ELSEVIER

Biotechnology Advances 18 (2000) 481–497

BIOTECHNOLOGY ADVANCES

Research review paper

Plants as 'chemical factories' for the production of polyunsaturated fatty acids

D. López Alonso[a,*], F. García Maroto[b]

[a]*Dept. Biología Aplicada, Universidad de Almería, 04071 Almería, Spain*
[b]*Dept. Bioquímica, Universidad de Almería, 04071 Almería, Spain*

Abstract

Polyunsaturated fatty acids (PUFAs) are valuable products because of their involvement in several aspects of human health. Market demand for most PUFAs is growing continually and current sources are considered insufficient for satisfying this demand; alternative sources are actively sought after. Oilseed plants can be a potential source of PUFAs if they are appropriately gene engineered. Most of the basic tools for genetic engineering of oilseed plants for giving them the ability to produce PUFAs are already developed. Here we review the prospects of genetic engineering of oilseed plants for producing some valuable long-chain polyunsaturated fatty acids. Genetic transformation for GLA production seems to be a near-term possibility, but gene engineering seems considerably more difficult for the other long-chain PUFAs. Nevertheless, with the current rapid pace of biotechnological advancement, the remaining difficulties may be surmounted in the near future.

Keywords: Polyunsaturated fatty acids; Oilseed plants; Genetic engineering; Desaturases

1. Polyunsaturated fatty acids (PUFAs)

1.1. What are PUFAs?

PUFAs are fatty acids with more than one double bond. They are usually named in abbreviated form as X:YnZ, where X stands for the number of carbon atoms of the chain, Y the number of double bonds, and Z the position of the first double bond counted from the methyl end (the n system of numbering). The positions of the remaining double bonds are easily deduced because they always follow the pattern called 'methylene interrupted.' For example, linoleic acid (LA), 18:2n6, has 18 carbon atoms, two double bonds, and the first double bond

* Corresponding author. Tel: +34-950-215033; fax: +34-950-215484.
E-mail address: dlopez@ualm.es (D. López Alonso).

0734-9750/00/$ – see front matter
PII: S0734-9750(00)00048-3

counting from the methyl end is at carbon 6, and the second is at carbon 9. There are other methods of naming PUFAs but they are more complex and less used. We will follow the nomenclature system described above. Some of the major PUFAs are listed in Table 1.

1.2. Uses of PUFAs

Several PUFAs are recognized as 'essential fatty acids' (Table 2) in the normal diet for preventing nutrition-related illnesses. These are LA, GLA, ALA, and AA (WHO/FAO, 1977; Gurr and Harwood, 1991). Beside these, there are other PUFAs that are being investigated for their role in human health. In the latter category we have the fatty acids OTA, DHGLA, EPA, and DHA (Table 2).

GLA has been claimed to play a role in development and prevention of some skin diseases, diabetes, reproductive disorders, and others (Gunstone, 1992, 1998; Horrobin, 1992). The role of AA and DHA in the development of nervous system is well supported (Innis, 1991; Nettleton, 1993; Singh and Chandra, 1988), and DHA is additionally related to the development of retina (Brown, 1994). In fact, several health agencies have recommended that infant feed formulae be fortified with DHA and AA (Gill and Valivety, 1997). The role of EPA in the proper functioning of the circulatory system was first identified nearly 30 years ago (Nelson, 1972) and is now well supported (Dyerberg, 1986; Iacono and Dougherty, 1993; Nettleton, 1993; Simonopoulos, 1991; Singh and Chandra, 1988). There is growing interest in the putative involvement of EPA in some cancers and other diseases (Simonsen et al., 1998; Wingmore et al., 1996a,b). An overview of claimed activities for the main PUFAs is shown in Table 2.

It is hypothesized that there are few direct actions of these PUFAs, and their activities are mostly mediated by their transformation in a number of metabolically active compounds collectively known as 'eicosanoids' (tromboxanes, leukotrienes, and prostaglandins). Eicosanoids are physiologically highly active and play diverse roles in human metabolism (Dyerberg, 1986; Gill and Valivety, 1997; Innis, 1991; Parent et al., 1992; Peck, 1994; Singh and Chandra, 1988).

1.3. Market

Because of their significance in human health, many PUFAs and PUFA-based products are on the market (Table 3). The market size is not precisely known. Production of some PUFAs is relatively well established, and intense research is underway to develop commercially

Table 1
The main polyunsaturated fatty acids

Formula	Common name	Abbreviation
18:2n6	Linoleic acid	LA
18:3n6	γ-linolenic acid	GLA
18:3n3	α-linolenic acid	ALA
18:4n3	Octadecatetraenoic acid	OTA
20:3n6	Dihomo-γ-linolenic acid	DHGLA
20:4n6	Arachidonic acid	AA
20:5n3	Eicosapentaenoic acid	EPA
22:6n3	Docosahexaenoic acid	DHA

Table 2
Real or putative role of main PUFAs

PUFA	Metabolic role	Reference
LA	Essential for diet	WHO/FAO, 1977
ALA	Essential for diet	WHO/FAO, 1977
GLA	Essential for diet	WHO/FAO, 1977
	Skin diseases and others	Horrobin, 1992
OTA	Anti-inflammatory	Coupland et al., 1996
DHGLA	Virus infections, cancer, inflammatory diseases, and atopy of the skin and mucosa	Jareonkitmongkol et al., 1993
AA	Essential for diet	WHO/FAO, 1977
	Eicosanoid precursor	Singh and Chandra, 1989
	Development of the nervous system	Singh and Chandra, 1989; Innis, 1991
EPA	Eicosanoid precursor	Dyerberg, 1986
	Circulatory system	Singh and Chandra, 1989; Innis, 1991
	Anti-inflammatory	Dyerberg, 1986
	Anti-tumoral	Wingmore et al., 1996; Simonsen et al., 1998
DHA	Development of the nervous system and retina	Singh and Chandra, 1989; Innis, 1991

viable production methods for others. For instance, GLA production is relatively well known, and this fatty acid is commonly obtained from plant sources such as evening primrose (*Oenothera biennis*), borago (*Borago officinalis*), and black-currant (*Ribes nigrum*) (Gunstone, 1998). Also, attempts have been made to produce GLA from fungi (Ratledge, 1989, 1993). The market demand of GLA is estimated to be about 2000 tons per year (Gunstone, 1998). GLA sells for ≈$100/kg of crude oil. Another fatty acid, EPA, has an estimated demand of 125 tons in Japan alone (Corden, personal communication) and it sells for ≈$600/kg of pure EPA. EPA has proved beneficial in treatment of several important ailments, and its demand is expected to increase rapidly in the near term (Belarbi et al., 2000).

1.4. Current sources and potential sources

PUFAs are currently obtained from a number of sources including higher plants, animal viscera, and oily fish (Table 4), but it has become evident that PUFA production from current sources is inadequate for supplying the expanding PUFA market (Gill and Valivety, 1997; Napier et al., 1999). Additional and significant problems of the current sources are: (1) seasonal and climatic variation in oil composition, which can result in inconsistencies in oil supply and quality (Gill and Valivety, 1997; Yongmanitchai and Ward, 1989); (2) low productivity of used cultivated plants (Gill and Valivety, 1997; Sayanova et al., 1997); (3) complex and expensive downstream processing (Barclay et al., 1994; Belarbi et al., 2000; Gill and Valivety, 1997). Because of these drawbacks, new sources of PUFAs are needed that can compete in cost with the current sources.

