Production of unusual fatty acids in rapeseed

Oilseeds as a source of novel fatty acids for the industry

Oléagineux, Corps Gras, Lipides. Volume 9, Numéro 1, 24-30, Janvier - Février 2002, Dossier : Lipides des plantes

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Résumé : Les manipulations sur la qualité de l'huile chez les plantes cultivées sont d'un intérêt majeur et dépendent des modifications de la composition en huile des graines. Les acides gras habituels sont stockés sous forme de triacylglycérols. De nombreuses espèces accumulent des acides gras inhabituels (AGI) qui peuvent servir comme précurseurs à de nombreux produits chimiques ou encore conférer de nouvelles propriétés physiques ou chimiques aux huiles. L'isolement de gènes contrôlant la synthèse d'AGI a permis la biosynthèse d'AGI par génie métabolique dans des graines de plantes cultivées. Nous présentons, ici quelques exemples de modifications de la composition en TAG chez le colza et tentons de comprendre quels peuvent être les facteurs limitant l'accumulation des AGI. Nous concluons que pour produire une huile homogène à un taux élevé, avec une nouvelle composition en acide gras chez une plante cultivée, il sera nécessaire de fournir un environnement optimal au transgène, de s'assurer que la plante est capable d'incorporer des AGI au niveau des TAG et enfin, de contrôler le métabolisme des lipides de stockage dans les graines en cours de développement chez ces plantes transgéniques.

Mots-clés : acides gras inhabituels, triacylglycérols, ségrégation, Brassica napus.

Summary : Vegetable-derived oils are of interest for industrial applications partly because of the chemical similarity of plant oils to mineral oils but also because of the economic need to reduce overproduction of seed oils for nutritional use. Complex oils can be produced in seeds as a low cost agricultural product based on renewable solar energy that requires less refining and is biodegradable and thus produces less adverse effects on the environment. In addition, biotechnologies have accelerated selection programmes and increased the genetic diversity available for the development of new varieties of oilseeds with specific fatty acid compositions. In the developing oilseed, energy and carbon are stored as lipid under the form of triacylglycerol, that is, a glycerol molecule to which three fatty acids are esterified. Fatty acids comprise a linear chain of carbon atoms, the first of which carries an organic acid group. The chain length and the presence of double bonds determine the properties of the fatty acid which in turn determine the physical and chemical properties of the oil of storage lipids and hence their economic value. In addition to the common C16- and C18-saturated and unsaturated fatty acids of membrane lipids, the seed storage lipids of many plant species contain unusual fatty acids (UFAs) which can vary in chain length, in the degree of unsaturation, possess double bonds in unusual positions, or can contain additional functional groups such as hydroxy,

epoxy, cyclic and acetylenic groups [1]. These unusual fatty acids are of value as industrial feedstocks and their uses include the production of fuels and lubricants, soap and detergents, paints and varnishes, adhesives and plastics (Figure 1).

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Advantages of rapeseed for oilseed biotechnology

Although there are some attempts to cultivate plants that accumulate unusual fatty acids, in general these species are not agronomically suitable. Thus, an alternative is to develop transgenic crop plants engineered to produce high levels of specific unusual fatty acids as components of seed triacylglycerols. Rapeseed offers several advantages for the application of biotechnological approaches to improve agronomic quality traits such as seed oil or protein composition [2]. The primary advantage of *Brassica napus* is the simplicity of transformation and regeneration. In addition, the close phylogenetic association between rapeseed and *Arabidopsis* allows rapeseed biotechnology to benefit from *Arabidopsis* genomic resources. The availability of the *Arabidopsis* genomic sequence [3] and a vast expressed sequence data set [4, 5] together with extensive mutant collections have led to the identification and isolation of many genes encoding enzymes of lipid biosynthesis [6]. The extensive co-linearity between the *Arabidopsis* and *Brassica* genomes at the individual gene and at the large chromosome segment level allows the manipulation of major genes contributing to the important characters such as glucosinolate content and oil content. The analysis of QTLs in *Brassica* followed by comparative mapping will allow the isolation of *Arabidopsis* genes. *Arabidopsis* transgenes may then be used to alter levels of gene expression in rapeseed in order to manipulate agronomically important characters.

Storage lipid biosynthesis in oilseeds

In developing rapeseed, the synthesis of triacylglycerols can be described in three stages, *de novo* synthesis of fatty acids in the plastid, modification of fatty acids and assembly into lipids at the endoplasmic reticulum followed by storage in the oleosomes (described in [7]). However, the synthesis and modification of fatty acids and the assembly of triacylglycerols in seeds is highly divergent among plant species [8].

