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# Plant Lipid Metabolism

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## Abstract

In plants, the synthesis of fatty acids takes place in the chloroplast and the fatty acid synthase is prokaryotic type. In plants, the structure of membrane lipids is different from that of eukaryotic cells. The membranes of the chloroplasts are essentially formed of galatolipids. This chapter will also focus on the structure and biosynthesis of fatty acids and membrane lipids in plants. Lipids of seeds are essentially composed of TAG; it would be interesting to describe their synthesis during the maturation of the seeds. Some plants contain in their reserve lipids unconventional fatty acids such as gamma linolenic acid in *Borrigo officinalis* L., short-chain fatty acids C: 12 and C: 10, fatty acids with very long chains, and fatty acids that are cyclical. All of these fatty acids can have industrial and/or pharmaceutical applications.

**Keywords:** fatty acid, lipids, biosynthesis, plant

## 1. Introduction

Plants produce the majority of lipids in the world. These lipids are the main source of calories and essential fatty acids for men and animals. Plants synthesize a huge variety of fatty acids although only a few are major and common constituents [1] like palmitic, oleic, linoleic, and linolenic acids. Like other eukaryotes, lipids are necessary for the biogenesis of cell membranes, as signal molecules and especially as a source of carbon and energy. In plants, carbon, energy, and reducing power are needed for fatty acid biosynthesis derived from photosynthesis in chloroplasts [2]. This chapter will describe lipid biosynthesis in plants by signaling differences with other organisms and highlighting the specificity of plants.

Fatty acid biosynthesis in plants occurs in the chloroplasts of green tissue and in the plastids of nonphotosynthetic tissues and not in the cytosol as in the animal cell. Although *de novo* synthesis is located in the stroma, plant mitochondria are capable of limited fatty acid synthesis [3]. The plastid membranes are mainly composed of galactolipids, while those of extrachloroplast membranes consist of phospholipids as in the animal cell [4]. Fatty acids in cell membranes consist mainly of palmitic, stearic, oleic, linoleic, and linolenic acids. All double bonds are of *cis* type. However, in the chloroplast, phosphatidyl glycerol (PG) is acylated with an unusual acid having a *trans*-type double bond:  $\Delta^3$  16: 1*t* [5].

Photosynthetic tissues of higher plants contain 60–70% trienoic fatty acids. The so-called “C18: 3” plants are generally the most advanced families of angiosperms (pea, spinach, etc.) whose position *sn*-2 of the galactolipids is esterified exclusively by polyunsaturated fatty acids with 18 carbon atoms. The “C16: 3” plants are generally the less evolved families of angiosperms (Brassicaceae) whose position *sn*-2 of the galactolipids is esterified by polyunsaturated fatty acids with 16 or 18 carbon atoms [6].

Plant lipids have a substantial impact on the world economy and human nutrition. The majority of oils used by humans are triacylglycerols derived from seeds or fruits. Indeed, the seeds are subdivided into three categories according to their reserve. Seeds that contain more than 45% protein are called protein seeds. Starchy seeds contain more than 70% of carbohydrates like cereals. Oleaginous seeds contain more than 50% of lipids in the form of triacylglycerol esterified generally by palmitic, oleic, linoleic, and linolenic acids in majority of seeds. Some plants can produce unusual fatty acids like hydroxyl fatty acids, cyclopropane fatty acids, epoxy fatty acids, and conjugated unsaturated fatty acids in their seed oils, many of which have useful industrial applications [7]. These unusual fatty acids accumulate preferentially in triacylglycerols.

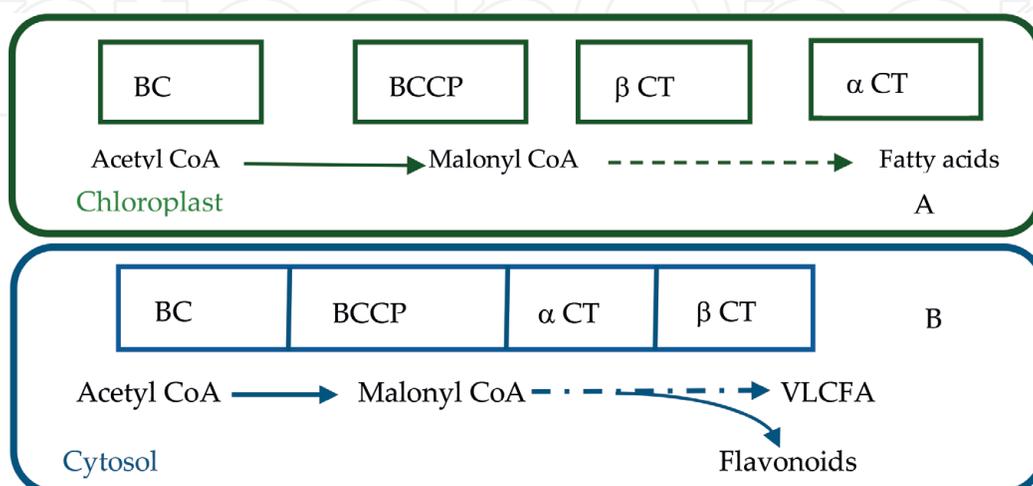
## 2. Fatty acid synthesis

In plants, *de novo* fatty acid biosynthesis mainly takes place in the plastidial compartment [8] from acetyl CoA, which is a direct product of photosynthesis. Plastid pyruvate dehydrogenase (EC 1.2.4.1) is the main route for a rapid and stable supply of acetyl CoA through its action on pyruvate (resulting from glycolysis or the pentose phosphate pathway). Another possible source is the import and activation of free acetate by acetyl CoA synthase (ACS, EC 6.2.1.1) [9]. The major product of FAS is palmitic acid, except the elongation of palmitic acid and the desaturation of stearic acid which take place in the chloroplast. Other changes (elongation, desaturation, hydroxylation, and epoxidation) occur mainly in the endoplasmic reticulum.