Some microorganisms, including bacteria, fungi, and microalgae, are considered alternative sources of PUFAs (Table 4) (Bajpai and Bajpai, 1993; Barclay et al., 1994; Gill and Valivety, 1997; Gunstone, 1998; López Alonso and Segura, 1999; López Alonso et al., 1992,

Table 3
Sources of commercial PUFAs

PUFA	Source	Co.	Product
GLA	Evening primrose	Clover Co., Australia	Milkarra
	Evening primrose	Croda Oleochemicals,	Crossesential EPO
	Borage	England	Crossesential GLA
	Evening primrose	Scotia Lipids, Scotland	Efamol®
OTA	*Trichodesma zeylanicum*	Croda Oleochemicals, England	
	Echium plantagineum		
AA	Fermentation[a]	Suntory Ltd., Japan	SUN-TGA
	Single-cell oil	Hoffmann-La Roche Ltd, Switzerland	ROPUFA
	Fermentation	Gist-brocades, Holland	ARASCO
	Mortierella alpina	Martek Biosciences Co.	
EPA	Fish oil	Croda Oleochemicals, England	Incromega
		Scotia Lipids, Scotland	Hi-EPA Oil
		BASF, Denmark	Dry n-3®
		PRONOVA, Norway	EPAX 0626 TG
DHA	Tuna	Clover Co., Australia	Milkarra™
		Hoffmann-La Roche	
	Fish oil	Ltd., Switzerland	ROPUFA
	Fish oil	Croda Oleochemicals,	Incromega
		England	
		Scotia Lipids, Scotland	Hi-DHA Oil
	Crypthecodinium cohnii	Martek Biosciences Co.	DHASCO
	Fish oil	BASF, Denmark	Dry n-3®
	Fish oil	PRONOVA, Norway	EPAX 0626 TG

[a] The organism is not reported.

1996; Shimizu et al., 1988; Yazawa et al., 1988; Yongmanitchai and Ward, 1991). Already, companies such as Martek Biosciences, USA, are marketing PUFA oils or PUFA concentrates obtained from microbial culture (Table 3).

Other potential sources are the genetically modified oilseed crops (Gill and Valivety, 1997; Napier et al., 1999). Oleaginous plants are attractive because of their high oil productivity. Also, well-established methods exist for commercial agronomic production of oilseeds and processing of the crop for recovery of the oil. In addition, the knowledge of plant molecular biology has advanced rapidly and this has opened up new possibilities for production of PUFAs in plants. Existing crops, already engineered for high agronomic productivity, could be turned into ‘chemical factories’ for PUFAs because many of the genes necessary for altering the lipid biosynthesis are now known (Budziszewski et al., 1996; Lassner, 1997). Thus, the genetic modification of oilseed crops to produce PUFAs is an attractive option (Budziszewski et al., 1996; Gill and Valivety, 1997).

2. Genetic engineering of oilseed crops: the tools

According to many sources, the basics for engineering of oilseed crops for producing special or improved quality oils and industrial oleochemicals have been already developed

Table 4
Current and alternative sources of PUFAs

PUFA	Current sources	Alternative sources
GLA	Plants: *Oenothera, Borago, Ribes*	Plants: Macaronesian *Echium* Fungi: *Mucor javanicus, Mortierella isabelina* Microalgae: *Spirulina*
ALA	Plants: *Glycine, Linum* (many others)	Not needed
AA	Animal viscera (brain)	Fungi: *Mortierella alpina* Microalgae: *Porphyridium cruentum*
EPA	Oil fish: *Sardina, Engraulis*, etc.	Fungi: *M. alpina, Saprolegnia* sp. Microalgae: *Phaeodactylum tricornutum, Monodus subterraneous* Mosses: *Phytium irregulare* Bacteria: *Shewanella* sp.
DHA	Oil fish: *Sardina, Engraulis*, etc.	Fungi: Traustochitrids Microalgae: Dynophyceae

(Budziszewski et al., 1996; Gunstone, 1998; Harwood, 1996; Kinney, 1994, 1998; Lassner, 1997; Murphy, 1993, 1995, 1996; Ohlrogge, 1994; Somerville and Browse, 1991). In the last few years several papers have reviewed the possibilities of engineering oilseed crops to produce 'designer' oils by genetic transformation, but these publications have mostly focused on common fatty acids and fats, i.e. saturated or monounsaturated fatty acids, butter and confectionery fats, and industrial oils (lubricants, polymers) (Budziszewski et al., 1996; Kinney, 1994, 1998; Murphy, 1995, 1996). Not much has been written about the production of PUFAs by plants.

There are several basic requirements for engineering oleaginous plants for making PUFAs: (1) acknowledgment of the metabolic pathways and, therefore, of the enzymes/genes involved; (2) cloning of the critical genes involved in the synthesis of a specific PUFA; (3) availability of suitable *cis*-regulatory elements (i.e. promoters) to achieve expression in the appropriate tissue; (4) availability of transformation methods for the oleaginous plants and stability of the trait (Budziszweski et al., 1996; López Alonso and Segura, 1999; Nishida and Murata, 1996). We shall briefly review the state of the art of the above requirements.

2.1. Metabolic pathways

The knowledge of lipids and fatty acids biosynthesis in plants has greatly expanded in the last decade, as is summarized in several good reviews (Browse and Somerville, 1991; Harwood, 1996; Murphy, 1993; Ohlrogge and Jaworsky, 1997; Slabas and Fawcett, 1992; Somerville and Browse, 1991). Plant fatty acid biosynthesis occurs almost exclusively into the plastid (Somerville and Browse, 1991), and is carried out by the fatty acid synthetase complex, which is a Type II multienzyme complex (Harwood, 1988). Fatty acids are then used directly in the plastid for the production of plastid glycerolipids (prokaryotic pathway) or exported to the cytoplasm. The cytoplasmic acyl lipid pool is used to produce other glycerolipids via endoplasmic reticulum processing (eukaryotic pathway) (Browse and Somerville, 1991). Desaturation reactions take place in both cellular compartments with the fatty acids in the form of acyl lipids (linked to the glycerol backbone), except for the desaturation of 18:0

