extracted in hexane and analysed by GC. Individual FAME were quantified on the basis of the amount of 17:0 TAG-FAME. Individual FAME weights, after subtraction of weights of the esterified methyl groups from FAME, were converted into moles by dividing by molecular weights of individual FAME. Total moles of all FAME were divided by three to calculate moles of TAG and therefore glycerol. Then, moles of TAG were converted in to weight of TAG. Finally, the percentage oil content on a seed weight basis was calculated using seed weights, assuming that all of the extracted lipid was TAG or equivalent to TAG for the purpose of calculating oil content. This method was based on Li et al. (2006). Seeds other than *Camelina* or canola seeds that are of a similar size can also be analysed by this method.

Canola and other seed oil content can be measured by nuclear magnetic resonance techniques (Rossell and Pritchard, 1991) by a pulsed wave NMS 100 Minispec (Bruker Pty Ltd Scientific Instruments, Germany). The NMR method can simultaneously measured moisture content. Seed oil content can also be measured by near infrared reflectance (NIR) spectroscopy such as using a NIRSystems Model 5000 monochromator. Moisture content can also be measured on a sample from a batch of seeds by drying the seeds in the sample for 18 hours at about 100ºC, according to Li et al. (2006).

Analysis of lipids from leaf lysate assays

Lipids from the lysate assays were extracted using chloroform:methanol:0.1 M KCl (2:1:1) and recovered. The different lipid classes in the samples were separated on Silica gel 60 thin layer chromatography (TLC) plates (MERCK, Dermstadt, Germany) impregnated with 10% boric acid. The solvent system used to fractionate TAG from

the lipid extract was chloroform/acetone (90/10 v/v). Individual lipid classes were visualized by exposing the plates to iodine vapour and identified by running parallel authentic standards on the same TLC plate. The plates were exposed to phosphor imaging screens overnight and analysed by a Fujifilm FLA-5000 phosphorimager before liquid scintillation counting for DPM quantification.

Total lipid isolation and fractionation of lipids from vegetative tissues

Fatty acid composition of total lipid in leaf and other vegetative tissue samples was determined by direct methylation of the fatty acids in freeze-dried samples. For total lipid quantitation, fatty acids in a known weight of freeze-dried samples, with 17:0 FFA, were directly methylated. To determine total TAG levels in leaf samples, TAG was fractionated by TLC from extracted total lipids, and methylated in the presence of 17:0 TAG internal standard, because of the presence of substantial amounts of polar lipids in leaves. This was done as follows. Tissues including leaf samples were freeze-dried, weighed (dry weight) and total lipids extracted as described by Bligh and Dyer (1959) or by using chloroform:methanol:0.1 M KCl (CMK; 2:1:1) as a solvent. Total lipids were extracted from *N. benthamiana* leaf samples, after freeze dying, by adding 900 µL of a chloroform/methanol (2/1 v/v) mixture per 1 cm diameter leaf sample. 0.8 µg DAGE was added per 0.5 mg dry leaf weight as internal standard when TLC-FID analysis was to be performed. Samples were homogenized using an IKA ultra-turrax tissue lyser after which 500 µL 0.1 M KCl was added. Samples were vortexed, centrifuged for 5 min and the lower phase was collected. The remaining upper phase was extracted a second time by adding 600 µL chloroform, vortexing and centrifuging for 5 min. The lower phase was recovered and pooled into the previous collection. Lipids were dried under a nitrogen flow and resuspended in 2 µL chloroform per mg leaf dry weight. Total lipids of *N. tabacum* leaves or leaf samples were extracted as above with some modifications. If 4 or 6 leaf discs (each approx 1 cm^2 surface area) were combined, 1.6 ml of CMK solvent was used, whereas if 3 or less leaf discs were combined, 1.2 ml CMK was used. Freeze dried leaf tissues were homogenized in an eppendorf tube containing a metallic ball using a Reicht tissue lyser (Qiagen) for 3 minutes at 20 frequency/sec.

Separation of neutral lipids via TLC and transmethylation

Known volumes of total leaf extracts such as, for example, 30 µL were loaded on a TLC silica gel 60 plate (1x20 cm) (Merck KGaA, Germany). The neutral lipids were fractionated into the different types and separated from polar lipids via TLC in an

equilibrated development tank containing a hexane/DEE/acetic acid (70/30/1 v/v/v/) solvent system. The TAG bands were visualised by primuline spraying, marked under UV, scraped from the TLC plate, transferred to 2 mL GC vials and dried with N_2. 750 µL of 1N methanolic-HCl (Supelco analytical, USA) was added to each vial together with a known amount of C17:0 TAG as an internal standard, depending on the amount of TAG in each sample. Typically, 30 µg of the internal standard was added for low TAG samples whilst up to 200 µg of internal standard was used in the case of high TAG samples.

Lipid samples for fatty acid composition analysis by GC were transmethylated by incubating the mixtures at 80ºC for 2 hours in the presence of the methanolic-HCl. After cooling samples to room temperature, the reaction was stopped by adding 350 µl H_2O. Fatty acyl methyl esters (FAME) were extracted from the mixture by adding 350 µl hexane, vortexing and centrifugation at 1700 rpm for 5 min. The upper hexane phase was collected and transferred into GC vials with 300 µl conical inserts. After evaporation, the samples were resuspended in 30 µl hexane. One µl was injected into the GC.

The amount of individual and total fatty acids (TFA) present in the lipid fractions was quantified by GC by determining the area under each peak and calculated by comparison with the peak area for the known amount of internal standard. TAG content in leaf was calculated as the sum of glycerol and fatty acyl moieties in the TAG fraction using a relation: % TAG by weigh = 100x ((41x total mol FAME/3)+(total g FAME- (15x total mol FAME)))/g leaf dry weight, where 41 and 15 are molecular weights of glycerol moiety and methyl group, respectively.

Capillary gas-liquid chromatography (GC)

FAME were analysed by GC using an Agilent Technologies 7890A GC (Palo Alto, California, USA) equipped with an SGE BPX70 (70% cyanopropyl polysilphenylene-siloxane) column (30 m x 0.25 mm i.d., 0.25 µm film thickness), an FID, a split/splitless injector and an Agilent Technologies 7693 Series auto sampler and injector. Helium was used as the carrier gas. Samples were injected in split mode (50:1 ratio) at an oven temperature of 150°C. After injection, the oven temperature was held at 150°C for 1 min, then raised to 210°C at 3°C.min^{-1} and finally to 240°C at 50°C.min^{-1}. Peaks were quantified with Agilent Technologies ChemStation software (Rev B.04.03 (16), Palo Alto, California, USA) based on the response of the known amount of the external standard GLC-411 (Nucheck) and C17:0-Me internal standard.

Quantification of TAG via Iatroscan

One µL of lipid extract was loaded on one Chromarod-SII for TLC-FID Iatroscan™ (Mitsubishi Chemical Medience Corporation – Japan). The Chromarod rack was then transferred into an equilibrated developing tank containing 70 mL of a hexane/$CHCl_3$/2-propanol/formic acid (85/10.716/0.567/0.0567 v/v/v/v) solvent system. After 30 min of incubation, the Chromarod rack was dried for 3 min at 100°C and immediately scanned on an Iatroscan MK-6s TLC-FID analyser (Mitsubishi Chemical Medience Corporation – Japan). Peak areas of DAGE internal standard and TAG were integrated using SIC-480II integration software (Version:7.0-E SIC System instruments Co., LTD – Japan).

TAG quantification was carried out in two steps. First, DAGE was scanned in all samples to correct the extraction yields after which concentrated TAG samples were selected and diluted. Next, TAG was quantified in diluted samples with a second scan according to the external calibration using glyceryl trilinoleate as external standard (Sigma-Aldrich).

Quantification of TAG in leaf samples by GC

The peak area of individual FAME were first corrected on the basis of the peak area responses of known amounts of the same FAMEs present in a commercial standard GLC-411 (NU-CHEK PREP, Inc., USA). The corrected areas were used to calculate the mass of each FAME in the sample by comparison to the internal standard. Since oil is stored primarily in the form of TAG, the amount of oil was calculated based on the amount of FAME in each sample. Total moles of glycerol were determined by calculating the number of moles of FAMEs and dividing total moles of FAMEs by three. The amount of TAG was calculated as the sum of glycerol and fatty acyl moieties using the formula: % oil by weight = 100x ((41x total mol FAME/3)+(total g FAME-(15x total mol FAME)))/g leaf dry weight, where 41 and 15 were the molecular weights of glycerol moiety and methyl group, respectively.

Total Lipid Extraction and Fatty Acid Profile Analysis

Total lipids were extracted from freeze-dried *N. benthamiana* leaves. During the extraction of total lipids, TAG 51:0 (tri-C17:0) was added as the internal standard for the quantification of both the TAG and total fatty acid (TFA) contents. Freeze dried leaf tissue was ground to powder in a microcentrifuge tube containing a metallic ball using Reicht tissue lyser (Qiagen) for 3 min. at 20 frequency/s. Chloroform:methanol (2:1, v/v) was added and mixed for a further 3 min. on the tissue lyser before the

addition of 1:3 (v/v) of 0.1 M KCl. The sample was then mixed for a further 3 min. before centrifugation (5 min. at 14,000 g), after which the lower lipid phase was collected. The remaining phase was washed once with chloroform, and the lower phase extracted and pooled with the earlier extract. Lipid phase solvent was then evaporated completely using N_2 gas flow and the lipids resuspended in 5 µL chloroform per mg of original dry leaf weight.

Fatty acid methyl esters (FAMEs) of total lipids (equivalent to 10mg dry weight) were produced by incubating extracted lipid in 1 N methanolic-HCl (Supelco, Bellefonte, PA) at 80°C for 3 hours. FAMEs were analyzed by an Agilent 7890A gas chromatograph coupled with flame ionisation detector (GC-FID, Agilent Technologies, Palo Alto, CA), on a BPX70 column (30m, 0.25 mm inner diameter, 0.25 µm film thickness, SGE) essentially as described previously (Zhou et al., 2011), except the column temperature program. The column temperature was programmed as an initial temperature at 100ºC holding for 3 min, ramping to 240°C at a rate of 7°C/min and holding for 1 min. NuChek GLC-426 was used as the external reference standard. Peaks were integrated with Agilent Technologies ChemStation software (Rev B.04.03 (16)).

TLC Analysis

From the total lipid extracts (equivalent to 10mg dry weight of plant tissue), TAG and polar lipids were fractionated by TLC (Silica gel 60, MERCK) using hexane:diethylether:acetic acid (70:30:1 v/v/v) and visualized by spraying Primuline (Sigma, 5 mg/100 ml acetone:water (80:20 v/v)) and exposing plate under UV. TLC analysis was primarily used for the identification of fatty acid composition of TAG and phospholipids from lipid extraction samples. This also enabled the determination of the total TAG content for each sample. The TAG and phospholipid fractions were scraped from the TLC plates and methylated according to the FAME preparation protocol described previously.

LC-MS Analysis

Lipids extracted from 1 mg dry leaf weight were dissolved and diluted to 1 mg/ml in mL butanol:methanol (1:1, v/v) and analyzed by liquid chromatography-mass spectrometry (LC-MS), based on previously described methods (Petrie et al., 2012). Briefly, lipids were chromatographically separated using a Waters BEH C8 (100 mm x 2.1 mm, 2.7 µm) fitted to an Agilent 1290 series LC and 6490 triple quadrupole LC-MS with Jet Stream ionisation with a binary gradient flow rate of 0.2 mL/min. The

mobile phases were: A. H_2O:acetonitrile (10:90, v/v) with 10 mM ammonium formate and 0.2 % acetic acid; B. H_2O:acetonitrile:isopropanol (5:15:80, v/v) with 10 mM ammonium formate and 0.2 % acetic acid. For the phosphatidylcholine (PC) and lysophosphatidylcholine (LPC) species hydrogen adducts were quantified by the characteristic 184 *m/z* phosphatidyl head group ion under positive ionisation mode. The ammonium adducts of monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG), diacylglycerol (DAG) and TAG lipid species were analyzed by the neutral loss of singular fatty acids C_{12} to C_{18}. Multiple reaction monitoring (MRM) lists were based on the following major fatty acids: 12:0, 14:0, 16:0, 16:3, 18:0, 18:1, 18:2, 18:3, using a collision energy of 28 V for all lipid classes except for DAG where a collision energy of 14 V was used. Individual MRM TAG was identified based on ammoniated precursor ion and product ion from neutral loss.

Example 2. Modifying traits in vegetative parts of monocotyledonous plants

Chimeric DNA constructs were designed to increase oil content in monocotyledonous plants, for example the C4 plant *S. bicolor* (sorghum), by expressing a combination of genes encoding WRI1, *Z. mays* LEC1 (Accession number AAK95562; SEQ ID NO:32), DGAT and Oleosin in the transgenic plants. Several pairs of constructs for biolistic co-transformation were designed and produced by restriction enzyme-ligation cloning, as follows.

The genetic construct pOIL136 was a binary vector containing three monocot expression cassettes, namely a selectable marker gene encoding phosphinothricin acetyltransferase (PAT) for plant selection, a second cassette for expressing DGAT and a third for expressing Oleosin. pJP136 was first produced by amplifying an Actin-1 gene promoter from *Oryza sativa* (McElroy et al., 1990) and inserting it as a blunt-*Cla*I fragment into pORE04 (Coutu et al., 2007) to produce pOIL094. pOIL095 was then produced by inserting a version of the *Sesamum indicum* Oleosin L gene which had been codon optimised for monocot expression into pOIL094 at the *Kpn*I site. pOIL093 was produced by cloning a monocot (*Triticum aestivum*) codon optimised version of the *Umbelopsis ramanniana* DGAT2a gene (Lardizabal et al., 2008) as a *Sma*I-*Kpn*I fragment into a vector already containing a *Zea mays* Ubiquitin gene promoter. pOIL134 was then produced by cloning the *Not*I DGAT2a expression cassette from pOIL093 into pOIL095 at the *Not*I sites. pOIL141 was produced by inserting the selectable marker gene coding for PAT as a *BamH*I-*Sac*I fragment into a vector containing the *Z. mays* Ubiquitin-1 promoter. Finally, pOIL136 was produced by cloning the *Z. mays* Ubiquitin::PAT expression cassette as a blunt-*Asc*I fragment into

the *Zra*I-*Asc*I of pOIL096. The genetic construct pOIL136 therefore contained the following expression cassettes: promoter *O. sativa* Actin::*S. indicum* Oleosin, promoter *Z. mays* Ubiquitin::*U. ramanniana* DGAT2a and promoter *Z. mays* Ubiquitin::PAT.

A similar vector pOIL197, containing NPTII instead of PAT was constructed by subcloning of the *Z. mays* Ubiquitin::NPTII cassette from pUKN (Liu and Godwin, 2012) as a *Hind*III-*Sma*I fragment into the *Asc*I (blunted) and *Hind*III sites of pJP3343. The resulting vector, pOIL196, was then digested with *Hind*III (blunted) and *Age*I. The resulting 3358bp fragment was cloned into the *Zra*I - *Age*I sites of pOIL134, yielding pOIL197.

A set of constructs containing genes encoding the *Z. mays* WRI1 (ZmWRI) or the LEC1 (ZmLEC1) transcription factors under the control of different promoters were designed and produced for biolistic co-transformation in combination with pOIL136 or pOIL197 to test the effect of promoter strength and cell specificity on the function of WRI1 or LEC1, or both if combined, when expressed in vegetative tissues of a C4 plant such as sorghum. This separate set of constructs did not contain a selectable marker gene, except for pOIL333 which contained NPTII as selectable marker. The different promoters tested were as follows. The *Z. mays* Ubiquitin gene promoter (pZmUbi) was a strong constitutive monocot promoter while the enhanced CaMV 35S promoter (e35S) having a duplicated enhancer region was reported to result in lower transgene expression levels (reviewed in Girijashankar and Swathisree, 2009). Whilst the *Z. mays* phosphoenolpyruvate carboxylase (pZmPEPC) gene promoter was active in leaf mesophyl cells (Matsuoka and Minami, 1989), the site of photosynthesis in C4 plant species, the *Z. mays* Rubisco small subunit (pZmSSU) gene promoter was specific for the bundle sheath cell layer (Nomura et al., 2000; Lebrun et al., 1987), the cells where carbon fixation takes place in C4 plants.

The expression of the *Z. mays* gene encoding the SEE1 cysteine protease (Accession number AJ494982) was identified as similar to that of the *A. thaliana* SAG12 senescence-specific promoter during plant development. Therefore a 1970bp promoter from the SEE1 gene (SEQ ID NO:53) was also selected to drive expression of the genes encoding the *Z. mays* WRI1 and LEC1 transcription factors. Further, the promoter from the gene encoding *Aeluropus littoralis* zinc finger protein AlSAP (Ben Saad et al., 2011; Accession number DQ885219; SEQ ID NO:54), the promoter from the gene encoding the *Saccharum* hybrid DIRIGENT (DIR16) (Damaj et al., 2010; Accession number GU062718; SEQ ID NO:82), the promoter from the gene encoding the *Saccharum* hybrid O-Methyl transferase (OMT) (Damaj et al., 2010; Accession number GU062719; SEQ ID NO:83), the A1 promoter allel from the gene encoding the

Saccharum hybrid R1MYB1 (Mudge et al., 2013; Accession number JX514703.1; SEQ ID NO:84), the promoter from the gene encoding the *Saccharum* hybrid Loading Stem Gene 5 (LSG5) (Moyle and Birch, 2013; Accession number JX514698.1; SEQ ID NO:85) and the promoter from the sucrose-responsive ArRolC gene from *A. rhizogenes* (Yokoyama et al., 1994; Accession number DQ160187; SEQ ID NO:55) were also selected for expression of ZmWRI1 expression in stem tissue. Therefore, each of these promoters was individually joined upstream of the ZmWRI1 or ZmLEC1 coding regions, as follows.

An intermediate vector, pOIL100, was first produced by cloning the *Z. mays* WRI1 coding sequence and a *Glycine max* lectin gene transcription terminator/polyadenylation region, flanked by *Asc*I-*Nco*I sites, into the same sites in the binary vector pJP3343. The WRI1 coding sequence was codon optimized using *T. aestivum* codon preferences. The different versions of the constructs for WRI1 expression were based on pOIL100 and were produced by cloning the various promoters into pOIL100. pOIL101 was produced by cloning a *Xho*I-*Sal*I fragment containing the e35S promoter with duplicated enhancer region into the *Xho*I site of pOIL100. pOIL102 was produced by cloning a *Hind*III-*Avr*II fragment containing the *Z. mays* Ubiquitin gene promoter (Christensen et al., 1992) into the *Hind*III-*Xba*I sites of pOIL100. pOIL103 was produced by cloning a *Hind*III-*Nco*I fragment containing a *Z. mays* PEPC gene promoter (Matsuoka and Minami, 1989) into the *Hind*III-*Nco*I sites of pOIL100. pOIL104 was produced by cloning a *Hind*III-*Avr*II fragment containing a *Z. mays* SSU gene promoter into the *Hind*III-*Avr*II sites of pOIL100.

A synthetic fragment containing the *Z. mays* SEE1 promoter region flanked by *Hind*III-*Xho*I unique sites was synthesized. This fragment was cloned upstream of the *Z. mays* WRI1 protein coding region using the *Hind*III-*Xho*I sites in pOIL100. The resulting vector was designated pOIL329. A synthetic fragment containing the *A. littoralis* AlSAP promoter region flanked by *Xho*I-*Xba*I unique sites was synthesized. This fragment was cloned upstream of the *Z. mays* WRI1 coding region using the *Xba*I-*Xho*I sites in pOIL100. The resulting vector was designated pOIL330. A synthetic fragment containing the *A. rhizogenes* ArRolC promoter region flanked by *PspOM*I-*Xho*I unique sites was synthesized. This fragment was cloned upstream of the *Z. mays* WRI1 coding region using the *PspOM*I-*Xho*I sites in pOIL100. The resulting vector was designated pOIL335. Finally, a binary vector (pOIL333) containing the *Z. mays* SEE1::ZmLEC1 expression cassette was obtained in three steps. First, a 35S::GUS expression vector was constructed by amplifying the *GUS* coding region with flanking primers containing *Avr*II and *Kpn*I sites. The resulting fragment was subsequently

cloned into the *Spe*I-*Kpn*I sites of pJP3343. The resulting vector was designated pTV111. Next, the 35S promoter region of pTV111 was replaced by the *Z. mays* SEE1 promoter. To this end, the *Z. mays* SEE1 sequence was amplified using flanking primers containing *Hind*III and *Xho*I unique sites. The resulting fragment was cut with the respective restriction enzymes and subcloned into the *Sal*I-*Hind*III sites of pTV111. The resulting vector was designated pOIL332. Next the ZmLEC1 coding sequence was amplified using flanking primers containing *Not*I and *EcoR*V sites. This resulting fragment was subcloned into the respective sites of pOIL332, yielding pOIL333.

A 2673bp synthetic fragment containing the *Saccharum* DIR16 promoter region flanked by *Hind*III-*Xba*I sites was synthesized. This fragment was cloned upstream of the *Z. mays* WRI1 protein coding region using the *Hind*III-*Xba*I sites in pOIL100. The resulting vector was designated pOIL337. A 2947bp synthetic fragment containing the *Saccharum* OMT promoter region flanked by *Xho*I-*Xba*I sites was synthesized. This fragment was cloned upstream of the *Z. mays* WRI1 protein coding region using the *Xho*I-*Xba*I sites in pOIL100. The resulting vector was designated pOIL339. A 1181bp synthetic fragment containing the *Saccharum* R1MYB1 promoter region flanked by *Hind*III-*Xho*I sites was synthesized. This fragment was cloned upstream of the *Z. mays* WRI1 protein coding region using the *Hind*III-*Xho*I sites in pOIL100. The resulting vector was designated pOIL341. A 4482bp synthetic fragment containing the *Saccharum* LSG5 promoter region flanked by *Xba*III-*Sma*I sites was synthesized. This fragment was cloned as an *Xba*III-*Sma*I fragment upstream of the *Z. mays* WRI1 protein coding region using the *Stu*I-*Nhe*I sites in pOIL100. The resulting vector was designated pOIL343.

Two putative *S. bicolor* SDP1 genes were identified by a BLASTn search using an *A. thaliana SDP1* nucleotide sequence (Accession number NM_120486; SEQ ID NO:37) as query. The Accession numbers of the two *S. bicolor* SDP1 homologs are XM_002458486 (SEQ ID NO:38) and XM_002463620 (SEQ ID NO:73). A 7991bp synthetic fragment was synthesized and contained the following genetic components in order: a matrix association region (MAR), a *Z. mays* promoter, a TMV 5' UTR sequence, a 2198bp hairpin RNA encoding region (SEQ ID NO:75) directed against both *S. bicolor SDP1* genes, an *OCS* gene polyadenylation/transcription terminator, an *O. sativa* Actin-1 gene promoter, TMV 5' UTR sequence, and a *NOS* gene polyadenylation/transcription terminator. The hairpin RNA encoding region contained a *Pdk* intron (Wesley et al., 2001) and a *Cat* intron, the second in reverse orientation. The entire fragment was synthesized and inserted into an *E. coli* expression vector. The resulting vector was designated pOIL385.

Whole plasmid DNA was prepared from pOIL101, pOIL102, pOIL103, pOIL104, pOIL197, pOIL136, pOIL329 and pOIL385 for biolistic transformation. pOIL197 DNA was then mixed with DNA from either pOIL101, pOIL102, pOIL103, pOIL104, pOIL329 or pOIL385 and introduced by biolistics into *S. bicolor* (TX430) differentiating embryonic calli (DEC) cells to produced transformed plants as described in Example 1. Alternatively, constructs for expression of the same combinations of genes are introduced separately or co-transformed by *Agrobacterium*-mediated methods (Gurel et al., 2009; Wu et al., 2014) into DEC tissues.

Between 9 and 47 transgenic plants were regenerated and selected by antibiotic resistance for the pairs of constructs including pOIL197 with each of pOIL101 (p35SSWRI1); pOIL102 (pZmUbi::WRI1), pOIL103 (pZmPEPC::WRI1), pOIL104 (pSSU::WRI1) and pOIL329 (pSEE1::WRI1). Transformations were also carried out with pOIL197 or pOIL102 alone, and for the transformation vector without an insert (empty vector control). The presence of the introduced transgenes in plants that were resistant to the selective agent was demonstrated by PCR. The copy number of each transgene was also determined by digital droplet PCR (ddPCR).

Total leaf lipids were quantified in a first subset of transgenic *S. bicolor* plants prior to their transfer from MS medium to soil. This preliminary screening suggested slightly elevated total lipid levels in leaf tissue of some events at this very early stage. The line with the highest total lipid content, pOIL136 (2), was further analyzed by thin layer chromatography (TLC) to determine the effect of transgene expression on TAG accumulation. Leaf tissue of this particular line was sampled at vegetative stage following transfer to soil in the glasshouse. When compared to the wildtype and empty vector negative controls, pOIL136 (2) exhibited increased TAG levels in leaf tissue after TLC separation and iodine staining. Subsequent quantification revealed 10-fold increased TAG in the transgenic line compared to the negative controls. The TAG profile was dominated by the polyunsaturated fatty acids linoleic and α-linolenic acid. The presence or absence of all three transgenes was determined by digital PCR analysis. Of note, up to 30% mortality rate was observed for plantlets at rooting stage during tissue culture following transformation with the pOIL103 and pOIL197 combination due to unknown reasons.

Confirmed transgenic plants were transferred to soil in pots in the glasshouse and leaves were sampled from primary transformants at vegetative stage of growth (i.e. prior to the appearance of the boot leaf), at the boot leaf stage (defined as when the boot leaf has fully emerged, the boot leaf is the last leaf formed on the plant and from which the panicle (head) emerges) and at the mature seed-setting stage. Total fatty acid

(TFA) and triacylglycerol (TAG) contents (% leaf dry weight) were quantified by TLC-GC as described in Example 1.

TFA levels in wild-type and empty vector negative controls decreased during plant development and were in the range 0.05-2.9% (weight/dry weight). The highest TFA levels were detected prior to the appearance of the boot leaf (termed the vegetative stage of growth) and were below 3%. TAG levels in the same plants were consistently low in the range 0-0.2% during the entire plant life cycle. Both the TFA content and the TAG content had fatty acid compositions of predominantly C16:0, C18:$2^{\Delta 9,12}$ (LA) and C18:$3^{\Delta 9,12,15}$(ALA). In particular, ALA was present at about >70% of the TFA content, reflecting the use of this fatty acid in wild-type plastid membranes. ALA also was the predominant fatty acid in the small amount of TAG present in the wild-type leaves.

27 confirmed transgenic plants which had been transformed with pOIL197 or pOIL136, comprising both pZmUbi:DGAT and pZmUbi:Oleosin genes in addition to the selectable marker genes, were analysed at the vegetative, boot leaf and mature seed setting stages. Some data are presented in Table 5. Generally, the pOIL197 and pOIL136 primary transformants displayed increased TFA and TAG accumulation compared to the negative control lines, but only to about triple for the TFA level compared to the controls. The highest TFA levels were detected at the vegetative stage of growth. Similar to the wild-type and negative control lines, TFA levels decreased as the plants grew and developed. Maximum TFA levels at vegetative, boot leaf and mature seed setting stages equalled 4.3%, 3.3% and 2.2%, respectively. The highest TAG levels detected varied between 0.8 and 1.4% depending on the age of the plant at the time of sampling (Table 3), so were increased up to 7-fold relative to the very low levels in the wild-type leaves. The TFA composition remained largely unchanged at the different stages and was dominated by ALA. The TAG composition displayed a higher degree of variation between the different transgenic lines. Compared to the fatty acid composition of the TFA content, the level of LA (18:$2^{\Delta 9,12}$) was consistently increased in TAG throughout all plant stages investigated.

Nine primary regenerated plants made by transformation with the single vector pOIL102 (pZmUbi:WRI1) were generated by co-bombardment of pOIL102 and pUKN, containing the *NPTII* selectable marker gene. Table 4 shows some of the data for TFA and TAG contents and fatty acid compositions were measured at the three growth stages. When compared to the plants transformed with the constructs encoding DGAT2 and Oleosin (pOIL197 or pOIL136), TFA and TAG levels in the pOIL102 transgenic events were generally lower. Indeed, levels of TFA and TAG were similar to

the levels in the wild-type and negative control plants at vegetative stage. Maximum TFA levels at vegetative, boot leaf and mature seed setting stages were 2.6%, 2.5% and 2.0%, respectively (Table 4). Maximum TAG levels observed were 0.2%, 0.4% and 0.9% at vegetative, boot leaf and mature seed setting stages, respectively.

Thirty-seven primary regenerated plants were obtained after co-bombardment with both pOIL197 (pZmUbi:DGAT and pZmUbi:Oleosin) and pOIL102 (pZmUbi:WRI1). Four of the regenerated events were found to be non-transgenic. In addition, 2 plants did not contain pOIL102 while 2 other plants did not contain the *DGAT2* transgene. All of the transgenic plants were analysed for TFA and TAG contents and fatty acid composition at the three growth stages, as above. Representative data are presented in Table 5. Some of the plants exhibited greatly increased TFA and TAG levels compared to the plants transfomred with single vectors pOIL197, pOIL136 or pOIL102. The maximum TFA levels at vegetative, boot leaf and mature seed setting stages in the pOIL102+pOIL197 transformed plants equalled 7.2%, 6.4% and 8.7% (w/dry weight), respectively. Importantly, the maximum observed TAG levels increased during plant development from 2.7% (vegetative stage) to 3.5% (boot leaf stage) and 6.1% (mature seed setting stage). Compared with the data obtained for the separate transformations with the DGAT and WRI1 transgenes, this exemplified the synergism for co-expressing DGAT and WRI1 transgenes to increase non-polar lipid accumulation in vegetative plant tissues. High levels of TAG and TFA were in most cases associated with a substantial reduction in the C18:$3^{\Delta 9,12,15}$ content, which was reduced by about 50% in the lines with the highest levels of TAG.

Forty-seven primary transformants were obtained following transformation with both pOIL197 (pZmUbi:DGAT and pZmUbi:Oleosin) and pOIL103 (pZmPEPC:WRI1). Copy number analysis by ddPCR revealed one non-transgenic plant and 3 plants that did not contain *DGAT2* and/or *OLEOSIN* transgenes. All events were subsequently analysed for TFA and TAG contents and fatty acid composition during the three stages of plant development. Some plants with this gene combination exhibited the highest TFA and TAG levels detected in this experimental series. TFA levels were observed at vegetative, boot leaf and mature seed setting stages in the pOIL103+pOIL197 population at 8.3%, 8.3% and 9.7%, respectively. Maximum TAG levels observed at vegetative, boot leaf and mature seed setting stages were at 2.3%, 6.6% and 7.6%, respectively. Of note, the highest TAG (6.6%) and TFA (8.3%) levels amongst all transgenic lines were detected in event TX-03-31 at mature seed setting stage. While C18:$3^{\Delta 9,12,15}$ typically dominated the TFA fraction other than TAG, the TAG in this population of transgenic plants displayed a high degree of variability in

fatty acid composition. Of note, some plants exhibited increases in levels of palmitic acid (C16:0) and/or linoleic acid (LA, C18:2$^{\Delta 9,12}$) at the expense of ALA. Indeed, the ALA level in both TFA and TAG contents was reduced below 40% in some plants as a percentage of the total fatty acid content, while less than 30% in other selected events. The ALA level in TAG was even less than 20% in some selected plants, as a percentage of the total fatty acid content.

Due to the use of biolistic transformation in this experiment, many of the transgenic sorghum plants contained high transgene copy numbers as determined by digital PCR. In addition, varying degrees of male and female sterility were observed amongst the transgenic lines, likely a result of the multiple transgene insertions. The inventors therefore did not pursue homozygosity of the transgenes in subsequent generations but rather performed a detailed analysis on vegetative progeny plants obtained from selected primary transformants. To this end, tillers were propagated allowing for triplicate analyses of TAG and TFA levels. Furthermore, the analyses focussed on the boot leaf stage of growth as this was a distinct and easily identified time point during development that allowed for good comparison between the different transgenic lines, grown under the same environmental conditions. Plants containing the higher levels of TFA and TAG were propagated by separating tillers and transplanting them into soil in new pots. The tillers produced new roots and continued to grow.

Quantitation of the total lipid content in triplicate leaves from established tillers confirmed elevated TAG and TFA contents in several independent lines co-transformed with either pOIL102+pOIL197 or pOIL103+pOIL197. The highest levels were observed in progeny plants of line 03-31, confirming the earlier results. Leaves of this line contained on average 6.9% TFA and 4.6% TAG (% DW) at boot leaf stage. This corresponded to an 89.4-fold increase in TAG content compared to wild-type control leaves at the same developmental stage.

Twenty primary regenerated plants were obtained following transformation with both pOIL197 (pZmUbi:DGAT and pZmUbi:Oleosin) and pOIL104 (pSSU:WRI1). Five plants were found to be non-transgenic and four other plants had only the gene(s) from one of the genetic constructs. All plants were analysed for TFA and TAG contents and fatty acid composition. Leaves of primary transformants containing both pOIL197 and pOIL104 T-DNA regions, sampled at vegetative, mature and seed setting stages of growth contained up to 4.1%, 5.9% and 5.89% TFA, respectively. Surprisingly, the highest TFA levels detected in this population were accompanied by a relatively low TAG content. TAG levels in pOIL104+pOIL197 transgenic plants at vegetative, boot leaf and seed setting stages reached only to 0.7%,

2.8% and 3.4%. Increased TAG levels were typically associated with a reduction in C18:3$^{\Delta 9,12,15}$ and an increase in both palmitic acid and LA.

Forty-three primary regenerated plants were obtained following transformation with both pOIL197 (pZmUbi:DGAT and pZmUbi:Oleosin) and pOIL101 (p35S:WRI1). One plant was non-transgenic, another lacked the *WRI1* transgene and another lacked the *DGAT1* transgene. All plants were analysed for TFA and TAG contents and fatty acid composition at boot leaf stage. Leaves of primary transformants containing both pOIL197 and pOIL104 T-DNA regions contained up to 4% TFA while TAG levels were low with a maximum of 1.4%. Increased TAG levels were associated with a reduction in C18:3$^{\Delta 9,12,15}$as a percentage of the total fatty acid content.

Twenty primary transformants were obtained following transformation with both pOIL197 (pZmUbi:DGAT and pZmUbi:Oleosin) and pOIL329 (pSEE1:WRI1). All plants were confirmed to be transgenic by ddPCR. TFA and TAG levels in leaves of 10 plants at vegetative growth stage were increased up to 3.6% and 0.3%, respectively. Maximum TFA and TAG levels at boot leaf stage equalled 3.8% and 1.5%, respectively. The low TFA and TAG levels were likely the result of the senescence-specific expression patterns of the SEE1 promoter used to drive *WRI1* transgene expression. Increased TAG levels were typically associated with a reduction in C18:3$^{\Delta 9,12,15}$ as a percentage of the total fatty acid content.

Thirty-six primary regenerated plants were obtained following transformation with both pOIL197 (pZmUbi:DGAT and pZmUbi:Oleosin) and pOIL385 (SDP1hpRNAi). Two plants lacked pOIL197 and another two lacked pOIL385. The highest TFA level detected in transgenic leaves at the vegetative growth stage was 4.2%. TAG levels in this particular event at the same growth stage was only 1.0%. TFA and TAG levels in leaves sampled at boot leaf stage were increased up to 3.9% and 1.6%, respectively. The lower TFA and TAG levels could be due to the absence of a *WRI1* transgene in this transgenic population. No changes in TAG or TFA fatty acid composition were detected relative to the wild-type plants.

Transgene expression levels were determined in propagated tillers of selected lines by RT-PCR. In the majority of transgenic lines, the *DGAT2a* transgene was typically expressed at a higher levels than the *WRI1* transgene. *Oleosin L* gene expression was either low or not detected. Total lipid and TAG contents at the boot leaf stage were used to calculate correlation coefficients with gene expression. Both *WRI1* and *DGAT2a* gene expression showed a significant positive correlation with TAG levels amongst pOIL102+pOIL197 and pOIL103+pOIL197 transgenic populations. Significant, albeit slightly weaker, correlation was observed for TFA content and *WRI1*

or *DGAT2a* expression. *Oleosin L* expression was not correlated with either TAG or TFA accumulation in transgenic leaf tissues. It was observed that plant TX-03-31 which had a relatively high TTQ had the highest level of expression of *DGAT* amongst the tested plants. It was concluded that high levels of *DGAT* expression were beneficial for increasing the TAG level and also the TTQ.

The most surprising and unexpected observation made in these experiments was the relatively high level of TFA accompanied by the low levels of TAG in most of the transformed sorghum plants, except in a few exceptional plants such as plant TX-03-31. That is, although fatty acid synthesis and accumulation were significantly increased, much of the fatty acid was appearing as TFA but not as TAG. This observation was the opposite of what had been seen with the WRI1 + DGAT transgenic plants for *Nicotiana* including tobacco. To quantitate this in the sorghum plants, the quotient of the TAG to TFA level was calculated for all of the above mentioned transgenic sorghum populations (Tables 3-6). The TAG/TFA Quotient (TTQ) parameter was calculated as the level of TAG (%) divided by the level of TFA (%), each as a percentage of the dry weight of the plant material (leaf in this case). It was observed that for many of the sorghum lines, the TTQ was in the range of 0.01 to 0.6, i.e. less than 60% of the TFA was present as TAG. Addition of one or more further genetic modifications to the combination of WRI1 and DGAT genes such as, for example, which provide for a reduction in the expression of endogenous *SDP1*, *TGD* or *TST* genes, or an increase in the levels of one or more of PDAT, PDCT or CPT polypeptides increases the TTQ to above 0.6 for a larger proportion of the plant lines. In particular, reduction in TAG lipase in combination with at least WRI1 and DGAT increases the TTQ to up to 0.95.

Due to the large difference in absolute TFA and TAG levels in many transgenic lines, the inventors selected two pOIL102+pOIL197 events (02-10, 02-19) and two pOIL103+pOIL197 events (03-31 and 03-48) for quantitation of the major neutral and polar lipid classes, to determine the type of lipid other than TAG in which the high level of fatty acids was present. The types of lipid were separated by TLC and quantitated. The propagated tillers were smaller compared to tillers obtained from wild-type controls plants grown under the same conditions with the exception of line 03-48. Quantitation by GC-FID of TAG and TFA levels in triplicate leaves confirmed increases in both lipid fractions. Maximum average TAG levels in triplicate leaves (% DW) of lines 02-19 and 03-31 sampled at boot leaf stage were 2.8% and 5.2%, respectively. For all of the transgenic lines, linoleic acid was increased at the expense of α-linolenic acid. However, differences were observed in the levels of palmitic acid

and oleic acid. Lines 02-10 and 02-19 contained increased proportions of oleic acid, whereas palmitic acid was elevated in the TFA and TAG fractions of 03-31 and 03-48 leaves. Lipid quantitation in leaf and stem tissues at seed setting stage revealed considerable leaf-to-leaf variation. Lower TFA and TAG contents were observed in older leaves of wild-type and transgenic propagated tillers. The TFA and TAG levels in the flag leaf of line 03-31 at seed setting equalled 9.9% and 8.4% on a DW basis, respectively. Transgenic stem tissues contained up to about 3% total lipids on a dry weight basis compared to 0.3% in wild-type stems.

Total lipid extracts from the wild-type and transgenic leaves sampled at boot leaf stage were subjected to LC-MS to analyse different neutral and polar lipid classes in more detail. Plants of all four transgenic lines exhibited elevated TAG, amounting to a 100-fold increase in line 03-31 compared to the wild-type control leaves. Small increases in levels of PC were detected in plants of the 03-31 and 03-48 transgenic lines while levels of the plastidial galactolipids MGDG and DGDG were variable, increased in some, decreased in other plants. Both LPC and DAG constituted minor lipid classes. TAG molecular species in plants of lines 03-31 and 03-48 were enriched in palmitic acid and linoleic acid. Major TAG species included TAG (50:2) and TAG (50:3) which contained two palmityl groups and TAG (52:4) and TAG (52:5) which contained palmitoyl and linoloyl groups. In contrast, plants of lines 02-10 and 02-19 exhibited distinctly different TAG profiles. Leaf tissues of both lines preferentially accumulated TAG comprising one or more linolyl chains such as TAG (52:3-5) and TAG (54:4-8). The distinct differences in TAG profiles between the two transgenic populations were consistent with earlier GC-FID results.

Changes in TAG compositions were also reflected in the precursor DAG. Dominant DAG (34:2) and DAG (34:3) molecular species in plants 03-31 and 03-48 were enriched in palmitic acid while both 02-10 and 02-19 plants had DAG molecules containing two C18 acyl chains (DAG 36:2-6). Abundant eukaryotic galactolipid species such as MGDG (36:6) and DGDG (36:6) were either reduced or not significantly affected. Two prokaryotic galactolipid species, MGDG (34:3) and DGDG (34:2) were increased slightly in plants 03-31 and 03-48. The dominant prokaryotic DGDG species (34:3) was either unchanged or reduced in transgenic leaves. PC molecular species containing palmitic or linoleic acid including PC (34:1-2) and PC (36:4) were elevated, particularly in lines 03-31 and 03-48. Di-palmitoyl PC (32:0) was increased in line 03-31, reflecting the higher levels of palmitic acid as detected by GC-FID.

Taken together, these results indicated an increased flux of acyl chains into TAG from PC in the transgenic lines whilst galactolipid biosynthesis mainly occurred via the eukaryotic pathway. These data also led the inventors to understand that reduction of TGD activity or increases in PDCT and/or CPT in the plants in addition to the present transgenes would likely enhance the TFA and TAG levels.

TAG accumulation affects starch and amino acid content

Transitory starch levels in transgenic leaves of lines 03-31 and 03-48 were reduced 7.4- and 15.3-fold on average, respectively. In contrast, starch levels in leaves of 02-10 and 02-19 plants were not significantly affected. Sucrose constituted the dominant leaf soluble sugar in all plants. Sucrose levels were 2-fold lower in line 03-48 while similar to the wild-type control in line 02-19. Raffinose was reduced by 19.6-fold in line 03-48 while monosaccharides such as glucose, fructose and galactose displayed smaller reductions.

A metabolite quantitation by GC-MS identified 36 compounds that were significantly different in leaves of wild-type and transgenic plants. Twenty metabolites were detected at higher levels in TAG-accumulating leaves, including multiple amino acids, urea and the citric acid cycle (TCA) intermediate, α-ketoglutarate. Several dicarboxylic acids, sugar alcohols, fructose, xylose and shikimate were amongst the metabolites that were less abundant in transgenic leaves. Principle component analysis revealed clear separations of both transgenic events and the wild-type control.

Sorghum leaves accumulate TAG as cytosolic lipid droplets

To examine transgenic leaves microscopically to see whether the increased TAG was accumulated in oil droplets, flag leaves of re-established side tillers from transgenic *S. bicolor* plants were harvested at the beginning of flowering and kept on ice until sections were prepared for imaging. Fresh, thin hand sections were stained for 10 min with a solution of 50 mM PIPES pH7 supplemented with 2 μg/ml of BODIPY 505-515 (4,4-Difluoro-1,3,5,7-Tetramethyl-4-Bora-3a,4a-Diaza-s-Indacene, ThermoFisher Scientific). They were then rinsed in a solution of PIPES pH7 and imaged right away. Control sections were placed directly in PIPES pH7 for 10 min before being mounted on slides and imaged.

All samples were imaged with a confocal laser scanning microscope (Leica TCS SP8) equipped with a white light laser and a 40x water immersion objective ([NA]=1.1), and controlled by the LAS X software (Leica Microsystems). Imaging was done in a sequential manner: BODIPY was excited at 505 nm and its emission was

collected at 520-540 nm, while in a separate track, chloroplasts were excited at 633 nm and their auto-fluorescence was collected at 650-690 nm. Maximum projections were generated with the LAS X software. Confocal imaging settings were optimized to distinguish cell types in which oil accumulated by minimizing chloroplast auto-fluorescence in the bundle sheath cells as opposed to the surrounding mesophyll cells.

Leaf cross sections of line 03-10 revealed an abundance of small lipid droplets that preferentially accumulated in the cytosol of mesophyll cells. The unequal distribution likely reflected the tissue specificity of the PEPC promoter used to generate this particular transgenic line. Some lipid accumulation was also visible in the bundle sheath cells of transgenic lines and the wild-type control. Line 02-10 contained an intermediate number of lipid droplets, confirming previous LC-MS and GC-FID TAG quantitation results. Transmission electron micrographs showed densely packed small lipid droplets in the cytosol of mesophyll cells in line 03-31. Mesophyll cells of the wild-type control plants were largely devoid of cytosolic oil droplets.

The chimeric DNA constructs for *Agrobacterium*-mediated transformation are used to transform *Zea mays* (corn) as described by Gould et al. (1991). Briefly, shoot apex explants are co-cultivated with transgenic *Agrobacterium* for two days before being transferred onto a MS salt media containing kanamycin and carbenicillin. After several rounds of sub-culture, transformed shoots and roots spontaneously form and are transplanted to soil. The constructs are similarly used to transform *Hordeum vulgare* (barley) and *Avena sativa* (oats) using transformation methods known for these species. Briefly, for barley, the *Agrobacterium* cultures are used to transform cells in immature embryos of barley (cv. Golden Promise) according to published methods (Tingay et al., 1997; Bartlett et al., 2008) with some modifications in that embryos between 1.5 and 2.5 mm in length are isolated from immature caryopses and the embryonic axes removed. The resulting explants are co-cultivated for 2-3 days with the transgenic *Agrobacterium* and then cultured in the dark for 4-6 weeks on media containing timentin and hygromycin to generate embryogenic callus before being moved to transition media in low light conditions for two weeks. Calli are then transferred to regeneration media to allow for the regeneration of shoots and roots before transfer of the regenerated plantlets to soil. Transformed plants are obtained and grown to maturity in the glasshouse.

Table 3. TFA and TAG levels, fatty acid composition and TTQ in sorghum leaves transformed with pOIL197 or pOIL136 (pZmUbi:DGAT; pZmUbi:Oleosin) during the boot leaf stage of growth. The lines are listed in order of increasing TFA levels.

Line	TAG or TFA	C16:0	C18:0	C18:1	C18:2	C18:3 n3	Other	TFA	TAG	TTQ
TX-197-14	**TFA**	12.7	5.2	2.0	14.4	57.7	8.1	1.2		
TX-197-14	**TAG**	8.8	7.1	3.1	22.7	54.7	3.6		0.3	0.266
TX-197-15	**TFA**	14.5	5.0	2.3	14.7	55.8	7.7	1.2		
TX-197-15	**TAG**	12.7	7.1	3.2	21.0	51.7	4.3		0.3	0.262
TX-197-19	**TFA**	13.1	3.2	2.0	14.3	60.9	6.4	1.2		
TX-197-19	**TAG**	10.6	4.3	3.4	24.4	54.0	3.2		0.2	0.203
TX-136-03	**TFA**	14.1	1.8	1.7	12.6	65.0	4.8	1.2		
TX-136-03	**TAG**	14.5	4.3	4.5	32.9	42.2	1.6		0.1	0.045
TX-197-08	**TFA**	14.4	3.5	1.3	14.2	62.2	4.4	1.2		
TX-197-08	**TAG**	13.7	5.2	2.7	22.4	50.5	5.5		0.3	0.211
TX-197-11	**TFA**	14.1	3.8	2.0	15.0	57.0	8.2	1.3		
TX-197-11	**TAG**	10.3	4.8	3.0	22.8	55.9	3.1		0.3	0.267
TX-136-24	**TFA**	15.5	2.2	2.2	16.9	58.1	5.2	1.3		
TX-136-24	**TAG**	14.7	3.3	4.0	32.4	42.9	2.7		0.2	0.164
TX-136-02	**TFA**	12.3	1.5	1.4	14.7	65.7	4.4	1.5		
TX-136-02	**TAG**	13.9	2.7	3.0	28.7	46.6	5.1		0.7	0.444
TX-197-30	**TFA**	13.1	2.3	1.3	9.3	65.1	8.8	2.0		
TX-197-30	**TAG**	10.0	3.0	2.2	15.0	65.3	4.5		0.4	0.223
TX-197-46	**TFA**	13.2	2.5	0.8	7.9	71.2	4.5	2.0		
TX-197-46	**TAG**	17.3	18.6	3.2	14.7	42.5	3.7		0.1	0.033
TX-197-45	**TFA**	13.6	2.7	0.6	6.7	71.7	4.5	2.1		
TX-197-45	**TAG**	22.7	17.7	4.4	12.9	38.6	3.6		0.1	0.030
TX-197-39	**TFA**	12.6	3.6	1.1	9.0	66.2	7.4	2.1		
TX-197-39	**TAG**	9.5	4.0	1.6	12.8	66.7	5.5		0.6	0.291
TX-197-22	**TFA**	13.6	2.0	0.8	7.3	71.3	4.9	2.1		
TX-197-22	**TAG**	13.8	3.3	1.8	14.2	64.6	2.3		0.1	0.056
TX-197-34	**TFA**	12.0	3.2	1.2	9.6	67.9	5.9	2.2		
TX-197-34	**TAG**	9.1	4.6	2.3	18.4	63.2	2.3		0.4	0.190
TX-197-50	**TFA**	13.0	2.5	1.1	9.1	66.8	7.5	2.5		
TX-197-50	**TAG**	11.4	4.6	2.1	15.3	59.8	6.9		0.5	0.183
TX-197-43	**TFA**	12.4	2.3	0.7	8.0	71.9	4.7	2.5		
TX-197-43	**TAG**	11.0	4.4	1.8	15.7	62.3	4.8		0.2	0.065
TX-197-32	**TFA**	12.5	2.1	1.1	9.0	70.0	5.3	2.5		
TX-197-32	**TAG**	12.8	3.7	2.1	16.1	60.3	5.0		0.6	0.220
TX-197-33	**TFA**	12.1	2.7	0.7	7.9	71.0	5.6	2.5		
TX-197-33	**TAG**	11.1	4.8	1.4	15.4	62.4	4.9		0.3	0.130
TX-197-41	**TFA**	12.8	1.9	0.7	8.1	72.8	3.7	2.6		
TX-197-41	**TAG**	15.1	5.9	2.4	16.7	53.7	6.3		0.2	0.065
TX-197-36	**TFA**	12.2	2.0	0.8	7.7	71.6	5.6	2.6		
TX-197-36	**TAG**	11.4	3.4	1.6	13.9	65.6	4.1		0.4	0.158
TX-197-42	**TFA**	12.4	2.1	0.8	8.2	70.3	6.3	2.7		
TX-197-42	**TAG**	12.4	5.4	2.3	17.8	57.1	5.0		0.2	0.060

TX-197-51	**TFA**	13.6	2.1	1.0	9.9	66.8	6.6	2.7		
TX-197-51	**TAG**	13.1	4.6	3.0	18.8	53.4	7.0		0.5	0.175
TX-197-49	**TFA**	15.2	2.9	1.0	9.3	65.3	6.3	2.7		
TX-197-49	**TAG**	17.3	5.0	2.0	16.7	52.7	6.3		0.5	0.192
TX-197-48	**TFA**	13.0	2.3	1.0	8.8	68.5	6.4	2.8		
TX-197-48	**TAG**	13.0	4.7	2.2	16.1	58.0	6.0		0.4	0.144
TX-197-38	**TFA**	12.2	2.0	1.0	7.7	72.1	5.0	2.9		
TX-197-38	**TAG**	11.2	3.4	2.2	14.9	63.8	4.5		0.5	0.160
TX-197-35	**TFA**	12.8	1.8	0.9	8.5	69.4	6.6	2.9		
TX-197-35	**TAG**	12.7	2.9	1.7	14.5	63.3	4.9		0.7	0.227
TX-197-40	**TFA**	12.7	1.9	0.7	7.7	73.9	3.1	2.9		
TX-197-40	**TAG**	16.3	4.7	3.3	20.8	52.4	2.6		0.1	0.031
TX-197-47	**TFA**	13.9	2.4	0.6	6.9	72.2	3.9	2.9		
TX-197-47	**TAG**	24.6	19.8	5.2	10.7	34.8	4.9		0.0	0.017
TX-136-01	**TFA**	11.6	1.4	1.3	14.1	67.2	4.3	3.3		
TX-136-01	**TAG**	14.6	2.9	3.0	29.5	44.1	5.9		0.7	0.199
TX-197-44	**TFA**	13.5	2.1	1.4	14.7	63.1	5.1	3.4		
TX-197-44	**TAG**	14.4	4.3	3.1	25.0	45.0	8.2		0.8	0.245
TX-136-25	**TFA**	13.6	2.2	0.7	10.8	67.4	5.2	3.4		
TX-136-25	**TAG**	16.6	4.2	1.4	20.1	51.5	6.1		1.0	0.286
TX-197-28	**TFA**	11.5	1.3	0.4	7.8	75.3	3.6	3.4		
TX-197-28	**TAG**	17.4	4.5	1.6	19.5	50.2	6.9		0.1	0.035
TX-197-37	**TFA**	12.6	3.4	6.3	17.4	54.1	6.2	4.5		
TX-197-37	**TAG**	13.4	5.0	10.1	27.4	40.2	3.9		1.9	0.426

Table 4. TFA and TAG levels, fatty acid composition and TTQ in sorghum leaves transformed with pOIL102 (pZmUbi:WRI1) during the boot leaf stage of growth.

Line	TAG or TFA	C16:0	C18:0	C18:1	C18:2	C18:3 n3	Other	TFA	TAG	TTQ
TX-102-8	**TFA**	16.9	4.2	2.3	12.3	57.7	6.5	0.9		
TX-102-8	**TAG**	14.5	6.2	13.5	25.7	36.8	3.4		0.2	0.243
TX-102-4	**TFA**	17.1	4.2	2.0	12.5	57.5	6.7	0.9		
TX-102-4	**TAG**	10.5	4.4	3.0	20.0	59.6	2.6		0.2	0.182
TX-102-1	**TFA**	16.6	4.3	3.9	15.4	50.7	9.1	1.1		
TX-102-1	**TAG**	10.7	4.4	5.3	21.9	54.1	3.6		0.3	0.273
TX-102-5	**TFA**	16.7	4.1	1.7	11.6	60.2	5.8	1.1		
TX-102-5	**TAG**	11.7	5.5	2.8	21.4	56.1	2.5		0.1	0.118
TX-102-6	**TFA**	17.8	3.8	15.9	17.0	38.8	6.6	1.5		
TX-102-6	**TAG**	19.6	7.0	29.4	25.4	13.9	4.7		0.4	0.267
TX-102-2	**TFA**	15.0	1.9	1.7	19.1	56.5	5.9	1.7		
TX-102-2	**TAG**	10.6	1.9	2.7	30.2	51.2	3.4		0.4	0.258
TX-102-7	**TFA**	15.0	3.1	7.0	13.9	56.1	4.9	2.4		

TX-102-7	**TAG**	16.1	6.5	20.5	28.0	24.4	4.5		0.3	0.111
TX-102-3	**TFA**	14.4	3.5	9.5	13.4	50.9	8.2	2.5		
TX-102-3	**TAG**	16.9	6.7	23.9	24.7	22.5	5.2		0.4	0.150

Table 5. TFA and TAG levels, fatty acid composition and TTQ in sorghum leaves transformed with pOIL102 (pZmUbi:WRI1) and pOIL197 (pZmUbi:DGAT and pZmUbi:Oleosin) during the boot leaf stage of growth. The lines are listed in order of increasing TFA levels.

Line	TAG or TFA	C16 :0	C18:0	C18:1	C18:2	C18:3 n3	Other	TFA	TAG	TTQ
TX-02-27	**TFA**	17.3	3.8	1.4	10.1	60.1	7.2	1.0		
TX-02-27	**TAG**	11.9	4.4	2.1	19.4	61.2	0.8		0.2	0.164
TX-02-21	**TFA**	15.9	2.3	2.0	19.3	53.3	7.3	1.2		
TX-02-21	**TAG**	12.6	3.7	2.7	27.0	51.0	3.0		0.4	0.318
TX-02-01	**TFA**	15.2	4.2	5.1	14.7	53.2	7.5	1.3		
TX-02-01	**TAG**	11.7	5.6	9.3	26.1	42.9	4.5		0.3	0.199
TX-02-12	**TFA**	15.3	3.2	2.0	13.6	58.9	6.9	1.3		
TX-02-12	**TAG**	13.7	4.2	3.6	25.1	50.4	2.9		0.1	0.111
TX-02-33	**TFA**	15.9	4.3	1.0	10.1	59.7	9.1	1.4		
TX-02-33	**TAG**	14.3	5.4	2.7	18.9	54.7	4.0		0.1	0.107
TX-02-13	**TFA**	15.4	5.1	11.4	19.4	39.1	9.5	1.4		
TX-02-13	**TAG**	12.9	6.5	20.3	25.2	28.6	6.4		0.5	0.389
TX-02-36	**TFA**	16.2	3.4	1.8	12.3	58.5	7.8	1.4		
TX-02-36	**TAG**	15.4	5.8	3.3	21.5	48.9	5.1		0.3	0.209
TX-02-37	**TFA**	13.3	3.5	1.3	9.9	65.3	6.7	1.4		
TX-02-37	**TAG**	9.6	3.6	3.8	20.4	60.6	2.1		0.2	0.137
TX-02-18	**TFA**	14.6	3.0	1.4	9.8	65.5	5.7	1.4		
TX-02-18	**TAG**	12.5	5.6	4.3	20.6	54.8	2.3		0.1	0.077
TX-02-34	**TFA**	16.6	2.2	2.2	17.6	54.7	6.7	1.4		
TX-02-34	**TAG**	14.1	2.8	4.1	30.3	44.7	4.1		0.3	0.231
TX-02-31	**TFA**	13.3	3.1	1.8	10.1	64.7	7.0	1.5		
TX-02-31	**TAG**	5.4	1.8	3.2	17.8	71.1	0.7		0.3	0.171
TX-02-29	**TFA**	13.2	3.2	1.1	8.2	68.6	5.6	1.6		
TX-02-29	**TAG**	10.5	4.7	2.9	18.1	62.0	1.8		0.1	0.082
TX-02-35	**TFA**	17.8	3.4	6.5	14.0	50.3	8.0	1.6		
TX-02-35	**TAG**	18.8	5.3	19.1	28.4	22.4	6.1		0.2	0.108
TX-02-09	**TFA**	14.0	3.3	0.9	9.9	66.0	6.0	1.6		
TX-02-09	**TAG**	11.2	4.7	1.9	19.6	58.7	3.9		0.1	0.036
TX-02-24	**TFA**	12.9	3.5	0.6	7.9	67.3	7.7	1.8		
TX-02-24	**TAG**	10.7	3.5	1.6	11.8	69.0	3.4		0.1	0.044
TX-02-126	**TFA**	13.8	2.7	1.1	9.9	66.4	6.0	1.8		

TX-02-126	**TAG**	12.8	4.3	2.1	17.0	58.6	5.2		0.5	0.247
TX-02-23	**TFA**	13.6	2.7	0.7	8.9	68.3	5.8	1.9		
TX-02-23	**TAG**	10.0	3.3	2.2	18.2	63.9	2.4		0.1	0.047
TX-02-07	**TFA**	17.5	2.3	10.9	17.5	44.5	7.3	1.9		
TX-02-07	**TAG**	21.0	3.9	24.5	27.4	15.2	8.0		0.4	0.225
TX-02-28	**TFA**	12.8	2.9	0.5	7.7	68.4	7.8	2.0		
TX-02-28	**TAG**	13.0	5.5	1.2	11.1	64.3	4.8		0.1	0.063
TX-02-04	**TFA**	13.6	2.9	1.2	12.1	65.3	4.9	2.1		
TX-02-04	**TAG**	12.0	4.4	2.4	21.6	55.9	3.6		0.4	0.206
TX-02-25	**TFA**	12.2	2.8	0.5	9.4	68.8	6.3	2.5		
TX-02-25	**TAG**	10.3	4.2	1.0	15.4	62.5	6.6		0.4	0.159
TX-02-05	**TFA**	13.6	3.6	3.2	14.7	59.8	5.1	2.5		
TX-02-05	**TAG**	12.2	5.5	7.0	26.8	43.4	5.1		0.6	0.220
TX-02-14	**TFA**	15.9	5.7	30.9	12.7	26.0	8.9	2.8		
TX-02-14	**TAG**	17.9	8.5	42.6	14.9	7.8	8.4		1.4	0.514
TX-02-131	**TFA**	12.6	1.4	0.6	8.3	73.1	3.9	2.9		
TX-02-131	**TAG**	16.0	3.9	1.9	18.0	53.9	6.3		0.2	0.061
TX-02-129	**TFA**	12.1	1.6	1.0	10.4	70.5	4.3	2.9		
TX-02-129	**TAG**	12.8	3.6	2.5	22.0	53.6	5.5		0.3	0.106
TX-02-08	**TFA**	17.6	2.6	5.6	17.2	51.2	5.8	3.0		
TX-02-08	**TAG**	24.4	5.9	15.8	29.3	15.8	8.8		0.6	0.183
TX-02-02	**TFA**	17.9	3.1	7.2	15.5	49.6	6.7	3.1		
TX-02-02	**TAG**	23.7	6.5	17.7	22.8	19.6	9.7		0.6	0.194
TX-02-11	**TFA**	25.1	4.1	9.0	16.3	36.3	9.1	3.2		
TX-02-11	**TAG**	33.3	6.6	13.9	20.9	16.0	9.3		1.1	0.341
TX-02-127	**TFA**	11.4	1.6	0.3	8.9	75.4	2.4	3.5		
TX-02-127	**TAG**	21.0	5.8	1.4	20.6	47.4	3.9		0.1	0.016
TX-02-30	**TFA**	16.4	3.1	3.7	17.1	53.8	5.9	4.0		
TX-02-30	**TAG**	21.3	5.0	7.6	27.1	30.5	8.5		0.9	0.236
TX-02-19	**TFA**	13.5	2.7	25.4	22.6	30.8	5.0	4.2		
TX-02-19	**TAG**	14.0	3.3	34.3	27.0	16.6	4.8		2.3	0.548
TX-02-06	**TFA**	24.0	4.8	14.3	19.6	29.7	7.7	4.8		
TX-02-06	**TAG**	29.7	6.9	19.2	23.0	13.4	7.7		2.7	0.555
TX-02-10	**TFA**	22.0	3.3	10.3	22.7	33.7	7.9	6.3		
TX-02-10	**TAG**	24.8	4.1	12.9	27.0	22.4	8.8		3.5	0.551
TX-02-38	**TFA**	24.8	4.4	13.9	24.5	23.7	8.7	6.4		
TX-02-38	**TAG**	21.5	5.3	8.6	25.2	39.3	0.0		2.5	0.392

Table 6. TFA and TAG levels, fatty acid composition and TTQ in pOIL103+pOIL197 primary transformants at boot leaf stage.

