

Vegetable Oil: Nutritional and Industrial Perspective

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Abstract: Oils of plant origin have been predominantly used for food-based applications. Plant oils not only represent a non-polluting renewable resource but also provide a wide diversity in fatty acids (FAs) composition with diverse applications. Besides being edible, they are now increasingly being used in industrial applications such as paints, lubricants, soaps, biofuels etc. In addition, plants can be engineered to produce fatty acids which are nutritionally beneficial to human health. Thus these oils have potential to 1) substitute ever increasing demand of non-renewable petroleum sources for industrial application and 2) also spare the marine life by providing an alternative source to nutritionally and medically important long chain polyunsaturated fatty acids or 'Fish oil'. The biochemical pathways producing storage oils in plants have been extensively characterized, but the factors regulating fatty acid synthesis and controlling total oil content in oilseed crops are still poorly understood. Thus understanding of plant lipid metabolism is fundamental to its manipulation and increased production. This review on oils discusses fatty acids of nutritional and industrial importance, and approaches for achieving future designer vegetable oil for both edible and non-edible uses. The review will discuss the success and bottlenecks in efficient production of novel FAs in non-native plants using genetic engineering as a tool.

Keywords: Omega-3 fatty acids, Hydroxy fatty acids, Stearidonic acid, γ -linolenic acid, Oleic acids, Erucic acid.

1. INTRODUCTION

Oils have a variety of uses besides edible applications. There is now an increasing evidence that fatty acids (FAs) play a crucial role in human nutrition that include therapeutic and prophylactic prevention of diseases, in growth and development of human embryo, brain function and provide protection against many serious diseases such as cardiovascular, inflammation etc. Many FAs are now known to have anticancer potential. Importance of role of fats and fatty acids in human nutrition is gaining attention as more and more research is being done. Besides an essential component of human diet, FAs also find importance in various industrial applications such as soaps and detergents, cosmetics, lubricants, ink, varnish, paints etc. Thus an ever expanding market exists for oilseed crops from both nutritional and industrial perspectives. In addition, plants produce a wide variety of fatty acids with different structures that confer unique physico-chemical properties on them and make them useful.

With increasing petroleum prices and depleting natural resources, there is a longstanding need to explore and develop new sources of fatty acids of both industrial and nutritional importance. With advancement of understanding the steps in metabolic pathways in the synthesis of fatty acids, attempts have been intensified in engineering the pathways for the production of useful and/or novel fatty acids in a cost effective way. Designer oils that preferentially produce these

fatty acids can be created and will be economically feasible and competitive to petroleum based products.

2. BIOSYNTHESIS OF FATTY ACIDS AND TRIACYLGLYCEROLS

Normally plants produce FAs which may have zero to three double bonds. These commonly found usual FAs include palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3). In oilseed plants, these fatty acids are predominantly stored as triacylglycerols (TAG) which is the major storage form in seed. These lipids may be stored in cotyledon or endosperm which are used to supply energy during germination. In addition to TAG, fatty acids also exist in the form of wax esters, for example jojoba fruit (*Simmondsia chinensis*).

The fatty acids are synthesized in plastids from acetyl-CoA as starting substrate and on acyl carrier protein (ACP) (Fig. 1). The fatty acids are then removed from ACP by action of enzyme, thioesterase. Free fatty acids move to cytosol where they are further incorporated into acyl-CoA pool and/or phosphatidylcholine (PC) pool, which then undergo modifications such as desaturation or hydroxylation, epoxylation etc. and their inclusion into TAG takes place. These later processes occur in endoplasmic reticulum (ER) of plant cells [1, 2]. TAGs are the major storage form found in seeds. It is synthesized in ER, using acyl-CoA and glycerol-3-phosphate as substrates by the Kennedy pathway. The first enzyme is glycerol-3-phosphate acyltransferase (GPAT), that acylates sn-1 position of glycerol backbone to form lysophosphatidic acid (LPA). The second enzyme of the pathway is lysophosphatidic acid acyltransferase (LPAAT) that

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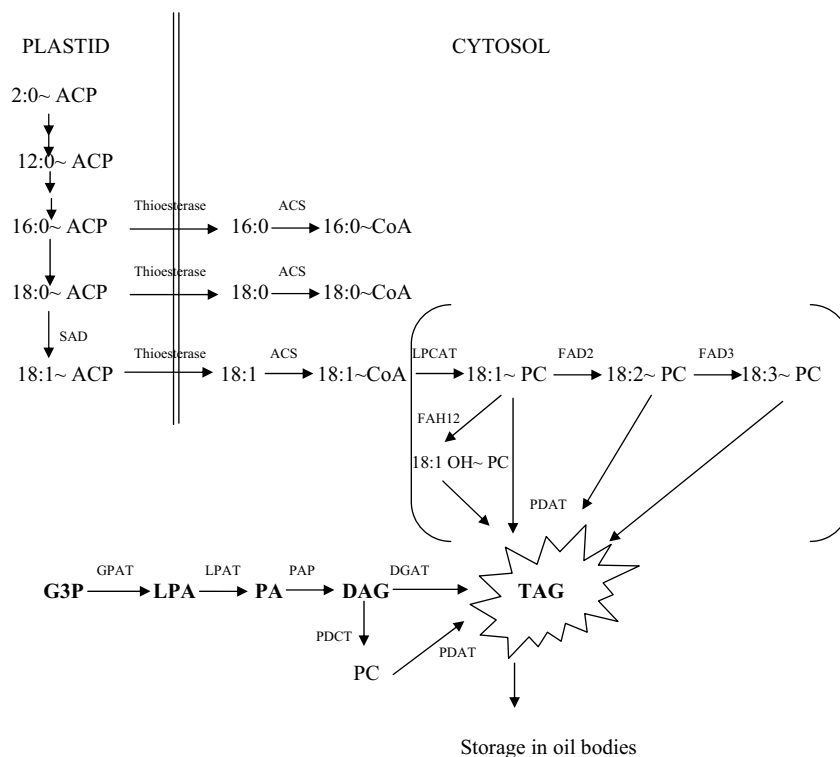