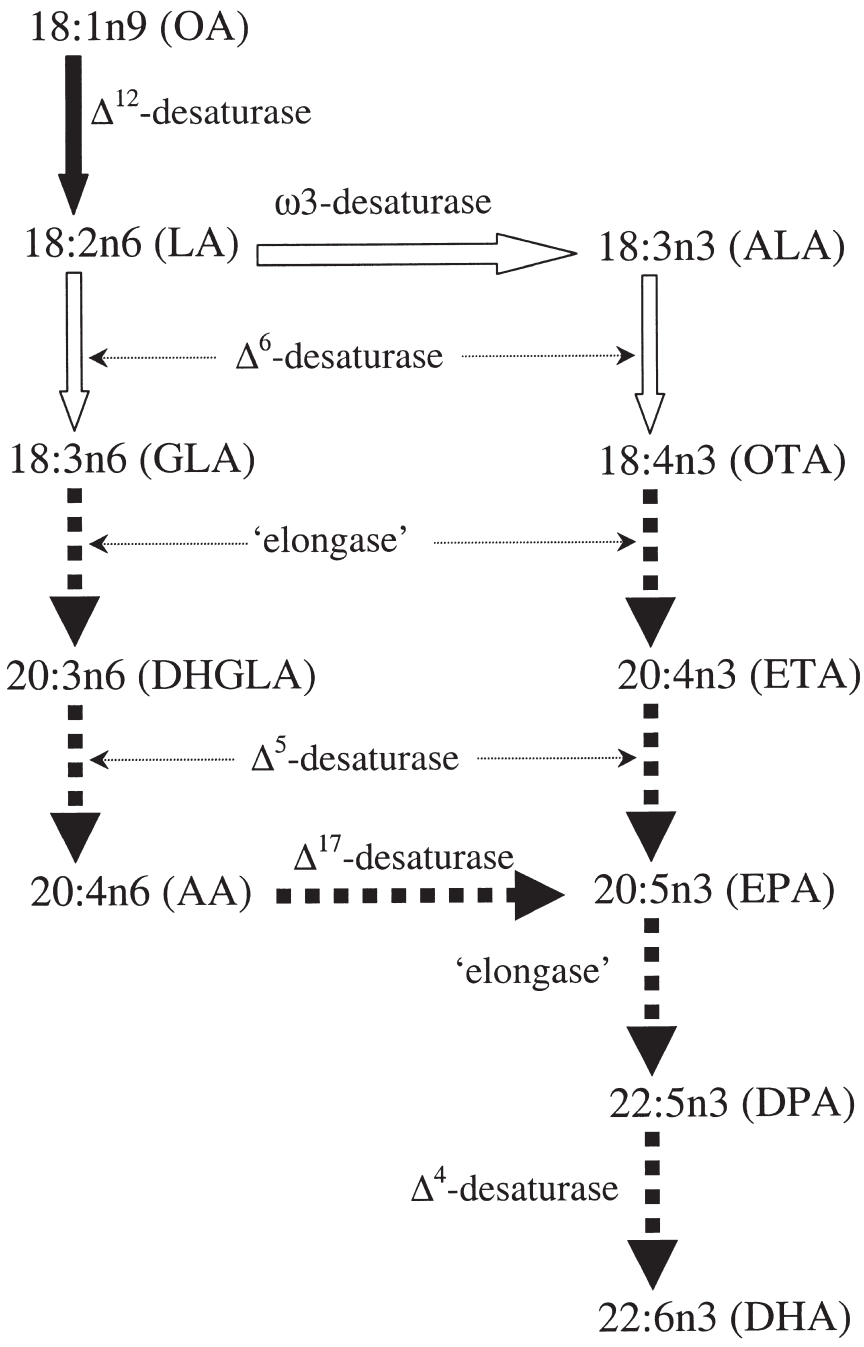

Fig. 1. The general biochemical pathway of in vivo synthesis of PUFAs in various organisms. Solid arrows represent reactions that are very common in higher plants; open arrows represent reactions that occur only in a few plants; broken arrows represent reactions that occur only in some lower organisms.

to 18:1n9 (Harwood, 1996). The enzymes involved in the desaturation steps in plants are well known (Fig. 1); usually there are at least two isoforms of each desaturase: one in the plastid, and another one in the microsomes (Harwood, 1996; Heppard et al., 1996). In some oilseed crops there seem to be an additional seed-specific desaturase in the microsomes (Kinney, 1994). Except for the Δ^9-desaturase, which is soluble, the remaining desaturases are all membrane-bound enzymes (Harwood, 1996; Slabas and Fawcett, 1992; Somerville and Browse, 1991).

Although some higher plants are able to synthesize very long chain fatty acids such as erucic acid (22:1) (Harwood, 1996; Millar et al., 1998), they do not synthesize very long chain PUFAs (Fig. 1) such as AA, EPA, and DHA (Harwood, 1996), and even GLA and ALA are not widely encountered in plants (Gill and Valivety, 1997; Gunstone, 1992, 1998). Recent work in which *Arabidopsis* was transformed with a fatty acid elongation gene, showed that very long chain fatty acids appeared in all lipid classes, implying that many of the enzymes involved in leaf glycerolipid metabolism have relatively broad substrate specificities (Millar et al., 1998) with the exception of the desaturases. Plant desaturases seem to exhibit low activity on C20 fatty acids (Millar et al., 1998; Spychalla et al., 1997) and, therefore, very long-chain PUFAs are not synthesized despite a substantial accumulation of very long-chain fatty acids in plants.

Some fungi, mosses, and microalgae have the ability to synthesize these very long-chain PUFAs (see Table 4) by pathways that are poorly understood (Fig. 1) (Arao et al., 1994; Arao and Yamada, 1994; Bajpai and Bajpai, 1993; Girke et al., 1998). These organisms contain all desaturases found in higher plants and several additional desaturases (Fig. 1). These additional desaturases, unlike those of higher plants, can specifically desaturate C20 and C22 fatty acids (Fig. 1).

Both the prokaryotic and the eukaryotic pathways—also known as ‘chloroplastic’ and ‘extra-chloroplastic’ pathways, respectively (Harwood, 1996)—serve the cellular needs for structural lipids (i.e. membrane lipids). In addition, the extra-chloroplastic pathway is the major route for the storage lipids, mainly triacylglycerol (TAG). The latter pathway is of special interest in the context of this review. Storage TAG are synthesized by the Kennedy pathway in developing seeds (Gurr and Harwood, 1991; Murphy, 1993). In principle, TAG synthesis requires only one additional enzyme compared to those required for the synthesis of structural lipids (Murphy, 1995; Ohlrogge and Jaworski, 1997; Somerville and Browse, 1991). This enzyme, diacylglycerol acyltransferase, usually appears to have a wide fatty acid specificity (Gurr and Harwood, 1991). Thus, the enzymes that control fatty acid composition affect membrane lipids as well as storage lipids (Miquel and Browse, 1994; Murphy, 1993; Wada et al., 1990). In fact, some genetic transformations of plants may pose problems because of their broader impact on all plant tissues and not only on seeds (Kinney, 1994). However, the positions *sn*-1 and *sn*-2 of the TAGs are usually occupied by specific fatty acids because of the specificity of the two first enzymes of the Kennedy pathway (Budziszewski et al., 1996; Gurr and Harwood, 1991; Harwood, 1996; Murphy, 1993; Slabas and Fawcett, 1992).

2.2. Gene cloning

Recent years have witnessed extraordinary advances in the cloning of the many genes involved in the biosynthesis of lipids. Within the PUFA pathway, all the genes have been

cloned from at least one organism (Table 5) with two exceptions: the Δ^{17}-desaturase gene, and the Δ^{4}-desaturase gene (Fig. 1). Although, a so-called Δ^{4}-desaturase gene was isolated from coriander endosperm, which catalyzed the formation of Δ^{4}-hexadecenoate (16:1n12) and Δ^{6}-octadecenoate (18:1n12) in transgenic tobacco (Harwood, 1996), its specificity was not of Δ^{4}-desaturation and, therefore, its utility for the synthesis of DHA (Fig. 1) is doubtful.