Line	**TFA or TAG**	**C16:0**	**C18:0**	**C18:1**	**C18:2**	**C18:3n3**	**Other**	**TFA**	**TAG**	**TTQ**

TX-03-20	**TFA**	12.2	2.6	1.7	10.3	67.5	5.7	2.1		
TX-03-20	**TAG**	9.4	3.6	3.3	18.1	63.0	2.5		0.4	0.217
TX-03-54	**TFA**	13.6	3.5	3.0	12.1	61.5	6.4	2.1		
TX-03-54	**TAG**	14.1	6.9	7.0	22.5	43.5	6.0		0.4	0.207
TX-03-61	**TFA**	23.9	3.1	1.7	19.0	43.9	8.3	2.2		
TX-03-61	**TAG**	31.4	6.6	3.4	28.3	19.6	10.8		0.4	0.159
TX-03-02	**TFA**	14.9	3.0	2.8	12.1	60.6	6.6	2.2		
TX-03-02	**TAG**	14.8	5.5	5.6	20.6	46.7	6.8		0.5	0.222
TX-03-53	**TFA**	18.5	3.7	8.9	15.4	43.1	10.4	2.3		
TX-03-53	**TAG**	20.1	6.8	16.7	24.5	23.3	8.6		0.6	0.275
TX-03-01	**TFA**	13.4	3.0	3.0	12.5	61.8	6.4	2.3		
TX-03-01	**TAG**	13.9	5.5	7.5	23.0	42.6	7.4		0.4	0.164
TX-03-47	**TFA**	12.8	2.1	1.6	7.5	70.7	5.3	2.4		
TX-03-47	**TAG**	14.8	5.1	5.0	19.3	52.1	3.7		0.1	0.050
TX-03-07	**TFA**	18.4	2.8	7.6	15.6	47.1	8.5	2.5		
TX-03-07	**TAG**	25.8	6.4	18.7	25.5	15.2	8.5		0.3	0.127
TX-03-05	**TFA**	21.4	2.3	1.4	9.7	59.1	6.1	2.6		
TX-03-05	**TAG**	36.4	5.6	3.9	17.1	28.4	8.6		0.4	0.168
TX-03-49	**TFA**	18.1	3.7	8.2	13.2	52.0	4.9	2.6		
TX-03-49	**TAG**	24.1	8.2	18.3	20.9	18.8	9.7		0.5	0.212
TX-03-34	**TFA**	19.0	2.7	6.0	15.4	50.6	6.4	2.6		
TX-03-34	**TAG**	24.8	10.5	10.9	23.9	20.6	9.3		0.8	0.287
TX-03-32	**TFA**	18.2	2.2	1.6	12.4	60.2	5.4	2.8		
TX-03-32	**TAG**	20.8	14.6	3.2	21.4	31.5	8.5		0.6	0.204
TX-03-04	**TFA**	18.8	3.1	5.8	13.4	50.3	8.6	2.9		
TX-03-04	**TAG**	26.7	7.5	14.6	23.1	19.0	9.1		0.3	0.118
TX-03-23	**TFA**	18.9	1.7	1.0	7.9	63.2	7.3	2.9		
TX-03-23	**TAG**	25.0	4.6	2.5	18.1	39.6	10.2		0.2	0.070
TX-03-25	**TFA**	14.5	1.8	0.4	6.4	73.5	3.4	3.0		
TX-03-25	**TAG**	20.3	5.1	1.0	12.3	53.6	7.7		0.3	0.110
TX-03-18	**TFA**	21.1	2.9	1.2	17.8	46.3	10.7	3.0		
TX-03-18	**TAG**	22.6	5.9	4.5	31.1	22.6	13.3		0.4	0.143
TX-03-50	**TFA**	16.5	2.6	6.1	12.9	53.9	8.0	3.0		
TX-03-50	**TAG**	20.2	19.9	12.9	19.6	20.6	6.8		0.7	0.217
TX-03-60	**TFA**	20.2	2.9	0.8	14.1	55.7	6.2	3.1		
TX-03-60	**TAG**	30.5	6.2	1.6	21.6	30.2	9.9		0.6	0.202
TX-03-21	**TFA**	12.3	1.7	0.5	6.8	74.4	4.4	3.2		
TX-03-21	**TAG**	16.1	4.7	1.6	13.1	57.0	7.5		0.2	0.067
TX-03-40	**TFA**	17.1	1.4	0.4	8.0	68.2	4.9	3.2		
TX-03-40	**TAG**	34.5	4.4	0.9	14.5	39.8	5.9		0.4	0.112
TX-03-62	**TFA**	25.3	2.9	1.7	14.7	47.9	7.6	3.3		
TX-03-62	**TAG**	40.3	5.6	3.5	22.3	18.7	9.5		0.6	0.171
TX-03-36	**TFA**	19.5	2.0	2.0	11.4	58.3	6.8	3.5		
TX-03-36	**TAG**	31.2	4.0	4.4	20.0	29.4	11.0		0.6	0.160
TX-03-63	**TFA**	25.4	3.6	2.6	18.2	42.0	8.2	3.5		
TX-03-63	**TAG**	33.1	6.1	3.8	24.9	21.6	10.4		1.4	0.383
TX-03-45	**TFA**	16.4	1.4	0.5	8.1	69.1	4.5	3.5		
TX-03-45	**TAG**	30.8	4.6	1.4	16.2	40.7	6.3		0.2	0.058
TX-03-17	**TFA**	14.2	1.8	0.8	6.9	71.2	5.2	3.6		

TX-03-17	**TAG**	18.7	4.5	2.2	13.5	52.8	8.3		0.4	0.120
TX-03-57	**TFA**	18.7	3.4	1.5	13.8	55.8	6.8	3.6		
TX-03-57	**TAG**	23.4	6.3	3.0	21.0	36.2	10.1		1.2	0.330
TX-03-11	**TFA**	29.1	6.4	2.1	22.4	33.0	7.1	3.6		
TX-03-11	**TAG**	30.6	8.5	2.8	27.0	19.7	11.4		1.9	0.510
TX-03-48	**TFA**	27.1	3.7	3.7	20.6	37.2	7.6	3.7		
TX-03-48	**TAG**	31.2	5.0	5.5	27.1	23.0	8.1		2.1	0.569
TX-03-29	**TFA**	20.1	2.3	1.7	13.4	55.5	7.1	3.7		
TX-03-29	**TAG**	33.0	5.0	4.1	24.3	26.4	7.2		0.4	0.104
TX-03-26	**TFA**	15.3	1.6	0.4	5.9	71.3	5.5	3.9		
TX-03-26	**TAG**	25.2	4.6	1.7	13.3	49.7	5.5		0.3	0.074
TX-03-10	**TFA**	28.6	6.8	2.1	21.8	33.0	7.7	3.9		
TX-03-10	**TAG**	31.0	8.5	2.9	26.7	18.6	12.2		1.9	0.491
TX-03-58	**TFA**	16.3	2.6	1.3	14.5	60.3	5.0	4.1		
TX-03-58	**TAG**	20.4	5.2	2.8	24.3	39.2	8.2		1.1	0.278
TX-03-08	**TFA**	19.8	2.0	0.7	6.6	64.9	5.9	4.1		
TX-03-08	**TAG**	34.8	5.2	2.7	14.3	34.5	8.5		0.2	0.051
TX-03-33	**TFA**	27.4	2.4	1.5	16.3	46.0	6.4	4.2		
TX-03-33	**TAG**	39.2	5.4	2.3	21.9	20.8	10.5		1.6	0.386
TX-03-22	**TFA**	19.8	2.8	3.1	11.8	53.4	9.1	4.2		
TX-03-22	**TAG**	28.4	5.3	5.4	19.4	38.3	3.2		1.2	0.287
TX-03-41	**TFA**	18.1	2.6	3.1	11.1	58.0	7.1	4.8		
TX-03-41	**TAG**	27.8	6.0	6.8	19.3	34.9	5.3		0.7	0.139
TX-03-46	**TFA**	24.6	2.0	0.6	7.9	57.4	7.4	4.9		
TX-03-46	**TAG**	44.7	4.2	1.3	13.4	31.4	5.0		1.1	0.220
TX-03-28	**TFA**	28.5	2.1	1.3	23.4	33.7	11.0	6.2		
TX-03-28	**TAG**	36.0	2.9	3.1	29.6	18.5	10.0		3.7	0.596
TX-03-31	**TFA**	33.4	2.9	4.3	28.6	25.5	5.5	8.3		
TX-03-31	**TAG**	38.0	3.6	4.9	30.6	14.8	8.1		6.6	0.789

Example 3. Increasing expression of thioesterase in plant cells

De novo fatty acid synthesis takes place in the plastids of eukaryotic cells where the fatty acids are synthesized while bound to acyl carrier protein as acyl-ACP conjugates. Following chain elongation to C16:0 and C18:0 acyl groups and then desaturation to C18:1 while linked to ACP, the fatty acids are cleaved from the ACP by thioesterases and enter the eukaryotic pathway by export from the plastids and transport to the ER where they participate in membrane and storage lipid biogenesis. In chloroplasts, the export process has two steps: firstly, acyl chains are released as free fatty acids by the enzymatic activity of acyl-ACP thioesterases (fatty acyl thioesterase; FAT), secondly by reaction with CoA to form acyl-CoA esters which is catalysed by long chain acyl-CoA synthetases (LACS). *A. thaliana* contains 3 fatty acyl thioesterases which can be distinguished based on their acyl chain specificity. FATA1 and FATA2 preferentially hydrolyze unsaturated acyl-ACPs while saturated acyl-ACP chains are typically cleaved by FATB.

To explore the effect upon total fatty acid content, TAG content, and fatty acid composition of the co-expression of a thioesterase and genes encoding the WRI1 and/or DGAT polypeptides, chimeric genes were made for each of the three *A. thaliana* thioesterases by insertion of the coding regions into the pJP3343 binary expression vector for transient expression in *N. benthamiana* leaf cells from the 35S promoter. Protein coding regions for the *A. thaliana* FATA1 (Accession No. NP_189147.1, SEQ ID NO:43) and FATA2 (Accession No. NP_193041.1, SEQ ID NO:44) thioesterases were amplified from silique cDNA using primers containing *EcoR*I and *Pst*I sites and subsequently cloned into pJP3343 using the same restriction sites. The resulting expression vectors were designated pOIL079 and pOIL080, respectively. The protein coding region of the *A. thaliana FATB* gene (Accession No. NP_172327.1, SEQ ID NO:45) was amplified using primers containing *Not*I and *Sac*I flanking sites and cloned into the corresponding restriction sites of pJP3343, resulting in pOIL081. Constructs pOIL079, pOIL080 and pOIL081 are infiltrated into *N. benthamiana* leaf tissue, either individually or in combination with constructs containing the genes for the *A. thaliana* WRI1 transcription factor (AtWRI1) (pJP3414) and/or DGAT1 acyltransferase (AtDGAT1) (pJP3352). For comparison, chimeric genes encoding the *Cocos nucifera* FatB1 (CnFATB1) (pJP3630), *C. nucifera* FatB2 (CnFATB2) (pJP3629) were introduced into *N. benthamiana* leaf tissue in parallel with the *Arabidopsis* thioesterases, to compare the effect of the FatB polypeptides having MCFA specificity to the *Arabidopsis* thioesterases which do not have MCFA specificity. All of the infiltrations included a chimeric gene for expression of the p19 silencing suppressor as described in Example 1. The negative control infiltrated only the p19 T-DNA.

A synergistic effect was observed between thioesterase expression and WRI1 and/or DGAT over-expression on TAG levels in *N. benthamiana* leaves. Expression of the thioesterase genes without the WRI1 or DGAT genes significantly increased TAG levels above the low level in the negative control (p19 alone). For example, expression of the coconut FATB2 thioesterase resulted in an 8.2-fold increase in TAG levels in the leaves compared to the negative control. Co-expression of the *A. thaliana* WRI1 transcription factor with each of the thioesterases further increased TAG levels compared to the AtWRI1 control. Co-expression of each of the coconut thioesterases CnFATB1 and CnFATB2 with WRI1 resulted in higher TAG levels than each of the three *A. thaliana* thioesterases with WRI1. Interestingly, the converse was observed when the *A. thaliana* DGAT1 acyltransferase was co-expressed in combination with a thioesterase and WRI1. This suggested a better match in acyl-chain specificity of the *A. thaliana* thioesterases and the *A. thaliana* DGAT1 acyltransferase, resulting in a greater

flux of acyl-chains from the acyl-ACP into TAG. The non-MCFA thioesterases were also considerably more effective in elevating the percentage of oleic acid in the total fatty acid content in the leaves. Co-expression of the AtWRI1, AtDGAT1 and AtFATA2 resulted in the greatest level of TAG in the leaves, providing a level which was 1.6-fold greater than when AtWRI1 and AtDGAT1 were co-expressed without the thioesterase. In another experiment, transient overexpression of FATA2 in combination with WRI1 and DGAT1 led to a 2.5-fold increase in TAG level relative to a p19+WRI1+DGAT1 control, which represented a 50-fold increase in TAG levels relative to p19 alone. Addition of FATA1 increased TAG levels 2-fold compared to p19+WRI1+DGAT1, a 40-fold increase compared to p19 alone. Addition of FATB increased TAG levels by 1.6-fold relative to p19+WRI1+DGAT1, a 32-fold increase relative to p19 control.

Co-expression of thioesterase FATA or FATB together with WRI1 and DGAT1 resulted in modified leaf fatty acid composition relative to WRI1 and DGAT1 without thioesterase. Addition of FATA1 increased the percentages of C16:0 and C18:0 at the expense of saturated fatty acids. Addition of FATA2 also increased the proportion of C18:0 but did not have as great an effect on C16:0. In contrast, addition of FATB increased C16:0 but not C18:0 levels. In each case, addition of FATA1, FATA2 and FATB reduced C18:1 levels. Notably, the C16:0 percentage increased from 28.4% in p19+WRI1+DGAT1 without thioesterase to 43.8% with the addition of FATA1, to 34.4% with the addition of FATA2 and to 46.3% with the addition of FATB.

These experiments confirmed the synergistic increase in oil synthesis and accumulation when both WRI1 and DGAT were co-expressed as well as showing the further synergistic increase obtained by adding a thioesterase to the combination.

Effect of transient thioesterase expression in a high oil background

The three *A. thaliana* thioesterase genes were also tested by transient expression in leaves of *N. benthamiana* plants (transgenic line AT001) which were transgenic for and stably expressing WRI1, DGAT1 and OLEOSIN genes (El Tahchy et al., 2017). Thirty plants from homozygous, T2 generation, transgenic AT001 seeds were grown in a randomised design alongside wild-type (WT) controls. At a vegetative stage of growth, 53 days after sowing (DAS), the transgenic leaves contained about 8.7% (DW) TAG compared to about 0.03% (DW) TAG in the wild-type plants. After further growth of the transgenic plants, TAG levels increased from about 11.2% to about 21.3% (DW) during flowering stages. They continued to increase, reaching about

31.4% (DW) TAG at maturity (late seed development stage). As the plants senesced, the TAG level in at least some plants decreased to about 19.6% DW.

The genes encoding the thioesterases were introduced into leaves of young plants (49 DAS) when the leaves typically had about 3.1% (DW) TAG, and sampled 5 days after infiltration with the *Agrobacterium* strains. Leaf samples were harvested and analyzed for TAG content. FATA2 overexpression in AT001 *N. benthamiana* leaves significantly increased TAG to 4.4% (DW) compared to the p19 control (3.1% TAG). FATA1 increased TAG content to 3.9% (DW). FATB transient expression did not appear to increase TAG accumulation in this experiment.

Samples were also used in radiolabel feeding assays with [^{14}C]-acetate. [^{14}C]-acetate was added in a 10 minute pulse to leaf discs of AT001 leaves, infiltrated previously with genes encoding p19 and one of FATA1, FATA2 and FATB. This pulse was followed by a 20 minute chase. Lipid extracts were prepared at each time point followed by separation of labelled lipid classes on TLC. Quantitation of the labelled reaction products showed increases in the rate of TAG production in the AT001 leaves transiently expressing FATA1 (602 DPM), FATA2 (762 DPM) and FATB (559 DPM) compared to the p19 control (283 DPM).

Three different binary expression vectors were constructed to test the effect of co-expression of genes encoding WRI1, DGAT1 and FATA on TAG levels and fatty acid composition in stably transformed *N. tabacum* leaves. The vector pOIL121 contained an *SSU::AtWRI1* gene for expression of AtWRI1 from the SSU promoter, a *35S::AtDGAT1* gene for expression of AtDGAT from the 35S promoter, and an *enTCUP2::AtFATA2* gene for expression of AtFATA2 from the enTCUP2 promoter which is a constitutive promoter. These genetic constructs were derived from pOIL38 by first digesting the DNA with *Not*I to remove the gene coding for the *S. indicum* oleosin. The protein coding region of the *A. thaliana FATA2* gene was amplified and flanked with *Not*I sites using pOIL80 DNA as template. This fragment was then inserted into the *Not*I site of pOIL38. pOIL121 then served as a parent vector for pOIL122 which contained an additional enTCUP2::SDP1 hairpin RNA cassette for RNAi-mediated silencing of the endogenous SDP1 gene in the transgenic plants. To do this, the entire *N. benthamiana* SDP1 hairpin cassette was isolated from pOIL51 (Vanhercke et al., 2017) as an *Sfo*I-*Sma*I fragment and cloned into the *Sfo*I site of pOIL121, producing pOIL122 (Figure 2). A third vector, pOIL123, containing the *SSU::WRI1* and *35S::DGAT1* genes and the *enTCUP2::SDP1* hairpin RNA gene was obtained in a similar way by cloning the enTCUP2::SDP1 hairpin RNA cassette as a *Sfo*I-*Sma*I fragment into the *Sfo*I site of pOIL36.

In summary, the vectors contained the gene combinations:

pOIL121: SSU::AtWRI1, 35S::AtDGAT1, enTCUP2::AtFATA2.

pOIL122: SSU::AtWRI1, 35S::AtDGAT1, enTCUP2::AtFATA2, enTCUP2::SDP1 hairpin.

pOIL123: SSU::AtWRI1, 35S::AtDGAT1, enTCUP2::SDP1 hairpin.

The three constructs were each used to produce transformed *N. tabacum* plants (cultivar Wi38) by *Agrobacterium*-mediated transformation. Co-expression of the *A. thaliana* FATA2 thioesterase or silencing of the endogenous SDP1 TAG lipase in combination with AtWRI1 and AtDGAT1 expression each resulted in further elevated TAG levels compared to expression of AtWRI1 and AtDGAT1 in the absence of both of the thioesterase gene and the SDP1-silencing gene. The greatest TAG yields were obtained using pOIL122 by the combined action of all four chimeric genes. In absence of SDP1, pOIL121 lines yielded 13.3% TAG which was included increased palmitate (16:0) levels (36%) and reduced ALA (18:3ω3) levels (7%).

It is noted that *N. benthamiana* is an 18:3 plant. The same constructs pOIL079, pOIL080 and pOIL081 are used to transform *A. thaliana*, a 16:3 plant.

The inventors conceived of the model that increasing plastidial fatty acid export such as by increased fatty acyl thioesterase activity reduces acyl-ACP accumulation in the plastids, thereby increasing fatty acid biosynthesis as a result of reduced feedback inhibition on the acetyl-CoA carboxylase (ACCase) (Andre et al., 2012; Moreno-Perez et al., 2012). Thioesterase over-expression increases export of acyl chains from the plastids into the ER, thereby providing an efficient link between so-called ‘Push’ and ‘Pull’ metabolic engineering strategies.

Example 4. The effect of different transcription factor polypeptides on plant traits

Previously reported experiments with WRI1 and DGAT (Vanhercke et al., 2013) used a synthetic gene encoding *A. thaliana* AtWRI1 (Accession No. AAP80382.1) and a synthetic gene encoding AtDGAT1, also from *A. thaliana* (Accession No. AAF19262; SEQ ID NO: 1). To compare other WRI polypeptides with AtWRI1 for their ability to combine with DGAT to increase oil content, other WRI coding sequences were identified and used to generate constructs for expression in *N. benthamiana* leaves. Nucleotide sequences encoding the *A. thaliana* WRI3 (Accession No. AAM91814.1, SEQ ID NO:46) and WRI4 (Accession No. NP_178088.2, SEQ ID NO:47) transcription factors (To et al., 2012) were synthesized and inserted as *Eco*RI fragments into pJP3343 under the control of the 35S promoter. The resulting binary expression vectors were designated pOIL027 and pOIL028, respectively. The coding

sequence for the oat (*Avena sativa*) WRI1 (AsWRI1, SEQ ID NO:48) was PCR amplified from a vector provided by Prof. Sten Stymne (Swedish University of Agricultural Sciences) using flanking primers containing additional *Eco*RI sites. The amplified fragment was inserted into pJP3343 resulting in pOIL055. A WRI1 candidate sequence from *S. bicolor* (Accession No. XP_002450194.1, SEQ ID NO:49) was identified by a BLASTp search on the NCBI server using the *Zea mays* WRI1 amino acid sequence (Accession No. NP_001137064.1, SEQ ID NO:50) as query. The protein coding region of the *S. bicolor WRI1* gene (*SbWRI1*) was synthesized and inserted as an *Eco*RI fragment into pJP3343, yielding pOIL056. A gene candidate encoding a WRI1 was identified from the Chinese tallow (*Triadica sebifera*; TsWRI1, SEQ ID NO:51) transcriptome (Uday et al., submitted). The protein coding region was synthesized and inserted as an *Eco*RI fragment into pJP3343 resulting in pOIL070. The pJP3414 and pJP3352 binary vectors containing the coding sequences for expression of the *A. thaliana* WRI1 and DGAT1 polypeptides were as described by Vanhercke et al. (2013).

Plasmids containing the various WRI coding sequences were introduced into *N. benthamiana* leaf tissue for transient expression using a gene encoding the p19 viral suppressor protein in all inoculations as described in Example 1. The genes encoding the WRI polypeptides were either tested alone or in combination with the DGAT1 acyltransferase gene, the latter to provide greater TAG biosynthesis and accumulation. The positive control in this experiment was the combination of the genes encoding *A. thaliana* WRI1 transcription factor and AtDGAT1. All infiltrations were done in triplicate using three different plants and TAG levels were analyzed as described in Example 1. Expression of most of the individual WRI polypeptides in the absence of exogenously added DGAT1 resulted in increased, yet still low, TAG levels (< 0.23% on dry weight basis) in infiltrated leaf spots, compared to the control which had only the p19 construct (Figure 3). The exception was TsWRI1 which, by itself, did not appear to increase TAG levels significantly. In addition, differences in TAG levels produced by expression of the different WRI transcription factors on their own were not great. Both AsWRI1 and SbWRI1 yielded TAG levels similar to AtWRI1 on its own. Analysis of the TAG fatty acid composition revealed only minor changes except for increased C18:1Δ9 levels from expression of AtWRI3 in the infiltrated leaf tissues (Table 7).

In contrast, differences in0 TAG yields from expression of the different WRI polypeptides were more pronounced upon co-expression with the AtDGAT1 acyltransferase. This again demonstrated the synergistic effect of WRI1 and DGAT co-expression on TAG biosynthesis in infiltrated *N. benthamiana* leaf tissue, as reported

by Vanhercke et al. (2013). Intermediate TAG levels were observed upon co-expression of DGAT1 with AtWRI3, AtWRI4 and TsWRI1 expressing vectors while levels obtained with the AsWRI1 and AtWRI1 were significantly lower. In a result that could not have been predicted beforehand, the highest TAG yields were obtained with co-expression of DGAT with SbWRI1, even though the assay was done in dicotyledonous cells. TAG fatty acid composition analysis revealed increased levels of C18:1$^{\Delta 9}$ and decreased levels of C18:3$^{\Delta 9,12,15}$ (ALA) in the case of SbWRI1, AsWRI1 and the AtWRI1 positive control. Unlike AtWRI1, however, expression of AsWRI1 and SbWRI1 both displayed increased C16:0 levels compared to the p19 negative control. Interestingly, AtWRI3 infiltrated leaf samples exhibited a distinct TAG profile with C18:1$^{\Delta 9}$ being enriched while C16:0 and ALA were only slightly affected.

This experiment showed that the *S. bicolor* WRI1 transcription factor, SbWRI1, was superior to AtWRI1 when co-expressed with DGAT to increase TAG levels in vegetative plant parts. The inventors also concluded that a transcription factor, for example a WRI1, from a monocotyledonous plant could function well in a dicotyledonous plant cell, indeed might even have superior activity compared to a corresponding transcription factor from a dicotyledonous plant. Likewise, a transcription factor from a dicotyledonous plant could function well in a monocotyledonous plant cell.

Table 7. TAG fatty acid composition in *N. benthamiana* leaf samples infiltrated with different chimeric genes for expression of WRI (n=3). All samples were also infiltrated with the P19 construct. The TAG samples also contained 0.1-0.4% C14:0; 0.5-1.2% C16:3 and; 0.1-0.7% C18:1Δ11.

Infiltrated genes	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3n3	C20:0	C20:1	C22:0	C24:0
Control (P19)	33.6 ± 4.7	0.5 ± 0.4	8.9 ± 2.2	4.7 ± 0.6	16.9 ± 1.0	32.2 ± 7.8	1.1 ± 0.2	0.8 ± 1.5	0.0	0.0
WRI1	35.5 ± 3.4	0.7 ± 0.2	5.2 ± 0.8	5.4 ± 1.3	17.1 ± 1.0	33.1 ± 2.7	0.8 ± 0.1	0.5 ± 0.6	0.3 ± 0.0	0.0
WRI3	27.3 ± 1.6	0.9 ± 0.2	4.8 ± 0.3	10.2 ± 1.5	16.1 ± 1.0	37.8 ± 1.2	0.8 ± 0.1	0.6 ± 0.7	0.1 ± 0.2	0.0
WRI4	30.1 ± 0.4	1.0 ± 0.4	5.2 ± 0.8	4.6 ± 0.6	17.2 ± 0.4	38.1 ± 1.6	0.8 ± 0.1	1.3 ±1.3	0.0	0.0
AsWRI	35.7 ± 3.0	1.7 ± 0.4	5.3 ± 0.7	6.5 ± 0.3	15.4 ± 0.4	31.6 ± 1.6	0.8 ± 0.1	0.4 ± 0.7	0.3 ± 0.1	0.0
SbWRI	37.4 ± 0.8	1.9 ± 0.3	4.8 ± 0.3	7.0 ± 1.2	15.2 ± 0.3	30.8 ± 0.3	0.8 ± 0.1	0.4 ± 0.6	0.3 ± 0.0	0.0
TsWRI	34.5 ± 4.8	0.0	9.4 ± 8.2	5.9 ± 1.7	16.0 ± 0.7	29.3 ± 12.4	0.0	n.d.	0.0	0.0
Control (P19)	31.0 ± 2.1	0.9 ± 0.1	8.7 ± 1.3	8.0 ± 2.3	24.9 ± 1.5	22.1 ± 4.7	2.0 ± 0.1	0.0	0.6 ± 0.6	0.2 ± 0.4
WRI1+DGAT	27.7 ± 0.1	0.3 ± 0.0	7.0 ± 0.1	17.2 ± 0.7	27.9 ± 0.9	14.7 ± 0.3	2.4 ± 0.2	0.3 ± 0.0	1.1 ± 0.1	0.8 ± 0.2
WRI3+DGAT	30.0 ± 0.8	0.6 ± 0.1	5.9 ± 0.4	13.9 ±2.9	21.5 ± 1.1	21.3 ± 0.8	2.8 ± 0.1	0.2 ± 0.0	1.8 ± 0.1	1.0 ± 0.2
WRI4+DGAT	27.0 ± 0.5	0.2 ± 0.1	8.5 ± 0.2	5.8 ± 0.7	23.9 ± 0.8	25.2 ± 1.3	3.5 ± 0.1	0.2 ± 0.0	2.1 ± 0.2	1.7 ± 0.2
AsWRI+DGAT	33.8 ± 0.5	1.1 ± 0.1	5.5 ± 0.9	12.2 ± 1.6	26.0 ± 1.9	16.3 ± 1.3	2.2 ± 0.2	0.2 ± 0.0	1.2 ± 0.1	0.8 ± 0.1
SbWRI+DGAT	34.6 ± 0.5	1.3 ± 0.1	5.6 ± 0.4	13.9 ± 1.6	23.6 ± 1.3	15.8 ± 0.6	2.2 ± 0.1	0.2 ± 0.0	1.2 ± 0.1	0.9 ± 0.1
TsWRI+DGAT	25.4 ± 0.5	0.2 ± 0.0	9.4 ± 0.1	7.7 ± 1.0	27.0 ± 1.3	22.1 ± 2.4	3.6 ± 0.2	0.2 ± 0.0	1.8 ± 0.2	1.3± 0.2

Use of other transcription factors

Genetic constructs were prepared for expression of each of 24 different transcription factors in plant cells to test their ability to function for increasing TAG levels in combination with other genes involved in TAG biosynthesis and accumulation. These transcription factors were candidates as alternatives for WRI1 or for addition to combinations including one or more of WRI1, LEC1 and LEC2 transcription factors for use in plant cells, particularly in vegetative plant parts. Their selection was largely based on their reported involvement in embryogenesis (reviewed in Baud and Lepiniec (2010), and Ikeda et al. (2006)), similar to LEC2, or plant storage lipid metabolism. Experiments were therefore carried out to assay their function, using the *N. benthamiana* expression system (Example 1), as follows.

Nucleotide sequences of the protein coding regions of the following transcription factors were codon optimized for expression in *N. benthamiana* and *N. tabacum*, synthesized and subcloned as *Not*I-*Sac*I fragments into the respective sites of pJP3343: *A. thaliana* FUS3 (pOIL164) (Luerssen et al., 1998; Accession number AAC35247; SEQ ID NO:34), *A. thaliana* LEC1L (pOIL165) (Kwong et al. 2003; Accession number AAN15924; SEQ ID NO:33), *A. thaliana* LEC1 (pOIL166) (Lotan et al., 1998; Accession number AAC39488; SEQ ID NO:31), *G. max* MYB73 (pOIL167) (Liu et al., 2014; Accession number ABH02868; SEQ ID NO:57), *A. thaliana* bZIP53 (pOIL168) (Alonso et al., 2009; Accession number AAM14360; SEQ ID NO:58), *A. thaliana* AGL15 (pOIL169) (Zheng et al., 2009; Accession number NP_196883; SEQ ID NO:59), *A. thaliana* MYB118 (Accession number AAS58517; pOIL170; SEQ ID NO:60), MYB115 (Wang et al., 2002; Accession number AAS10103; pOIL171; SEQ ID NO:61), *A. thaliana* TANMEI (pOIL172) (Yamagishi et al., 2005; Accession number BAE44475; SEQ ID NO:62), *A. thaliana* WUS (pOIL173) (Laux et al., 1996; Accession number NP_565429; SEQ ID NO:63), *A. thaliana* BBM (pOIL174) (Boutilier et al., 2002; Accession number AAM33893, SEQ ID NO:64), *B. napus* GFR2a1 (Accession number AFB74090; pOIL177; SEQ ID NO:64), GFR2a2 (Accession number AFB74089; pOIL178; SEQ ID NO:65) (Liu et al. (2012)), *E. guineensis* NF-YB1 (pOIL405) (Geurin et al., 2016; Accession number XM_010907896; SEQ ID NO:143), *E. guineensis* ZFP1 (pOIL406) (Geurin et al., 2016; Accession number XM_010930940; SEQ ID NO:144), *A. thaliana* NF-YB2 (pOIL407) (Geurin et al., 2016; Accession number NM_124138; SEQ ID NO:145), *A. thaliana* NF-YB3 (pOIL408) (Geurin et al., 2016; Accession number NM_117534; SEQ ID NO:146), *A. thaliana* ZFP2 (pOIL409) (Geurin et al, 2016; Accession number NM_125133; SEQ ID NO:147), *E. guineensis* ABI5 (pOIL410) (Yeap et al., 2017; Accession number XM_010909282; SEQ ID NO:148), *E. guineensis* NF-YC2 (pOIL411) (Yeap et al., 2017; Accession number

XM_010911913; SEQ ID NO:149), and *E. guineensis* NF-YA3 (pOIL412) (Yeap et al., 2017; Accession number XM_010941630; SEQ ID NO:150). In addition, a codon optimized version of the *A. thaliana* PHR1 transcription factor involved in adaptation to high light phosphate starvation conditions was similarly subcloned into pJP3343 (pOIL189) (Nilsson et al (2012); Accession number AAN72198; SEQ ID NO:221). The sequence coding for the *G. max* DOF4 (Wang et al., 2007; Accession number DQ857254; SEQ ID NO:151) was codon optimized for expression in *N. benthamiana* and *N. tabacum*, synthesized as a *Not*I-*Spe*I fragment and subcloned into pJP3343. The resulting vector was designated pOIL379. Finally, the gene coding for the *G. max* ZF351 transcription factor (Li et al., 2017; Accession number XM_003526219; SEQ ID NO:152) was synthesized as a *Not*I-*Eco*RI fragment and cloned into pJP3343, resulting in pOIL420. These transcription factors are summarised in Table 8.

As a screening assay to determine the function of these transcription factors, the genetic constructs and a gene encoding DGAT1were co-infiltrated into *N. benthamiana* leaf cells as described in Example 1, either with or without a gene encoding WRI1. Total lipid content and fatty acid composition of the leaf cells were analysed 5 days post-infiltration. Among the various embryogenic transcription factors tested, only overexpression of FUS3 resulted in significantly increased TAG levels in *N. benthamiana* leaf tissue when compared to DGAT and DGAT1+WRI1 control infiltrations (Table 9).

Table 8. Additional transcription factors and the genetic constructs for their expression

Plasmid	Transcription factor	Species	Length (amino acid)	Accession number
pOIL164	FUS3	*A. thaliana*	312	AAC35247
pOIL165	LEC1L	*A. thaliana*	234	AAN15924
pOIL166	LEC1	*A. thaliana*	208	AAC39488
pOIL167	MYB73	*G. max*	74	ABH02868
pOIL168	bZIP53	*A. thaliana*	146	AAM14360
pOIL169	AGL15	*A. thaliana*	268	NP_196883
pOIL170	MYB118	*A. thaliana*	437	AAS58517
pOIL171	MYB115	*A. thaliana*	359	AAS10103
pOIL172	TANMEI	*A. thaliana*	386	BAE44475
pOIL173	WUS	*A. thaliana*	292	NP_565429
pOIL174	BBM	*A. thaliana*	584	AAM33803
pOIL177	GFR2a1	*B. napus*	453	AFB74090
pOIL178	GFR2a2	*B. napus*	461	AFB74089

pOIL189	PHR1	*A. thaliana*	409	AAN72198
pOIL379	DOF4	*G. max*	300	DQ857254
pOIL405	NF-YB1	*E. guineensis*	215	XM_010907896
pOIL406	ZFP1	*E. guineensis*	142	XM_010930940
pOIL407	NF-YB2	*A. thaliana*	190	NM_124138
pOIL408	NF-YB3	*A. thaliana*	161	NM_117534
pOIL409	ZFP2	*A. thaliana*	150	NM_125133
pOIL410	ABI5	*E. guineensis*	398	XM_010909282
pOIL411	NF-YC2	*E. guineensis*	272	XM_010911913
pOIL412	NF-YA3	*E. guineensis*	352	XM_010941630
pOIL420	ZF351	*G. max*	351	003526219

Table 9. TAG level (% leaf dry weight) and fatty acid profile of infiltrated *N. benthamiana* leaves.

	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	TAG
P19	27.1 ± 1.5	0.3 ± 0.1	9.6 ± 1.7	4.4 ± 1.2	22.4 ± 4.0	30.5 ± 0.9	0.0
P19+DGAT1	26.3 ± 1.0	0.1 ± 0.0	10.7 ± 0.6	3.7 ± 0.7	26.1 ± 1.6	26.4 ± 1.4	0.2 ± 0.0
P19+DGAT1+FUS3	24.1 ± 1.0	0.1 ± 0.0	6.3 ± 0.4	5.2 ± 1.6	27.9 ± 1.8	30.0 ± 1.8	0.6 ± 0.1
P19+DGAT1+LEC1L	26.0 ± 1.4	0.1 ± 0.0	10.3 ± 0.8	3.9 ± 1.0	26.6 ± 2.1	26.4 ± 0.7	0.2 ± 0.0
P19	30.3 ± 0.7	0.0	12.4 ± 0.7	6.8 ± 0.9	22.9 ± 0.2	26.0 ± 0.9	0.0
P19+DGAT1	25.8 ± 1.1	0.0	10.1 ± 0.4	4.4 ± 0.9	26.1 ± 1.3	26.2 ± 1.4	0.2 ± 0.0
P19+DGAT1+WRI1	22.7 ± 0.9	0.0	10.1 ± 0.4	14.9 ± 0.5	27.9 ± 1.3	18.5 ± 0.8	0.3 ± 0.1
P19+DGAT1+FUS3	23.9 ± 0.7	0.2 ± 0.1	7.6 ± 0.4	5.3 ± 0.7	29.1 ± 0.8	26.8 ± 0.7	0.4 ± 0.1
P19+DGAT1+LEC1	24.9 ± 0.4	0.1 ± 0.2	11.1 ± 0.2	4.0 ± 0.1	25.9 ± 0.5	26.1 ± 0.6	0.1 ± 0.0
P19+DGAT1+MYB73	25.8 ± 0.3	0.0	10.9 ± 0.7	4.3 ± 1.0	26.2 ± 0.8	25.2 ± 1.8	0.1 ± 0.0
P19	34.2 ±	0.0	10.6 ±	8.3 ±	19.5 ±	23.2 ±	0.1 ±

	4.9		3.1	4.1	1.4	0.8	0.1
P19+DGAT1	27.7 ± 0.1	0.3 ± 0.1	9.9 ± 1.1	4.2 ± 0.3	26.4 ± 1.8	22.5 ± 0.4	0.2 ± 0.1
P19+DGAT1+WRI1	24.8 ± 1.0	0.2 ± 0.0	8.8 ± 1.0	14.7 ± 0.6	27.6 ± 1.0	17.2 ± 0.3	0.4 ± 0.1
P19+DGAT1+bZIP53	29.3 ± 0.8	0.1 ± 0.2	8.7 ± 0.4	2.9 ± 0.3	22.0 ± 0.5	25.9 ± 0.5	0.1 ± 0.1
P19+DGAT1+AGL15	29.2 ± 1.4	0.2 ± 0.0	4.9 ± 0.9	7.0 ± 1.9	19.8 ± 0.8	30.0 ± 1.3	0.3 ± 0.1
P19+DGAT1+MYB118	31.6 ± 1.7	0.2 ± 0.1	5.8 ± 1.2	4.8 ± 0.8	20.7 ± 0.3	28.2 ± 1.6	0.2 ± 0.1
P19	27.4 ± 1.2	0.0	6.9 ± 1.0	4.8 ± 2.6	20.0 ± 1.5	39.0 ± 4.1	0.1 ± 0.0
P19+DGAT1	26.0 ± 1.1	0.0	8.0 ± 0.6	4.2 ± 1.6	22.3 ± 2.4	33.9 ± 4.3	0.2 ± 0.0
P19+DGAT1+WRI1	23.4 ± 0.8	0.1 ± 0.1	8.5 ± 0.6	17.0 ± 2.4	23.3 ± 1.8	23.3 ± 4.3	0.5 ± 0.1
P19+DGAT1+MYB115	26.3 ± 0.4	0.1 ± 0.1	6.6 ± 0.3	2.8 ± 0.4	22.5 ± 1.8	35.7 ± 2.9	0.2 ± 0.0
P19+DGAT1+TANMEI	25.6 ± 0.9	0.1 ± 0.2	8.5 ± 1.2	2.6 ± 0.5	21.9 ± 2.0	35.3 ± 3.8	0.2 ± 0.0
P19+DGAT1+WUS	24.3 ± 0.9	0.1 ± 0.1	5.5 ± 0.6	1.7 ± 0.2	16.8 ± 1.6	47.9 ± 3.3	0.2 ± 0.0
P19	30.5 ± 1.3	0.0	8.1 ± 0.9	8.2 ± 6.0	21.8 ± 1.2	28.3 ± 7.3	0.1 ± 0.1
P19+DGAT1+WRI1	25.9 ± 1.7	0.2 ± 0.0	8.3 ± 0.7	19.9 ± 2.8	24.5 ± 1.1	16.0 ± 0.6	0.8 ± 0.1
P19+DGAT1+WRI1+BBM	27.7 ± 0.7	0.2 ± 0.0	6.7 ± 0.2	21.2 ± 0.7	19.8 ± 0.5	18.5 ± 0.6	0.5 ± 0.1
P19+DGAT1+WRI1+GFR2a1	29.2 ± 1.3	0.4 ± 0.0	6.1 ± 0.1	12.9 ± 1.5	24.3 ± 0.4	20.9 ± 0.5	0.4 ± 0.1
P19+DGAT1+WRI1+GFR2a2	29.9 ± 2.4	0.4 ± 0.1	5.5 ± 0.6	13.5 ± 2.7	23.0 ± 0.5	21.3 ± 1.2	0.5 ± 0.1
P19+DGAT1+WRI1+PHR1	26.2 ± 0.3	0.2 ± 0.0	4.9 ± 0.0	7.6 ± 0.2	19.2 ± 0.3	36.0 ± 0.7	0.3 ± 0.0

P19	32.0 ± 1.9	1.6 ± 2.7	11.1 ± 2.7	5.5 ± 2.2	23.3 ± 1.1	25.4 ± 3.3	0.0
P19+DGAT1+WRI1	27.5 ± 1.2	0.7 ± 0.8	6.8 ± 0.4	16.6 ± 2.1	26.7 ± 0.8	16.5 ± 0.3	1.2 ± 0.2
P19+DGAT1+WRI1+FUS3	23.6 ± 1.1	2.1 ± 3.5	6.5 ± 0.5	13.3 ± 0.9	32.1 ± 2.6	15.6 ± 1.5	1.6 ± 0.1
P19+GFP	35.8 ± 1.8	0.0 ± 0.0	8.5 ± 0.8	2.0 ± 1.3	19.7 ± 1.2	32.1 ± 2.2	0.03 ± 0.0
P19+GFP+DGAT1+WRI1	24.6 ± 1.4	0.2 ± 0.0	10.3 ± 0.5	22.7 ± 2.7	23.0 ± 1.7	14.0 ± 0.6	0.99 ± 0.2
P19+GFP+DGAT1+NF-YB2	27.6 ± 0.6	0.1 ± 0.0	10.2 ± 0.2	3.0 ± 0.2	24.1 ± 1.1	27.1 ± 1.2	0.25 ± 0.0
P19+GFP+DGAT1+NF-YB3	27.4 ± 0.5	0.1 ± 0.0	10.8 ± 0.5	3.1 ± 1.0	24.6 ± 0.9	26.0 ± 0.7	0.27 ± 0.1
P19+GFP+DGAT1+NF-YA3	28.9 ± 0.8	0.2 ± 0.0	8.3 ± 0.4	3.6 ± 0.5	22.7 ± 1.0	29.2 ± 0.9	0.17 ± 0.0
P19+GFP	38.3 ± 1.3	0.0 ± 0.0	11.1 ± 1.2	2.9 ± 1.4	21.3 ± 1.0	26.4 ± 3.8	0.0 ± 0.0
P19+GFP+DGAT1+WRI1	29.8 ± 1.1	0.3 ± 0.0	7.6 ± 1.7	18.3 ± 0.6	23.9 ± 1.4	15.0 ± 0.7	1.1 ± 0.5
P19+GFP+DGAT1+DOF4	32.5 ± 0.5	0.0 ± 0.0	5.1 ± 0.7	3.6 ± 0.2	20.5 ± 0.9	32.6 ± 1.2	0.2 ± 0.1
P19+GFP+DGAT1+NF-YB1	27.9 ± 0.7	0.0 ± 0.0	10.8 ± 0.5	2.9 ± 0.5	27.0 ± 1.3	23.7 ± 1.4	0.3 ± 0.1
P19+GFP+DGAT1+ZFP1	25.4 ± 1.4	0.1 ± 0.2	4.1 ± 0.3	5.2 ± 1.2	22.8 ± 0.8	36.2 ± 0.8	0.3 ± 0.1
P19+GFP	37.7 ± 1.7	0.0 ± 0.0	11.5 ± 1.5	2.6 ± 2.3	22.2 ± 1.9	24.1 ± 4.7	0.0 ± 0.0
P19+GFP+DGAT1+WRI1	28.0 ± 2.1	0.2 ± 0.0	9.3 ± 1.0	17.2 ± 3.1	27.3 ± 1.2	13.0 ± 0.3	0.8 ± 0.3
P19+GFP+DGAT1+ZF351	30.8 ± 0.5	0.2 ± 0.1	9.5 ± 0.7	2.6 ± 1.5	25.4 ± 1.1	25.4 ± 2.1	0.2 ± 0.0
P19	18.9 ± 2.9	0.4 ± 0.3	5.6 ± 1.7	6.1 ± 4.8	18.3 ± 1.7	45.8 ± 9.5	0.4 ± 0.1
P19+DGAT1+WRI1	21.4 ±	0.2 ±	9.9 ±	19.4 ±	20.5 ±	23.8 ±	1.7 ±

	2.3	0.0	0.8	0.9	0.9	2.7	0.6
P19+DGAT1+WRI1+ZFP2	23.1 ± 1.2	0.3 ± 0.1	5.3 ± 0.5	9.3 ± 1.7	16.2 ± 0.7	40.5 ± 4.1	1.0 ± 0.4
P19+DGAT1+WRI1+ABI5	21.4 ± 1.1	0.2 ± 0.0	8.4 ± 0.7	11.4 ± 1.3	23.2 ± 1.4	29.9 ± 2.9	1.2 ± 0.4
P19+DGAT1+WRI1+NF-YC2	20.5 ± 0.7	0.2 ± 0.1	9.6 ± 0.4	18.1 ± 0.6	21.2 ± 0.6	25.4 ± 1.5	1.6 ± 0.4

For stable transformation of plants using genes encoding the alternative transcription factors, the following binary constructs are made. The genes for expression of the transcription factors use either the SSU promoter or the SAG12 promoter. Over-expression of embryogenic transcription factors such as LEC1 and LEC2 has been shown to induce a variety of pleotropic effects, undesirable in the present context, including somatic embryogenesis (Feeney et al. (2012); Santos-Mendoza et al. (2005); Stone et al. (2008); Stone et al. (2001); Shen et al. (2010)). To minimize possible negative impact on plant development and biomass yield, tissue or developmental-stage specific promoters are preferred over constitutive promoters to drive the ectopic expression of master regulators of embryogenesis.

Example 5. Stem-specific expression of a gene encoding a transcription factor

Leaves of *N. tabacum* plants expressing transgenes encoding WRI1, DGAT and Oleosin contain about 16% TAG at seed setting stage of development. However, the TAG levels were much lower in stems (1%) and roots (1.4%) of the plants (Vanhercke et al., 2014a and b). The inventors considered whether the lower TAG levels in stems and roots were due to poor promoter activity of the Rubisco SSU promoter used to express the gene encoding WRI1 in the transgenic plants. The DGAT transgene in the T-DNA of pJP3502 was expressed by the CaMV35S promoter which is expressed more strongly in stems and roots and therefore was unlikely to be the limiting factor for TAG accumulation in stems and roots.

In an attempt to increase TAG biosynthesis in stem tissue, a construct was designed in which the gene encoding WRI1 was placed under the control of an *A. thaliana* SDP1 promoter. A 3.156kb synthetic DNA fragment was synthesized comprising 1.5kb of the *A. thaliana* SDP1 promoter (SEQ ID NO:41) (Kelly et al., 2013a and b), followed by the coding region for the *A. thaliana* WRI1 polypeptide and the *G. max* lectin terminator/polyadenylation region. This fragment was inserted between the *Sac*I and *Not*I sites of pJP3303. The resulting vector was designated pOIL050, which was then used to transform cells from the *N. tabacum* plants homozygous for the T-DNA from pJP3502 by *Agrobacterium*-mediated

transformation. Transgenic plants were selected for hygromycin resistance and a total of 86 independent transgenic plants were grown to maturity in the glasshouse. Samples were taken from transgenic leaf and stem tissue at seed setting stage and contain increased TAG levels compared to the *N. tabacum* parental plants transformed with pJP3502.

Example 6. Effect of oil body protein expression on plant traits

N. tabacum plants transformed with the T-DNA of pJP3502 and expressing transgenes encoding *A. thaliana* WRI1, DGAT1 and *S. indicum* Oleosin had increased TAG levels in vegetative tissues. As shown in Example 2 above, when the endogenous gene encoding SDP1 TAG lipase was silenced in those plants, the leaf TAG levels further increased, which indicated to the inventors that substantial TAG turnover was occurring in the plants that retained SDP1 activity. Therefore, the level of expression of the transgenes in the plants was determined. While Northern hybridisation blotting confirmed strong WRI1 and DGAT1 expression and some oleosin mRNA expression, expression analysis by digital PCR and qRT-PCR detected only very low levels of oleosin transcripts. The expression analysis revealed that the gene encoding the Oleosin was poorly expressed compared to the WRI1 and DGAT1 transgenes. From these experiments, the inventors concluded that the oil bodies in the leaf tissue were not completely protected from TAG breakdown because of inadequate production of Oleosin protein when encoded by the T-DNA in pJP3502. To improve stable accumulation of TAG throughout plant development, several pJP3502 modifications were designed in which the Oleosin gene was substituted. These modified constructs were as follows.

1. pJP3502 contains a gene (SEQ ID NO:42 provides the sequence of its complement) encoding the *S. indicum* oleosin which was poorly expressed. That gene has an internal UBQ10 intron which might be reducing the expression level. To test this, a 502bp synthetic DNA fragment containing the *S. indicum* oleosin gene and lacking the internal UBQ10 intron was synthesized and inserted into pJP3502 as a *Not*I fragment, to substitute the oleosin gene containing the intron in pJP3502. The resultant plasmid was designated pOIL040.
2. The Rubisco small subunit (SSU) promoter driving expression of the oleosin gene in pJP3502 was replaced by the constitutive enTCUP2 promoter. To this end, a 2321bp fragment containing the enTCUP2 promoter, Oleosin protein coding region, *G. max* lectin terminator/polyadenylation region and the first 643bp of the downstream SSU promoter driving *wri1* expression was

synthesized and subcloned into the *Asc*I and *Spe*I sites of pJP3502 resulting in pOIL038.

3. A similar strategy was followed for the expression of an engineered version of the *S. indicum* oleosin gene containing 6 introduced cysteine residues (o3-3) under the control of the enTCUP2 promoter (Winichayakul et al., 2013). A 2298bp fragment containing the enTCUP2 promoter, Oleosin o3-3 protein coding region, *G. max* lectin terminator/polyadenylation region and the first 643bp of the downstream SSU promoter driving *wri1* expression was synthesized and subcloned into the *Asc*I and *Spe*I sites of pJP3502 resulting in pOIL037.
4. The *Not*I sites flanking the *S. indicum* oleosin gene in pJP3502 were used to exchange the protein coding region for one encoding peanut Oleosin3 (Accession No. AAU21501.1) (Parthibane et al., 2012a and b). A 528bp fragment containing the *oleosin3* gene, flanked by *Not*I sites, was synthesized and subcloned into the respective site of pJP3502. The resulting vector was designated pOIL041.
5. Similarly, a 1077bp *Not*I flanked fragment containing the gene coding for the *A. thaliana* steroleosin (Arab-1) (Accession No. AAM10215.1) (Jolivet et al., 2014) was synthesized and subcloned into the *Not*I site of pJP3502, resulting in pOIL043.
6. The *Nannochloropsis oceanic* lipid droplet surface protein (LDSP) (Accession No. AFB75402.1) (Vieler et al., 2012) was synthesized as a 504bp *Not*I-flanked fragment and subcloned into the *Not*I site of pJP3502, yielding pOIL044.
7. Finally, the *A. thaliana* caleosin (CLO3) (Accession No. O22788.1) (Shimada et al., 2014) was synthesized as a 612bp *Not*I flanked fragment and subcloned into pJP3502, resulting in pOIL042.

Each of these constructs was introduced into *N. benthamiana* leaf cells as described in Example 1. Transient expression of both pJP3502 and pOIL040 in *N. benthamiana* leaf tissue resulted in elevated TAG levels and similar changes in the TAG fatty acid profile but pOIL040 increased the TAG level more (1.3% compared to 0.9%). Each of the constructs pOIL037, pOIL038, pOIL041, pOIL042 and pOIL043 were used to stably transform *N. tabacum* plants (cultivar W38) by *Agrobacterium*-mediated methods. Transgenic plants were selected on the basis of kanamycin resistance and are grown to maturity in the glasshouse. Samples are taken from transgenic leaf tissue at different stages during plant development and contain increased TAG levels compared to wild-type *N. tabacum* and *N. tabacum* plants transformed with pJP3502.

Cloning and characterisation of LDAP polypeptides from Sapium sebifera

Oleosins are not highly expressed in non-seed oil accumulating plant tissues such as the mesocarp of olive, oil palm, and avocado (Murphy, 2012). Instead, lipid droplet associated proteins (LDAP) have been identified in these tissues that may play a similar role to that of oleosin in seed tissues (Horn et al., 2013). The inventors therefore considered it possible that oleosin might not be the optimal packaging protein to protect the accumulated oil from TAG lipase or other cytosolic enzyme activities in vegetative tissues of plants. LDAP polypeptides were therefore identified and evaluated for enhancement of TAG accumulation, as follows.

The fruit of Chinese tallow tree, *Sapium sebifera*, a member of the family Euphorbiaceae, was of particular interest to the inventors as it contains an oil-rich tissue outside of the seed. A recent study (Divi et al, submitted for publication) indicated that this oleoginous tissue, called a tallow layer, might be derived from the mesocarp of its fruit. Therefore, the inventors queried the transcriptome of *S. sebifera* for LDAP sequences. A comparative analysis of expressed genes in the fruit coat and seed tissues revealed a group of three previously unidentified LDAP genes which were highly expressed in the tallow layer.

Nucleotide sequences encoding the three LDAPs were obtained by RT-PCR using RNAs derived from tallow tissue using three pairs of primers. The primer sequences were based on the DNA sequences flanking the entire coding region of each of the three genes. The primer sequences were: for LDAP1, 5'-TTTTAACGATATCCGCTAAAGG-3' (SEQ ID NO:76) and 5'-AATGAATGAACAAGAATTAAGTC-3' (SEQ ID NO:77) AT-3'; LDAP2, 5'-CTTTTCTCACACCGTATCTCCG-3' (SEQ ID NO:78) and 5'-AGCATGATATA CTTGTCGAGAAAGC-3' (SEQ ID NO:79); LDAP3, 5'-GCGACAGTGTAGCGTTTT-3' (SEQ ID NO:80) and 5'-ATACATAAAATGAAAACTATTGTGC-3' (SEQ ID NO:81).

Analysis of the *S. sebifera* transcriptome revealed multiple orthologs for each of the LDAP genes, including eight LDAP1, six LDAP2, and six LDAP3 genes, with less than 10% sequence divergence within each gene family. The putative peptide sequences were aligned and a phylogenetic tree was constructed using Genious software (Figure 4), together with LDAPs homologs from other plant species, including two from avocado (Pam), one from oil palm, one from *Parthenium argentatum* (Par), two from Arabidopsis(Ath), five from *Taraxacum brevicorniculatum* (Tbr), three from *Hevea brasiliensis* (Hbr), as presented in Figure 4. The phylogenetic tree was revealed that the SsLDAP3 shared greater amino acid sequence identity to the LDAP1 and LDAP2 polypeptides from avocado and the

LDAP from oil palm, while the SsLDAP1 and SsLDAP2 polypeptides were more divergent.

Genetic constructs for over-expression of LDAP

In order to test the function of the LDAPs from *S. sebifera*, expression vectors were made to express each of these polypeptides under the control of the 35S promoter in leaf cells. The full length SsLDAP cDNA sequences were inserted into the pDONR207 destination vector by recombination reactions, replacing the CcdB and Cm(R) regions of the destination vector with the SsLDAP cDNA fragments. Following confirmation by restriction digestion analysis and DNA sequencing, the constructs were introduced into *Agrobacterium tumefaciens* strain AGL1 and used for both transient expression in *N. benthamiana* leaf cells and stable transformation of *N. tabacum*.

The expression of each of the three SsLDAP genes under the transcriptional control of the 35S promoter in *N. benthamiana* leaves in combination with the expression of 35S::AtDGAT1 and 35S::AtWRI1 yielded substantially higher levels of TAG accumulation relative to the cells infiltrated with the 35S::AtDGAT1 and 35S::AtWRI1 genes without the LDAP construct. The TAG level was increased about 2-fold above the TAG level in the control cells. A significant increase in the level of α-linolenic acid (ALA) and a reduced level of saturated fatty acids was observed in the cells receiving the combination of genes, relative to the control cells.

Co-localisation of YFP-fused LDAP polypeptides with lipid droplets in leaf cells

In order to characterise SsLDAPs *in vivo* and observe their dynamic behaviour, expression constructs were made for expression of fusion polypeptides consisting of the LDAP polypeptides fused to yellow fluorescent protein (YFP). For each fusion polypeptide, the YFP was fused in-frame to the C-terminus of the SsLDAP. The full open reading frame of each of the three LDAP genes without a stop codon, at its 3' end, was fused to the YFP sequence and the chimeric genes inserted into pDONR207. Following confirmation of the resultant constructs by restriction digestion and DNA sequencing, the constructs were introduced into *A. tumefaciens* strain AGL1 and used for both transient expression in *N. benthamiana* leaf cells and stable transformation of *N. tabacum*. Three days following infiltration of the leaf cells with the LDAP-YFP constructs, leaf discs from the infiltrated zones were stained with Nile Red, which positively stained lipid droplets, and observed under a confocal microscope to detect both the red stain (lipid droplets) and fluorescence from the YFP polypeptide. Co-localisation of LDAP-YFP with the lipid droplets was observed, indicating that the LDAP associated with the lipid droplets in the leaf cells.

Example 7. Modifying traits in monocotyledonous plants - Expression in leaves and stems

A series of binary expression vectors was designed for *Agrobacterium*-mediated transformation of sorghum (*S. bicolor*) and wheat (*Triticum aestivum*) to increase the oil content in vegetative tissues. The starting vectors for the constructions were pOIL093-095, pOIL134 and pOIL100-104 (see Example 5 of WO 2016/004473). Firstly, a DNA fragment encoding the *Z. mays* WRI1 polypeptide was amplified by PCR using pOIL104 as a template and primers containing *Kpn*I restriction sites. This fragment was subcloned downstream of the constitutive *Oryza sativa* Actin1 promoter of pOIL095, using the *Kpn*I site. The resulting vector was designated pOIL154. The DNA fragment encoding the *Umbelopsis ramanniana* DGAT2a under the control of the *Z. mays* ubiquitin promoter (pZmUbi) was isolated from pOIL134 as a *Not*I fragment and inserted into the *Not*I site of pOIL154, resulting in pOIL155. An expression cassette consisting of the PAT coding region under the control of the pZmUbi promoter and flanked at the 3' end by the *A. tumefaciens* NOS terminator/polyadenylation region was constructed by amplifying the *PAT* coding region using pJP3416 as a template. Primers were designed to incorporate *BamH*I and *Sac*I restriction sites at the 5' and 3' ends, respectively. After *BamH*I + *Sac*I double digestion, the PAT fragment was cloned into the respective sites of pZLUbi1casNK. The resulting intermediate was designated pOIL141. Next, the PAT selectable marker cassette was introduced into the pOIL155 backbone. To this end, pOIL141 was first cut with *Not*I, blunted with Klenow fragment of DNA polymerase I and subsequently digested with *Asc*I. This 2622bp fragment was then subcloned into the *Zra*I – *Asc*I sites of pOIL155, resulting in pOIL156. Finally, the Actin1 promoter driving WRI1 expression in pOIL156 was exchanged for the *Z. mays* Rubisco small subunit promoter (pZmSSU) resulting in pOIL157. This vector was obtained by PCR amplification of the *Z. mays* SSU promoter using pOIL104 as a template and flanking primers containing *Asi*SI and *Pml*I restriction sites. The resulting amplicon was then cut with *Spe*I + *Mlu*I and subcloned into the respective sites of pOIL156.

These vectors therefore contained the following expression cassettes:

pOIL156: promoter *O. sativa* Actin1::*Z. mays* WRI1, promoter *Z. mays* Ubiquitin::*U. rammaniana* DGAT2a and promoter *Z. mays* Ubiquitin::PAT

pOIL157: promoter *Z. mays* SSU::*Z. mays* WRI1, promoter *Z. mays* Ubiquitin::*U. rammaniana* DGAT2a and *Z. mays* Ubiquitin::PAT.

A second series of binary expression vectors containing the *Z. mays* SEE1 senescence promoter (Robson et al., 2004, see Example 5 of WO 2016/004473), *Z. mays* LEC1 transcription factor (Shen et al., 2010) and a *S. bicolor* SDP1 hpRNAi

fragment were constructed as follows. First, a matrix attachment region (MAR) was introduced into pORE04 by *Aat*II+*Sna*BI digest of pDCOT and subcloning into the *Aat*II+*EcoR*V sites of pORE04. The resulting intermediate vector was designated pOIL158. Next, the PAT selectable marker gene under the control of the *Z. mays* Ubiquitin promoter was subcloned into pOIL158. To this end, pOIL141 was first digested with *Not*I, treated with Klenow fragment of DNA polymerase I and finally digested with *Asc*I. The resulting fragment was inserted into the *Asc*I+*Zra*I sites of pOIL158, resulting in pOIL159. The original RK2 oriV origin of replication in pOIL159 was exchanged for the RiA4 origin by *Swa*I+*Spe*I restriction digestion of pJP3416, followed by subcloning into the *Swa*I+*Avr*II sites of pOIL159. The resulting vector was designated pOIL160. A 10.019kb 'Monocot senescence part1' fragment containing the following expression cassettes was synthesized: *O. sativa* Actin1::*A. thaliana* DGAT1, codon optimized for *Z. mays* expression, *Z. mays* SEE1::*Z. mays* WRI1, *Z. mays* SEE1::*Z. mays* LEC1. This fragment was subcloned as a *Spe*I-*EcoR*V fragment into the *Spe*I-*Stu*I sites of pOIL160, resulting in pOIL161. A second 7.967kb 'Monocot senescence part2' fragment was synthesized and contains the following elements: MAR, *Z. mays* Ubiquitin::hpRNAi fragment targeted against *S. bicolor*/*T. aestivum* SDP1, empty cassette under the control of the *O. sativa* Actin1 promoter. The sequences of two *S. bicolor* SDP1 TAG lipases (Accession Nos. XM_002463620; SEQ ID NO:73 and XM_002458486; SEQ ID NO:38) and one *T. aestivum* SDP1 sequence (Accession No. AK334547) (SEQ ID NO:74) were obtained by a BLAST search with the *A. thaliana* SDP1 sequence (Accession No. NM_120486). A synthetic hairpin construct (SEQ ID NO:75) was designed including four fragments (67bp, 90bp, 50bp, 59bp) of the *S. bicolor* XM_002458486 sequence that showed highest degree of identity with the *T. aestivum* SDP1 sequence. In addition, a 278bp fragment originating from the *S. bicolor* XM_002463620 SDP1 lipase was included to increase silencing efficiency against both *S. bicolor* SDP1 sequences. The 'Monocot senescence part2' fragment is subcloned as a *BsiW*I-*EcoR*V fragment into the *BsiW*I-*Fsp*I sites of pOIL161. The resulting vector is designated pOIL162.

The genetic constructs pOIL156 pOIL157, pOIL161 and pOIL162 are used to transform *S. bicolor* and *T. aestivum* using *Agrobacterium*-mediated transformation. Transgenic plants are selected for hygromycin resistance and contain elevated levels of TAG and TFA in vegetative tissues compared to untransformed control plants. Such plants are useful for providing feed for animals as hay or silage, as well as producing grain, or may be used to extract oil.

Further genetic constructs are made for expression of combinations of polypeptides in leaves and stems of monocotyledonous plants, including the C4-

photosynthesis plants *S. bicolor* and *Z. mays*. Several constructs are made containing genes for expression of WRI1, DGAT and oleosin, with each gene under the control of a constitutive promoter such as a maize Ubiquitin gene promoter or a rice actin gene promoter, and containing an NPTII gene as selectable marker gene. In one particular construct, the WRI1 is sorghum WRI1. In another, the oleosin is SiOleosinL (see Example 9). In other particular constructs, the oleosin gene is replaced with a gene encoding either LDAP2 or LDAP3 from *S. sebifera* (Example 6). These constructs are used as the "core constructs" for transformation of *S. bicolor* and *Z. mays* and are deployed on their own or in combination with genetic constructs for expression of a hairpin RNA targeting one or more SDP1 genes in sorghum or maize (see above), a construct encoding Lec2 under the control of a SEE1 promoter (senescence specific), or both. Another construct is made comprising three genes, namely for expression of a hairpin RNA targeting the endogenous *TGD5* gene to reduce its expression, a FatA fatty acyl thioesterase and a PDAT, which is used to increase the level of TAG and/or the TTQ parameter for plants transformed with this construct.

Example 8. Extraction of oil

Extraction of lipid from leaves

Transgenic tobacco leaves which had been transformed with the T-DNA from pJP3502 were harvested from plants grown in a glasshouse during the summer months. The leaves were dried and then ground to 1-3mm sized pieces prior to extraction. The ground material was subject to soxhlet (refluxing) extraction over 24 hours with selected solvents, as described below. 5 g of dried tobacco leaf material and 250ml of solvent was used in each extraction experiment.

Hexane solvent extraction

Hexane is commonly used as a solvent commercially for oil extraction from pressed oil seeds such as canola, extracting neutral (non-polar) lipids, and was therefore tried first. The extracted lipid mass was 1.47g from 5 g of leaf material, a lipid recovery of 29% by weight. 1H NMR analysis of the hexane extracted lipid in DMSO was preformed. The analysis showed typical signals for long chain triglyceride fatty acids, with no aromatic products being present. The lipid was then subjected to GCMS for identification of major components. Direct GCMS analysis of the hexane extracted lipid proved to be difficult as the boiling point was too high and the material decomposed in the GCMS. In such situations, a common analysis technique is to first make methyl esters of the fatty acids, which was done as follows: 18mg lipid extract was dissolved in 1mL toluene, 3mL of dry 3N methanolic HCL

was added and stirred overnight at 60 ºC. 5mL of 5% NaCl and 5mL of hexane were added to the cooled vial and shaken. The organic layer was removed and the extraction was repeated with another 5mL of hexane. The combined organic fractions were neutralized with 8mL of 2% KHCO3, separated and dried with Na2SO4. The solvent was evaporated under a stream of N2 and then made up to a concentration of 1mg/mL in hexane for GCMS analysis. The main fatty acids present were 16:0 (palmitic, 38.9%) and 18:1 (oleic, 31.3%) (Table 10).

Table 10. Fatty acid composition in transgenic tobacco leaves

FA	16:0	16:1	18:0	18:1	18:2	20:0	22:0
% wt	38.9	4.6	6.4	31.3	2.5	1.5	0.6

Acetone solvent extraction

Acetone was used as an extraction solvent because its solvent properties should extract almost all lipid from the leaves, i.e. both non-polar and polar lipids. The acetone extracted oil looked similar to the hexane extracted lipid. The extracted lipid mass was 1.59g from 5 g of tobacco leaf, i.e. 31.8% by weight. 1H NMR analysis of the lipid in DMSO was performed. Signals typical of long chain triglyceride fatty acids were observed, with no signal for aromatic products.

Hot water solvent extraction

Hot water was attempted as an extraction solvent to see if it was suitable to obtain oil from the tobacco leaves. The water extracted material was gel like in appearance and gelled when cooled. The extracted mass was 1.9 g, or 38% by weight. This material was like a thick gel and was likely to have included polar compounds from the leaves such as sugars and other carbohydrates. The 1H NMR analysis of the material in DMSO was preformed. The analysis showed typical signals for long chain triglyceride fatty acids, with no aromatic products being extracted. The left over solid material was extracted with hexane, yielding 20% of lipid by weight, indicating that the water extraction had not efficiently extracted non-polar lipids.

Ethanol solvent extraction

Ethanol was used as an extraction solvent to see if it was suitable to obtain oil from the tobacco leaves. The ethanol extracted lipid was similar in appearance to both the water- and hexane-extracted lipid, being yellow-red in colour, had a gel-like appearance and gelled when cooled. The extracted lipid mass was 1.88g from 5 g

tobacco, or 37.6% by weight. The ethanol solvent would also have extracted some of the polar compounds in the tobacco leaves.

Ether solvent extraction

Diethyl ether was attempted as an extraction solvent since it was thought that it might extract less impurities than other solvents. The extraction yielded 1.4 g, or 28% by weight. The ether extracted lipid was similar to the hexane extracted material in appearance, was yellowish in colour, and it did appeared a little cleaner than the hexane extract. While the diethyl ether extraction appeared to have given the cleanest oil, the NMR analysis showed a mixture of more organic compounds.

Example 9. Expression of oil body proteins in plant vegetative tissues

A protein coding region encoding a *Rhodococcus opacus* TadA lipid droplet associated protein (MacEachran et al. 2010; Accession number HM625859), codon optimized for expression in dicotyledonous plants such as *Nicotiana benthamiana*, was synthesized as a NotI-SpeI DNA fragment. The fragment was inserted downstream of the 35S promoter in pJP3343 using the NotI-SpeI sites. The resultant plasmid was designated pOIL380. A protein coding region encoding a Sesame indicum OleosinL lipid droplet associated protein (Tai et al. 2002; Accession number AF091840; SEQ ID NO:86) was synthesized as a NotI-SacI DNA fragment and inserted downstream of the 35S promoter in pJP3343 using the same sites. The resultant plasmid was designated pOIL382. A protein coding region encoding a Sesame indicum OleosinH1 lipid droplet associated protein (Tai et al., 2002; Accession number AF302807) was synthesized as a NotI-SacI DNA fragment and cloned downstream of the 35S promoter in pJP3343 using the same sites. The resultant plasmid was designated pOIL383. A variant of the protein coding region encoding *S. indicum* OleosinH1 having three amino acid substitutions to remove ubiquitination sites (K130R, K143R, K145R) (Hsiao and Tzen, 2011) was generated by targeted mutagenesis. The coding region was inserted downstream of the 35S promoter in pJP3343 as a NotI-SacI fragment. The resultant plasmid was designated pOIL384. A protein coding region encoding a *Vanilla planifolia* leaf OleosinU1 lipid droplet associated protein (Huang and Huang, 2016; Accession number SRX648194) was codon optimized for expression in *N. benthamiana*, synthesized as a SpeI-EcoRI DNA fragment and inserted downstream of the 35S promoter in pJP3343 using the same sites. The resultant plasmid was designated pOIL386. A protein coding region encoding a *Persea americana* mesocarp OleosinM lipid droplet associated protein (Huang and Huang 2016; Accession number SRX627420) was codon optimized for expression in *N. benthamiana*, synthesized as a SpeI-EcoRI DNA fragment and

inserted downstream of the 35S promoter in pJP3343 using the same restriction sites. The resultant plasmid was designated pOIL387. A protein coding region encoding an *Arachis hypogaea* Oleosin 3 lipid droplet associated protein (Parthibane et al., 2012a; Accession number AY722696) was codon optimized for expression in *N. benthamiana*, flanked by NotI sites and inserted into the binary expression vector pJP3502. The resulting plasmid, pOIL041, was digested with NotI and the resultant 520 bp DNA fragment was inserted downstream of the 35S promoter of pJP3343. The resultant plasmid was designated pOIL190. Similarly, the protein coding region for the *A. thaliana* Caleosin3 lipid droplet associated protein (Shen et al., 2014; Laibach et al., 2015; Accession number AK317039) was codon optimized for expression in *N. benthamiana*, flanked by NotI sites and inserted into pJP3502. The resulting plasmid, pOIL042, was digested with NotI and the resulting 604 bp DNA fragment was inserted downstream of the 35S promoter of pJP3343. The resultant plasmid was designated pOIL191. A protein coding region encoding an *A. thaliana* steroleosin lipid droplet associated protein (Accession number AT081653) was codon optimized for expression in *N. benthamiana*, flanked by NotI sites and inserted into pJP3502. The resultant plasmid, pOIL043, was digested with NotI and the resultant 1069 bp DNA fragment was inserted downstream of the 35S promoter of pJP3343. The resultant plasmid was designated pOIL192. A protein coding region encoding a *Nannochloropsis oceanica* LSDP oil body protein (Vieler et al., 2012; Accession number JQ268559) was codon optimized for expression in *N. benthamiana*, flanked by NotI sites and inserted into the pJP3502 binary expression vector. The resultant plasmid, pOIL044, was digested with NotI and the 496 bp DNA fragment was inserted downstream of the 35S promoter of pJP3343. The resultant plasmid was designated pOIL193. A protein coding region encoding a *Trichoderma reesei* HFBI hydrophobin (Linder et al., 2005; Accession number Z68124) was codon optimized for expression in *N. benthamiana*, flanked by NotI sites and inserted into pJP3502. The resultant plasmid, pOIL045, was digested with NotI and the 313 bp DNA fragment was inserted downstream of the 35S promoter of pJP3343. The resultant plasmid was designated pOIL194. An ER-targeted variant of the *Trichoderma reesei* HFBI hydrophobin was created by amending the KDEL ER retention peptide to the C-terminus (Gutierrew et al., 2013). This variant was codon optimized for expression in *N. benthamiana* and cloned as a NotI fragment into pJP3502, resulting in pOIL046. Subsequently, pOIL046 was digested with NotI and the 325 bp fragment was inserted into pJP3343. The resulting vector was designated pOIL195.