Two enzyme systems are required for fatty acid formation: acetyl CoA carboxylase (ACCase, EC 6.4.1.2) of which two forms have been identified in plants [10] and fatty acid synthase which is a multienzyme complex present in the stroma of chloroplasts [11].

### 2.1 Acetyl CoA carboxylase (ACCase)

The first enzyme complex is the ACCase that catalyzes an ATP-dependent carboxylation of acetyl CoA to malonyl CoA. For plants, acetyl CoA carboxylase (ACCase) directs the flow of carbon from photosynthesis to primary and secondary metabolites. Two distinct molecular forms of ACCase have been identified, a multiprotein complex and a multifunctional protein [12] (**Figure 1**).



**Figure 1.** Structure of the two types of ACCase. (A) The multisubunit (MS complex) ACCase and (B) the multifunctional (MF) ACCase. BCCP, biotin carboxyl carrier protein; BC, biotin carboxylase;  $\alpha$  and  $\beta$  CT,  $\alpha$  and  $\beta$  carboxyl transferase; VLCFA, very long-chain fatty acids.

The multisubunit (MS complex) ACCase, present in plastids of all plants, except *Poaceae* and *Geraniaceae*, is involved in *de novo* fatty acid synthesis [13]. It is composed of four independent polypeptides: biotin carboxyl carrier protein (BCCP), biotin carboxylase (BC) and  $\alpha$  and  $\beta$  carboxy transferase ( $\alpha$  and  $\beta$  CT). The biotin carboxylase (BC) subunit catalyzes the ATP-dependent carboxylation of the biotinyl moiety on biotin carboxyl carrier protein (BCCP), and the carboxytransferase (CT) subunits catalyze the transfer of activated carboxyl groups from BCCP to acetyl CoA to form malonyl CoA.

The multifunctional (MF) ACCase, consisting of a single 220–240 kDa polypeptide with BCCP, BC, and CT domains, is nuclear encoded except the  $\alpha$ CT subunit which is encoded by the plastidial genome [13]. In all plants, MF ACCase is involved in very long-chain fatty acid and flavonoid biosynthesis in the cytosol [13].

The sensitivity of plastidial ACCase to sethoxydim and the presence of a 220-kDa biotinylated polypeptide in soybean plastids provide a biochemical indication for the possible presence of two ACCase isoforms, one resistant (MS) and one sensitive (MF), in soybean leaf chloroplasts [14].

## 2.2 Fatty acid synthase (FAS)

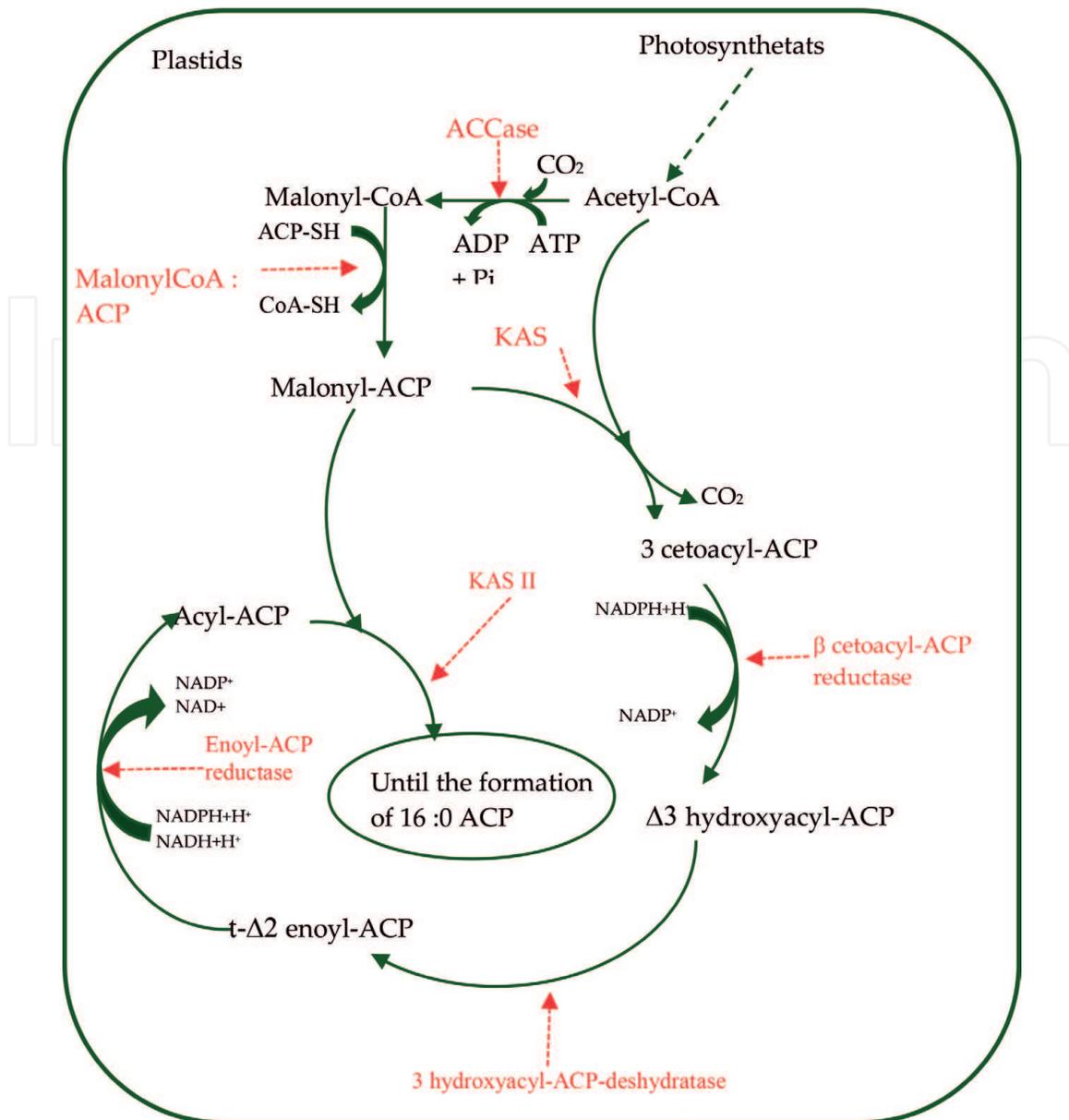
The second enzyme complex involved in *de novo* synthesis is the fatty acid synthase (FAS). In nature, fatty acid synthases are subdivided into two groups. The fatty acid synthase type I which is characterized by a large, multifunctional proteins typical of yeast and mammals and the fatty acid synthase type II, found in prokaryotes which is composed of four dissociable proteins that catalyze individual reactions. Although plant cells are eukaryotic, the fatty acid synthase found in plastids is of type II [15]. Plant fatty acid synthase has inherited from photosynthetic prokaryotes; plastids being considered by the endosymbiotic theory as an old cyanobacteria. In plants, acyl carrier protein (ACP) is used as the acyl carrier for the various intermediate for fatty acid synthase unlike other eukaryotic cells where fatty acids are in acyl CoA form [15].