In all plants, *de novo* fatty acid biosynthesis occurs in the plastid and is controlled by the fatty acid synthase complex *(Figure 2)*. The 2-carbon unit of malonyl-ACP produced from acetylCoA by acetylCoA carboxylase is used to elongate an acyl primer by successive condensations controlled by ketoacyl (ACP) synthases to C16:0-ACP and C18:0-ACP. The majority of the C18:0 is desaturated to oleoyl-ACP by stearoyl desaturase and in the seeds of certain species additional desaturases may produce unusual C18:1 isomers. The elongation of fatty acids may be terminated by the action of two acyl-ACP thioesterases, the C16:0-preferring isoform is encoded by the FatB gene and the C18:1-preferring isoform encoded by FatA, which hydrolyse the acyl-ACPs to release free fatty acids which are then exported to the cytoplasm. The seeds of certain species possess additional thioesterases that terminate elongation and result in the production of short and medium-chain fatty acids. The fatty acids are exported to the cytoplasm, and esterified to CoA. The acylCoA thioesters are used for the synthesis of glycerolipids *via* the eukaryotic (Kennedy) pathway located at the endoplasmic reticulum. The fatty acyl groups are incorporated by the successive acylation of glycerol-3-phosphate by the action of glycerol-3-phosphate acyltransferase (GPAT) to form lysophosphatidic acid (LPA), 1-

acyl-glycerol-3-phosphate acyltransferase (LPAAT) to form phosphatidic acid (PA) which is dephosphorylated by phosphatidic acid phosphatase (PAP) to form diacylglycerol (DAG) and in developing seeds, finally acylated to form triacylglycerol (TAG) by diacylglycerol acyltransferase (DAGAT). The seeds of species that accumulate short- and medium-chain saturated or unusual monounsaturated fatty acids in their triacylglycerols possess additional LPAATs with substrate specificities for these UFAs. Fatty acids esterified to phosphatidylcholine are subject to modification by endoplasmic reticulum associated DELTA-12 and DELTA-15 desaturases to produce polyunsaturated fatty acids linoleic (C18:2) and linolenic (C18:3) or may be subject to additional modifications by including hydroxylation, acetylation and epoxidation or elongation in the seeds that synthesise UFAs.

Engineering unusual fatty acid production in rapeseed

The majority of genes encoding enzymes involved in storage lipid biosynthesis in plants have now been isolated, and include genes encoding ketoacyl-ACP synthases, acyl-ACP thioesterases, desaturases and acyltransferases from *Arabidopsis* and from crop species but also from species that accumulate certain UFAs. It appears that the synthesis of a particular UFA may require only one additional enzymatic reaction to that of primary lipid synthesis and that all enzymes identified to date that are involved in UFA synthesis are structurally related to enzymes of primary metabolism. Therefore a process of duplication and specialisation of housekeeping genes has given rise to enzymes which have variant substrate preferences that catalyse closely related but modified reactions [9].

We summarise below the progress in attempts to produce UFAs in rapeseed. Results obtained with *Arabidopsis* transformations frequently serve as an intermediate model to test rapeseed transformations and are included. Genes encoding enzymes controlling the synthesis of diverse products including polyhydroxybutyrate, industrial enzymes and pharmaceutical peptides have also been transferred to rapeseed [2].

Medium-chain fatty acids

The most successful example to date, that of a transgenic crop with a modified oil composition is that of lauric acid rapeseed, commercially cultivated in the USA since 1995. In plants that accumulate medium-chain length (C8-C14) fatty acids, the plastidial elongation pathway is modified to produce shorter chains by action of an additional acyl-ACP thioesterase. In the case of high laurate rapeseed, an acyl-ACP thioesterase of *Umbellularia californica* that possesses a substrate preference for C12:0 acyl groups was expressed in the developing seeds and resulted in the production of oils containing up to 40% laurate [10]. The incorporation of laurate was restricted to the sn-1 and sn-3 positions of the triacylglycerol because the rapeseed LPAAT does not use C12:0CoA as a substrate. In species such as coconut and *Cuphea lanceolata*, additional, seed-specific LPAATs are present that use medium-chain saturated fatty acids as a substrate to allow the synthesis of trisaturate TAG [11]. Thus, the co-expression of a coconut C12:0-CoA-preferring lysophosphatidic acid acyltransferase in high laurate rapeseed allowed the accumulation of laurate at sn-2 of the triacylglycerol and resulted in further increases in laurate content and in the production of trilaurin up to 40% [12].