Each of the genetic constructs encoding the lipid droplet associated polypeptides were introduced into *N. benthamiana* leaves in combination with genetic constructs encoding WRI1, DGAT1 and p19 as described in Example 1 with some

minor modifications. *Agrobacterium tumefaciens* cultures containing the gene coding for the p19 silencing suppressor protein and the chimeric genes of interest were mixed such that the final OD600 of each culture was equal to 0.125 prior to infiltration. Samples being compared were located on the same leaf. After infiltration, *N. benthamiana* plants were grown for a further five days before leaf discs were harvested, pooled across three leaves from the same plant, freeze-dried, weighed and stored at −80°C. Total lipids were extracted from freeze-dried tissues using chloroform:methanol:0.1 M KCl (2:1:1 v/v/v) and aliquots loaded on a thin layer chromatography (TLC) plate and developed in hexane:diethyl ether:acetic acid (70:30:1, v/v/v). TAG was recovered, converted to FAME in the presence of a known amount of triheptadecanoin (Nu-Chek PREP, Inc. USA) as internal standard for lipid quantitation, and analyzed by GC-FID.

The assays showed a range of TAG levels compared to the WRI1 + DGAT1 control. Some constructs encoding lipid droplet associated polypeptides increased the TAG level relative to the control in some assays whereas others did not. A consistent and statistically significant increase in TAG content was observed when the construct expressing SiOleosinL (pOIL382) was introduced (Figure 5); this construct was superior to all the others tested in these assays. In one experiment, the increase was 2.27-fold compared to p19+WRI+DGAT and 121.7-fold compared to the p19 control. An increase in the levels of C18:2 and C18:1 (about 22% increased) and a decrease in C16:0 (about 23% decreased) was also observed in the TAG for this construct, relative to the p19+WRI1+DGAT1 control (Figure 5). Microscopic analyses to visualise lipid droplets in the leaf cells expressing SiOleosinL showed a decrease in lipid droplet size and an increase in abundance compared to the control.

The lipid droplets in leaf cells transiently expressing the genes encoding SiOleosinL together with p19 + WRI1 + DGAT1 were examined by microscopy. *N. benthamiana* treated leaf discs were collected 4 days after infiltration. Each leaf sample was prepared, stained and imaged within 30-45 minutes, to ensure the samples were imaged fresh. More specifically, immediately after collection, the abaxial epidermis was peeled off in 50 mM PIPES pH7. One half of each disc was stained for 10 minutes in 2 µg/ml BODIPY505/515 in 50 mM PIPES pH7, followed by 2-3 washes in 50 mM PIPES pH7. During this time, the other disc half was kept in 50 mM PIPES pH7. Leaf tissue was mounted in 50 mM PIPES pH7 and imaged immediately, using a Leica SP8 Laser-Scanning Confocal Microscope, a 20x objective (NA = 0.75), and the LAS X software. Lipid droplets and chloroplasts were imaged by exciting the leaf discs with a 505 nm laser. BODIPY 505/515 signal was collected between 510 and 540 nm, while chloroplast signal was collected between

650 nm and 690 nm. Unstained half discs were imaged to determine tissue auto-fluorescence.

Microscopy of cells in the leaf discs having the introduced SiOleosinL showed an accumulation of smaller lipid droplets compared to the discs having the p19 + WRI1 + DGAT1 without SiOleosinL. In contrast, leaf cells expressing genes encoding the p19 + WRI1 + DGAT1 + SiOleosinH combination showed larger lipid droplets which looked about the same as those observed in leaves expressing p19 + WRI1 + DGAT1 without an oleosin. Finally, when genes encoding both SiOleosinH and SiOleosinL were co-expressed with p19 + WRI1 + DGAT1, the lipid droplets were smaller and looked similar to those observed in leaves expressing p19 + WRI1 + DGAT1 + SiOleosinL. Interestingly, expression of the vanilla leaf oleosin (pOIL386) resulted in a different pattern in which lipid droplets appeared compacted in a smear form.

Further assays were carried out using radiolabelled [^{14}C]-acetate to measure the rate of TAG synthesis for the different gene combinations including each of the lipid droplet associated polypeptides. The [^{14}C]-acetate was infiltrated into the same leaf tissues at 3 days post-infiltration of the genetic constructs i.e. after the genes had been expressed for three days. Leaf discs were sampled after 5 min, 10 min and 3 hr after addition of the radiolabel, and total lipids in the tissues were extracted and fractionated by TLC. The amount of radioactivity in different lipid types was quantitated using a Fujifilm FLA-5000 phosphorimager or using a Beckman-Coulter LS 6500 Multipurpose Scintillation Counter.

These assays demonstrated an increase in TAG synthesis rates in the leaves expressing SiOleosinL (pOIL382) as well as an increase in PC and PA synthesis rates over the three hours in leaves expressing SiOleosinL. SiOleosinL expression increased TAG accumulation already at 15 minutes (789 dpm) compared to p19 (198 dpm). In *N. benthamiana* leaf cells expressing genes encoding the p19+WRI1+DGAT1 combination, TAG accumulated rapidly, reaching 3865 dpm after 5 min of [^{14}C]-acetate incorporation compared to 293 dpm in the p19 control. This accumulation reached a maximum at 10 minutes after [^{14}C]-acetate addition (4519 dpm). However, the radiolabel in TAG quickly reduced thereafter to reach 1013 dpm at 15 minutes, indicating TAG catabolism. When the gene encoding SiOleosinL was added, the TAG was stabilised, indicating protection (i.e. TAG packaging) in the leaf cells. TAG rapidly accumulated at 5 minutes of infiltration (2855 dpm) and the level remained the same at 10 and 15 min after [^{14}C]-acetate addition. At the 15 min timepoint, TAG accumulation was equivalent to 2690 dpm for the p19+WRI1+DGAT1+SiOleosinL combination compared to 1013 dpm for the p19+WRI1+DGAT1 combination.

TAG degradation was not correlated with free fatty acid (FFA) levels, presumably because of further catabolism or of incorporation into lipids other that TAG. In order to study TAG degradation and chase the resulting derivatives, [^{14}C]-acetate incorporation into TAG and and its stability at 3 hr post-addition was studied. This experiment showed an increase in [^{14}C] in PC (2579 dpm) and PA (1270 dpm) in leaf cells expressing the SiOleosinL construct compared to 1495 dpm PC and 899 PA in both p19 and p19+WRI1+DGAT1 controls.

In another experiment, pOIL191 (AtCaleosin 3) was transiently expressed in *N. benthamiana* leaves. The expression of this gene increased TAG content by 3.6 fold (Figure 6) compared to p19 control. The expression of AtCaleosin3 with WRI1 and DGAT1 resulted in a further increase in TAG content by up to 15.3 fold compared p19 control, and up to 1.6 fold compared to WRI1 and DGAT1 control. TAG yields are comparable with *Si*Oleosin co-expression with WRI1 and DGAT1.

Example 10. Medium-chain fatty acid production in vegetative plant cells

Eccleston et al. (1996) studied the accumulation of C12:0 and C14:0 fatty acids in both seeds and leaves of transgenic *Brassica napus* plants transformed with a constitutively expressed gene encoding California Bay Laurel 12:0-ACP thioesterase (*Umbellularia californica*). That study reported that substantial levels of C12:0 accumulated in mature *B. napus* seeds but only very low levels of C12:0 were observed in leaf tissue, despite high levels of 12:0-ACP thioesterase expression and activity. The same results were obtained when the gene was transformed into *A. thaliana* (Voelker et al., 1992). That research was extended by the co-expression of the *Cocos nucifera* LPAAT and *Umbellularia californica* thioesterase which resulted in an increased accumulation of total C12:0 as well as an increased fraction of trilaurin in the seeds of *B. napus* (Knutzon et al., 1999). The prior art therefore indicated that medium chain fatty acids (MCFA) synthesis in vegetative plant cells was problematic.

To test the effect of introducing thioesterases having specificity for MCFA in combination with other genes described herein, chimeric DNAs for expressing several different thioesterases were synthesized and introduced into plant cells either singly or in combinations. The protein coding regions for thioesterases from organisms known to produce MCFAs (Jing et al., 2011) were synthesised and inserted as *Eco*RI fragments into the binary vector pJP3343 which contained a 35S-promoter expression cassette (Vanhercke et al., 2013). The thioesterases were: *Cinnamomum camphora* 14:0-ACP thioesterase (referred to as Cinca-TE) (Yuan et al., 1995; Accession No. Q39473.1; SEQ ID NO:43), *Cocos nucifera* acyl-ACP thioesterase FatB1 (Cocnu-TE1; Accession No. AEM72519.1; SEQ ID NO:88), *Cocos nucifera* acyl-ACP

thioesterase FatB2 (Cocnu-TE2; Accession No. AEM72520.1; SEQ ID NO: 89), *Cocos nucifera* acyl-ACP thioesterase FatB3 (Cocnu-TE3; Accession No. AEM72521.1; SEQ ID NO: 90), *Cuphea lanceolata* acyl-(ACP) thioesterase type B (Cupla-TE) (Topfer et al., 1995; Accession No. CAB60830.1; SEQ ID NO: 91), *Cuphea viscosissima* FatB1 (Cupvi-TE; Accession No. AEM72522.1; SEQ ID NO: 92) and *Umbellularia californica* 12:0-ACP thioesterase (Umbca-TE) (Voelker et al., 1992; Accession No. Q41635.1; SEQ ID NO: 93). These thioesterases were all in the FATB class and had specificity for MCFA. The protein coding regions for *C. nucifera* LPAAT (Cocnu-LPAAT, MCFA type) (Knutzon et al., 1995; Accession No. Q42670.1; SEQ ID NO:94) and *A. thaliana* plastidial LPAAT1 (Arath-PLPAAT; Accession No. AEE85783.1; SEQ ID NO:95), were also cloned. Cocnu-LPAAT had previously been shown to increase MCFA incorporation on the *sn*-2 position of TAG in seeds (Knutzon et al., 1995) whilst *A. thaliana* plastidial LPAAT (Arath-PLPAAT) (Kim et al., 2014) was used as a control LPAAT to determine the effect of any MCFA specificity that the Cocnu-LPAAT might have. The former LPAAT uses acyl-CoA as one substrate and operates in the ER in its native context, whereas the latter PLPAAT uses acyl-ACP as substrate and works in the plastid.

The thioesterase genes were introduced into *Nicotiana benthamiana* leaves by *Agrobacterium*-mediated infiltration as described in Example 1 along with the gene for co-expression of the p19 silencing suppressor and either the Cocnu-LPAAT or Arath-PLPAAT to determine whether MCFA could be produced in *N. benthamiana* leaf tissue. Infiltrated leaf zones were harvested and freeze-dried five days after infiltration with the *Agrobacterium* mixtures, after which the total fatty acid content and composition were determined by GC as described in Example 1 (Table 11). For the data shown in Table 11, errors are the standard deviation of triplicate infiltrations. The infiltrated zones of control leaves contained only trace (<0.1%) or zero levels of fatty acids C12:0 and C14:0 whereas C16:0 was present at 14.9%±0.6 of the TFA in the total leaf lipids. C12:0 levels were only increased significantly by expression of the Cocnu-TE3 (1.2%±0.1) and Umbca-TE (1.6%±0.1). Expression of each of the tested thioesterases resulted in the accumulation of C14:0 in the *N. benthamiana* leaves, with Cinca-TE giving the highest level of 11.3%±1.0. Similarly, expression of each of the thioesterases with the exception of Umbca-TE resulted in increased C16:0 levels. The highest level of C16:0 accumulation (35.4%±4.7) was observed with expression of Cocnu-TE1. Substantial necrosis of the infiltrated zones was observed in the leaves when the *FATB* genes were expressed alone, which appeared to correlate with the level of MCFA production. The inventors considered that the necrosis was probably due to levels of free fatty acids (FFA) greater than optimum, and also due to the extensive accumulation of MCFA in phospholipid lipid pools rather than in TAG.

Table 11. Total leaf fatty acid composition (% total leaf fatty acid) of selected fatty acids in *Nicotiana benthamiana* leaves infiltrated with various thioesterases (TE) and LPAATs. Results are grouped by the co-infiltrated gene (single genes (other than p19 present in all samples), Arath-LPAAT + various TE, Cocnu-LPAAT + various TE). 'Control' denotes uninfiltrated *N. benthamiana* leaf whereas 'p19 only' contains the silencing suppressor gene alone. 16:3 is $16:3^{\Delta7,10,13}$; 18:3 is $18:3^{\Delta9,12,15}$. Gene identities are defined in the text.

		12:0	14:0	16:0	16:3	18:3
	Control	0.2±0	0.1±0	14.0±0.2	8.1±0.1	57.2±0
	p19 only	0.2±0	0.1±0	14.9±0.6	7.0±0.8	53.1±0.7
Single-gene tests	**Cinca-TE**	0.4±0	11.3±1.0	21.9±0.7	5.0±0.2	38.5±1.0
	Cocnu-TE1	0.2±0	6.3±0.6	35.4±4.7	4.2±1.4	29.9±5.5
	Cocnu-TE2	0.2±0	7.1±0.3	31.9±2.2	4.7±0.5	32.9±2.8
	Cocnu-TE3	1.2±0.1	7.2±1.3	19.6±1.6	5.7±0.5	44.8±2.9
	Cupla-TE	0.2±0	1.1±0.2	21.8±2.9	6.0±0.6	48.2±3.1
	Cupvi-TE	0.2±0	0.6±0.1	17.3±1.3	6.4±0.4	52.9±2.1
	Umbca-TE	1.6±0.1	1.1±0.2	14.4±0.8	6.5±0.3	52.7±0.1
	Arath-LPAAT	0.2±0	0.4±0.5	17.4±1.0	6.2±0.3	51.4±1.3
	Cocnu-LPAAT	0.1±0.1	0.1±0	15.1±1.5	6.7±0.5	52.2±4.2
+Arath-LPAAT	**Cinca-TE**	0.2±0	7.8±0.1	24.6±0.4	5.3±0.2	39.2±1.5
	Cocnu-TE1	0.2±0	4.6±1.3	35.3±1.4	4.4±0.7	32.7±2.0
	Cocnu-TE2	0.2±0	6.1±0.4	32.5±1.8	4.7±0.1	34.1±0.6
	Cocnu-TE3	0.9±0.2	8.5±0.4	21.4±1.9	5.6±0.2	41.7±0.6
	Cupla-TE	0.2±0	1.0±0.1	23.4±2.7	5.9±0.5	47.3±1.2
	Cupvi-TE	0.2±0	0.6±0	19.0±0.2	6.3±0.1	51.4±1.0
	Umbca-TE	1.2±0.2	1.1±0.1	15.4±0.2	6.5±0.2	52.3±1.3
+Cocnu-LPAAT	**Cinca-TE**	0.7±0.2	14.9±1.6	23.0±3.7	4.8±1.4	35.4±3.3
	Cocnu-TE1	0.2±0	5.4±0.9	40.2±2.8	3.3±0	27.8±1.1
	Cocnu-TE2	0.2±0	6.6±1.0	38.3±1.1	3.7±0.2	28.2±1.1
	Cocnu-TE3	2.0±0.3	10.9±1.0	24.4±1.8	4.9±0.5	37.7±0.9
	Cupla-TE	0.5±0.1	1.6±0.3	22.2±0.6	6.0±0.3	46.9±2.0
	Cupvi-TE	0.5±0	1.1±0	19.6±0.8	6.0±0.2	49.8±0.3
	Umbca-TE	3.3±0.5	1.2±0.1	13.9±0.4	6.4±0.2	51.3±1.7

Co-infiltration of the chimeric gene for expressing Arath-PLPAAT with the thioesterases tended to reduce the accumulation of both C12:0 and C14:0 compared to the absence of the LPAAT, whilst slightly increasing the accumulation of C16:0. In

contrast, co-infiltration of the genes for expressing Cocnu-LPAAT or Umbca-TE increased the accumulation of C12:0 to 3.3%±0.5 whilst C14:0 was found to accumulate to 14.9%±1.6 in the Cinca-TE + Cocnu-LPAAT sample. The highest C16:0 levels were observed after co-expression of Cocnu-TE1 and Cocnu-LPAAT (40.2%±2.8). Addition of an LPAAT to each inoculated zone decreased the degree of necrosis of the leaf tissue. Surprisingly, both C8:0 and C10:0 fatty acids were also produced in the plant cells in the transient expression studies. The accumulation of C8:0 and C10:0 was not observed when the thioesterase was expressed alone. However, when thioesterase expression was combined with the co-expression of CuphoFatB with CnLPAAT and AtWRI1, C8:0 was found to be present at a concentration of 0.27±0.09% of the total fatty acid content in the plant cells. Similarly, when CuplaFatB was co-expressed with CnLPAAT and AtWRI1, C10:0 was found to be present at 0.54±0.16% of the total fatty acid content.

These results indicated that the previously-reported acyl specificities of the thioesterases, observed from seed expression, were essentially maintained in *N. benthamiana* leaves and that this expression system was a valid system for testing acyl specificity. The addition of the plastidial *A. thaliana* PLPAAT did not increase the accumulation of MCFAs although it did result in slightly increased accumulation of C16:0 in *A. thaliana* cells. In contrast, the *C. nucifera* LPAAT increased the accumulation of C12:0, C14:0 and C16:0 in *N. benthamiana* leaves, which fatty acids are found in *C. nucifera* oil (Laureles et al., 2002). This indicated that the native *N. benthamiana* LPAAT was either not highly expressed in leaf tissue or did not have high activity on C12:0, C14:0 and C16:0 substrates.

Medium-chain fatty acid production in vegetative plant cells accumulating high levels of TAG

The inventors previously obtained the production of 15% TAG in *N. tabacum* leaves by the coordinate expression of chimeric genes encoding *A. thaliana* WRI1, *A. thaliana* DGAT1 and *S. indicum* Oleosin (Vanhercke et al., 2014a and b). To test whether the accumulation of MCFA that was observed after expression of thioesterases in combination with an LPAAT would also occur or be increased in plant cells producing high levels of TAG (Vanhercke et al., 2013), these genes were co-expressed. The best performing C12:0, C14:0 and C16:0 thioesterase/LPAAT combinations (Cocnu-LPAAT plus Umbca-TE, Cinca-TE and Cocnu-TE2 thioesterases, respectively) were infiltrated with and without the Arath-WRI1+DGAT combinations previously described (Vanhercke et al., 2013). The data are shown in Figure 7.

The accumulation of the relevant MCFA (C12:0 for Umbca-TE, C14:0 for Cinca-TE and C16:0 for Cocnu-TE2) was consistently and substantially increased most by the addition of Arath-WRI1 to the combinations: C12:0 comprised 9.5%±0.9 of total leaf fatty acids in the Umbca-TE+Cocnu-LPAAT+Arath-WRI1 samples, the C14:0 level was 18.5%±2.6 in the Cinca-TE+Cocnu-LPAAT+Arath-WRI1 samples and the C16:0 level was 38.3%±3.0 in the Cocnu-TE2+Cocnu-LPAAT+Arath-WRI1 samples. Thioesterase plus Arath-WRI1 infiltrations were found to have a significantly greater effect on C12:0 in the presence of Umbca-TE, C14:0 in the presence of Cinca-TE and C16:0 in the presence of Cocnu-TE2 relative to infiltration with thioesterase plus Cocnu-LPAAT in the absence of WRI1 (Figure 8). The addition of the Cocnu-LPAAT to the thioesterase plus Arath-WRI1 mixtures did have an effect on the fatty acid composition with relatively small increases in C12:0 and C14:0 observed in the Umbca-TE and Cinca-TE sets and a small decrease in C16:0 in the Cocnu-TE2 set. The maximum levels observed were: 8.8%±1.1 of C12:0 in total leaf fatty acids observed in the Umbca-TE + Arath-WRI1 + Cocnu-LPAAT samples, 14.1%±3.5 of C14:0 in the Cinca-TE + Arath-WRI1 + Cocnu-LPAAT samples and 48.6%±3.7 of C16:0 in the Cocnu-TE2 + Arath-WRI1 sample.

Interestingly, the only thioesterase in which the Arath-WRI1 did not increase MCFA accumulation as much was the Cocnu-TE2, although it still increased significantly. The addition of this gene alone resulted in the increased accumulation of C16:0 from 16.0%±0.4 to 37.3%±0.6 whereas the further addition of Arath-WRI1 only increased this to 48.6%±1.7. This may have been due to the C12:0 and C14:0 intermediates being relatively transient during plastidial fatty acid synthesis compared to C16:0.

Other effects that were noted included the increase in C16:0 and C18:1$^{\Delta 9}$ and decrease in C18:3$^{\Delta 9,12,15}$ levels in the presence of Arath-WRI1. The further addition of the Cinca-TE and Cocnu-TE2 decreased C18:3$^{\Delta 9,12,15}$ levels further still. In contrast, the extra C12:0 produced following the addition of Arath-WRI1 to Umbca-TE appeared to come at the cost of C16:0 rather than additional C18:3$^{\Delta 9,12,15}$ (Figure 9).

A subset of samples were also analysed by LC-MS to gain a better understanding of MCFA accumulation. The plastidial galactolipids monogalactosyl diacylglycerol (MGDG) and digalactosyl diacylglycerol (DGDG) contained only low levels of C12:0 and C14:0 and reduced levels of C16:0 relative to the p19 control infiltration. The major C12:0-containing MGDG species in the Umbca-TE samples was 30:3 indicating that one C18:3 and one C12:0 were co-located on the monogalactosyl backbone. The other main C12:0-containing MGDG species was 28:0, indicating that the second fatty acid was C16:0. The major C14:0-containing MGDG species in the Cinca-TE samples were 28:0 and 30:0, indicating that a

significant proportion of the C14:0 in MGDG was either di-C14:0 or with C16:0. The C12:0-containing and C14:0-containing MGDG species were not detected in the p19 control sample. In contrast, C16:0-containing MGDG species tended to be reduced in the Cocnu-TE2 samples. The major MGDG species in the wildtype samples (C16:3-containing 34:6, C18:3-containing 34:6, and C18:3-containing 36:6) all tended to be reduced by the expression of the transgenes. This reduction was greatest in the presence of the WRI+DGAT combination.

Only trace levels of C12:0-containing DGDG species were observed in the Umbca-TE samples. The major C14:0-containing species observed in the Cinca-TE samples were 28:0 and 30:0, both of which were absent in the control. These species were also observed at elevated levels in the Cocnu-TE2 samples but only at trace levels in the Umbca-TE samples. The major DGDG species in the wildtype samples (C16:0-containing 34:3, C18:3-containing 34:3, and C18:3-containing 36:6) all tended to be reduced by the expression of the transgenes. This reduction was greatest in the presence of WRI.

Similarly, TAG species were generally increased considerably in all the samples containing WRI + DGAT as previously described (Vanhercke et al., 2013). C12:0 species were found to be dominant in the high TAG Umbca-TE sample, C14:0 in the high TAG Cinca-TE sample and C16:0 in the high TAG Cocnu-TE2 sample. LC-MS analysis of the TAG fraction showed that the C12:0-containing 36:0 was found to be the dominant TAG species, twice the level of TAG species containing C18:3, in all Umbca-TE samples containing the WRI transcription factor. Similarly, C14:0-containing 42:0 was the dominant TAG species in the Cinca-TE samples co-transformed with either LPAAT, DGAT, WRI or WRI+DGAT, although the response was considerably higher in the case of the samples containing WRI. Several C16:0-containing TAG species were significantly elevated in both the high TAG Cinca-TE (e.g. 44:0 and 50:3) and Cocnu-TE2 (e.g. 46:0, 48:0, 50:2 and 50:3) samples. Again, the greatest C16:0 increases were observed in the presence of WRI.

Stable transformation for production of MCFA in vegetative tissues.

A series of genetic constructs were made in a binary vector in order to stably transform plants such as tobacco with combinations of genes for production of MCFA in vegetative tissues, to identify optimal combinations of genes. These constructs included a gene for expression of WRI1 under the control of either the SSU promoter (see Example 3, pOIL121) or the senescence-specific SAG12 promoter, a gene encoding an oil palm DGAT (below), a gene encoding the coconut LPAAT (CocnuLPAAT, see above) under the control of an enTCUP promoter and several

genes expressing a variety of fatty acyl thioesterases (FATB) expressed from either a 35S promoter or a SAG12 promoter. These are described below.

Cloning of a gene encoding Elaeis guineensis (oil palm) DGAT

In order to firstly test different DGAT enzymes, including representative DGAT1, DGAT2 and DGAT3 enzymes, candidate oil palm DGAT sequences were identified from the published transcriptome (Dussert et al., 2013) and codon optimised for expression in *Nicotiana tabacum*. The protein coding regions were then each cloned individually into binary expression vectors under the control of the 35S promoter for testing in transient *N. benthamiana* leaf assays as described in Example 1. The gene combinations tested were as follows:

1 P19 (negative control)
2 P19+CnLPAAT+WRI1
3 P19+CnLPAAT+AtWRI1+AtDGAT1
4 P19+CnLPAAT+AtWRI1+EgDGAT1
5 P19+CnLPAAT+AtWRI1+EgDGAT2
6 P19+CnLPAAT+AtWRI1+EgDGAT3
7 P19+CincaFatB
8 P19+CincaFatB+CnLPAAT+WRI1
9 P19+CincaFatB+CnLPAAT+AtWRI1+AtDGAT1
10 P19+CincaFatB+CnLPAAT+AtWRI1+EgDGAT1
11 P19+CincaFatB+CnLPAAT+AtWRI1+EgDGAT2
12 P19+CincaFatB+CnLPAAT+AtWRI1+EgDGAT3

The results for the TFA and TAG levels, and the levels of total MCFA in the TFA or the TAG contents, are shown in Figure 10. Compared to AtDGAT1, the expression of EgDGAT1 led to greater accumulation of total fatty acids and increased TAG levels. The total MCFA content in the total fatty acid content was reduced with the expression of EgDGAT1 relative to AtDGAT1, but the levels of MCFA present in TAG remained about the same (Figure 10).

Preparation of genetic constructs

Genetic constructs for stable transformation (Table 12) were assembled through the sequential insertion of gene cassettes through the use of compatible restriction enzyme sites. The four gene constructs (Table 12) each contained a gene encoding the oil palm DGAT1 (EgDGAT1) expressed from the 35S promoter, a gene encoding the *C. nucifera* LPAAT (CnLPAAT) expressed from the constitutive enTCUP2 promoter, and a gene encoding AtWRI1 expressed from either the SSU promoter or the SAG12 promoter in addition to one of a series of genes encoding FATB enzymes.

The five gene constructs also contained a gene for expression of a hairpin RNA for reducing expression of an endogenous gene encoding acyl-activating enzyme (AAE). The hairpin was constructed based on sequence similarity with the identified AAE15 from *Arabidopsis lyrata* (EFH44575.1) and the *N. benthamiana* genome. AAE has been shown to be involved in the reactivation of MCFA, and hence further elongation. It was considered that silencing of AAE might increase MCFA accumulation. The hairpin cassette was constructed in the vector pKANNIBAL and then subcloned into the expression vector pWBVec2 with the expression of the hairpin being driven by the 35S promoter.

Table 12. Summary of assembled genetic constructs.

	Construct	Gene Combination
Single Gene Constructs	pKR1	35S::UmbcaFATB
	pKR2	35S::CincaFATB
	pKR3	35S::CocnuFATB2
	pOIL115	SAG12::CincaFATB
	pOIL116	SAG12::UmbcaFATB
	pOIL117	SAG12::CocnuFATB2
Construction Components	pOIL300	35S::EgDGAT1
	pOIL301	enTCUP::CnLPAAT inFATBrmediaFATB construct
	pOIL302	35S::EgDGAT1 + enTCUP::CnLPAAT
	pOIL303	35S::EgDGAT1 + enTCUP::CnLPAAT + SSU:AtWRI1
	pOIL304	35S::EgDGAT1 + enTCUP::CnLPAAT + SAG12:AtWRI1
Four Gene Constructs	pOIL305	35S::EgDGAT1 + enTCUP::CnLPAAT + SSU:AtWRI1 + 35S::UmbcaFATB
	pOIL306	35S::EgDGAT1 + enTCUP::CnLPAAT + SSU:AtWRI1 + 35S::CincaFATB
	pOIL307	35S::EgDGAT1 + enTCUP::CnLPAAT + SSU:AtWRI1 + 35S::CocnuFATB2
	pOIL308	35S::EgDGAT1 + enTCUP::CnLPAAT + SSU:AtWRI1 + SAG12::UmbcaFATB
	pOIL309	35S::EgDGAT1 + enTCUP::CnLPAAT + SSU:AtWRI1 + SAG12::CincaFATB
	pOIL310	35S::EgDGAT1 + enTCUP::CnLPAAT + SSU:AtWRI1 + SAG12::CocnuFATB2
	pOIL311	35S::EgDGAT1 + enTCUP::CnLPAAT + SAG12:AtWRI1 + 35S::UmbcaFATB
	pOIL312	35S::EgDGAT1 + enTCUP::CnLPAAT + SAG12:AtWRI1 + 35S::CincaFATB
	pOIL313	35S::EgDGAT1 + enTCUP::CnLPAAT + SAG12:AtWRI1 + 35S::CocnuFATB2
	pOIL314	35S::EgDGAT1 + enTCUP::CnLPAAT + SAG12:AtWRI1 + SAG12::UmbcaFATB
	pOIL315	35S::EgDGAT1 + enTCUP::CnLPAAT + SAG12:AtWRI1 + SAG12::CincaFATB
	pOIL316	35S::EgDGAT1 + enTCUP::CnLPAAT + SAG12:AtWRI1 + SAG12::CocnuFATB2

Five Gene Constructs	pOIL317	35S::EgDGAT1 + enTCUP::CnLPAAT + SSU:AtWRI1 + 35S::UmbcaFATB + 35S::hpNbAAE
	pOIL318	35S::EgDGAT1 + enTCUP::CnLPAAT + SSU:AtWRI1 + 35S::CincaFATB + 35S::hpNbAAE
	pOIL319	35S::EgDGAT1 + enTCUP::CnLPAAT + SSU:AtWRI1 + 35S::CocnuFATB2 + 35S::hpNbAAE
	pOIL320	35S::EgDGAT1 + enTCUP::CnLPAAT + SSU:AtWRI1 + SAG12::UmbcaFATB + 35S::hpNbAAE
	pOIL321	35S::EgDGAT1 + enTCUP::CnLPAAT + SSU:AtWRI1 + SAG12::CincaFATB + 35S::hpNbAAE
	pOIL322	35S::EgDGAT1 + enTCUP::CnLPAAT + SSU:AtWRI1 + SAG12::CocnuFATB2 + 35S::hpNbAAE
	pOIL323	35S::EgDGAT1 + enTCUP::CnLPAAT + SAG12:AtWRI1 + 35S::UmbcaFATB + 35S::hpNbAAE
	pOIL324	35S::EgDGAT1 + enTCUP::CnLPAAT + SAG12:AtWRI1 + 35S::CincaFATB + 35S::hpNbAAE
	pOIL325	35S::EgDGAT1 + enTCUP::CnLPAAT + SAG12:AtWRI1 + 35S::CocnuFATB2 + 35S::hpNbAAE
	pOIL326	35S::EgDGAT1 + enTCUP::CnLPAAT + SAG12:AtWRI1 + SAG12::UmbcaFATB + 35S::hpNbAAE
	pOIL327	35S::EgDGAT1 + enTCUP::CnLPAAT + SAG12:AtWRI1 + SAG12::CincaFATB + 35S::hpNbAAE
	pOIL328	35S::EgDGAT1 + enTCUP::CnLPAAT + SAG12:AtWRI1 + SAG12::CocnuFATB2 + 35S::hpNbAAE

These genetic constructs were used to produce transformed tobacco plants of cultivars Wisconsin 38 and a high oil line transformed with the T-DNA from pJP3502. It was observed that plants transformed with the single gene FATB constructs expressed from the 35S promoter were significantly smaller than those transformed with the corresponding FATB construct expressed from the SAG12 promoter or from the four gene constructs. The smaller plant size was considered to be caused by a buildup of MCFA which was not incorporated efficiently into TAG.

Discussion

The present study found that C12:0 production in leaf cells was only about 1.6% of the total fatty acid content after expression of Umbca-TE alone (Table 11). The addition of a gene for expression of Arath-WRI had a much stronger effect on C12:0 and C14:0 accumulation in leaf tissue than the addition of the coconut LPAAT (Figures 7 and 9). This indicated that WRI1 in combination with the thioesterase greatly increased MCFA accumulation in leaf cells, acting synergistically. Importantly, much of the C12:0, C14:0 and C16:0 was found to accumulate in the leaves in TAG, which lipid does not accumulate at substantial levels in wild-type leaves. These experiments showed that the cells in the vegetative parts of plants could be modified to produce MCFA, particularly C12:0 and C14:0 in TAG at high levels. C16:0 levels were also increased substantially.

Example 11: Gene selection and vector construction

Fatty acyl thioesterases were identified from *Cinnamomum camphora* 14:0-ACP thioesterase (referred to as '*CcTE'*, Accession No. Q39473.1, (Yuan et al., 1995)), *Umbellularia californica* 12:0-ACP thioesterase (*UcTE*, Accession No. Q41635.1, (Voelker et al., 1992)), and *Cocos nucifera* acyl-ACP thioesterase *FatB2* (*CnTE2*, Accession No. AEM72520.1, (Jing et al., 2011)). A *C. nucifera LPAAT* (*CnLPAAT*, Accession No. Q42670.1, (Knutzon et al., 1995)) was also identified. Coding regions were synthesized using codon optimised nucleotide sequences for expression in *Nicotiana* plant cells. Expression vectors encoding WRI1 and DGAT were produced as previously described by Vanhercke et al. (2013).

Three *DGAT* candidate sequences were identified in the transcriptome of African oil palm (*Elaeis guineensis*) (Dussert et al., 2013) and selected to be tested in their utilisation of MCFA for the assembly of leaf lipids. The *DGAT*s from oil palm were selected based on the fatty acid compositions of palm oil and palm kernel oil (Edem, 2002), being high in MCFA content.

A gene encoding glycerol-3-phosphate acyltransferase 9 (GPAT9) from *C. nucifera* (coconut, *CnGPAT9*) was identified from a transcriptome. A genetic construct to express this enzyme was made from RNA isolated from developing coconut endosperm, as described below.

Each gene was cloned into the *Eco*RI site of the binary vector pJP3343 which contained a constitutive 35S promoter with duplicated enhancer region (Vanhercke et al., 2013) for expression in plant cells. *Agrobacterium tumefaciens* strain AGL1 was transformed with each of the constructs.

Example 12: Increasing medium chain fatty acid production in vegetative plant cells

GPAT9 has recently been identified as functioning in *Arabidopsis thaliana* seed to transfer acyl groups from acyl-CoA to the *sn*-1 position of glycerol-3-phosphate (G3P) (Shockey et al., 2016; Singer et al., 2016). The inventors hypothesized that a GPAT9 from coconut might assist in increasing the MCFA content of transgenic oils produced in vegetative plant cells. A GPAT9 gene from coconut was identified by searching an assembled coconut endosperm transcriptome using the *Arabidopsis thaliana GPAT9* nucleotide sequence (*AtGPAT9*) (Shockey et al., 2016) as the BLAST query. A candidate for *GPAT9* from coconut was identified, namely NCBI Accession number KX235871. High fidelity PCR was used to amplify the full length *CnGPAT9* cDNA sequence from coconut. Following isolation and sequencing of the full length transcript of interest, the open reading frame for the

predicted *CnGPAT9* was identified. The predicted amino acid sequence was aligned with the sequence of AtGPAT9, revealing that the sequences were 78% identical. Sequence alignment with other annotated *GPAT* nucleotide sequences showed that the identified *CnGPAT9* nucleotide sequence clustered with other *GPAT9* sequences (Figure11).

A nucleotide sequence encoding the candidate CnGPAT9 was synthesized and inserted into pJP3343 in order to test its enzymatic function using the transient *N. benthamiana* infiltration assay as described in Example 1, in particular to test its ability to increase TAG content. AtGPAT9 was used as a positive control. Total lipids were extracted from infiltrated leaf zones and analysed to determine the effect of the GPAT9s on TAG content (Figure 12). From comparison with the samples where p19 alone was infiltrated, which provided a TAG level of about 0.1%, expression of either *AtGPAT9* or *CnGPAT9* provided significant increases in the TAG content in the leaf, to 0.5 ± 0.2% and 0.7 ± 0.1% on a dry weight basis, respectively. There was no significant difference in the TAG levels between the two GPAT9s. It was concluded from these data and the phylogeny (Figure 11) that the isolated *CnGPAT9* sequence from coconut encoded a functional GPAT9.

Example 13: DGAT1 promotes production of MCFA-enriched oils

It has been previously demonstrated that MCFA-containing oils could be produced in the leaves of *N. benthamiana* (Reynolds et al., 2015). However, chlorosis of the leaves was observed with some gene combinations when MCFA accumulated in membrane lipids such as PC. The inventors wanted to test whether the introduction of a DGAT capable of esterifying MCFA into TAG might increase the MCFA content and perhaps reduce the chlorosis phenotype.

Gene candidates that might be involved in lipid synthesis pathways were identified in the *Elaeis guineensis* (African oil palm) transcriptome (Dussert et al., 2013) as described above. The fatty acid profile of the oils from oil palm (palm oil and palm kernel oil) (Edem, 2002) suggested that some DGATs from oil palm might exhibit preference for MCFA substrates. Sequences for three candidate DGAT1 cDNAs were identified from the *E. guineensis* transcriptome. Alignment of the predicted amino acid sequences after translation of the cDNAs revealed that the isoforms designated EgDGAT1.2 and EgDGAT1.3 lacked highly conserved C- and N- terminal motifs (Cao, 2011) which are responsible for the catalytic and regulatory activities of DGAT1, respectively (Liu et al., 2012; Xu et al., 2008), suggesting these isoforms would be non-functional. The third candidate EgDGAT1.1 had these conserved motifs and was further tested.

A genetic construct with codon optimization for expressing EgDGAT1.1 in *N. tabacum* was synthesized and infiltrated into *N. benthamiana* in combination with genetic constructs to express *Arabidopsis thaliana* WRI1 and CnLPAAT. The infiltrations were either with or without a gene for co-expression of a thioesterase from *Cinnamomum camphora* (CcTE), to measure levels of both TAG production and the incorporation of MCFA into TAG. Five days after infiltration, a strong chlorosis phenotype was observed to be associated with several gene combinations, correlated in particular with the presence of CcTE. Surprisingly, the chlorosis phenotype was alleviated by the addition of the gene encoding EgDGAT1.1 (hereinafter referred to as EgDGAT1) moreso than with AtDGAT1. It was hypothesized that the alleviation of the negative chlorosis phenotype was due to the increased capacity of EgDGAT1 to sequester MCFA into TAG relative to AtDGAT1.

Total lipids were extracted and analysed in order to better understand the relationship between chlorosis and the particular gene combinations. The total fatty acid profile revealed that in the absence of CcTE, the TFA content was similar in the presence of either AtDGAT1 or EgDGAT1. In the presence of CcTE, the TFA content was significantly greater for treatments including EgDGAT1 relative to AtDGAT1. The same correlation was observed for TAG content. Although the TAG content was similar for the AtWRI1 + AtDGAT1 and AtWRI1 + EgDGAT1.1 samples, the TAG content was significantly increased for samples expressing CcTE and EgDGAT1, compared to samples expressing AtDGAT1. These results suggested that following CcTE expression, in the presence of AtDGAT1, fatty acid synthesis was inhibited due to inefficient assembly of the MCFA into glycerolipids. Conversely, there appeared to be no inhibition of fatty acid synthesis following the addition of EgDGAT highlighted by increases in both the TFA and TAG content, implying improved incorporation efficiency for MCFAs.

The fatty acid composition of the phospholipid fraction in the infiltrated leaf zones was also analysed. Total phospholipids were fractionated by TLC and prepared for analysis by the preparation of FAME. Analysis of the fatty acid composition of the phospholipids revealed a significant reduction in the accumulation of MCFA, particularly C14:0 and C16:0, following the expression of the *EgDGAT1* construct, compared to *AtDGAT1*. This suggested that the reduced accumulation of MCFA into membrane lipids assisted in reducing the chlorosis phenotype.

Example 14: Reconfiguration of Kennedy Pathway for efficient MCFA accumulation

Following confirmation of CnGPAT9 activity, its capability to use various MCFA acyl-CoAs as substrates for TAG assembly was tested. This was done in the

context of the Kennedy pathway components LPAAT and DGAT1, as well as WRI1 to increase the level of fatty acid synthesis. The fatty acid composition of TAG and the TAG content were determined by GC-FID (Figure 13, Tables 13-15). When combined with co-expression of UcTE, the sequential addition of each acyltransferase resulted in both significantly increased total TAG content, and a significantly increased accumulation of laurate (C12:0) in the TAG as a percentage of the total fatty acid content of the TAG. C12:0 levels were up to 51.6 ± 2.0% in the presence of the combined expression of UcTE + AtWRI1 + CnGPAT9 + CnLPAAT + EgDGAT1, at a total TAG content in the leaf tissue of 2.4 ± 0.7%. It was also observed that this combination was associated with a reduction of the chlorosis phenotype, thought by the inventors to be a result of efficient sequestering of laurate into TAG, i.e. less inclusion in membrane lipids such as PC. Similar results were observed with the co-expression of CcTE. C14:0 accumulated to 40.3 ± 1.2% in the presence of the combination of CcTE + AtWRI1 + CnGPAT9 + CnLPAAT. There was an increase in the TAG content but not significantly compared to CcTE + CnGPAT9. The greatest TAG production was achieved following the further addition of the EgDGAT1, with a total TAG content of 2.8 ± 0.2%. The fatty acid composition of TAG was altered following the additional combination with EgDGAT1, with a significant reduction in C14:0 and a significant increase in C16:0 content, each as a percentage of the total fatty acid content of the TAG. This shift in profile suggested that EgDGAT1 exhibited a stronger substrate preference for C16:0 compared to C14:0. Consistent with the observations with UcTE, a significant improvement in the chlorotic phenotype was observed following the addition of EgDGAT1. When CnTE2 was used, the sequential addition of the acyltransferases did not result in any significant differences in either the fatty acid profile of TAG, or the total TAG content. This may have been due to the native acyltransferases' ability to efficiently utilise the increased flux of C16:0 acyl-CoA associated with the activity of CnTE2.

Further investigations into the effects of the sequential addition of acyltransferases on the utilization of acyl-CoAs for the assembly of MCFA-enriched glycerolipids was performed using QQQ-LCMS as described in Example 1, to reveal any differences in MCFA assembly and distribution. The integrated analysis including DAG, PC and TAG revealed much information about the assembly process of lipids in the leaf cells. When CnGPAT9 was expressed with UcTE + AtWRI1, it was observed that CnGPAT9 used C12:0 substrate for assembly, based on the presence of PC 30:3 (C12:0 plus C18:3). It was reasoned that the *sn*-2 position of the PC was most likely occupied by C18:3, due to either the esterification of C12:0 to the *sn*-1 position via CnGPAT9 or from the absence of CnLPAAT. The presence of some TAG 42:3 suggested that the native DGATs exhibited some capability of utilising

C12:0 for TAG assembly (12:0/18:3/12:0). With the addition of CnLPAAT, a significant amount of PC 24:0 (di-C12:0) was produced, indicating that C12:0 was efficiently esterified to both the *sn*-1 and *sn*-2 positions of the G3P backbone. However, without a strong substrate preference for C12:0, most of the produced laurate remains sequestered in membrane lipids. However, further addition of EgDGAT1 increased laurate accumulation. This shift involved the reduction of MCFAs accumulating in PC and increased production of MCFA-enriched TAG. Most notable was the shift from PC 24:0 (without EgDGAT1) to the accumulation of TAG 36:0 (tri-C12:0) (with EgDGAT1), highlighting that laurate was being efficiently incorporated into all three position of the G3P backbone in the presence of EgDGAT1. Significant increases were also observed for other MCFA-enriched TAG species including TAG 38:0, TAG 40:0 and TAG 42:0. These results confirmed that the expression of an appropriate DGAT1 was effective for the efficient incorporation of the unusual fatty acids of interest (in this instance, C12:0 and other MCFA) into TAG. These results highlighted that the expression of the EgDGAT1 in the enzyme combination effectively relieved the accumulation of MCFA in PC and promoted efficient production of MCFA-enriched TAG in plant leaf lipids.

A similar pattern was also observed in the case study involving combinations including CcTE. When CnGPAT9 was combined with CcTE + AtWRI1, it was observed that CnGPAT9 utilised C14:0 substrate, based on the accumulation of PC 28:0 (di-C14:0) and PC 30:0 (C14:0 plus C16:0). It appeared that the native LPAAT genes were somewhat capable of utilising C14:0-CoA as substrate based on the presence of PC 28:0, indicating that C14:0 was being esterified at both the *sn*-1 and *sn*-2 positions of the PC. Similarly, the native DGATs also appeared capable of utilising C14:0-CoA for TAG assembly, based on the production of TAG 42:0 (tri-C14:0). However, the subsequent addition of CnLPAAT to the system increased utilisation of C14:0 acyl-CoA, evident from the significantly increased abundance of PC 28:0, which indicated an increased efficiency of esterification to the *sn*-2 position of PC. This increased accumulation of MCFA was also correlated with a more severe chlorosis phenotype then compared to the CnGPAT9 alone, most likely attributed to the increased accumulation in the membrane lipids. The further addition of the EgDGAT1 to the combination resulted in almost complete absence of MCFA from PC. This was associated with an increased production of MCFA-enriched TAG species, particularly TAG 40:0, TAG 42:0, TAG 44:0 and TAG 46:0, all of which include the incorporation of C14:0.

When CnGPAT9 was combined with CnTE2 + AtWRI1, it was observed that CnGPAT9 also utilised C16:0-CoA as substrate, based on the accumulation of PC 32:0 (di-C16:0). Based on the fatty acid profile of *N. benthamiana* leaves, it was

expected that the native LPAATs and DGATs would exhibit substrate preference for the incorporation of C16:0 into glycerolipids, evidenced from the increased production of C16:0-enriched TAG species, through simply over-expressing a thioesterase with C16:0 specificity. Although the subsequent additions of the CnLPAAT and EgDGAT1 did not appear to significantly affect the overall TAG composition, there was a significant reduction in the total MCFA accumulation in PC lipids. Importantly, the addition of the EgDGAT1 to CnTE2 was associated with a reduction in the degree of leaf chlorosis, although not as complete as in the presence of the other TEs.

It was concluded that a GPAT9 like CnGPAT9 having a preference for MCFA substrates was an important factor in contributing towards both MCFA accumulation and increasing the total production of TAG in plant leaves. In the absence of a DGAT having substrate preference for MCFA, the low abundance of MCFA-containing DAG species suggested that DAG containing the MCFA was efficiently converted to PC through the activities of either PDCT or CPT (Bates and Browse, 2011; Bates and Browse, 2012; Bates et al., 2012). The addition of EgDGAT1 changed the metabolic flux of the system, pushing MCFA towards TAG accumulation via the Kennedy pathway, and thus away from incorporation of the MCFA into membrane lipids through reducing conversion of DAG to PC.

Table 13. Total leaf fatty acid composition of TAG (% total TAG) of C6:0, C8:0 and C10:0 fatty acids in *Nicotiana benthamiana* leaves infiltrated with various constructs.

Genotype	**C6:0**	**C8:0**	**C10:0**
REPLICATE 1			
P19	0.000	0.000	4.317
P19+ CincaTE + CnGPAT9 + AtWRI	0.000	0.000	0.000
P19+ CuplaTE + CnGPAT9 + AtWRI	0.000	0.000	5.687
P19+ UmbcaTE + CnGPAT9 + AtWRI	0.000	0.000	0.000
P19+ CocnuTE2 + CnGPAT9 + AtWRI	0.000	0.000	0.000
P19+ CincaTE + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.000	0.000
P19+ CuplaTE + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.000	0.947
P19+ UmbcaTE + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.000	0.000
P19+ CocnuTE2 + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.000	0.000

P19+ CincaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	0.000
P19+ CuplaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	1.533
P19+ UmbcaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	0.000
P19+ CocnuTE2 + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	0.000
P19+ CincaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	0.000
P19+ CuplaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	1.643
P19+ UmbcaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	0.000
REPLICATE 2			
P19	0.000	0.000	0.000
P19+ CincaTE + CnGPAT9 + AtWRI	0.000	0.000	0.000
P19+ CuplaTE + CnGPAT9 + AtWRI	0.000	0.000	4.368
P19+ UmbcaTE + CnGPAT9 + AtWRI	0.000	0.000	0.000
P19+ CocnuTE2 + CnGPAT9 + AtWRI	0.000	0.000	0.000
P19+ CincaTE + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.000	0.000
P19+ CuplaTE + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.000	3.523
P19+ UmbcaTE + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.000	0.000
P19+ CocnuTE2 + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.000	0.000
P19+ CincaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	0.000
P19+ CuplaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	0.000
P19+ UmbcaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	0.000

P19+ CocnuTE2 + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	0.000
P19+ CincaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	0.000
P19+ CuplaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	0.000
P19+ UmbcaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	0.000
REPLICATE 3			
P19	0.000	0.000	0.000
P19+ CincaTE + CnGPAT9 + AtWRI	0.000	0.000	0.000
P19+ CuplaTE + CnGPAT9 + AtWRI	0.000	0.000	0.000
P19+ UmbcaTE + CnGPAT9 + AtWRI	0.000	0.000	0.000
P19+ CocnuTE2 + CnGPAT9 + AtWRI	0.000	0.000	0.000
P19+ CincaTE + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.000	0.000
P19+ CuplaTE + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.000	0.000
P19+ UmbcaTE + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.000	0.000
P19+ CocnuTE2 + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.000	0.000
P19+ CincaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	0.000
P19+ CuplaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	0.000
P19+ UmbcaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	0.000
P19+ CocnuTE2 + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	0.000
P19+ CincaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	0.000
P19+ CuplaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	0.000

P19+ UmbcaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	0.000

Table 14. Total leaf fatty acid composition of TAG (% total TAG) of 12:0, C14:0, C14;1, C15:0, C16:0 and C16:1 fatty acids in *Nicotiana benthamiana* leaves infiltrated with various constructs.

Genotype	C12:0	C14:0	C14:1	C15:0	C16:0	C16:1
REPLICATE 1						
P19	3.882	11.116	0.000	1.380	41.258	0.000
P19+ CincaTE + CnGPAT9 + AtWRI	3.332	35.333	0.000	0.226	27.276	0.203
P19+ CuplaTE + CnGPAT9 + AtWRI	2.119	10.647	0.000	0.000	47.322	0.000
P19+ UmbcaTE + CnGPAT9 + AtWRI	32.957	9.794	0.000	0.000	16.217	0.000
P19+ CocnuTE2 + CnGPAT9 + AtWRI	0.000	17.998	0.000	0.343	56.230	0.578
P19+ CincaTE + CnGPAT9 + AtWRI + CnLPAAT	7.219	41.154	0.000	0.261	24.586	0.334
P19+ CuplaTE + CnGPAT9 + AtWRI + CnLPAAT	1.491	7.331	0.000	0.241	57.931	0.315
P19+ UmbcaTE + CnGPAT9 + AtWRI + CnLPAAT	44.742	10.476	0.000	0.000	10.207	0.270
P19+ CocnuTE2 + CnGPAT9 + AtWRI + CnLPAAT	0.465	14.889	0.000	0.335	56.250	0.481
P19+ CincaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	4.620	30.554	0.000	0.177	37.511	0.475
P19+ CuplaTE + CnGPAT9 +	4.598	5.250	0.000	0.221	51.742	0.320

AtWRI + CnLPAAT + EgDGAT						
P19+ UmbcaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	53.604	7.690	0.000	0.157	11.120	0.094
P19+ CocnuTE2 + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.654	14.116	0.000	0.256	53.942	0.306
P19+ CincaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	4.499	35.151	0.000	0.196	33.202	0.314
P19+ CuplaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	4.943	5.716	0.000	0.262	50.177	0.542
P19+ UmbcaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	49.589	7.781	0.000	0.105	12.284	0.252
REPLICATE 2						
P19	6.485	10.998	0.000	0.000	46.160	0.000
P19+ CincaTE + CnGPAT9 + AtWRI	0.000	0.000	0.000	0.000	0.000	0.000
P19+ CuplaTE + CnGPAT9 + AtWRI	1.758	10.767	0.000	0.000	49.728	0.583
P19+ UmbcaTE + CnGPAT9 + AtWRI	32.530	10.553	0.000	0.000	15.254	0.544
P19+ CocnuTE2 + CnGPAT9 + AtWRI	0.628	16.693	0.000	0.327	49.863	0.466
P19+ CincaTE + CnGPAT9 +	3.660	40.701	0.000	0.264	28.736	0.333

AtWRI + CnLPAAT						
P19+ CuplaTE + CnGPAT9 + AtWRI + CnLPAAT	2.472	10.374	0.000	0.364	49.195	0.635
P19+ UmbcaTE + CnGPAT9 + AtWRI + CnLPAAT	43.462	10.775	0.000	0.206	10.328	0.225
P19+ CocnuTE2 + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.000	0.000	0.000	0.000	0.000
P19+ CincaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	4.101	33.380	0.000	0.000	35.431	0.000
P19+ CuplaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	8.061	5.606	0.000	0.000	47.901	0.000
P19+ UmbcaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	49.552	6.800	0.000	0.000	12.602	0.000
P19+ CocnuTE2 + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	14.374	0.000	0.000	50.723	0.000
P19+ CincaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	2.758	26.757	0.000	0.000	38.082	0.000
P19+ CuplaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	2.672	4.771	0.000	0.000	53.725	0.000
P19+ UmbcaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	49.847	6.988	0.000	0.000	11.945	0.000

REPLICATE 3						
P19	0.000	0.000	0.000	0.000	55.478	0.000
P19+ CincaTE + CnGPAT9 + AtWRI	0.000	32.975	0.000	0.000	29.893	0.000
P19+ CuplaTE + CnGPAT9 + AtWRI	0.000	9.743	0.000	0.000	55.084	0.000
P19+ UmbcaTE + CnGPAT9 + AtWRI	29.807	9.939	0.000	0.000	15.215	0.000
P19+ CocnuTE2 + CnGPAT9 + AtWRI	0.000	20.098	0.000	0.000	48.646	0.000
P19+ CincaTE + CnGPAT9 + AtWRI + CnLPAAT	4.924	38.894	0.000	0.000	22.078	0.000
P19+ CuplaTE + CnGPAT9 + AtWRI + CnLPAAT	0.000	9.483	0.000	0.000	57.458	0.000
P19+ UmbcaTE + CnGPAT9 + AtWRI + CnLPAAT	46.258	8.809	0.000	0.000	9.487	0.000
P19+ CocnuTE2 + CnGPAT9 + AtWRI + CnLPAAT	0.000	18.294	0.000	0.000	56.968	0.000
P19+ CincaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	3.909	34.512	0.000	0.000	36.091	0.000
P19+ CuplaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	4.605	0.000	0.000	56.818	0.000
P19+ UmbcaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	51.506	7.067	0.000	0.000	11.083	0.000
P19+ CocnuTE2 + CnGPAT9 + AtWRI + CnLPAAT +	0.000	10.744	0.000	0.000	55.660	0.000

EgDGAT						
P19+ CincaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	3.697	26.670	0.000	0.000	37.159	0.000
P19+ CuplaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	1.737	4.336	0.000	0.000	54.136	0.000
P19+ UmbcaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	49.371	6.898	0.000	0.000	10.168	0.000

Table 15. Total leaf fatty acid composition of TAG (% total TAG) of C17:0, C17:1, C18:0, C18:1, C19:0, C18:2, C18:3, C20:0, C20:1, C22:0 and C24:0 fatty acids in *Nicotiana benthamiana* leaves infiltrated with various constructs.

Genotype	C17:0	C17:1	C18:0	C18:1	C19:0	C18:2	C18:3	C20:0	C20:1	C22:0	C24:0
REPLICATE 1											
P19	0.000	0.000	7.003	3.505	0.000	7.516	13.827	1.204	1.260	1.303	2.428
P19+ CincaTE + CnGPAT9 + AtWRI	0.000	0.381	2.160	1.542	0.363	6.795	21.636	0.382	0.000	0.208	0.163
P19+ CuplaTE + CnGPAT9 + AtWRI	0.000	0.768	3.491	1.051	0.000	7.046	20.841	0.607	0.000	0.000	0.421
P19+ UmbcaTE + CnGPAT9 + AtWRI	0.000	1.251	2.658	1.439	0.000	9.993	25.690	0.000	0.000	0.000	0.000
P19+ CocnuTE2 + CnGPAT9 + AtWRI	0.000	0.485	3.864	1.427	0.000	5.547	13.027	0.503	0.000	0.000	0.000
P19+ CincaTE + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.296	1.824	2.156	0.000	4.653	17.211	0.307	0.000	0.000	0.000
P19+ CuplaTE + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.242	2.812	5.820	0.616	6.643	14.892	0.515	0.000	0.203	0.000
P19+ UmbcaTE + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.191	1.359	3.790	0.514	8.779	19.329	0.204	0.000	0.140	0.000
P19+ CocnuTE2 + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.333	3.431	2.297	0.000	4.517	16.271	0.552	0.000	0.179	0.000
P19+ CincaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.244	2.274	6.967	0.414	7.193	8.577	0.560	0.000	0.297	0.137
P19+ CuplaTE + CnGPAT9 +	0.000	0.283	3.698	7.203	0.473	9.780	13.19	0.895	0.000	0.535	0.272

AtWRI + CnLPAAT + EgDGAT							8				
P19+ UmbcaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.237	1.704	6.638	0.460	8.261	8.641	0.521	0.117	0.470	0.286
P19+ CocnuTE2 + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.457	3.117	1.071	0.324	4.844	20.248	0.459	0.000	0.205	0.000
P19+ CincaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.299	2.232	4.203	0.290	5.963	12.828	0.500	0.000	0.233	0.089
P19+ CuplaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.321	4.172	5.766	0.479	8.508	15.845	0.902	0.000	0.501	0.224
P19+ UmbcaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.185	1.873	6.977	0.608	9.240	9.823	0.532	0.095	0.425	0.230
REPLICATE 2											
P19	0.000	0.000	5.724	0.000	0.000	9.527	21.105	0.000	0.000	0.000	0.000
P19+ CincaTE + CnGPAT9 + AtWRI	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
P19+ CuplaTE + CnGPAT9 + AtWRI	0.000	0.450	4.115	0.919	0.000	7.307	18.646	0.635	0.000	0.440	0.285
P19+ UmbcaTE + CnGPAT9 + AtWRI	0.000	0.432	3.015	2.565	0.000	10.355	23.801	0.580	0.000	0.370	0.000
P19+ CocnuTE2 + CnGPAT9 + AtWRI	0.000	0.324	3.210	1.170	0.467	5.649	20.593	0.447	0.000	0.163	0.000

P19+ CincaTE + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.206	2.042	3.281	0.299	4.527	15.495	0.334	0.000	0.122	0.000
P19+ CuplaTE + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.342	3.649	1.568	0.000	6.412	20.615	0.553	0.000	0.298	0.000
P19+ UmbcaTE + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.197	1.653	3.620	0.431	8.552	20.205	0.201	0.000	0.145	0.000
P19+ CocnuTE2 + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
P19+ CincaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	1.023	2.522	4.695	0.000	7.688	11.161	0.000	0.000	0.000	0.000
P19+ CuplaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	3.790	5.402	0.000	11.197	17.104	0.939	0.000	0.000	0.000
P19+ UmbcaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	1.950	6.434	0.000	10.572	10.877	0.615	0.000	0.598	0.000
P19+ CocnuTE2 + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	2.364	2.998	1.381	0.000	6.570	21.589	0.000	0.000	0.000	0.000
P19+ CincaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.962	2.526	6.126	0.000	9.898	12.288	0.603	0.000	0.000	0.000
P19+ CuplaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	1.141	3.660	6.542	0.000	11.577	14.693	0.794	0.000	0.424	0.000
P19+ UmbcaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.000	1.909	6.636	0.000	11.998	10.677	0.000	0.000	0.000	0.000

REPLICATE 3											
P19	0.000	0.000	0.000	0.000	0.000	19.966	24.557	0.000	0.000	0.000	0.000
P19+ CincaTE + CnGPAT9 + AtWRI	0.000	1.600	2.296	2.966	0.000	10.225	20.046	0.000	0.000	0.000	0.000
P19+ CuplaTE + CnGPAT9 + AtWRI	0.000	0.000	3.044	2.041	0.000	10.711	19.376	0.000	0.000	0.000	0.000
P19+ UmbcaTE + CnGPAT9 + AtWRI	0.000	0.000	2.821	3.186	0.000	14.113	24.919	0.000	0.000	0.000	0.000
P19+ CocnuTE2 + CnGPAT9 + AtWRI	0.000	1.637	2.992	1.264	0.000	6.611	18.753	0.000	0.000	0.000	0.000
P19+ CincaTE + CnGPAT9 + AtWRI + CnLPAAT	0.000	2.465	1.645	1.269	0.000	6.427	22.298	0.000	0.000	0.000	0.000
P19+ CuplaTE + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.000	2.864	3.499	0.000	7.993	18.702	0.000	0.000	0.000	0.000
P19+ UmbcaTE + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.000	1.356	4.320	0.000	10.699	19.070	0.000	0.000	0.000	0.000
P19+ CocnuTE2 + CnGPAT9 + AtWRI + CnLPAAT	0.000	0.000	3.467	0.000	0.000	6.091	15.180	0.000	0.000	0.000	0.000
P19+ CincaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.875	2.053	5.925	0.000	7.047	9.165	0.422	0.000	0.000	0.000
P19+ CuplaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	1.103	3.685	8.285	0.000	10.471	15.034	0.000	0.000	0.000	0.000
P19+ UmbcaTE + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.631	1.506	6.663	0.000	11.367	9.290	0.462	0.000	0.425	0.000

P19+ CocnuTE2 + CnGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	1.705	3.665	2.985	0.000	7.058	18.182	0.000	0.000	0.000	0.000
P19+ CincaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.816	2.447	7.987	0.000	9.927	10.359	0.598	0.000	0.339	0.000
P19+ CuplaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	1.020	3.588	7.767	0.000	11.969	14.237	0.765	0.000	0.445	0.000
P19+ UmbcaTE + AtGPAT9 + AtWRI + CnLPAAT + EgDGAT	0.000	0.647	1.538	8.125	0.000	12.136	10.070	0.433	0.000	0.377	0.236

Discussion

In the seeds of native plants, the incorporation of unusual fatty acids is almost exclusively confined to TAG and typically excluded from membrane lipids, most likely because they interfere with proper membrane functions and are often deleterious to the plant cells (Millar et al., 2000). A different scenario has been observed in transgenic plants that have attempted to modify the oil fatty acid profiles, such as increasing the lauric acid content (Knutzon et al., 1999). Although high levels of laurate accumulation in plant oils have been achieved in the seeds of transgenic canola, there was a significant level of laurate being sequestered in PC during seed development (Wiberg et al., 1997). In that work, *de novo* DAG containing laurate was not efficiently converted to TAG by the resident DGAT but was instead converted to the membrane lipid PC. The native canola LPCAT lacked the capability to handle MCFAs (Zhang et al., 2015) so the route to PC could be through PDCT or CPT activities. Consequently, this inefficient utilization of laurate for TAG synthesis was also associated with a negatively correlated penalty in total oil yields (Knutzon et al., 1999).

Similar to the expression of MCFA in seed oil, the over expression of MCFA in the leaf cells described here with the co-expression of CnGPAT9 and CnLPAAT identified a metabolic bottleneck through the sequestering of MCFA in PC. The low abundance of MCFA-containing DAG species suggested that *de novo* DAG containing MCFA was quickly converted to PC through the activities of PDCT or CPT or both, due to the absence of a DGAT capable of using the MCFA-containing DAG for TAG assembly. The inventors showed that the addition to the enzyme combination of a DGAT with a preference for MCFA as substrate, relative to one or more C18 substrates such as oleic acid, LA or ALA, promoted synthesis of MCFA-enriched TAG and relieved this bottleneck. Endogenous PDAT may also be involved in the maintenance of membrane homeostasis, through the removal of unusual fatty acids from the membrane lipids and sequestering them into TAG (Fan et al., 2014; Fan et al., 2013a and b). This study demonstrated that the expression of the DGAT from a species such as *E. guineensis* (EgDGAT1) was sufficient to restore membrane homeostasis by reducing the accumulation of MCFA in PC. The expression of EgDGAT1 proved that a DGAT with MCFA substrate preference was beneficial for the efficient assembly of TAG and increased TAG content in the plant cells. The reconfigured Kennedy pathway for improving MCFA incorporation into TAG is expected to benefit seedoil composition and TAG content as well.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

All publications discussed and/or referenced herein are incorporated herein in their entirety.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

REFERENCES

Adhikari et al. (2016) Plant Physiol 171:179-191.