Fig. (1). Biosynthesis of commonly found fatty acids in plants. ACP: Acyl carrier protein, SAD: Stearoyl ACP desaturase, CoA: Coenzyme A, PC: Phosphatidylcholine, FAH12: Fatty acid hydroxylase 12, FAD2: Fatty acid desaturase 2, FAD3: Fatty acid desaturase 3, G3P: Glyceraldehyde-3-phosphate, LPA : Lysophosphatidic acid, PA: Phosphatidic acid, PC : Phosphatidylcholine, DAG: Diacylglycerol, TAG: Triacylglycerol, PDAT: Phospholipid: diacylglycerol acyltransferase, DGAT: acyl-CoA: Diacylglycerol acyltransferase, GPAT: acyl-CoA: Glyceraldehyde-3-phosphate acyltransferase, LPAT: Lysophosphatidic acid acyltransferase, LPCAT : Lysophosphatidic acid acyltransferase, PAP: Phosphatidic acid phosphatase, ACS: Acyl-CoA synthetase.

acylates at the sn-2 position to form phosphatidic acid (PA), which is then converted into diacylglycerol (DAG) by the enzyme phosphatidic acid phosphatase (PAP). Another acyltransferase, diacylglycerol acyltransferase (DGAT), forms TAG from DAG using an acyl-CoA as a substrate [1, 2].

Vegetable oils constitute an important component of human diet. Major edible vegetable oils in terms of production include those from soybean, canola, sunflower and peanut. They are sources of edible fatty acids (saturated, monounsaturated or polyunsaturated), which play an important role in cellular metabolism as a way to store energy and also by providing energy when required. FAs are known to play an important role in cell division and growth. They are an integral component of cell membranes, hormones, neurotransmitters etc. Intake of different fatty acids has a direct influence on human health. For example, increased intake of saturated fatty acids has been linked to cardiovascular diseases. Thus it is considered desirable to have diets low in saturated fatty acids. Besides these, some very long chain-polyunsaturated fatty acids (VLC-PUFA; C20-C22) such as arachidonic acid (ARA; 20:4), eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6), which are usually derived from marine resources, have been shown to play an important role in human nutrition.

Long-chain polyunsaturated fatty acids (LC-PUFAs) play a variety of roles in human nutrition. Based on the position of the first double bond, PUFA are of two types: omega-3 and omega-6 fatty acids. Of PUFA, linolenic acid (LA; 18:2) is a major omega-6 fatty acid while α -linolenic acid (ALA; 18:3), is a major omega-3 fatty acid. These fatty acids are synthesized by higher plants. Oleic acid is converted into LA by Δ 12-desaturase and LA, is then converted to ALA by Δ 15-desaturase. However, human body cannot synthesize these fatty acids *de novo*. Thus, these are essential fatty acids that need to be supplemented regularly in diet [3, 4]. The major source of these fatty acids in human diet is marine fishes. These fishes feed on other marine organisms such as algae and diatoms which are the primary source of these LC-PUFAs [5]. In human body, LA and ALA can be further metabolized to form longer chain fatty acids which play a crucial role in human growth and development. The linoleic acid is converted into arachidonic acid (ARA; 20:4), and α -linolenic acid into eicosapentaenoic acid (EPA; 20:5), and docosahexaenoic acid (DHA; 22:6) [3, 6-8]. However, these cannot be efficiently made by human body and need to be regularly supplemented to the diet [3, 9]. Though VLC-PUFAs cannot be synthesized by higher plants, there are some plants reported to produce stearidonic acid (SDA; 18:4) and γ -linolenic acid (GLA; 18:3), which are intermediates in the pathway of synthesis of these VLC-PUFA and have similar health benefits [10-14].

Different pathways of VLC-PUFAs have been identified in different organisms [7, 8, 15, 16]. In the conventional or $\Delta 6$ -desaturation pathway (Fig. 2), linoleic acid is first converted into γ -linolenic acid (GLA; 18:3) by $\Delta 6$ -desaturase [15]. The same enzyme also converts ALA into stearidonic acid (SDA; 18:4). The next step involves the synthesis of dihomono- γ -linolenic acid (DGLA; 20:3) and eicosatetraenoic acid (ETA; 20:4) by C2 elongation. In the final step, $\Delta 5$ -desaturase generates ARA (20:4) and EPA (20:5), respectively. EPA is further converted into DHA, via C2 elongation by enzyme $\Delta 5$ -elongase, followed by desaturation by $\Delta 4$ -specific desaturase. Pathway leading to the synthesis of DHA also varies in some organisms. The other pathway that has been characterized is $\Delta 9$ -pathway or also known as alternative pathway. This pathway is found in *Tetrahymena pyriformis*, *Pavlova sp.* *Isochrysis sp.* etc, where LA and ALA undergo elongation by $\Delta 9$ -elongase enzyme to give eicosadienoic acid and ETA, respectively. A specific $\Delta 8$ -desaturase acts on these substrates to form DGLA and ETA, respectively and as in the conventional $\Delta 6$ -pathway mentioned above, $\Delta 5$ -desaturase converts these fatty acids into ARA and EPA [7, 8].