The initial substrates for fatty acid biosynthesis are acetyl CoA and malonyl-ACPs. The transfer of malonyl moiety from CoA to ACP is catalyzed by malonyl CoA:ACP transacylase (MAT). After the initial condensation of acetyl CoA and malonyl-ACP, all the intermediates for each step of the fatty acid biosynthetic pathway are acyl-ACPs.

AGS is composed of four enzymes: ketoacyl-ACP synthase (KAS, EC 2.3.1.41),  $\beta$ -ketoacyl-ACP reductase (EC 1.1.1.100), hydroxy acyl-ACP dehydrase (EC 4.2.1.59), and enoylacyl-ACP reductase (EC 1.3.1.9). All components of fatty acid synthase occur in plastids, although they are encoded in the nuclear genome and synthesized on cytosolic ribosomes. There are four sequential reactions involved in two-carbon addition (**Figure 2**).

The first condensation takes place between acetyl CoA and malonyl-ACP. This reaction is catalyzed by 1,3-ketoacyl-ACP synthase III (KAS, EC 2.3.1.41), one of three ketoacyl synthases in plant systems [15]. KAS I is responsible for the condensations in each elongation cycle up through that producing palmitoyl-ACP (16:0-ACP). KAS II is dedicated to the final plastidial elongation, that of palmitoyl-ACP (16:0-ACP) to stearoyl-ACP (18:0-ACP).

The  $\beta$ -ketoacyl-ACP formed during the condensation reaction successively undergoes a reduction reaction by  $\beta$ -ketoacyl-ACP reductase (EC 1.1.1.100), dehydration by the  $\beta$  hydroxyacyl-ACP dehydratase (EC 4.2.1.59) and a further reduction by enoylacyl-ACP reductase (EC 1.3.1.9) to give butyryl-ACP. The coenzyme of the two oxidation-reduction reactions is NADPH (**Figure 3**). The butyryl-ACP formed



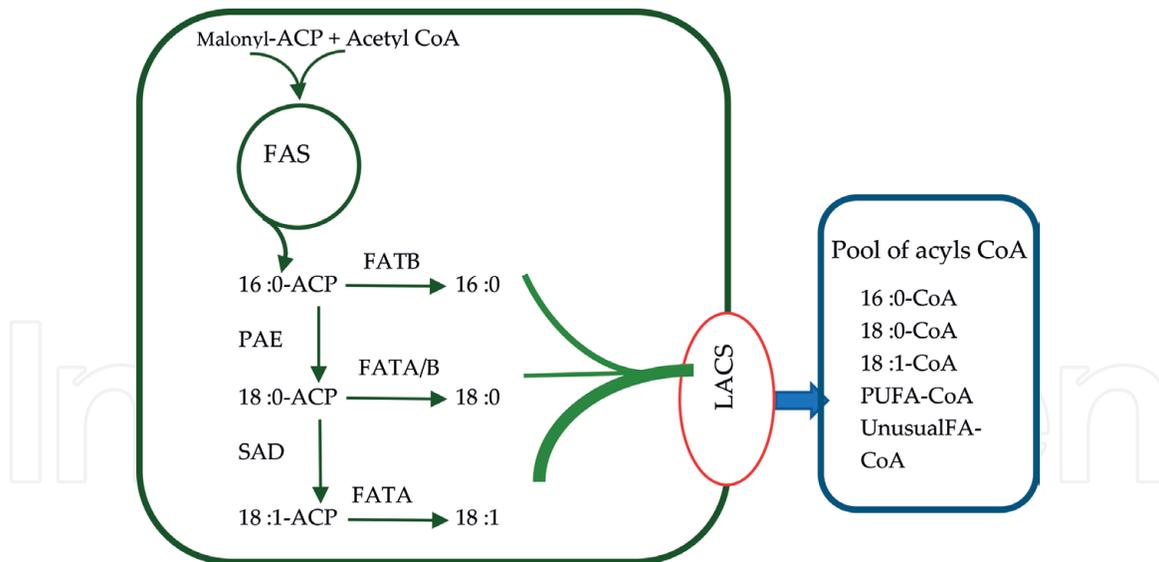
**Figure 2.** Plant fatty acid biosynthesis. This chain requires a carboxylation reaction of acetyl CoA to malonyl CoA, an activation reaction of malonyl CoA to malonyl-ACP, a condensation reaction between acetyl CoA and malonyl-ACP to form  $\beta$ -ketoacyl-ACP, which undergoes in turn a reduction reaction, dehydration, and a second reduction extending the fatty acid of two carbon atoms.

will be extended by two further C2 units after further condensation with malonyl-ACP. The  $\beta$ -ketoacyl-ACP synthase I (KASI) catalyzes this reaction. After seven rounds of cycle, palmitoyl-ACP is formed.