Unusual monounsaturated fatty acids

Unusual monounsaturated fatty acids exist with double bonds at positions other than DELTA-9 as in oleic acid. The coriander seeds *(Coriandrum sativum)* contain petroselinic acid (C18:6) which is formed by the action of a plastidial DELTA-4 desaturase that introduces a double bond between carbons 4 and 5 of palmitoyl-ACP to produce DELTA-4 hexadecanoyl-ACP which is subsequently elongated to petroselenyl-ACP [13]. This desaturase is structurally related to the soluble plastidial DELTA-9 desaturase encoded by the *AAD1* gene but possesses a different chain length and regiospecificity. Expression of the coriander gene encoding the D4 palmitoyl ACP desaturase in rapeseed was reported to lead to the accumulation of up to 3% petroselinic acid in mature seeds [14].

Hydroxy, epoxy and acetylenic fatty acids

The unusual fatty acids containing hydroxy, epoxy and acetylenic functional groups are synthesised by enzymes that are structurally similar to the endoplasmic reticulum-bound DELTA-12 desaturases encoded by the *FAD2* gene for example, the gene encoding the hydroxylase from *Ricinus communis* [15]. The structural similarities between desaturases and hydroxylases allowed the isolation of other *FAD2*-like enzymes from other species that accumulate UFAs, for example the epoxygenase and acetylenase from Crepis [16]. The expression of *ricinus* and *Lesquerella* hydroxylases in *Arabidopsis* led to the production of four hydroxylated fatty acids present at levels up to 17% of the seed oil [17]. In an attempt to maximise ricinoleic acid production, the two hydroxylase genes were expressed separately in a *fad2/fae1* double mutant of *Arabidopsis* lacking the DELTA-12 desaturase and oleate elongase activities [18]. Transformants produced both ricinoleic and densipolic acids (C18:2-OH) at levels up to 42% in their seed oils. When expressed in a *fad3* mutant lacking DELTA-15 desaturase activity, ricinoleic acid was produced but densipolic acid was absent.

Very long chain monounsaturated fatty acids

The very long chain monounsaturated fatty acids (VLCMFA) possess chain lengths greater than C18 and are synthesised in the cytoplasm by the elongation of a C18:1 acyl precursor. Each elongation cycle requires four enzymatic reactions: condensation of the two carbon moiety of malonyl-CoA, reduction, dehydration and a second reduction controlled by an endomembrane-bound acylCoA elongase complex [19]. Erucic acid, a VLCFA, is present in seed oil of *Brassica napus* but only at positions sn-1 and sn-3 of the triacylglycerol. In contrast, VLCMFAS are present at each position of the glycerol skeleton of the TAG of *Limnanthes douglasii* because of the presence of a seed-specific, VLCFA-prefering LPAAT gene [20]. In order to increase the erucic acid content of rapeseed oil, the erucic acid-prefering LPAAT of *Limnanthes* was expressed in rapeseed. In lines possessing high levels of elongase activity up to 40% erucic acid was incorporated at position sn-2 [21]. The failure to increase the total erucic acid content in these lines suggested that erucic production was limited by elongase activity. The expression of the *FAE1* (fatty acid elongation 1) gene encoding the condensing enzyme, a ketoacylCoA synthase alone is sufficient to direct the synthesis of VLCFAs since the other activities necessary for elongation seem to be ubiquitously expressed [22]. However, although rapeseed coexpressing the *Limnanthes* LPAAT gene and a *Brassica napus FAE1* gene accumulated 60% erucic acid at sn-2, the total erucic acid content was only slightly increased [23].

Accumulation of VLCFAs to a level of 60-70% may reflect possible limitation by the availability of a C18:1 substrate for C18:1CoA elongation or by the cytosolic pool of malonyl CoA or alternatively, it is possible that the elongation may be limited by the activity of the additional acylCoA elongase subunits.

Liquid waxes

Waxes comprise esters of long chain alcohols and fatty acids. Waxes are formed by elongation of oleoylCoA to produce very long chain CoA precursors. Fatty alcohols are formed by reduction of very long chain acyl-CoAs under the control of a fatty acyl-CoA reductase. A wax synthase (fatty acylCoA: fatty alcohol acyltransferase) activity then transfers an acyl moiety from acylCoA to the fatty alcohol to produce a wax molecule [24]. The coexpression of a *FAE1* gene of *Lunaria annua*, together with the fatty alcohol reductase and wax synthase genes from jojoba led to the accumulation in *Arabidopsis* seeds of long chain wax esters representing up to 70% by weight of the oil content [25]. This example, where carbon has been diverted from TAG formation to wax synthesis illustrates the enormous extent to which seed storage lipid compositions can be altered by transgenesis.