2.3. Transformation

Wada et al. (1990) reported the first case of lipid alteration by genetic engineering. Although this was done in cyanobacteria ,it proved the feasibility of this approach to modifying the lipid composition. At the same time, transformation methods for higher plants have been developed, although at present they are concentrated in a few species. The focus has been on nonoleaginous model plants such as *Arabidopsis* (Millar et al., 1998; Voelker et al., 1992)

Table 5
List of the desaturase genes with potential biotechnological applications that have been cloned[a]

Gene/enzyme	Biological source	Reference
Δ^{9}-desaturase	*Anabaena variabilis*	Sakamoto et al., 1994
	Synechocystis sp.	Sakamoto et al., 1994
	Rosa hybrida	Fukuchi-Mizutani et al., 1995
	Arabidopsis thaliana	Fukuchi-Mizutani et al., 1995
n-3 desaturase (microsomal)	*Arabidopsis thaliana*	Yadav et al., 1993
	Glycine max	Yadav et al., 1993
	Brassica napus	Yadav et al., 1993
	Limnanthes douglasii	Bhella et al., 1995
	Nicotiana tabaccum	Hamada et al., 1994
	Triticum aestivum	Horiguchi et al., 1998
	Perilla frutescens	Chung et al., 1999
n-3 desaturase (plastidial)	*A. thaliana (FAD-7)*	Yadav et al., 1993
	A. thaliana (FAD-8)	Gibson et al., 1994
	G. max	Yadav et al., 1993
	B. napus	Yadav et al., 1993
	N. tabaccum	Hamada et al., 1994
	T. aestivum	Horiguchi et al., 1998
	P. frutescens	Lee et al., unpublished
Δ^{12}-desaturase (microsomal)	*A. thaliana*	Okuley et al., 1994
	G. max	Heppard et al., 1996
	Borago officinalis	Sayanova et al., unpublished
Δ^{12}-desaturase (plastidial)	*A. thaliana*	Falcone et al., 1994
	A. thaliana	Hitz et al., 1994
	Spinacia oleracea	Schmidt et al., 1994
Δ^{6}-desaturase	*Borago officinalis*	Sayanova et al., 1997
	Physcomitrella patens	Girke et al., 1998
	Synechocystis sp.	Reddy et al., 1993
	Helianthus annuus	Sperling et al., 1995
	Mortierella alpina	Huang et al., 1999
Δ^{5}-desaturase	*Mortierella alpina*	Michaelson et al., 1998
		Knutzon et al., 1998

[a] This list is not exhaustive.

and tobacco (Nunberg et al., 1994; Sayanova et al., 1997; Takaiwa et al., 1991), which are of little direct practical value for agronomic-based oil production.

Rapeseed is surely the major oilseed crop amenable to routine transformation at high efficiency via *Agrobacterium tumefaciens* (Facciotti et al., 1999; Falco et al., 1995; Friedt and Lühs, 1998; Murphy, 1995). Efficient transformation methods have also been developed for other oilseed crops (e.g. soybean, maize). For example, soybean has been successfully transformed using the well-known *A. tumefaciens* (Falco et al., 1995; Heppard et al., 1996) or the gold particle bombardment (Stewart et al., 1996), and maize was also transformed at high efficiency via *Agrobacterium* (Ishida et al., 1996). It also appears that gene constructs and binary vectors developed for modification in rapeseed can be used directly for transforming other species that are susceptible to *Agrobacterium*-mediated transformation (Murphy, 1996). Great effort is underway for engineering of other oilseed crops (e.g. peanut, sunflower). In summary, efficient transformation methods are already available for several oilseed crops and the range of oil crops amenable to genetic manipulation is set to increase.

2.4. Genetic stability of the trait

The stability of the transformed trait seems not to be a problem in transgenic plants, and there are many reports of extended expression of the transgene in modified plants (Friedt and Lühs, 1998). For example, rapeseed transformed for high lauric content is currently cultivated in the United States (Friedt and Lühs, 1998; Gunstone, 1998), and other oils, from oleaginous plants, are at various stages of development (Gunstone, 1998). Notwithstanding this, some pitfalls have also occurred, for example, with high-stearate canola lines, in which the trait was not inherited in a stable way (Lassner, 1997).

2.5. Appropriate expression

The expression of the transgene in the appropriate tissue is of paramount importance (Kinney, 1994; Lassner, 1997; Murphy, 1995; Reddy and Thomas, 1996). The constitutive expression of some fatty acid transgenes has proven detrimental for the performance of the organism (Kinney, 1994; Millar et al., 1998) especially under certain conditions (Kinney, 1994; Miquel and Browse, 1994). Other characteristics are sometimes poorly expressed or not expressed in seeds (Reddy and Thomas, 1996; Sayanova et al., 1999) where the transgene is designed for expression in the oilseed plant. These circumstances can invalidate the usefulness of the transgenic plant as production vehicle, and great effort is necessary for assuring the correct expression (i.e. in the seeds) without other adverse effects (Budziszewski et al., 1996). Fortunately, the expectations remain good in view of several reports of seed-specific promoters that work satisfactorily when used with heterologous genes (Falco et al., 1995; Nunberg et al., 1994; Sarmiento et al., 1997; Takaiwa et al., 1991).

Some lipid-related genes are specifically expressed in seeds. For example, two genes for the ω6-desaturase (Δ^{12}-desaturase) have been reported, and one of them is specific for seeds (Heppard et al., 1996); also the fatty acid elongation gene of *Arabidopsis* is specifically expressed in developing seeds (James et al., 1995). Although it is not known if all these genes share common motifs in their regulatory elements (Heppard et al., 1996), there are indications that the enzymes of fatty acid synthesis are coordinatedly regulated, which suggests

that there are common motifs and global transcription factors that control many of the genes of the pathway (Ohlrogge and Jaworski, 1997). If these transcription factors can be identified in the near future, almost complete control may be exerted over the expression of the entire pathway (Ohlrogge and Jaworski, 1997).

3. Genetic engineering of oil crops: the practice

As we have discussed, the basic requirements of a genetic engineering approach for tailoring oilseed crops are reasonably well established, and the way is open for engineering oleaginous plants for PUFA production (Gill and Valivety, 1997; Gunstone, 1998; Lassner, 1997; Napier et al., 1999; Sayanova et al., 1997).

Curiously, so far, the genetic transformation of oilseed plants has been focused on eliminating or reducing the PUFA content. This makes sense, because of the specific requirements of oils used in cooking application where PUFAs can be undesirable. For instance, soybean oil is naturally rich in LA and ALA, which make the oil unstable at high temperatures and inappropriate for margarines because of the formation of *trans*-fatty acids during hydrogenation (Gunstone, 1998; Kinney, 1998). Therefore, the usual target for oilseed crops has been to reduce the PUFAs to a minimum (Budzizevski et al., 1996; Gunstone, 1998; Kinney, 1994, 1998). Now we are facing a new market: the use of the oils as a source of specific PUFAs for therapeutic and nutraceutical applications. PUFAs are envisaged for therapeutic use as oils, as pure fatty acids, or as fatty acid derivatives (e.g. ethyl-esters, structured lipids, etc.). Therefore, the objective now is to tailor the oilseed crops to produce oils with high contents of specific PUFAs.