Although the final product of fatty acid synthase is palmitic acid, two other common fatty acids are synthesized in the chloroplast stroma. These are stearic and oleic acids. The palmitoyl-ACP (C16:0-ACP) will be extended by two new units to form a stearyl-ACP (C18:0-ACP) chain by a plastid soluble stearyl-ACP synthase which is a multienzymatic complex composed of four enzymes (KASII, enoyl-ACP reductase, hydroxyacyl-ACP dehydrase, and enoylacyl-ACP reductase) [16].

The formed stearyl-ACP is then desaturated with a plastidial soluble stearyl-ACP desaturase (SAD, EC 1.14. 19.2) in oleoyl-ACP (C18:1 $\Delta$ 9-ACP) [17]. This enzyme is a nuclear-encoded, plastid-localized soluble desaturase that introduces the first  $\Delta$ 9 double bond into the saturated fatty acid resulting in the conversion of 18:0-ACP into 18:1 $\Delta$ 9-ACP [18].

The lack of structural similarity between plant and mammalian desaturase reflects the facts that the fatty acid substrates are on different carriers (ACP and



**Figure 3.**  
*Schematic representation of the export of fatty acids from the plastid to the cytosol. ACC, acetyl CoA carboxylase; ACP, acyl carrier protein; FA, fatty acid; CoA, coenzyme A; FAS, fatty acid synthase; FAT A/B, fatty acyl-ACP thioesterase A/B; LACS, long-chain acyl CoA synthetase; PAE, palmitoyl-ACP elongase; SAD, stearoyl-ACP desaturase.*

CoA, respectively), that the enzymes utilize different electron donors (ferredoxin vs. cytochrome b5), and that the plant enzyme is soluble, whereas the animal and fungal enzymes are integral membrane protein [19]. This enzyme plays a key role in determining the ratio of saturated to unsaturated fatty acids [17].

### 3. Glycerolipids as substrates for desaturation

In addition to the soluble acyl-ACP desaturases, the fatty acids synthesized in the chloroplast (palmitate, stearate, and oleate) are desaturated by membrane-bound desaturases that utilize complex lipid substrates such as phosphatidylcholine (PC) in the endoplasmic reticulum (ER) or monogalactosyl-diacylglycerol (MGDG) in the plastid [16]. These fatty acids were used for the synthesis of glycerolipids by two distinct metabolic pathways (prokaryotic and eukaryotic pathway) and in different cellular compartments (plastids and ER) [20].

The importance of both biosynthetic pathway depends on the plant species. In the “C18:3” plants photosynthetically active, only the extrachloroplast galactolipid pathway is functional; in the case of “C16:3” plants, the two pathways coexist and their importance differs according to the species and the conditions of the environment.

#### 3.1 Importance of thioesterases

The flow of fatty acids (palmitoyl-ACP, oleoyl-ACP, and to a lesser extent stearoyl-ACP) through the two pathways would be subject to severe control. Two acyl-ACPs thioesterase enzymes and a chloroplast glycerol 3-phosphate acyl transferase play a very important role. Indeed, the acyl residues enter directly into the extrachloroplast pathway after having been hydrolyzed by fatty acyl-ACPs thioesterases FAT (A EC 3.1.2.14 and B EC 3.1.2.22) [22, 23] or in the chloroplast pathway after being acylated by acyl transferases [21].

The released palmitic, oleic, and stearic acids are then activated into coenzyme A ester by the action of long-chain acyl-CoA synthetase (LACS, EC 6.2.1.3) [24] and are exported to the cytosol (Figure 3).

Plants export sufficient fatty acid (16: 0-CoA, 18: 0-CoA, 18: 1-CoA) for lipid synthesis of extraplastid membranes and TAG of seed lipids of all plants.

### 3.2 Prokaryotic pathway

The prokaryotic pathway uses acyl-ACPs for PA and PG synthesis in all plants, and galactolipids (MGDG, DGDG, and SQDG) of so-called “C16:3” plants. This pathway is similar to the pathway demonstrated in photosynthetic prokaryotes [22].

The prokaryotic pathway is distinguished from the eukaryotic one by the presence of C16 fatty acids at the *sn*-2 position of the glycerol backbone. This pathway is characterized by the presence of molecular species 18: 3/16: 3 MGDG [23].

The major molecular species of MGDG synthesized by the prokaryotic pathway generally contain  $\alpha$ -linolenic acid (C18:3), exclusively on the *sn*-1 position of glycerol backbone, while the *sn*-2 position is esterified by hexadecatrienoic acid (C16:3), resulting in desaturation of palmitic acid. The prokaryotic pathway, exclusively localized in plastids, therefore requires desaturation steps.

The DAG, precursor of prokaryotic MGDG, is an 18:1/16:0 DAG (**Figure 5**). The first molecular species synthesized by MGDG synthase is 18:1/16:0 MGDG. The palmitoyl residue is desaturated to a *cis*-hexadecenoyl residue by an  $\omega$ 9 desaturase, which is specific for both, the *sn*2 position of the fatty acid on glycerol and lipids (MGDG) [24, 25]. The  $\omega$ 9 desaturase is much more active on the palmitoyl residue in the *sn*2 position of glycerol of the MGDG than on the one located in the position *sn*2 of the DGDG.