Factors limiting unusual fatty acid production in crop plant seeds

The enormous natural diversity of seed TAGs implies that it is theoretically possible to produce UFAs in rapeseed and other crop species. Economically viable development of industrial oils will require the production of uniform TAG compositions containing 80-90% of a single fatty acid. Certain species such as *Ricinus communis* accumulate up to 90% of the acyl groups of their TAG as ricinoleic acid and thus, in principle, there is no genetic barrier to producing near uniform TAG compositions. It was initially perceived that traits controlling seed oil quality would be controlled by a single or few genes and that metabolic pathways to store lipid synthesis were sufficiently well characterised to allow manipulation by single gene transfer in order to develop new rapeseed varieties with desired TAG compositions. In the majority of cases, the transfer of genes coding for enzymes of UFA synthesis to rapeseed and other oilseed crops has led to only modest levels of novel storage lipids containing the desired UFA. What are then the limitations to the accumulation of high levels of UFAs in the seeds of crop plants? Factors that influence the biosynthesis of modified triacylglycerols include: the optimisation of UFA synthesis, the stability of the UFA controlled by efficient segregation into TAG and the stability of the novel storage lipid.

Environment for transgene function-protein cofactors

In order to produce UFAs in rapeseed in quantities similar to the level found in the native species, the expression of proteins complementary to the enzyme responsible for the synthesis of the fatty acid may be necessary to ensure the optimal synthesis and incorporation into triacylglycerol. For example, *Culphea* species that produce high levels of medium-chain saturated fatty acids in their seed oils possess a keto-acyl (ACP) synthase II activity that is expressed in seeds and optimises the production of medium-chain length acyl groups for interception and termination of elongation by the specialised acyl-ACP thioesterases co-expression with the C8:0, C10:0 or the C12:0, the ACP thioesterase resulted in an enhanced accumulation of medium-chain fatty acids compared to lines expressing the acyl-ACP thioesterases only [26, 27].

Similarly, the accumulation of petroselenic acid in the endosperm of coriander seeds requires, in addition to the plastidial DELTA-4 ACP desaturase, at least four additional specialised components including a variant petroselenyl-ACP thioesterase [28] and a specialised ACP [29], a variant ketoacyl-ACP synthase I specific for DELTA-4 hexadecanoyl-ACP that controls the elongation to petroselinic acid [30]) and a specialised ferredoxin [31]. This degree of complexity of the cofactors involved suggests that a specialised complex may exist that controls the synthesis of petroselinic acid in coriander. The optimal production of petroselinic acid in transgenic rapeseed may therefore require the co-expression of these specialised enzymes as a functional "metabolon".

Segregation of storage and membrane lipid biosynthesis

The chemical and physical properties of UFAs are potentially incompatible with the structure and functioning of cellular bilayer membranes. Plants are able to tolerate UFAs in the form of storage lipids since they are partitioned into lipid bodies in seed tissue and are absent from membrane lipids of other plant tissues. In developing seeds, storage and membrane lipid biosynthesis occur simultaneously in the endoplasmic reticulum and share common enzymes and intermediates. Nevertheless, UFAs are excluded from the phospholipids of seed tissue indicating an efficient segregation between TAG and membrane lipid synthesis [32]. This discrimination is remarkable since in many cases the acyl group substrates of the modification enzymes that control the synthesis of UFAs are esterified to the membrane lipid, phosphatidylcholine. In contrast, the developing seeds of transgenic plants expressing genes encoding enzymes of UFA synthesis appear to be less efficient at excluding UFAs from membrane lipids during triacylglycerol accumulation. The best documented example is that of *Brassica napus* seeds engineered to synthesize medium-chain fatty acids where lines that produced laurate in excess of 50% accumulated more than 30% laurate in phosphatidylcholine [33]. By comparison, species that naturally accumulate C12:0 contained only 1-4% laurate in phosphatidylcholine. Thus, the exclusion of laurate from phospholipids is less efficient in developing rapeseed than in species that naturally accumulate laurate. Remarkably, the mature seeds of these high laurate transformants contained less than 7% laurate in phosphatidylcholine, indicating that an editing process is present in rapeseed during seed maturation. Other UFAs, including short, medium, long and oxygenated fatty acids may also be removed from membrane lipids during seed maturation [34].

A consequence of this inability of transformed rapeseed to channel large quantitites of medium-chain fatty acids during the phase of TAG accumulation is the induction of beta-oxidation and glyoxylate pathways that recycle excess fatty acids to acetylCoA and sucrose [35]. In these transformants, oil content was unchanged since the loss of fatty acids to beta-oxidation was compensated by an increase in fatty acid biosynthesis. Thus, much of the potential substrate for TAG biosynthesis produced in the developing seed of high lauric acid transgenic plants is lost to a futile cycle of synthesis and breakdown of lauric acid. A second, potentially serious, consequence of the incapacity of developing seeds of transgenic plants to remove UFAs from membrane lipids during rapid TAG synthesis may disrupt membrane integrity and physiological functioning of seed membranes and impair seed development and germination.