Alemanno et al. (2008) Planta 227:853–866.

Almeida and Allshire (2005) TRENDS Cell Biol. 15:251-258.

Alonso et al. (2009) Plant Cell 21: 1747-1761.

Alonso et al. (2010) Green Chem. 12:1493-1513.

Alvarez et al. (2000) Theor. Appl. Genet. 100:319-327.

Andre at al (2012) Proc. Natl. Acad. Sci. U.S.A. 109:10107-10112.

Arkcoll (1988) Lauric Oil Resources. Economic Botany 42:195-205.

Bartlett et al. (2008) Plant Methods 4:22.

Basiron and Weng (2004) Journal of Oil Palm Research 16.

Bates et al. (2014) PNAS USA 111:1204-1209.

Bates and Browse (2011) Plant J 68:387-399.

Bates and Browse (2012) Frontiers in Plant Science 3:147.

Baud et al. (2007) Plant J. 50:825-838.

Baud and Lepiniec (2010) Progr. Lipid Res. 49: 235-249.

Baumlein et al. (1991) Mol. Gen. Genet. 225:459-467.

Baumlein et al. (1992) Plant J. 2:233-239.

Belide et al. (2013) Plant Cell Tiss. Org. Cult. DOI 10.1007/s11240-013-0295-1.

Ben Saad et al. (2011) Transgenic Res 20: 1003-1018.

Bibikova et al. (2002) Genetics 161:1169-1175.

Bihmidine et al. (2015) BMC Plant Biology 15:186.

Bihmidine et al. (2016) Plant Signaling & Behaviour 11: e1117721.

Bligh and Dyer (1959) Canadian Journal of Biochemistry and Physiology 37:911-917.

Boutilier et al. (2002) Plant Cell 14:1737–1749.

Bouvier-Nave et al. (2000) European Journal of Biochemistry / FEBS 267:85-96.

Bradford (1976) Anal. Biochem. 72:248-254.

Broothaerts et al. (2005) Nature 433:629-633.

Broun et al. (1998) Plant J. 13:201-210.

Browse et al. (1986) Biochem J 235: 25-31.

Buchanan-Wollaston (1994) Plant Physiol. 105:839-846.

Burgal et al. (2008) Plant Biotechnol J 6:819-831.

Busk et al. (1997) Plant J. 11:1285-1295.

Cai et al. (2015) Plant Cell 27:2616-2636.

Cao (2011) BMC Research Notes 4:249.

Cao et al. (2007) J. Lipid Res. 48:583-591.

Capuano et al. (2007) Biotechnol. Adv. 25:203-206.

Chapman and Ohlogge (2012) J. Biol. Chem. 287:2288-2294.
Chen et al (2011) Plant Physiol. 155:851-865.
Chen et al. (2016) International Journal of Molecular Sciences 17:507.
Chikwamba et al. (2003) Proc. Natl. Acad. Sci. U.S.A. 100:11127-11132.
Christensen et al. (1992) Plant Mol Biol 18:675-689.
Christie (1993) Advances in Lipid Methodology–Two, Oily Press, Dundee, pp195-213.
Chung et al. (2006) BMC Genomics 7:120.
Comai et al. (2004) Plant J 37: 778-786.
Cong et al. (2013) Science 339:819-823.
Corrado and Karali (2009) Biotechnol. Adv. 27:733-743.
Coutu et al. (2007) Transgenic Res. 16:771-781.
Dahlqvist et al. (2000), Proc. Natl. Acad. Sci. U.S.A. 97: 6487-6492.
Damaj et al., (2010) Planta 231:1439-1458.
Dandik and Aksoy (1998) Fuel Process Technol. 57: 81-92.
Dauk et al (2007) Plant Sci. 173:43-49.
Deruyffelaere et al. (2015) Plant Cell Physiol 56:1374-1387.
Dehesh (2001) European Journal of Lipid Science and Technology 103 :688-697.
Durrett et al. (2008) Plant J. 54:593-607.
Dussert et al.. (2013) Plant Physiol 162:1337-1358.
Dyer et al. (2002) Plant Physiol. 130:2027-2038.
Eastmond et al. (2006) Plant Cell 18: 665-675.
Eccleston et al. (1996) Planta 198:46-53.
Eccleston and Ohlrogge (1998) The Plant Cell Online 10:613-621.
Edem (2002) Plant Foods for Human Nutrition 57:319-341.
Ellerstrom et al. (1996) Plant Mol. Biol. 32:1019-1027.
El Tahchy et al. (2017) FEBS Letters 591:448-456.
Endalew et al. (2011) Biomass and Bioenergy 35:3787-3809.
Fan et al. (2013a) Plant Cell 25: 3506-3518.
Fan et al. (2013b) Plant Journal 76: 930-942.
Fan et al. (2014) Plant Cell 26: 4119-4134.
Fan et al. (2015) Plant Cell 27: 2941-2955.
FAO Animal Production and Health Proceedings (2002) Protein sources for the animal feed industry, Expert Consultation and Workshop, Bangkok.
Feeney et al. (2012) Plant Physiol 162: 1881-1896.
Finkelstein et al. (1998) Plant Cell 10:1043-1054.
Froissard et al. (2009) FEMS Yeast Res 9:428-438.

Gan (1995) Molecular characterization and genetic manipulation of plant senescence. PhD thesis. University of Wisconsin, Madison.
Gan and Amasino (1995) Science 270:1986-1988.
Gazzarrini et al. (2004) Dev. Cell 7:373-385.
Geurin et al. (2016) Plant Biotech. J 87: 423-441.
Ghosal et al. (2007) Biochimica et Biophysica Acta 1771:1457-1463.
Ghosh et al. (2009) Plant Physiol. 151:869-881.
Gidda et al (2013) Plant Signaling Behav. 8:e27141.
Girijashankar and Swathisree, (2009) Physiol. Mol. Biol. Plants 15: 287-302.
Gong and Jiang (2011) Biotechnol. Lett. 33:1269-1284.
Gould et al. (1991) Plant Physiol. 95:426-434.
Greenwell et al. (2010) J. R. Soc. Interface 7:703-726.
Guan et al. (2015) Lipids 50:407–416.
Gurel et al. (2009) Plant Cell Rep. 28:429-444.
Gutierrew et al. (2013) BMC Biotechnol. 13: 40.
Hedrich et al. (2015) Curr Opin Plant Biol 25: 63-70.
Henikoff et al. (2004) Plant Physiol. 135:630-636.
Hershey and Stoner (1991) Plant Mol. Biol. 17:679–690.
Hinchee et al. (1988) Biotechnology 6:915-922.
Hom et al. (2007) Euphytica 153:27-34.
Horn et al. (2013). Plant Physiol 162:1926-1936.
Horvath et al. (2000) Proc. Natl. Acad. Sci. U.S.A. 97:1914-1919.
Hsiao and Tzen (2011) Plant Physiol. Biochem. 49: 77-81.
Hsieh and Huang (2004) Plant Physiol 136:3427-3434.
Huang (1996) Plant Physiol. 110:1055-1061.
Huang and Huang (2016) Plant Physiol. 171: 1867-1878.
Ichihara et al (1988) Biochim. Biophys. Acta 958:125-129.
Ikeda et al. (2006) Pl Biotech J. 23: 153-161.
Iwabuchi et al. (2003) J. Biol. Chem. 278:4603-4610.
James et al. (2010) Proc. Natl. Acad. Sci. USA 107:17833-17838.
Jepson et al. (1994) Plant Mol. Biol. 26:1855-1866.
Jing et al. (2011) BMC Biochemistry 12:44.
Jolivet et al. (2014) Plant Physiol. Biochem. 42:501-509.
Jones et al. (1995) Plant Cell 7: 359-371.
Karmakar et al. (2010) Bioresource Technology 101:7201-7210.
Kelly et al. (2011) Plant Physiol. 157: 866-875.
Kelly et al (2013a) Plant Biotech. J. 11:355-361.
Kelly et al. (2013b) Plant Physiol. 162:1282-1289.

Kereszt et al. (2007) Nature Protocols 2:948-952.
Knutzon et al. (1995) Plant Physiol 109:999-1006.
Knutzon et al. (1999) Plant Physiology 120:739-746.
Kim et al. (2014) Biotechnology for Biofuels 7:36.
Kim et al. (2015a) Plant J 84:1021-1033.
Kim et al. (2015b) Journal of Experimental Botany 66:4251-4265.
Kim et al. (1996) Proc. Natl. Acad. Sci. USA 93:1156-1160.
Kim et al. (2016) Plant Physiol 171: 1951-1964.
Klemens et al. (2013) Plant Physiol 163: 1338-1352.
Koziel et al. (1996) Plant Mol. Biol. 32:393-405.
Kuhn et al. (2009) J. Biol. Chem. 284:34092-102.
Kunst et al. (1988) Proc. Natl. Acad. Sci. U.S.A. 85:4143-4147.
Kwong et al. (2003) Plant Cell 15:5-18.
Lacroix et al. (2008) Proc. Natl. Acad. Sci.U.S.A. 105: 15429-15434.
Laibach et al. (2015). J. Biotechnol. 201: 15-27.
Lardizabal et al. (2008) Plant Physiol. 148: 89-96.
Laureles et al. (2002) J Agric Food Chem 50:1581-1586.
Lebrun et al. (1987) Nucl. Acids Res. 15:4360.
Laux et al. (1996) Development 122: 87-96.
Lazo et al. (1991) Bio/Technology 9 :963-967.
Lee et al. (1998) Science 280:915-918.
Lee et al. (2003) Proc. Natl. Acad. Sci. U.S.A. 100:2152-2158.
Li et al. (1996) FEBS Lett. 379:117-121.
Li et al. (2006) Phytochemistry 67: 904-915.
Li et al. (2017) Plant Phsyiol 173:2208-2224.
Lin et al. (2005) Plant Physiol. Biochem. 43:770-776.
Linder et al. (2005). FEMS Microbiol. Rev. 29: 877-896.
Liu and Godwin (2012). Plant Cell Reports 31, 999-1007.
Liu et al. (2010) Plant Physiol. Biochem. 48: 9-15.
Liu et al. (2012) Prog Lipid Res 51 :350-377.
Liu et al. (2012) J Exp Bot 63: 3727-3740.
Liu et al. (2014) BMC Plant Biol. 14: 73.
Lotan et al. (1998) Cell 93: 1195-1205.
Lu et al. (2009) Proc Natl Acad of Sci USA 106:18837–18842.
Luerssen et al. (1998) Plant J. 15: 755-764.
Lui et al. (2009) J. Agric. Food Chem. 57: 2308-2313.
Ma et al. (2016) Plant Journal doi: 10.1111/tpj.13244.
MacEachran et al. (2010). Appl. Environ. Microbiol. 76: 7217-7225.

Maher and Bressler (2007) Bioresource Technology 98:2351-2368.
Matsuoka et al. (1994) Plant J. 6:311-319.
Matsuoka and Minami (1989) Eur. J. Biochem. 181: 593-598.
McCleary et al. (2013) J AOAC Int 93:221-233.
McCleary et al. (2015) Starch 67:860-883.
McElroy et al. (1990) Plant Cell 2: 163-171.
McKinley et al. (2016) Plant Journal: doi:10.1111/tpj.13269.
Meier et al. (1997) FEBS Lett. 415:91-95.
Millar et al. (2000) Trends in Plant sScience 5:95-101.
Millar and Waterhouse (2005). Funct Integr Genomics 5:129-135.
Miller (1984). Crop Sci 24:1224–1224.
Mojica et al. (2000) Mol Microbiol 36:244-246.
Moreno-Perez (2012) PNAS 109:10107-10112.
Mongrand et al. (1998) Phytochemistry 49:1049-1064.
Moyle and Birch (2013) Theor. Appl. Genet. 126:1775-1782.
Mu et al. (2008) Plant Physiol. 148:1042-1054.
Mudge et al. (2013) Plant Biotechnol. J. 11:502-509.
Murashige and Skoog (1962). Physiol Plant 15:473–497.
Murphy et al. (2012). Protoplasma 249:541-585.
Needleman and Wunsch (1970) J. Mol Biol. 45: 443-453.
Nilsson et al. (2012) Physiol. Plantarum 144: 35-47.
Nomura et al. (2000) Plant Mol. Biol. 44: 99-106.
OECD/FAO (2015) OECD-FAO Agricultural Outlook (Edition 2015). Paris:OECD Publishing.
Ohlrogge and Browse (1995) Plant Cell 7: 957–970.
Padidam (2003) Curr. Opin. Plant Biol. 6:169-77.
Padidam et al. (2003) Transgenic Res. 12:101-9.
Parthibane et al. (2012a) J. Biol. Chem. 287:1946-1965.
Parthibane et al. (2012b) Plant Physiol. 159:95-104.
Pasquinelli et al. (2005). Curr. Opin. Genet. Develop. 15:200-205.
Perez-Vich et al. (1998) J.A.O.C.S. 75:547-555.
Perrin et al. (2000) Mol. Breed. 6:345-352.
Petrie et al. (2012) PLOS One 7: e35214.
Phillips et al. (2002) Journal of Food Composition and Analysis 12:123-142.
Potenza et al. (2004) In Vitro Cell Dev. Biol. Plant 40:1-22.
Poxleitner et al. (2006) Plant J. 47:917-933.
Prosky et al. (1985) J AOAC Chem 68:677-679.
Pyc et al. (2017) Trends in Plant Sci. 22:596-609.

Qazi et al. (2012) Journal of Plant Physiology 169: 605-613.
Qiu et al. (2001) J. Biol. Chem. 276:31561-3156.
Reynolds et al. (2015) Frontiers in Plant Science 6.
Robson et al. (2004) Plant Biotechnol J 2:101-112.
Rossell and Pritchard (1991) Analysis of Oilseeds, Fats and Fatty Foods. Elsevier
Ruuska et al. (2002) Plant Cell 14:1191-1206.
Saha et al. (2006) Plant Physiol. 141:1533-1543.
Sanjaya et al. (2011) Plant Biotechnol J 9:874-883.
Santos-Mendoza et al. (2005) FEBS Lett. 579:4666-4670.
Santos-Mendoza et al. (2008) Plant J. 54:608-620.
Schneider et al. (2012) Plant Biol 14: 325-336.
Schnurr et al. (2002) Plant Physiol 129:1700-1709.
Shaw et al. (1959) J Soil Sci 10:316-326.
Shen et al. (2010) Plant Phys. 153: 980-987.
Shen et al. (2014). Biochem. Biophys. Res. Comm. 448: 365-371.
Semwal et al. (2011) Bioresource Technology 102:2151-2161.
Shen et al. (2010) Plant Physiol. 153:980-987.
Shiina et al. (1997) Plant Physiol. 115:477-483.
Shimada and Hara-Nishimura (2010) Biol. Pharm. Bull. 33:360-363.
Shimada et al. (2014) Plant Physiol. 164:105-118.
Shockey et al. (2002) Plant Physiol 129:1710-1722.
Shockey et al. (2016) Plant Physiol 170:163-179.
Singer et al. (2016) Journal of Experimental Botany 67:4627-4638.
Slade and Knauf (2005) Transgenic Res. 14: 109-115.
Smith et al. (2000) Nature 407:319-320.
Somerville et al. (2000) Lipids. In BB Buchanan, W Gruissem, RL Jones, eds, Biochemisty and Molecular Biology of Plants. American Society of Plant Physiologists, Rockville, MD, pp 456–527.
Sorokin et al. (2009) Biochemistry Biokhimiia 74:1411-1442.
Srinivasan et al. (2007) Planta 225:341-51.
Stalker et al. 1988 Science 242: 419-423.
Stone et al. (2001) Proc. Natl. Acad. Sci. U.S.A.98: 11806-11811.
Stone et al. (2008) Proc. Natl. Acad. Sci. U.S.A.105: 3151-3156.
Tai et al. (2002). Biosci. Biotechnol. Biochem. 66: 2146-2153.
Tan et al. (2011) Plant Physiol. 156:1577-1588.
Taylor (1997) The Plant Cell 9:1245-1249.
Thillet et al. (1988) J. Biol. Chem 263:12500-12508.
Tingay et al. (1997) Plant J. 11:1369-1376.

Tjellstrom et al. (2013) FEBS Lett 587:936-942.
To et al. (2012) Plant Cell 24:5007-5023.
Ulmasov et al. (1995) Plant Physiol. 108:919-927.
van de Loo et al. (1995) Proc Natl Acad Sci U S A. 92:6743-6747.
van Erp et al. (2011) Plant Physiol 155:683-693.
van Erp et al. (2015) Plant Physiol 168:36-46.
Vanhercke et al. (2013) FEBS Letters 587:364-369.
Vanhercke et al. (2014a). Plant Biotech. J. 12:231-239.
Vanhercke et al. (2014b) Biocatalysis and Agricultural Biotechnology 3:75-80.
Vanhercke et al. (2017) Metabolic Engineering 39:237-246.
Vieler et al. (2012) Plant Physiol. 158:1562-1569.
Voinnet et al. (2003) Plant J. 33:949-956.
Voelker et al. (1992) Science 257:72-74.
Voelker et al. (1996) Plant J 9:229-241.
Wang et al. (2002) Plant J 32:831-843.
Wang et al. (2007) Plant J 52: 716-729.
Waterhouse et al. (1998). Proc. Natl. Acad. Sci. U.S.A. 95:13959-13964.
Weissbach and Weissbach, (1989) Methods for Plant Mol Biol, Academic Press.
Weissbach et al., In: Methods for Plant Molecular Biology, Academic Press, San Diego, Calif., (1988).
Weissman (2001) Molec Cell Biol. 2:169-178.
Wesley et al. (2001) Plant J. 27:581-590.
Wiberg et al. (1997) Planta 203:341-348.
Wiberg et al. (2000) Planta 212:33-40.
Winichayakul et al. (2013) Plant Physiol. 162:626-639.
Wood (2014) EMBO Reports 15:201-202.
Wood et al. (2009) Plant Biotech. J. 7: 914-924.
Wu et al. (2014) In Vitro Cellular and Dev. Biol.-Plant 50:9-18.
Xu et al. (2008) Plant Biotechnol J 6:799-818.
Xu et al (2010) Plant and Cell Physiol. 51:1019-1028.
Xu et al (2005) Plant Cell 17:3094-3110.
Xu et al (2008) Plant Cell 20:2190-2204.
Yamagishi et al. (2005) Pl Physiol 139: 163-173.
Yamasaki et al. (2004) Plant Cell 16 :3448-3459.
Yang et al. (2003) Planta 216:597-603.
Yang et al. (2010) Proc. Natl. Acad. Sci. U.S.A.107:12040-12045.
Yeap et al. (2017) Plant Journal 91: 97-113.
Yen et al. (2002) Proc. Natl. Acad. Sci. U.S.A. 99:8512-8517.

Yen et al. (2005) J. Lipid Res. 46: 1502-1511.
Yokoyama et al. (1994) Mol Gen Genet 244: 15-22.
Yuan et al. (1995) Proc. Natl. Acad. Sci. U.S.A. 92 :10639-10643.
Zale et al. (2016) Plant Biotech J. 14: 661-669.
Zhang et al. (2015) PLoS ONE 10, e0144653.
Zheng et al. (2009) Pl Physiol 21: 2563-2577.
Zhou et al. (2011) J Biol Chem 286:43644-43650.
Zolman et al. (2001) Plant Physiol. 127:1266-1274.

CLAIMS

1. A process for producing extracted plant lipid, comprising the steps of:

a) obtaining one or more plant parts comprising lipid, preferably vegetative plant parts, the lipid comprising a total fatty acid content which comprises fatty acids in an esterified form, the fatty acids comprising a level of total, or new, medium chain fatty acids (MCFA) that is at least 25% of the total fatty acid content on a weight basis, and

b) extracting lipid from the plant part(s),

thereby producing the extracted plant lipid.

2. The process of claim 1, wherein the plant part comprises one or more exogenous polynucleotides which encode polypeptides having fatty acid thioesterase (TE) activity, and either glycerol-3-phosphate acyltransferase (GPAT) activity, preferably GPAT9 activity, or diacylglycerol acyltransferase (DGAT) activity, preferably DGAT1 activity, or both GPAT and DGAT,

wherein the exogenous polynucleotide is operably linked to a promoter which is capable of directing expression of the polynucleotide in a cell of the plant part.

3. The process of claim 2, wherein the plant part further comprises one or more or all of:

vi. an exogenous polynucleotide which encodes a second polypeptide having glycerol-3-phosphate acyltransferase (GPAT) activity, preferably GPAT9 activity, or diacylglycerol acyltransferase (DGAT) activity, preferably DGAT1 activity;

vii. an exogenous polynucleotide which encodes a third polypeptide having 1-acyl-glycerol-3-phosphate acyltransferase (LPAAT) activity;

viii. an exogenous polynucleotide which encodes a transcription factor polypeptide that increases the expression of one or more glycolytic and/or fatty acid biosynthetic genes in a cell of the plant part compared to a corresponding cell lacking the exogenous polynucleotide;

ix. an exogenous polynucleotide which encodes a polypeptide which increases the export of fatty acids out of plastids of a cell in the plant part when compared to a corresponding cell lacking the exogenous polynucleotide; and

x. an exogenous polynucleotide which encodes an oil body coating (OBC) polypeptide,

wherein each exogenous polynucleotide is operably linked to a promoter which is capable of directing expression of the polynucleotide in a cell of the plant part.

4. The process of claim 3, wherein the OBC polypeptide is an oleosin, such as a polyoleosin or a caleosin, or a lipid droplet associated protein (LDAP).

5. The process of claim 3 or claim 4, wherein the transcription factor polypeptide is selected from the group consisting of Wrinkled 1 (WRI1), Leafy Cotyledon 1 (LEC1), LEC1-like, Leafy Cotyledon 2 (LEC2), BABY BOOM (BBM), FUS3, ABI3, ABI4, ABI5, Dof4 and Dof11, preferable WRI1.

6. The process of any one of claims 3 to 5, wherein the polypeptide which increases the export of fatty acids out of plastids of the cell is a fatty acid thioesterase such as a FATA polypeptide or a FATB polypeptide, a fatty acid transporter such as an ABCA9 polypeptide or a long-chain acyl-CoA synthetase (LACS), preferably a FATB polypeptide.

7. The process of claim 6, wherein the fatty acid thioesterase is capable of hydrolysing a substrate which is an acyl carrier protein (ACP) esterified to a medium chain fatty acid and/or a C16:0, preferably wherein the MCFA is a C10, C12 and/or C14.

8. The process of any one of claims 2 to 7, wherein the plant part further comprises one or more or all of:

iv. a genetic modification which down-regulates endogenous production and/or activity of a polypeptide involved in the catabolism of triacylglycerols (TAG) in a cell of the plant part when compared to a corresponding cell lacking the genetic modification;

v. a genetic modification which down-regulates endogenous production and/or activity of a polypeptide involved in importing fatty acids into plastids of a cell in the plant part when compared to a corresponding cell lacking the genetic modification; and

vi. a genetic modification which down-regulates endogenous production and/or activity of a polypeptide involved in diacylglycerol (DAG) production in the plastid when compared to a corresponding cell in the plant part lacking the genetic modification.

9. The process of claim 8, wherein the polypeptide involved in the catabolism of triacylglycerols (TAG) in the plant, or part thereof, is an SDP1 lipase, a Cgi58 polypeptide, an acyl-CoA oxidase such as ACX1 or ACX2, or a polypeptide involved

in β-oxidation of fatty acids in the plant or part thereof such as a PXA1 peroxisomal ATP-binding cassette transporter, preferably an SDP1 lipase.

10. The process of any one of claims 1 to 9, wherein the plant part comprises an increased level or activity of polypeptides which are:

xxix. a GPAT, a LPAAT, and a WRI1 polypeptide;

xxx. a GPAT, a LPAAT, a DGAT and a WRI1 polypeptide;

xxxi. a GPAT9, a LPAAT, and a WRI1 polypeptide;

xxxii. a GPAT9, a LPAAT, a DGAT, and a WRI1 polypeptide;

xxxiii. a GPAT, a LPAAT, a DGAT1, and a WRI1 polypeptide;

xxxiv. a GPAT9, a LPAAT, a DGAT1, and a WRI1 polypeptide;

xxxv. a GPAT, a LPAAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xxxvi. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xxxvii. a GPAT9, a LPAAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xxxviii. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xxxix. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xl. a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xli. a GPAT, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xlii. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xliii. a GPAT9, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xliv. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xlv. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xlvi. a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xlvii. a GPAT, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xlviii. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xlix. a GPAT9, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

l. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

li. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

lii. a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

liii. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

liv. a GPAT9, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

lv. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP, and a silencing RNA which

reduces the expression of an endogenous gene which encodes a SDP1 lipase;

lvi. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin, or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase; or a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin, or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase.

11. A process for producing extracted plant lipid, comprising the steps of:

a) obtaining one or more plant parts comprising lipid, preferably vegetative plant parts, the lipid comprising a total fatty acid content which comprises fatty acids in an esterified form, the fatty acids comprising an increased level of medium chain fatty acids (MCFA) relative to a corresponding wild-type plant part, wherein the plant part comprises an increased level or activity of polypeptides which are:

xxx. a GPAT, a LPAAT, and a WRI1 polypeptide;

xxxi. a GPAT, a LPAAT, a DGAT and a WRI1 polypeptide;

xxxii. a GPAT9, a LPAAT, and a WRI1 polypeptide;

xxxiii. a GPAT9, a LPAAT, a DGAT, and a WRI1 polypeptide;

xxxiv. a GPAT, a LPAAT, a DGAT1, and a WRI1 polypeptide;

xxxv. a GPAT9, a LPAAT, a DGAT1, and a WRI1 polypeptide;

xxxvi. a GPAT, a LPAAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xxxvii. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xxxviii. a GPAT9, a LPAAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xxxix. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xl. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xli. a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xlii. a GPAT, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xliii. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xliv. a GPAT9, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xlv. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xlvi. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xlvii. a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xlviii. a GPAT, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xlix. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

l. a GPAT9, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

li. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

lii. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

liii. a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which

reduces the expression of an endogenous gene which encodes a SDP1 lipase;

liv. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

lv. a GPAT9, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

lvi. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

lvii. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin, or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase; or

lviii. a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin, or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase,

and

b) extracting lipid from the plant part(s),

thereby producing the extracted plant lipid.

12. The process of claim 10 or 11, wherein one or more or all of the polypeptides are encoded by one or more exogenous polynucleotides in the plant parts.

13. The process of claim 11 or claim 12, wherein the level of total, or new, MCFA is increased relative to a corresponding wild-type plant part, preferably the level is at least 25% of the total fatty acid content on a weight basis.

14. The process of any of claims 2 to 12, wherein one or more or all of the encoded GPAT, LPAAT and DGAT have a preference for utilising medium chain fatty acid substrates.

15. The process of any one of claims 1 to 14, wherein the extracted lipid has one or more or all of the following features:

xxi. the level of medium chain fatty acids in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid, is at least about 30%, at least about 35%, at least about 40%, at least about 50%, at least about 55%, or between about 25% and about 55%, between about 25% and about 50%, between about 30% and about 50%, between about 35% and about 50%, between about 25% and about 40%, or between about 30% and about 40%;

xxii. the level of lauric acid (C12:0) in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid, is, or is increased by, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 50%, at least or about 55%, or between about 15% and about 55%, between about 20% and about 50%, between about 30% and about 50%, between about 35% and about 50%, between about 15% and about 25%, or between about 20% and about 30%;

xxiii. the level of myristic acid (C14:0) in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid, is, or is increased by, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, or between about 25% and about 45%, between about 20% and about 50%, between about 30% and about 50%, between about 35% and about 50%, between about 30% and about 40%, between about 15% and about 25%, or between about 20% and about 30%;

xxiv. the level of palmitic acid (C16:0) in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid, is, or is increased by, between about 2% and about 18%, or between about 2% and about 16%, or between about 2% and about 15%, or between about 15% and about 50%;

xxv. the level of lauric acid (C12:0) in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid is, or is increased by, at least about 25%, at least about 30%, at least about 40%, at least about 45%, or at least about 50%, and the level of myristic acid (C14:0) in the total fatty acid content of the extracted lipid and/or in the total

fatty acid content of the TAG of the extracted lipid is, or is increased by, at least about 1%, at least about 2%, at least about 5%, or at least about 10%, or between about 1% and about 10%, or between about 2% and 10%, or between about 2% and about 6%, or less than about 10%, or less than about 8% or less than about 5%, or less than about 2%;

xxvi. the level of myristic acid (C14:0) in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid is, or is increased by, at least about 20%, at least about 25%, at least about 30%, or at least about 40%, and the level of lauric acid (C12:0) in the total fatty acid content of the extracted lipid and/or in the total fatty acid content of the TAG of the extracted lipid is, or is increased by, at least about 1%, at least about 2%, at least about 5%, or at least about 10%, or between about 1% and about 10%, or between about 2% and about 10%, or between about 2% and about 6%, or less than about 10%, or less than about 8% or less than about 5%, or less than about 2%;

xxvii. the level of myristic acid (C14:0) in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid is, or is increased by, at least about 20%, at least about 25%, at least about 30%, and the level of palmitic acid (C16:0) in the total fatty acid content of the extracted lipid and/or in the total fatty acid content of the TAG of the extracted lipid is, or is increased by, at least about 2%, at least about 3%, at least about 4%, or at least about 5%.

xxviii. the ratio of lauric acid (C12:0):myristic acid (C14:0) in the fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid, is increased, or is about 1:4, about 1:5, about 1:10, about 1:15, about 1:20, about 1:25, or about 4:1, about 5:1, about 10:1, about 15:1, about 20:1, about 30:1, about 40:1, or about 45:1;

xxix. the ratio of lauric acid (C12:0):palmitic acid (C16:0) in the fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid, is increased, or is about 1:2, about 1:3, about 1:4, about 1:5, about 1:10, about 1:15, about 10:1, about 20:1, about 30:1, about 40:1, or about 45:1;

xxx. the ratio of myristic acid (C14:0):palmitic acid (C16:0) in the fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid, is increased, or is about 1:2, about 1:3, about 1:4, about 1:5, about 1:10, about 1:15, about 10:1, about 20:1, about 30:1, or about 40:1;

xxxi. the level of oleic acid in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid, is

decreased, or is less than about 10%, less than about 8%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, less than about 1%;

xxxii. the level of linoleic acid (LA) in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid, is increased or decreased, or is less than about 20%, less than about 15%, less than about 10%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1%;

xxxiii. the level of α-linolenic acid (ALA) in the total fatty acid content of the extracted lipid, or in the total fatty acid content of the TAG of the extracted lipid, is decreased or is less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 10%, less than about 8%, less than about 6%, less than about 5%, less than about 2%, or less than about 1%;

xxxiv. the level of total unsaturated fatty acids in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid, is decreased, or is less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 15%, less than about 10%, less than about 8%, less than about 6%, less than about 5%, less than about 2%, or less than about 1%;

xxxv. the level of total monounsaturated fatty acids in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid, is decreased, or is less than about 10%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1%;

xxxvi. the level of total polyunsaturated fatty acids in the total fatty acid content of the extracted lipid, and/or in the total fatty acid content of the TAG of the extracted lipid, is less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 15%, less than about 10%, less than about 8%, less than about 6%, less than about 5%, less than about 2%, or less than about 1%;

xxxvii. the triacylglycerol (TAG) content of the extracted lipid is at least about 80%, at least about 85%, at least about 90%, or least about 95%, and about 98%, or between about 95% and about 98%, by weight of the extracted lipid;

xxxviii. the TAG content of the extracted lipid comprises, or is increased in a level of, one or more or all of the TAG species 36:0, 38:0, 40:0 and 42:0;

xxxix. the extracted lipid comprises tri-laurin (tri-C12:0) and/or tri-myristin (tri-C14:0); and

xl. the phosphocholine (PC) content of the extracted lipid comprises one or both of the PC species 28:0 and 30:0,

wherein any increase or decrease is relative to a corresponding wild-type plant part.

16. The process of any one of claims 1 to 15, wherein the extracted lipid is in the form of an oil, wherein at least about 90%, or least about 95%, at least about 98%, or between about 95% and about 98%, by weight of the oil is the lipid.

17. The process of any one of claims 1 to 16, wherein the plant part is a vegetative plant part such as a plant leaf or stem, or the plant part is a seed or a fruit.

18. The process of any one of claims 1 to 17, wherein the plant part is from a species selected from a group consisting of a *Acrocomia aculeata* (macauba palm), *Arabidopsis thaliana*, *Aracinis hypogaea* (peanut), *Astrocaryum murumuru* (murumuru), *Astrocaryum vulgare* (tucumã), *Attalea geraensis* (Indaiá-rateiro), *Attalea humilis* (American oil palm), *Attalea oleifera* (andaiá), *Attalea phalerata* (uricuri), *Attalea speciosa* (babassu), *Avena sativa* (oats), *Beta vulgaris* (sugar beet), *Brassica sp.* such as *Brassica carinata*, *Brassica juncea*, *Brassica napobrassica*, *Brassica napus* (canola), *Camelina sativa* (false flax), *Cannabis sativa* (hemp), *Carthamus tinctorius* (safflower), *Caryocar brasiliense* (pequi), *Cocos nucifera* (Coconut), *Crambe abyssinica* (Abyssinian kale), *Cucumis melo* (melon), *Elaeis guineensis* (African palm), *Glycine max* (soybean), *Gossypium hirsutum* (cotton), *Helianthus sp.* such as *Helianthus annuus* (sunflower), *Hordeum vulgare* (barley), *Jatropha curcas* (physic nut), *Joannesia princeps* (arara nut-tree), *Lemna sp.* (duckweed) such as *Lemna aequinoctialis*, *Lemna disperma*, *Lemna ecuadoriensis*, *Lemna gibba* (swollen duckweed), *Lemna japonica*, *Lemna minor*, *Lemna minuta*, *Lemna obscura*, *Lemna paucicostata*, *Lemna perpusilla*, *Lemna tenera*, *Lemna trisulca*, *Lemna turionifera*, *Lemna valdiviana*, *Lemna yungensis*, *Licania rigida* (oiticica), *Linum usitatissimum* (flax), *Lupinus angustifolius* (lupin), *Mauritia flexuosa* (buriti palm), *Maximiliana maripa* (inaja palm), *Miscanthus sp.* such as *Miscanthus x giganteus* and *Miscanthus sinensis*, *Nicotiana sp.* (tabacco) such as *Nicotiana tabacum* or *Nicotiana benthamiana*, *Oenocarpus bacaba* (bacaba-do-azeite), *Oenocarpus bataua* (patauã), *Oenocarpus distichus* (bacaba-de-leque), *Oryza sp.* (rice) such as *Oryza sativa* and *Oryza glaberrima*, *Panicum virgatum* (switchgrass), *Paraqueiba paraensis* (mari), *Persea amencana* (avocado), *Pongamia pinnata* (Indian beech), *Populus trichocarpa*, *Ricinus communis* (castor), *Saccharum sp.* (sugarcane), *Sesamum indicum* (sesame), *Solanum tuberosum* (potato), *Sorghum*

sp. such as *Sorghum bicolor*, *Sorghum vulgare, Theobroma grandifor um* (cupuassu), *Trifolium sp.*, *Trithrinax brasiliensis* (Brazilian needle palm), *Triticum sp.* (wheat) such as *Triticum aestivum* and *Zea mays* (corn).

19. The process of any one of claims 1 to 18, wherein the plant part is from a monocotyledonous plant, preferably a plant from the family Poaceae, more preferably a *Sorghum* sp., a *Zea mays*, *Miscanthus sp.* such as *Miscanthus x giganteus* and *Miscanthus sinensis*, and/or a *Panicum virgatum* (switchgrass) plant.

20. The process of any one of claims 2 to 10, or 12 to 19, wherein one or more or all of the promoters are expressed at a higher level in a vegetative plant part relative to seed of a plant.

21. Extracted plant lipid produced by the process of any one of claims 1 to 20, preferably comprising plant leaf lipid.

22. Extracted plant lipid, comprising fatty acids in an esterified form, wherein the level of medium chain fatty acids in the total fatty acid content of the lipid in the vegetative plant part is at least about 25%.

23. The lipid of claim 22, wherein the lipid has one or more of the features defined in claims 2 to 20.

24. A cell comprising an increased level or activity of polypeptides which are:

xxx. a GPAT, a LPAAT, and a WRI1 polypeptide;

xxxi. a GPAT, a LPAAT, a DGAT and a WRI1 polypeptide;

xxxii. a GPAT9, a LPAAT, and a WRI1 polypeptide;

xxxiii. a GPAT9, a LPAAT, a DGAT, and a WRI1 polypeptide;

xxxiv. a GPAT, a LPAAT, a DGAT1, and a WRI1 polypeptide;

xxxv. a GPAT9, a LPAAT, a DGAT1, and a WRI1 polypeptide;

xxxvi. a GPAT, a LPAAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xxxvii. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xxxviii. a GPAT9, a LPAAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xxxix. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xl. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xli. a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, and a fatty acid thioesterase, preferably a FATB polypeptide;

xlii. a GPAT, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xliii. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xliv. a GPAT9, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xlv. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xlvi. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xlvii. a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP;

xlviii. a GPAT, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

xlix. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

l. a GPAT9, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

li. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

lii. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which

reduces the expression of an endogenous gene which encodes a SDP1 lipase;

liii. a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

liv. a GPAT, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

lv. a GPAT9, a LPAAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

lvi. a GPAT9, a LPAAT, a DGAT, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase;

lvii. a GPAT, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin, or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase; or

lviii. a GPAT9, a LPAAT, a DGAT1, a WRI1 polypeptide, a fatty acid thioesterase, preferably a FATB polypeptide, an OBC polypeptide such as an oleosin, preferably a caleosin, or a LDAP, and a silencing RNA which reduces the expression of an endogenous gene which encodes a SDP1 lipase.

25. The cell of claim 24, wherein one or more or all of the polypeptides are encoded by one or more exogenous polynucleotides in the plant parts.

26. The cell of claim 24 or claim 25, wherein the level of total, or new, MCFA is increased relative to a corresponding wild-type plant part, preferably at least 25% of the total fatty acid content on a weight basis.

27. The cell of any one of claims 24 to 26, wherein one or more or all of the encoded GPAT, LPAAT and/or DGAT have a preference for utilising medium chain fatty acid substrates.

28. The cell of any one of claims 24 to 27, wherein the cell further comprises one or more or all of:

iv. a genetic modification which down-regulates endogenous production and/or activity of a polypeptide involved in the catabolism of triacylglycerols (TAG) in the cell when compared to a corresponding cell lacking the genetic modification;

v. a genetic modification which down-regulates endogenous production and/or activity of a polypeptide involved in importing fatty acids into plastids of the cell when compared to a corresponding cell lacking the genetic modification; and

vi. a genetic modification which down-regulates endogenous production and/or activity of a polypeptide involved in diacylglycerol (DAG) production in the plastid when compared to a corresponding cell lacking the genetic modification.

29. The cell according to claim 28, wherein the genetic modification is a mutation of an endogenous gene which partially or completely inactivates the gene, such as a point mutation, an insertion, or a deletion, or the genetic modification is an exogenous polynucleotide encoding an RNA molecule which inhibits expression of the endogenous gene, wherein the exogenous polynucleotide is operably linked to a promoter which is capable of directing expression of the polynucleotide in the cell.

30. The cell of any one of claims 25 to 29, wherein one or more or all of the promoters are expressed at a higher level in a vegetative plant part relative to seed of a plant.

31. The cell of any one of claims 26 to 30 which has one or more or all of the following features:

xxiv. the level of medium chain fatty acids in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is at least about 30%, at least about 35%, at least about 40%, at least about 50%, at least about 55%, or between about 25% and about 55%, between about 25% and about 50%, between about 30% and about 50%, between about 35% and about 50%, between about 25% and about 40%, or between about 30% and about 40%;

xxv. the level of lauric acid (C12:0) in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is, or is increased by, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 50%, at least or about 55%, or between about 15% and about 55%, between about 20% and about 50%, between about 30% and about 50%, between about 35% and about 50%, between about 15% and about 25%, or between about 20% and about 30%;

xxvi. the level of myristic acid (C14:0) in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is, or is increased by, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, or between about 25% and about 45%, between about 20% and about 50%, between about 30% and about 50%, between about 35% and about 50%, between about 30% and about 40%, between about 15% and about 25%, or between about 20% and about 30%;

xxvii. the level of palmitic acid (C16:0) in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is, or is increased by, between about 2% and about 18%, or between about 2% and about 16%, or between about 2% and about 15%, or between about 15% and about 50%;

xxviii. the level of lauric acid (C12:0) in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell is, or is increased by, at least about 25%, at least about 30%, at least about 40%, at least about 45%, or at least about 50%, and the level of myristic acid (C14:0) in the total fatty acid content of the cell and/or in the total fatty acid content of the TAG of the cell is, or is increased by, at least about 1%, at least about 2%, at least about 5%, or at least about 10%, or between about 1% and about 10%, or between about 2% and 10%, or between about 2% and about 6%, or less than about 10%, or less than about 8% or less than about 5%, or less than about 2%;

xxix. the level of myristic acid (C14:0) in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell is, or is increased by, at least about 20%, at least about 25%, at least about 30%, or at least about 40%, and the level of lauric acid (C12:0) in the total fatty acid content of the cell and/or in the total fatty acid content of the TAG of the cell is, or is increased by, at least about 1%, at least about 2%, at least about 5%, or at least about 10%, or between about 1% and about 10%, or between about 2% and about 10%, or between about 2% and about 6%, or less than about 10%, or less than about 8% or less than about 5%, or less than about 2%;

xxx. the level of myristic acid (C14:0) in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell is, or is increased

by, at least about 20%, at least about 25%, at least about 30%, and the level of palmitic acid (C16:0) in the total fatty acid content of the cell and/or in the total fatty acid content of the TAG of the cell is, or is increased by, at least about 2%, at least about 3%, at least about 4%, or at least about 5%.

xxxi. the ratio of lauric acid (C12:0):myristic acid (C14:0) in the fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is increased, or is about 1:4, about 1:5, about 1:10, about 1:15, about 1:20, about 1:25, or about 4:1, about 5:1, about 10:1, about 15:1, about 20:1, about 30:1, about 40:1, or about 45:1;

xxxii. the ratio of lauric acid (C12:0):palmitic acid (C16:0) in the fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is increased, or is about 1:2, about 1:3, about 1:4, about 1:5, about 1:10, about 1:15, about 10:1, about 20:1, about 30:1, about 40:1, or about 45:1;

xxxiii. the ratio of myristic acid (C14:0):palmitic acid (C16:0) in the fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is increased, or is about 1:2, about 1:3, about 1:4, about 1:5, about 1:10, about 1:15, about 10:1, about 20:1, about 30:1, or about 40:1;

xxxiv. the level of oleic acid in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is decreased, or is less than about 10%, less than about 8%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, less than about 1%;

xxxv. the level of linoleic acid (LA) in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is increased or decreased, or is less than about 20%, less than about 15%, less than about 10%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1%;

xxxvi. the level of α-linolenic acid (ALA) in the total fatty acid content of the cell, or in the total fatty acid content of the TAG of the cell, is decreased or is less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 10%, less than about 8%, less than about 6%, less than about 5%, less than about 2%, or less than about 1%;

xxxvii. the level of total unsaturated fatty acids in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is decreased, or is less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 15%, less than about 10%, less than about 8%, less than about 6%, less than about 5%, less than about 2%, or less than about 1%;

xxxviii. the level of total monounsaturated fatty acids in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is

decreased, or is less than about 10%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1%;

xxxix. the level of total polyunsaturated fatty acids in the total fatty acid content of the cell, and/or in the total fatty acid content of the TAG of the cell, is less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 15%, less than about 10%, less than about 8%, less than about 6%, less than about 5%, less than about 2%, or less than about 1%;

xl. the triacylglycerol (TAG) content of the cell is at least about 80%, at least about 85%, at least about 90%, or least about 95%, and about 98%, or between about 95% and about 98%, by weight of the cell;

xli. the TAG content of the cell comprises, or is increased in a level of, one or more or all of the TAG species 36:0, 38:0, 40:0 and 42:0;

xlii. the cell comprises tri-laurin (tri-C12:0) and/or tri-myristin (tri-C14:0);

xliii. the phosphocholine (PC) content of the cell comprises one or both of the PC species 28:0 and 30:0,

xliv. the cell has a reduced level of medium chain fatty acids in membrane lipids relative to a corresponding cell;

xlv. the cell has less chlorosis relative to a corresponding cell which comprises the exogenous polynucleotide encoding the thioesterase but lacks the exogenous polynucleotide encoding the DGAT; and

xlvi. the cell is in a vegetative plant part and the part has an alleviated chlorosis phenotype relative to a corresponding vegetative plant part,

wherein any increase or decrease is relative to a corresponding wild-type cell.

32. A plant or a part thereof comprising the cell of any one of claims 24 to 31, or which is transgenic for one or more exogenous polynucleotides as defined in any one of claims 2 to 10.

33. A population of at least about 1000 plants, each being a plant according to claim 32, growing in a field, or a collection of at least about 1000 plant parts, each being a plant part according to claim 32, wherein the plant parts have been harvested from plants growing in a field.

34. Seed of, or obtained from, a plant according to claim 32.

35. A process for obtaining a cell according to any one of claims 24 to 31, the process comprising the steps of:

i) introducing into a cell at least one exogenous polynucleotide and/or at least one genetic modification as defined in any one of claims 24 to 31 to produce a cell as defined in any one of claims 24 to 31,

ii) expressing the exogenous polynucleotide(s) in the cell or a progeny cell thereof,

iii) analysing the lipid content of the cell or progeny cell, and

iv) selecting a cell according to any one of claims 24 to 31.

36. A method of producing a plant which has integrated into its genome a set of exogenous polynucleotides and/or genetic modifications as defined in any one of claims 24 to 31, the method comprising the steps of:

i) crossing two parental plants, wherein one plant comprises at least one of the exogenous polynucleotides and/or at least one genetic modifications as defined in any one of claims 24 to 31; and the other plant comprises at least one of the exogenous polynucleotides and/or at least one genetic modifications as defined in any one of claims 24 to 31, and wherein between them the two parental plants comprise a set of exogenous polynucleotides and/or genetic modifications as defined in any one of claims 24 to 31,

ii) screening one or more progeny plants from the cross for the presence or absence of the set of exogenous polynucleotides and/or genetic modifications as defined in any one of claims 24 to 31, and

iii) selecting a progeny plant which comprise the set of exogenous polynucleotides and/or genetic modifications as defined in any one of claims 24 to 31, thereby producing the plant.

37. A process for producing an industrial product, the process comprising the steps of:

i) obtaining a cell of any one of claims 24 to 31, a plant or a part thereof of claim 32, or seed of claim 34, and

ii) either

a) converting at least some of the lipid in the cell, plant or part thereof, or seed, of step i) to the industrial product by applying heat, chemical, or enzymatic means, or any combination thereof, to the lipid *in situ* in the cell, or plant or vegetative part thereof, or seed, or

b) physically processing the cell, plant or part thereof, or seed, of step i), and subsequently or simultaneously converting at least some of the lipid in the processed cell, plant or part thereof, or seed, to the industrial product by

applying heat, chemical, or enzymatic means, or any combination thereof, to the lipid in the processed cell, plant or part thereof, or seed, and

iii) recovering the industrial product,

thereby producing the industrial product.

38. The process of claim 37, further comprising steps of:

(a) extracting at least some of the non-polar lipid content of the cell, or plant or part thereof, or seed, as non-polar lipid, and

(b) recovering the extracted non-polar lipid,

wherein steps (a) and (b) are performed prior to the step of converting at least some of the lipid in the cell, plant or part thereof, or seed, to the industrial product.

39. A process for producing extracted lipid, the process comprising the steps of:

i) obtaining a plant cell of any one of claims 24 to 31, or a plant or a part thereof of claim 32, or seed of claim 34,

ii) extracting lipid from the cell, or plant or part thereof, or seed, and

iii) recovering the extracted lipid,

thereby producing the extracted lipid.

40. The process of claim 38 or claim 39 which comprises recovering the extracted lipid by collecting it in a container and/or one or more of degumming, deodorising, decolourising, drying, fractionating the extracted lipid, removing wax esters from the extracted lipid, or analysing the fatty acid composition of the extracted lipid.

41. The process of any one of claims 38 to 40, wherein the process further comprises converting the extracted lipid to an industrial product.

42. The process of any one of claims 37, 38 or 41 wherein the industrial product is a hydrocarbon product such as fatty acid esters, preferably fatty acid methyl esters and/or a fatty acid ethyl esters, an alkane such as methane, ethane or a longer-chain alkane, a mixture of longer chain alkanes, an alkene, a biofuel, carbon monoxide and/or hydrogen gas, a bioalcohol such as ethanol, propanol, or butanol, biochar, or a combination of carbon monoxide, hydrogen and biochar.

43. A process for producing seed, the process comprising:

i) growing a plant according to claim 32, and

ii) harvesting seed from the plant.

44. Recovered or extracted lipid obtainable from a cell according to any one of claims 24 to 31, a plant or a part thereof of claim 32, seed of claim 34, or obtainable by the process of any one of claims 1 to 20, 39, or 40.

45. An industrial product produced by the process according to any one of claims 37, 38 or 41, which is a hydrocarbon product such as fatty acid esters, preferably fatty acid methyl esters and/or a fatty acid ethyl esters, an alkane such as methane, ethane or a longer-chain alkane, a mixture of longer chain alkanes, an alkene, a biofuel, carbon monoxide and/or hydrogen gas, a bioalcohol such as ethanol, propanol, or butanol, biochar, or a combination of carbon monoxide, hydrogen and biochar.

46. Use of a cell according to any one of claims 24 to 31, a plant or part thereof of claim 32, seed of claim 34, or the lipid of any one of claims 21, 22, 23, or 44, for the manufacture of an industrial product.

47. A process for producing fuel, the process comprising:

i) reacting the lipid of any one of claims 21, 22, 23, or 44 with an alcohol, optionally, in the presence of a catalyst, to produce alkyl esters, and

ii) optionally, blending the alkyl esters with petroleum based fuel.

48. A process for producing a synthetic diesel fuel, the process comprising:

i) converting the lipid in a cell of any one of claims 24 to 31, or a plant or a part thereof of claim 32, or seed of claim 34, to a bio-oil by a process comprising pyrolysis or hydrothermal processing or to a syngas by gasification, and

ii) converting the bio-oil to synthetic diesel fuel by a process comprising fractionation, preferably selecting hydrocarbon compounds which condense between about 150ºC to about 200ºC or between about 200ºC to about 300ºC, or converting the syngas to a biofuel using a metal catalyst or a microbial catalyst.

49. A process for producing a biofuel, the process comprising converting the lipid in a cell of any one of claims 24 to 31, a plant or a part thereof of claim 32 or seed of claim 34, to bio-oil by pyrolysis, a bioalcohol by fermentation, or a biogas by gasification or anaerobic digestion.

50. A process for producing a feedstuff, the process comprising admixing a plant cell of any one of claims 24 to 31, a plant or a part thereof of claim 32, seed of clam 34, or the lipid of any one of claims 21, 22, 23 or 44, or an extract or portion thereof, with at least one other food ingredient.

51. Feedstuffs, cosmetics or chemicals comprising a plant cell of any one of claims 24 to 31, a plant or a part thereof of claim 32, seed of claim 34, or the lipid of any one of claims 21, 22, 23 or 44, or an extract or portion thereof.

5

52. A process for feeding an animal, the process comprising providing to the animal a plant or a part thereof of claim 32, seed of claim 34, or the lipid of any one of claims 21, 22, 23 or 44.

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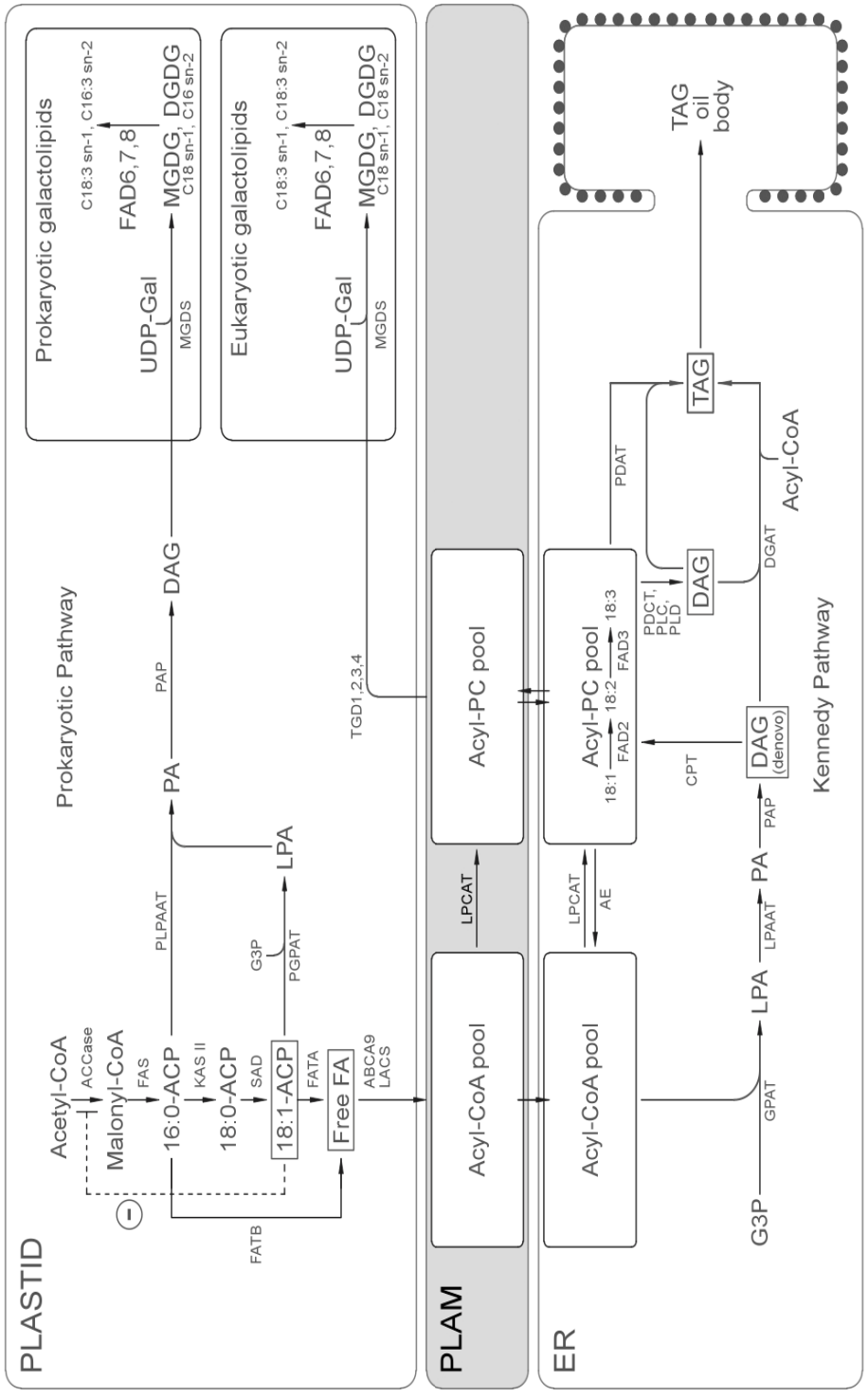


FIGURE 1

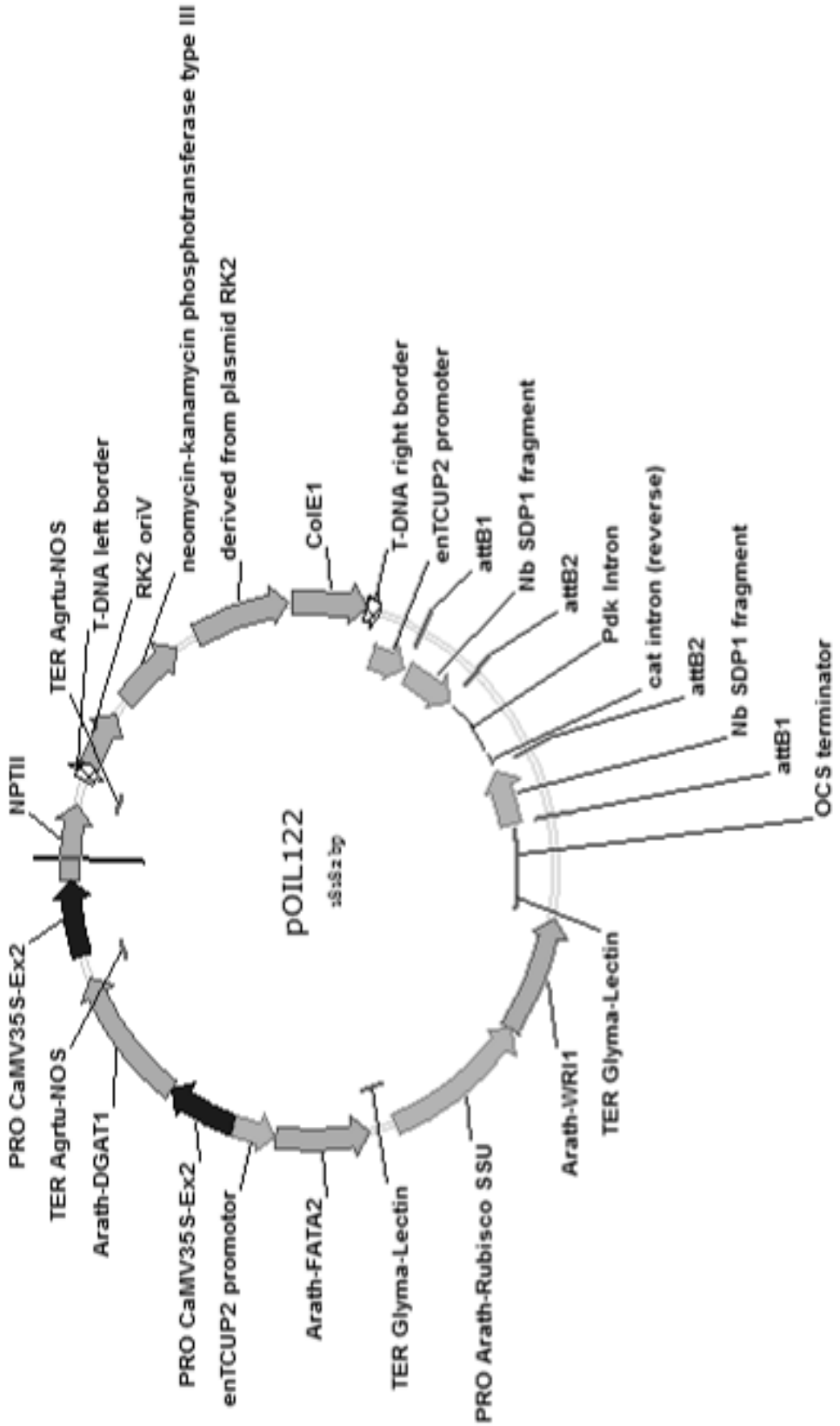


FIGURE 2

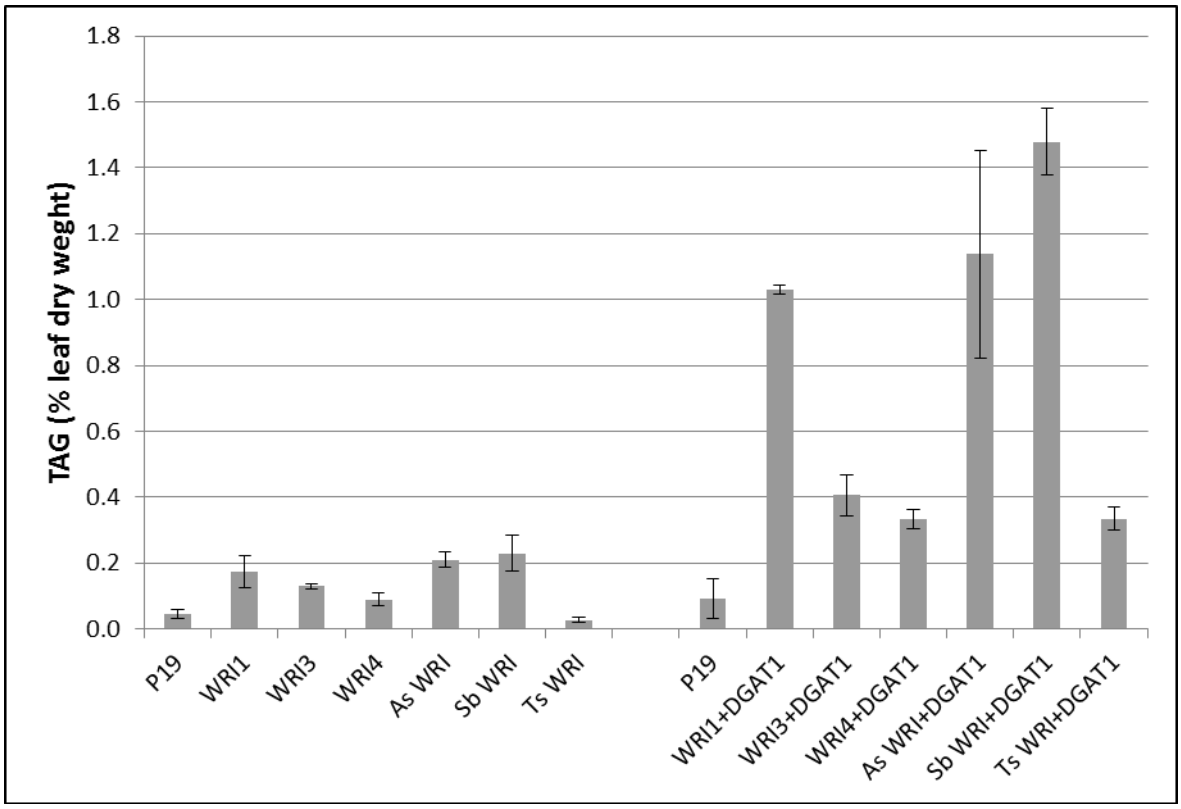


FIGURE 3

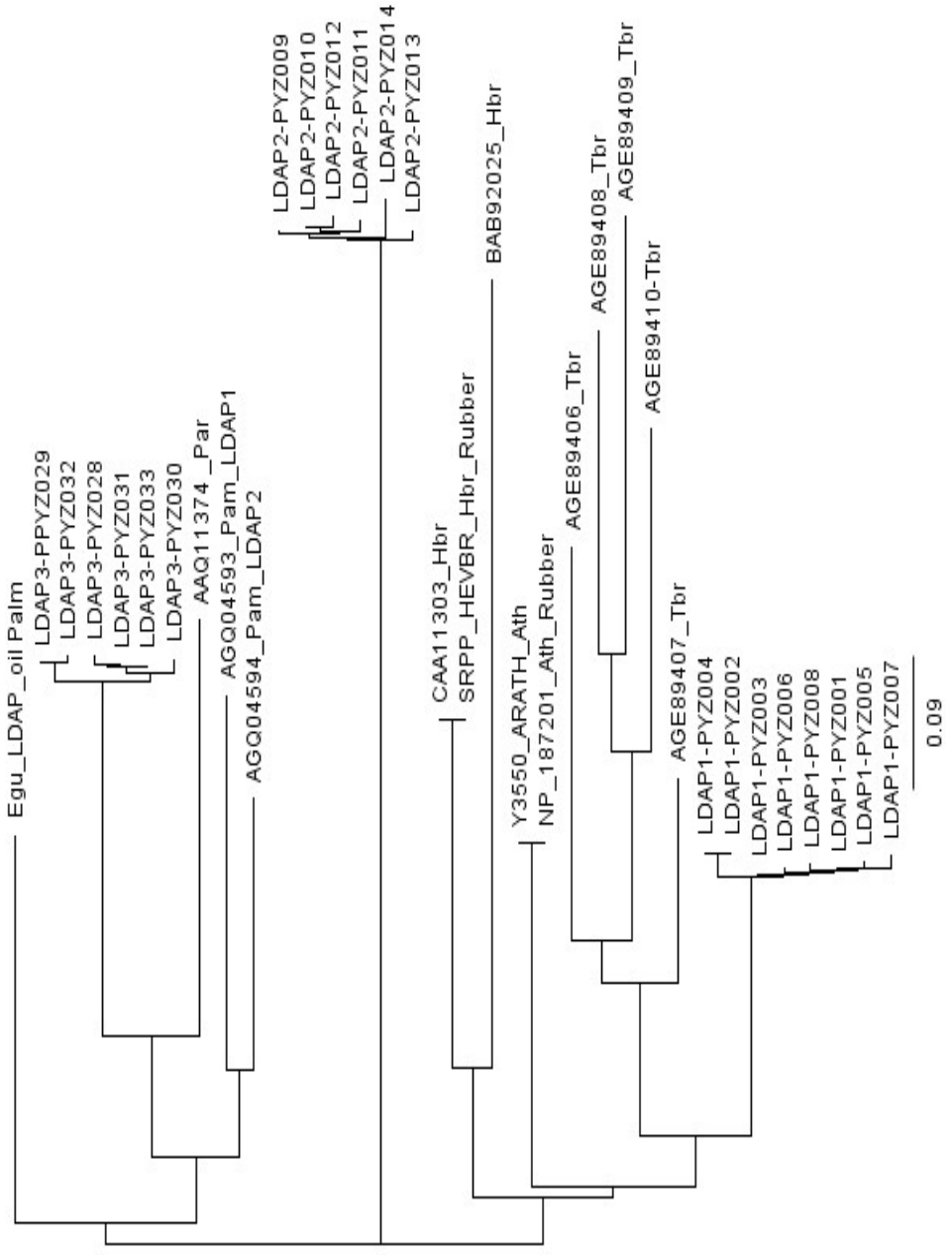


FIGURE 4

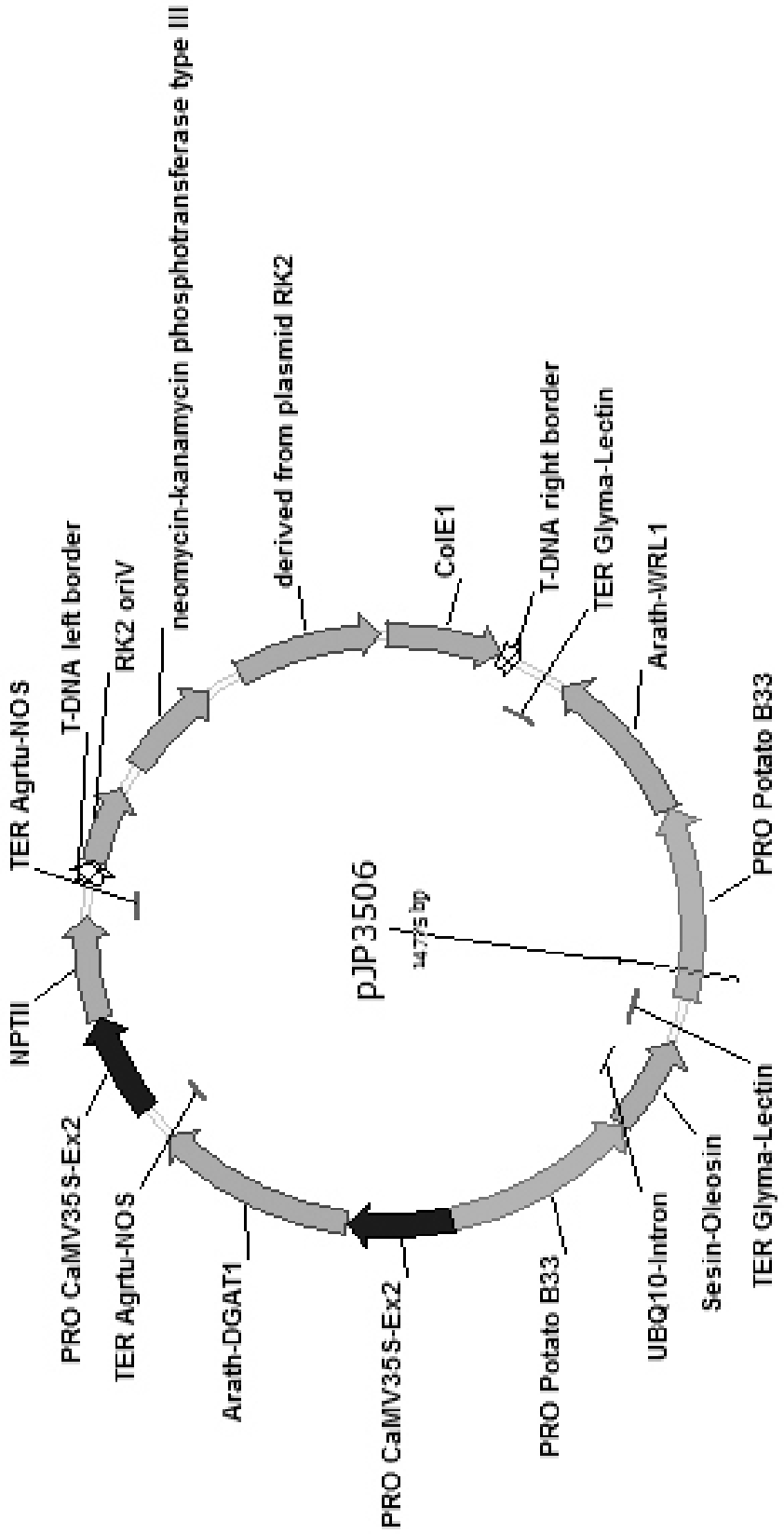


FIGURE 5

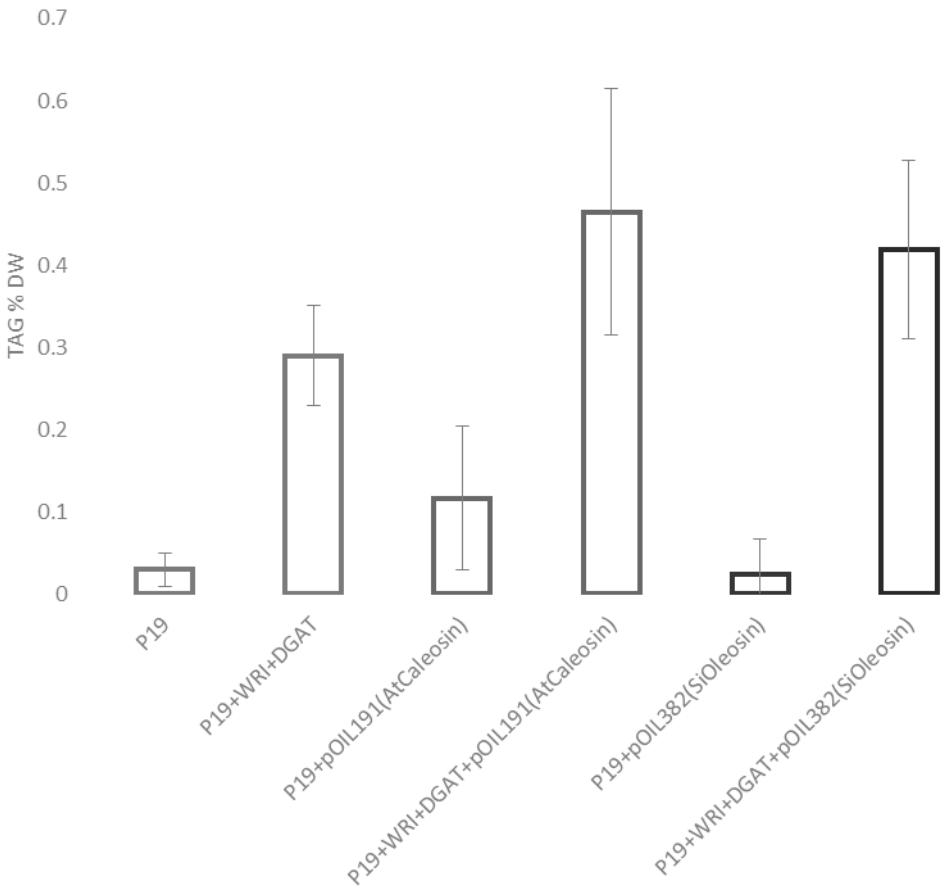
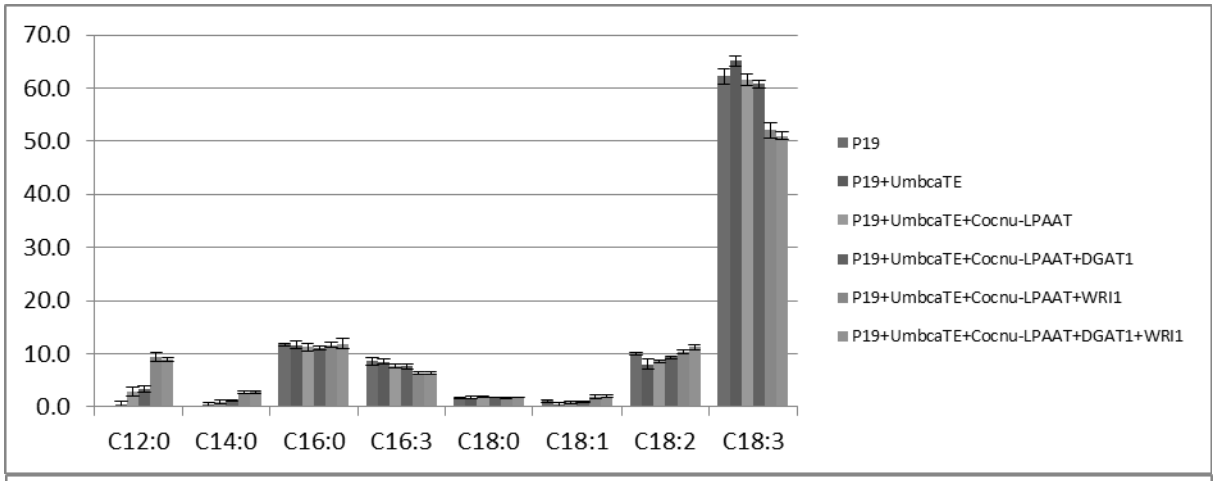
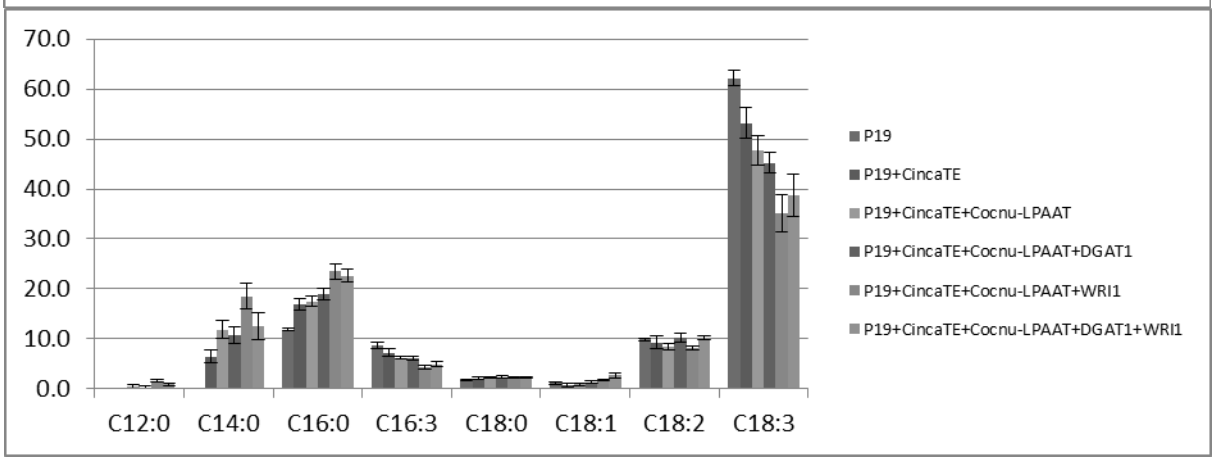