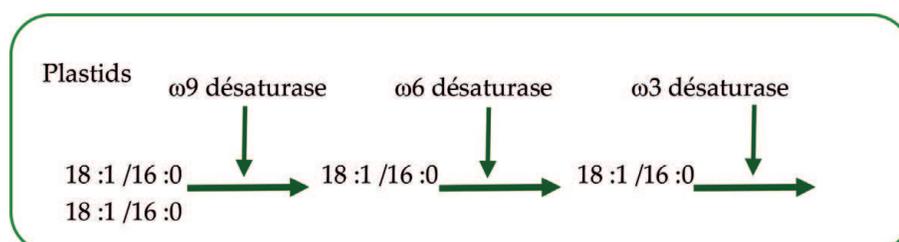
The  $\omega$ 6 and  $\omega$ 3 desaturases, respectively, catalyze the desaturation of the monounsaturated acyls (hexadecenoyl and oleoyl) and diunsaturated (hexadecadienoyl and linoleoyl) residues. These desaturases have no specificity with respect to the length of the fatty acid chain or its position on glycerol. The  $\omega$ 6 desaturase acts equally well on the hexadecenoyl and oleoyl residues located at the *sn*2 and *sn*1 positions of the MGDGs and DGDGs. The desaturation of palmitic acid to hexadecenoyl acid is a prerequisite for other desaturations [26] (**Figure 4**).

This desaturation scheme is similar to that proposed for the desaturation of lipid acyls in the blue seaweed *Anabaena variabilis* [22].

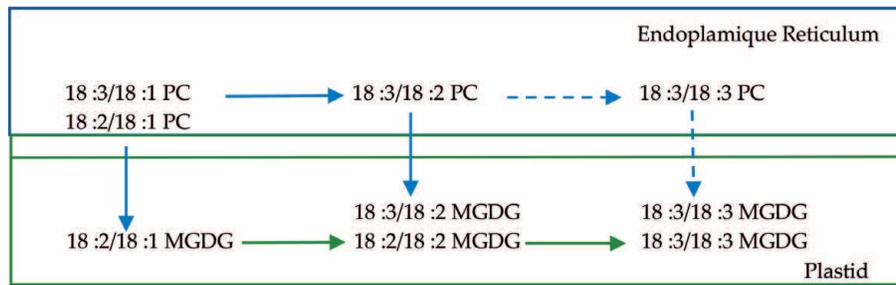
Phosphatidyl glycerol synthesis occurs in both “C16:3” and “C18: 3” plants in the chloroplast. It involves in a first step the phosphatidic acid (PA) and CDP DAG which is of prokaryotic type. About 30–40% of the palmitoyl residue at position *sn*-2 of PG is desaturated at carbon 3 to form 3-*trans*-hexadecenoic acid [27]. The structure of this fatty acid is unusual; in plants, all the double bonds of the fatty acids of the membrane lipids are of *cis* type with the exception of this fatty acid.

### 3.3 Eukaryotic pathway

The second pathway, called the “eukaryotic” pathway, leads to the formation of two types of MGDG molecular species; one of them contains  $\alpha$ -linoleate in both



**Figure 4.** Possible desaturation scheme of prokaryotic MGDG in plastids.



**Figure 5.** Scheme of possible ways for the synthesis of eukaryotic-type MGDG in spinach, C16: 3 plants according to [25].

positions of glycerol and the other molecular species contains  $\alpha$ -linoleate in position *sn*-2 and palmitate in position *sn*-1. This pathway requires cooperation between plastids and the endoplasmic reticulum for the formation of glyceroglycolipids in chloroplasts [23, 28].

The oleate integrated into PC molecules at the position *sn*-2 of glycerol backbone undergoes a succession of desaturations catalyzed by the ( $\omega$ -6,  $\Delta$ 12) oleate desaturase, still identified by the *fad2* mutation of *Arabidopsis* and allowing the synthesis of linoleic acid and the ( $\omega$ -3,  $\Delta$ 15) linoleate desaturase, identified by the *fad3* mutation of *Arabidopsis*, which allows the synthesis of  $\alpha$ -linoleic acid. Mutants deficient for the lysophosphatidylcholine acyltransferase (LPCAT1 and LPCAT2 genes) have reduced levels of polyunsaturated FA (PUFA) in TAGs [29].

After desaturation as acyl-PC, a part of them, probably in the form of DAG, returns to the chloroplast and contributes to the formation of chloroplast galactolipids (Figure 5). These DAGs can be desaturated by chloroplast desaturases [30, 31].

It is therefore possible to judge the relative contributions of the prokaryotic and eukaryotic pathways by comparing the proportions of eukaryotic 18/18 and 16/18 glycerolipids with prokaryotic 18/16 glycerolipids.

## 4. Synthesis of membrane glycerolipids

Membranes of eukaryotic cells have multiple functions in ensuring physical compartmentalization at the cellular and subcellular levels, the regulation of exchanges by the transport of metabolites and macromolecules, cellular communication (hormone receptors, surface antigens, signal transduction, etc.), and in some specific metabolic reactions. In the same cell, it is therefore not surprising to encounter different types of membranes with a specific lipid and protein composition that will determine their respective functions [32]. This synthesis is mainly carried out by two metabolic pathways described as prokaryotic and eukaryotic [33].

### 4.1 Synthesis of plastid lipids

Eukaryotic DAGs and prokaryotic DAG structures are the precursors of glycolipid synthesis (SQDG, MGDG, and DGDG). There are therefore two types of glycolipids: prokaryotic glycolipids whose DAG backbone is of the C18/C16 type and which are desaturated exclusively in the plastid and eukaryotic glycolipids including DAGs of the type (C18:1/C18:1 and C16:0/C18:1) are derived from phosphatidylcholine and are desaturated in RE and plastid [34]. The synthesis of glycolipids, being localized in the membranes of the plastid envelope, thus requires a mechanism for importing DAGs of eukaryotic structure. These differences in DAG structure are due to different specificities of the chloroplast and ER acyl transferases. The first step of the prokaryotic pathway is the transfer of the oleate to