3. BENEFICIAL EFFECTS OF OMEGA-3 FATTY ACIDS

As mentioned earlier, PUFA or more specifically the omega-3 fatty acids find more importance as human dietary supplement. Omega-3 fatty acids have been shown to decrease the risk of cardiovascular diseases or occurrence of type-2 diabetes (17,18,19). There is increasing evidence that omega-3 fatty acids also play a helpful role in treating ailments like depression- a widespread problem [20]. DHA has shown to improve sensitivity of brain tumor cells to anticancer drug, etoposide (VP16) and therapy [21]. The fatty acids are very crucial for retina and neural development, and for

overall fetal development [19, 22]. Thus milk powders are being fortified or supplemented with DHA and ARA for brain development in infants [22]. Omega-6 fatty acids such as γ -linolenic acid and dihomono- γ -linolenic acid (DGLA) have shown anticancer activities and have inhibitory effect on proliferation of cells [23].

Due to their health benefits, it is recommended to take omega-3 FAs as regular dietary supplement. On an average an adult needs to consume 250–2000 mg per day of EPA + DHA (FAO) [9]. There is therefore a growing demand for these fatty acids. Currently, as stated earlier the major source of VLC-PUFA is fish oil which is largely derived from marine resources. Marine fishes feed on marine microalgae like diatoms that are primary source of PUFA. The high demand of these FAs is leading to overfishing. Also, there are concerns of environmental pollutants [8]. Other sources such as aquaculture itself is dependent on fish meal as a source for feed to maintain levels of PUFA in cultured fishes. Thus, fish oil is not able to meet the current demand for omega-3 fatty acids, necessitating development of alternative and sustainable sources. In addition, linseed oil which is rich in omega-3 FA is also being promoted as a nutritional supplement.

In order to gain benefits of PUFA, one has to consider the intake of omega-3 FA in relation to omega-6 fatty acid consumption. This is because conversion of ALA to EPA and DHA also depends on the amount of intake of linoleic acid. It has been observed that increasing the ratio of intake of linoleic acid to α -linolenic acid competitively reduces the conversion of ALA to longer chain omega-3 fatty acid [22, 24]. There is a competition between omega-6 and omega-3 fatty acids for desaturation by the same $\Delta 6$ -desaturase i.e. LA to GLA and ALA into SDA. A ratio of 2:1 to 6:1 of omega-6 and omega-3 fatty acids is considered good and

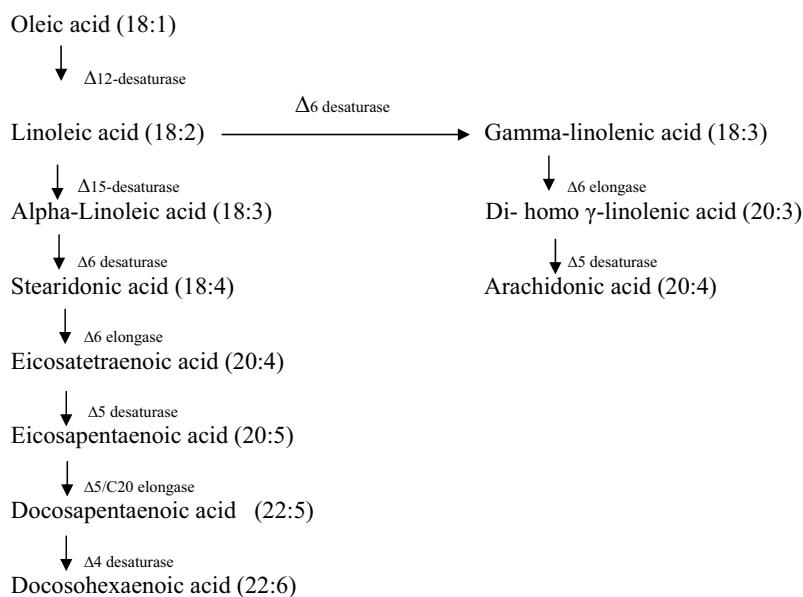


Fig. (2). Conversion of the linoleic acid to arachidonic acid (ARA) and α -linolenic acid to eicosapentaenoic acid (EPA) / Docosohexaenoic acid (DHA).

recommended, possibly improving cardiovascular health, asthma etc [25, 26]. This can be achieved by dietary intake of oils which are rich in α -linolenic acid such as flaxseed, walnut or monounsaturated rich vegetable oils such as olive oil. There are plants like *Camelina sativa* having very high content of α -linolenic acid.

4. FATTY ACIDS OF INDUSTRIAL IMPORTANCE

In addition to usual fatty acids discussed earlier, certain plants also synthesize several other FAs which deviate significantly in their physico-chemical properties. They are often termed “unusual fatty acids” (UFA) and may differ in carbon chain length, number and position of double bond, or they may have different functional groups such as hydroxy, epoxy, conjugated or acetylenic bonds etc. These UFAs are also stored mainly in TAG fraction inside seed. There are different types of unusual fatty acids reported that find their use in various applications. Unusual fatty acids have distinct chemical and physical properties which make them useful in industrial applications such as soaps, plastics, nylon, lubricants, paints, coatings and adhesives. For example, α -eleostearic acid found in tung oil has applications in paints and printing inks. Lauric acid, which is derived from coconut, is used for making soaps and detergents. Calendic acid is another type of UFA produced via desaturation of linoleic acid and is an important component of marigold seed oil. Besides these, another important industrial application of vegetable oil is as biofuel. A huge market therefore exists for these fatty acids. Normally the raw material for these applications is derived from petroleum-based resources. But due to depleting oil reserves and concerns for environmental pollution, there is an ensuing need of developing an alternative source.