FIGURE 6





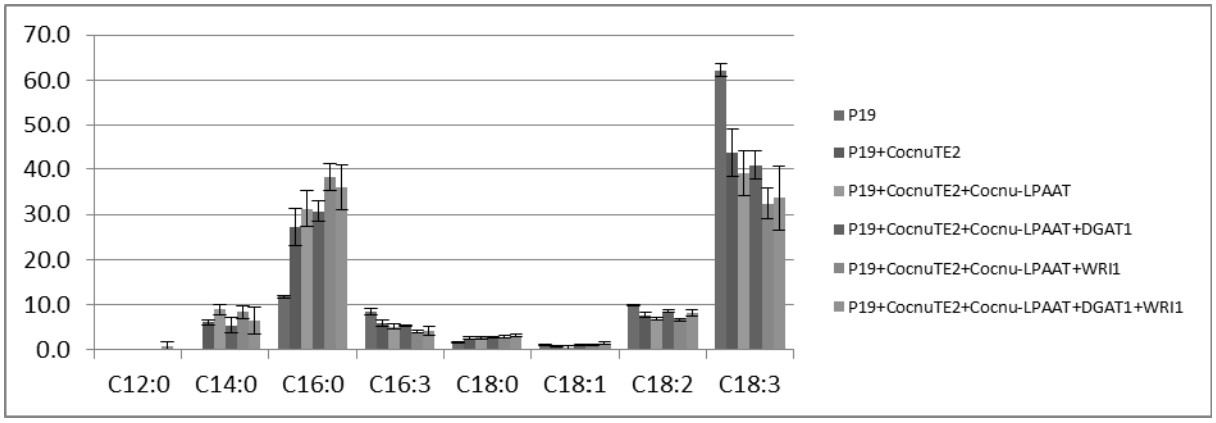


FIGURE 7

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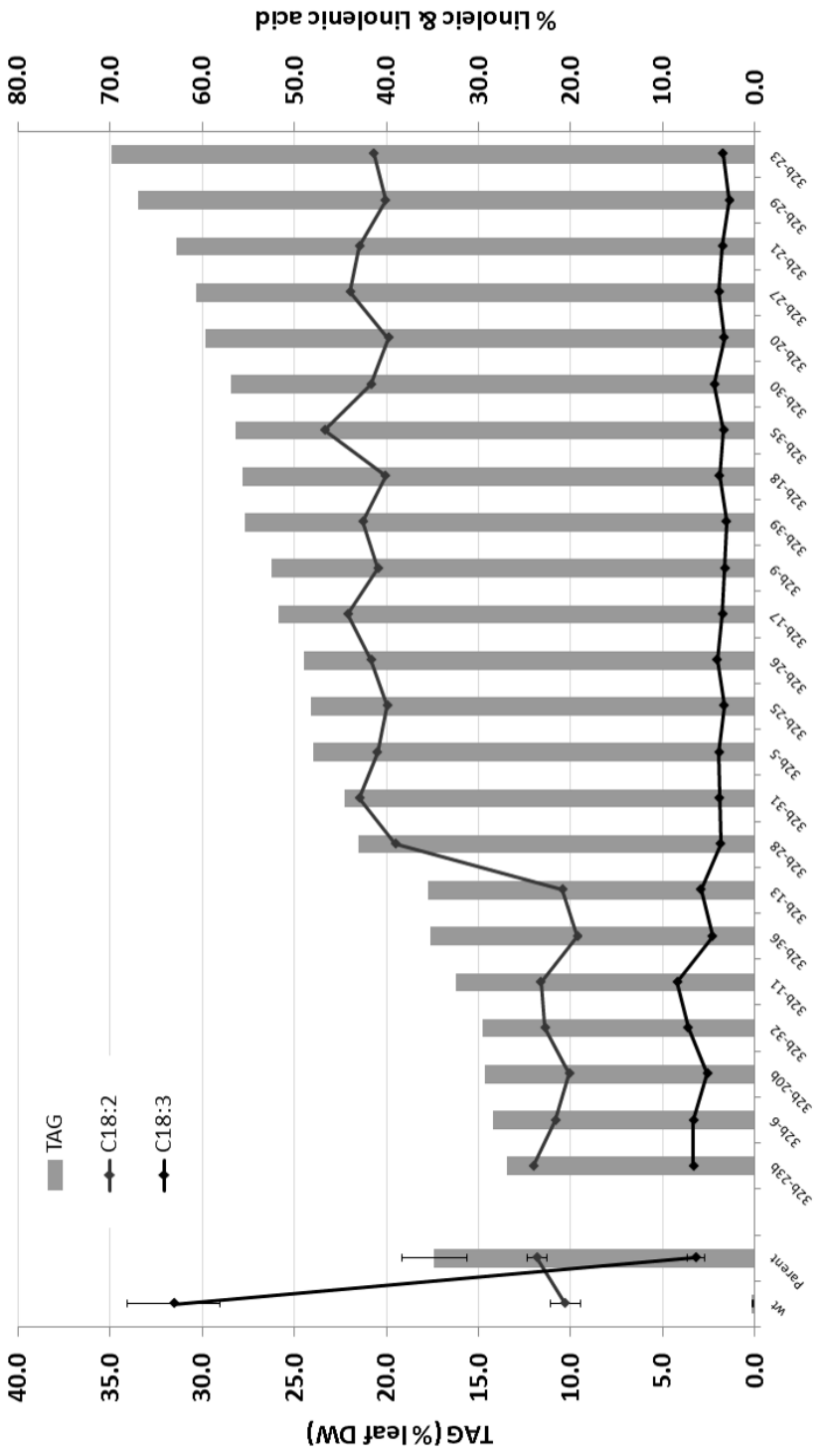

FIGURE 8

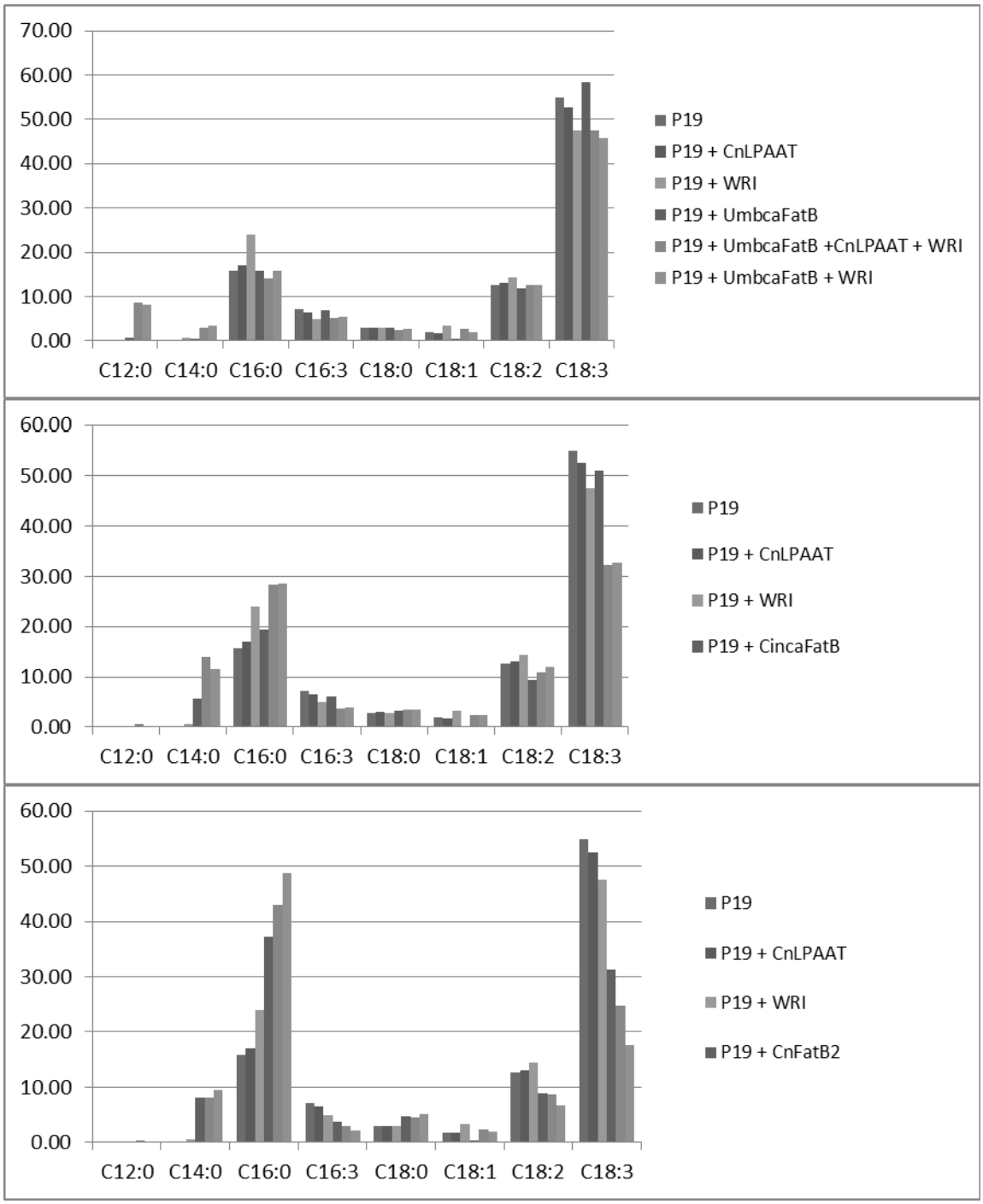

FIGURE 9

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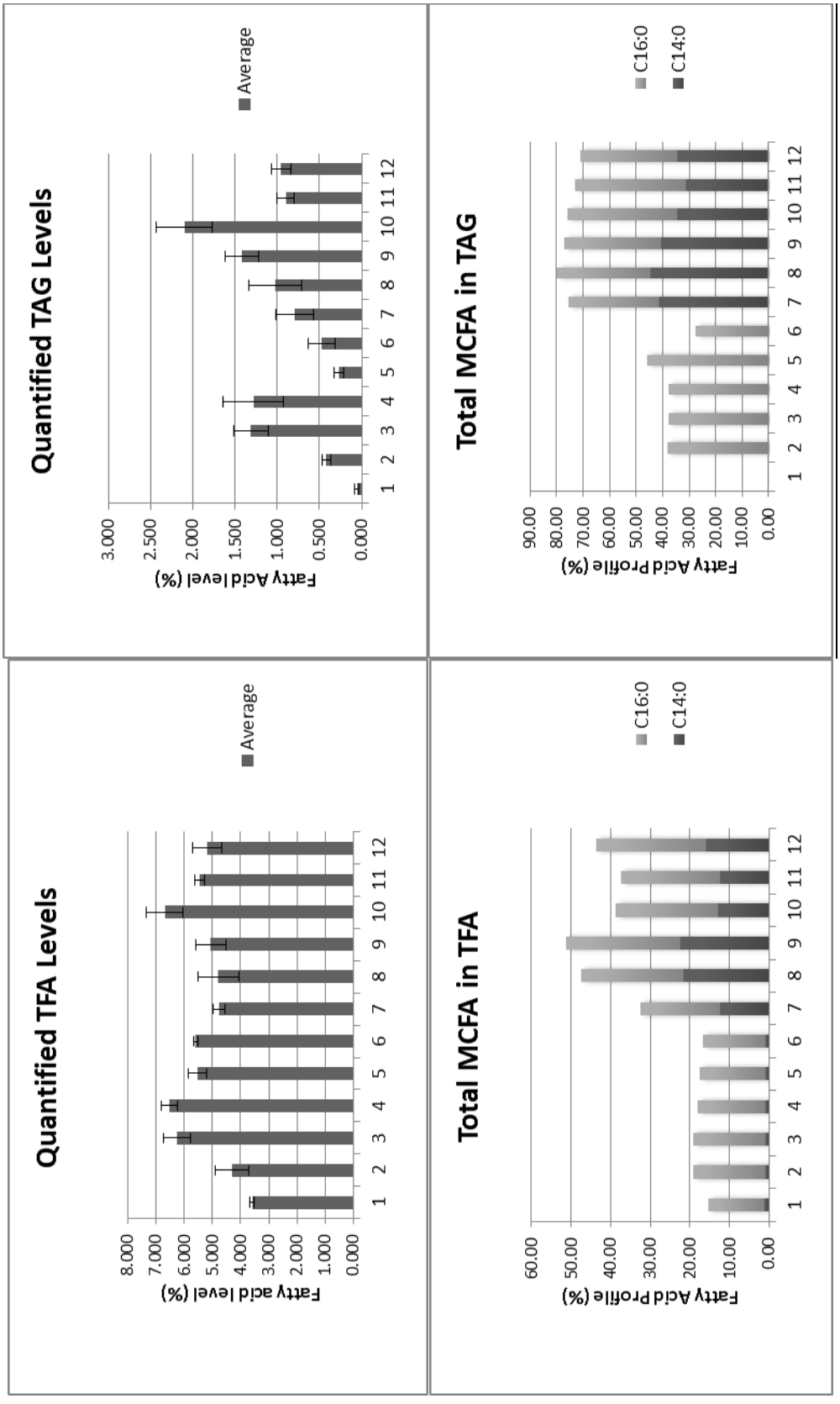

FIGURE 10

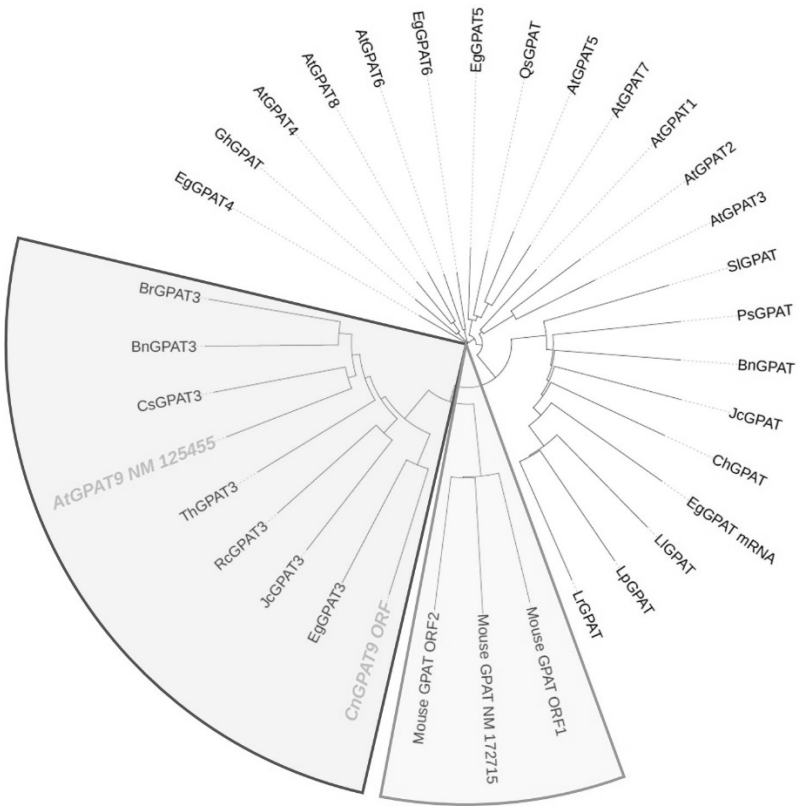

FIGURE 11

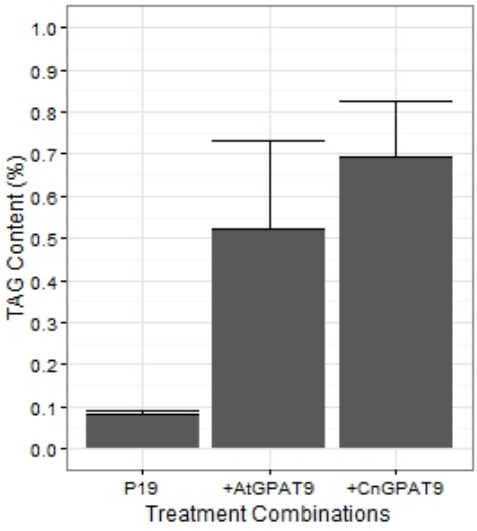

FIGURE 12

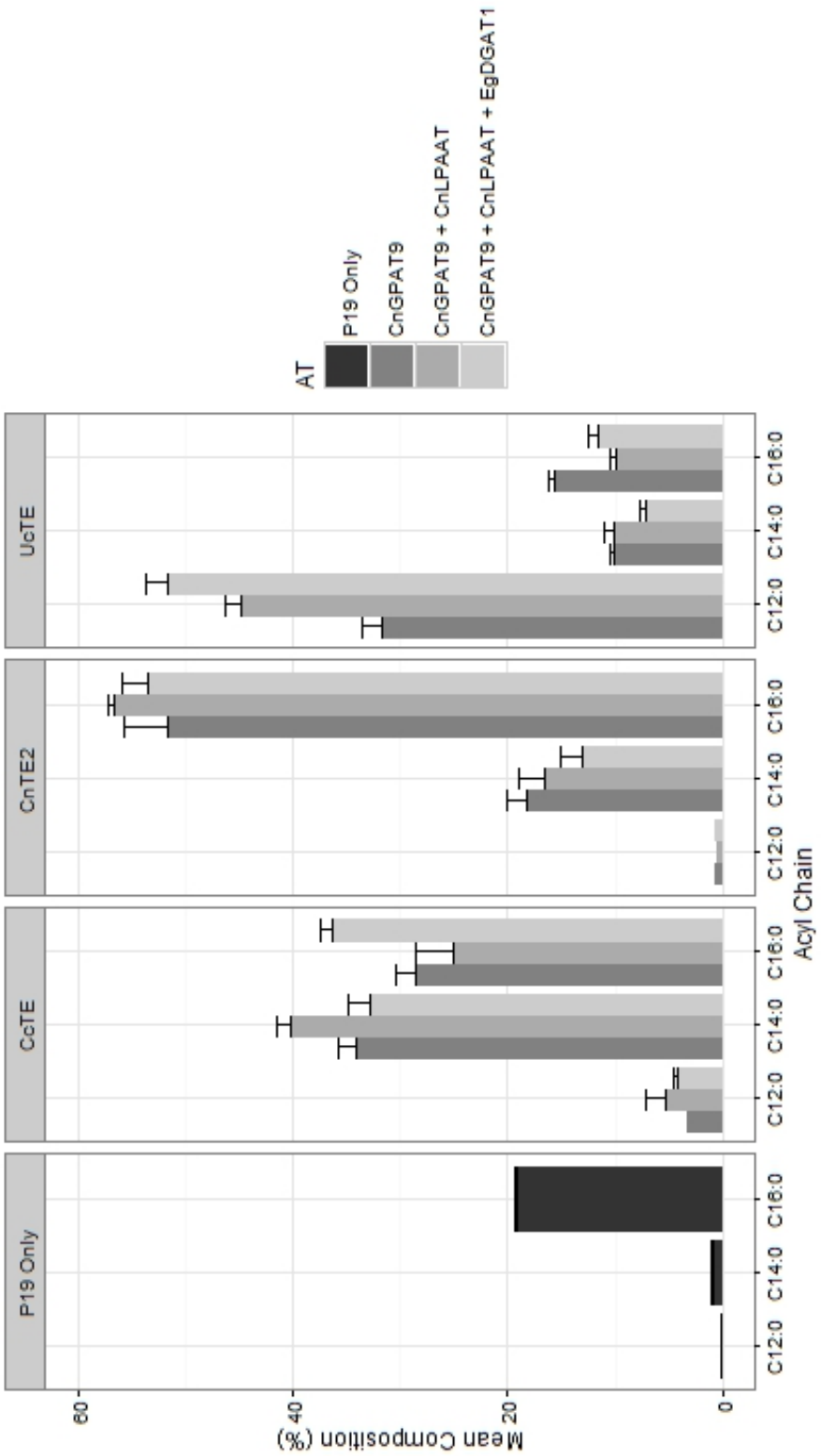

FIGURE 13

Sequence Listing

1	**Sequence Listing Information**	
1-1	File Name	524739AUP01.xml
1-2	DTD Version	V1_3
1-3	Software Name	WIPO Sequence
1-4	Software Version	2.3.0
1-5	Production Date	2026-03-15
1-6	Original free text language code	
1-7	Non English free text language code	
2	**General Information**	
2-1	Current application: IP Office	AU
2-2	Current application: Application number	
2-3	Current application: Filing date	
2-4	Current application: Applicant file reference	543981AUP01
2-5	Earliest priority application: IP Office	AU
2-6	Earliest priority application: Application number	20180201932
2-7	Earliest priority application: Filing date	2018-03-16
2-8en	Applicant name	Commonwealth Scientific and Industrial Research Organisation
2-8	Applicant name: Name Latin	
2-9en	Inventor name	
2-9	Inventor name: Name Latin	
2-10en	Invention title	Plants Producing Modified Levels of Medium Chain Fatty Acids
2-11	Sequence Total Quantity	152

3-1	**Sequences**	
3-1-1	Sequence Number [ID]	1
3-1-2	Molecule Type	AA
3-1-3	Length	520
3-1-4	Features	**source 1..520**
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		organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-1-5	Residues	MAILDSAGVT TVTENGGGEF VDLDRLRRRK SRSDSSNGLL LSGSDNNSPS DDVGAPADVR 60 DRIDSVVNDD AQGTANLAGD NNGGGDNNGG GRGGGEGRGN ADATFTYRPS VPAHRRARES 120 PLSSDAIFKQ SHAGLFNLCV VVLIAVNSRL IIENLMKYGW LIRTDFWFSS RSLRDWPLFM 180 CCISLSIFPL AAFTVEKLVL QKYISEPVVI FLHIIITMTE VLYPVYVTLR CDSAFLSGVT 240 LMLLTCIVWL KLVSYAHTSY DIRSLANAAD KANPEVSYYV SLKSLAYFMV APTLCYQPSY 300 PRSACIRKGW VARQFAKLVI FTGFMGFIIE QYINPIVRNS KHPLKGDLLY AIERVLKLSV 360 PNLYVWLCMF YCFFHLWLNI LAELLCFGDR EFYKDWWNAK SVGDYWRMWN MPVHKWMVRH 420 IYFPCLRSKI PKTLAIIIAF LVSAVFHELC IAVPCRLFKL WAFLGIMFQV PLVFITNYLQ 480 ERFGSTVGNM IFWFIFCIFG QPMCVLLYYH DLMNRKGSMS 520
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3-2-1	Sequence Number [ID]	2
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3-2-3	Length	
3-2-4	Features	
	Location/Qualifiers	
	NonEnglishQualifier Value	
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3-3-1	Sequence Number [ID]	3
3-3-2	Molecule Type	AA
3-3-3	Length	4
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	NonEnglishQualifier Value	
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3-4-2	Molecule Type	AA
3-4-3	Length	4
3-4-4	Features	**REGION 1..4**
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		source 1..4
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-4-5	Residues	EPHS 4
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3-5-2	Molecule Type	AA
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3-5-4	Features	**REGION 1..24**
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		note=X - any amino acid
		SITE 5
		note=X - any amino acid
		SITE 6
		note=X - Lysine (K) or Arginine (R)
		SITE 7
		note=X - any amino acid
		REGION 9..11
		note=X - any amino acid
		REGION 13..15
		note=X - any amino acid
		SITE 16
		note=X - Leucine (L) or Valine (V)
		REGION 19..21
		note=X - any amino acid

		SITE 24	
		note=X - Glutamic Acid (E) or Glutamine (Q)	
		source 1..24	
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		organism=synthetic construct	
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3-6-2	Molecule Type	AA	
3-6-3	Length	8	
3-6-4	Features	**REGION 1..8**	
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		REGION 5..7	
		note=X - any amino acid	
		source 1..8	
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		organism=synthetic construct	
	NonEnglishQualifier Value		
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3-7-2	Molecule Type	AA	
3-7-3	Length	15	
3-7-4	Features	**REGION 1..15**	
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		source 1..15	
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		organism=synthetic construct	
	NonEnglishQualifier Value		
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3-8-2	Molecule Type		
3-8-3	Length		
3-8-4	Features		
	Location/Qualifiers		
	NonEnglishQualifier Value		
3-8-5	Residues	000	3
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3-9-1	Sequence Number [ID]	9	
3-9-2	Molecule Type		
3-9-3	Length		
3-9-4	Features		
	Location/Qualifiers		
	NonEnglishQualifier Value		
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3-10-1	Sequence Number [ID]	10	
3-10-2	Molecule Type	AA	
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3-10-4	Features	**source 1..393**	
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3-11-3	Length	428	
3-11-4	Features	**source 1..428**	

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	NonEnglishQualifier Value	
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3-12-3	Length	440
3-12-4	Features	**source 1..440**
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	NonEnglishQualifier Value	
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3-13-3	Length	428
3-13-4	Features	**source 1..428**
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	NonEnglishQualifier Value	
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		source 1..11
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	NonEnglishQualifier Value	
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3-15-2	Molecule Type	AA
3-15-3	Length	8
3-15-4	Features	**REGION 1..8**
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		note=X - Phenylalanine (F) or Tyrosine (Y)
		source 1..8
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
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3-16-3	Length	9	
3-16-4	Features	**REGION 1..9**	
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		organism=synthetic construct	
	NonEnglishQualifier Value		
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3-17-3	Length	8	
3-17-4	Features	**REGION 1..8**	
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		SITE 5	
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		note=X - any amino acid	
		source 1..8	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
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		SITE 6	
		note=X - Arginine (R) or Lysine (K)	
		SITE 12	
		note=X - Arginine (R) or Lysine (K)	
		SITE 13	
		note=misc_feature - Xaa can be any naturally occurring amino acid	
		source 1..14	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
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3-19-1	Sequence Number [ID]	19	
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3-19-3	Length	9	
3-19-4	Features	**REGION 1..9**	
	Location/Qualifiers	note=conserved sequence	
		SITE 7	
		note=X - any amino acid	
		source 1..9	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
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3-20-1	Sequence Number [ID]	20	
3-20-2	Molecule Type	DNA	
3-20-3	Length	11142	
3-20-4	Features	**misc_feature 1..11142**	
	Location/Qualifiers	note=TDNA sequence	
		source 1..11142	
		mol_type=other DNA	
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	NonEnglishQualifier Value	
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		tggtcccaaa gatggacccc cacccacgag gagcatcgtg gaaaaagaag acgttccaac 15540 cacgtcttca aagcaagtgg attgatgtga taacatggtg gagcacgaca ctctcgtcta 15600 ctccaagaat atcaaagata cagtctcaga agaccaaagg gctattgaga cttttcaaca 15660 aagggtaata tcgggaaacc tcctcggatt ccattgccca gctatctgtc acttcatcaa 15720 aaggacagta gaaaaggaag gtggcaccta caaatgccat cattgcgata aaggaaaggc 15780 tatcgttcaa gatgcctctg ccgacagtgg tcccaaagat ggacccccac ccacgaggag 15840 catcgtggaa aaagaagacg ttccaaccac gtcttcaaag caagtggatt gatgtgatat 15900 ctccactgac gtaagggatg acgcacaatc ccactatcct tcgcaagacc ttcctctata 15960 taaggaagtt catttcattt ggagaggaca cgctgaaatc accagtctct ctctacaaat 16020 ctatctctct cgagatgatt gaacaagatg gattgcacgc aggttctccg gccgcttggg 16080 tggagaggct attcggctat gactgggcac aacagacaat cggctgctct gatgccgccg 16140 tgttccggct gtcagcgcag gggaggccgg ttctttttgt caagaccgac ctgtccggtg 16200 ccctgaatga acttcaagac gaggcagcgc ggctatcgtg gctggccacg acgggcgttc 16260 cttgcgcagc tgtgctcgac gttgtcactg aagcgggaag ggactggctg ctattgggcg 16320 aagtgccggg gcaggatctc ctgtcatctc accttgctcc tgccgagaaa gtatccatca 16380 tggctgatgc aatgcggcgg ctgcatacgc ttgatccggc tacctgccca ttcgaccacc 16440 aagcgaaaca tcgcatcgag cgagcacgta ctcggatgga agccggtctt gtcgatcagg 16500 atgatctgga cgaagagcat caggggctcg cgccagccga actgttcgcc aggctcaagg 16560 cgcgcatgcc cgacggcgag gatctcgtcg tgactcatgg cgatgcctgc ttgccgaata 16620 tcatggtgga aaatggccgc ttttctggat tcatcgactg tggccggctg ggtgtggcgg 16680 accgctatca ggacatagcg ttggctaccc gtgatattgc tgaagagctt ggcggcgaat 16740 gggctgacc 16749
3-22	**Sequences**	
3-22-1	Sequence Number [ID]	22
3-22-2	Molecule Type	DNA
3-22-3	Length	137
3-22-4	Features	**misc_feature 1..137**
	Location/Qualifiers	note=linker sequence
		source 1..137
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-22-5	Residues	atttaaatgc ggccgcgaat tcgtcgattg aggacgtccc tactagacct gctggacctc 60 ctcctgctac ttactacgat tctctcgctg tgcatatggt cagtcatgcc cgggcctgca 120 ggcggccgca tttaaat 137
3-23	**Sequences**	
3-23-1	Sequence Number [ID]	23
3-23-2	Molecule Type	DNA
3-23-3	Length	434
3-23-4	Features	**misc_feature 1..434**
	Location/Qualifiers	note=hpRNAi
		source 1..434
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-23-5	Residues	gtgagcaatg aaccaagatt tatcaatacc gttacttttg atagcaaaga gggttctcct 60 actcttgtta tggtccacgg atatggtgcc tctcagggtt tcttctttcg gaatttttat 120 gcccttgcga ggcatttcaa agttattgct attgatcagc ttggctgggg tggttcaagc 180 aggcctgact tcacatgcag aagtacagaa gagactgaag attggtttat tgattccttt 240 gaggagtggc gcaaagccaa aaaccttagc aactttattt tgcttgggca ctcctttgga 300 gggtatgtcg ctgcaaaata tgctctcaag catccagagc atgttcagca gttgattctg 360 gtaggaccag ctggatttac atcagagact gaacatatgt ccgagcggct tacccagttt 420 agagcaacat ggaa 434
3-24	**Sequences**	
3-24-1	Sequence Number [ID]	24
3-24-2	Molecule Type	DNA
3-24-3	Length	593
3-24-4	Features	**misc_feature 1..593**
	Location/Qualifiers	note=hpRNAi
		source 1..593
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-24-5	Residues	actgctgatg ctgtcaggca gtatctatgg ttgtttgagg agcataatgt tcttgaattc 60 ctcgtacttg ctggagatca tctatatcga atggattatg aaaagttcat tcaagcccac 120 agagaaacag atgctgatat tactgttgcc gcactgccaa tggatgaaaa gcgagccact 180 gcatttggtc tcatgaagat tgacgaagaa ggacgcatta ttgaatttgc agagaaaccg 240 aaaggagagc aattgaaagc aatgaaagtg gatactacca ttttaggtct tgatgatgag 300 agagctaaag agatgccttt tatcgcaagt atgggtatat atgtcattag caaagatgtg 360 atgttaaact tacttcgtga taagttccct ggtgccaatg attttggcag tgaagttatt 420 cctggtgcaa cttcgcttgg gatgagagtg caagcttatt tatatgatgg atactgggaa 480 gatattggta ccatcgaagc tttctacaat gccaatttgg gcattaccaa aaagccagtc 540

		ccagatttta gcttctatga ccgatcagct ccaatctaca cccaacctcg ata	593
3-25	**Sequences**		
3-25-1	Sequence Number [ID]	25	
3-25-2	Molecule Type		
3-25-3	Length		
3-25-4	Features		
	Location/Qualifiers		
	NonEnglishQualifier Value		
3-25-5	Residues	000	3
3-26	**Sequences**		
3-26-1	Sequence Number [ID]	26	
3-26-2	Molecule Type		
3-26-3	Length		
3-26-4	Features		
	Location/Qualifiers		
	NonEnglishQualifier Value		
3-26-5	Residues	000	3
3-27	**Sequences**		
3-27-1	Sequence Number [ID]	27	
3-27-2	Molecule Type	AA	
3-27-3	Length	7	
3-27-4	Features	**REGION 1..7**	
	Location/Qualifiers	note=probable lipid binding motif	
		REGION 2..4	
		note=X - any amino acid	
		source 1..7	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-27-5	Residues	VXXXHGF	7
3-28	**Sequences**		
3-28-1	Sequence Number [ID]	28	
3-28-2	Molecule Type	AA	
3-28-3	Length	584	
3-28-4	Features	**source 1..584**	
	Location/Qualifiers	mol_type=protein	
		organism=Arabidopsis thaliana	
	NonEnglishQualifier Value		
3-28-5	Residues	MNSMNNWLGF SLSPHDQNHH RTDVDSSTTR TAVDVAGGYC FDLAAPSDES SAVQTSFLSP	60
		FGVTLEAFTR DNNSHSRDWD INGGACNTLT NNEQNGPKLE NFLGRTTTIY NTNETVVDGN	120
		GDCGGGDGGG GGSLGLSMIK TWLSNHSVAN ANHQDNGNGA RGLSLSMNSS TSDSNNYNNN	180
		DDVVQEKTIV DVVETTPKKT IESFGQRTSI YRGVTRHRWT GRYEAHLWDN SCKREGQTRK	240
		GRQVYLGGYD KEEKAARAYD LAALKYWGPT TTTNFPLSEY EKEVEEMKHM TRQEYVASLR	300
		RKSSGFSRGA SIYRGVTRHH QHGRWQARIG RVAGNKDLYL GTFGTQEEAA EAYDIAAIKF	360
		RGLSAVTNFD MNRYNVKAIL ESPSLPIGSS AKRLKDVNNP VPAMMISNNV SESANNVSGW	420
		QNTAFQHHQG MDLSLLQQQQ ERYVGYYNGG NLSTESTRVC FKQEEEQQHF LRNSPSHMTN	480
		VDHHSSTSDD SVTVCGNVVS YGGYQGFAIP VGTSVNYDPF TAAEIAYNAR NHYYYAQHQQ	540
		QQQIQQSPGG DFPVAISNNH SSNMYFHGEG GGEGAPTFSV WNDT	584
3-29	**Sequences**		
3-29-1	Sequence Number [ID]	29	
3-29-2	Molecule Type	DNA	
3-29-3	Length	336	
3-29-4	Features	**misc_feature 1..336**	
	Location/Qualifiers	note=inducible promoter	
		source 1..336	
		mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-29-5	Residues	tcgatagttg tgatagttcc cacttgtccg tccgcatcgg catccgcagc tcgggatagt	60
		tccgacctag gattggatgc atgcggaacc gcacgagggc ggggcggaaa ttgacacacc	120
		actcctctcc acgcaccgtt caagaggtac gcgtatagag ccgtatagag cagagacgga	180
		gcactttctg gtactgtccg cacgggatgt ccgcacggag agccacaaac gagcggggcc	240
		ccgtacgtgc tctcctaccc caggatcgca tccccgcata gctgaacatc tatataaaga	300
		cccccaaggt tctcagtctc accaacatca tcaacc	336
3-30	**Sequences**		
3-30-1	Sequence Number [ID]	30	
3-30-2	Molecule Type	DNA	
3-30-3	Length	2466	
3-30-4	Features	**misc_feature 1..2466**	
	Location/Qualifiers	note=inducer	

		source 1..2466
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-30-5	Residues	atggccgaca ctagaagaag gcagaaccac tcttgtgacc catgccgtaa gggcaagaga 60 agatgtgatg ctccagagaa ccgtaacgag gctaatgaga acggatgggt gtcatgctct 120 aactgcaaga ggtggaacaa ggactgcacc ttcaactggc ttagctccca aaggtctaag 180 gctaagggtg ctgctccaag agctaggact aagaaggcta ggactgctac tactacctcc 240 gagccttcta cttccgctgc tactattcca actcccgagt ccgataatca cgatgctcca 300 ccagtgatca actcccacga tgctttgcca tcttggactc agggacttct ttctcaccct 360 ggcgatctct tcgacttctc ccattctgct attccagcta acgctgagga tgctgctaac 420 gtgcaatctg atgctccatt cccatgggat cttgctatcc caggcgattt ctctatggga 480 cagcaacttg agaagcccct ctccccattg tctttccagg ctgttcttct tccaccacac 540 tccccaaaca ctgatgatct cattcgtgag cttgaggaac agactaccga tccagattcc 600 gtgactgaca ctaactccgt tcagcaagtt gctcaggatg gctctctttg gtctgatagg 660 cagtctccac tcctcccaga aaacagtttg tgcatggctt ccgactctac cgctagaagg 720 tatgctaggt ccaccatgac caagaacctc atgaggatct accacgactc catggaaaac 780 gcccttтctt gctggcttac tgagcacaac tgcccatact ccgaccagat ttcttacctc 840 ccaccaaagc aaagggctga gtggggacca aattggtcta acaggatgtg cattagggtg 900 tgcaggctcg atagggtgtc aacttctctt agaggaaggg ctctctccgc tgaagaagat 960 aaggctgctg ctagggcact tcaccttgct attgtggctt tcgcttctca gtggactcaa 1020 catgctcaaa ggggagctgg acttaacgtc ccagctgata ttgctgctga cgagcgttct 1080 attaggcgta acgcttggaa tgaggctagg catgcacttc agcacactac tggaatccca 1140 tccttcaggg tgatcttcgc caacatcatc ttcagcctca ctcagtccgt gctcgatgat 1200 gatgagcaac atggaatggg agctaggctc gataagcttc tcgagaatga tggtgctcca 1260 gtgttcctcg agactgctaa taggcagctc tacaccttca ggcacaagtt cgctaggatg 1320 cagagaaggg gtaaggcttt caataggctt cctggtggat ccgtggcttc tactttcgct 1380 ggaattttcg agactcccac cccctcatct gagtctccac aacttgatcc agtggtggct 1440 tctgaggaac acaggtctac tctgtctctc atgttctggc tcgggatcat gttcgacact 1500 ctgtctgctg ctatgtacca gaggccactt gttgtgtccg atgaggactc ccagatctct 1560 tctgcttctc caccaagaag aggtgccgag actcctatta accttgattg ctgggagcca 1620 ccaaggcagg tcccatctaa tcaagagaag tctgatgtgt ggggcgacct gttccttagg 1680 acttctgatt ctttgcccga ccacgagtcc cacactcaaa tttctcaacc agctgctagg 1740 tggccatgca cttatgaaca agctgctgct gctctctcct ctgctactcc tgttaaggtg 1800 ttgctttaca ggcgtgtgac tcagctccag actttgttgt ataggggagc ttctccagct 1860 aggcttgagg ctgctattca gaggactctc tacgtgtaca accactggac tgctaagtac 1920 cagccattca tgcaggattg cgttgccaac catgagcttc tcccatccag gatccagtct 1980 tggtacgtga tccttgatgg acactggcac cttgctgcta tgcttttggc tgatgtgctc 2040 gagtccatcg acagggattc ctactccgat atcaaccaca tcgacctcgt gactaagctc 2100 aggcttgata acgctcttgc tgtgtctgct ctcgctaggt catctcttag aggccaagaa 2160 ctcgatccag gcaaggcttc tccaatgtac aggcacttcc acgactccct tactgaggtt 2220 gcattccttg ttgagccatg gactgtggtg ctcatccact catttgctaa ggctgcttac 2280 atcctcctcg attgccttga tcttgatggt cagggaaacg ctctcgctgg ataccttcaa 2340 cttaggcaga actgcaacta ctgcatcagg gctctccagt tccttggccg taagtctgat 2400 atggctgctc tcgtggctaa ggatcttgag aggggactca acggaaaggt cgacagcttc 2460 ctctaa 2466
3-31	**Sequences**	
3-31-1	Sequence Number [ID]	31
3-31-2	Molecule Type	AA
3-31-3	Length	208
3-31-4	Features	**source 1..208**
	Location/Qualifiers	mol_type=protein
		organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-31-5	Residues	MTSSVIVAGA GDKNNGIVVQ QQPPCVAREQ DQYMPIANVI RIMRKTLPSH AKISDDAKET 60 IQECVSEYIS FVTGEANERC QREQRKTITA EDILWAMSKL GFDNYVDPLT VFINRYREIE 120 TDRGSALRGE PPSLRQTYGG NGIGFHGPSH GLPPPGPYGY GMLDQSMVMG GGRYYQNGSS 180 GQDESSVGGG SSSSINGMPA FDHYGQYK 208
3-32	**Sequences**	
3-32-1	Sequence Number [ID]	32
3-32-2	Molecule Type	AA
3-32-3	Length	278
3-32-4	Features	**source 1..278**
	Location/Qualifiers	mol_type=protein
		organism=Zea mays
	NonEnglishQualifier Value	
3-32-5	Residues	MDSSSFLPAA GAENGSAAGG ANNGGAAQQH AAPAIREQDR LMPIANVIRI MRRVLPAHAK 60 ISDDAKETIQ ECVSEYISFI TGEANERCQR EQRKTITAED VLWAMSRLGF DDYVEPLGAY 120 LHRYREFEGD ARGVGLVPGA APSRGGDHHP HSMSPAAMLK SRGPVSGAAM LPHHHHHHDM 180 QMHAAMYGGT AVPPPAGPPH HGGFLMPHPQ GSSHYLPYAY EPTYGGEHAM AAYYGGAAYA 240 PGNGGSGDGS GSGGGGGSAS HTPQGSGGLE HPHPFAYK 278
3-33	**Sequences**	
3-33-1	Sequence Number [ID]	33

3-33-2	Molecule Type	AA
3-33-3	Length	234
3-33-4	Features	**source 1..234**
	Location/Qualifiers	mol_type=protein
		organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-33-5	Residues	MERGGFHGYR KLSVNNTTPS PPGLAANFLM AEGSMRPPEF NQPNKTSNGG EEECTVREQD 60 RFMPIANVIR IMRRILPAHA KISDDSKETI QECVSEYISF ITGEANERCQ REQRKTITAE 120 DVLWAMSKLG FDDYIEPLTL YLHRYRELEG ERGVSCSAGS VSMTNGLVVK RPNGTMTEYG 180 AYGPVPGIHM AQYHYRHQNG FVFSGNEPNS KMSGSSSGAS GARVEVFPTQ QHKY 234
3-34	**Sequences**	
3-34-1	Sequence Number [ID]	34
3-34-2	Molecule Type	AA
3-34-3	Length	312
3-34-4	Features	**source 1..312**
	Location/Qualifiers	mol_type=protein
		organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-34-5	Residues	MVDENVETKA STLVASVDHG FGSGSGHDHH GLSASVPLLG VNWKKRRMPR QRRSSSSFNL 60 LSFPPPMPPI SHVPTPLPAR KIDPRKLRFL FQKELKNSDV SSLRRMILPK KAAEAHLPAL 120 ECKEGIPIRM EDLDGFHVWT FKYRYWPNNN SRMYVLENTG DFVNAHGLQL GDFIMVYQDL 180 YSNNYVIQAR KASEEEEVDV INLEEDDVYT NLTRIENTVV NDLLLQDFNH HNNNNNNNSN 240 SNSNKCSYYY PVIDDVTTNT ESFVYDTTAL TSNDTPLDFL GGHTTTTNNY YSKFGTFDGL 300 GSVENISLDD FY 312
3-35	**Sequences**	
3-35-1	Sequence Number [ID]	35
3-35-2	Molecule Type	AA
3-35-3	Length	321
3-35-4	Features	**source 1..321**
	Location/Qualifiers	mol_type=protein
		organism=Brassica napus
	NonEnglishQualifier Value	
3-35-5	Residues	MMADENVETK ASTLIASVGH QGHGFGSGSG GHHGLSASVP LLGVNSKKRR MPRQRRSSSS 60 FNLLSLPPPM PLSPHVPTPL SARKIDPRKL RFLFQKELKN SDVSSLRRMI LPKKAAEAHL 120 PALECKEGIP IRMEDLDGLH VWTFKYRYWP NNNSRMYVLE NTGDFVNAHG LQLGDFIMVY 180 LDLDSNNYVI QARKASEEEE EEEDVTIIEE DDVYTNLTKI ENTVVNDLLI QDFNHHNDNS 240 SNNNSNNNIN NNKCSYYYPV IDDITTNTAS FVYDTTTLTS NDSPLDFLGG HTTTTTNTYY 300 SKFGSFEGLG SVENISLDDF Y 321
3-36	**Sequences**	
3-36-1	Sequence Number [ID]	36
3-36-2	Molecule Type	AA
3-36-3	Length	314
3-36-4	Features	**source 1..314**
	Location/Qualifiers	mol_type=protein
		organism=Medicago truncatula
	NonEnglishQualifier Value	
3-36-5	Residues	MMMDEGEGKK KVVVQKTEAC GFMAGVEDEL GFVNVKGDNN NGSGQRIHHD HGFVAAAFGT 60 VHRKKRMARQ RRSSSSTITI HLKNLPSSTT TTTTTTTSHV PISPIPPLFH SLPPAREIDH 120 RRLRFLFQKE LKNSDVSSLR RMVLPKKAAE AFLPVLESKE GILLSMDDLD GLHVWSFKYR 180 FWPNNNSRMY VLENTGDFVS THGLRFGDSI MVYQDNQNHN YVIQAKKACD QDEYMEEAND 240 TINHIFVDDY EVNKSCFDVA YPAMNDTSMS FIYDTTISND SPLDFLGGSM TNYSRIGSVE 300 TFGSVENLSL DDFY 314
3-37	**Sequences**	
3-37-1	Sequence Number [ID]	37
3-37-2	Molecule Type	DNA
3-37-3	Length	3275
3-37-4	Features	**source 1..3275**
	Location/Qualifiers	mol_type=genomic DNA
		organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-37-5	Residues	ggttggctat atggtccaaa ttttgatttg caatatgaga ttgcacagag agaacaatct 60 ttcattatga ttaattattg tacaagtaac aaacaccaat ctccgatata ctttggctct 120 ttagcacatt gttatgctag aagttagcgg aaatctatat gttgttaaac gcagcgttta 180 aattgaacag tgtaatttac cttgaaattt taagactaca tgctgtttag aatttcagat 240 gaaaacatct tgatgtttta gaaatccacg tgggaatagc gtaaaatctt atccaacgaa 300 cttattttgg ttttgttgta tttgtgcaag tcgtcacgct aatcgaaaaa agaaaagaaa 360 aaaagaagcc gtcatgatcg gccatttctc ggccgagtct gagtctgact ctgcgtccgt 420 gtcaccatta tcagatcgag cctgtcttat ctcgttgcga ttcctatgc aaaaatcttc 480 ttctttttt tattccccca tttatctctg atctcttctc tcttctcaag taaacctctc 540 tgcttcacgt ctcttctttt cttgtcgatt ttccccagat aatcagttga aaacacaccc 600 aaattcatct tcgaatcaat aatggatata agtaatgagg ctagtgtcga tccctttcg 660

		attggaccat catctatcat gggtcgaacc attgctttca gagtcttgtt ctgtagatca 720 atgtcacagc ttaggcgtga tctctttcgg ttcttgttgc attggtttct tagatttaag 780 ctgaccgttt caccgtttgt gtcgtggttt catcctcgga accctcaagg gattttagcg 840 gtggttacaa tcattgcctt tgtgttgaaa cgatacacga atgtgaaaat aaaggcggaa 900 atggcttacc ggaggaagtt ttggaggaat atgatgcgga cggctttgac ttatgaggaa 960 tgggctcatg ctgctaagat gttagagaag gaaacaccaa agatgaatga atctgatctt 1020 tatgatgaag agttggttaa gaacaagctt caggagcttc gtcatcgtcg ccaagaaggc 1080 tcacttagag acattatgtt ttgtatgaga gctgatttgg tgaggaatct cggtaatatg 1140 tgtaattcgg agcttcataa aggtagactt caggttccta gacatatcaa agagtacatt 1200 gatgaggtgt ctactcagtt gagaatggtt tgtaactctg attcagagga gctttcttta 1260 gaagagaagc tttcttttat gcatgaaaca cggcatgcct ttggtagaac ggctttgctt 1320 ttgagtggtg gggcttctct tggtgcgttt catgttggtg tggttaggac tttggttgag 1380 cataagcttt tacctcgaat aattgctggt tctagtgttg gatccatcat ttgtgctgtt 1440 gtggcctcaa ggtcttggcc agaactacag agtttctttg agaattcttt gcattcttta 1500 cagttctttg atcagctcgg aggcgtgttc tcaatagtga aacgggtaat gacacaaggg 1560 gctctacacg atatcagaca gttgcaatgt atgcttagaa acctcacaag caatctcaca 1620 ttccaagaag cttatgacat gacaggaagg attctcggga tcaccgtttg ctccccaaga 1680 aagcatgaac ctcctcggtg tcttaactat ttgacttcgc ctcatgtggt tatatggagc 1740 gcagtgactg cttcttgtgc ttttcctggt ctctttgaag ctcaagagct aatggctaaa 1800 gatcgaagtg gagagatcgt accgtatcat ccacctttca atttggatcc agaagtaggc 1860 actaaatcat catctggacg ccggtggaga gatggtagtt tggaggttga tttaccaatg 1920 atgcagctta aagaactgtt caatgtcaat cattttattg tgagccaagc caatcctcac 1980 attgctccat tactgcgtct aaaggattta gttcgagctt atggtggtag attcgcagct 2040 aagctcgcgc atctagtgga gatggaggtc aaacatagat gcaaccaggt attagagctc 2100 ggttttcctc tcggtggact cgcaaagctt tttgctcagg agtgggaagg tgatgttaca 2160 gttgtaatgc ctgctactct tgctcagtac tcgaagatta tacaaaatcc gactcatgtc 2220 gagcttcaga aagcggctaa ccaaggaaga agatgcactt gggagaagct ctcagccata 2280 aaatcaaact gcgggatcga gcttgcgctt gatgattctg tagctattct taaccatatg 2340 cggaggctca agaaaagtgc ggagagagcc gccactgcca cgtcttcgtc tcatcacgga 2400 ttggcttcaa ccaccagatt caatgcttca agaagaatcc catcttggaa cgtccttgcc 2460 agagagaact caacaggctc actggatgat ctagtcactg acaataacct ccacgcttct 2520 tcgggcagga atttaagcga cagtgaaaca gagagcgtgg agttgagttc ttggacaaga 2580 actggtggac ctttaatgag aacagcttct gctaataagt tcattgattt tgttcagagt 2640 cttgatatcg acattgcatt ggtcagagga tttagtagca gtcccaattc tccagcagtt 2700 cctcctggtg gctcgtttac tccaagcccg agatccatag cggctcattc ggatatcgaa 2760 tcaaacagca atagcaacaa tcttggaaca agcacttcaa gcataacagt tactgaaggt 2820 gatcttctac agcctgagag aacgagtaac ggatttgtgt taaacgtcgt taaaagagag 2880 aacttgggaa tgccatcgat tgggaaccaa aatacagagt taccagagag tgtacagctc 2940 gatataccgg agaaggagat ggattgtagc tctgtatcag aacacgaaga agatgataac 3000 gacaatgaag aagaacataa cggctcgagt ctggttactg tttcttcaga agattccggt 3060 ttacaagaac cggtgtctgg tagtgttata gatgcttaga gtgtgattga ttcaagtgag 3120 tatagattct taattaaatt tgcagagttt ccaaagggtt tagtgcacca cttgtgtatg 3180 tttgtattgc ttattgtttg aaattcattt gtgaaatcga aatatatctg taaattcaga 3240 aaatattctc tcatccatta caaaatattt gagtc 3275
3-38	**Sequences**	
3-38-1	Sequence Number [ID]	38
3-38-2	Molecule Type	DNA
3-38-3	Length	2724
3-38-4	Features	**source 1..2724**
	Location/Qualifiers	mol_type=genomic DNA organism=Sorghum bicolor
	NonEnglishQualifier Value	
3-38-5	Residues	atggatgaca tcgccagcga ggcgccggtg ggggcgttcg ccatcggccc gtccacggcg 60 ctgggccgcg ccgtcgcgct ccgggtgctg ctctgcggct ccgcggcgcg cctgcggcac 120 cgcctggccg cggcgctccg cgccgcgctg cccgtcgcgg cggcgtggct gcacccgcgc 180 gacaacacgc gcgggatcct gctcgccgtc tgcgccgtcg cgctcctgct gcggggccga 240 cgcggcaggg ccgggctgcg ggcgagggtg cagtccgcct accgccgcaa gttctggcgg 300 aacatgatgc gcgccgcgct cacctacgag gagtgggcgc acgcggcgcg gatgctggag 360 cgcgaggccg ccccgcgccg cgccagcgac gccgacctct acgacgagga gctcgtccgc 420 aataagctcc gcgagctcag gcaccggcgc cacgagggat cgctcaggga catcgtcttc 480 tgcatgcgcg cggacctgct caggaacctc ggcaatatgt gcaaccccga actgcacaaa 540 gggaggctgc aggtgcctag actcataaag gaatacattg aggaagtatc tactcaactg 600 aaaatggtct gtgattctga ttcagatgag ttgcctcttg aagagaaact cgcatttatg 660 catgagacaa ggcatgcctt tggtagaaca gccttgctgc taagtggagg tgcttcattg 720 ggatcctttc atgtgggtgt tgttaaaacc ttggtagagc ataaactttt gccaaggata 780 atttcaggat caagtgttgg ctcgataatg tgttctatag tagcaacaag atcatggcct 840 gagctggaga gctttttga agagtggcat tccctgaaat tttttgatca gatgggtgga 900 atctttcctg tggttaaaag aattttgacg caaggcgctg ttcatgatat aaggcacttg 960 caggtgcttt tgagaaacct taccagcaat ttgacatttc aagaagctta tgacatgact 1020 ggtcggattc ttgttgtcac cgtgtgttct ccaaggaagc atgagccgcc tcgatgccta 1080 aactatttaa catcacctca tgttcttatc tggagtgcag taacagcttc ctgtgctttt 1140 cctggacttt ttgaggccca agaattgatg gcaaaagata gatttggtca aaccattcct 1200 ttccatgctc cattcttatt aggcatagaa gaacgaactg ttgctccaac ccgccgctgg 1260 agagatggga gcttagaaag cgatttaccc atgaagcaat tgaaggaact attcaatgtg 1320 aatcatttca tagtaagcca agccaatcct cacatagctc cgctgttgag actaaaggaa 1380

		atcgtcaggg cttatggagg cagcttcgct gccaagcttg ctgaacttgc tgagatggaa 1440 gtcaaacata ggtgtaatca agttttggaa cttggatttc ctctaggagg attagctaaa 1500 ttatttgctc aagattggga aggcgatgtt acagttgtta tgccagccac tcttgcgcag 1560 tattccaaga tgatacagaa cccatcttat gctgagcttc agaaggctgc gaatcaaggt 1620 aggagatgca cttgggaaaa gctatcagcc atcagggcaa attgtgctat tgagcttgca 1680 ctggatgaat gtgttgccct cctgaaccac ttgcgtaggc taaagaggag tgcagaaaga 1740 gcatccgcat cgcaaggata tggtccagca atcaggttct gcccatctag gaggattcca 1800 tcctggaatc tcatagcaag agaaaattca actggttctc ttgaagaaga aatgcttaca 1860 tctcctcaag gacctggagg agttgctgga acatctacca gaaaccagta tcctcagaga 1920 agtgcacatg agagcagcga cagtgaatct gagagtattg atttacactc ttggacaaga 1980 agtggtggcc ctcttatgag gacaacctca gccaataaat tcatcagctt tgttcagaat 2040 cttgagatcg acacagaatc cagaacaatt ccatcgaggg aagacataac tgatcttgtg 2100 acaccaaatg ctggtacctt ggcagctcat gcagtgagta gagaagcaat cgataggagc 2160 ttggacaatt cagctttaga tatccatgat accagtaccc ctagatcgac atttggccct 2220 tcaacaagta ttgtggtttc tgaaggtgac ttgttgcagc ctgaaaagat tgaaaatggt 2280 attttgttta atgttgtaag gagggatact ctgctcgggt ctagtagtgg agttgagtct 2340 caaggatctc ctcgggaacc agatgttgaa acagtacaga cggagtgcct tgatggcgtg 2400 tctacttctg atgatgatga tgacaaggaa ctaaatgcca ttgatgatgg aggaactagt 2460 cccatgagca gaaataatct acaacatcag gggtcctcac tggaagaaaa attataccat 2520 ccctcttcct taaattctga agacgagaca aacacaaaca aaccagaagc tgcatcgatt 2580 tttgatatat gtacagatat gcatccggca tctattagcc tacctgaagg gtcttcagaa 2640 aagacagaac tggaaacaac aaagattcct gatgacaatt cagctgttat gaatgatgaa 2700 gttgcctcag gtgctggtaa ctaa 2724
3-39	**Sequences**	
3-39-1	Sequence Number [ID]	39
3-39-2	Molecule Type	DNA
3-39-3	Length	3470
3-39-4	Features	**source 1..3470**
	Location/Qualifiers	mol_type=genomic DNA
		organism=Nicotiana benthamiana
	NonEnglishQualifier Value	
3-39-5	Residues	gttatctgat ccaaacttct gacttttct attttccgaa tccctatgtt ttttaataaa 60 tccatctctg ccattgcagt gatatattca tttattgtta tcaccttctt catttattgg 120 tccctctgtg ttttccatat attgaaggag aaaacattaa ctttatgcga ttttgtagtt 180 tttctggttg attcctacaa ccccttttga cattgatctt gtgggttaca aaaaacattg 240 aatctttatg tcaaaatttg atctttgtat ttcattttaa attgaaattt gatttttggg 300 ggtattaagg attcttttgt cggttgattt tgtgcctttt ttgccaagtt cttgtcggtc 360 tctgagctga atttccataa tttgacaaaa agaaaaggct aaagcagaaa ggttgggagt 420 ttctttcttt gactttcaga aactaaggta ttttctttga tctaattctt gttaatatct 480 ggttcaatct gattccgttg aatcttgtga atagcctttg tttccctatt gtcagaaaat 540 tatttccttt tcactttcct cgactctcag aagttagtac aatctttgtt ctgctaaatc 600 ttgtgaataa cctttagctt agagttttag gtatctgtat attgggttct cttaacattt 660 agcctagaag ccttctctag gattagtccc ccttttcatt gagatggata taagtaatga 720 ggctacaatt gacttctttt ccattggacc tactacgata ttgggtcgaa caatcgcctt 780 tagagtgttg ttctgtaaat caatttcaca attgaagcat cacctatttc atttcttgat 840 atattacttg tacaaattca agaatggttt gtcatactac ttgacaccct tgatctcgtg 900 gttgcaccct cgtaatccac aaggaatatt ggcattggta acgcttctcg ccttcttgtt 960 gaggcgatac acgaatgtaa aaatcaaggc tgagatggcc tataggagga agttttggag 1020 gaatatgatg agatctgcat tgacttatga ggagtgggct catgctgcca agatgctaga 1080 taaagagacc cctaaaatga atgaggcaga tctttatgat gtagaattag ttcgaaataa 1140 actccaagag cttcgacatc gtaggcaaga gggttctatg agggatatca tattctgtat 1200 gagagctgac cttgttagga atcttggtaa tatgtgtaat ccagaacttc acaagggaag 1260 gcttcatgtg cctagactga ttaaggatta tattgatgag gtttcaactc agttgagaat 1320 ggtatgcgac tctgattcgg aggagcttct cttggaagag aagcttgctt tcatgcatga 1380 aacaagacat gcctttggta ggacagcttt gcttttaagt ggaggtgctt ctttaggagc 1440 tttccatgtg ggcgtggtga aaacacttgt agaacacaaa ctgatgccac ggataattgc 1500 tggttcaagt gtcggctcga ttatgtgctc catagttgca actcgatctt ggcctgagct 1560 ccagagtttt ttcgaggact cctggcactc tttgcaattt ttcgatcagt tgggtgggat 1620 ttttactatt ttcaggaggg tcatgaccca gggtgctgta catgagatca gacagctgca 1680 ggtgctgtta cgtaatctca cgaataatct tactttccaa gaagcctatg acatgactgg 1740 tagagttctg gggattactg tttgctcgcc taggaaacat gaacctccta gatgcttgaa 1800 ctacttgact tcacctcatg ttgttatatg gagtgccgtt accgcttctt gtgcctttcc 1860 tggtctcttc gaagctcaag aacttatggc aaaggataga agtggagatc ttgttccata 1920 tcacccacca tttcatttgg gtcctgatgc cacttctagt gcatctgctc gtcgttggag 1980 ggatggtagc ttggaggttg atttgccaat gatgcagcta aaggagctct tcaatgtcaa 2040 tcactttatt gtgagccagg cgaatccgca tattgctcca ctgctgagga tcaaagagtt 2100 tgtaagagct tatggaggca actttgctgc caagcttgct caacttacgg aaatggaggt 2160 gaagcacaga tgcaatcagg tattagaact tggttttccc ttgggaggat tagcaaagct 2220 ttttgctcaa gaatgggagg gtgatgtaac tgttgtaatg cctgccactc tagctcagta 2280 ctcaaaaatc atacagaatc cctcgactct ggagctgcaa aaagcagcaa atcaaggaag 2340 aaggtgcact tgggaaaaac tctcagccat gaaagcaaac tgtggaattg agcttgcact 2400 tgatgaatgc gttgctatac tgaatcacat gcgtagactg aaaaggagtg ctgagagggc 2460 ggctgctgct tcacatggct tggcaagcac tgtcagattt aacacttcca gaagaattcc 2520 ttcttggaac tgcattgcac gagagaactc aacaggctcc cttgaagatt ttcttgcgga 2580 tgttgctgct tcacatcatc aaggaggcag tggttcgggg gcgcatgtta accgtagttg 2640