Mechanisms that exclude unusual fatty acids from membrane phospholipids

If transgenic rapeseed can accumulate large amounts (greater than 80-90%) of UFAs in the TAGs, it may be necessary to improve the capacity to prevent contamination of seed membrane lipids by UFAs. Thus, an understanding of how certain plants exclude unusual fatty acids from membrane lipids may be central to the problem of producing high levels of novel lipids in the seeds of transgenic crop plants. In plants, the synthesis of membrane and storage lipids occurs simultaneously in the endoplasmic reticulum of the developing seeds using a common pathway. How then are the diverse acyl group compositions of storage and membrane lipids established and maintained? Three possible mechanisms include the spatial separation of membrane and storage lipid synthesis, channelling determined by the specificity of enzymes of DAG utilisation, and editing of phospholipid acyl group composition.

Compartmentation of TAG biosynthesis

The hypothesis that membrane and storage lipid synthesis are spatially separated in the endoplasmic reticulum using distinct subsets of enzymes is based on the observation that the enzymes that metabolise diacylglycerol and triacylglycerol do not possess a sufficiently strong substrate specificity to channel diacylglycerol containing common or unusual fatty acids into either membrane or storage lipids. The observation that enzymes of TAG and membrane lipid biosynthesis do not co-localise after fractionation of the ER from developing rapeseed [36] and that isoforms exist for each Kennedy pathway enzyme supports this hypothesis (TJ Roscoe, unpublished results) as does the identification of a subset of enzymes specialised for the synthesis of petroselinic acid [30, 31]. However, the fact that the synthesis of fatty acyl groups occurs when esterified to phosphatidylcholine argues against separation of phospholipid and TAG synthesis in the endomembrane system.

Segregation (channelling) of UFAs to TAG biosynthesis

The channelling of unusual fatty acids to TAG biosynthesis is strongly influenced by the acyltransferases that synthesize and utilise intermediates of the Kennedy pathway *(figure 3)*. In particular, lysophosphatidic acid acyltransferase (LPAAT) and diacylglycerol acyltransferase (DAGAT) exhibit variations in substrate preferences for both donor acyl groups and acceptor glycerolipids. For example, the glycerol-3-phosphate acyltransferase of *Culphea lanceolata* seeds can incorporate a medium- or long-chain fatty acids into position sn-1 of the glycerol-3-phosphate, but the acylCoA specificity of the LPAAT is influenced by the composition of the lysophosphatidic acid (LPA) acceptor substrate. When caproylate is present in LPA then a C10:0-CoA-preferring LPAAT isoform is used to acylate at position sn-2. Furthermore, the *Culphea lanceolata* DAGAT exhibits a strong selectivity for diacylglycerol containing C10:0 and uses C10:0-CoA to acylate position sn-3. Hence, the synthesis of diacylglycerol containing C18:1 at sn-1 and C10:0 at sn-2 is prevented, and the TAG is enriched in C10:0 acyl groups [37]. The DAGAT of certain species exhibits a selectivity for UFAs, and therefore plays an important role in the removal of UFAs from phosphatidylcholine. Since the diacylglycerol and phosphatidylcholine pools are in equilibrium, UFAs present in the diacylglycerol moiety of phosphatidylcholine may be channelled into TAG by DAGAT [38].

The equilibrium between the diacylglycerol and phosphatidylcholine pools is controlled by CDP-choline phosphotransferase (CPT) *(Figure 4)*. This enzyme could therefore also play a role in channelling UFAs that are esterified to phosphatidylcholine into diacylglycerol for TAG synthesis or

could prevent diacylglycerol that contains UFAs from contaminating the phosphatidylcholine pool. The substrate preference of CPT was examined in microsomes of seeds that accumulate medium-chain, ricinoleic acid and erucic acid but none of the enzymes showed selectivities for diacylglycerol containing either common or UFAs and therefore CPT does not influence channelling to TAG [39].

Editing of phospholipids

The modified acyl groups that are synthesised on, or that cycle through, phosphatidylcholine are present in a lower abundance in membrane lipids than in the TAGs of mature seeds in species that accumulate UFAs implying a selective removal or editing. This process could be mediated by the action of phospholipases or by acyltransferases and transacylases. Certain plants possess seed phospholipases that remove UFAs from membrane lipids and are specific for the UFAs synthesized by that plant. For example, medium-chain fatty acids are removed from phosphatidylcholine in developing seeds of *Ulmus* which accumulates capric and caproic acids, by a phospholipase A2 activity [40]. Similarly, *Ricinus, Culphea* and *Euphorbia* possess phospholipase A2 activities that are selective for individual UFAs. Although the action of PLA2 would reduce contamination of membrane lipids by UFAs, it is not clear how editing by specific phospholipases would direct UFAs to TAG.