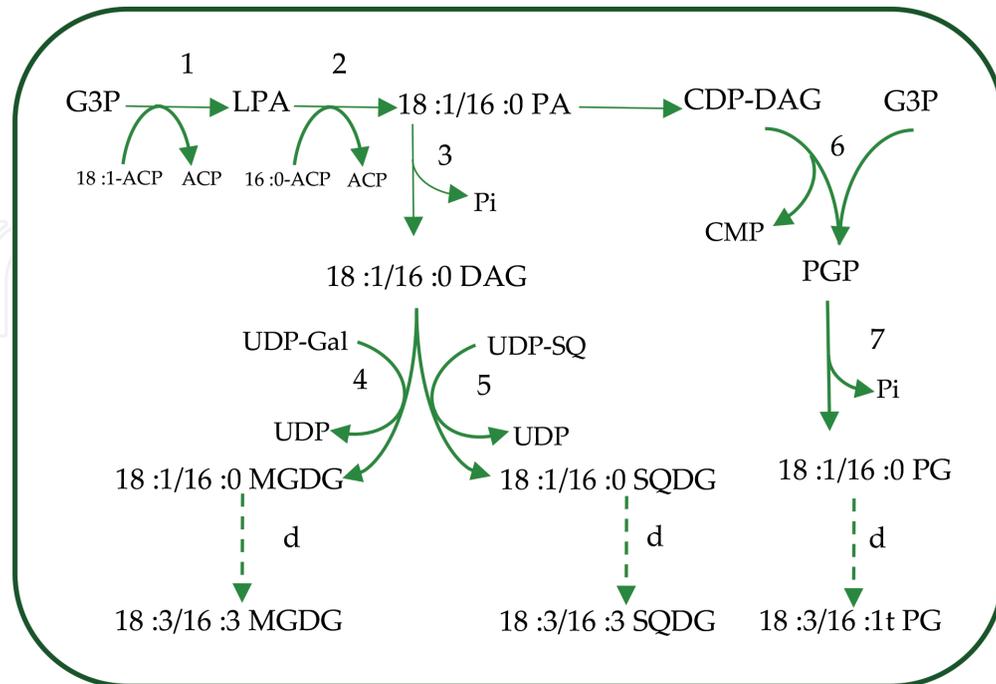
a glycerol-3-phosphate at position *sn*-1 by an acyl ACP-glycerol 3 phosphate acyltransferase (EC 2.3.1.1), soluble in the stroma of the plastid [35]. Lysophosphatidic acid (LPA) is thus formed. A second plastid-related plastid acyltransferase, the LPA-ACP acyltransferase, catalyzes the esterification of palmitoyl-ACP at the *sn*-2 position (LPAAT1; EC 2.3.1.51) [36]. This results in the synthesis of 18:1/16:0-PA.

Phosphatidic acid (PA) can either be converted to CDP-DAG by the action of a CTP-phosphatidate cytidyltransferase (EC 2.7.7.41) which catalyzes the reaction between PA and CTP to form CDP-DAG and pyrophosphate or dephosphorylated to diacylglycerol (DAG) by phosphatidate phosphatase (PAP; EC 3.1.3.4). CDP-DAG will be used for the synthesis of phosphatidyl glycerol (PG) of the plastid [35] and DAG can be used for the synthesis of galactolipids (MGDG, DGDG) or a sulfolipid (sulfoquinovosyldiacylglycerol) (**Figure 6**).

From the phylogenetic point of view, the difference between so-called “C16:3” and “C18:3” plants is related to the presence of plastid phosphatidate phosphatase in “C16:3” plants, lost during evolution in “C18:3” plants. The chloroplast enzyme is clearly different from other phosphatidate phosphatases in the cell because it is membrane-bound, strongly associated with the inner membrane of the envelope, has an optimum alkaline pH, and is inhibited by cations such as Mg<sup>2+</sup> [37]. The DAG thus produced (18/16 DAG) is at the origin of the glycolipids of prokaryotic structure, SQDG, MGDG, and DGDG (**Figure 5**).

#### 4.1.1 Synthesis of monogalactosyl diacylglycerol (MGDG)

MGDG is synthesized in a single step by a 1,2-DAG 3-β-galactosyltransferase (or MGDG synthase) that transfers galactose from UDP-Gal to DAG via a β1 → 3 glycosidic linkage [38]. MGDG synthase 1 catalyzes the synthesis of eukaryotic and prokaryotic MGDG molecules in vitro with no apparent specificity for either structure [38] and is at the origin of the majority of the MGDG synthesized in standard



**Figure 6.** Biosynthesis of glycerolipids according to the prokaryotic pathway (MGDG, DGDG, SQDG, and PG). The enzymes involved are: (1) glycerol-3-phosphate acyl transferase; (2) 1-acyl-glycerol-3-phosphoacyltransferase; (3) phosphatidate phosphatase; (4) MGDG synthase; (5) SQDG synthase; (6) phosphatidate cytidyl transferase; (7) CDP-DAG: glycerol-3-phospho-cytidyltransferase; (8) phosphatidate glycerophosphatase; d: desaturase.

condition. In contrast, MGDG synthase 2 and 3 would be localized in the outer membrane [38]. These two enzymes have a better affinity for eukaryotic DAG (C18: 2/C18: 2) [38] and would likely be in the supply of MGDG for synthesis of DGDG [39].

#### 4.1.2 Synthesis of the DGDG

A small proportion of MGDGs are again glycosylated by DGDG synthase (EC 2.4.1.241) to form DGDG. Two enzymes catalyze DGDG synthesis by adding Gal from UDP-Gal to MGDG via  $\alpha 1 \rightarrow 6$  glycosidic linkage [40]. DGDG synthase1 acts preferentially on MGDG C18/C18, whereas DGDG synthase2 seems to have an affinity for MGDG with C16/18 [41]. These two enzymes would be localized in plastids, presumably in the outer membrane of the envelope [41].