		gcgaacgcac cggaatgcac atgatggtag tgacagtgag ccggaaaatg tggaccttaa 2700 ttcttggaca agatcgggtg gtcctttgat gaggacaaca tcagctgata agtttattga 2760 ctttgtccag aacttggaaa ttggttcgcg attgaacaaa ggattgacta ttgacctcaa 2820 caatattatt cctcagatgg caagcaggga ccatttctcc ccgagcccaa gggtaacaac 2880 acctgataga agttcagata cagaatttga tcaaagagat tttagttaca gggtccctgc 2940 gagtagttca agcattatgg taggcgaagg tgaccttctg cagcctgaaa ggactaacag 3000 cggtattgtc ttcaatgtgg taaggaaagg agacttgacc ccatcgaaca gaagccttga 3060 ttcagaaaat aatagttccg tgcaggatgc agttgctgag tgcgtgcaac ttgaaagtcc 3120 agaaaaggag atggatatta gctcagtatc ggaggatggt gagaatgatg ttgggcaagg 3180 aagtagggta aatgaagttg attgtagtaa aaatcgttca tcaatcggtg atggcaacga 3240 taagcaagtt attgatactt gagagtttag ctttgattat tctacacagg ccattcgaat 3300 tatttttat actcaaatgg agcttctttc agagctaaca cactcagaat tggggttgta 3360 aatagtgcaa gtagcaaatc tgtaataaat gtttagtgta gtcatcaccc ttctactagt 3420 tcaaagtggc tcagttcaat tcaaattcag aacttcgata attcatgttt 3470
3-40	**Sequences**	
3-40-1	Sequence Number [ID]	40
3-40-2	Molecule Type	DNA
3-40-3	Length	713
3-40-4	Features	**source 1..713**
	Location/Qualifiers	mol_type=genomic DNA organism=Nicotiana benthamiana
	NonEnglishQualifier Value	
3-40-5	Residues	tgtatgagag ctgaccttgt taggaatctt ggtaatatgt gtaatccaga acttcacaag 60 ggaaggcttc atgtgcctag actgattaag gattatattg atgaggtttc aactcagttg 120 agaatggtat gcgactctga ttcggaggag cttctcttgg aagagaagct tgctttcatg 180 catgaaacaa gacatgcctt tggtaggaca gctttgcttt taagtggagg tgcttcttta 240 ggagctttcc atgtgggcgt ggtgaaaaca cttgtagaac acaaactgat gccacggata 300 attgctggtt caagtgtcgg ctcgattatg tgctccatag ttgcaactcg atcttggcct 360 gagctccaga gtttttcga ggactcctgg cactctttgc aatttttcga tcagttgggt 420 gggattttta ctattttcag gagggtcatg acccagggtg ctgtacatga gatcagacag 480 ctgcaggtgc tgttacgtaa tctcacgaat aatcttactt tccaagaagc ctatgacatg 540 actggtagag ttctggggat tactgtttgc tcgcctagga aacatgaacc tcctagatgc 600 ttgaactact tgacttcacc tcatgttgtt atatggagtg ccgttaccgc ttcttgtgcc 660 tttcctggtc tcttcgaagc tcaagaactt atggcaaagg atagaagtgg aga 713
3-41	**Sequences**	
3-41-1	Sequence Number [ID]	41
3-41-2	Molecule Type	DNA
3-41-3	Length	1500
3-41-4	Features	**source 1..1500**
	Location/Qualifiers	mol_type=genomic DNA organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-41-5	Residues	cgaaaaaaga agtagaatat atatatatat atatatatat atatatatat atatatattc 60 gtgtggacat cataaatgcc taaatgataa tagttgattt cgagttttat tttcgttact 120 tccaatcaaa ttctccttgc accatattta tttttttact gtgagaacat atataagtat 180 atattggaat tacgtatccg agaggttttt gcatatttcg tttatttatt ttcgatatcc 240 acactactgt attattaaaa atttgaaaaa ttcaactagg gcttttcatc ttctctagaa 300 ttattcgttt atttatgtcg atgtccacac tattattaaa ataaaacgag aggatatggt 360 tggatcatcc aagtttcgtt tatgactctt tgttcattta caaacgttta gttttccact 420 taagttttga aaagagttaa tttccaatat attcggcaca gtttttcaag tgtattcatc 480 tgttttttt ttttttggtt ggctatatgg tccaaatttt gatttgcaat atgagattgc 540 acagagagaa caatctttca ttatgattaa ttattgtaca agtaacaaac accaatctcc 600 gatatacttt ggctctttag cacattgtta tgctagaagt tagcggaaat ctatatgttg 660 ttaaacgcag cgtttaaatt gaacagtgta atttaccttg aaattttaag actacatgct 720 gtttagaatt tcagatgaaa acatcttgat gttttagaaa tccacgtggg aatagcgtaa 780 aatcttatcc aacgaactta ttttggtttt gttgtatttg tgcaagtcgt cacgctaatc 840 gaaaaaagaa aagaaaaaaa gaagccgtca tgatcggcca tttctcggcc gagtctgagt 900 ctgactctgc gtccgtgtca ccattatcag atcgagcctg tcttatctcg ttgcgattcc 960 ctatgcaaaa atcttcttct tttttttatt cccccattta tctctgatct cttctctctt 1020 ctcaagtaaa cctctctgct tcacgtctct tcttttcttg tcgattttcc ccagataatc 1080 aggtaaataa ggctactttc ttatttgatc tggtggtctt tgtgttgaaa tctctgggtt 1140 ttctctgttg atttcaaagt tctctctttt ttttttgttt tactgggtgc tgtgaaaaat 1200 gatcttgtca aagtctcctc ttttcatcga attgaaactc taattagaaa aaagatcata 1260 acttttatta aaaaaatgag tttgctttgc ttaattttgc gaattgcttc atagattcat 1320 tgattagcct atttggggta acaaaaaaaa gctgacacgg tttcagattc caaaaataga 1380 tcatgactct gtttcttctc tgcagaggtt ttaataaata tatgcttctt ctcatgagtt 1440 ctcgtttttt ttgtcacctt cgcagttgaa aacacaccca aattcatctt cgaatcaata 1500
3-42	**Sequences**	
3-42-1	Sequence Number [ID]	42
3-42-2	Molecule Type	DNA
3-42-3	Length	2871
3-42-4	Features	**misc_feature 1..2871**
	Location/Qualifiers	note=Nucleotide sequence of the complement of the pSSU-Oleosin gene i n the T-DNA of

		pJP3502. In order (complementary sequences): Glycine max Lectin terminator 348nt, 3' exon 255nt, UBQ10 intron 304nt, 5' e xon 213nt, SSU promoter
		source 1..2871
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-42-5	Residues	ggcccctaga atctaattat tctattcaga ctaaattagt ataagtattt ttttaatcaa 60 taaataataa ttaataattt attagtagga gtgattgaat ttataatata ttttttttaa 120 tcatttaaag aatcttatat ctttaaattg acaagagttt taaatgggga gagtgttatc 180 atatcacaag taggattaat gtgttatagt ttcacatgca ttacgataag ttgtgaaaga 240 taacattatt atatataaca atgacaatca ctagcgatcg agtagtgaga gtcgtcttat 300 tacactttct tccttcgatc tgtcacatgg cggcggcccg cggccgcttc attactcgag 360 ccaggaggat ggatcgatgc tggtctgaga ccctgctacc ggttgctgac tgaactgctc 420 ggcacggtcc ttcatttcac gggccttgct cgccaacttt gtcttggccg actccaactg 480 atccgctccg ggtggatgtt tccccgtcag gtaacggtag atccaggaca gcacagacag 540 agcggcaaca ccaaatcccc cgcttgccag aaaacccgct cccaacagga agatggtgat 600 gactgcagat cagaaaaact cagattaatc gacaaattcg atcgcacaaa ctagaaacta 660 acaccagatc tagatagaaa tcacaaatcg aagagtaatt attcgacaaa actcaaatta 720 tttgaacaaa tcggatgata tctatgaaac cctaatcgag aattaagatg atatctaacg 780 atcaaaccca gaaaatcgtc ttcgatctaa gattaacaga atctaaacca aagaacatat 840 acgaaattgg gatcgaacga aaacaaaatc gaagattttg agagaataag gaacacagaa 900 atttacctgc agggaccagt acaggcgaga agatcaccag gagaggtgtg gcgattgtca 960 gcgcaatgac cgttccagcc agggtcaacc cggataacac caacaggcta cctccggcag 1020 taaccgcggt cgctgccttt acaacacgct gagcacgcgg ttgcagttgc aagtgggggg 1080 cacgtgtttg ttgctgctgc ccgtagtgct ctgccatggt ttttttaac ggagcaagcg 1140 gccgctgttc ttctttactc tttgtgtgac tgaggtttgg tctagtgctt tggtcatcta 1200 tatataatga taacaacaat gagaacaagc tttggagtga tcggagggtc taggatacat 1260 gagattcaag tggactagga tctacaccgt tggattttga gtgtggatat gtgtgaggtt 1320 aattttactt ggtaacggcc acaaaggcct aaggagaggt gttgagaccc ttatcggctt 1380 gaaccgctgg aataatgcca cgtggaagat aattccatga atcttatcgt tatctatgag 1440 tgaaattgtg tgatggtgga gtggtgcttg ctcattttac ttgcctggtg gacttggccc 1500 tttccttatg gggaatttat attttactta ctatagagct ttcatacctt ttttttacct 1560 tggatttagt taatatataa tggtatgatt catgaataaa aatgggaaat ttttgaattt 1620 gtactgctaa atgcataaga ttaggtgaaa ctgtggaata tatatttttt tcatttaaaa 1680 gcaaaatttg ccttttacta gaattataaa tatagaaaaa tatataacat tcaaataaaa 1740 atgaaaataa gaactttcaa aaaacagaac tatgtttaat gtgtaaagat tagtcgcaca 1800 tcaagtcatc tgttacaata tgttacaaca agtcataagc ccaacaaagt tagcacgtct 1860 aaataaacta aagagtccac gaaaatatta caaatcataa gcccaacaaa gttattgatc 1920 aaaaaaaaaa aacgcccaac aaagctaaac aaagtccaaa aaaaacttct caagtctcca 1980 tcttccttta tgaacattga aaactataca caaaacaagt cagataaatc tctttctggg 2040 cctgtcttcc caacctccta catcacttcc ctatcggatt gaatgtttta cttgtacctt 2100 ttccgttgca atgatattga tagtatgttt gtgaaaacta atagggttaa caatcgaagt 2160 catggaatat ggatttggtc caagattttc cgagagcttt ctagtagaaa gcccatcacc 2220 agaaatttac tagtaaaata aatcaccaat taggtttctt attatgtgcc aaattcaata 2280 taattataga ggatatttca aatgaaaacg tatgaatgtt attagtaaat ggtcaggtaa 2340 gacattaaaa aaatcctacg tcagatattc aactttaaaa attcgatcag tgtggaattg 2400 tacaaaaatt tgggatctac tatatatata taatgcttta caacacttgg attttttttt 2460 ggaggctgga atttttaatc tacatatttg ttttggccat gcaccaactc attgtttagt 2520 gtaatacttt gattttgtca aatatatgtg ttcgtgtata tttgtataag aatttctttg 2580 accatataca cacacacata tatatatata tatatatatt atatatcatg cacttttaat 2640 tgaaaaaata atatatatat atatagtgca ttttttctaa caaccatata tgttgcgatt 2700 gatctgcaaa aatactgcta gagtaatgaa aaatataatc tattgctgaa attatctcag 2760 atgttaagat tttcttaaag taaattcttt caaattttag ctaaaagtct tgtaataact 2820 aaagaataat acacaatctc gaccacggaa aaaaaacaca taataaattt g 2871
3-43	**Sequences**	
3-43-1	Sequence Number [ID]	43
3-43-2	Molecule Type	AA
3-43-3	Length	362
3-43-4	Features	**source 1..362**
	Location/Qualifiers	mol_type=protein
		organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-43-5	Residues	MLKLSCNVTD SKLQRSLLFF SHSYRSDPVN FIRRRIVSCS QTKKTGLVPL RAVVSADQGS 60 VVQGLATLAD QLRLGSLTED GLSYKEKFVV RSYEVGSNKT ATVETIANLL QEVGCNHAQS 120 VGFSTDGFAT TTTMRKLHLI WVTARMHIEI YKYPAWGDVV EIETWCQSEG RIGTRRDWIL 180 KDSVTGEVTG RATSKWVMMN QDTRRLQKVS DDVRDEYLVF CPQEPRLAFP EENNRSLKKI 240 PKLEDPAQYS MIGLKPRRAD LDMNQHVNNV TYIGWVLESI PQEIVDTHEL QVITLDYRRE 300 CQQDDVVDSL TTTTSEIGGT NGSATSGTQG HNDSQFLHLL RLSGDGQEIN RGTTLWRKKP 360 SS 362
3-44	**Sequences**	
3-44-1	Sequence Number [ID]	44
3-44-2	Molecule Type	AA
3-44-3	Length	367
3-44-4	Features	**source 1..367**

	Location/Qualifiers	mol_type=protein
		organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-44-5	Residues	MLKLSCNVTD HIHNLFSNSR RIFVPVHRQT RPISCFQLKK EPLRAILSAD HGNSSVRVAD 60 TVSGTSPADR LRFGRLMEDG FSYKEKFIVR SYEVGINKTA TIETIANLLQ EVACNHVQNV 120 GFSTDGFATT LTMRKLHLIW VTARMHIEIY KYPAWSDVVE IETWCQSEGR IGTRRDWILK 180 DCATGEVIGR ATSKWVMMNQ DTRRLQRVTD EVRDEYLVFC PPEPRLAFPE ENNSSLKKIP 240 KLEDPAQYSM LGLKPRRADL DMNQHVNNVT YIGWVLESIP QEIIDTHELK VITLDYRREC 300 QQDDIVDSLT TSETPNEVVS KLTGTNGSTT SSKREHNESH FLHILRLSEN GQEINRGRTQ 360 WRKKSSR 367
3-45	**Sequences**	
3-45-1	Sequence Number [ID]	45
3-45-2	Molecule Type	AA
3-45-3	Length	412
3-45-4	Features	**source 1..412**
	Location/Qualifiers	mol_type=protein
		organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-45-5	Residues	MVATSATSSF FPVPSSSLDP NGKGNKIGST NLAGLNSAPN SGRMKVKPNA QAPPKINGKK 60 VGLPGSVDIV RTDTETSSHP APRTFINQLP DWSMLLAAIT TIFLAAEKQW MMLDWKPRRS 120 DMLVDPFGIG RIVQDGLVFR QNFSIRSYEI GADRSASIET VMNHLQETAL NHVKTAGLLG 180 DGFGSTPEMF KKNLIWVVTR MQVVVDKYPT WGDVVEVDTW VSQSGKNGMR RDWLVRDCNT 240 GETLTRASSV WVMMNKLTRR LSKIPEEVRG EIEPYFVNSD PVLAEDSRKL TKIDDKTADY 300 VRSGLTPRWS DLDVNQHVNN VKYIGWILES APVGIMERQK LKSMTLEYRR ECGRDSVLQS 360 LTAVTGCDIG NLATAGDVEC QHLLRLQDGA EVVRGRTEWS SKTPTTTWGT AP 412
3-46	**Sequences**	
3-46-1	Sequence Number [ID]	46
3-46-2	Molecule Type	AA
3-46-3	Length	345
3-46-4	Features	**source 1..345**
	Location/Qualifiers	mol_type=protein
		organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-46-5	Residues	MFIAVEVSPV MEDITRQSKK TSVENETGDD QSATSVVLKA KRKRRSQPRD APPQRSSVHR 60 GVTRHRWTGR YEAHLWDKNS WNETQTKKGR QVYLGAYDEE DAAARAYDLA ALKYWGRDTI 120 LNFPLCNYEE DIKEMESQSK EEYIGSLRRK SSGFSRGVSK YRGVAKHHHN GRWEARIGRV 180 FGNKYLYLGT YATQEEAAIA YDIAAIEYRG LNAVTNFDIS RYLKLPVPEN PIDTANNLLE 240 SPHSDLSPFI KPNHESDLSQ SQSSSEDNDD RKTKLLKSSP LVAEEVIGPS TPPEIAPPRR 300 SFPEDIQTYF GCQNSGKLTA EEDDVIFGDL DSFLTPDFYS ELNDC 345
3-47	**Sequences**	
3-47-1	Sequence Number [ID]	47
3-47-2	Molecule Type	AA
3-47-3	Length	303
3-47-4	Features	**source 1..303**
	Location/Qualifiers	mol_type=protein
		organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-47-5	Residues	MAKVSGRSKK TIVDDEISDK TASASESASI ALTSKRKRKS PPRNAPLQRS SPYRGVTRHR 60 WTGRYEAHLW DKNSWNDTQT KKGRQVYLGA YDEEEAAARA YDLAALKYWG RDTLLNFPLP 120 SYDEDVKEME GQSKEEYIGS LRRKSSGFSR GVSKYRGVAR HHHNGRWEAR IGRVFATQEE 180 AAIAYDIAAI EYRGLNAVTN FDVSRYLNPN AAADKADSDS KPIRSPSREP ESSDDNKSPK 240 SEEVIEPSTS PEVIPTRRSF PDDIQTYFGC QDSGKLATEE DVIFDCFNSY INPGFYNEFD 300 YGP 303
3-48	**Sequences**	
3-48-1	Sequence Number [ID]	48
3-48-2	Molecule Type	AA
3-48-3	Length	445
3-48-4	Features	**source 1..445**
	Location/Qualifiers	mol_type=protein
		organism=Avena sativa
	NonEnglishQualifier Value	
3-48-5	Residues	MKRSPPPAPP AAPPPPQPSP SSSSPACSPS PSSSSCPSSS DSSSIVIPRK RARTQKAASG 60 KPKAKASAKR PKKDASRSSK ETDANGAAAA AGKRSSIYRG VTRHRWTGRF EAHLWDKNCF 120 TSVQNKKKGR QVYLGAYDTE DAAARAYDLA ALKYWGSETI LNFSVEDYAK EMPEMEAVSR 180 EEYLAALRRR SSGFSRGVSK YRGVARHHHN GRWEARIGRV LGNKYLYLGT FDTQEEAAKA 240 YDLAAIEYRG ANAVTNFDIS CYLDQPQLLA QLQQGPQVVP ALQEELQHDV QHDLQNDNAV 300 QELNSGEVQM PGAMDEPIAL DDSTECINTP FEFDFSVEEN LWSPCMDYEL DAILGNNTSN 360 SANMNEWFND STFESNIGCL FEGCSNIDDC SSSKHCADLA AFDFFKEGDD NDFSNMEMEI 420 TPQANDVSCP PNDVSCPPKM ITVCN 445
3-49	**Sequences**	
3-49-1	Sequence Number [ID]	49

3-49-2	Molecule Type	AA
3-49-3	Length	420
3-49-4	Features	**source 1..420**
	Location/Qualifiers	mol_type=protein
		organism=Sorghum bicolor
	NonEnglishQualifier Value	
3-49-5	Residues	MDMERSQQQK SPTESPPPPS PSSSSSSVSA DTVLPPPGKR RRAATTAKAK AGAKPKRARK 60 DAAAAADPPP PPAAAAAGKR SSVYRGVTRH RWTGRFEAHL WDKHCLAALH NKKKGRQVYL 120 GAYDSEEAAA RAYDLAALKY WGPETLLNFP VEDYSSEMPE MEGVSREEYL ASLRRRSSGF 180 SRGVSKYRGV ARHHHNGRWE ARIGRVFGNK YLYLGTFDTQ EEAAKAYDLA AIEYRGVNAV 240 TNFDISCYLD HPLFLAQLQQ EPQVVPALNQ EAQPDQSETE TIAQESVSSE AKTPDDNAEP 300 DDNAEPDDIA EPLITVDDSI EESLWSPCMD YELDTMSRSN FGSSINLSEW FNDADFDSNI 360 GCLFDGCSAV DEGGKDGVGL ADFSLLEDFS LFEAGDGQLK DVLSDMEEGI QPPTMISVCN 420
3-50	**Sequences**	
3-50-1	Sequence Number [ID]	50
3-50-2	Molecule Type	AA
3-50-3	Length	395
3-50-4	Features	**source 1..395**
	Location/Qualifiers	mol_type=protein
		organism=Zea mays
	NonEnglishQualifier Value	
3-50-5	Residues	MERSQRQSPP PPSPSSSSSS VSADTVLVPP GKRRRAATAK AGAEPNKRIR KDPAAAAAGK 60 RSSVYRGVTR HRWTGRFEAH LWDKHCLAAL HNKKKGRQVY LGAYDSEEAA ARAYDLAALK 120 YWGPETLLNF PVEDYSSEMP EMEAVSREEY LASLRRRSSG FSRGVSKYRG VARHHHNGRW 180 EARIGRVFGN KYLYLGTFDT QEEAAKAYDL AAIEYRGVNA VTNFDISCYL DHPLFLAQLQ 240 QEPQVVPALN QEPQPDQSET GTTEQEPESS EAKTPDGSAE PDENAVPDDT AEPLTTVDDS 300 IEEGLWSPCM DYELDTMSRP NFGSSINLSE WFADADFDCN IGCLFDGCSA ADEGSKDGVG 360 LADFSLFEAG DVQLKDVLSD MEEGIQPPAM ISVCN 395
3-51	**Sequences**	
3-51-1	Sequence Number [ID]	51
3-51-2	Molecule Type	AA
3-51-3	Length	430
3-51-4	Features	**source 1..430**
	Location/Qualifiers	mol_type=protein
		organism=Triadica sebifera
	NonEnglishQualifier Value	
3-51-5	Residues	MASSSSDPVL KAELGSSGGG CSSGGGGESS EAVIANDQLL LYRGLKKPKK ERGCTAKERI 60 SKMPPCTAGK RSSIYRGVTR HRWTGRYEAH LWDKSTWNQN QNKKGKQVYL GAYDDEEAAA 120 RAYDLAALKY WGPGTLINFP VTDYTRDLEE MQNMSREEYL ASLRRKSSGF SRGISKYRGL 180 SSRWESSVGR MPGSEYFSSI NYVDDPAAES EYVGSLCFER KIDLTSYIKW WGLNKTRQAE 240 SISKSAEETK PGCAEDIGGE LKTTEWAIQP TEPYQMPRLG MPVHVKKHKG SKISALSVLS 300 QSAAFKSLQE KASKKQENST DNDENENKNT NTNKIDYGKA VETSASHDSS NERPVTALGM 360 SGGLSLKRNV YQLTPFLSAP LLTNYGTIDQ LVDPILWASL VPVLPTGLSR NPEVTKTETS 420 STYTFFRPEE 430
3-52	**Sequences**	
3-52-1	Sequence Number [ID]	52
3-52-2	Molecule Type	DNA
3-52-3	Length	1531
3-52-4	Features	**source 1..1531**
	Location/Qualifiers	mol_type=genomic DNA
		organism=Solanum tuberosum
	NonEnglishQualifier Value	
3-52-5	Residues	ttttaaatca ttgttttatt ttctctttct ttttacaggt ataaaaggtg aaaattgaag 60 caagattgat tgcaagctat gtgtcaccac gttattgata ctttggaaga aatttttact 120 tatatgtctt tgtttaggag taatatttga tatgttttag ttagattttc ttgtcattta 180 tgctttagta taatttttagt tatttttatt atatgatcat gggtgaattt tgatacaaat 240 atttttgtca ttaaataaat taatttatca caacttgatt actttcagtg acaaaaaatg 300 tattgtcgta gtacccttttt ttgttgaata tgaataattt tttttatttt gtgacaattg 360 taattgtcac tacttatgat aatatttagt gacatatatg tcgtcggtaa aagcaaacac 420 tttcagtgac aaaataatag atttaatcac aaaattatta acctttttta taataataaa 480 tttatcccta atttatacat ttaaggacaa agtatttttt ttatatataa aaaatagtct 540 ttagtgacga tcgtagtgtt gagtctagaa atcataatgt tgaatctaga aaaatctcat 600 gcagtgtaaa ataaacctca aaaaggacgt tcagtccata gaggggtgt atgtgacacc 660 ccaacctcag caaaagaaaa cctcccttca acaaggacat ttgcggtgct aaacaatttc 720 aagtctcatc acacatatat ttattatata atactaataa agaatagaaa aggaaaggta 780 aacatcatta aatcgtcttt gtatattttt agtgacaact gattgacgaa atctttttcg 840 tcacacaaaa tttttagtga cgaaacatga tttatagatg atgaaattat ttgtccctca 900 taatctaatt tgttgtagtg atcattactc ctttgtttgt tttatttgtc atgttagtcc 960 attaaaaaaa aatatctctc ttcttatgta cgtgaatggt tggaacggat ctattatata 1020 atactaataa agaatagaaa aaggaaagtg agtgaggttc gagggagaga atctgtttaa 1080 tatcagagtc gatcatgtgt caattttatc gatatgaccc taacttcaac tgagtttaac 1140 caattccgat aaggcgagaa atatcatagt attgagtcta gaaaaatctc atgtagtgtg 1200

		gggtaaacct cagcaaggac gttgagtcca tagaggggg tgtatgtgac accccaacct 1260 cagcaaaaga aaacctcccc tcaagaagga catttgcggt gctaaacaat ttcaagtctc 1320 atcacacata tatatatatt atataatact aataaataat agaaaaagga aaggtaaaca 1380 tcactaacga cagttgcggt gcaaactgag tgaggtaata aacagcacta acttttattg 1440 gttatgtcaa actcaaagta aaatttctca acttgtttac gtgcctatat ataccatgct 1500 tgttatatgc tcaaagcacc aacaaaattt a 1531
3-53	**Sequences**	
3-53-1	Sequence Number [ID]	53
3-53-2	Molecule Type	DNA
3-53-3	Length	1970
3-53-4	Features	**source 1..1970**
	Location/Qualifiers	mol_type=genomic DNA
		organism=Zea mays
	NonEnglishQualifier Value	
3-53-5	Residues	ggtacctttt ttcccagaga taaatgtgga atagctctac aaacaaacgg catgatgctg 60 acacttggat ggcgaccttg caatcccaag aactattgca tacggttgcc agtcgacaaa 120 tatctacgcc atgcatggct acggtcggaa tacaccgtag cggcgggtaa ctcgccgata 180 ccgtccacgt gtccttggat gcccggtcgc tgatacttct ggtcttctgg acatgcacca 240 agacaatcaa gtgattcaac cttaatttaa cataatataa ataatacgta acatccaact 300 gacgtgttca cctatagaga atattccttc tgattctact ttcagaatga tgccgttgcc 360 gtgtatcgag caagtactct cactcgaagt atcttatctc ccacatccag cacaaaaatc 420 ttctgttcgt ggcaaatctt gtggcggttg aacgaaagaa tgctatataa gtagctatag 480 agaacgtatt atgtgtaaac caaccgttca gtgtaaatcg tgtgtaaata gtcatgttaa 540 ttttttggcg gcagatcaag tacaaactgt atgcctcgga taaacatgta caaaccacaa 600 cactggccac tagatctata tccaacgttc ataaccatcc atccctctct gctgcactct 660 gcaaacaagc acccccatct cgtagcaaca tcttgtctcc gacaagctct cgatgtagtg 720 gaggccctcc accgcaatat cctagtgtat gatgttggag aagcgactcc taaataatgg 780 tggcaagatg ttgctaggtt tgtagccata gcctcaatct aagatcatcc caagccatgg 840 gacctgattc tacgaggcct acaaccaggc atgacacgtc gtctacccac tcttgtgcat 900 catcggtcac ttgatctgac ttggttccta accacttacc ctaggttcca aagccctaag 960 tttctcgtat attgttagtc attcttagtg ggagttttat gtgtatttca ttcctgttaa 1020 atagcatgcc aactaagcaa acatgatgat ataatatgca atctaataaa aagatatatg 1080 agtgggtttc ataaaaaagg gagagagttt catgaggagt gaaactctga atacagatac 1140 tgatatgaca gctttaaaag tagtgttatg aaatcatcat tgagaaatgg tattagcact 1200 caatcgattt ctacgctgtc aattgtcatg agcacaattt tcacccaaag aggcacacca 1260 gcaatgtccg cttgtagtgt ccgagacgtt gctccatcgc cgtcgtcttg tttctgtgcg 1320 ctccattcaa tgcggcaagt ggctcaatcc caagcggtcg tcgcctccca gccccagcag 1380 caaaatatct tcccatgcgg ccatgccttg aaaattggaa tagattctct agattcaccg 1440 ccgcgtcatc ttcactactt tctcactggc ccaatcagca tctccttctc cgagctcaat 1500 catgctcagt caagcgtcac caatggcgtc acggttggtt ttgtcactgt ctgcatgcaa 1560 gggtattttg cttcgcaagt gtaaatggaa aatggatcta aacaactgca ctgcaccaat 1620 tttggaacgc ggaaccgaga gtctgtttgg gttcgtttga aacgcgctga tgtttctcat 1680 tttttaatag atgtagttac ctgatactat ttaagttgga cgatcaaacg actgtgtcaa 1740 gtgtgattaa gaaaagcatc gaaaataaaa tttatcgcca taaaaagtta aaaacagtgg 1800 ataatagtag gacctcataa tagaaaaaat tatcaaacgg aatggagggg cccaacgcag 1860 tatatagcag ccgggtggtg ccggacatcc gacgctcgtg ccagcaggcc attcttctcg 1920 ccttactccc tcacagaacc cagtaaaata tcgccagtcc cgccgtcgag 1970
3-54	**Sequences**	
3-54-1	Sequence Number [ID]	54
3-54-2	Molecule Type	DNA
3-54-3	Length	584
3-54-4	Features	**source 1..584**
	Location/Qualifiers	mol_type=genomic DNA
		organism=Aeluropus littoralis
	NonEnglishQualifier Value	
3-54-5	Residues	cccaagcttg accgatgcac acgctacctg ccaaggctcc ctccatccgc actctgcatc 60 gtcgcttcgg cgtaaacttc cacgtagtac ttgtacgatt ctagctagac ccagtgcgcc 120 caccctaccg ccggcgagcg ggcccccatc tcgcgccagg cttccatgcg ggtccaccgt 180 ggaccagccc tacgccgaac cgagcccatc cctccaccct ttcaccgcca agcgggaccc 240 gcgttggacc tttccgcttg gctggccccc accagcgtcc acgcgggcca acggcctcgc 300 gaaatggatc tccacacgac aaaccaaaac gagaagaaaa taaatggaaa ggaaagaaac 360 ggatcgccac gcgttccaga ggcgtccgct aaccacccga ttatgcttgc gcagcgtgcg 420 taacctcgtc gtggggttaa tccgggtggc cggatcggga aagccacggc ctttataacc 480 catccctgcc ggatcgaacc ggtaccggaa acaaaaacag ggggagaaaa aaagttcttc 540 gcgaggaagg aaaaggaaaa gtcgcgtgcc gtcctcgccc acag 584
3-55	**Sequences**	
3-55-1	Sequence Number [ID]	55
3-55-2	Molecule Type	DNA
3-55-3	Length	928
3-55-4	Features	**source 1..928**
	Location/Qualifiers	mol_type=genomic DNA
		organism=Agrobacterium rhizogenes
	NonEnglishQualifier Value	

3-55-5	Residues	ttagcgaaag gatgtcaaaa aaggatgccc ataattggga ggagtggggt aaagcttaaa 60 gttggcccgc tattggattt cgcgaaagcg gcattggcaa acgtggagat tgctgcattc 120 aagatacttt ttctattttc tggttaagat gtaaagtatt gccacaatca tattaattac 180 taacattgta tatgtaatat agtgcggaaa ttatctatgc caaaatgatg tattaataat 240 agcaataata atatgtgtta atctttttca atcgggaata cgtttaagcg attatcgtgt 300 tgaataaatt attccaaaag gaaatacatg gttttggaga acctgctata gatatatgcc 360 aaatttacac tagtttagtg ggtgcaaaac tattatctct gtttctgagt ttaataaaaa 420 ataaataagc agggcgaata gcagttagcc taagaaggaa tggtggccat gtacgtgctt 480 ttaagagacc ctataataaa ttgccagctg tgttgctttg gtgccgacag gcctaacgtg 540 gggtttagct tgacaaagta gcgcctttcc gcagcataaa taaaggtagg cgggtgcgtc 600 ccattattaa aggaaaaagc aaaagctgag attccataga ccacaaacca ccattattgg 660 aggacagaac ctattccctc acgtgggtcg ctagctttaa acctaataag taaaaacaat 720 taaaagcagg caggtgtccc ttctatattc gcacaacgag gcgacgtgga gcatcgacag 780 ccgcatccat taattaataa atttgtggac ctatacctaa ctcaaatatt tttattattt 840 gctccaatac gctaagagct ctggattata aatagtttgg atgcttcgag ttatgggtac 900 aagcaacctg tttcctactt tgttacca 928
3-56	**Sequences**	
3-56-1	Sequence Number [ID]	56
3-56-2	Molecule Type	AA
3-56-3	Length	512
3-56-4	Features	**source 1..512**
	Location/Qualifiers	mol_type=protein organism=Elaeis guineensis
	NonEnglishQualifier Value	
3-56-5	Residues	MAVSKNPETL APDQEPSKES DLRRRPASSP SSTAASPAVP DSSSRTSSSI TGSWTTALDG 60 DSGAGAVRIG DPKDRIGEAN DIGEKKKACS GEVPVGFVDR PSAPVHVRVV ESPLSSDTIF 120 QQSHAGLLNL CVVVLIAVNS RLIIENLMKY GLLIGSGFFF SSRLLRDWPL LICSLTLPVF 180 PLGSYMVEKL AYKKFISEPV VVSLHVILII ATIMYPVFVI LRCDSPILSG INLMLFVSSI 240 CLKLVSYAHA NYDLRSSSNS IDKGIHKSQG VSFKSLVYFI MAPTLCYQPS YPRTTCIRKG 300 WVICQLVKLV IFTGVMGFII EQYIDPIIKN SQHPLKGNVL NAMERVLKLS IPTLYVWLCV 360 FYCTFHLWLN ILAELLCFGD REFYKDWWNA KTIEEYWRMW NMPVHKWMLR HVYLPCIRNG 420 IPKGVAMVIS FFISAIFHEL CIGIPCHIFK FWAFIGIMFQ VPLVILTKYL QNKFKSAMVG 480 NMIFWFFFSI YGQPMCVLLY YHDVMNRKVG TE 512
3-57	**Sequences**	
3-57-1	Sequence Number [ID]	57
3-57-2	Molecule Type	AA
3-57-3	Length	74
3-57-4	Features	**source 1..74**
	Location/Qualifiers	mol_type=protein organism=Glycine max
	NonEnglishQualifier Value	
3-57-5	Residues	MADIDRSFDN NVSAVSTEKS SQVSDVEFSE AEEILIAMVY NLVGERWSLI AGRIPGRTAE 60 EIEKYWTSRF STSQ 74
3-58	**Sequences**	
3-58-1	Sequence Number [ID]	58
3-58-2	Molecule Type	AA
3-58-3	Length	146
3-58-4	Features	**source 1..146**
	Location/Qualifiers	mol_type=protein organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-58-5	Residues	MGSLQMQTSP ESDNDPRYAT VTDERKRKRM ISNRESARRS RMRKQKQLGD LINEVTLLKN 60 DNAKITEQVD EASKKYIEME SKNNVLRAQA SELTDRLRSL NSVLEMVEEI SGQALDIPEI 120 PESMQNPWQM PCPMQPIRAS ADMFDC 146
3-59	**Sequences**	
3-59-1	Sequence Number [ID]	59
3-59-2	Molecule Type	AA
3-59-3	Length	268
3-59-4	Features	**source 1..268**
	Location/Qualifiers	mol_type=protein organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-59-5	Residues	MGRGKIEIKR IENANSRQVT FSKRRSGLLK KARELSVLCD AEVAVIVFSK SGKLFEYSST 60 GMKQTLSRYG NHQSSSASKA EEDCAEVDIL KDQLSKLQEK HLQLQGKGLN PLTFKELQSL 120 EQQLYHALIT VRERKERLLT NQLEESRLKE QRAELENETL RRQVQELRSF LPSFTHYVPS 180 YIKCFAIDPK NALINHDSKC SLQNTDSDTT LQLGLPGEAH DRRTNEGERE SPSSDSVTTN 240 TSSETAERGD QSSLANSPPE AKRQRFSV 268
3-60	**Sequences**	
3-60-1	Sequence Number [ID]	60
3-60-2	Molecule Type	AA
3-60-3	Length	437

3-60-4	Features	**source 1..437**
	Location/Qualifiers	mol_type=protein
		organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-60-5	Residues	MEFESVFKMH YPYLAAVIYD DSSTLKDFHP SLTDDFSCVH NVHHKPSMPH TYEIPSKETI 60 RGITPSPCTE AFGACFHGTS NDHVFFGMAY TTPPTIEPNV SHVSHDNTMW ENDQNQGFIF 120 GTESTLNQAM ADSNQFNMPK PLLSANEDTI MNRRQNNQVM IKTEQIKKKN KRFQMRRICK 180 PTKKASIIKG QWTPEEDKLL VQLVDLHGTK KWSQIAKMLQ GRVGKQCRER WHNHLRPDIK 240 KDGWTEEEDI ILIKAHKEIG NRWAEIARKL PGRTENTIKN HWNATKRRQH SRRTKGKDEI 300 SLSLGSNTLQ NYIRSVTYND DPFMTANANA NIGPRNMRGK GKNVMVAVSE YDEGECKYIV 360 DGVNNLGLED GRIKMPSLAA MSASGSASTS GSASGSGSGV TMEIDEPMTD SWMVMHGCDE 420 VMMNEIALLE MIAHGRL 437
3-61	**Sequences**	
3-61-1	Sequence Number [ID]	61
3-61-2	Molecule Type	AA
3-61-3	Length	359
3-61-4	Features	**source 1..359**
	Location/Qualifiers	mol_type=protein
		organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-61-5	Residues	MYHQNLISST PNQNSNPHDW DIQNPLFSIH PSAEIPSKYP FMGITSCPNT NVFEEFQYKI 60 TNDQNFPTTY NTPFPVISEG ISYNMHDVQE NTMCGYTAHN QGLIIGCHEP VLVHAVVESQ 120 QFNVPQSEDI NLVSQSERVT EDKVMFKTDH KKKDIIGKGQ WTPTEDELLV RMVKSKGTKN 180 WTSIAKMFQG RVGKQCRERW RNHLRPNIKK NDWSEEEDQI LIEVHKIVGN KWTEIAKRLP 240 GRSENIVKNH WNATKRRLHS VRTKRSDAFS PRNNALENYI RSITINNNAL MNREVDSITA 300 NSEIDSTRCE NIVDEVMNLN LHATTSVYVP EQAVLTWGYD FTKCYEPMDD TWMLMNGWN 359
3-62	**Sequences**	
3-62-1	Sequence Number [ID]	62
3-62-2	Molecule Type	AA
3-62-3	Length	386
3-62-4	Features	**source 1..386**
	Location/Qualifiers	mol_type=protein
		organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-62-5	Residues	MSKRPPPDPV AVLRGHRHSV MDVSFHPSKS LLFTGSADGE LRIWDTIQHR AVSSAWAHSR 60 ANGVLAVAAS PWLGEDKIIS QGRDGTVKCW DIEDGGLSRD PLLILETCAY HFCKFSLVKK 120 PKNSLQEAES HSRGCDEQDG GDTCNVQIAD DSERSEEDSG LLQDKDHAEG TTFVAVVGEQ 180 PTEVEIWDLN TGDKIIQLPQ SSPDESPNAS TKGRGMCMAV QLFCPPESQG FLHVLAGYED 240 GSILLWDIRN AKIPLTSVKF HSEPVLSLSV ASSCDGGISG GADDKIVMYN LNHSTGSCTI 300 RKEITLERPG VSGTSIRVDG KIAATAGWDH RIRVYNYRKG NALAILKYHR ATCNAVSYSP 360 DCELMASASE DATVALWKLY PPHKSL 386
3-63	**Sequences**	
3-63-1	Sequence Number [ID]	63
3-63-2	Molecule Type	AA
3-63-3	Length	292
3-63-4	Features	**source 1..292**
	Location/Qualifiers	mol_type=protein
		organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-63-5	Residues	MEPPQHQHHH HQADQESGNN NNNKSGSGGY TCRQTSTRWT PTTEQIKILK ELYYNNAIRS 60 PTADQIQKIT ARLRQFGKIE GKNVFYWFQN HKARERQKKR FNGTNMTTPS SSPNSVMMAA 120 NDHYHPLLHH HHGVPMQRPA NSVNVKLNQD HHLYHHNKPY PSFNNGNLNH ASSGTECGVV 180 NASNGYMSSH VYGSMEQDCS MNYNNVGGGW ANMDHHYSSA PYNFFDRAKP LFGLEGHQEE 240 EECGGDAYLE HRRTLPLFPM HGEDHINGGS GAIWKYGQSE VRPCASLELR LN 292
3-64	**Sequences**	
3-64-1	Sequence Number [ID]	64
3-64-2	Molecule Type	AA
3-64-3	Length	453
3-64-4	Features	**source 1..453**
	Location/Qualifiers	mol_type=protein
		organism=Brassica napus
	NonEnglishQualifier Value	
3-64-5	Residues	MDLGSVTGNV NGSPSLKELR ESKQDRSEFD GEDCLQQSSK LARTIAEDKH LPSSYAAAYS 60 RPMSFHQGIP LARSASLLSS DSRRQEHMLS FSDKPEAFDF SKYVGLDNNK NSLSPFLHQL 120 PPPYCRTPGG GYGSGGMMMS MQGKGPFTLT QWAELEQQAL IYKYITANVP VPSSLLISIQ 180 KSFYPYRSFP PSSFGWGTFH LGFAGGKMDP EPGRCRRTDG KKWRCSKDAV PDQKYCERHI 240 NRGRHRSRKP VEVQPGQTAA SKAAAVASRN TASQIPNNRV QNVIYPSTVN LPPKEQRNNN 300 NSSFGFGHVT SPSLLTSSYL DFSSNQNKPE ELKSDWTQLS MSIPVASSSP SSTAQDKTTL 360 SPLRLDLPIQ SQQETLEAVR KVNTWIPISW GNSLGGPLGE VLNSTTSSPT LGSSPTGVLQ 420 KSTFCSLSNS SSVTSPVADN NRNNNVDYFH YTT 453
3-65	**Sequences**	

3-65-1	Sequence Number [ID]	65
3-65-2	Molecule Type	AA
3-65-3	Length	461
3-65-4	Features	**source 1..461**
	Location/Qualifiers	mol_type=protein
		organism=Brassica napus
	NonEnglishQualifier Value	
3-65-5	Residues	MDLGSVTGNV NGSPGLKELR GSKQDRSGFD GEDCLQQSSK LARTIAEDKH LPSSYAAYSR 60 PMSFHQGIPL TRSASLLSSD SRRQEHMLSF SDKPEAFDFS KYVGLDNNKN SLSPFLHQLP 120 PPYCRSSGGG YGSGGMMMSM QGKGPFTLTQ WAELEQQALI YKYITANVPV PSSLLISIQK 180 SFYPYRSFPP SSFGWGTFHL GFAGGKMDPE PGRCRRTDGK KWRCSKDAVP EQKYCERHIN 240 RGRHRSRKPV EVQPGQTAAS KAVASRDTAS QIPSNRVQNV IYPSNVNLQP KEQRNNDNSP 300 FGFGHVTSSS LLTSSYLDFS SNQEKPSGNH HNQSSWPEEL KSDWTQLSMS IPVASSSPSS 360 TAQDKTALSP LRLDLPIQSQ QETLESARKV NTWIPISWGN SLGGPLGEVL NSTTSSPTLG 420 SSPTGVLQKS TFCSLSNSSS VTSPIADNNR NNNVDYFHYT T 461
3-66	**Sequences**	
3-66-1	Sequence Number [ID]	66
3-66-2	Molecule Type	AA
3-66-3	Length	409
3-66-4	Features	**source 1..409**
	Location/Qualifiers	mol_type=protein
		organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-66-5	Residues	MEARPVHRSG SRDLTRTSSI PSTQKPSPVE DSFMRSDNNS QLMSRPLGQT YHLLSSSNGG 60 AVGHICSSSS SGFATNLHYS TMVSHEKQQH YTGSSSNNAV QTPSNNDSAW CHDSLPGGFL 120 DFHETNPAIQ NNCQIEDGGI AAAFDDIQKR SDWHEWADHL ITDDDPLMST NWNDLLLETN 180 SNSDSKDQKT LQIPQPQIVQ QQPSPSVELR PVSTTSSNSN NGTGKARMRW TPELHEAFVE 240 AVNSLGGSER ATPKGVLKIM KVEGLTIYHV KSHLQKYRTA RYRPEPSETG SPERKLTPLE 300 HITSLDLKGG IGITEALRLQ MEVQKQLHEQ LEIQRNLQLR IEEQGKYLQM MFEKQNSGLT 360 KGTASTSDSA AKSEQEDKKT ADSKEVPEEE TRKCEELESP QPKRPKIDN 409
3-67	**Sequences**	
3-67-1	Sequence Number [ID]	67
3-67-2	Molecule Type	DNA
3-67-3	Length	1173
3-67-4	Features	**source 1..1173**
	Location/Qualifiers	mol_type=genomic DNA
		organism=Sapium sebiferum L.
	NonEnglishQualifier Value	
3-67-5	Residues	tgccaatagc cagccaataa aacatctaca cgttttcaca cggcttttca tcacagccgt 60 tgtttttctc atctcactcc gtgccttcat cttcatcctc ttctcctctc tctctgtctc 120 tatatgtata gaagcgttag atgtcttgcg ttgttaacca attcattttt cgctttctgc 180 ttcttctaat attataagaa agtttgattc ttcttcttgt caatctttgt tcgcggcttt 240 taacgatatc cgctaaagga aatttgaaat ttcaattatg gccgatggaa acgtcaattc 300 gcaagaacag atggctaagc aggaggaaca gaggctgaag tatttggagt ttgtacaagt 360 ggctgcaata catgctgtgg tgaccttcac aaacctctat gtttatgcca aaaacaagtc 420 gggtccattg aagcccggtg ttgagactgt tgaaggtacg gtcaagagtg tggttggacc 480 tgtttatggc aagttccatg atgttcccat tgaggttctc aagtttgtcg atcgcaagat 540 tgatcaatct gtaagcagcc tagacagccg tgtgcctcca gttgtgaagc agttatcggc 600 ccaagcattt tcagtggctc gcgaagcccc agtggctgct cgtgctgtgg cttctgaagt 660 gcagactgct ggagtgaagg aaactgcatc tgggttggca agaactctgt acttcaaata 720 tgaacccaag gccaaggagc tatacaccaa gtatgaacca aaagcggaac agtgtgctgc 780 ctctgcctgg cgtaagctca atcaactccc agtcttccct catgtagctc aggttgttat 840 gccaacagca gcttattgtt ctgaaaagta caaccaggca gtacttacca ccgctgagaa 900 aggatacaga gtgtcctctt atttgccttt tgtgcccact gagagaattg ctaagttgtt 960 taggaatgag gcacctgaat ctaccccttt cctttccaat tgagcaagat gctgataaat 1020 gattcacaat ggacatgtgg acagaataaa aatctttgga tattatatgg tactgtgtat 1080 ttcaaggttc aagattactc tctacaatgt gtgaattttt gtttcagatg acttaattct 1140 tgttcattca ttatatatat atatatatat ata 1173
3-68	**Sequences**	
3-68-1	Sequence Number [ID]	68
3-68-2	Molecule Type	AA
3-68-3	Length	241
3-68-4	Features	**source 1..241**
	Location/Qualifiers	mol_type=protein
		organism=Sapium sebiferum L.
	NonEnglishQualifier Value	
3-68-5	Residues	MADGNVNSQE QMAKQEEQRL KYLEFVQVAA IHAVVTFTNL YVYAKNKSGP LKPGVETVEG 60 TVKSVVGPVY GKFHDVPIEV LKFVDRKIDQ SVSSLDSRVP PVVKQLSAQA FSVAREAPVA 120 ARAVASEVQT AGVKETASGL ARTLYFKYEP KAKELYTKYE PKAEQCAASA WRKLNQLPVF 180 PHVAQVVMPT AAYCSEKYNQ AVLTTAEKGY RVSSYLPFVP TERIAKLFRN EAPESTPFLS 240 N 241
3-69	**Sequences**	

3-69-1	Sequence Number [ID]	69
3-69-2	Molecule Type	DNA
3-69-3	Length	1252
3-69-4	Features	**source 1..1252**
	Location/Qualifiers	mol_type=genomic DNA
		organism=Sapium sebiferum L.
	NonEnglishQualifier Value	
3-69-5	Residues	ctacttttcc ctagcattag tattctaggc cccactctgt agattcctcc agctgcctga 60 tctaattttt tatcaactct tgaccgttcg atcatcccaa cggctcagat tcactagtac 120 ttttctcaca ccgtatctcc gattctccat gactccatcg atataaatcg cagtgctcat 180 caactgaatt ctcgaaattg cggttacaag ctgctataag aagcgaaaag aaacgctgag 240 aaacaggatc cgttcctcct ccctcgtttt ttactcctta caagatggag accgagaaga 300 agattcctga attgaagcac ttagggttcg tgaggatggc tgctattcag tcactgattt 360 gcgtctcgaa tctctacgat tacgcgaagc ataactcagg acctttgaga tccactgttg 420 gaaccgtgga gggtgccgta accaccgtag taggtccagt ttaccagaaa ttcaaagacc 480 ttcctgatga tcttcttgta tatgttgata agaaggtgga tgaaggaaca cacaagtttg 540 ataagcatgc tccacctatt gctaagaagg ctgcgagcca agcccatagt ttgtttcata 600 tagccttgga gaaggtcgaa aaactcgtgc aggaggctcg tgcaggagga cctcgtgctg 660 ctctgcattt tgtggctaca gagtcgaagc acttggcgtt gacccaatct gtgaagctgt 720 atagtaaact taatcagttc cctgtcattc acactgttac agatgtaacc cttcccacag 780 ctactcactg gtcagataag tataaccata cccttatgga cctgacccgg aagggttata 840 cgatctttgg ttatttgcct ttggttccta ttgatgacat atctaagaca tttaaacaaa 900 gtaaagcaga ggagaaagaa aatgcaacta cgcataaatc tgattcatcg gattccgact 960 aaacggttgc catcatgtct aatgggtgtg gtttgttaag tatagtggtt tgcgaaaatg 1020 ttctagggtt tatgagcctg ctcgaaagat gctgagaaat ggaaatctgt actatttagg 1080 agtttttccg tactataata atgagtatga atggtttgta aattctgcct tgtgctttct 1140 cgacaagtat atcatgcttc tatttttac tactacttac tggactactg aattgtctca 1200 taattgtccc tagtgtctaa ttaaatatca cctccaaaat attattgaaa aa 1252
3-70	**Sequences**	
3-70-1	Sequence Number [ID]	70
3-70-2	Molecule Type	AA
3-70-3	Length	225
3-70-4	Features	**source 1..225**
	Location/Qualifiers	mol_type=protein
		organism=Sapium sebiferum L.
	NonEnglishQualifier Value	
3-70-5	Residues	METEKKIPEL KHLGFVRMAA IQSLICVSNL YDYAKHNSGP LRSTVGTVEG AVTTVVGPVY 60 QKFKDLPDDL LVYVDKKVDE GTHKFDKHAP PIAKKAASQA HSLFHIALEK VEKLVQEARA 120 GGPRAALHFV ATESKHLALT QSVKLYSKLN QFPVIHTVTD VTLPTATHWS DKYNHTLMDL 180 TRKGYTIFGY LPLVPIDDIS KTFKQSKAEE KENATTHKSD SSDSD 225
3-71	**Sequences**	
3-71-1	Sequence Number [ID]	71
3-71-2	Molecule Type	DNA
3-71-3	Length	938
3-71-4	Features	**source 1..938**
	Location/Qualifiers	mol_type=genomic DNA
		organism=Sapium sebiferum L.
	NonEnglishQualifier Value	
3-71-5	Residues	gagtattcac actctggcct gattgggttt gctataaagg gcgatcgttg caacgctcca 60 tattgtctac ttggttttgt ttcaaatctc atcattttgt aaatttgcga cagtgtagcg 120 ttttctagga aaaaggttgc taaaggaaag tagttatcaa accgcagaaa tggcggaatc 180 cgaacttaat caacacacag atatggttca agatgatgat aaaaaactca agtatctaga 240 ttttgtacaa gtggccgcga tctatgttgt ggtttgtttc tctagtatct atgaatatgc 300 taaggaaaac tccggtccac taaaaccagg ggtccaagcc gttgagtgta ccgtcaaaac 360 tgtaataagt ccggtttacg agaagtttcg cgacgtacct tttgaactcc ttaaattcgt 420 cgatcgtaaa gttgacaact ctctaggcga gttggacagg cacgtgccgt cgctggtgaa 480 gcaggcatca agccaagctc gagctgtggc tagtgaaatt caacatgctg gattggtaga 540 cgcaactaag aacattgcga agacgatgta tacaaagtat gaactgacgg cttggcagct 600 ctactgcaaa tacaagccgg tggctaagcg ttacgcggtg tcgacctggc gctcattgaa 660 ccagcttcct ctgtttcctc aagcggctca gattgcaatc ccaactgctg cttcgtggtc 720 tgagaaatac aataagatgg ttcgttacac gaaagataga ggatatccag cggcggtgta 780 tctgccattg atctcggttg agaggattgc caaggtgttc aatgaagact taaacgggcc 840 caccgtccct accaatggat catccgccgc agcacaatag ttttcatttt atgtatttat 900 gtcagattga agacgctccg gagattttga aaacctga 938
3-72	**Sequences**	
3-72-1	Sequence Number [ID]	72
3-72-2	Molecule Type	AA
3-72-3	Length	194
3-72-4	Features	**source 1..194**
	Location/Qualifiers	mol_type=protein
		organism=Sapium sebiferum L.
	NonEnglishQualifier Value	

3-72-5	Residues	MAESELNQHT DMVQDDDKKL KYLDFVQVAA IYVVVCFSSI YEYAKENSGP LKPGVQAVEC 60 TVKTVISPVY EKFRDVPFEL LKFVDRKVDN SLGELDRHVP SLVKQASSQA RAVASEIQHA 120 GLVDATKNIA KTMYTKYELT AWQLYCKYKP VAKRYAVSTW RSLNQLPLFP QAAQIAIPTA 180 ASWSEKYNKM VRYT 194
3-73	**Sequences**	
3-73-1	Sequence Number [ID]	73
3-73-2	Molecule Type	DNA
3-73-3	Length	2526
3-73-4	Features	**source 1..2526**
	Location/Qualifiers	mol_type=genomic DNA organism=Sorghum bicolor
	NonEnglishQualifier Value	
3-73-5	Residues	atggacgagt ccggggaagc gagcgtcggc tccttcagga tcggcccgtc gacgctgctg 60 ggccgcgggg tggcgctccg cgtgcttctc ttcagctcgc tgtggcgcct gcgggcgcgc 120 gcgtacgccg ccatctcgcg cgtgcgcagc gcggtgctgc cggtggcggc gtcctggctt 180 cacctcagga acacccacgg cgtcctcctc atggtcgtcc tcttcgccct ctccctgagg 240 aagctctccg gcgcgcggtc gcgggcggcg ctcgcgcgcc ggcgcaggca gtacgagaag 300 gccatgctgc atgccgggac gtacgaggtc tgggcccgcg ccgccaatgt gctcgacaag 360 atgtctgatc aggtccatga ggcggatttc tatgacgagg agctgatcag gaacaggctt 420 gaggacctcc ggaggcggag ggaggacggg tcgctgcggg acgtggtgtt ctgtatgcgc 480 ggcgatcttg ttaggaactt ggggaacatg tgcaatcctg aacttcacaa gggcaggcta 540 gaggttccta agcttataaa ggaatacatt gaagaggttt ctattcaact aagaatggtg 600 tgcgaatctg acactgatga gttgctattg ggagagaagc ttgcctttgt tcaggagacc 660 aggcatgcct ttgggaggac agccctactc ttaagtgggg gtgcttcact ggggtctttc 720 catgtaggtg tagtgaaaac attggttgag cataagcttc tgcctcggat tatagcagga 780 tcaagcgttg gttccattat atgttcgatt gttgctaccc ggacatggcc tgagattgag 840 agcttcttca cagactcatt acagaccttg cagttctttg ataggatggg tggaattttt 900 gcagtgatga ggcgagtcac cactcatggt gcactgcatg acattagcca gatgcaaagg 960 cttctgaggg atctcacaag taacttaaca tttcaagagg cttatgacat gactggccgt 1020 gtccttggga tcaccgtttg ctctcctaga aaaaatgagc caccccgctg cctcaactat 1080 ctgacgtcgc cgcacgttgt tatttggagt gctgtaactg cctcttgtgc atttcctggg 1140 ctctttgaag ctcaggaact gatggcgaag gatagattcg gcaacatagt tcccttccat 1200 gcacccttg ccacagatcc tgaacaaggt cctggagcat caaagcgccg gtggagagat 1260 gggagcctgg aaatggattt gcccatgatg agactcaagg agttgtttaa tgtaaaccat 1320 ttcattgtga gccaaactaa tcctcacatt tctcccctcc tccgaatgaa agagcttgtt 1380 agagtctatg gagggcgctt tgctggaaag cttgctcgtc ttgctgagat ggaggttaag 1440 tatcgatgta accaaatcct agagattggt cttccaatgg gaggacttgc aaaattgttt 1500 gctcaggact gggagggtga tgtcaccatg gttatgccgg caacagtagc tcagtatttg 1560 aagattattc agaatccaac atatgcggag ctccaaatgg ctgccaacca aggccgcagg 1620 tgtacatggg agaagctctc tgcaatcaga gcaaactgtg ccatcgaact tgcattggat 1680 gaatctatag cagttttaaa ccacaaacgg aggctaaaaa gaagcatgga gaggacagag 1740 gctgctttgc agggtcattc taactatgtt cgactcaaaa ctccaaggag ggtaccatca 1800 tggagctgca tcagtcgaga gaattcttca gaatctctct cggaagagat ttcagcagtt 1860 gctacttcaa ccgcgcagca aggtgctgct cttgttgtcg gcacagccac tctttctcac 1920 catgttcgac gcaattctca tgacggaagt gagagtgaat cagaaaccat tgaccttaat 1980 tcctggacca ggagtggtgg gcctctaatg aggacagcat ctgctgacat gttcatcagt 2040 ttcatccata accttgagat tgacacggaa ttaagtaggc cctgtactgt ggagggtggt 2100 actgcaggta tttcgtcaga atctaccttc ccaaatgatc cacaaccgaa caatggctca 2160 agtgttacta ctccaggtag atgcacagaa aattctgaga ccgaggcata cgacactgtc 2220 aacaccagag ccagtcaggc ttctactccc acaagcatcg ctgtttctga aggagatttg 2280 ctgcagcctg aaagcattgc tgacggtatc ctgcttaaca ttgtgaaaag agatgccttg 2340 caggctcaaa atgacagcgt aactgaattg gccgaaagct cctgcactga aacatatgcg 2400 gaaacttgtg acaccatctc agggtctggc actgctgaag ataacaagga tactgctgac 2460 tcaagcaatc actcacttga tattgatgct tttgtagttt cgcatcaacc ttcagctgat 2520 gattag 2526
3-74	**Sequences**	
3-74-1	Sequence Number [ID]	74
3-74-2	Molecule Type	DNA
3-74-3	Length	3099
3-74-4	Features	**source 1..3099**
	Location/Qualifiers	mol_type=genomic DNA organism=Triticum aestivum
	NonEnglishQualifier Value	
3-74-5	Residues	atgcccgcgc ctgcaggtgc gtgcagccaa gccccaccgc tcgccttcta ttccgcgtcc 60 cctagcttgg cccggccctg ctccgatcca aggccgcggc ggtggcccag tgccctctcc 120 ctcctgccac gccgtccgcc gcccatggac gtcatcacca acgaggcgcg cgtgggggcg 180 ttcgcgatcg gcccgtccac ggcggcgggg cgcgcgctcg cgctgcgcgt gctcctctgc 240 ggctcgctgg cgcggctgcg gcaccgcctc gccgccgcgc tgcgcgccgc ggcgccgctg 300 gcggcggcct ggctgcaccc gcgccacaac acgcgggga tcctgctcgc cgtctgcgcc 360 gtcgcgctgc tgctgcgcgg ccgcgggggc cgcgccgggg tgcgcgcgcg ggtgcagtcc 420 gcctaccgcc gcaagttctg gcggaacatg atgcgcgccg cgctcaccta cgaggagtgg 480 gcgcacgccg cgcggatgct cgagcgggag acgccgcgcc gcgtcaccga cgccgacctc 540 tacgacgagg agctcgtgtg caacaagctc cgtgagctca ggcaccgccg tcaggagggc 600 tcgctcaggg acatcgtctt ctgcatgcgc gccgatctgc tcaggaacct tggtaacatg 660

		tgcaaccccg agctccacaa gttgaggctg caggtgccta aaaccatcaa ggagtacatt 720 gaggaggtat ctactcaact gaaaatggtt tgcaattctg attcggacga gttacccctt 780 gaagagaaac tggcatttat gcatgagaca agacatgcct ttggtagatc ggccctactg 840 ctaagtggag gtgcttcatt tggctctttc catgtgggtg ttgtgaaaac cttggtagag 900 cataagcttc tacctaggat tatttcagga tcaagcgttg gcgcaataat gtgtgctatt 960 gtagccacac ggtcatggcc agaactagag agtttttttg aggagtggca ttccttgaaa 1020 ttctttgacc agatgggtgg gatctttcct gtatttaaaa gaattttgac gcatggagcg 1080 gttcatgaca ttaggcactt gcagacgcag ttgagaaatc ttacaagcaa tttgacattt 1140 caagaggcat atgacatgac tggccgggtt ctcgttgtta ctgtgtgttc tccaagaaaa 1200 catgagccac cacgatgcct gaactatttg acatcacctc atgttctcat ttggagtgca 1260 gtaactgctt cctgtgcttt tcctggactt tttgaggccc aggagttgat ggccaaagat 1320 agattcggag aaacagttcc ttttcatgct ccattcttgt tgggtgtgga ggaacgagct 1380 gacgctgcta cacggcgctg gagagatggc agcttagaaa gtgatttacc catgaagcaa 1440 ttgaaggaat tattcaacgt aaatcacttc atagtaagcc aagccaatcc tcacattgct 1500 ccattactga gactaaagga gatcatcagg gcttacggag gcagctttgc tgcaaagctt 1560 gctgaacttg ctgagatgga agttaagcat aggttcaatc aagttctgga acttggattt 1620 ccattaggag gaatagctaa gttgtttgct caacattggg aaggtgatgt gacaatcgtt 1680 atgccagcca cacttgctca gtattcgaag atcatacaga atccttcgta ttctgagctt 1740 cagaaagccg caagtcaggg taggcgatgc acttgggaaa agctctctgc tatcagggca 1800 aactgcgcta ttgagcttgc attagatgaa tgtgttgccc tcctgaacca catgcgtagg 1860 ctgaagagaa gtgcagaaag agcagctgct tcacaaggat atggtgctac aattagactc 1920 tgtccatcta gaaggattcc atcatggaat ctcatagcaa gagaaaattc aactggttct 1980 ctcgatgagg aaatgctcac atgtcccact gttacgagcc atcaagcagt tggagggact 2040 gctgggccat ctaacagaaa tcaccatctc caacatagta tgcatgatag cagtgacagt 2100 gaatctgaga gtatagactt gaactcatgg acgagaagtg gtggccctct catgagaaca 2160 gcctcagcta ataaattcat cagctttgtt cagaaccttg agattgacac agaattcaga 2220 acaatttcac caaggggggag cgaaggtgat attgttacac cgaatagtaa cttatttgct 2280 ggtcacccaa ttggtagaga gccagttgat aaccatccag ggcctgctac tcctggtagg 2340 acctcaggca attcaggttg cgatcctcat gatactcctg ttcctaggtc tccatttggt 2400 ctttccacaa gtatcatggt ccctgaaggt gacttgctgc agccggaaaa gattgagaat 2460 ggtattttat tcaatgttgt gagaagggat gctcttgtag cgactactag cggagttgaa 2520 cctcatggat cttcacagga agcagatgtg gaaactgtac cgaccgagtg cctttatggt 2580 gcttcggatg acgacgacga caacgtggaa ctgaatgctg atcatgaagc attatctgac 2640 cctggagatc agagatcctc agttgcagga aacctagatc cgtccacttc catggattgt 2700 caagctgatg aaacaagtac tactcgatca gaagctccat ctctctttaa tatctgtgtg 2760 gagattcctc cagcaaccat gatcagagaa aatagtcggc ccgacgagcc ttcttcagac 2820 ataagactgg agattgtaaa gacagaatgc cctgatgaga attcagctgc tgggaacgat 2880 gaagttggct cagttcctgc caataaagaa tcttcctatt gttctcagac agctgaaaat 2940 agacagcagc atcaagttga tatgggatct gtgaactcct gtagtgtttc agtttcagaa 3000 gatgataggc atgtcagcct catttcgaac gagaaaccag ttactacttc cagtggcgga 3060 gcggagagta tgacatctgg aagaaatgaa gctgactag 3099
3-75	**Sequences**	
3-75-1	Sequence Number [ID]	75
3-75-2	Molecule Type	DNA
3-75-3	Length	2198
3-75-4	Features	**misc_feature 1..2198**
	Location/Qualifiers	note=S. bicolor SDP1 hpRNAi fragment
		source 1..2198
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-75-5	Residues	gcggcggcgt ggctgcaccc gcgcgacaac acgcgcggga tcctgctcgc cgtctgcgcc 60 gtcgcgctgg gtgcagtccg cctaccgccg caagttctgg cggaacatga tgcgcgccgc 120 gctcacctac gaggagtggg cgcacgcggc gcggatgctt ggagtgcagt aacagcttcc 180 tgtgcttttc ctggactttt tgaggcccac catctaggag gattccatcc tggaatctca 240 tagcaagaga aaattcaact ggttctctat gtgcaatcct gaacttcaca agggcaggct 300 agaggttcct aagcttataa aggaatacat tgaagaggtt tctattcaac taagaatggt 360 gtgcgaatct gacactgatg agttgctatt gggagagaag cttgcctttg ttcaggagac 420 caggcatgcc tttgggagga cagccctact cttaagtggg ggtgcttcac tggggtcttt 480 ccatgtaggt gtagtgaaaa cattggttga gcataagctt ctgcctcgga ttatagcagg 540 atcaagaagg gtggacccag ctttcttgta caaagtggtc tcgaggaatt cggtacccca 600 gcttggtaag gaaataatta ttttcttttt tccttttagt ataaaatagt taagtgatgt 660 taattagtat gattataata atatagttgt tataattgtg aaaaaataat ttataaatat 720 attgtttaca taaacaacat agtaatgtaa aaaaatatga caagtgatgt gtaagacgaa 780 gaagataaaa gttgagagta agtatattat ttttaatgaa tttgatcgaa catgtaagat 840 gatatactag cattaatatt tgttttaatc ataatagtaa ttctagctgg tttgatgaat 900 taaatatcaa tgataaaata ctatagtaaa aataagaata aataaattaa aataatattt 960 ttttatgatt aatagtttat tatataatta aatatctata ccattactaa atattttagt 1020 ttaaaagtta ataaatattt tgttagaaat tccaatctgc ttgtaattta tcaataaaca 1080 aaatattaaa taacaagcta aagtaacaaa taatatcaaa ctaatagaaa cagtaatcta 1140 atgtaacaaa acataatcta atgctaatat aacaaagcgc aagatctatc attttatata 1200 gtattatttt caatcaacat tcttattaat ttctaaataa tacttgtagt tttattaact 1260 tctaaatgga ttgactatta attaaatgaa ttagtcgaac atgaataaac aaggtaacat 1320 gatagatcat gtcattgtgt tatcattgat cttacatttg gattgattac agttgggaag 1380 ctgggttcga aatcgataag cttgcgctgc agttatcatc atcatcatag acacacgaaa 1440

		taaagtaatc agattatcag ttaaagctat gtaatatttg cgccataacc aatcaattaa	1500
		aaaatagatc agtttaaaga aagatcaaag ctcaaaaaaa taaaaagaga aaagggtcct	1560
		aaccaagaaa atgaaggaga aaaactagaa atttacctgc acaagcttgg atcctctaga	1620
		ccactttgta caagaaagct gggtccaccc ttcttgatcc tgctataatc cgaggcagaa	1680
		gcttatgctc aaccaatgtt ttcactacac ctacatggaa agaccccagt gaagcacccc	1740
		cacttaagag tagggctgtc ctcccaaagg catgcctggt ctcctgaaca aaggcaagct	1800
		tctctcccaa tagcaactca tcagtgtcag attcgcacac cattcttagt tgaatagaaa	1860
		cctcttcaat gtattccttt ataagcttag gaacctctag cctgcccttg tgaagttcag	1920
		gattgcacat agagaaccag ttgaattttc tcttgctatg agattccagg atggaatcct	1980
		cctagatggt gggcctcaaa aagtccagga aaagcacagg aagctgttac tgcactccaa	2040
		gcatccgcgc cgcgtgcgcc cactcctcgt aggtgagcgc ggcgcgcatc atgttccgcc	2100
		agaacttgcg gcggtaggcg gactgcaccc agcgcgacgg cgcagacggc gagcaggatc	2160
		ccgcgcgtgt tgtcgcgcgg gtgcagccac gccgccgc	2198
3-76	**Sequences**		
3-76-1	Sequence Number [ID]	76	
3-76-2	Molecule Type	DNA	
3-76-3	Length	22	
3-76-4	Features	**misc_feature 1..22**	
	Location/Qualifiers	note=Oligonucleotide primer	
		source 1..22	
		mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-76-5	Residues	ttttaacgat atccgctaaa gg	22
3-77	**Sequences**		
3-77-1	Sequence Number [ID]	77	
3-77-2	Molecule Type	DNA	
3-77-3	Length	23	
3-77-4	Features	**misc_feature 1..23**	
	Location/Qualifiers	note=Oligonucleotide primer	
		source 1..23	
		mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-77-5	Residues	aatgaatgaa caagaattaa gtc	23
3-78	**Sequences**		
3-78-1	Sequence Number [ID]	78	
3-78-2	Molecule Type	DNA	
3-78-3	Length	22	
3-78-4	Features	**misc_feature 1..22**	
	Location/Qualifiers	note=Oligonucleotide primer	
		source 1..22	
		mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-78-5	Residues	cttttctcac accgtatctc cg	22
3-79	**Sequences**		
3-79-1	Sequence Number [ID]	79	
3-79-2	Molecule Type	DNA	
3-79-3	Length	25	
3-79-4	Features	**misc_feature 1..25**	
	Location/Qualifiers	note=Oligonucleotide primer	
		source 1..25	
		mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-79-5	Residues	agcatgatat acttgtcgag aaagc	25
3-80	**Sequences**		
3-80-1	Sequence Number [ID]	80	
3-80-2	Molecule Type	DNA	
3-80-3	Length	18	
3-80-4	Features	**misc_feature 1..18**	
	Location/Qualifiers	note=Oligonucleotide primer	
		source 1..18	
		mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-80-5	Residues	gcgacagtgt agcgtttt	18
3-81	**Sequences**		