4. Strategies

To manipulate gene expression and modify oil composition, two basic strategies have been commonly used, as discussed next.

4.1. Expression knock-out (shutdown)

This has been done by antisense expression, i.e. the introduction of the gene in an opposite direction to give an aberrant mRNA, which triggers sequence-specific degradation of the homologous mRNA (Gura, 2000; Kooter et al., 2000; Stam et al., 2000). This approach was first used to increase the stearic acid level in rapeseed by introducing the cDNA for the stearoyl ACP desaturase in antisense orientation (Knutzon et al., 1992). A similar strategy was successfully used to decrease ALA and LA by antisense expression of the ω-3 and ω-6 desaturases in rapeseed (Voelker et al., 1992) and soybean (Hitz et al., 1995; Topfer et al., 1995). Downregulation of several plant genes has also been achieved by overexpression of the corresponding cDNAs (cosuppression) through a poorly understood mechanism. One can envisage the usefulness in the near future of this strategy in the shutdown of genes involved in oil biosynthesis.

4.2. Expression or overexpression

This approach is a direct one. The idea is to introduce the gene or genes needed for the production of a fatty acid or a specialty oil into a plant that does not have the gene or genes.

Thus, by gain of function, the transformed plant becomes able to synthesize the target product. This methodology has been proven with several nonoleaginous plants. For example, transgenic tobacco plants have been transformed by insertion of a Δ^6-desaturase gene from cyanobacteria (Reddy and Thomas, 1996) and from borage (Sayanova et al., 1997, 1999). This way, some unusual fatty acids such as GLA (produced from LA, see Fig. 1) and OTA (produced from ALA, see Fig. 1) could be made to appear in transformed tobacco plants (Reddy and Thomas, 1996; Sayanova et al., 1997, 1999).

The greatest example of this approach with current commercial application has been the high-laureate canola (rapeseed) (Gunstone, 1998; Lassner, 1997). A thioesterase gene from the California Bay tree was introduced into rapeseed. This thioesterase had a preference for hydrolyzing 12:0-ACP, enabling the export of laureate (12:0) from the plastid to the cytoplasm and then incorporation to triacylglycerol (Voelker et al., 1992). In this way high-laureate canola produced an oil with more than 50% of 12:0 at the expense of the C18 fatty acids that are commonly present in rapeseed oil (Gunstone, 1998; Lassner, 1997). Recently, a similar transformation, with a thioesterase from *Garcinia mangostana*, which has a high specific activity of 18:0-ACP, produced an improved stearate phenotype in transgenic canola (Facciotti et al., 1999).

Another possibility is to overexpress a gene that is already present in the genome of the plant. An example of this approach is the transgenic *Arabidopsis* plants overexpressing the *Arabidopsis* fatty acid elongation gene causing accumulation of very long-chain fatty acids throughout the plant (Millar et al., 1998).

5. Prospects

Using the various approaches of genetic manipulation and regulation most of the objectives of PUFA production may be achieved (Budziszewski et al., 1996). Here we focus on the prospects of engineering oilseeds for the production of some specific PUFAs (GLA, AA, EPA, and DHA) that are commercially the most attractive.

5.1. GLA

GLA can be produced from its precursor LA by the action of a Δ^6-desaturase (Fig. 1), but this reaction is not common in nature. As noted above, among higher plants borage, evening primrose, and blackcurrant have been reported to use this route (Table 4). All three species are nonoleaginous, and there is an opportunity for transforming an oleaginous source for GLA production. Potentially, GLA may be produced economically from oilseed plants by a relatively simple transformation (i.e. the introduction of a single Δ^6-desaturase gene), providing that the gene is expressed stably in seeds and at a high rate, and the transformed plants are easy to cultivate. Transformation with a Δ^6-desaturase gene has succeeded in tobacco (Reddy and Thomas, 1996; Sayanova et al., 1997, 1999) and cyanobacteria (Reddy et al., 1993) and, apparently, this approach has been tried in some oilseeds (Murphy, 1995) but the results are not known. A possible problem with transformation of rapeseed for GLA is the presence of ALA, which is also a substrate for the Δ^6-desaturase and could lead to the pro-

duction of OTA instead of GLA (Sayanova et al., 1997). Maize and sunflower, which are naturally deficient in ALA (Kinney, 1994), are probably better candidates for GLA production after genetic transformation.

5.2. AA

AA is produced directly from DHGLA by a Δ^5-desaturase (Fig. 1). This process takes place only in some fungi and microalgae because DHGLA is uniquely present in these organisms. DHGLA is probably synthesized from GLA by an 'elongase,' which adds two carbons to GLA (Fig. 1). In contrast, oilseed crops are able to synthesize only LA, the precursor of GLA. Therefore, an oleaginous plant for producing AA would likely require multiple transformation, with at least three genes: the Δ^6-desaturase gene, the Δ^5-desaturase gene, and the 'elongase' gene.

As noted earlier, the former gene has been successfully cloned and used (Reddy et al., 1993; Reddy and Thomas, 1996; Sayanova et al., 1997, 1999). The Δ^5-desaturase gene has also been cloned (Michaelson et al., 1998), and might be available for plant transformation; however, the 'elongase' gene is of an uncertain identity. In fact, it is not known whether the 'elongase' activity is associated with one or multiple genes. However, it seems that the introduction of a single gene, a very long-chain fatty acid sythetase gene, can lead to elongation of C18 fatty acids to C20 and C22 fatty acids, at least in *Arabidopsis* plants. Although transformation of a single plant with several different genes is still not routine, there are good expectations using the *A. tumefaciens* system. Cotransformation of *Arabidopsis* using two different binary vectors each containing up two genes on a single plasmid has been possible (Poirier et al., 2000). Therefore, it is conceivable that AA may be produced from a multiple transformed plant, i.e. one with several different genes inserted. Whether this is successful is uncertain.

5.3. EPA

EPA is naturally produced only by some microalgae, fungi, and mosses (Table 4). Oily fish accumulate EPA and DHA obtained in their food chain, but do not synthesize these fatty acids. In micro-organisms, EPA is synthesized either from ALA (the n-3 pathway) or from AA (the n-6 pathway) (Fig. 1). To produce EPA from ALA, if an oilseed crops naturally produce ALA (e.g. soybean and rapeseed), at least three genes need to be inserted into the plant genome. These genes are: a Δ^6-desaturase gene (to convert ALA into OTA), a VLCFA gene (to elongate OTA into 20:4n3) and, a Δ^5-desaturase gene (to convert OTA into EPA) (Fig. 1). If the aim is to produce EPA by the n-6 pathway, we would need to insert four genes: a Δ^6-desaturase gene (to convert LA to GLA), a VLCFA gene (to convert GLA into DHGLA), a Δ^5-desaturase gene (to convert DHGLA into AA), and, a Δ^{17}-desaturase gene (to convert AA into EPA) (Fig. 1).