Of all the unusual fatty acids, hydroxy fatty acids (HFA) are the most important from industrial standpoint. Ricinoleic acid is a kind of hydroxy-fatty acid produced by castor bean plants (*Ricinus communis*) of family Euphorbiaceae. It is produced by addition of hydroxyl group to oleic acid by enzyme oleate hydroxylase or fatty acid hydroxylase-12 (FAH12). Castor oil is composed of ~90% ricinoleic acid which is stored as TAGs in the seed. Ricinoleic acid has various industrial applications such as in nylons, paints, coatings, lubricants etc. Castor bean plants are the only commercial source for this fatty acid. However, one major drawback with castor plant is the presence of a protein toxin ricin, which is present in the endosperm of castor seed. Ricin has an enzymatic activity that catalyzes removal of an adenine moiety from a conserved specific region of 28S rRNA and thereby inhibiting protein synthesis by ribosomes containing depurinated 28S rRNA [27]. India is the largest producer of castor oil followed by China and Brazil. The other HFA is lesquerolic acid (20:1-OH), produced by lesquerella (*Physaria fendleri*), of Brassicaceae family which also has industrial applications.

Erucic acid is a very long chain fatty acid which finds its use in many industrial applications such as lubricant, plastic films, cosmetics etc. This fatty acid is produced from oleic acid through a series of reactions catalyzed by the enzyme fatty acid elongase, FAE1. It is normally produced in high levels in special cultivars of *Brassica napus*, also known as

high erucic acid rapeseed (HEAR). Other sources include *Crambe abyssinica* which produces up to 55%–60% erucic acid in its seed oil. The *Brassica* species produce a maximum of 45-50% of erucic acid, which is usually incorporated at sn-1 and sn-3 position of TAG.

5. GENETIC ENGINEERING OF PLANTS FOR PRODUCTION OF NUTRITIONALLY IMPORTANT FATTY ACIDS

Recently, there has been an interest to genetically modify plants to produce fatty acids from nutritional standpoint as well. With the advent of genetic engineering technology, it is now possible to produce designer oils with desirable fatty acid composition. One of the major advantages of using genetic engineering is to produce nutritionally beneficial fatty acids, which are normally produced in non-cultivated plant species or derived from marine sources, in agriculturally amenable crops. Once these designer plants are created, production of these FAs can become economically viable and can substitute limited and overexploited resources like marine life. Following are some examples where genetic manipulations have been carried out keeping nutrition as a focal point.

6. PRODUCTION OF OMEGA-3 FAs

As discussed earlier, PUFAs have beneficial effect on human health, and are largely derived from marine sources such as fish oil. There is a huge gap in the current demand and supply of fish oil and there is a need to identify and develop alternative sources to produce these FAs. Cultivation of microalgae directly for production of PUFA has a potential. The microalgae such as *Mortierella alpina* that are the primary producer of PUFA have been used to derive PUFA for commercial production [28]. Production of PUFA have also been detected in various microalgae that include *Phaeodactylum tricorutum*, *Fistulifera* species [29]. However, many of these microalgae are not suitable for large scale production and the technology needs further optimization, so that they become economically viable and cost effective [30]. Use of iterative metabolic engineering also holds potential in accumulation of omega-3 FAs as seen in diatoms, *Phaeodactylum tricorutum* by expressing gene from another alga, *Ostreococcus tauri* [31]. Metabolic engineering of yeast and *Yarrowia lipolytica* have been done to obtain sustainable production of EPA (reviewed in [32]). Still the global demand far exceeds the supply from above mentioned sources including marine or aquaculture. Other alternative approach is to identify and develop unconventional sources for production of PUFAs. Biotechnology can provide us a mean to genetically engineer oilseed plants to produce these PUFAs, in an economically viable way.

7. TRANSGENIC PRODUCTION OF STEARIDONIC ACID AND γ -LINOLENIC ACID

Besides fish oil, humans can also consume flaxseed, walnut etc, which have very high levels of α -linolenic acid (18:3). This ALA can be converted into EPA and DHA in the human body. However, it is more efficient to convert SDA to EPA than ALA to EPA and supports the suggestion that $\Delta 6$ -desaturase activity is rate limiting [33] and provides a step

which can be modulated by genetic engineering. As mentioned previously stearidonic acids give similar health benefits as EPA [13]. Some plants such as *Borago officinalis* can produce SDA in their seeds [34, 35]. However, these plants are not part of human diet and agronomically not amenable to cultivation. Oilseeds can therefore be engineered to produce this nutritionally important fatty acid by transferring $\Delta 6$ -desaturase gene to produce SDA. The $\Delta 6$ -desaturase can use both linoleic and α -linolenic acid as a substrate and convert them into γ -linolenic acid (GLA) and stearidonic acid (SDA) respectively (see Fig. 2). When $\Delta 6$ -desaturase from *Borago officinalis* was expressed constitutively in tobacco, it led to production of both 13.2% GLA and 9.6% SDA in leaves and up to 27% GLA in stem [34, 35]. When a borage $\Delta 6$ -desaturase gene was expressed along with an *Arabidopsis* $\Delta 15$ -desaturase gene in soybean seed under seed-specific β -conglycinin promoter, SDA content of as high as 29% was observed. The overall omega-3 fatty acid profile was increased to 60% in the transgenic seeds [36]. When $\Delta 6$ -desaturase gene from *M. alpina* was expressed in low α -linolenic canola along with $\Delta 12$ -desaturase gene, it resulted in production of up to 40% w/w GLA in seeds [37]. Expression of $\Delta 6$ -desaturase (*PiD6*), from an oleaginous fungus, *Pythium irregulare* gene in *Brassica juncea* under seed-specific napin promoter resulted in production of GLA up to 40% of the total seed fatty acids [38]. Similarly, when the $\Delta 6$ -desaturase gene from *Saprolegnia diclina* was expressed in cultivars of safflower producing high levels of LA, the transgenic plants produced >70% (v/v) of GLA whereas when the $\Delta 6$ -desaturase gene from *M. alpina* was expressed, 50% GLA levels were achieved. The difference in level of accumulation was due to differences in $\Delta 6$ -desaturase activity [39]. However, the effectiveness of SDA content in plant oils when compared to EPA/DHA containing oils as a nutritional supplement is less and depends upon human capacity to convert LA/ALA into VLC-PUFA [33, 40]. Thus the focus now has to shift to directly engineer plants to produce and accumulate EPA/DHA as an alternative source.