3-81-1	Sequence Number [ID]	81
3-81-2	Molecule Type	DNA
3-81-3	Length	25
3-81-4	Features	**misc_feature 1..25**
	Location/Qualifiers	note=Oligonucleotide primer
		source 1..25
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-81-5	Residues	atacataaaa tgaaaactat tgtgc 25
3-82	**Sequences**	
3-82-1	Sequence Number [ID]	82
3-82-2	Molecule Type	DNA
3-82-3	Length	2631
3-82-4	Features	**source 1..2631**
	Location/Qualifiers	mol_type=genomic DNA
		organism=Saccharum hybrid
	NonEnglishQualifier Value	
3-82-5	Residues	ctgcgacagc tagaggcgcc accgcgtcct agcttcctcc aacttctcgt cggagatccc 60 ttcagggatg cccaatgcca ccgcccctaa gtcaacctgc gggagctgga gcttcgccag 120 ggtcagagct gcggcagcac cctggtagac cgcattcctg atgacccgcg gggtgcgctc 180 catgaagaag tgcattcgcc caaccaagtc gagtgggtcg cctggagggg gcggggaagc 240 aaaacgttgc atgcacctag cgccctggca gcgagctcct gtagtatcac ctgcgtcgcc 300 tccagctcat gctcgcaagc ctccagggcg gcccggcagt gctccaacac tttcgcctcc 360 tcctacagct ccttccacat gcagtcgtgc tccgcacgca ccttctccac ctttttactc 420 ttttctttct cttttcttgg cccatctttg gtattttcac aaatgtcccc ctacaaatga 480 taaatcacca aaactcatgg agcttgctag ttataaactc taattctaag tttggtgttt 540 atttgagtgg attttgtgtg aaagttggtg gttagaaata ggagttaagg accgccaaca 600 agatccccca cacttagccc tttgctcatc ctcgagtaaa gttcaaggac taaggtggaa 660 catctcctca aatggtacga tgcctgcata taagttattc caagcctcac ctatacatgt 720 gaactttgaa gtgtctacca cgccatcttg ggtggttgag aaatggaaca gatcagaatc 780 cagtcatctt tacctctctt gcttagataa cttgggtttt tgtaaggttt tcaaatttaa 840 aacatagtct tgctcctcaa atgattctct catatagctc aatgtgtatg gtttctcacc 900 aaggcaatgt tttgcctctt ttcatcctac ttctaatatt tcttttgtgg agcttagggt 960 agggaatgaa aaggaagcat acttgcattg catatgttac taagtcaaaa accaaatctg 1020 aggagaagca agtcatacaa tctgatcaag atgtgcaagt gtgtggatat gtggattaag 1080 ataactcctg tttattcatg ctctcctcct taataaactt tagagggcat ggcaatcttt 1140 gcatgggcct tcatgagctc atcgtatgtc taagcatgga gctcatcatt tatataagca 1200 tggtgatacc aaaattactc cttttgagca tgtttatatt taggaggacg ttttacctgt 1260 tgaggtaaat ctgaacgcta ataaatcggc taagcaaaat aatttatcac ctgttgattc 1320 taacaatttg atgatggaca atattgatga ggtgactgac aaatgattga aggctttaaa 1380 ggagattgag aaggataaat ctacaataaa aatgtaaaga agaaagcatt caaagtgtga 1440 gatctggtgt ggaagactat tttgcctctt gggggtaaaa gacaacaagt ttagtaagtg 1500 gcctcaaaat tgggagggcc catgcaagat tgttaaagta attgttttgg attgacggag 1560 gcatttcaag gtgatcatct acctagagct ctcaatggga ggtgctcgaa gacatattac 1620 ccatgtgtat ggcaagatgt ttagctagta actgactgat agtgtaaacg atctccaatg 1680 gggcaagaca tattacctaa ggccaggctg gtttttgcaa gttcgagtag gatatagaga 1740 ttctcgtgcg agttgtaaac gatctccaat ggggcaagac atcctaccct atatatagtg 1800 aaggggcagt agctgattga gaatcaatca atcaagcaca atataattta ttaatttttt 1860 attcaaaccc aatttttttc cttttccaac cctaattata gttttccttt tgcctctagg 1920 acaaattgac gtgttccggg tatcctgctg aattaagaac aaccctaggt gcacctgtcc 1980 cgatagagtc ccacctgggt aggcattcat agggattcgt gtatttcctg caaaaaagcg 2040 attaagctgg cttctaaaac tggctaggcc ggattctgtg gccttcacta ccaggtgatt 2100 ttcatgtgat ccgtgcattc tagcactttg ctatgtaacc caaacttaag tcgacaacta 2160 taaatatgct acttgcagga tgttatcacg acacaactcc taatctacgg aagcctaagt 2220 ttagttttgc tcggagacaa gcaattgtgg ccagtcacta tagctacgtc agagggtagt 2280 gggagcagtt gcgtcgttgg attgaaaaca ggtggatcgt atcagatatt atgcattcac 2340 atggacagta aatgtggtac agtaacttcg caaacaataa aatctgtcac aatttattag 2400 tgcactcctc tgacgtaaat gcttctacgt cagaggattt gattccgagg gccgctgcac 2460 ccatcactaa tgacggtctt tacccatcat catggaccat tgttcacatc catgctatca 2520 ctgtcgtcct gtccatgcac tgcagccctc tataaatact ggcatccctc ccccgttcac 2580 agatcacaca acacaagcaa gaaataaacg gtagctgcca taactagtac a 2631
3-83	**Sequences**	
3-83-1	Sequence Number [ID]	83
3-83-2	Molecule Type	DNA
3-83-3	Length	2907
3-83-4	Features	**source 1..2907**
	Location/Qualifiers	mol_type=genomic DNA
		organism=Saccharum hybrid
	NonEnglishQualifier Value	
3-83-5	Residues	gcataggcat tgtaaaagcg gtatgcctct tcttcagtgc agaatttcat accaacctta 60 ggtatcctgt cttccataga attttctacc tgagtaggtt cggtctggtt ggatttgtag 120 cgggtttcat gcaaaataag ttagaaatcg tgcaaacttg caatggaggt taaatttgaa 180

		atatatttgc atagacaaaa caaatataga ttatgaatgg taatccaata tgacttgcat 240 tttctaactc tattgctact gtgccagatg aagaatgttg atctggagaa gttttgtgag 300 aatgtgacaa caacgggagg tcatatcaag attctgggta cccgcggaga atcggcctcc 360 atgtagttag cctcgtcagg catgggggga attggctgag atgcccccat gtagtcgtca 420 ggcatggaga gtactggctg agatgccatt gttgtgtaga tcgagagaaa cgagaagaat 480 gctagtctaa taataccctt ccgtatgcta accaactatt ataattggca ccatttttca 540 catgctagcg ccttttgcct gctttattta attcaattgg gtccgataag catgtgaacg 600 tgggagacgg ttccgtcgga cggctccgtt ttcttgtagc gtacggcgtg gacggagaaa 660 aggtgagggc ctatctctaa aggggaacga atggatggtg gacacgtgtg gggagacacc 720 gaagggacat gccgaggagg cacacaagct tcagcaggcg tctccagact ctcagaagaa 780 gaagaagctc acggcacggt tgcggctggt tcttgctgtc gctgtctcgt ggtgcacgtt 840 tctgtgatca cgctgaaatc gaccggccgg cggaccaaca ggaggtcagc tcggccactc 900 cgtctccgag cgcatgagtg caccgttcgt ccgcggttcc ttttctcgtg gtgccgtgca 960 cgcctctgcg ttcaccggca ccctgaaacc aatcagaacg ttccctttac aggggaaagg 1020 gacaagtctg ataacctctc tgtttccatc gtcctctaac cgcgaagagc ggacgcacaa 1080 gacttagagt ctatttgttc gaaatttttt actctcacaa aagctagctt ttatagacgg 1140 gcataaaagc tatcatgtcg accggcacgt ttaatattta acttatacca tatgaatatc 1200 atgtcgaact atgaggatga tacttttctg aacgtgattg cgtgagttat taaattgtac 1260 ttttagttgt ttgagcatga aggtctgaac tatgaattta tgatgtattg tggcttgtga 1320 gctactccgc tctacattta gttggtatca taaatattat tatattatca tataaatttg 1380 atcaacttga gatgctttga ctcttcaaga ttcttggaat gacttatcat ttggggtagg 1440 gagtaggttt ctaaggccag tctcagtggg gtttcatcag agtttcatgg acattaaata 1500 agctgatgtg acaccgtatt gatgaagaga gagatgataa gagtttcatg cgagtagaga 1560 gagtttcatg gggatgaaac tcttcttcac tgtttccaaa atatagatgc attggtaaga 1620 gggccatgaa atctctagtg acactgacct aagatgagat tgactctagc actatgtttc 1680 aaaatctgca tgcatgcatg ctttgaatat tgtaacctca cattaactcc cctcacacat 1740 gcatgcaaac gggcggtgca cgcaaaagaa ttgagtgaag atgcacatga aaaataagta 1800 aaatgctttg gcttcatcac ccggcttaaa tgctcgacag aaaaacacgt cggtagtcaa 1860 ggttgtgcct aacaaactgg ggttcacatg taaaacacgt tcatgcctta gaaacggcct 1920 ggagggatta gatacaactt caattatatc ttagggcccc tccaatattg tcagctctaa 1980 actagtttta tgtgtcacgg tggaggagag ggaggctaaa aatataatct tgagctaacg 2040 tgaagagaag agctatttt ttttgctccc caatacatga tagatacaat atgagagaaa 2100 aaatatatga ataaagaaca ctttacatgc cagccataca atatgagatt tcatctaaga 2160 gccaacacct gactcgtact gttgaaggtg tcctagttgg agtggtcgat cttttagttg 2220 ttagtagtgt aagacctagt ttagtgctct tttcttgtct aggtttatgt tgtgttttgg 2280 ctgccaagtg ttgaacaact caaggtaagg tcccatctaa ttctaaaatg atgccaaata 2340 aagatagatt acaaagttaa acgacggaaa aactctaaaa taggatggaa agttttgtag 2400 agtaataatt ggtatgaagt ggcgaagtcg accacaacca aacataaaga gttaaatgca 2460 tggtaggctc ttgatcttgt ctggaggtgc cacttaggtc cacaaactct caaattgcat 2520 ttttgacacc ctaatgttat tcaagtgtgc cacttagatc tacaaactct caaaatgcat 2580 ttctgatacc ctagtgttgt tcaagtgtgt cacttaggca agaaaagtta gataattttg 2640 ataagctatg ggaccaaatt aatttatgta tgcatgctcg aactagttga tgatgatgga 2700 ccccataata gacactagtt catgggctgg tttccttgta tagtactagc tagtataact 2760 ttttcaagtt gtagctacta ctttagctta tactccgcat attacaatca aatagaattc 2820 ggaagtacta taaacgggag cctataaatg gagacgtttt gcatcatgag gctataacaa 2880 cttgagcaaa aacagaagcc gtgcgcc 2907
3-84	**Sequences**	
3-84-1	Sequence Number [ID]	84
3-84-2	Molecule Type	DNA
3-84-3	Length	1141
3-84-4	Features	**source 1..1141**
	Location/Qualifiers	mol_type=genomic DNA organism=Saccharum hybrid
	NonEnglishQualifier Value	
3-84-5	Residues	actatagggc acgcgtggtc gacggcccgg gctggtctgg ttttggcctc ttttagttac 60 taaattgcca aaaagagtga ctaaaaagtg actaaactga tttagtcctc tagtcaaggg 120 actaaaccag ctaaaagaca tccgctgccc ctcattaatg cacagaagga gagagagagg 180 gagagggagg acattttggt ctttatatag tagctttaat ggactttagt acctagatcc 240 aaaccggtag tgactaaagt ttagtccttg aactgaactt taatccaggg acatggaacc 300 aaacatgccc ttaacttttt tttattctaa tccctcttac attcacttgt ctcacaaagt 360 ggcaagtcat ttgccaccct cactaccagt ggcgactggt taaatatcct catgtttggt 420 ttttttttagt aaccaaatac tgcaagctat tgggaaaaaa ggcaaaaaat tatctccttg 480 cttatagttg tataatccat gatccggcaa ttgtttgtta cggagatcct gaatcctctg 540 acgtagagtt taatcaattt tagctcaaga ataatacact ataaagtgga tatgacaatc 600 accgtagtac ttatttatct tgtagtagta tactgtattc gacctgcgat tatgataaag 660 gcatcagaaa ctagagtact ttctagaatc tttagtcagt ttctgtaaga tgaacgtgac 720 taggaaactt atactgttgc aatcctctga cattctctga ttgaaactcg gtttccaaaa 780 atcatatgtt actaaacaaa acatatctaa ccaaatacta tgtggtagtg tagatttata 840 tgctgtgtac tgaaagtgac gtcaagtata gtagtggcag agactcaaaa gatacctgcg 900 gattctgaat accacaacca taaaaaacag gatgatgtta tacttgtccc cttccatgat 960 acaggactgt ttagtaattt cccaaacagc ccataataca ttctgcaccc tttattaaac 1020 ctctactagc tacaacatct tactccatct tgtctagttg gacaagttct ctctttcttg 1080 gctgactcca acttactaca ccgcaacttc ttgtgccctt gttccaacca tcacaattga 1140 g 1141
3-85	**Sequences**	

3-85-1	Sequence Number [ID]	85
3-85-2	Molecule Type	DNA
3-85-3	Length	4438
3-85-4	Features	**source 1..4438**
	Location/Qualifiers	mol_type=genomic DNA
		organism=Saccharum hybrid
	NonEnglishQualifier Value	
3-85-5	Residues	aagtacaaac gtagactctg acatacacgc acgtagactc tgacatacac gcataaacga 60 acgaagaatg ttattattta tgttttgagt gggaatattt ggtactgcta tgattcacgt 120 gtgtaaggaa ggattcaaga agaaaggatg cgtttagttc gcgaaaattt ttgactttta 180 ccactatagc actttcgttt gtatttgtta attagtgtcc aatcatggac taattagact 240 caaaagatcc gtctcgtggt tttaaaccaa actgtgtaat taatttttt tatctatatt 300 taatgctcca tatatgtgtc aaatattcga tataacgaag aatcttgaaa atttttagga 360 actaaacatg gccaaagtgt tgtcccgact gagaaacttt ggaagcagaa taaaggctca 420 aaggaacatt taaaagaaga ggatgatata taatcaaaag tggcgacaaa gaagtgtgta 480 cgacccactc gagattgacg aaggacagct tctttgttct tttgtgtgtt actgaatatg 540 taataatctt gtatagattg gtttttaaaa tacggtggca aattaaagac gatatcactt 600 acaaagacat ggacaatgtg gaggggccaa aagttatata aacgacacgc cgaatcggtg 660 atgaacacca catgcctccc ataaagacgg tgtatcaatc tttgatataa tgggtatccg 720 tttgaggcgg catttatact tgatctagtg aaattacaag gagaggaaaa gaagtttaag 780 agaatgataa tgataatgaa aaaaatcgga ggaaaaagaa catgaacaaa gcaagaggag 840 atagccgtgc acacaaaata gagataattt cctcttagaa ctatgaaaac ttcctcttct 900 ttctgcaaca ctgatttgag tttttgttct ctatctagca tttcagtcca tcttgatgtc 960 aagtgacatg taaaaagacg tattgccccc attgctgttt taaattgtct ccacacttga 1020 caacaattta atgagttgtt aaaatattat gtgtgtttat ggccaattat acttttagt 1080 tttgagtttt tcatgaagtc attaagatgc taaaaataat ataaagttgt caatgcttgt 1140 cggaagcccc aatatgtgac taaaatgctg ctaaaagttt atagcatttt tttaaaaatc 1200 taaacaaatt gaaaaaagaa atccaaacta gaaattgtag atcttatcga aaactataag 1260 ttttatataa aaggcgactt tatctaacac cacacaagaa agatgtgctt tttctaagaa 1320 gacaagtctt agtatgtgat taatatgcta ctgaaaattt atattatttt taagcatttt 1380 aataacctca aatggaaaca tacaaaacta agttgcagat cttatcaaga gctataattt 1440 ttatataaaa tgtatattta aataacacca tacaagaaag atatatgatt ttttctaaga 1500 cgacaaagct ttgtatgcaa tttaatatgt tgctaaaaaa tcatattatt ttttttatca 1560 tcttaacgtc ctcaaataaa aaaaaatcag actagttggt atagacctca tcgaggctac 1620 aatttttata aaaactcaac ttcatccggt gttgtataaa aatgatataa tttttcctag 1680 atagagcgtt gccataagtg tattttggtc aagaaatata tgtatactta ttaatgaaat 1740 cctaacaaaa tatactttaa aatctgacgg aaatgttgga taggaaacaa aagcttaaat 1800 caatgctaaa tagggaagtt ttcatcatag ttataatgag tgatttctcc acaaaatatg 1860 atgtaccaca tgttaaatat tactcgcgca caaataatca gagcatatta ctttcatagc 1920 gtggtcgtgg ccatggccta gacttggttg tggacgtctc acttcaccaa ttgatagaaa 1980 aaaaacattt ataagaaaga aaagatacag aaaccatcac acgcgacaac atgacttgcc 2040 gaaacacaaa accaaaaccc aaactcgaga agatgctttc gagaaaaagc ctgaaaagaa 2100 aaaaaatttg cacgtaaaat caaattcgga cggcgaagag ggcaaacgag acagacaact 2160 gggtccactt gctgataaaa aagagagaga ggagggccca cttgccggcg ggcacccctc 2220 agactgtctc caacaatact gacgcaaaca gaagacgcat tggatgcaat gcgttgcgct 2280 gtggcaaaaa attaggtacc tatttctagt gtattccaac agagaacgca aaagaagatg 2340 ccgtactgcg ccatgcattc atgtgggacc ggggaggatg cgggcaacag cagtttgcac 2400 gacccattgg ccggagcatg cgacgtatat ttgcgttgcg cctcgcttcc tacgcaaaat 2460 gtgtcgttgg tatgcctacc ctgttggagg gcgttttctt ctgctaaagt aacgtggagc 2520 acgcatttgc gtaggctgtt ggagatagtc tcaccacgcg gtgaccggac caggccaatt 2580 cccgagccca aaaagaaaaa agcacacaca cagagacaca cgctctcgct ctcgcctccc 2640 tgacgctgga tttaagcaga gcagggagca gaggtgcaac cgcccaccac gatctcccct 2700 cccgcacgcc ccgcgggcag acccagccaa ggcaaggcag ccgcgaaccg gagcacgccg 2760 gccggtgtcg cctcccgcgc cggcggcctg ctgctcgctc gccctcgctt ccgcattgga 2820 tcacgcggcg gttggcgact tggtggtgtc tgctgctggt gattgcgcct agccggccga 2880 cgcggagacg gtgaggcgct gctcttcgct tctctcccca ctgctcccct cagcggtttc 2940 tctctccctg ttatgcgtgg aggagccctg cccccgcgga acggaagcct ccgccggatc 3000 tctgttacgc cgcggttact gcctcgccct ggatttgaac ttgtttcgta attttccctt 3060 gctgcgcttc tcgatttcgg ggaggggttc tgccggcagc tctgccgctc cacctgactt 3120 ggggaccttt ctatgttccg cgacagcagc attgatgatc tgcttgtctc ttgagttttt 3180 ttttcgtgcg atgcatcgag cgcgtgggga cacgatcacg cctgatgggc ggtagtccgc 3240 gatccgcatt tctgaatccc ggcgcctagc cgaggtgcct cggtgcttcc tggttgcctt 3300 gctgctattc ccttcttcgg atccgctctc gtacggctgg cacggtggtt gcggccttag 3360 aatttcgtgg cggcggtttg gttggattgg tgatgctgct ccgtccgcat ttatgaagga 3420 atgttctcca aacttttaag ctgctcgtgt actcggagta ttgaattgcc tgttccttgc 3480 cgctatagga ggccctgggc cagcctaccc cgctttgggt tgtgattggt gatttccggc 3540 agctgttatt gtttcatgat tcgtgtgggg aaaaaaagtt tttttggttc acgagtggtt 3600 tctggtgcat gttttgacaa gttttctatg atgctggtac tgtctttacc cctgctagag 3660 tagtttggtg gtgcgttttc ctattaggtg ggaatttaat cactttccca ctttatcgta 3720 tctctactat ggtaaccatc ttttggcaat tttgattggt atagtcatgt ttaagataag 3780 cttttgaatt caatgatctt gccgttcatt agctagcact taattttgta gagctgcttg 3840 gatcaccaaa gtgccgctca atcttgttca agtgcctatg atatatggga ttctgatgga 3900 actcttagca gtcgtgtcct taggcagtcg gcaccttgat aaggttccaa gagttcaatc 3960 ttacggaaga aatagtgagc ttgatctgag ttcagatcgg ttgtcttcac acttcacgat 4020 taattaccac gtttttaagg tgtgcattct cacttcttta cttccatcgt caatcttctt 4080

		aactggttgg gttggaggtg tggtcatgca cccaaccaca taggttgagt cctcttcaac 4140 tcgaatttag gtgcctattt ttttcttaat aaaaaaggcc acctgattct ccttggttgg 4200 tcacattttt ttcttaataa aaaaaggcca cctcaatgtt tctcctttta gcttgagcac 4260 tttttctgga tctcctcttt cttcttaatt ctgatccaag tgtcatcagc gttatattta 4320 tttgaacctg cttgcttttg taagcctgat cagtttgcaa aagttactag aacaatttaa 4380 ccatctgtgc ttgttatttc tgcaggcatc aagtttctaa caatttgaag tacctaaa 4438
3-86	**Sequences**	
3-86-1	Sequence Number [ID]	86
3-86-2	Molecule Type	AA
3-86-3	Length	145
3-86-4	Features	**source 1..145**
	Location/Qualifiers	mol_type=protein
		organism=Sesamum indicum
	NonEnglishQualifier Value	
3-86-5	Residues	MAEHYGQQQQ TRAPHLQLQP RAQRVVKAAT AVTAGGSLLV LSGLTLAGTV IALTIATPLL 60 VIFSPVLVPA VITIFLLGAG FLASGGFGVA ALSVLSWIYR YLTGKHPPGA DQLESAKTKL 120 ASKAREMKDR AEQFSQQPVA GSQTS 145
3-87	**Sequences**	
3-87-1	Sequence Number [ID]	87
3-87-2	Molecule Type	AA
3-87-3	Length	382
3-87-4	Features	**source 1..382**
	Location/Qualifiers	mol_type=protein
		organism=Cinnamomum camphora
	NonEnglishQualifier Value	
3-87-5	Residues	MATTSLASAF CSMKAVMLAR DGRGMKPRSS DLQLRAGNAQ TSLKMINGTK FSYTESLKKL 60 PDWSMLFAVI TTIFSAAEKQ WTNLEWKPKP NPPQLLDDHF GPHGLVFRRT FAIRSYEVGP 120 DRSTSIVAVM NHLQEAALNH AKSVGILGDG FGTTLEMSKR DLIWVVKRTH VAVERYPAWG 180 DTVEVECWVG ASGNNGRRHD FLVRDCKTGE ILTRCTSLSV MMNTRTRRLS KIPEEVRGEI 240 GPAFIDNVAV KDEEIKKPQK LNDSTADYIQ GGLTPRWNDL DINQHVNNIK YVDWILETVP 300 DSIFESHHIS SFTIEYRREC TMDSVLQSLT TVSGGSSEAG LVCEHLLQLE GGSEVLRAKT 360 EWRPKLTDSF RGISVIPAES SV 382
3-88	**Sequences**	
3-88-1	Sequence Number [ID]	88
3-88-2	Molecule Type	AA
3-88-3	Length	417
3-88-4	Features	**source 1..417**
	Location/Qualifiers	mol_type=protein
		organism=Cocos nucifera
	NonEnglishQualifier Value	
3-88-5	Residues	MVASVAASAF FPTPSFSSTA SAKASKTIGE GSESLDVRGI VAKPTSSSAA MQGKVKAQAV 60 PKINGTKVGL KTESQKAEED AAPSSAPRTF YNQLPDWSVL LAAVTTIFLA AEKQWTLLDW 120 KPRRPDMLTD AFSLGKIVQD GLIFRQNFSI RSYEIGADRT ASIETLMNHL QETALNHVRN 180 AGLLGDGFGA TPEMSKRNLI WVVTKMQVLV EHYPSWGDVV EVDTWVGASG KNGMRRDWHV 240 RDYRTGQTIL RATSVWVMMN KHTRKLSKMP EEVRAEIGPY FVEHAAIVDE DSRKLPKLDD 300 DTADYIKWGL TPRWSDLDVN QHVNNVKYIG WILESAPISI LENHELASMT LEYRRECGRD 360 SVLQSLTAIS NDCTGGLPEA SIECQHLLQL ECGAEIVRGR TQWRPRRASG PTSAGSA 417
3-89	**Sequences**	
3-89-1	Sequence Number [ID]	89
3-89-2	Molecule Type	AA
3-89-3	Length	423
3-89-4	Features	**source 1..423**
	Location/Qualifiers	mol_type=protein
		organism=Cocos nucifera
	NonEnglishQualifier Value	
3-89-5	Residues	MVASIAASAF FPTPSSSSSA ASAKASKTIG EGPGSLDVRG IVAKPTSSSA AMQEKVKVQP 60 VPKINGAKVG LKVETQKADE ESSPSSAPRT FYNQLPDWSV LLAAVTTIFL AAEKQWTLLD 120 WKPRRPDMLA DAFGLGKIVQ DGLVFKQNFS IRSYEIGADR TASIETLMNH LQETALNHVK 180 SAGLMGDGFG ATPEMSKRNL IWVVTKMRVL IERYPSWGDV VEVDTWVGPT GKNGMRRDWH 240 VRDHRSGQTI LRATSVWVMM NKNTRKLSKV PEEVRAEIGP YFVERAAIVD EDSRKLPKLD 300 EDTTDYIKKG LTPRWGDLDV NQHVNNVKYI GWILESAPIS ILENHELASM SLEYRRECGR 360 DSVLQSLTAV SNDLTDGLVE SGIECQHLLQ LECGTELVKG RTEWRPKHSP ALGNMGPTPG 420 GSA 423
3-90	**Sequences**	
3-90-1	Sequence Number [ID]	90
3-90-2	Molecule Type	AA
3-90-3	Length	414
3-90-4	Features	**source 1..414**
	Location/Qualifiers	mol_type=protein
		organism=Cocos nucifera
	NonEnglishQualifier Value	

3-90-5	Residues	MVASVAASSS FFPVPSSSSS ASAKASRGIP DGLDVRGIVA KPASSSGWMQ AKASARAIPK 60 IDDTKVGLRT DVEEDAASTA RRTSYNQLPD WSMLLAAIRT IFSAAEKQWT LLDSKKRGAD 120 AVADASGVGK MVKNGLVYRQ NFSIRSYEIG VDKRASVEAL MNHFQETSLN HCKCIGLMHG 180 GFGCTPEMTR RNLIWVVAKM LVHVERYPWW GDVVQINTWI SSSGKNGMGR DWHVHDCQTG 240 LPIMRGTSVW VMMDKHTRRL SKLPEEVRAE ITPFFSERDA VLDDNGRKLP KFDDDSAAHV 300 RRGLTPRWHD FDVNQHVNNV KYVGWILESV PVWMLDGYEV ATMSLEYRRE CRMDSVVQSL 360 TAVSSDHADG SPIVCQHLLR LEDGTEIVRG QTEWRPKQQA RDLGNMGLHP TESK 414
3-91	**Sequences**	
3-91-1	Sequence Number [ID]	91
3-91-2	Molecule Type	AA
3-91-3	Length	419
3-91-4	Features	**source 1..419**
	Location/Qualifiers	mol_type=protein
		organism=Cuphea lanceolata
	NonEnglishQualifier Value	
3-91-5	Residues	MVAAAATSAF FPVPAPGTSP KPGKSGNWPS SLSPTFKPKS IPNAGFQVKA NASAHPKANG 60 SAVNLKSGSL NTQEDTSSSP PPRAFLNQLP DWSMLLTAIT TVFVAAEKQW TMLDRKSKRP 120 DMLVDSVGLK SIVRDGLVSR QSFLIRSYEI GADRTASIET LMNHLQETSI NHCKSLGLLN 180 DGFGRTPGMC KNDLIWVLTK MQIMVNRYPT WGDTVEINTW FSQSGKIGMA SDWLISDCNT 240 GEILIRATSV WAMMNQKTRR FSRLPYEVRQ ELTPHFVDSP HVIEDNDQKL HKFDVKTGDS 300 IRKGLTPRWN DLDVNQHVSN VKYIGWILES MPIEVLETQE LCSLTVEYRR ECGMDSVLES 360 VTAVDPSENG GRSQYKHLLR LEDGTDIVKS RTEWRPKNAG TNGAISTSTA KTSNGNSAS 419
3-92	**Sequences**	
3-92-1	Sequence Number [ID]	92
3-92-2	Molecule Type	AA
3-92-3	Length	419
3-92-4	Features	**source 1..419**
	Location/Qualifiers	mol_type=protein
		organism=Cuphea viscosissima
	NonEnglishQualifier Value	
3-92-5	Residues	MVAAAATSAF FPVPAPGTSP KPGKSGNWPS SLSPTFKPKS IPNGGFQVKA NASAHPKANG 60 SAVNLKSGSL NTQEDTSSSP PPRAFLNQLP DWSMLLTAIT TVFVAAEKQW TMLDRKSKRP 120 DMLVDSVGLK SIVRDGLVSR HSFSIRSYEI GADRTASIET LMNHLQETTI NHCKSLGLHN 180 DGFGRTPGMC KNDLIWVLTK MQIMVNRYPT WGDTVEINTW FSQSGKIGMA SDWLISDCNT 240 GEILIRATSV WAMMNQKTRR FSRLPYEVRQ ELTPHFVDSP HVIEDNDQKL RKFDVKTGDS 300 IRKGLTPRWN DLDVNQHVSN VKYIGWILES MPIEVLETQE LCSLTVEYRR ECGMDSVLES 360 VTAVDPSENG GRSQYKHLLR LEDGTDIVKS RTEWRPKNAG TNGAISTSTA KTSNGNSVS 419
3-93	**Sequences**	
3-93-1	Sequence Number [ID]	93
3-93-2	Molecule Type	AA
3-93-3	Length	382
3-93-4	Features	**source 1..382**
	Location/Qualifiers	mol_type=protein
		organism=Umbellularia californica
	NonEnglishQualifier Value	
3-93-5	Residues	MATTSLASAF CSMKAVMLAR DGRGMKPRSS DLQLRAGNAP TSLKMINGTK FSYTESLKRL 60 PDWSMLFAVI TTIFSAAEKQ WTNLEWKPKP KLPQLLDDHF GLHGLVFRRT FAIRSYEVGP 120 DRSTSILAVM NHMQEATLNH AKSVGILGDG FGTTLEMSKR DLMWVVRRTH VAVERYPTWG 180 DTVEVECWIG ASGNNGMRRD FLVRDCKTGE ILTRCTSLSV LMNTRTRRLS TIPDEVRGEI 240 GPAFIDNVAV KDDEIKKLQK LNDSTADYIQ GGLTPRWNDL DVNQHVNNLK YVAWVFETVP 300 DSIFESHHIS SFTLEYRREC TRDSVLRSLT TVSGGSSEAG LVCDHLLQLE GGSEVLRART 360 EWRPKLTDSF RGISVIPAEP RV 382
3-94	**Sequences**	
3-94-1	Sequence Number [ID]	94
3-94-2	Molecule Type	AA
3-94-3	Length	308
3-94-4	Features	**source 1..308**
	Location/Qualifiers	mol_type=protein
		organism=Cocos nucifera
	NonEnglishQualifier Value	
3-94-5	Residues	MDASGASSFL RGRCLESCFK ASFGMSQPKD AAGQPSRRPA DADDFVDDDR WITVILSVVR 60 IAACFLSMMV TTIVWNMIML ILLPWPYARI RQGNLYGHVT GRMLMWILGN PITIEGSEFS 120 NTRAIYICNH ASLVDIFLIM WLIPKGTVTI AKKEIIWYPL FGQLYVLANH QRIDRSNPSA 180 AIESIKEVAR AVVKKNLSLI IFPEGTRSKT GRLLPFKKGF IHIALQTRLP IVPMVLTGTH 240 LAWRKNSLRV RPAPITVKYF SPIKTDDWEE EKINHYVEMI HALYVDHLPE SQKPLVSKGR 300 DASGRSNS 308
3-95	**Sequences**	
3-95-1	Sequence Number [ID]	95
3-95-2	Molecule Type	AA
3-95-3	Length	356
3-95-4	Features	**source 1..356**

	Location/Qualifiers	mol_type=protein
		organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-95-5	Residues	MDVASARSIS SHPSYYGKPI CSSQSSLIRI SRDKVCCFGR ISNGMTSFTT SLHAVPSEKF 60 MGETRRTGIQ WSNRSLRHDP YRFLDKKSPR SSQLARDITV RADLSGAATP DSSFPEPEIK 120 LSSRLRGIFF CVVAGISATF LIVLMIIGHP FVLLFDPYRR KFHHFIAKLW ASISIYPFYK 180 INIEGLENLP SSDTPAVYVS NHQSFLDIYT LLSLGKSFKF ISKTGIFVIP IIGWAMSMMG 240 VVPLKRMDPR SQVDCLKRCM ELLKKGASVF FFPEGTRSKD GRLGSFKKGA FTVAAKTGVA 300 VVPITLMGTG KIMPTGSEGI LNHGNVRVII HKPIHGSKAD VLCNEARSKI AESMDL 356
3-96	**Sequences**	
3-96-1	Sequence Number [ID]	96
3-96-2	Molecule Type	DNA
3-96-3	Length	1539
3-96-4	Features	**misc_feature 1..1539**
	Location/Qualifiers	note=Codon optimised sequence
		source 1..1539
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-96-5	Residues	atggctgtgt ccaagaaccc agagactctc gctccagatc aagagccatc caaagagtct 60 gatcttaggc gtaggccagc ttcctctcca tcttctactg ctgcttctcc agctgtgcca 120 gattcctcat ctaggacttc cagttccatc actggctctt ggactactgc tctcgatggt 180 gattctggtg ctggtgctgt taggatcgga gatccaaagg ataggatcgg cgaggctaac 240 gatatcggcg aaaagaaaaa ggcttgctcc ggtgaggttc cagtgggatt tgttgataga 300 ccatctgctc cagtgcacgt gagagttgtt gagtctcctc tctcctccga tacaatcttc 360 cagcagtctc acgctggact cctcaatctt tgcgtggtgg tgcttatcgc tgtgaactcc 420 aggctcatta tcgagaacct tatgaagtac ggcctcctca tcggctccgg atttttcttc 480 tcatctcgtt tgctcaggga ttggcctctc cttatctgct ctcttactct cccagtgttc 540 ccactcggat cctacatggt tgagaagctc gcttacaaga agttcatctc cgagccagtg 600 gtggtgtctc ttcacgtgat cctcatcatt gctactatca tgtaccctgt gttcgtgatt 660 ctcaggtgcg attccccaat cctctccgga atcaacctca tgcttttcgt gtcctccatc 720 tgcctcaagc tcgtttctta cgctcacgct aactacgatc tcaggtcctc ctccaactcc 780 atcgataagg gaatccacaa gtcccagggc gtgtccttca agtctctcgt gtactttatc 840 atggctccaa cactctgcta ccagccatct tacccaagga ctacttgcat taggaagggc 900 tgggttatct gccagcttgt gaagctcgtg atcttcactg gtgtgatggg cttcatcatc 960 gagcagtaca tcgatccaat catcaagaac tcccagcacc cactcaaggg aaacgtgttg 1020 aacgctatgg aaagggtgct caagctctcc atcccaacac tttacgtgtg gctctgcgtg 1080 ttctactgca ctttccacct ctggctcaat atcctcgctg agcttctttg cttcggcgat 1140 cgtgagttct acaaggattg gtggaacgct aagactatcg aagagtactg gcgtatgtgg 1200 aacatgccag tgcacaagtg gatgcttagg cacgtttacc tcccatgcat ccgtaacggt 1260 attccaaagg gtgtggctat ggtgatctcc ttcttcatct ctgctatctt ccacgagttg 1320 tgcatcggaa tcccatgcca catcttcaag ttctgggctt tcatcggcat catgttccag 1380 gtgccactcg ttatcctcac taagtacctc cagaacaagt tcaagtccgc tatggtgggc 1440 aacatgattt tctggttctt tttctcaatc tacggccagc caatgtgcgt gctcctttac 1500 taccacgatg tgatgaatag gaaggtgggc actgagtaa 1539
3-97	**Sequences**	
3-97-1	Sequence Number [ID]	97
3-97-2	Molecule Type	AA
3-97-3	Length	371
3-97-4	Features	**source 1..371**
	Location/Qualifiers	mol_type=protein
		organism=Cocos nucifera
	NonEnglishQualifier Value	
3-97-5	Residues	MVELRSSSSE MDLDRPNIEE YLTTDSIQES PKKLHLRDLL DISPTLTEAT GAIVDDSFTR 60 CFKSNPPEPW NWNVYLFPLW CLGVIIRYGI LFPLRVAILT AGWLVFFAAF IPVHFLLTAH 120 NKWRRKIERK LVEMICSVFV ASWTGVVKYH GPRPSMRPQQ VFVANHTSMI DFIILEQMTA 180 FAVIMQKHPG WVGFIQKTIL EGVGCIWFNR TESKDREVVA RKLREHIHGA DNNPLLIFPE 240 GTCVNNHYTV MFKKGAFELG CAVCPVAIKY NKIFVDAFWN SKKQSFTMHL FHLMTSWAVV 300 CDVWYLEPQY IRPGETPIEF AERVRDMISV RAGLKKVPWD GYLKYFRPSP KLTERKQQIF 360 AESVLQRLEE K 371
3-98	**Sequences**	
3-98-1	Sequence Number [ID]	98
3-98-2	Molecule Type	AA
3-98-3	Length	376
3-98-4	Features	**source 1..376**
	Location/Qualifiers	mol_type=protein
		organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-98-5	Residues	MSSTAGRLVT SKSELDLDHP NIEDYLPSGS SINEPRGKLS LRDLLDISPT LTEAAGAIVD 60 DSFTRCFKSN PPEPWNWNIY LFPLYCFGVV VRYCILFPLR CFTLAFGWII FLSLFIPVNA 120 LLKGQDRLRK KIERVLVEMI CSFFVASWTG VVKYHGPRPS IRPKQVYVAN HTSMIDFIVL 180 EQMTAFAVIM QKHPGWVGLL QSTILESVGC IWFNRSEAKD REIVAKKLRD HVQGADSNPL 240

		LIFPEGTCVN NNYTVMFKKG AFELDCTVCP IAIKYNKIFV DAFWNSRKQS FTMHLLQLMT 300 SWAVVCEVWY LEPQTIRPGE TGIEFAERVR DMISLRAGLK KVPWDGYLKY SRPSPKHSER 360 KQQSFAESIL ARLEEK 376
3-99	**Sequences**	
3-99-1	Sequence Number [ID]	99
3-99-2	Molecule Type	AA
3-99-3	Length	371
3-99-4	Features	**source 1..371**
	Location/Qualifiers	mol_type=protein
		organism=Elaeis guineensis
	NonEnglishQualifier Value	
3-99-5	Residues	MVELRSSSSE MDLDRPNIEE YLPPTPSKNP PKKLHLRDLL DISPTLTEAA GAIVDDSFTR 60 CFKSNPPEPW NWNVYLFPLW CLGVIIRYGI LFPLRVAILT AGWLVFFAAF IPVHFLLTAH 120 NKWRRKIERK LVEMICSVFV ASWTGVVKYH GPRPSMRPQQ VFVANHTSMI DFIILEQMTA 180 FAVIMQKHPG WVGFIQKTIL EGVGCIWFNR TESKDREVVA RKLREHIHGA DNNPLLIFPE 240 GTCVNNHYTV MFKKGAFELG CAVCPVAIKY NKIFVDAFWN SKKQSFTMHL FHLMTSWAVV 300 CDVWYLEPQY IRPGETPIEF AERVRDMISI RAGLKKVPWD GYLKYFRPSP KLTERKQQIF 360 AESVLQRLEE K 371
3-100	**Sequences**	
3-100-1	Sequence Number [ID]	100
3-100-2	Molecule Type	AA
3-100-3	Length	371
3-100-4	Features	**source 1..371**
	Location/Qualifiers	mol_type=protein
		organism=Phoenix dactylifera
	NonEnglishQualifier Value	
3-100-5	Residues	MVGLRSSSSE MDLDRPNIEE YLTTDSIEES PKKLHLRDLL DISPTLTEAA GAIVDDSFTR 60 CFKSNPPEPW NWNVYLFPLW CLGVIIRYGI LFPLRVAVLT AGWLVFFAAF IPAHFLLTAH 120 NKWRRKIERK LVEMICSVFV ASWTGVVKYH GPRPSMRPQQ VFVANHTSMI DFIILEQMTA 180 FAVIMQKHPG WVGFIQKTIL EGVGCIWFNR TESKDREVVA RKLREHIQGA DNNPLLIFPE 240 GTCVNNHYTV MFKKGAFELG CAVCPVAIKY NKIFVDAFWN SKKQSFTMHL FHLMTSWAVV 300 CDVWYLEPQY IRPGETPIEF AERVRDMISV RAGLRKVPWD GYLKYFRPSP KLTERKQQIF 360 AESVLQRLEE K 371
3-101	**Sequences**	
3-101-1	Sequence Number [ID]	101
3-101-2	Molecule Type	AA
3-101-3	Length	371
3-101-4	Features	**source 1..371**
	Location/Qualifiers	mol_type=protein
		organism=Musa acuminata
	NonEnglishQualifier Value	
3-101-5	Residues	MAGLATSSTE MDLDRPNIDE YLTVESIREA PKKLHLRDLL DISPTLKEAA GAIVDDSFTR 60 CFKSNPSEPW NWNIYLFPLW CLGVVIRYGI LFPFRVIILV AGWIVFFAAF SLVHFLLGEH 120 NKWKREIERK LVEMICSVFV ASWTAVIKYH GPRPSMRPQQ VFVANHTSMI DFIILEQMTA 180 FAVIMQKHPG WVGFIQKIIV ESLGCIWFNR TEAKDREIVA RKLREHIQGV DNNPLLIFPE 240 GTCVNNHYTV MFKKGAFELG CAVCPVAIKY NKIFVDAFWN SKKQSFTMHL VQLMTSWAVV 300 CDVWYLEPQY IRPGETPIEF AERVQDMISV RAGLKKVPWD GYLKYFRPSP KLIERKQQIF 360 AESVLQRLEE K 371
3-102	**Sequences**	
3-102-1	Sequence Number [ID]	102
3-102-2	Molecule Type	AA
3-102-3	Length	372
3-102-4	Features	**source 1..372**
	Location/Qualifiers	mol_type=protein
		organism=Ananas comosus
	NonEnglishQualifier Value	
3-102-5	Residues	MAEALGSSSA EMDLDRPNLE EYLPTDSIQD SPKNLHLRDL LDISPTLTEA AGAIVDDSFT 60 RCFKSNPPEP WNWNIYLFPL WCLGVVVRYG ILFPLRVAVL AIGWIVFFSA FFPVHFLLKG 120 YPKWRRKLER KLVEMMCSVF VASWTGVVKY HGPRPSTRPH QVFVANHTSM IDFIILEQMT 180 AFAVIMQKHP GWVGFIQKTI LESVGCIWFN RTESKDRGVV GRKLREHVQG VDNNPLLIFP 240 EGTCVNNHYT VMFKKGAFEL GCAVCPIAIK YNKIFVDAFW NSKKQSFTMH LVRLMTSWAV 300 VCDVWYLEPQ YLRPGETPIE FAERVRDMIS ARAGLKKVPW DGYLKYFRPS PKHTERKQQI 360 FAESILRRLE RK 372
3-103	**Sequences**	
3-103-1	Sequence Number [ID]	103
3-103-2	Molecule Type	AA
3-103-3	Length	370
3-103-4	Features	**source 1..370**
	Location/Qualifiers	mol_type=protein
		organism=Asparagus officinalis
	NonEnglishQualifier Value	

3-103-5	Residues	MAGLESSSAG IDVDPPNIED YLTSDALHQP HKKLQLKDLL DISPTLTEAA GAIVDDSFTR 60 CFKSNPPEPW NWNVYLFPLW CLGVVVRYGI LFPLRVMTLA AGWIVFFSAF LPVHYLMKGQ 120 NKWKNNIERK LVEMICSVFV ASWTGVVRYH GPRPSMRPQQ VFVANHTSMI DFIILEQMAA 180 FAVIMQKHPG WVGFLQTTIL ESIGSIWFNR TEAKDREVVA RKLREHTEGD NNPLLIFPEG 240 TCVNNDYTVM FKKGAFELGC AVCPVAIKYN KIFVDAFWNS KKQSFTMHLM RLMTSWAVVC 300 DVWYLEPQYL KPGETSIEFA ERVRDMISVR AGLRKVPWDG YLKYFRPSPK LTERKQQIFA 360 ESVLRRLEEK 370
3-104	**Sequences**	
3-104-1	Sequence Number [ID]	104
3-104-2	Molecule Type	AA
3-104-3	Length	370
3-104-4	Features	**source 1..370**
	Location/Qualifiers	mol_type=protein
		organism=Oryza brachyantha
	NonEnglishQualifier Value	
3-104-5	Residues	MASSSVAGDI ELDRPNLEDY LPPDSLPQES PGNLHLRDLL DISPVLTEAA GAAVDDSFTR 60 CFKSNSPEPW NWNIYLFPLW CLGVVIRYGI LFPLRGLTLL VGWIAFFAAF FSVHFLFKGQ 120 KMRSKIERKL VEMMCSVFVA SWTGVIKYHG PRPSTRPHQV FVANHTSMID FIILEQMTAF 180 AVIMQKHPGW VGFIQKTILE SVGCIWFNRN DLKDREVVAK KLRDHVQHPD NNPLLIFPEG 240 TCVNNQYTVM FKKGAFELGC AVCPIAIKYN KIFVDAFWNS KKQSFTMHLV RLMTSWAVVC 300 DVWYLEPQYL KEGETAIQFA ERVRDMIAAR AGLKKVPWDG YLKHNRPSPK HTEEKQRIFA 360 DSVLQRLEES 370
3-105	**Sequences**	
3-105-1	Sequence Number [ID]	105
3-105-2	Molecule Type	AA
3-105-3	Length	370
3-105-4	Features	**source 1..370**
	Location/Qualifiers	mol_type=protein
		organism=Oryza sativa
	NonEnglishQualifier Value	
3-105-5	Residues	MATSSVAGDI ELDRPNLEDY LPSDSLPQEF PRNLHLRDLL DISPVLTEAA GAIVDDSFTR 60 CFKSNSPEPW NWNIYLFPLW CLGVVIRYGI LFPLRGLTLL VGWLAFFAAF FPVHFLLKGQ 120 KMRSKIERKL VEMMCSVFVA SWTGVIKYHG PRPSTRPHQV FVANHTSMID FIILEQMTAF 180 AVIMQKHPGW VGFIQKTILE SVGCIWFNRN DLKDREVVAK KLRDHVQHPD SNPLLIFPEG 240 TCVNNQYTVM FKKGAFELGC AVCPIAIKYN KIFVDAFWNS KKQSFTMHLV RLMTSWAVVC 300 DVWYLEPQYL RDGETAIEFA ERVRDMIAAR AGLKKVPWDG YLKHNRPSPK HTEEKQRIFA 360 DSVLRRLEES 370
3-106	**Sequences**	
3-106-1	Sequence Number [ID]	106
3-106-2	Molecule Type	AA
3-106-3	Length	363
3-106-4	Features	**source 1..363**
	Location/Qualifiers	mol_type=protein
		organism=Nelumbo nucifera
	NonEnglishQualifier Value	
3-106-5	Residues	MDLDRPNIEE YLPSEAIQES NEKLHLRDLL DISPTLTEAA GAIVDDSFTR CFKSNPSEPW 60 NWNVYLFPLW CFGVVVRYGI LFPVRVLVLT IGWIIFLSSF IPAHFLLRSH DKWRKKIERY 120 LVELICSFFV ASWTGVVKYH GPRPSMRPKQ VFVANHTSMI DFIVLEQMTA FAVIMQKHPG 180 WVGLLQSTIL ESVGCIWFNR AEAKDREIVA RKLRDHIQGV DNNPLLIFPE GTCVNNHYTV 240 MFKKGAFELG CTVCPIAIKY NKIFVDAFWN SKKQSFTMHL LHLMTSWAVV CDVWYLEPQN 300 IRPGETPIEF AERVRDIISV RAGLKKVPWD GYLKYSRPSP KHRERKQQRF VESVLQRLEK 360 KGK 363
3-107	**Sequences**	
3-107-1	Sequence Number [ID]	107
3-107-2	Molecule Type	AA
3-107-3	Length	376
3-107-4	Features	**source 1..376**
	Location/Qualifiers	mol_type=protein
		organism=Vitis vinifera
	NonEnglishQualifier Value	
3-107-5	Residues	MANAPDNKLT SSSSELDLDR PNLEDYLPSG SMQEPRGKLR LRDLLDISPT LTEAAGAIVD 60 DSFTRCFKSN PPEPWNWNVY LFPLWCLGVV IRYGILFPTR VLVLTLGWII FLSSFIPVHF 120 LLKGNDKLRK KLERCLVELI CSFFVASWTG VVKYHGPRPS RRPQQVFVAN HTSMIDFIVL 180 EQMTAFAVIM QKHPGWVGLL QSTILESVGC IWFNRTEAKD REIVARKLRD HVQGADNNPL 240 LIFPEGTCVN NHYTVMFKKG AFELGCTVCP IAIKYNKIFV DAFWNSKKQS FTMHLLQLMT 300 SWAVVCDVWY LEPQTLKPGE TPIEFAERVR DIISLRAGLK KVPWDGYLKY SRPSPKHREQ 360 KQQSFADSVL RRLEEK 376
3-108	**Sequences**	
3-108-1	Sequence Number [ID]	108
3-108-2	Molecule Type	AA
3-108-3	Length	373

3-108-4	Features	**source 1..373**
	Location/Qualifiers	mol_type=protein
		organism=Nicotiana tomentosiformis
	NonEnglishQualifier Value	
3-108-5	Residues	MNMNKLKTSS SELDLDRPNL EDYLPTGSIP EPHGKLRLRD LIDISPTLTE AAGAIVDDSF 60 TRCFKSNPPE PWNWNIYLFP LWCLGVVVRY GILFPIRVIV LTIGWIIFLS CYIPVHFLLK 120 GHDKFRKKLE RCLVELICSF FVASWTGVVK YHGPRPSIRP KQVFVANHTS MIDFIVLEQM 180 TAFAVIMQKH PGWVGLLQST ILEGVGCIWF NRSEAKDREI VARKLRQHVE GADNNPLLIF 240 PEGTCVNNHY TVMFKKGAFE LGCTVCPVAI KYNKIFVDAF WNSRKQSFTM HLLQLMTSWA 300 VVCDVWYLEP QNIRPGETPI EFAERVRDII SARAGLKKVP WDGYLKYSRP SPKHRERKQQ 360 SFAESVLRRL EEK 373
3-109	**Sequences**	
3-109-1	Sequence Number [ID]	109
3-109-2	Molecule Type	AA
3-109-3	Length	375
3-109-4	Features	**source 1..375**
	Location/Qualifiers	mol_type=protein
		organism=Jatropha curcas
	NonEnglishQualifier Value	
3-109-5	Residues	MATPGKLKTS SSELDLDRPN IEDYLPSGVS IQEPRGKLRL RDLLDISPTL TEAAGAIVDD 60 TFTRCFKSNP PEPWNWNIYL FPLWCCGVVC RYGILFPIRV LVLTIGWIIF LSCYIPVHFL 120 LKGHDKLRKK LERCLVELIC SFFVASWTGV VKYHGPRPSI RPKQVFVANH TSMIDFIILE 180 QMTAFAVIMQ KHPGWVGLLQ STILESVGCI WFNRSEAKDR EIVTKKLRDH VQGADNNPLL 240 IFPEGTCVNN HYTVMFKKGA FELGCTVCPI AIKYNKIFVD AFWNSRKQSF TTHLLQLMTS 300 WAVVCDVWYL EPQNLKPGET PIEFAERVRD IISVRAGLKK VPWDGYLKYS RPSPKHRERK 360 QQSFAESVLQ RLEEK 375
3-110	**Sequences**	
3-110-1	Sequence Number [ID]	110
3-110-2	Molecule Type	AA
3-110-3	Length	376
3-110-4	Features	**source 1..376**
	Location/Qualifiers	mol_type=protein
		organism=Glycine max
	NonEnglishQualifier Value	
3-110-5	Residues	MNNSGTPKSS SSELDLDRPN IEDYLPSGST IQQEPHGKLF LHDLLNISPT LSEAAGAIVD 60 DSFTRCFKSN PPEPWNWNVY LFPLWCFGVV IRYLILFPIR VIGLTIGWII FLSSFIPVHF 120 LLKGHDKLRR SIERSLVEMM CSFFVASWTG VVKYHGPRPS RRPKQVFVAN HTSMIDFIIL 180 EQMTAFAVIM QKHPGWVGLL QSTILESLGC IWFNRTEAKD REIVARKLRD HVQGADNNPL 240 LIFPEGTCVN NHYTVMFKKG AFELGCTVCP VAIKYNKIFV DAFWNSRKQS FTMHLLQLMT 300 SWAVVCDVWY LEPQNLKPGE TPIEFAERVR DIISVRAGLK KVPWDGYLKY SRPSPKHRER 360 KQQNFAESVL RRWEEK 376
3-111	**Sequences**	
3-111-1	Sequence Number [ID]	111
3-111-2	Molecule Type	AA
3-111-3	Length	371
3-111-4	Features	**source 1..371**
	Location/Qualifiers	mol_type=protein
		organism=Sesamum indicum
	NonEnglishQualifier Value	
3-111-5	Residues	MSKLNTSSSE LDFDRPNIED YLPSGSIQEP HGKLRLRDLL DISPTLTEAA GAIVDDSFTR 60 CFKSNPPEPW NWNIYLFPLW CLGVVIRYGL LFPLRVIVLT IGWIIFLSCY FPVHFLLRGH 120 DKLRKRLERG LVELICSFFV ASWTGVVKYH GPRPSMRPKQ VFVANHTSMI DFIVLEQMTA 180 FAVIMQKHPG WVGLLQSTIL ESLGCIWFNR SESKDREIVA KKLREHVHDA DNNPLLIFPE 240 GTCVNNHYTV MFKKGAFELG CTVCPIAIKY NKIFVDAFWN SRKQSFTTHL LQLMTSWAVV 300 CDVWYLEPQN LKPGETPIEF AERVRDIISV RAGLRKVPWD GYLKYSRPSP KHRERKQQSF 360 AESILRRLEE K 371
3-112	**Sequences**	
3-112-1	Sequence Number [ID]	112
3-112-2	Molecule Type	AA
3-112-3	Length	364
3-112-4	Features	**source 1..364**
	Location/Qualifiers	mol_type=protein
		organism=Brachypodium distachyon
	NonEnglishQualifier Value	
3-112-5	Residues	MASSLDAPNL DDYLPTDSLP QEPPRSLNLR DLLDISPVLT EAAGAIVDDS FTRCFKSNSP 60 EPWNWNIYLF PLWCFGVVVR YGLLFPLRVL TLGLGWMVFF AAFFPVHFLL KGQNKLRSKI 120 ERKLVEMMCS VFVASWTGVI KYHGPRPSSR PYQVFVANHT SMIDFIILEQ MTAFAVIMQK 180 HPGWVGFIQK TILESVGCIW FNRNDLKDRE VVGRKLRDHV QRPDNNPLLI FPEGTCVNNQ 240 YTVMFKKGAF ELGCAVCPIA IKYNKIFVDA FWNSKKQSFT MHLGRLMTSW AVVCDVWFLE 300 PQYLREGETS IAFTERVRDM IAARAGLKKV LWDGYLKHNR PSPKHTEEKQ RIFAESVLKR 360 LEES 364

3-113	**Sequences**	
3-113-1	Sequence Number [ID]	113
3-113-2	Molecule Type	AA
3-113-3	Length	371
3-113-4	Features	**source 1..371**
	Location/Qualifiers	mol_type=protein
		organism=Setaria italica
	NonEnglishQualifier Value	
3-113-5	Residues	MASSSVAADM ELDRPNLEDY LPPDSLPQEA PRNLHLRDLL DISPVLTEAA GAIVDDSFTR 60 CFKSNSPEPW NWNIYLFPLW CLGVVIRYGI LFPLRSLTLA IGWLAFFAAF FPVHFLLKGQ 120 DKLRSKIERK LVEMMCSVFV ASWTGVIKYH GPRPSTRPHQ VFVANHTSMI DFIILEQMTA 180 FAVIMQKHPG WVGFIQKTIL ESVGCIWFNR NDLRDREVTA RKLRDHVQQP DKNPLLIFPE 240 GTCVNNQYTV MFKKGAFELG CAVCPIAIKY NKIFVDAFWN SKKQSFTMHL VRLMTSWAVV 300 CDVWYLPPQY LREGETAIAF AERVRDMIAA RAGLKKVPWD GYLKHNRPSP KHTEEKQRIF 360 AESVLMRLEE K 371
3-114	**Sequences**	
3-114-1	Sequence Number [ID]	114
3-114-2	Molecule Type	AA
3-114-3	Length	376
3-114-4	Features	**source 1..376**
	Location/Qualifiers	mol_type=protein
		organism=Cicer arietinum
	NonEnglishQualifier Value	
3-114-5	Residues	MNSTGTLKSS SSELDLDRPN IEDYLPSGAA IQQEPRGKLR LHDLLDISPT LSEAAGAIVD 60 DSFTRCFKSN PPEPWNWNIY LFPLWCFGVV VRYLILFPTR VLGLTLGWII FLSAFIPVHL 120 LLKGHDKLRR NIERSLVEMM CGFFVASWTG VVKYHGPKPS RRPKQVFVAN HTSMIDFIIL 180 EQMTAFAVIM QKHPGWVGLL QSTILESVGC IWFNRTEAKD REIVARKLRE HVQGADNNPL 240 LIFPEGTCVN NHYTVMFKKG AFELGCTVCP VAIKYNKIFV DAFWNSRKQS FTKHLLQLMT 300 SWAVVCDVWY LEPQNLKPGE TPIEFAERVR DIISHRAGLK KVPWDGYLKY SRPSPKHRER 360 KQQNFAESVL RRLEEK 376
3-115	**Sequences**	
3-115-1	Sequence Number [ID]	115
3-115-2	Molecule Type	AA
3-115-3	Length	371
3-115-4	Features	**source 1..371**
	Location/Qualifiers	mol_type=protein
		organism=Zea mays
	NonEnglishQualifier Value	
3-115-5	Residues	MASSSVAADM ELDRPNLEDY LPPDSLPQEA PRNLHLRDLL DISPVLTEAA GAIVDDSFTR 60 CFKSNSPEPW NWNIYLFPLW CFGVVIRYGL LFPLRSLTLA IGWLAFFAAF FPVHFLLKGQ 120 DKLRNKIERK LVEMMCSVFV ASWTGVIKYH GPRPSTRPHQ VFVANHTSMI DFIILEQMTA 180 FAVIMQKHPG WVGFIQKTIL ESVGCIWFNR NDLRDREVTA RKLRDHVQHP DKNPLLIFPE 240 GTCVNNQYTV MFKKGAFELG CAVCPIAIKY NKIFVDAFWN SKKQSFTMHL VRLMTSWAVV 300 CDVWYLEPQY LREGETAIAF AERVRDMIAA RAGLKKVPWD GYLKHNRPSP KHTEEKQRIF 360 AESVLRRLEE K 371
3-116	**Sequences**	
3-116-1	Sequence Number [ID]	116
3-116-2	Molecule Type	AA
3-116-3	Length	378
3-116-4	Features	**source 1..378**
	Location/Qualifiers	mol_type=protein
		organism=Gossypium hirsutum
	NonEnglishQualifier Value	
3-116-5	Residues	MNSSEGKLKS SSSELDLDRP NIEDYLPSGS SIQEPHGKLR LRDLLDISPA LTEAAGAIVD 60 DSFTRCFKSN PPEPWNWNVY LFPLWCCGVV FRYLILFPMR ALILTIGWII FLSCFIPVHF 120 LLKGNDNLRK KMERALVELI CSFFVASWTG VVKYHGPRPS MRPKQVFVAN HTSMIDFIIL 180 EQMTAFAVIM QKHPGWVGLL QSTILESVGC IWFNRSEAKD REIVTRKLRE HSQGADNNPL 240 LIFPEGTCVN NQYSVMFKKG AFELGCTVCP IAIKYNKIFV DAFWNSRKQS FTMHLLQLMT 300 SWAVVCDVWY LEPQNLRPGE TPIEFAERIR DIISVRAGLK KVPWDGYLKY SRPSPKHRER 360 KQQSFAESVL RGLELEEK 378
3-117	**Sequences**	
3-117-1	Sequence Number [ID]	117
3-117-2	Molecule Type	AA
3-117-3	Length	375
3-117-4	Features	**source 1..375**
	Location/Qualifiers	mol_type=protein
		organism=Eucalyptus grandis
	NonEnglishQualifier Value	
3-117-5	Residues	MASPRKLPTS SSELDLDRLN IEDYLPSGSS IHEPPGQLRL RDLLDITPTL TEAAGAIVDD 60 SFTRCFKSNS QEPWNWNVYL FPLWCFGVVV RYLILFPARV LVLTIGWIIF LSSFAIVHFM 120 LKAHDALRRK LERLLVELIC SFFVASWTGV VKYHGPRPSI RPKQVFVANH TSMIDFIILE 180