The genes of the n-3 pathway have been cloned, but there is the problem of multiple gene insertion (three different genes). The n-6 pathway is even more problematic, because the Δ^{17}-desaturase gene, despite much effort, appears to be especially recalcitrant to cloning. Although, recently, a ω3-desaturase gene has been cloned from *Caenorabditis elegans*, and this produces Δ^{17}-desaturase activity. By heterologous expression of this gene from *C. elegans* into *A. thaliana*, the resulting enzyme was shown to desaturate exogenously supplied DH-

GLA (20:3n6) and AA (20:4n6) to their corresponding ω3-desaturated fatty acids, i.e. 20:4n3 and EPA, respectively (Spychalla et al., 1997).

5.4. DHA

As noted earlier, DHA and EPA are present in fish oils as a result of the bioaccumulation through the food chain. DHA is naturally synthesized only by some algae-like micro-organisms and some fungi (Table 4). DHA is synthesized from EPA by elongation and subsequent Δ^4-desaturation (Fig. 1). To our knowledge, the gene for this desaturation has not been cloned yet. Although a so-called Δ^4-desaturase gene have been cloned from coriander endosperm (Harwood, 1996), its true specificity is doubtful. Therefore, the engineering of oilseed crops to produce DHA is currently even less likely than for EPA because DHA synthesis would require more genes and one of the essential ones (i.e. the Δ^4-desaturase gene) has not yet been cloned.

6. Concluding remarks

Despite a general consensus about the great potential of engineered oilseed crops (Budziszewski et al., 1996), many growers and processors remain skeptical, mainly because most of the transgenic varieties are substitution products aimed at existing markets (Murphy, 1996) where it is not easy to displace current products. Development of PUFA production is advantageous because PUFAs command high prices, and at present, there are no PUFA producing plants in commercial use, except for GLA. Also, the plant producers of GLA are not oilseed plants, i.e. they are not high oil producers, and, therefore, they would not be major competitors if an oilseed crop is eventually developed for producing GLA (Sayanova et al., 1997).

Clearly, there is a great potential for using plants as ‘chemical factories’ for producing PUFAs. The basic knowledge about the genes/enzymes involved, the cloning, the transformation methods, and so on, are well developed. Within a few years, some engineered crops should be available for field testing and possible commercial use in production of GLA. Indeed, some time ago there was a report of transformation of rapeseed by Δ^6-desaturase gene from cyanobacteria, which led to the accumulation of GLA in the seed oil (Murphy, 1995). Although that report stated that ‘field assessment of GLA-containing, transgenic rapeseed plants is now underway’ (Murphy, 1995), no further developments have been reported. Obviously, genetic engineering for ‘designed’ oil crops is possible but not as easy as it initially appears. Many problems require solving at various stages of development from the laboratory to the field trials.

PUFA crops for AA, EPA, and DHA are even harder to develop (López Alonso and Segura, 1999; Napier et al., 1999). However, there is no fundamental reason why these important PUFAs could not be produced by plants. If, as expected, market demand continues to grow, sufficient funding should become available to support the development of engineered crops for these expensive PUFAs.

In the next few years, micro-organisms could be important competitors to PUFA production in plants (Gunstone, 1998). The market demand for EPA, AA, and DHA in the next few years probably will be supplied by microbial sources because the genetic engineered plants

are not currently sufficiently developed. However, the situation may change radically in another few years when the engineered plant crops catch up. Crop plants will offer several advantages relative to production in micro-organisms: easier reproduction of the transgenic organism without the need of a constant selection pressure; well-developed agronomic practices; and well-established industrial processing of the oil crops. The future certainly looks promising for PUFA production from plants.

References

Arao T, Sakaki T, Yamada M. Biosynthesis of polyunsaturated lipids in the diatom, *Phaeodactylum tricornutum*. Phytochemistry 1994;36:629–35.

Arao T, Yamada M. Biosynthesis of polyunsaturated fatty acids in the marine diatom, *Phaeodactylum tricornutum*. Phytochemistry 1994;35:1177–81.

Bajpai P, Bajpai PK. Eicosapentaenoic acid (EPA) production from microorganisms: a review. J Biotechnol 1993;30:161–83.

Barclay WR, Meager KM, Abril JR. Heterotrophic production of long chain omega-3 fatty acids utilizing algae and algae-like microorganisms. J Appl Phycol 1994;6:123–9.

Belarbi EH, Molina E, Chisti Y. A process for high yield and scaleable recovery of high purity eicosapentaenoic acid esters from microalgae and fish oil. Enzyme Microbiol Technol 2000;26:516–29.

Bhella RS, MacKenzie SL. Nucleotide sequence of a cDNA from *Limnanthes douglasii* L. encoding a delta-15 linoleic acid desaturase. Plant Physiol 1995;108:861.

Brown P. Baby formula needs essential extras. New Sci 1994;November:1952.

Browse J, Somerville C. Glycerolipid synthesis: biochemistry and regulation. Annu Rev Plant Physiol Plant Mol Biol 1991;42:467–506.

Budziszewski GJ, Croft KPC, Hildebrand DF. Uses of biotechnology in modifying plant lipids. Lipids 1996;31:557–69.

Chung CH, Kim JL, Lee YC, Choi YL. Cloning and characterization of a seed-specific 8.0 ω-3 fatty acid desaturase cDNA from *Perilla frutescens*. Plant Cell Physiol 1999;40:114–8.

Coupland K, Coupland D, Packer C. New sources of stearidonic acid and the moderation of inflammation by plant oils containing stearidonate. ISSFAL 1996.

Dyerberg J. Linolenate-derived polyunsaturated fatty acids and prevention of atherosclerosis. Nutr Rev 1986;44:125–34.

Facciotti MA, Bertain PB, Yuan L. Improved stearate phenotype in transgenic canola expressing a modified acyl-acyl carrier protein thioesterase. Nat Biotechnol 1999;17:593–7.

Falco SC, Guida T, Locke M, Mauvais J, Snaers C, Ward RT, Webber P. Transgenic canola and soybean seeds with increased lysine. Biotechnology 1995;13:577–82.

Falcone DL, Gibson S, Lemieux B, Somerville C. Identification of a gene that complements an *Arabidopsis* mutant deficient in chloroplast ω-6 desaturase activity. Plant Physiol 1994;106:1453–9.

Friedt W, Lühs W. Recent developments and perspectives of industrial rapeseed breeding. Fett/Lipid 1998;100:219–26.

Fukuchi-Mizutani M, Savin K, Cornish E, Tanaka Y, Ashikari T, Kusumi T, Murata N. Senescence-induced expression of a homologue of Δ^9-desaturase in rose petals. Plant Mol Biol 1995;29:627–35.

Gibson S, Arondel V, Iba K, Somerville C. Cloning of a temperature-regulated gene encoding a chloroplast ω-3 desaturase from *Arabidopsis thaliana*. Plant Physiol 1994;106:1615–21.

Gill I, Valivety R. Polyunsaturated fatty acids, part 1: occurrence, biological activities and applications. Trends Biotechnol 1997;15:401–9.

Girke T, Schmidt H, Zähringer U, Reski R, Heinz E. Identification of a novel delta6-acyl-group desaturase by targeted gene disruption in *Physcomitrella patens*. Plant J 1998;15:39–48.