#### 4.1.3 Synthesis of sulfoquinovosyl-diacylglycerol SQDG

Similarly, a sulfolipid synthase (EC 3.13.1.1) catalyzes the attachment of UDP-sulfoquinovose (UDP-SQ) to the *sn*-3 position of DAG to form SQDG. The first step in the synthesis of SQDG or sulfolipid is the formation of UDP-SQ, a polar donor group [32]. The second reaction is catalyzed by a sulfolipid synthase (EC 3.13.1.1) that transfers SQ from UDP-SQ to a DAG molecule [42].

#### 4.1.4 Synthesis of phosphatidylglycerol (PG)

Phosphatidic acid (PA) is also a substrate for CDP-DAG synthase (EC 2.7.7.41) to form CDP-DAG, the precursor of PG synthesis (**Figure 5**). In chloroplasts, PG is generated in the inner membrane of the envelope where phosphatidylglycerol-phosphate synthase and phosphatidylglycerol-phosphate phosphatase (EC 3.1.3.27) activities have been detected [43].

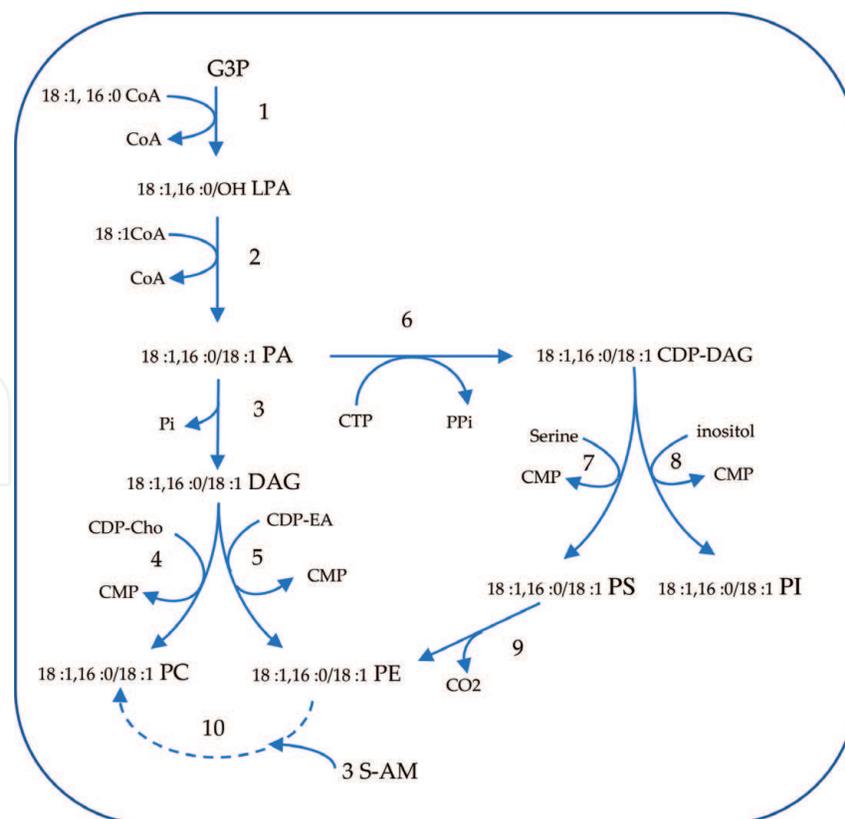
The fatty acids that make up the various glycerolipids formed in the plastid are characterized by a high degree of unsaturations introduced by the various fatty acid desaturases (FAD6, FAD7 and FAD8, EC 1.14.19) to generate polyunsaturated fatty acids (PUFA) necessary for the proper functioning of plastids [43].

## 4.2 Synthesis of glycerophospholipids in the endoplasmic reticulum

A major proportion of palmitic and oleic acids are transported as CoA esters outside the chloroplast to be incorporated at the endoplasmic reticulum (ER) into the phospholipids (PC, PE, PI, and PS) (**Figure 6**). ER is the main site for the synthesis of phospholipids and triacylglycerol, which derive from lysophosphatidic acid (LPA) as for the prokaryotic pathway (**Figure 7**).

In plant, the glycerophosphate acyltransferase (GPAT) family is involved in the first reaction leading to LPA synthesis of the eukaryotic pathway [35]. In the second reaction, cytosolic lysophosphatidic acid acyl transferase (LPAAT2, EC 2.3.1.23) specifically incorporates oleic acid at the *sn*-2 position of LPA, which is the specific signature glycerolipids from the eukaryotic pathway.

Most of the flow of chloroplast-exported fatty acids is incorporated in phosphatidylcholine (PC) by a mechanism called “acyl editing” [40]. This mechanism consists of a deacylation-reacylation cycle of the PC which makes it possible to exchange acyls present on the PC with activated FAs taken from a cytosolic pool of free acyl CoA. The oleate exported from plastids, in the form of oleoyl CoA, is used as a substrate for the synthesis of polyunsaturated fatty acids which are inserted either in membrane lipids (PC, PE, and PI) or in storage lipids (triacylglycerols TAG).



**Figure 7.**

Biosynthesis of glycerolipids according to the eukaryotic pathway (PC, PE, PI, and PS). The enzymes involved are: (1) glycerol-3-phosphate acyl transferase; (2) 1-acyl-glycerol-3-phosphoacyltransferase; (3) phosphatidate phosphatase; (4) CDP-choline: DAG choline phosphotransferase; (5) CDP-ethanolamine: DAG ethanolamine phosphotransferase or PE synthase; (6) phosphatidate cytidyl transferase; (7) PS synthase; (8) PI synthase; (9) PS decarboxylase; (10) N-methyltransferase.

In general, the synthesis of phospholipids is separated into three pathways: the phospholipids derived from cytidine diphosphate (CDP)-DAG (PI, PS), those derived from DAG (PC, PE) (**Figure 6**), and those from exchange of polar heads belonging to other phospholipids.