8. TRANSGENIC PRODUCTION OF ARA/EPA/DHA

Considering the growing list of health benefits of omega-3 fatty acids and limited fish oil source, there is a need to develop alternative plant-based source for production of these FAs. As described earlier, (Fig. 2) depicts major pathways for production of nutritionally important FAs. A preliminary demonstration of ETA biosynthesis involved use of alternative pathway using a combination of genes for three enzymes viz. $\Delta 9$ -elongase, $\Delta 8$ -desaturase and $\Delta 5$ -desaturase under a constitutive promoter. It resulted in production of low levels of ETA and ARA but showed that it is possible to engineer plants for VLC-PUFA [41]. Other preliminary attempts to produce EPA and DHA involved use of enzymes of $\Delta 6$ -desaturase pathway from *Phaeodactylum tricornerutum* ($\Delta 5$ - and $\Delta 6$ -desaturase) and $\Delta 6$ -elongase from *Physcomitrella patens* [42]. This study demonstrated that accumulation of intermediates and low levels of EPA or ARA in transgenic plants was due to low levels of acyl-CoA precursor pool of FAs, which are used as a substrate and is elongated by $\Delta 6$ -elongase (see Fig. 2). Thus this step is apparently a rate limiting. By coexpressing genes for $\Delta 9$ -elongase from *Isochrysis galbana* and $\Delta 8$ - and $\Delta 5$ -desaturase from *Pavlova salina* in *Arabidopsis* seeds resulted in accumula-

tion up to 20% ARA and 2% EPA in storage oil and ~10% ARA in *B. napus* [43]. Petrie *et al.* [44] engineered the pathway for DHA production in *A. thaliana* that resulted in accumulation of up to 15% DHA, almost similar (18%) to fish oil. The $\Delta 6$ -desaturase pathway to produce DHA from oleic acid involved coexpression of genes for $\Delta 12$ -desaturase (*Lachancea kluyveri*), $\Delta 15$ -desaturase (*Pichia pastoris*), along with $\Delta 6$ -desaturase (*Micromonas pusilla*), $\Delta 5$ - and $\Delta 4$ -desaturase (*Pavlova salina*) and $\Delta 6$ - and $\Delta 5$ -elongase (*Pyramimonas cordata*) (see Fig. 2). A greater success was also achieved using acyl-CoA-dependent $\Delta 6$ -desaturase from *Ostreococcus tauri*. Ruiz-Lopez *et al.* [45, 46] reported a 10-fold increase in EPA/DHA production. Petrie *et al.* [47] also engineered *Camelina sativa* to produce up to 15% of DHA in seed oil with a high w3/w6 ratio, which is more than the quantity found in bulk fish oil. The EPA and DHA were incorporated at sn-1,3 position of TAG. Betancore *et al.* [48] generated seeds of transgenic *C. sativa* that produces up to 20% of EPA. The oil from such transgenic plants can replace fish meal in aquaculture without affecting the nutritional quality of farmed fishes. This, therefore, has potential to decrease the load on marine life by serving as an alternate source of omega fatty acids. Attempts have also been made to produce EPA and ETA in seeds of *C. sativa* using alternative pathway [16]. Transgenic seeds accumulate EPA and ETA up to 26.4% confirming that it is possible to produce these PUFAs at significant levels.

9. PRODUCTION OF COMMON FATTY ACIDS

Besides omega-3, there are other fatty acids with potential health benefits. FAs such as stearic acid and monounsaturated oleic acid can substitute palm oil and partially hydrogenated oils in applications such as baking. Oleic acid has increased shelf life and higher oxidative stability than linoleic acid due to presence of one less double bond. Thus, it is desirable to develop vegetable oils with high level of monounsaturated fatty acids such as oleic acid and low level of linoleic acid. Oleic acid is produced by dehydrogenation of stearic acid by action of enzyme stearoyl-CoA 9-desaturase (SAD). The *fad2* gene codes for enzyme oleate desaturase ($\Delta 12$ -desaturase), which converts oleic acid into linoleic acid. High oleic acid lines have been developed in maize, canola, and soybean. All of these lines have mutations in the *fad2* gene [49-51]. Seed-specific silencing of *fad2* gene resulted in increasing levels of oleic acid in *Arabidopsis* and *Brassica napus* [52-54]. The oleic acid content was increased along with reduction of linolenic acid. When *fad2* gene is also silenced in soybean, along with *fatB* gene that codes for a thioesterase, oleic acid is increased up to 85% from 75% in plants in which only *fad2* alone is silenced [55]. By down regulating both *fad2* and *fae1* genes, Peng *et al.* [56] were able to increase oleic acid content to 75%, in addition to reduction of PUFA to 10% and complete elimination of erucic acid. Similarly the saturated fatty acid levels can be manipulated in seed oils so that it becomes nutritionally superior replacement in baking.