Gunstone FD. Gamma linolenic acid-occurrence and physical and chemical properties. Prog Lipid Res 1992;31:145–61.

Gunstone FD. Movements towards tailor-made fats. Prog Lipid Res 1998;37:277–305.
Gura T. A silence that speaks volumes. Nature 2000;404:804–8.
Gurr MI, Harwood JL. Lipid Biochemistry. London: Chapman & Hall, 1991.
Hamada T, Kodama H, Nishimura M, Iba K. Cloning of a cDNA encoding tobacco omega-3 fatty acid desaturase. Gene 1994;147:293–4.
Harwood JL. Fatty acid metabolism. Annu Rev Plant Physiol Plant Mol Biol 1988;39:101–88.
Harwood JL. Recent advances in the biosynthesis of plant fatty acids. Biochim Biophys Acta 1996;1301:7–56.
Heppard EP, Kinney AJ, Stecca KL, Miao G-H. Developmental and growth temperature regulation of two different microsomal omega-6 desaturase genes in soybeans. Plant Physiol 1996;110:311–9.
Hitz WD, Carlson TJ, Booth JR, Kinney AJ, Stecca KL, Yadav NS. Cloning of a higher-plant plastid ω-6 fatty acid desaturase cDNA and its expression in a cyanobacterium. Plant Physiol 1994;105:635–41.
Hitz WD, Yadav NS, Reiter RS, Mauvais CJ, Kinney AJ. Reducing polyunsaturation in oils of transgenic canola and soybean. In: Kader JC, Mazliak P, editors. Plant Lipids Metabolism. The Netherlands: Kluwer Academic Press, 1995. pp. 506–8.
Horiguchi G, Kawakami N, Kusumi K, Kodama H, Iba K. Developmental regulation of genes from microsome and plastid omega-3 fatty acid desaturases in wheat (*Triticum aestivum* L.). Plant Cell Physiol 1998;39:540–4.
Horrobin DF. Nutritional and medical importance of gamma-linolenic acid. Prog Lipid Res 1992;31:163–94.
Huang YS, Chaudhary S, Thurmond JM, Bobik EG, Yuan L, Chan GM, Kirchner SJ, Mukerji P, Knutzon DS. Cloning of Δ12- and Δ6-desaturases from *Mortierella alpina* and recombinant production of gamma-linolenic acid in *Saccharomyces cerevisiae*. Lipids 1999;34:649–59.
Iacono JM, Dougherty RM. Effects of polyunsaturated fats on blood pressure. Annu Rev Nutr 1993;13:243–60.
Innis SM. Essential fatty acids in growth and development. Prog Lipid Res 1991;30:39–103.
Ishida Y, Saito H, Ohta S, Hiei Y, Komari T, Kumashiro T. High efficiency transformation of maize (*Zea mays* L.) mediated by *Agrobacterium tumefaciens*. Nat Biotechnol 1996;14:745–50.
James DW, Lim E, Keller J, Plooy I, Ralston E, Dooner HK. Direct tagging of the *Arabidopsis* fatty acid elongation 1 (FAE 1) gene with the maize transposon activator. Plant Cell 1995;7:309–19.
Jareonkitmongkol S, Sakuradani E, Shimizu S. A novel Δ5-desaturase-defective mutant of *Mortierella alpina* 1S–4 and its dihomo-γ-linolenic acid productivity. Appl Environ Microbiol 1993;59:4300–4.
Kinney AJ. Genetic modification of the storage lipids of plants. Curr Opinion Biotechnol 1994;5:144–51.
Kinney AJ. Plants as industrial chemical factories—new oils from genetically engineered soybeans. Fett/Lipid 1998;100:173–6.
Knutzon DS, Thompson GA, Radke SE, Johnson WB. Modification of *Brassica* seed oil by antisense expression of a stearoyl-acyl carrier protein desaturase gene. Proc Natl Acad Sci USA 1992;89:2624–8.
Knutzon DS, Thurmond JM, Huang Y-S, Chaudhary S, Bobik EM, Chan GM, Kirchner SJ, Mukerji P. Identification of Δ5-desaturase from *Mortierella alpina* by heterologous expression in bakers' yeast and canola. J Biol Chem 1998;273:29360–6.
Kooter JM, Matzke MA, Meyer P. Listening to the silent genes: transgene silencing, gene regulation and pathogen control. Trends Plant Sci 2000;4:340–7.
Lassner M. Transgenic oilseed crops: a transition from basic research to product development. Lipid Technol 1997;1:5–9.
López AD, Molina E, Sánchez JA, García JL, García F. Fatty acid variation among different isolates of a single strain of *Isochrysis galbana*. Phytochemistry 1992;31:3901–4.
López AD, Segura C, Molina E, Cohen Z. First insights into improvement of eicosapentaenoic acid content in *Phaeodactylum tricornutum* (Bacillariophyceae) by induced mutagenesis. J Phycol 1996;32:339–45.
López AD, Segura CI. Genetic improvement of EPA content in microalgae. In: Cohen Z, editor. Chemicals from Microalgae. London: Taylor & Francis, 1999. pp. 93–107.
Michaelson LV, Lazarus CM, Griffiths G, Napier JA, Stobart AK. Isolation of a Δ^5-fatty acid desaturase gene from *Mortierella alpina*. J Biol Chem 1998;273:19055–9.
Millar AA, Wrischer M, Kunst L. Accumulation of very-long-chain fatty acids in membrane glycerolipids is associated with dramatic alterations in plant morphology. Plant Cell 1998;11:1889–902.
Miquel MF, Browse J. High-oleate oilseeds fail to develop at low temperature. Plant Physiol 1994;106:421–7.
Murphy DJ. Structure, function and biogenesis of storage lipids bodies and oleosins in plants. Prog Lipid Res