		QMTAFAVIMQ KHPGWVGLLQ STILESVGCI WFNRSEAKDR EIVARKLRDH VLGTDNNPLL 240 IFPEGTCVNN HYTVMFKKGA FELGCTVCPI AIKYNKIFVD AFWNSRKQSF TMHLLQLMTS 300 WAVVCDVWYL EPQTLKPGET PIEFAERVRD IISVRAGLKK VPWDGYLKYS RPSPKHREGK 360 QRSFAEWVLQ RLEER 375
3-118	**Sequences**	
3-118-1	Sequence Number [ID]	118
3-118-2	Molecule Type	AA
3-118-3	Length	375
3-118-4	Features	**source 1..375**
	Location/Qualifiers	mol_type=protein organism=Cucumis sativus
	NonEnglishQualifier Value	
3-118-5	Residues	MSGAALLKSS ASELDLDRPN IEDYLPSGSS IQQPTAKLRL RDLLDISPTL TEAAGAIVDD 60 SFTRCFKSNP PEPWNWNIYL FPLWCCGVVI RYLFLFPARV LILTIGWIIF LSTFIPVNLL 120 LKGHPKLRAK LERFLVELIC SFFVASWTGV VKYHGPRPSI RPKQVFVANH TSMIDFIVLE 180 QMTAFAVIMQ KHPGWVGLLQ STILESIGCI WFNRTELKDR EIVAKKLNDH VQGADNNPLL 240 IFPEGTCVNN HYSVMFKKGA FELGCSVCPI AIKYNKIFVD AFWNSRKQSF TMHLLQLMTS 300 WAVVCDVWYL EPQVLKPGET PIEFAERVRD IICARAGLKK VPWDGYLKHS RPSPKYRERK 360 QQSFAESVLQ LLDNK 375
3-119	**Sequences**	
3-119-1	Sequence Number [ID]	119
3-119-2	Molecule Type	AA
3-119-3	Length	375
3-119-4	Features	**source 1..375**
	Location/Qualifiers	mol_type=protein organism=Gossypium arboreum
	NonEnglishQualifier Value	
3-119-5	Residues	MSGAALLKSS ASELDLDRPN IEDYLPSGSS IQQPTAKLRL RDLLDISPTL TEAAGAIVDD 60 SFTRCFKSNP PEPWNWNIYL FPLWCCGVVI RYLFLFPARV LILTIGWIIF LSTFIPVNLL 120 LKGHPKLRAK LERFLVELIC SFFVASWTGV VKYHGPRPSI RPKQVFVANH TSMIDFIVLE 180 QMTAFAVIMQ KHPGWVGLLQ STILESIGCI WFNRTELKDR EIVAKKLNDH VQGADNNPLL 240 IFPEGTCVNN HYSVMFKKGA FELGCSVCPI AIKYNKIFVD AFWNSRKQSF TMHLLQLMTS 300 WAVVCDVWYL EPQVLKPGET PIEFAERVRD IICARAGLKK VPWDGYLKHS RPSPKYRERK 360 QQSFAESVLQ LLDNK 375
3-120	**Sequences**	
3-120-1	Sequence Number [ID]	120
3-120-2	Molecule Type	DNA
3-120-3	Length	1116
3-120-4	Features	**source 1..1116**
	Location/Qualifiers	mol_type=genomic DNA organism=Cocos nucifera
	NonEnglishQualifier Value	
3-120-5	Residues	atggttgagc tgcggtcatc gagctcggag atggatctgg accgccccaa catcgaggag 60 tacctcacca cggactccat ccaagaatcc cccaagaagc tccacctgag ggacttgctc 120 gacatttctc ccacgctgac ggaggccacc ggcgccatcg ttgatgattc cttcactcgc 180 tgctttaaat cgaatcctcc agagccctgg aattggaatg tctatttatt tcccttatgg 240 tgcttgggag tgattattag atatggaatt ctttttcccc taagagttgc aatcttgaca 300 gcaggttggc tagtgttctt tgcagccttc attcctgtac atttcttgtt gacagcacat 360 aataagtgga ggcgtaaaat agagaggaag ttggttgaga tgatatgcag tgtctttgtt 420 gcttcatgga caggggtagt caagtatcat gggcctcgtc ctagcatgcg ccctcagcag 480 gtatttgttg ccaaccacac ttccatgatt gatttcatca tactagagca gatgacagca 540 tttgctgtta taatgcaaaa gcatcctgga tgggttggat ttattcaaaa gaccatattg 600 gaaggtgttg gttgtatttg gttcaaccgt acagaatcaa aggatcgtga agttgtggca 660 cgaaagttaa gagaacatat tcatggagct gacaacaacc ctcttctgat atttccagaa 720 ggaacttgtg ttaacaacca ttacactgtc atgttcaaga agggtgcttt tgaacttggt 780 tgtgctgttt gcccggttgc aataaagtac aacaaaattt ttgtggatgc cttctggaac 840 agtaagaagc aatcttttac aatgcatttg tttcacctta tgacatcgtg ggctgttgtt 900 tgcgatgttt ggtacctgga gcctcagtac ataagacctg gagagacgcc cattgaattt 960 gctgaaaggg ttagagacat gatatctgtt cgagctggtc tcaaaaaggt cccgtgggat 1020 ggatatttga agtacttccg ccccagtcct aagctaacag agaggaagca gcagatcttt 1080 gcggagtcgg tcttgcagag gttggaggaa aaataa 1116
3-121	**Sequences**	
3-121-1	Sequence Number [ID]	121
3-121-2	Molecule Type	DNA
3-121-3	Length	1131
3-121-4	Features	**source 1..1131**
	Location/Qualifiers	mol_type=genomic DNA organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-121-5	Residues	atgagcagta cggcagggag gctcgtgact tcaaaatccg agcttgacct cgatcaccct 60 aacatcgaag attaccttcc ttctggttct tccatcaatg aacctcgcgg caagctcagc 120 ctgcgtgatt tgctagacat ctctccaacg ctcactgaag ctgctggtgc cattgttgat 180

		gactcgttca caagatgttt caaatcaaat cctccagaac cttggaactg gaatatttac 240 ttattcccac tatactgctt tggggttgtt gttagatact gtatcctctt tcccttgagg 300 tgcttcactt tagcttttgg gtggattatt ttcctttcat tgtttatccc tgtaaatgcg 360 ttgctgaaag gtcaagatag gttgaggaaa aagatagaga gggtcttggt ggaaatgatt 420 tgcagctttt ttgtcgcctc atggaccgga gttgtcaaat atcacgggcc acgtcctagc 480 atccgtccta agcaggtcta tgttgccaac catacttcaa tgattgattt catcgtattg 540 gagcagatga ccgcatttgc tgttataatg cagaagcatc ctggttgggt tggtcttctg 600 caaagcacaa tattagagag tgtgggatgt atctggttca atcgttcaga ggcaaaggat 660 cgtgaaattg tagcaaaaaa gttaagggac catgtccaag gagctgacag taatcctctt 720 ctcatatttc ccgaagggac atgtgtaaat aataattaca cagtgatgtt taagaagggt 780 gcttttgaat tggactgcac tgtttgtcca attgcaatta aatacaacaa gatttttgtt 840 gacgccttct ggaatagcag aaaacaatca tttactatgc acttgctgca actcatgaca 900 tcatgggctg ttgtatgtga agtgtggtac ttggaaccac aaaccataag gcccggtgaa 960 acaggaattg aatttgcaga gagggtcaga gacatgatat ctcttcgggc gggtctcaaa 1020 aaggtccctt gggatggata cttgaagtat tcgagaccaa gccccaagca tagtgaacgc 1080 aagcaacaga gtttcgcaga gtcgatcctg gctagattgg aagagaagtg a 1131
3-122	**Sequences**	
3-122-1	Sequence Number [ID]	122
3-122-2	Molecule Type	DNA
3-122-3	Length	1116
3-122-4	Features	**source 1..1116**
	Location/Qualifiers	mol_type=genomic DNA organism=Elaeis guineensis
	NonEnglishQualifier Value	
3-122-5	Residues	atggttgagc tgcggtcatc gagctcggag atggatctgg accgccccaa catcgaggag 60 tacctcccac cgactccatc caagaatccc cccaagaagc tccacctgag ggacttgctc 120 gacatttctc ccacgctgac ggaggccgcc ggcgccatcg ttgatgattc cttcactcgc 180 tgctttaaat cgaatcctcc agagccctgg aattggaatg tctatttatt tcccttatgg 240 tgcttgggag tgattattag atacggaatt ctttttcccc taagagttgc aatcttgaca 300 gcagggtggc tagtattctt tgcagccttt attcctgtac atttcttgtt gacagcacat 360 aataagtgga ggcgtaaaat agagaggaag ttggttgaga tgatatgcag tgtctttgtt 420 gcttcatgga caggggtggt caagtatcat gggcctcgtc ctagcatgcg ccctcagcag 480 gtatttgttg ccaaccacac ttccatgatt gatttcatca tactagagca gatgacagca 540 tttgctgtta taatgcaaaa gcatcctgga tgggtcggat ttattcaaaa gactattttg 600 gaaggtgttg gttgtatttg gttcaaccgt acagaatcaa aggatcgtga agttgtggca 660 cgaaagttaa gagaacatat tcacggagct gacaacaacc ctcttctgat atttccagaa 720 ggaacctgcg tcaacaacca ttacactgtc atgttcaaga agggtgcttt tgaacttggt 780 tgtgctgttt gcccagtcgc aataaagtac aacaaaattt ttgtggatgc cttctggaac 840 agtaagaagc aatcttttac aatgcatttg tttcacctta tgacatcgtg ggctgttgtt 900 tgcgatgttt ggtacctgga gcctcagtac ataagacctg gagagacgcc tattgaattt 960 gctgaaaggg ttagggacat gatttccatt cgagctggtc tcaaaaaggt gccgtgggat 1020 ggatatttga aatacttccg ccccagtcct aagctcactg aaagaaagca gcagatattt 1080 gcagagtcag tcttgcagcg gttggaggaa aaataa 1116
3-123	**Sequences**	
3-123-1	Sequence Number [ID]	123
3-123-2	Molecule Type	DNA
3-123-3	Length	1057
3-123-4	Features	**source 1..1057**
	Location/Qualifiers	mol_type=genomic DNA organism=Phoenix dactylifera
	NonEnglishQualifier Value	
3-123-5	Residues	atggttggac tgcggtcatc gagctcggag atggatcttg accggccgaa cattgaggag 60 tacctcacca ccgactccat cgaagaatcc cccaagaagc tccacttgag ggacttgctc 120 gacatttctc ccacgctgac ggaggccgct ggtgctatcg ttgatgattc tttcactcgg 180 tgctttaaat cgaatcctcc agaaccctgg aattggaatg tctatttatt tcccttatgg 240 tgcttgggag tgattattag atacggaatt ctttttcccc taagagttgc agtcttgaca 300 gcagggtggc tagtattctt tgcagccttt attcctgcac atttcctgtt gacagctcat 360 aataagtgga ggcgtaaaat agagaggaag ttggttgaga tgatatgcag tgtctttgtt 420 gcttcatgga caggggtggt caagtatcat gggcctcgtc ctagcatgcg cccccagcag 480 gtatttgttg ccaaccacac ttcaatgatt gatttcatca tactagagca gatgacagca 540 tttgctgtta tcatgcaaaa gcatcctgga tgggtcggat ttattcagaa gactattttg 600 gaaggtgttg gttgtatttg gttcaaccgt acagaatcaa aggatcgtga agttgtggca 660 cgaaagttaa gagaacatat tcaaggagct gacaacaacc ctcttctgat atttccagaa 720 ggaacctgcg ttaacaacca ttacactgtc atgttcaaga agggtgcttt tgaacttggt 780 tgtgctgttt gcccagttgc aataaagtac aacaaaattt ttgtggatgc tttctggaac 840 agtaagaagc aatcttttac gatgcatttg tttcacctta tgacatcatg ggctgttgtt 900 tgtgatgttt ggtatctgga gcctcagtac ataagacctg gagagacgcc cattgaattt 960 gctgaaaggg ttagagacat gatttctgtt cgagctggtc tcagaaaggt cccatgggat 1020 ggatatttga aatacttccg cccgagtcct aagctaa 1057
3-124	**Sequences**	
3-124-1	Sequence Number [ID]	124
3-124-2	Molecule Type	DNA
3-124-3	Length	1116

3-124-4	Features	**source 1..1116**
	Location/Qualifiers	mol_type=genomic DNA
		organism=Musa acuminata
	NonEnglishQualifier Value	
3-124-5	Residues	atggctgggt tggctacctc gagcacggag atggatctcg accgccccaa catcgacgag 60 tacctcaccg tggagtcgat ccgggaggcc cccaagaagc tccacctgag ggacctcctc 120 gacatttctc ctactctcaa agaagctgcc ggcgccatcg tggacgactc cttcactcgt 180 tgctttaagt cgaatccttc agaaccctgg aattggaata tctatttgtt ccctttatgg 240 tgcttgggag tagttattag atatgggatt ctttttccat tcagagttat aatcttggtt 300 gcaggatgga tagtattctt tgcagccttt tcactggtgc atttcctttt aggagaacat 360 aataagtgga agcgtgaaat agagaggaaa ctggttgaga tgatatgcag cgtatttgtt 420 gcttcatgga cggcagtgat taaataccat ggacctcgtc ccagcatgcg ccctcaacag 480 gtcttcgttg ccaaccacac ttctatgatt gatttcatca tcttagaaca gatgacagca 540 tttgctgtca ttatgcaaaa gcatcctggt tgggttggat ttatccagaa gatcatcgta 600 gaaagtttag gttgtatatg gttcaaccgt acagaggcta aggaccgtga aattgttgct 660 agaaagttga gagaacacat tcaaggagtt gacaacaacc ctcttctgat atttcctgag 720 ggaacttgcg ttaacaacca ttatactgtt atgttcaaga agggtgcttt tgaacttggt 780 tgtgctgttt gtcctgtagc aatcaagtac aacaagattt ttgtggatgc tttctggaac 840 agcaaaaagc aatctttcac gatgcactta gtacagctta tgacatcatg ggctgttgtt 900 tgtgatgttt ggtacctgga gcctcagtat ataaggcctg gagagactcc tattgaattt 960 gctgaaaggg ttcaagacat gatctctgtt cgagctggtc tcaaaaaggt cccatgggat 1020 ggctatctaa agtacttccg ccccagtccc aagctcatag agcgcaagca gcagatcttt 1080 gcggagtcag tcttacagcg attggaggag aaatga 1116
3-125	**Sequences**	
3-125-1	Sequence Number [ID]	125
3-125-2	Molecule Type	DNA
3-125-3	Length	1119
3-125-4	Features	**source 1..1119**
	Location/Qualifiers	mol_type=genomic DNA
		organism=Ananas comosus
	NonEnglishQualifier Value	
3-125-5	Residues	atggctgaag ctctgggctc gtcgagcgcg gagatggatc tcgaccgtcc caacctcgag 60 gagtacctcc ccaccgactc catccaagac tcccccaaaa acctccacct gagggacctg 120 ctcgatatct cccccacgct caccgaggcc gcgggcgcca tcgttgatga ctcattcact 180 cgctgcttta aatcaaatcc tccagaacca tggaattgga atatatattt gttccctcta 240 tggtgcctcg gagtcgttgt aagatatggg attcttttttc cactcagagt tgcagtcttg 300 gcgatagggt ggatagtatt tttttctgcc ttcttccctg tacatttctt attgaaaggg 360 tatcccaagt ggaggcgcaa actagagaga aaattggttg agatgatgtg cagtgtattt 420 gttgcttcat ggactggagt cgtaaaatat catggaccac gcccaagcac gcgccctcat 480 caggtatttg ttgctaatca cacctccatg atcgatttca tcattttaga acaaatgact 540 gcatttgctg ttatcatgca aaaacatcct ggatgggttg gatttattca gaagaccatc 600 ttagaaagtg taggatgtat ttggttcaac cgaacggagt ctaaggatcg cggagttgtc 660 gggcggaagc taagagaaca tgttcaagga gtagacaaca accctcttct gatatttcca 720 gaaggaacct gcgtaaacaa tcactacact gtcatgttta agaagggtgc ttttgagctt 780 ggatgtgctg tttgcccaat agcaatcaaa tacaacaaaa tttttgtgga tgccttctgg 840 aacagtaaga agcaatcttt taccatgcat ctggtccgcc tcatgacgtc gtgggctgtt 900 gtctgtgatg tgtggtactt ggagcctcag tacctgagac ctggggagac gccaattgaa 960 tttgctgaaa gggttagaga catgatttct gctcgagctg gtctaaagaa ggttccatgg 1020 gatgggtatc tgaagtactt tcgtcctagc cccaagcata cagaacggaa gcagcagatc 1080 tttgcagagt caatcttgcg gcggttggag aggaaatga 1119
3-126	**Sequences**	
3-126-1	Sequence Number [ID]	126
3-126-2	Molecule Type	DNA
3-126-3	Length	1113
3-126-4	Features	**source 1..1113**
	Location/Qualifiers	mol_type=genomic DNA
		organism=Asparagus officinalis
	NonEnglishQualifier Value	
3-126-5	Residues	atggcggggc tggagtcctc gagcgcaggg atcgacgtcg accctccaaa tattgaagac 60 tatctcacat ccgatgccct ccatcaacct cataagaagc ttcaattgaa ggatttactc 120 gatatttctc ctacactaac tgaggctgca ggagcaattg ttgatgactc atttacacga 180 tgtttcaagt caaatcctcc cgaaccctgg aattggaatg tctacctatt tcccttgtgg 240 tgcttgggag tggttgttcg atatgggatc ctttttccct tgagagttat gactctggca 300 gctggatgga ttgtgttctt ttcagccttt cttcctgttc attatctaat gaaagggcag 360 aacaaatgga aaaataatat agagagaaaa ttggtggaga tgatatgtag tgtttttgtt 420 gcttcttgga ctggtgttgt caggtatcac ggacctcgtc ctagcatgcg ccctcaacag 480 gtatttgtgg cgaatcatac ttcgatgatt gatttcatca ttttagagca gatggctgca 540 tttgctgtaa tcatgcagaa gcatcctgga tgggttggtt tccttcagac gacaattttg 600 gaaagcatag gttctatttg gttcaatcgt accgaggcca aggatcgcga agttgtagca 660 agaaagttaa gagaacatac tgaaggggac aacaatcctt tactaatatt tccggaagga 720 acttgtgtga acaatgacta cactgttatg ttcaaaaagg gcgcatttga actgggatgt 780 gctgtttgtc ctgtagccat caagtacaat aaaattttcg tggacgcctt ctggaacagc 840 aagaagcaat cttttacgat gcatctgatg cgccttatga catcatgggc tgtggtatgt 900

		gatgtttggt atcttgaacc acagtatctg aaaccgggag agacttctat tgaattcgct 960 gaaagggtca gggatatgat ttcggtccga gctggtctca gaaaggtccc gtgggatgga 1020 tatttgaagt acttccgccc aagtcctaag cttacagagc gcaagcagca aatatttgcg 1080 gaatcagtcc tacggcggct ggaagaaaag tga 1113
3-127	**Sequences**	
3-127-1	Sequence Number [ID]	127
3-127-2	Molecule Type	DNA
3-127-3	Length	1113
3-127-4	Features	**source 1..1113**
	Location/Qualifiers	mol_type=genomic DNA
		organism=Oryza brachyantha
	NonEnglishQualifier Value	
3-127-5	Residues	atggcgtcct catcggtggc gggggacatc gagctggacc ggccgaacct ggaggattac 60 ctcccgcccg actcgctgcc gcaggaatcc cccgggaatc tccatctgcg cgatctgctt 120 gacatctcgc cggtgctcac tgaagcggcg ggggcggccg tcgatgattc attcacacgt 180 tgctttaagt ccaattctcc agagccatgg aattggaaca tttatttatt cccactatgg 240 tgcttgggag tagtgataag atatggaata ctcttcccac taaggggctt aactcttcta 300 gttggatgga tagctttctt cgctgccttt ttctctgtgc atttcttatt taaagggcaa 360 aagatgagaa gtaaaataga gagaaaactg gttgaaatga tgtgcagtgt ttttgttgct 420 tcttggactg gagtgatcaa gtatcatgga cctcgcccaa gcacacggcc tcatcaggta 480 tttgttgcaa accacacatc gatgatagac ttcattattc tggagcagat gacagcattt 540 gctgtcatta tgcaaaagca tcctggatgg gttggattta ttcagaagac tatattggaa 600 agtgtgggtt gcatctggtt caatcgtaac gatctcaagg atcgtgaagt agtagcaaaa 660 aagttacgag atcatgttca acatccagac aacaatcctc tcctaatttt ccctgaagga 720 acttgtgtta acaaccagta cactgtcatg ttcaagaagg gtgcttttga gcttggctgt 780 gctgtatgcc caatagctat caaatacaat aaaatatttg ttgatgcctt ctggaatagt 840 aagaagcaat cttttacaat gcacttggtt cggcttatga catcatgggc agttgtgtgt 900 gatgtatggt acttggagcc gcaatatcta aaggagggag aaacagcaat tcaatttgct 960 gaaagagtaa gagacatgat agctgctaga gctggtctta agaaggttcc atgggacgga 1020 tatctgaaac acaaccgccc tagccccaaa cacactgaag agaagcagcg catctttgct 1080 gattctgtgt tgcagagact ggaggaaagc taa 1113
3-128	**Sequences**	
3-128-1	Sequence Number [ID]	128
3-128-2	Molecule Type	DNA
3-128-3	Length	1113
3-128-4	Features	**source 1..1113**
	Location/Qualifiers	mol_type=genomic DNA
		organism=Oryza sativa
	NonEnglishQualifier Value	
3-128-5	Residues	atggcgacct cgtcggtggc gggggacatc gagctggacc ggccgaacct ggaggactac 60 ctcccatccg actcgctgcc gcaggagttc cccaggaatc tccatctgcg cgatctgctg 120 gacatctcgc cggtgctcac tgaagcggcg ggcgccatcg tcgatgattc attcacacgt 180 tgctttaagt caaattctcc agagccatgg aattggaaca tttatttatt cccattgtgg 240 tgcttgggag tagtgataag atacggaata ctattcccgc tgaggggcct aactcttcta 300 gttggatggt tagcattctt tgctgccttt tttcctgtac atttcttatt gaaaggtcaa 360 aagatgagaa gtaaaataga gagaaagctg gttgaaatga tgtgcagtgt ttttgttgct 420 tcttggactg gagtgatcaa gtatcatggg cctcgcccaa gcacacggcc tcatcaggta 480 tttgttgcaa accatacatc gatgatagat ttcattattc tggagcagat gacagcattt 540 gctgtcatta tgcaaaagca tcctggatgg gttggattta ttcagaagac tatcttggaa 600 agtgttggtt gcatctggtt taatcgcaat gatctcaagg atcgtgaagt ggttgcaaaa 660 aagttacgag atcatgttca acatccagac agcaatcctc tcctgatttt ccctgaagga 720 acttgtgtta acaaccagta cactgtcatg ttcaagaagg gtgcttttga gcttggctgt 780 gctgtatgcc caatagctat caaatacaat aaaatatttg ttgatgcctt ctggaatagt 840 aagaagcaat cgtttacaat gcacttggtt aggcttatga catcatgggc agttgtgtgt 900 gatgtatggt acttggagcc tcagtatctg agggatggag aaacagcaat tgaatttgct 960 gaaagagtaa gagacatgat agctgctaga gctggtctta agaaggttcc gtgggacggg 1020 tatctgaaac acaaccgccc tagtcccaaa cacactgaag agaagcagcg catctttgct 1080 gactctgtgt tgcggagact ggaggaaagc taa 1113
3-129	**Sequences**	
3-129-1	Sequence Number [ID]	129
3-129-2	Molecule Type	DNA
3-129-3	Length	1092
3-129-4	Features	**source 1..1092**
	Location/Qualifiers	mol_type=genomic DNA
		organism=Nelumbo nucifera
	NonEnglishQualifier Value	
3-129-5	Residues	atggacttgg atcgaccaaa catagaggaa tatttacctt cagaagccat tcaagagtct 60 aacgagaagc ttcacttgcg tgatttgctc gacatttcgc ctactctaac cgaggctgct 120 ggtgccattg ttgatgattc tttcactcgt tgtttcaagt caaatccgtc agaaccttgg 180 aattggaatg tatatttatt tccactttgg tgctttggag tggtggtaag atatgggatt 240 ctttttcctg ttagagttct agtgttaaca attgggtgga taatattcct ttcatccttc 300 attcctgcac atttcctatt gagaagtcat gataagtgga ggaagaagat agagagatat 360 ctggtggagt taatatgcag cttctttgtt gcatcatgga ctggggttgt caaatatcat 420

		gggccacggc caagcatgcg acccaagcag gtttttgtgg ccaatcatac ttccatgata 480 gattttattg ttttagaaca gatgactgca tttgctgtaa ttatgcagaa gcatcctgga 540 tgggttgggc ttttgcaaag cactattttg gagagtgtag gttgtatctg gttcaatcgt 600 gcagaagcaa aggaccgtga aattgtagca agaaagttaa gagaccacat tcaaggggtt 660 gacaacaatc ctcttcttat atttccagaa ggaacatgtg taaataacca ctatacagtc 720 atgttcaaga agggtgcatt tgaacttgga tgcactgttt gtccaatagc aatcaagtac 780 aataaaattt ttgttgatgc cttctggaat agtaagaagc aatcttttac catgcactta 840 ctgcacctta tgacttcatg ggctgttgtt tgtgatgttt ggtatttgga gccgcaaaat 900 attagacctg gagagacacc catagaattt gcagagaggg tacgagacat aatttctgtt 960 cgagcaggtc ttaaaaaggt tccatgggat ggatatttga aatattctcg tcctagcccc 1020 aaacacagag aaagaaagca acaaaggttt gtagagtcgg tattgcagcg cttggagaaa 1080 aagggaaaat ga 1092
3-130	**Sequences**	
3-130-1	Sequence Number [ID]	130
3-130-2	Molecule Type	DNA
3-130-3	Length	1131
3-130-4	Features	**source 1..1131**
	Location/Qualifiers	mol_type=genomic DNA organism=Vitis vinifera
	NonEnglishQualifier Value	
3-130-5	Residues	atggccaacg ctcccgataa taagctcact tcctcaagct ccgagctcga cttggatcgc 60 cccaatctcg aagactacct tccctccgga tccatgcaag aacctcgcgg caagcttcgc 120 ctgcgtgatt tattggacat ttcgccgacc ctaaccgagg ctgctggggc cattgttgac 180 gactctttca cacgatgttt caagtcgaac cctccggagc cttggaactg gaatgtgtat 240 ttgtttcctc tttggtgttt gggagtggta attcgatatg gaattttatt tcccacaagg 300 gttctagtac tcacactggg gtggataata ttcctttcat cctttattcc agtacatttt 360 ctattgaagg gaaacgataa gttgaggaaa aagttggaga gatgtctagt ggagttaatt 420 tgcagcttct ttgttgcatc atggactgga gttgtcaagt accatgggcc acggcctagc 480 aggaggcctc agcaggtttt tgttgccaat catacttcca tgattgattt tatcgtttta 540 gaacagatga ctgcatttgc agttattatg cagaagcatc ctggctgggt tggattgctg 600 caaagtacca ttttggagag tgtaggatgt atctggttca atcgtacaga agcaaaggac 660 cgtgaaattg ttgctaggaa gctaagggat catgttcagg gggctgacaa caaccctctt 720 ctcatattcc cagaaggaac ttgtgtgaat aaccactaca ctgtcatgtt caagaagggc 780 gcattcgaac ttggctgcac tgtttgccct attgcaataa agtacaataa gattttcgtt 840 gatgctttct ggaacagtaa gaagcaatcc tttacaatgc atcttctgca gcttatgaca 900 tcctgggctg tcgtttgtga tgtttggtac ttagagcccc aaacattgaa gccaggagag 960 acacccattg aatttgcaga gagggtcagg gacataattt ctcttcgagc tggtttgaaa 1020 aaggttcctt gggatggata tttgaagtac tctcgcccta gcccaaagca tagagagcag 1080 aagcagcaga gctttgctga ttcagtgtta cggcgcctgg aagagaagtg a 1131
3-131	**Sequences**	
3-131-1	Sequence Number [ID]	131
3-131-2	Molecule Type	DNA
3-131-3	Length	1122
3-131-4	Features	**source 1..1122**
	Location/Qualifiers	mol_type=genomic DNA organism=Nicotiana tomentosiformis
	NonEnglishQualifier Value	
3-131-5	Residues	atgaatatga ataagctaaa aacatcaagc tccgaattgg acttggatcg acccaatctc 60 gaagattatc ttccaactgg atccatccca gaaccccatg gcaagcttcg cctgcgtgat 120 ttaattgata tttctcccac cctaactgag gctgctggtg ccattgttga cgattctttc 180 accagatgct tcaagtcaaa tccaccagag ccttggaact ggaacattta tttgttccct 240 ttatggtgct tgggggttgt tgttagatat gggattcttt tccctataag agttattgtc 300 ttgacaatag gatggataat attcctctct tgctatatcc cggtgcattt cctgctgaaa 360 ggacacgata agttcaggaa aaagcttgag agatgtctgg tggagctgat atgcagtttc 420 tttgttgcat cttggactgg ggttgtcaaa taccatggtc cacggcctag catacgacct 480 aagcaggttt ttgtggcgaa tcacacgtca atgatagatt ttattgtcct agagcagatg 540 actgcatttg cagtgatcat gcagaagcat cctggatggg ttggactact gcagagtacc 600 attttagaag gtgttggatg tatctggttc aaccgctcag aagccaagga tcgtgaaatt 660 gtagcacgaa agttgaggca acatgttgaa ggggccgata acaaccctct tcttatattc 720 cccgagggaa cttgcgtaaa taaccactac actgtcatgt tcaaaaaggg agcatttgaa 780 ctcggttgca ctgtttgtcc tgttgcaatc aagtacaaca aaatttttgt tgacgccttt 840 tggaatagta gaaaacaatc cttcacaatg cacctcttgc agctcatgac atcttgggct 900 gttgtctgtg atgtttggta cctggagcct cagaacataa gacctgggga gactccaatc 960 gagtttgcag agagggtgag ggacatcatt tctgctagag caggtcttaa aaaggttcct 1020 tgggatggat atttaaaata ctctcgtcct agccccaagc atcgagagag gaagcaacag 1080 agttttgcag aatcagtgct gcgtcgcctg gaagagaagt ag 1122
3-132	**Sequences**	
3-132-1	Sequence Number [ID]	132
3-132-2	Molecule Type	DNA
3-132-3	Length	1128
3-132-4	Features	**source 1..1128**
	Location/Qualifiers	mol_type=genomic DNA organism=Jatropha curcas

	NonEnglishQualifier Value	
3-132-5	Residues	atggctactc caggtaagct aaagacctcg agctctgaat tggacttgga tcgacccaat 60 atcgaagact accttccttc tggagtctct attcaagaac ctcgtggcaa gcttcgtctg 120 cgtgatttgc ttgacatttc gccgacccta acggaggctg ctggtgccat tgttgatgac 180 acctttacaa ggtgtttcaa gtcaaatcct ccagaaccat ggaattggaa catatatcta 240 tttccccttt ggtgctgcgg tgtggtgtgt cgatatggga ttttgtttcc catcagggtt 300 ctagtactga caatagggtg gataattttc ctttcatgct acattcctgt gcatttccta 360 cttaaaggac atgacaagtt gagaaaaaag cttgagagat gtttggtgga gttaatttgc 420 agcttctttg tggcatcatg gaccggagtt gtcaagtacc atggtccacg gcctagcatc 480 cgacctaaac aggtttttgt ggccaatcat acctccatga ttgattttat catcttggaa 540 cagatgactg catttgctgt tattatgcag aagcatcctg gatgggttgg actactgcaa 600 agcactatat tagagagtgt cggatgtatc tggttcaatc gttcagaggc aaaggatcgt 660 gaaattgtaa caaaaaagtt aagggatcat gtacaggggg ctgacaataa ccctcttctc 720 atatttcctg aaggaacttg tgtaaataat cactatactg taatgttcaa gaagggtgca 780 ttcgaactgg gatgtactgt ttgtccaatt gcaatcaaat acaacaaaat ttttgttgat 840 gctttttgga acagccggaa gcagtcattt acaacgcatt tgctgcaact catgacttcc 900 tgggctgttg tttgtgatgt atggtacttg gagccacaaa atctgaaacc tggagagaca 960 cccattgagt ttgctgagag ggtcagggac ataatatctg tacgagcagg tctcaaaaag 1020 gttccttggg atggatatct aaagtattct cgccctagcc caaagcatag agagcgaaag 1080 caacaaagct ttgctgagtc agtgctgcag cgactggagg agaaatga 1128
3-133	**Sequences**	
3-133-1	Sequence Number [ID]	133
3-133-2	Molecule Type	DNA
3-133-3	Length	1131
3-133-4	Features	**source 1..1131**
	Location/Qualifiers	mol_type=genomic DNA organism=Glycine max
	NonEnglishQualifier Value	
3-133-5	Residues	atgaataact cagggacacc caagtcttca agttctgaat tggatcttga tcgacccaac 60 attgaggatt acctcccttc agggtccacc attcaacaag aacctcatgg aaagcttttc 120 ctgcatgatt tgctcaatat ttctcctact ttgtctgagg ctgcaggtgc tattgtagat 180 gactcattca caagatgctt caagtcaaat cctccagaac catggaattg gaatgtttat 240 ttgtttcctt tgtggtgttt tggagttgtg attcgatact tgattctgtt cccaatcagg 300 gttatagggt taacaatagg atggataata tttctttcat ccttcattcc ggtgcacttc 360 ctattgaaag gacatgacaa gttaaggaga agtattgaga ggtctttggt agagatgatg 420 tgcagtttct ttgttgcatc ttggactggg gttgttaagt atcatggacc caggcctagc 480 aggagaccaa agcaggtttt tgtagccaac catacttcca tgattgattt cattatctta 540 gaacagatga ctgcttttgc tgttattatg cagaagcatc ctggatgggt tggattattg 600 cagagtacca ttttggagag tctaggatgc atctggttca accgtacaga ggcaaaggat 660 cgggaaatag tagcaaggaa attgagggat catgtccagg gagctgataa caaccccctt 720 ctcatatttc ctgaaggaac ttgtgtaaat aatcactata cagtcatgtt caagaagggt 780 gcatttgaac ttggctgcac agtttgccca gttgcaatca agtacaataa gatttttgta 840 gatgcttttt ggaatagtcg aaagcaatca ttcactatgc atctgttgca gctaatgacg 900 tcttgggcag ttgtttgtga tgtttggtac ttggagccac aaaatctgaa gccaggagag 960 acgcctattg agttcgcaga gagggtgaga gacataatct cagttcgtgc tggccttaaa 1020 aaggttcctt gggatggata tctgaagtat tctcgtccta gcccaaagca tagagaaagg 1080 aagcaacaga actttgctga gtcagtgctg cggcgatggg aggaaaagtg a 1131
3-134	**Sequences**	
3-134-1	Sequence Number [ID]	134
3-134-2	Molecule Type	DNA
3-134-3	Length	1116
3-134-4	Features	**source 1..1116**
	Location/Qualifiers	mol_type=genomic DNA organism=Sesamum indicum
	NonEnglishQualifier Value	
3-134-5	Residues	atgagtaagc ttaacacatc cagctccgaa ttggattttg atcgccccaa catcgaggac 60 tatctcccat ccggatccat tcaagagcct cacggcaaac tccgcctgcg tgatttgctc 120 gatatttcac caactctcac tgaggccgct ggtgcaattg ttgatgactc tttcaccaga 180 tgcttcaagt caaatcctcc agaaccctgg aactggaaca tatacttgtt tcctttatgg 240 tgcttgggag tggtcatcag atatggcctt cttttcccat taagggtaat agtgttgaca 300 ataggatgga ttatatttct atcatgctat tttcctgtgc atttcctgtt aagagggcac 360 gacaaattga ggaaaagatt agagagaggt ctagtggagt tgatttgcag tttcttcgtt 420 gcatcatgga caggggttgt caagtatcat ggtccacggc cgtccatgcg acctaagcag 480 gtttttgtgg cgaatcacac atccatgatt gatttcattg ttttggaaca aatgactgct 540 tttgcagtga ttatgcagaa gcatcctggg tgggttggat tattgcagag cacaattttg 600 gaaagtctag gatgtatctg gttcaaccgc tcagagtcca aggatcgtga aattgttgca 660 aaaaagctaa gggaacatgt ccatgatgct gataacaatc ctcttcttat attcccggaa 720 ggaacttgtg tgaataacca ttacactgtt atgtttaaga agggtgcatt tgaacttggc 780 tgcactgtct gtccaatagc aatcaagtac aacaagatat ttgttgatgc cttctggaat 840 agccgaaagc aatccttcac tacacacttg ttgcagctta tgacatcctg ggctgttgtt 900 tgtgacgttt ggtacctaga gcctcaaaat ctgaaacctg gggaaacacc cattgaattt 960 gcagagaggg tgagggacat tatttctgtt cgggccggcc tcagaaaggt gccttgggat 1020 ggatatttga agtactctcg gcctagtccg aagcatcgtg aacgcaagca acaaagcttt 1080 gcagagtcaa ttctccgtcg cttggaagag aaatag 1116

3-135	**Sequences**	
3-135-1	Sequence Number [ID]	135
3-135-2	Molecule Type	DNA
3-135-3	Length	1095
3-135-4	Features	**source 1..1095**
	Location/Qualifiers	mol_type=genomic DNA
		organism=Brachypodium distachyon
	NonEnglishQualifier Value	
3-135-5	Residues	atggcgtcgt cgctcgacgc gccgaacctt gatgattacc tccccacgga ctcgctcccg 60 caggaacccc ccaggagcct caatctgcgc gatctgctgg acatctcgcc agtgctcact 120 gaagcggcgg gcgccatcgt ggatgattcg ttcacacgct gctttaagtc aaattctcca 180 gagccatgga actggaacat ttatttgttc ccgttatggt gcttcggagt agtcgtaaga 240 tacggactac tgtttccact cagggtatta acgcttggat taggatggat ggtattcttt 300 gctgcctttt ttcccgtgca tttcctattg aaagggcaaa ataaactgag aagtaaaata 360 gagagaaagc tcgttgaaat gatgtgcagt gtttttgttg cttcttggac tggagtaatc 420 aagtaccatg gaccacgccc aagctcacgg ccttatcagg tatttgttgc aaaccataca 480 tcaatgatag atttcattat tctggagcag atgacagcat ttgctgtcat tatgcaaaag 540 catcctggat gggttggatt tattcagaag actattttgg aaagtgtggg ttgcatctgg 600 tttaatcgta atgatcttaa ggatcgtgaa gtagttggca gaaagttacg tgatcatgtt 660 caacgtccag acaacaaccc tctcttgatt ttcccagaag gaacttgtgt taacaaccag 720 tacactgtaa tgttcaagaa gggtgctttt gagcttgggt gtgctgtatg tccgatagct 780 atcaagtata ataaaatatt tgttgatgcc ttctggaata gtaagaagca atctttcaca 840 atgcacttgg gtcggcttat gacatcatgg gctgtagtgt gtgatgtttg gttcttggaa 900 cctcaatatc tcagggaagg agagacatcg attgcattta ctgaaagagt aagggacatg 960 atagctgctc gagccggtct taagaaggtt ctgtgggatg ggtatctgaa gcataaccgt 1020 cctagcccca aacacactga ggagaagcag cgcatatttg cagaatcggt gttgaagaga 1080 ctagaggaaa gctaa 1095
3-136	**Sequences**	
3-136-1	Sequence Number [ID]	136
3-136-2	Molecule Type	DNA
3-136-3	Length	1116
3-136-4	Features	**source 1..1116**
	Location/Qualifiers	mol_type=genomic DNA
		organism=Setaria italica
	NonEnglishQualifier Value	
3-136-5	Residues	atggcgagct cctcggtggc ggcggacatg gagctggacc gccccaatct ggaggactac 60 ctcccgcccg actcgctccc gcaggaggcg ccccggaatc tccatctgcg cgatttgctg 120 gacatctcgc cagtgctcac cgaggcagca ggcgccatcg tcgatgactc cttcacgcgt 180 tgctttaagt caaattctcc agagccatgg aattggaaca tatatctgtt ccccttatgg 240 tgcttgggtg tagtaataag atatggaata ctcttcccac tgaggtcctt aacgcttgca 300 ataggatggt tagcattttt tgctgccttt tttcctgtcc atttcctatt gaaagggcaa 360 gacaagttga gaagtaaaat tgagaggaag ttggttgaaa tgatgtgcag tgtttttgtt 420 gcttcatgga ctggagtgat caagtatcat ggaccacgcc caagcacacg acctcatcag 480 gtattcgttg caaaccatac atcaatgata gatttcatta ttctggagca aatgacagca 540 tttgctgtca tcatgcagaa gcatcctgga tgggttggat ttattcagaa gactatcttg 600 gaaagtgtcg gttgcatctg gtttaatcgt aatgatcttc gggatcgtga agttacggca 660 cggaagttac gtgatcatgt tcaacaacca gacaaaaatc ctctcttgat ttttccggaa 720 ggaacttgtg ttaacaacca gtacacggtc atgttcaaga agggtgcctt tgagcttggc 780 tgcgctgtct gtccaatagc tatcaagtac aataaaatat ttgttgatgc cttttggaac 840 agtaagaagc aatcttttac aatgcacttg gtccggctga tgacatcatg ggctgttgtg 900 tgtgatgttt ggtacttacc tcctcaatat ctgagggagg gagagacggc aattgcattt 960 gctgagagag taagggacat gatagccgct agagctggac taaaaaaggt tccgtgggat 1020 ggctatctga aacacaaccg tcctagtccc aaacacactg aagagaaaca acgcatattt 1080 gccgaatctg tcctgatgag actggaggag aaatga 1116
3-137	**Sequences**	
3-137-1	Sequence Number [ID]	137
3-137-2	Molecule Type	DNA
3-137-3	Length	1131
3-137-4	Features	**source 1..1131**
	Location/Qualifiers	mol_type=genomic DNA
		organism=Cicer arietinum
	NonEnglishQualifier Value	
3-137-5	Residues	atgaatagca ctggaacact taagtcttca agttctgagt tggatcttga tcgacccaac 60 attgaggatt atctcccttc aggagccgcc attcaacaag aacctcgcgg caagcttcgc 120 ctgcatgact tgcttgatat ttctcctaca ctatctgagg cagctggtgc tattgtagat 180 gactcattca caagatgttt caagtcaaat cctccagaac catggaattg gaatatatat 240 ttgtttcctt tgtggtgttt tggagttgtt gttcgatatt tgatactgtt ccctacaagg 300 gttcttgggt taacattagg atggataata tttctttctg ctttcattcc agtgcacctc 360 ctattgaaag gacatgacaa gttgaggaga aatattgaga ggtctttggt agagatgatg 420 tgcggtttct ttgttgcatc ttggactggg gttgtcaagt accatgggcc aaagcccagc 480 aggcgaccaa aacaggtttt tgttgccaac cacacttcca tgattgattt cattatctta 540 gaacagatga ctgcttttgc tgttattatg cagaagcatc ctggatgggt tggattgttg 600 caaagcacca ttttggagag tgtaggatgt atctggttca atcgcacaga ggcaaaggat 660

		cgagaaattg tggcaagaaa attgagggaa catgtccagg gagctgacaa caatcctctt 720 ctcatatttc cagagggaac ttgtgtaaat aatcactaca cagtcatgtt taagaagggt 780 gcatttgaac ttggctgcac agtttgccct gttgcaatca aatacaacaa aatttttgtc 840 gatgcatttt ggaatagtcg aaagcaatca ttcactaaac atctgttgca gctaatgaca 900 tcatgggctg ttgtttgtga tgtttggtac ttggagccac aaaacctaaa gccaggagag 960 acaccaattg agtttgccga aagggtgaga gacataatct cacatcgtgc tggtcttaaa 1020 aaggttcctt gggatggata tctgaagtat tcgcgtccta gcccaaagca tagagaaaga 1080 aagcaacaga actttgctga gtcggtgctg cggcgtttgg aagaaaaata a 1131
3-138	**Sequences**	
3-138-1	Sequence Number [ID]	138
3-138-2	Molecule Type	DNA
3-138-3	Length	1116
3-138-4	Features	**source 1..1116**
	Location/Qualifiers	mol_type=genomic DNA organism=Zea mays
	NonEnglishQualifier Value	
3-138-5	Residues	atggcgagct cgtctgtggc ggcggacatg gagctggacc gccccaacct ggaggactac 60 ctcccgcccg actcgctccc gcaggaggcg cccaggaatc tccatctgcg cgatctgctt 120 gacatctcgc cggtgctaac cgaggcagcg ggtgccatag tcgatgattc attcacgcgc 180 tgctttaagt cgaattctcc agaaccatgg aactggaaca tatatttgtt ccctttatgg 240 tgcttcggtg tagtaattcg atatggatta ctcttcccac tgaggtcctt aacgcttgca 300 ataggatggt tagcattttt tgctgccttt ttccccgtgc atttcctatt gaaaggtcaa 360 gacaagttga gaaataaaat tgagaggaag ttggttgaaa tgatgtgcag tgtttttgtt 420 gcttcatgga ctggagtgat caagtaccat ggaccacgcc caagcacacg acctcatcag 480 gtatttgttg caaaccatac atcaatgata gatttcatta ttctggagca aatgacagca 540 tttgctgtca tcatgcagaa gcatcctgga tgggttggat ttattcagaa gactatcttg 600 gaaagtgtgg gttgcatctg gtttaaccgt aatgatctcc gggatcgtga agttacggca 660 cggaagttgc gtgatcatgt tcaacatcca gacaaaaacc ctctcttgat tttcccagaa 720 ggaacttgtg ttaacaacca gtatacggtc atgttcaaga agggtgcctt tgagcttggg 780 tgtgctgtct gtccaatagc tatcaaatac aataaaatat ttgttgatgc cttttggaac 840 agtaagaagc aatcttttac gatgcacttg gtccggttga tgacatcatg ggctgttgtg 900 tgtgatgttt ggtacttgga gcctcaatat ctgagggagg gagagactgc aattgcgttt 960 gctgagagag taagggacat gatagcagct agagctggtc ttaagaaggt cccgtgggat 1020 ggctatctga aacacaaccg ccctagtccc aaacacaccg aagagaagca acgcatattc 1080 gccgaatctg tcttgaggag actagaggag aaatga 1116
3-139	**Sequences**	
3-139-1	Sequence Number [ID]	139
3-139-2	Molecule Type	DNA
3-139-3	Length	1137
3-139-4	Features	**source 1..1137**
	Location/Qualifiers	mol_type=genomic DNA organism=Gossypium hirsutum
	NonEnglishQualifier Value	
3-139-5	Residues	atgaacagta gtgaagggaa gttgaaatca tcgagttccg aattggattt ggatcgaccc 60 aacatcgaag attatctccc ttctggatct tccattcaag aaccacatgg caagcttcgc 120 ctgcgggatt tgcttgatat ttctcccgct ttaactgaag ctgctggtgc tattgttgat 180 gattctttca cacggtgttt taagtcgaat cccccggaac cgtggaactg gaatgtgtat 240 ctttttcctc tctggtgttg tggtgtggta tttcggtact tgattttgtt ccctatgagg 300 gctttaattt tgacaatagg atggataata tttctgtcat gcttcattcc tgtgcacttt 360 cttctcaaag ggaacgataa cttgcggaaa aagatggaga gggcgttggt ggagctaatc 420 tgcagcttct ttgttgcatc ctggactgga gttgttaagt accatggacc gcggcctagc 480 atgcggccca agcaggtgtt tgtggccaat catacttcta tgattgattt catcatatta 540 gaacagatga ctgcatttgc tgtcattatg cagaagcacc ctggatgggt tggactgcta 600 cagagcacta ttttagagag tgtagggtgt atttggttta accgttcaga ggccaaagat 660 cgtgaaattg taacaaggaa gttaagggag catagtcagg gagctgacaa taaccctctt 720 ctcatatttc ccgaagggac atgtgtaaac aatcaataca gcgttatgtt caagaagggt 780 gcattcgaac ttggttgcac tgtttgcccg attgcaataa agtacaataa aatttttgtt 840 gatgcctttt ggaatagccg gaagcagtcc tttacaatgc atttattgca gcttatgaca 900 tcctgggctg ttgtttgcga tgtttggtac ctagagcccc aaaatctaag gcctggagaa 960 acacccatcg agtttgcaga gaggatcaga gacataatct ctgttcgagc aggtcttaaa 1020 aaggttccat gggacggata tttgaagtat tctcgcccga gccctaagca tagagagcga 1080 aaacaacaaa gttttgccga atctgttctt cggggactgg aactggaaga aaaatga 1137
3-140	**Sequences**	
3-140-1	Sequence Number [ID]	140
3-140-2	Molecule Type	DNA
3-140-3	Length	1128
3-140-4	Features	**source 1..1128**
	Location/Qualifiers	mol_type=genomic DNA organism=Eucalyptus grandis
	NonEnglishQualifier Value	
3-140-5	Residues	atggcgagcc ccaggaagct gccgacctcg agctccgagc tggacctgga tcgcctcaac 60 atcgaggatt acctcccttc cggatcctcc atccacgagc cccccggcca gctccgcctg 120 cgcgatttgc ttgatatcac gccgactctg accgaggccg ccggtgctat cgtcgatgac 180

		tcgttcacgc ggtgcttcaa gtcgaattcg caggaaccgt ggaactggaa cgtgtacctc 240 ttcccgctgt ggtgcttcgg ggtggtggtt cggtacttga tcctgttccc ggcaagggtt 300 ttagtgttga caattggatg gataatattc ctctcatcat ttgccattgt tcactttatg 360 cttaaagcac atgatgcact gagaaggaag ctggagaggt tgctggtgga gttaatttgc 420 agcttctttg ttgcttcatg gactggtgtc gtcaaatacc atgggccacg gcctagcatt 480 cggcctaaac aagtttttgt tgccaaccac acttccatga ttgatttcat catcttagag 540 caaatgactg ccttcgctgt tattatgcaa aagcatcctg gatgggttgg actactgcaa 600 agcactattt tggagagtgt aggatgcatc tggtttaatc gttctgaggc caaagatcgt 660 gaaattgtgg caagaaagtt gagagatcac gtactgggaa ctgataacaa tcctcttctc 720 atatttcctg aagggacttg tgtgaacaat cactatactg tcatgttcaa aaagggtgca 780 tttgagcttg ggtgcactgt ttgccctatc gcaatcaagt acaataagat cttcgtggat 840 gccttttgga acagcaggaa acaatctttc acaatgcatc tactgcaact tatgacatct 900 tgggctgttg tttgtgacgt ctggtacttg gaaccccaaa ccttgaaacc tggtgaaacg 960 ccaattgaat ttgcagagag ggtccgtgac atcatatctg ttcgagctgg tttgaagaag 1020 gttccttggg atggatatct gaagtactct cgccctagcc ccaagcatag agaagggaag 1080 caacgaagct ttgctgagtg ggtgctgcag cgacttgagg agaggtga 1128
3-141	**Sequences**	
3-141-1	Sequence Number [ID]	141
3-141-2	Molecule Type	DNA
3-141-3	Length	1128
3-141-4	Features	**source 1..1128**
	Location/Qualifiers	mol_type=genomic DNA organism=Cucumis sativus
	NonEnglishQualifier Value	
3-141-5	Residues	atgagtggtg ctgctcttct caaatcctcc gcctctgaat tggacttaga tcgacccaat 60 atcgaagatt acttgccttc cggatcctct atccaacaac ccactgccaa gcttcgcctt 120 cgtgatttgc tcgatatttc gccgaccctt accgaggctg ctggtgctat tgttgatgat 180 tcgtttacaa ggtgtttcaa atcaaaccca ccagagccat ggaattggaa tatttatttg 240 ttccctttgt ggtgctgtgg agtggtgatt cggtatttgt ttctcttccc ggcaagggtt 300 ctcatattga cgataggatg gataattttc ctttcaacgt tcattccagt gaatctcctt 360 ctgaaagggc atcctaaact gagagctaag ttagagaggt ttttggtgga gttgatttgc 420 agcttctttg ttgcatcttg gactggagtt gttaagtatc atgggccacg gcctagcatc 480 agaccaaaac aggttttcgt ggccaaccac acttccatga ttgatttcat agtcttggag 540 caaatgactg cctttgctgt tattatgcaa aaacatcctg ggtgggttgg actgttgcaa 600 agcactatat tggagagtat aggatgtata tggttcaacc gtacagagtt gaaggaccgt 660 gaaattgtag caaagaagtt aaatgaccac gttcaagggg ctgacaacaa tcctcttctt 720 atatttcctg aaggaacttg tgtaaataac cactactctg ttatgttcaa gaagggtgca 780 tttgaacttg gatgctctgt ttgcccaatt gcaatcaaat acaataaaat tttcgttgat 840 gctttttgga acagcaggaa gcagtcgttc actatgcatc tgctgcagct catgacctct 900 tgggctgttg tttgtgatgt ttggtacctg gagccccaag ttttgaagcc tggagaaaca 960 cccattgagt ttgcagaaag ggtcagggac ataatatgtg ctcgagcagg tcttaagaag 1020 gttccatggg atggatattt gaagcactcc cgtccgagcc caaaataccg agaacgtaaa 1080 caacaaagct tcgcggagtc agtgctgcag ctattggaca ataagtga 1128
3-142	**Sequences**	
3-142-1	Sequence Number [ID]	142
3-142-2	Molecule Type	DNA
3-142-3	Length	1137
3-142-4	Features	**source 1..1137**
	Location/Qualifiers	mol_type=genomic DNA organism=Gossypium arboreum
	NonEnglishQualifier Value	
3-142-5	Residues	atgaacagta gtgaagggaa gttgaaatca tcgagttccg aattggattt ggatcgaccc 60 aacatcgaag attatctccc ttctggatct tccattcaag aaccacatgg caagcttcgc 120 ctgcgggatt tgcttgatat ttctcccgct ttaactgaag ctgctggtgc tattgttgat 180 gattcattca cacggtgttt taagtcgaat cccccggaac cgtggaactg gaatgtgtat 240 ctgtttcctc tctggtgttg tggtgtggta tttcggtact tgattttgtt ccctatgagg 300 gctttagttt tgacaatagg atggataata tttctgtcat gcttcattcc tgtgcacttt 360 cttctcaaag ggaacgataa cttgcggaaa aagatggaga gggcgttggt ggagctaatc 420 tgtagcttct ttgttgcgtc ctggactgga gttgttaagt accatggacc acggcctagc 480 atgcggccca agcaggtgtt tgtggccaat catacttcta tgattgattt catcatatta 540 gaacagatga ctgcatttgc tgtcattatg cagaagcacc ctggatgggt tggactgcta 600 cagagcacta ttttagagag tgtagggtgt atttggttta accgttcaga ggccaaagat 660 cgtgaaattg taacaaggaa gttaagggag catagtcagg gagctgacaa taaccctctt 720 ctcatatttc ccgaagggac atgtgtaaac aatcaataca gcgttatgtt caagaagggt 780 gcattcgaac ttggttgcac tgtttgcccg attgcaataa agtacaataa aatttttgtt 840 gatgcctttt ggaatagccg gaagcagtcc tttacaatgc atttattgca gctaatgaca 900 tcctgggctg ttgtttgcga tgtttggtac ctagagcccc aaaatctaag gcctggagaa 960 acacccatcg agtttgcaga gaggatcaga gacataatct ctgttcgagc aggtcttaaa 1020 aaggttccat gggacggata tttgaagtat tctcgcccga gccctaagca tagagagcga 1080 aaacaacaaa gttttgccga atctgttctt cggggactgg aactggaaga aaaatga 1137
3-143	**Sequences**	
3-143-1	Sequence Number [ID]	143
3-143-2	Molecule Type	AA

3-143-3	Length	215
3-143-4	Features	**source 1..215**
	Location/Qualifiers	mol_type=protein
		organism=Elaeis guineensis
	NonEnglishQualifier Value	
3-143-5	Residues	MPDSDNESGG QNNSHNNNVG EYSSSREQDR FLPIANVSRI MKKALPANAK ISKDAKETVQ 60 ECVSEFISFI TGEAADKCQR EKRKTINGDD LLWAMTTLGF EDYVDPLKVY LHRFREMEGD 120 KCSAGASASS QPQHKDGGDG GGGGGGGAPS MGNNVVGLGG GGGGAGGMMM MMGQQMYATP 180 PSYHHHMSTM SGKSSMGGGS SASSSSPGFG RQGRV 215
3-144	**Sequences**	
3-144-1	Sequence Number [ID]	144
3-144-2	Molecule Type	AA
3-144-3	Length	133
3-144-4	Features	**source 1..133**
	Location/Qualifiers	mol_type=protein
		organism=Elaeis guineensis
	NonEnglishQualifier Value	
3-144-5	Residues	MEPENPELNL DLALQPSSPP EPARVFSCNY CQKKFYSSQA LGGHQNAHKL ERSLAKRSWE 60 LATALRPHAG STIGQHTSTV VLVERQREEC CYNGVGLATR GREASRASIR LGSRKESDDK 120 RELADGIDLS LRL 133
3-145	**Sequences**	
3-145-1	Sequence Number [ID]	145
3-145-2	Molecule Type	AA
3-145-3	Length	190
3-145-4	Features	**source 1..190**
	Location/Qualifiers	mol_type=protein
		organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-145-5	Residues	MGDSDRDSGG GQNGNNQNGQ SSLSPREQDR FLPIANVSRI MKKALPANAK ISKDAKETMQ 60 ECVSEFISFV TGEASDKCQK EKRKTINGDD LLWAMTTLGF EDYVEPLKVY LQRFREIEGE 120 RTGLGRPQTG GEVGEHQRDA VGDGGGFYGG GGGMQYHQHH QFLHQQNHMY GATGGGSDSG 180 GGAASGRTRT 190
3-146	**Sequences**	
3-146-1	Sequence Number [ID]	146
3-146-2	Molecule Type	AA
3-146-3	Length	161
3-146-4	Features	**source 1..161**
	Location/Qualifiers	mol_type=protein
		organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-146-5	Residues	MADSDNDSGG HKDGGNASTR EQDRFLPIAN VSRIMKKALP ANAKISKDAK ETVQECVSEF 60 ISFITGEASD KCQREKRKTI NGDDLLWAMT TLGFEDYVEP LKVYLQKYRE VEGEKTTTAG 120 RQGDKEGGGG GGGAGSGSGG APMYGGGMVT TMGHQFSHHF S 161
3-147	**Sequences**	
3-147-1	Sequence Number [ID]	147
3-147-2	Molecule Type	AA
3-147-3	Length	150
3-147-4	Features	**source 1..150**
	Location/Qualifiers	mol_type=protein
		organism=Arabidopsis thaliana
	NonEnglishQualifier Value	
3-147-5	Residues	MDYQPNTSLR LSLPSYKNHQ LNLELVLEPS SMSSSSSSST NSSSCLEQPR VFSCNYCQRK 60 FYSSQALGGH QNAHKLERTL AKKSRELFRS SNTVDSDQPY PFSGRFELYG RGYQGFLESG 120 GSRDFSARRV PESGLDQDQE KSHLDLSLRL 150
3-148	**Sequences**	
3-148-1	Sequence Number [ID]	148
3-148-2	Molecule Type	AA
3-148-3	Length	398
3-148-4	Features	**source 1..398**
	Location/Qualifiers	mol_type=protein
		organism=Elaeis guineensis
	NonEnglishQualifier Value	
3-148-5	Residues	MASASESRNV TSEETEVTSE RRPEEGKEER ELGLEFPLMR QSSIYSLTLD EIQNTVCEPG 60 KSFGSMNMDE FLTNIWNVEE GQIASANAQN QQHIGGGGPP AAPPLQRQGS IAVPAPLCRK 120 TVDEVWSDIH RGQNARRQNV DRPPPPSQQQ ESNCAAPRKP TFGEITLEDF LVKAGVVREG 180 YQPGSAPSAH APVPPATQYG MPAGYQMVGT EGAPVFGHVV GVQAYGDHQV TAANAMYPVV 240 GDGGGPGYAV GNGFGGRVGN GYGAVAAVGG SPASPGSSEG VGGGQVENSG AAEGGGGGKG 300 GRKRPLDGTV EKVVERRQRR MIKNRESAAR SRARKQAYTV ELEAELNQLK EENARLKEAE 360 KKMLALKKQL LMEAMAERAR VNAQKTILTM RRCNSSKW 398
3-149	**Sequences**	

3-149-1	Sequence Number [ID]	149
3-149-2	Molecule Type	AA
3-149-3	Length	272
3-149-4	Features	**source 1..272**
	Location/Qualifiers	mol_type=protein
		organism=Elaeis guineensis
	NonEnglishQualifier Value	
3-149-5	Residues	MEQSTQPSHP VMGIVTGAAQ IAYAAPTYQS AAMVTGAPAV IGAIPSPAQP TSTFPTSPAQ 60 LTSQHQLAYQ QVRQFHHQQQ QQQQQQLQTF WANQMLEIEH ATDFKNHSLP LARIKKIMKA 120 DEDVRMISAE APVIFAKACE MFILELTLRS WIHTEENKRR TLQKNDIAAA ITRTDIFDFL 180 VDIVPRDELK EEGIGIARAA LPTMGAPADS GPYYYVPAQH QLAGPGMIMG KPVDQATTAA 240 MYTAQPPHPV AYMWQQPQQQ QAQQQQQMPD SG 272
3-150	**Sequences**	
3-150-1	Sequence Number [ID]	150
3-150-2	Molecule Type	AA
3-150-3	Length	352
3-150-4	Features	**source 1..352**
	Location/Qualifiers	mol_type=protein
		organism=Elaeis guineensis
	NonEnglishQualifier Value	
3-150-5	Residues	MPLDNANAFD TQHFSNKDSE HSSVTSVHSA SNCVDNFPSL WKQSGSHFPQ STYFKNFCMN 60 MGFLAQPDNQ MKQLGGQMPD QDSSSSQSTG QSHQEVSGTS EGNLHEQSIS AQAGNDKTCG 120 KQVEGHVNSV LFLGTPEAAF VSPRLDYGQS FACVPYTYAD PSFGGVLAAY GSPAIIHPQM 180 VGVPPSSRVP LPLEPAAEEP IYVNAKQYRA ILRRRQLRAK LEAQNKLIKA RKPYLHESRH 240 LHAMKRARGS GGRFLNTKQL EQQQQRPLLP PPPSVSTGLG NLSASNLHFE NGPSGSSAAP 300 TSSADVVRVS TSGGMLEQQG HLSFLSADFH SHVRSTQGGG DSGSQPRITI MR 352
3-151	**Sequences**	
3-151-1	Sequence Number [ID]	151
3-151-2	Molecule Type	AA
3-151-3	Length	300
3-151-4	Features	**source 1..300**
	Location/Qualifiers	mol_type=protein
		organism=Glycine max
	NonEnglishQualifier Value	
3-151-5	Residues	MQQIHSMPGG RFFSGSGSAD RRLRPHHQNQ QALKCPRCDS LNTKFCYYNN YNLSQPRHFC 60 KNCRRYWTKG GVLRNVPVGG GCRKSKRSSK PNKITPSETA SPPPPPHPDH NNNSNSHSSS 120 ESSSLTAAVA TTTEAVSAPE TLNSDSNNNN NMQESKLLIP ALETNNPLEQ GTGDCGGIFS 180 EIGPFTSLIT TTTSTNEPLG SGFGFGNSTL PDASSFQWHY QKVSSNNEEL KLPENSFLDH 240 TVDLSGMHSK TSHGGGFGSL DWQGGADQGL FDLPNTVDHA YWSHTHWSDH DNSSSLFHLP 300
3-152	**Sequences**	
3-152-1	Sequence Number [ID]	152
3-152-2	Molecule Type	AA
3-152-3	Length	351
3-152-4	Features	**source 1..351**
	Location/Qualifiers	mol_type=protein
		organism=Glycine max
	NonEnglishQualifier Value	
3-152-5	Residues	MSSVFSEHKF QLQPSHQLLS LKKSLGDIDI PVPPRKLLTR RSAAVHDGSG DIYLPHSGST 60 DSSTDDDSDG DPYASDQFRM FEFKVRRCSR SRSHDWTDCP FVHPGEKARR RDPRRFYYSG 120 TVCPEFRRGQ CDRGDACEFS HGVFECWLHP SRYRTEACKD GKNCKRKVCF FAHTPRQLRV 180 FHSNDNSNKK KCTDISPHNN NNCCLVCHCS NSTRSPTSTL FGMSHFSPPL SPPSPSSPSM 240 FETNNHHHGV VKYNKDVFSE LVCSMEGLNF DEASSLLSAA SKPHHHNNLS SWLDVSKDHN 300 QKQFNTLNSP TITACGSFSN NGNGGFLRAE NGVVVDDVIA PDLAWVNELL M 351