10. GENETIC ENGINEERING FOR FATTY ACIDS OF INDUSTRIAL IMPORTANCE

In the past, plant breeders have made selection and developed high oil-yielding varieties largely for food-based

applications. Besides their use for edible purpose, the utilization of vegetable oil is now expanding from nutrition point of view to industrial-based applications to biofuels. It is now possible to modify plant metabolism especially fatty acid synthesis, accumulation and composition. In addition, many fatty acids of unique importance as mentioned earlier, which either come from non-plant sources or are produced by repertoire of plants which are not amenable for commercial cultivation. Thus genetic engineering provides an opportunity to transfer genes coding for production of novel fatty acids of industrial as well as nutritional importance to oilseed crops and produce application-based designer oils.

11. PRODUCTION OF HYDROXY FATTY ACIDS IN NON-NATIVE PLANTS

Unusual fatty acids like hydroxy fatty acids are of immense importance as they serve as a feedstock for various industrial applications. Ricinoleic acid is a type of hydroxy fatty acid that accumulates in seeds of castor bean. Ricinoleic acid is produced by action of enzyme $\Delta 12$ -hydroxylase (FAH-12) present in endoplasmic reticulum (ER), and is stored as triacylglycerols (TAG) [57]. The $\Delta 12$ -hydroxylase enzyme transfers hydroxyl group to the delta-12 position of oleic acid. Castor seeds contain up to 80-90% ricinoleic acid. When $\Delta 12$ -hydroxylase gene was expressed in *Arabidopsis* plant, the transgenic plants accumulated HFAs ~17% of the seed oil [58]. Similar results were observed in *Brassica napus* [59] and *Camelina* [60]. This accumulation is much less as compared to the castor plants indicating that there are other factors involved in accumulation of this unusual fatty acid. In fact, plants producing more than 20% HFA have reduced oil content and seed viability, indicating that this unusual fatty acid has a role in physiology of the seed [61]. Thus, expressing only gene for FAH-12 is insufficient to produce ricinoleic acid for industrial purpose as other genes play a role in hydroxy fatty acid synthesis and accumulation as well.

Mechanisms involved in synthesis and storage of hydroxy fatty acids are being studied using *Arabidopsis* and castor bean as model plants. Several genes have been characterized that are involved in fatty acid accumulation. These studies have led to an understanding that lack of corresponding acyltransferases that catalyze synthesis of TAG or enzymes involved in editing functions, may be the reason behind it. As mentioned earlier, TAG is the major storage form of the oilseeds. The DGAT enzyme catalyzes acylation of DAG and thus influences fatty acid accumulation by being involved in TAG synthesis. Over expressing these enzymes in seed-specific manner increases the quantity of oil content and seed weight [62]. Coexpression of genes for DGAT2, and $\Delta 12$ -hydroxylase from castor bean, increases the accumulation of HFA to 30% compared to 17% in transgenic plants expressing only the $\Delta 12$ -hydroxylase gene. Interestingly, oil content of seeds is also comparable to that of the control plants [61]. Bates *et al.* [63] reported that a high level of HFA reduces fatty acid synthesis by a post-translational mechanism affecting plastidial acetyl-CoA carboxylase (ACCase) activity. In transgenic plants for $\Delta 12$ -hydroxylase gene alone, inefficient incorporation of ricinoleic acid into TAG causes inhibition of ACCase activity. This bottleneck was alleviated by coexpressing gene for HFA-specific

DGAT2 that efficiently and specifically incorporates ricinoleic acid into TAG and in turn restores seed oil content. Similarly in tung tree that produces an unusual fatty acid; α -eleostearic acid, DGAT2 preferentially incorporates eleostearic acid into TAG [64]. Thus low levels of accumulation of unusual fatty acids in transgenic plants could be the result of lack of their incorporation into TAG.

Ricinoleic acid is normally produced in phospholipids in ER, and needs to be removed from phospholipids and transferred to TAGs for storage during seed development. This can occur by two known mechanisms: one mechanism involves an enzyme phospholipid:diacylglycerol acyltransferase (PDAT) which transfers an acyl group from phospholipid pool to DAG. This DAG is then used for TAG synthesis as well. In castor bean, there are three PDAT enzymes reported of which PDAT1-2 is predominantly expressed in seeds. This when coexpressed in conjunction with $\Delta 12$ -hydroxylase improves incorporation of hydroxy fatty acid into TAG in seeds of transgenic *Arabidopsis* plants. Moreover, elevated levels of HFA in these transgenic plants did not affect the seed physiology. It was also found that no further increase was seen in transgenic plants expressing three genes for FAH12, PDAT1-2, DGAT2 [65, 66]. Besides acyltransferases, castor bean electron donor cytochrome b5 (RcCb5) and NADH:cytochrome b5 reductase (RcCBR1) were found to be important in HFA accumulation in transgenic plants [67]. However, when RcCBR1 and RcCb5 were coexpressed in FAH12 transgenic plants, no increase in HFA levels was seen [68]. A study by van Erp *et al.* [69] showed that by reducing competition from endogenous acyltransferases (AtDGAT1), the levels of HFA can be further increased in transgenic plants expressing FAH12 and RcDGAT2. Another enzyme that seems to play a role in hydroxy fatty acid accumulation is phosphocholine diacylglycerol choline phosphotransferase (PDCT). This enzyme controls flux of interconversion of PC and DAG which is then incorporated into TAG for storage [70].