1993;32:247–80.
Murphy DJ. The use of conventional and molecular genetics to produce new diversity in seed oil composition for the use of plant breeders-progress, problems and future prospects. Euphytica 1995;85:433–40.
Murphy DJ. Engineering oil production in rapeseed and other oil crops. Trends Biotechnol 1996;14:206–13.
Napier JA, Michaelson LV, Stobart AK. Plant desaturases: harvesting the fat of the land. Curr Opin Plant Biol 1999;2:123–7.
Nelson AM. Diet therapy in coronary disease. Geriatrics 1972;27:103–16.
Nettleton JA. Are n-3 fatty acids essential nutrients for fetal and infant development? J Am Diet Assoc 1993;93:58–64.
Nishida I, Murata N. Chilling sensitivity in plants and cyanobacteria: the crucial contribution of membrane lipids. Annu Rev Plant Physiol Plant Mol Biol 1996;47:541–68.
Nunberg AN, Li Z, Bogue MA, Vivekananda J, Reddy AS, Thomas TL. Developmental and hormonal regulation of sunflower helianthinin genes: proximal promoter sequences confer regionalized seed expression. Plant Cell 1994;6:473–86.
Ohlrogge JB, Jaworski JG. Regulation of fatty acid biosynthesis. Annu Rev Plant Physiol Plant Mol Biol 1997;48:109–36.
Ohlrogge JB. Design of new plant products: engineering of fatty acid metabolism. Plant Physiol 1994;104:821–6.
Okuley J, Lightner J, Feldmann K, Yadav N, Lark E, Browse J. *Arabidopsis FAD2* gene encodes the enzyme that is essential for polyunsaturated lipid synthesis. Plant Cell 1994;6:147–58.
Parent CA, Lagarde M, Venton DL, LeBreton GC. Selective modulation of the human platelet thromboxane A2/prostaglandine H2 receptor by eicosapentaenoic and docosahexaenoic acids in intact platelets and solubilized platelet membranes. J Biol Chem 1992;267:6541–7.
Peck MD. Interactions of lipids with immune function II—experimental and clinical studies of lipids and immunity. J Nutr Biochem 1994;5:514–21.
Poirier Y, Ventre G, Nawrath C. High-frequency linkage of co-expressing T-DNA in transgenic *Arabidopsis thaliana* transformed by vacuum-infiltration of *Agrobacterium tumefaciens*. Theor Appl Genet 2000;100:487–93.
Ratledge C. Microbial routes to lipids. Biochem Soc Trans 1989;17:1139–41.
Ratledge C. Single cell oils—have they a biotechnological future? Trends Biotechnol 1993;11:278–84.
Reddy AS, Nuccio ML, Gross LM, Thomas TL. Isolation of a Δ^6-desaturase gene from the cyanobacterium *Synechocystis* sp. strain PCC 6803 by gain-of-function expression in *Anabaena* sp. strain PCC 7120. Plant Mol Biol 1993;27:293–300.
Reddy AS, Thomas TL. Expression of a cyanobacterial Δ^6-desaturase gene results in γ-linolenic acid production in transgenic plants. Nat Biotechnol 1996;14:639–42.
Sakamoto T, Wada H, Nishida I, Ohmori M, Murata N. Delta-9 acyl-lipid desaturases of cyanobacteria. J Biol Chem 1994;269:25576–80.
Sarmiento C, Ross JHE, Herman E, Murphy DJ. Expression and subcellular targeting of a soybean oleosin in transgenic rapeseed. Implications for the mechanism of oil-body formation in seeds. Plant J 1997;11:783–96.
Sayanova O, Davies GM, Smith MA, Griffiths G, Stobart AK, Shewry PR, Napier JA. Accumulation of Δ^6-unsaturated fatty acids in transgenic tobacco plants expressing a Δ^6-desaturase from *Borago officinalis*. J Exp Bot 1999;50:1647–52.
Sayanova O, Smith MA, Lapinskas P, Stobart AK, Dobson G, Christie WW, Shewry PR, Napier JA. Expression of a borage desaturase cDNA containing an N-terminal cytochrome b5 domain results in the accumulation of high levels of Δ^6-desaturated fatty acids in transgenic tobacco. Proc Natl Acad Sci USA 1997;94:4211–16.
Schmidt H, Dresselhaus T, Buck F, Heinz E. Purification and PCR-based cDNA cloning of a plastidial n-6 desaturase. Plant Mol Biol 1994;26:631–42.
Shimizu S, Kawashima H, Shinmen Y, Akimoto K, Yamada H. Production of eicosapentaenoic acid by *Mortierella* fungi. J Am Oil Chem Soc 1988;65:1455–9.
Simonopoulos AP. Omega-3 fatty acids in health and disease and in growth and development. Am J Clin Nutr 1991;54:438–63.
Simonsen S, van't Veer P, Strain JJ. Adipose tissue omega-3 and omega-6 fatty acid content and breast cancer in the EURAMIC study. Am J Epidemiol 1998;147:342–52.

Singh G, Chandra RK. Biochemical and cellular effects of fish and fish oils. Prog Food Nutr 1988;12:371–419.

Slabas AR, Fawcett T. The biochemistry and molecular biology of plant lipid biosynthesis. Plant Mol Biol 1992;19:169–91.

Somerville C, Browse J. Plant lipids: metabolism, mutants, and membranes. Science 1991;252:80–7.

Sperling P, Schmidt H, Heinz E. A cytochrome-b5-containing fusion protein similar to plant acyl lipid desaturases. Eur J Biochem 1995;232:798–805.

Spychalla JP, Kinney AJ, Browse J. Identification of an animal ω-3 fatty acid desaturase by heterologous expression in *Arabidopsis*. Proc Natl Acad Sci USA 1997;94:1142–7.

Stam M, de Bruin R, van Blokland R, van der Hoorn RAL, Mol JNM, Kooter JM. Distinct features of post-transcriptional gene silencing by antisense transgenes in single copy and inverted T-DNA repeat loci. Plant J 2000;21:27–42.

Stewart CN, Andang MJ, All JN, Boerma HR, Cardineau G, Tucker D, Parrott WA. Genetic transformation, recovery, and characterization of fertile soybean transgenic for a synthetic *Bacillus thuringiensis* cryIAc gene. Plant Physiol 1996;112:121–9.

Takaiwa F, Oono K, Kato A. Analysis of the 5' flanking region responsible for the endosperm-specific expression of a rice glutelin chimeric gene in transgenic tobacco. Plant Mol Biol 1991;16:49–58.

Topfer R, Martini N, Schell J. Modification of plant lipid synthesis. Science 1995;268:681–6.

Voelker TA, Worrell AC, Anderson L, Bleibaum J, Fan C, Hawkins DJ, Radke SE, Davies HM. Fatty acid biosynthesis redirected to medium chains in transgenic oilseed plants. Science 1992;257:72–4.

Wada H, Gombos Z, Murata N. Enhancement of chilling tolerance of a cyanobacterium by genetic manipulation of fatty acid desaturation. Nature 1990;347:200–3.

WHO/FAO. Dietary Fats and Oils in Human Nutrition. Report of an Expert Consultation. Rome: FAO, 1977.

Wingmore SJ, Ron JA, Barber MD, Faron KCH. Phase II trials of high purity eicosapentaenoic acid (EPA) in patient with pancreatic cancer cachexia. British Association of Parenteral and Enteral Nutrition, Blackpool Meeting (UK), December 5th, 1996a.

Wingmore SJ, Ron JA, Falconer JS, Plaster CE, Tisdale MJ, Carter DC, Faron KCH. The effect of PUFA on the progress of cachexia in patients with pancreatic cancer. Nutrition 1996b;12:27–30.

Yadav NS, Wierzbicki A, Aegerter M, Caster CS, Pérez-Grau L, Kinney AJ, Hitz WD, Russell J, Schweiger B, Stecca KL, Allen SM, Blackwell M, Reiter RS, Carlson TJ, Russell SH, Feldmann KA, Pierce J, Brown J. Cloning of higher plant omega-3 fatty acid desaturases. Plant Physiol 1993;103:467–76.

Yazawa K, Araki K, Okazaki N, Watanabe K, Ishikawa C, Inoue A, Numao N, Kondo K. Production of eicosapentaenoic acid by marine bacteria. J Biochem 1988;103:5–7.

Yongmanitchai W, Ward OP. Omega-3 fatty acids: alternative sources of production. Process Biochem 1989;24:117–25.

Yongmanitchai W, Ward OP. Screening of algae for potential alternative sources of eicosapentaenoic acid. Phytochemistry 1991;30:2963–7.