Evidence has accumulated for the existence of acylCoA-independent transacylation pathways for the synthesis of triacylglycerol which may provide a means to both remove UFAs from phospholipids and channel these towards storage lipid synthesis. An enzyme, phospholipid/diacylgylcerol acyltransferase, present in microsomal extracts of castor, sunflower and crepis, was shown to transfer UFAs from sn-2 of phosphatidylcholine to diacylglycerol to form triacylglycerol and lysophosphatidylcholine. Thus, UFAs would be removed from phosphatidylcholine and prevented from accumulating in diacylglycerol and the acylCoA pool [41].

It is probable that several enzymes are responsible for the removal of unusual fatty acids from membrane lipids and include phospholipases, transacylases and acyltransferases, the relative importance of which varies among species. The channelling of unusual fatty acids into TAG varies among species as a consequence of the substrate selectivities of the acylCoA-dependent acyltransferases and transacylases.

Remodeling of TAG composition

There is evidence that triacylglycerols are not metabolically inert but remain accessible to metabolism by transacylase enzymes that remodel the TAG acyl composition. In microsomes isolated from the developing seeds of castor and safflower, acyl groups from phosphotidylcholine are used in a reversible transacylation reaction where two diacylglycerol molecules are used to produce a TAG and a monoacylglycerol molecule. Rapid exchange between diacylglycerol and phosphatidylcholine *via* CPT could explain the remodelling of TAG with groups from phosphatidylcholine [42]. Such remodelling could either replace UFAs in TAG with acyl groups from phosphatidylcholine or enrich the TAG composition with UFAs and polyunsaturated acyl groups and thus influence oil quality [43].

CONCLUSION

Great progress has been made in identifying genes controlling the biosynthesis of unusual fatty acids and expressing these genes in rapeseed with the objective of modifying seed oil composition. The economic production of UFAs in the seeds of crop plants will require that the TAG composition is modified to produce a uniform oil containing a predominant UFA. This is a feasible goal since certain non-cultivated species produce oils with near uniform fatty acid compositions. For transgenic plants to accumulate high levels of UFAs in TAG, it will be necessary to optimize the synthesis of the UFA, prevent the degradation of the UFA and ensure that the UFA is incorporated and stabilised in the TAG.

The optimal production of UFA in rapeseed or other oilseed crops will require a knowledge of how the UFA is synthesised in the native plant in order to ensure an environment for the correct functioning of the biosynthetic enzyme encoded by the transgene. The study of the bioynthesis of UFAs in their native species has revealed a requirement for specialised protein cofactors. These results suggest the existence in native plants of enzymatic complexes specialised in the production of UFAs. Thus, to achieve high-level accumulation of certain UFAs in rapeseed it may be necessary to transfer proteins additional to the enzymes responsible for the synthesis of the UFA. A second problem related to the host environment is that of structural compatiblity of the transgene product with the enzymes of the biosynthetic pathway present in the host species. In certain cases it may be desirable to re-engineer the endogenous enzyme to produce a novel fatty acid. The feasibility of this approach has been demonstrated with the engineering of substrate preference and regiospecificity of soluble desaturase enzymes [44].

The problem of accumulation of excess UFAs and the potential contamination of membrane lipids may be circumvented by the creation of a lipid sink. The observation that both synthetic and catabolic activities are induced in plants producing excess medium-chain fatty acids and that oil content is unchanged suggests that seeds may be preprogrammed to produce a particular amount of oil with lipid biosynthetic enzyme activity adjusted to meet the prescribed demand for TAG biosynthesis. If the production of UFAs can be coordinated with their utilisation for TAG biosynthesis, perhaps through incorporation into TAGs, then it should be possible to avoid degradation of fatty acylCoAs by beta-oxidation. The reports of enhanced TAG production in plants transformed with acyltransferases suggest that by creating new sinks it may be possible to channel excess production of UFAs into TAG synthesis [45, 46].

As a conclusion, the biotechnological problems of producing unusual fatty acids and accumulating novel uniform lipids in rapeseed provide insight into two fundamental questions of lipid biology: how do plants regulate the acyl composition of membrane lipids and what is the basis of the genetic variation in seed storage lipid content.