It has also been proposed that low levels of unusual fatty acid accumulation in transgenic plants is due to futile cycle of β -oxidation as analyzed in transgenic plants expressing California bay lauroyl-acyl carrier protein thioesterase [71]. Thus multiple factors seem to influence fatty acid synthesis and accumulation.

12. GENETIC ENGINEERING OF PLANTS FOR PRODUCTION OF ERUCIC ACID

The major source of erucic acid is HEAR which produces a maximum of up to 50% in its seed oil. This fatty acid is not incorporated at the sn-2 position of the TAG. It was found that the *B. napus* LPAAT that is involved in DAG synthesis via the Kennedy pathway, lacks specificity for erucoyl-CoA. In order to further increase the erucic acid, genetic engineering of rapeseed using LPAAT enzyme having specificity for erucoyl-CoA as an acyl substrate was performed [72]. A gene coding for LPAAT was isolated from *Limnanthes* species and expressed in rapeseed [73]. Erucic acid was incorporated into sn-2 position and trierucin was obtained. However, the total erucic acid content did not increase. The other strategy used for erucic acid production revolves around elongation of oleic acid into erucic acid. When *fae1* gene,

Table 1. Genetic engineering of plants for production of FAs of nutritional and industrial importance.

Fatty Acids Specific Utility	Major Natural Source	Target Crop	Gene Source	Gene	Remarks	Reference
DHA/EPA/ARA	Fish, Algae	Tobacco	<i>Phaeodactylum tricornutum</i>	$\Delta 5$ - and $\Delta 6$ -desaturase	5% including ARA and EPA	[42]
		Linseed	<i>Physcomitrella patens</i>	$\Delta 6$ -elongase		
		<i>Arabidopsis</i>	<i>Isochrysis galbana</i> <i>Pavlova salina</i>	$\Delta 9$ -elongase $\Delta 8$ - and $\Delta 5$ -desaturases	>20% ARA and 2% EPA in seeds	[43]
		<i>A. thaliana</i> <i>Camelina sativa</i>	<i>Lachancea kluyveri</i> <i>Pichia pastoris</i> <i>Micromonas pusilla</i> <i>Pavlova salina</i> <i>Pyramimonas cordata</i>	$\Delta 12$ -desaturase $\Delta 15$ -desaturase $\Delta 6$ -desaturase $\Delta 5$ - and $\Delta 4$ -desaturases $\Delta 6$ - and $\Delta 5$ -elongases	15% DHA in seed	[44, 47]
GLA	<i>Borago officinalis</i>	Tobacco	<i>Borago officinalis</i>	$\Delta 6$ -desaturase	13.2% GLA in leaves 27% GLA in stem	[34, 35]
		<i>Brassica juncea</i>	<i>Pythium irregular</i>	$\Delta 6$ -desaturase	40% GLA in seeds	[37]
		low α -linolenic canola	<i>Mortierella alpina</i>	$\Delta 6$ -desaturase gene And $\Delta 12$ -desaturase	40% GLA in seeds	[38]
		Safflower (producing high levels of LA)	<i>Saprolegnia diclina</i> <i>M. alpina</i>	$\Delta 6$ -desaturase $\Delta 6$ -desaturase	>70% GLA 50% GLA	[39]
SDA	Seafood <i>B. officinalis</i> Black current	Tobacco	<i>Borago officinalis</i>	$\Delta 6$ -desaturase	9.6% SDA in stem	[34, 35]
		Soybean	<i>B. officinalis</i> <i>Arabidopsis</i>	$\Delta 6$ -desaturase and $\Delta 15$ -desaturase gene	29% SDA in seed	[36]
Hydroxy fatty acid	Castor, <i>Lesquerella</i>	Castor	<i>Arabidopsis</i>	Oleate-12-hydroxylase	17% HFA	[58]
				Oleate-12-hydroxylase And <i>DGAT2</i>	30% HFA	[61]
				Oleate-12-hydroxylase and <i>PDAT1-2</i>	27 % HFA	[65]
Erucic acid	Brassica Crambe	<i>Brassica napus</i> (HEAR)	<i>B. napus</i>	<i>Fatty acid elongase (Bn-FAE1.1)</i>	60% erucic acid	[74]
		<i>B. napus</i>	<i>B. napus</i> <i>Limnanthes douglasii</i>	<i>BnFAE1</i> , <i>LdLPAAT</i>	72% erucic acid	[75]
		<i>Crambe abyssinica</i>	<i>B. napus</i> <i>Limnanthes douglasii</i> <i>C. abyssinica</i>	<i>BnFAE1</i> , <i>LdLPAAT</i> and <i>CaFAD2-RNAi</i>	73.9% erucic acid	[76]

(Table 1) contd....

Fatty Acids Specific Utility	Major Natural Source	Target Crop	Gene Source	Gene	Remarks	Reference
Stearic acid	Palm kernel	<i>B. napus</i>	<i>B. rapa</i>	Stearoyl-ACP D-9 desaturase (antisense)	2%- 40% stearic acid	[81]
Lauric acid	Coconut Palm kernel	<i>B. napus</i>	<i>Umbellularia californica</i>	Acyl-ACP thioesterase	Upto 50% lauric acid	[79]
		<i>B. napus</i>	<i>Umbellularia californica</i> <i>Cocos nucifera</i>	Acyl-ACP thioesterase (<i>UcFatB1</i>) and (<i>CnLPAT</i>)	Further 5% increase	[80]
Capric acid	Coconut	<i>B. napus</i>	<i>Cuphea hookeriana</i>	Acyl-ACP thioesterase (<i>ChFatB2</i>)	11 and 27 Mol%	[82]
Palmitoleic acid	<i>Doxantha unguis-cati</i>	<i>Arabidopsis</i>	Castor <i>Aspergillus nidulans</i> <i>Stagonospora nodorum</i> <i>Arabidopsis</i>	Plastidial $\Delta 9$ -16:0-ACP desaturase (variant), $\Delta 9$ -16:0-ACP desaturases and ketoacyl-ACP synthase II 16:0 elongase (downregulation).	71% w-7 fatty acids	[78]

which is involved in fatty acid elongation from oleic acid to erucic acid, was overexpressed under seed specific promoter, an increase in erucic acid content was observed in transgenic plants [74]. When *fae1* was overexpressed along with LPAAT gene from *Limnanthes douglasii* in HEAR lines, which was further combined with plants carrying mutant alleles for PUFA i.e. linoleic and linolenic acid, plants producing up to 72% erucic acid and reduced PUFA were obtained [75]. Similar results were obtained with up to 73% upon genetic modification of crambe which is another example where genetic engineering has advanced production of fatty acids [76]. Jadhav *et al.* [77] used cosuppression and antisense method to down regulate *fad2* in *B. carinata*, and obtained increased erucic acid content in transgenic plants.

Besides HFA and erucic acid, genetic engineering has been used to modify plant oils for production of other industrially important fatty acids as well. For example, by expressing an engineered plastidial $\Delta 9$ -16:0-ACP desaturase from *Doxantha unguis-cati*, down-regulation of ketoacyl-ACP synthase II 16:0 elongase, and coexpression of fungal 16:0-ACP desaturases led to production of up to ~71% w-7 fatty acids in *Arabidopsis*. This level is comparable to that of *Doxantha* seeds [78]. Similarly, lauric acid (12:0) is a type of saturated, medium chain fatty acid that is widely used for production of soaps and detergents. Major sources of this FA are coconut and palm tree. As an alternative, vegetable oils rich in lauric acid have been obtained by expressing a heterologous thioesterase gene under seed-specific napin promoter. This enzyme which has a high specificity for lauroyl-ACP, releases the lauric acid into the lipid pool. This gene when expressed in canola yielded up to 50% lauric acid [79]. An additional increase of 5% in the lauric acid content was achieved by expressing a gene for lysophosphatidic acid acyltransferase (LPAAT) [80]. (Table 1) summarizes the attempts made for transgenic production of FAs of nutritional and industrial importance.

13. INCREASE IN OIL CONTENT

Other area of interest is to increase oil production. Several biochemical pathways involved in oil biosynthesis in plants have been characterized elucidating key steps and rate limiting enzymes [1]. As mentioned earlier several bottlenecks have been identified in fatty acid biosynthesis and their flux is under coordinated regulation between various pathways as well as carbohydrate metabolism. For example targeting acetyl-coenzyme A carboxylase (ACCase) to plastids results in an increase of ~5% oil content in rapeseed [83]. Expression of genes for DGAT, DGAT2A, DGAT1-2 in *Arabidopsis*, soybean, and maize increases overall oil content [62, 84, 85]. Besides known genes involved in fatty acid metabolism, other genes that may play a role in increasing storage reserves could also serve as important tools to boost the oil content. The gene encoding transcription factor, LEC1 is a potential candidate for controlling both quality and quantity of oil [86]. Another transcription factor in *B. napus*, *WRINKLED1*, seems to control metabolic processes that influences fatty acid accumulation. Ectopic expression of *WRINKLED1* under CAMV 35S promoter results in increase in TAG content in seedlings [87]. Several comparative transcriptomics studies are now identifying genes involved at rate limiting steps [88]. Chandrasekaran *et al.* [89] studied the role of abscisic acid (ABA) signaling during seed filling and storage of oil in castor bean seeds using transcriptomic studies. The focus now is not only seed specific metabolic engineering for fatty acid but its use for production of TAG in leaf tissue as well [90]. Petris *et al.* [91] have reported an alternative pathway for production of TAG in plants. This pathway involves enzyme GPAT4 or GPAT6 that synthesizes sn-2-monoacylglycerol (MAG) using dicarboxylic and w-hydroxyl acyl-CoA fatty acids. This MAG is converted to DAG by MGAT acyltransferases or even to TAG by action of a mouse bifunctional enzyme M/DGAT1 acyltransferases. This pathway that bypasses the Kennedy pathway may prove useful for increasing levels of TAG in transgenic plants.

CONCLUSIONS

Significant advancements have been made in understanding the biochemistry of plant lipid metabolism and several bottlenecks have been documented. Factors that may influence novel FA accumulation include substrate availability or substrate specificity of enzymes, their incorporation into TAG or negative post-translational regulatory controls within and between pathways. These bottlenecks may be bypassed by use of knowledge of alternative pathways or identifying enzymes from other sources that can overcome these bottlenecks. Use of engineered enzymes may also provide better resources for transgenic production. The other major constraint is where, when and how the transgenes and endogenous genes are integrated to give best results and avoid problems such as biosynthetic intermediates or futile cycles. Plant biotechnology has now opened new vistas with several promising demonstration of successful genetic manipulation using multiple genes for nutritionally important fatty acids. A fair progress has also been made regarding industrially important fatty acids with several reports of success. Use of master regulators (transcription factors) can be further explored to increase seed oil content. The future global demands of oilseed production will be influenced from industrial or nutritional perspectives. Designer oilseeds will help achieve the targets not earlier possible.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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