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Illustrations

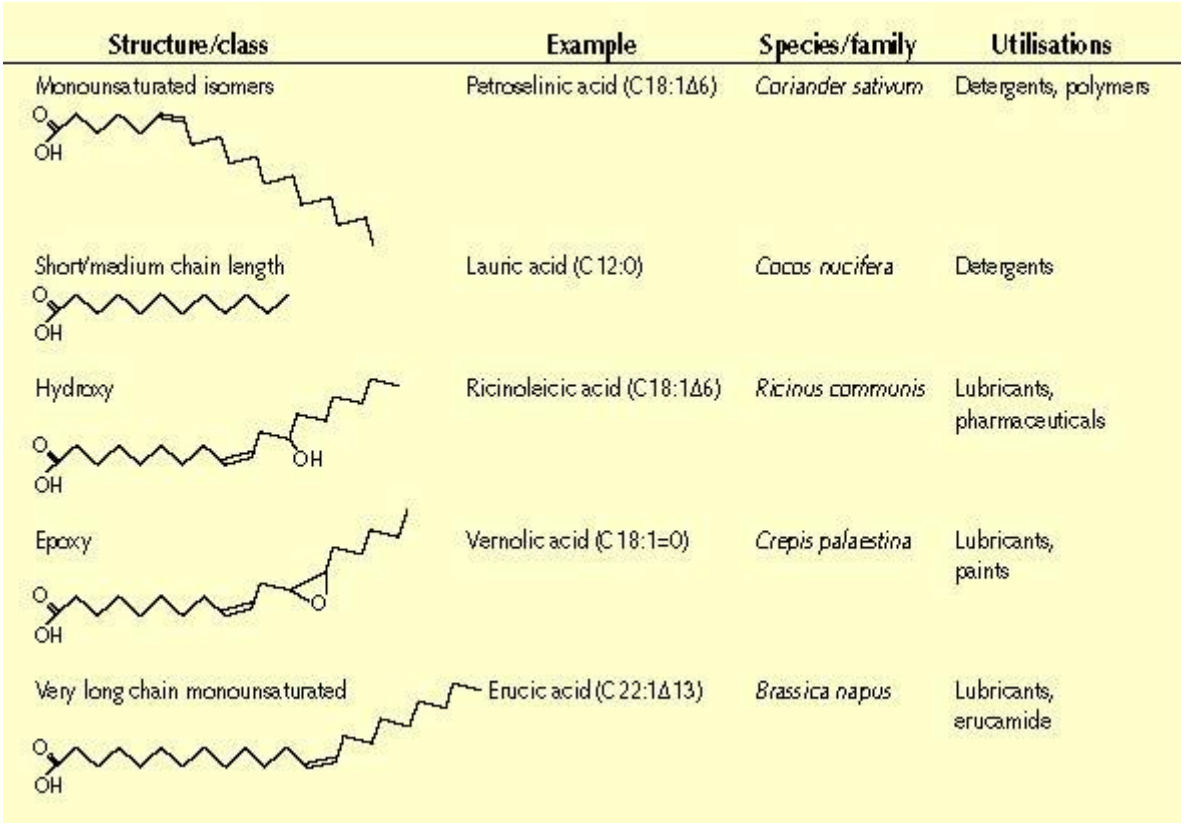
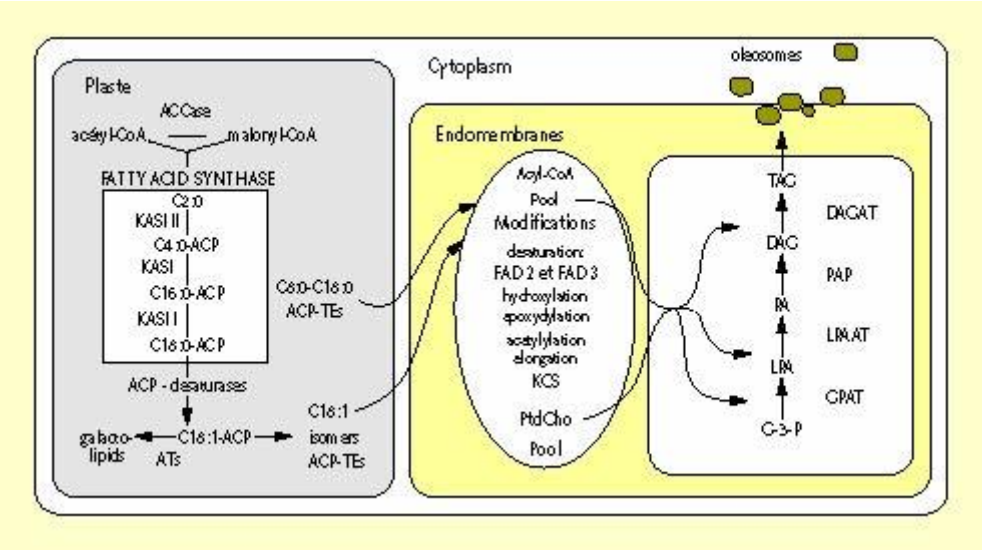
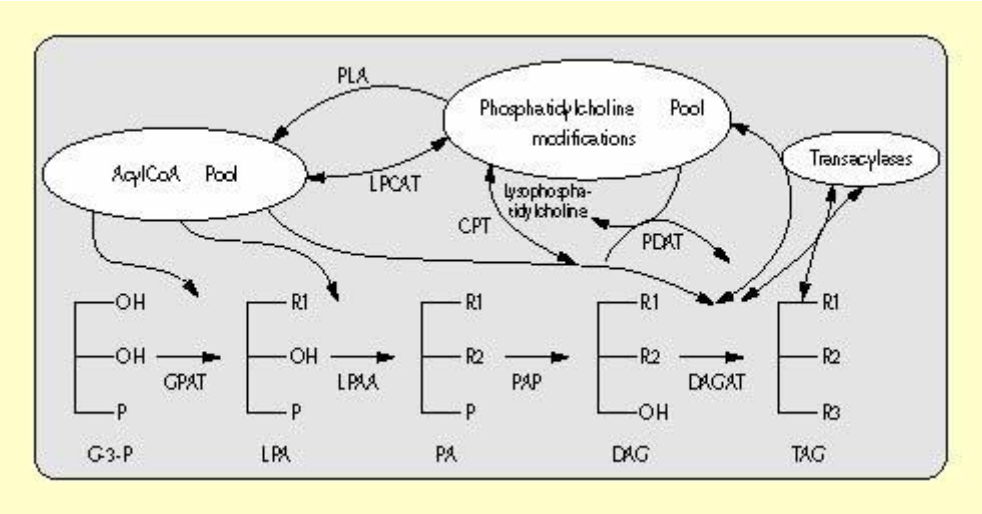


Figure 1. *Industrial uses for unusual fatty acids present in seed oils.*



AcetylCoA and malonylCoA are elongated to C16:0-ACP and C18:0-ACP controlled by ketoacyl (ACP) synthases and terminated by the action of two acyl-ACP thioesterases which hydrolyse the acyl-ACPs. The seeds of certain species possess additional thioesterases that terminate elongation and result in the production of short- and medium-chain fatty acids. The majority of the C18:0 is desaturated to oleoyl-ACP by stearoyl desaturase and in the seeds of certain species additional desaturases may produce unusual C18:1 isomers. The fatty acids are exported to the cytoplasm as acylCoA thioesters that are used for the synthesis of glycerolipids via the Kennedy pathway located at the endoplasmic reticulum. The fatty acyl groups are incorporated into glycerol-3-phosphate by the action of glycerol-3-phosphate acyltransferase (GPAT) to form lysophosphatidic acid (LPA), 1-acyl-glycerol-3-phosphate acyltransferase (LPAAT) to form phosphatidic acid (PA) which is dephosphorylated by phosphatidic acid phosphatase (PAP) to form diacylglycerol (DAG) and in developing seeds, finally acylated to form triacylglycerol (TAG) by diacylglycerol acyltransferase (DAGAT). The seeds of certain species possess additional LPAATs with substrate specificities for UFAs. Fatty acids esterified to phosphatidylcholine are subject to modification by endoplasmic reticulum-associatedDELTA-12 and DELTA-15 desaturases to produce polyunsaturated fatty acids linoleic (C18:2) and linolenic (C18:3) or may be subject to additional modifications by including hydroxylation, acetylation and epoxidation or elongation in the seeds that synthesise UFAs.

Figure 2. *Biosynthesis of storage lipids in oilseeds.*



Unusual fatty acids are channelled into storage lipids by the acyltransferases LPAAT and DAGAT. Diacylglycerol may be exchanged reversibly with phosphatidylcholine by CDP-choline diacylglycerol choline phosphotransferase (CPT). Fatty acids esterified to phosphatidylcholine may be subject to additional modifications. The unusual acyl groups may be exchanged with the acylCoA pool by the action of acylCoA lysophosphatidylcholine acyltransferase (LPCAT) or removed to the acylCoA pool by the editing action of phospholipases (PLA). Triacylglycerol may be formed by an alternative reaction using the fatty acid esterified at sn-2 of PtdCho for acylatation of DAG by the action of phospholipid diacylglycerol acyltransferase (PDAT). The acyl groups present may be exchanged with phosphatidylcholine and triacylglycerols may be formed directly from diacylglycerol by the action of transacylases.

Figure 3. S*egregation and remodelling of unusual fatty acids.*