#### 4.2.1 Phospholipids derived from CDP-DAG: PI and PS

PA can be converted to CDP-DAG by the action of a CTP phosphatidate cytidyltransferase. This enzyme catalyzes the reaction between a eukaryotic PA molecule and a CTP molecule to form CDP-DAG and pyrophosphate.

Phosphoinositides are an important group of complex structure. PI represents 93% of phosphoinositides, while PIP (mainly PI-3P and PI-4P) and PIP2 (PI-(4,5) P2) represent less than 1%. These phosphoinositides play a major role in signaling processes. PI synthesis is catalyzed by PI synthase from free inositol and CDP-DAG. PI-3P and PI-4P are formed by phosphorylation of PI, respectively, by PI 3- and PI 4-kinases. Finally, PIP2 is formed from PI-4P by PI-4P 5-kinase activity (**Figure 7**).

PS synthase catalyzes the addition of serine to CDP-DAG [44].

#### 4.2.2 Lipids derived from DAG: PE and PC

The plants synthesize ethanolamine by decarboxylation of serine [45], by serine decarboxylase which is a soluble, plant-specific enzyme. The synthesized free ethanolamine is then phosphorylated by an ethanolamine kinase, specific for ethanolamine different from choline kinase [46]. Phosphoethanolamine is then converted to CDP-ethanolamine by a CTP: phosphoethanolamine cytidyl transferase. The last step of PE synthesis is catalyzed by a CDP-ethanolamine: DAG ethanolamine

phosphotransferase. This enzyme is an amino alcohol phosphotransferase that synthesizes both PE and PC [47] (**Figure 7**).

PC can also be synthesized by two different ways, either by methylation of PE with PE-N-methyltransferase, or by the addition of CDP-choline on DAG. The pathway using CDP-choline is preponderant [48].

The synthesis of all these lipids, phospholipids, and glycolipids is localized in specific membranes. However, a large part of the lipids thus generated is present in other membranes than those in which they are synthesized (vacuole, plasma membrane, and thylakoid). The cell therefore has specific lipid transport mechanisms.

## 5. TAG biosynthesis

TAGs are neutral lipids and are the major component of oilseed oil. These storage lipids represent the main source of carbon and energy mobilized during germination. Other tissues can also accumulate TAGs, such as senescence leaves or pollen grains [49, 50].

Their biosynthesis occurs at the ER membrane during the storage accumulation phase after embryogenesis. The TAGs result from the esterification at the *sn*-3 position of DAG of fatty acid from the pool of cytosolic acyl CoA by the action of diacylglycerol acyltransferase (DGAT, EC 2.3.1.20) or phospholipid: diacylglycerol acyltransferase (PDAT, EC 2.3.1.158) [17]. The acyl CoAs can be released from PC after desaturation with lyso-PC acyltransferase and reincorporated into the DAG-TAG chain. This allows for a renewal of the fatty acid composition of TAGs [51].

### 5.1 Seed triacylglycerols often contain unusual fatty acids

More than 300 different fatty acids are known to occur in seed TAG. Chain length may range from less than 8 to over 22 carbons. The position and number of double bonds may also be unusual, and hydroxy, epoxy, or other functional groups can modify the acyl chain.

The synthesis of these unusual fatty acids involves just one additional or alternative enzymatic step from primary lipid metabolism. All the enzymes identified to date that are involved in unusual fatty acid biosynthesis are structurally related to enzymes of primary lipid metabolism. Many of the unusual fatty acids are found in taxonomically dispersed families implying that the recruitment of enzymes for the synthesis of these unusual fatty acids might have occurred a number of independent times during angiosperm evolution.

Plants that synthesize **unusual monounsaturated fatty acids** have an additional desaturase, which is closely related to the  $\Delta 9$ -desaturase but introduces a double bond at a different location on the acyl-ACP. In coriander (*Coriandrum sativum*), petroselinic acid is synthesized by a desaturase that introduces a double bond between carbons 4 and 5 of a C16 acyl-ACP ( $\Delta 4$ -desaturase). This fatty acid is then extended by two carbons and cleaved from ACP to produce the free fatty acid. These last two steps are thought to require a specialized condensing enzyme and a specialized acyl-ACP thioesterase [52].

Plants that synthesize **medium-chain fatty acids** have several thioesterases. Indeed, plants that produce seeds with high concentrations of 8 to 14 carbon atoms, like *Cuphea lanceolata* rich in decanoic acid (C10: 0) *Umbellularia californica* rich in laurate (C12: 0) contain specific thioesterase for medium fatty acid chains. By removing acyl groups from ACP prematurely, the medium-chain thioesterases simultaneously prevent their further elongation and release them for triacylglycerol synthesis outside the plastids [53].

Seeds of *Ricinus communis* L. are the source of castor oil, used for the production of high-quality lubricants due to its high proportion of the unusual fatty acid ricinoleic acid. Castor bean seed oil contains 90% of the unusual hydroxy-fatty acid. Castor bean seeds contain an oleate hydroxylase which is structurally similar to extraplastidial membrane-bound  $\Delta$ 12-desaturases (FAD2), and only four amino acid substitutions are needed to convert an 18:1-desaturase into an 18:1-hydroxylase [54]. The synthesis of these fatty acids is thought to take place on the endoplasmic reticulum and use fatty acids esterified to the major membrane lipid phosphatidylcholine as a substrate.

Borage (*Borago officinalis* L.) seeds and evening primrose (*Oenothera biennis* L.) seeds are rich in  $\gamma$ -linolenic acid ( $\Delta$ 6, 9, 12), respectively, from 22 to 25% and from 8 to 10%, an essential fatty acid. Its synthesis takes place in the RE during the formation of the seed. The precursor is a linoleoyl-PC and the desaturation is catalyzed by a D6 desaturase [55].

Very long-chain fatty acids (AGTLCs, containing more than 18 carbons) are used in the biosynthesis of many lipids involved in seed storage and waxes. Very long-chain fatty acids (VLCFAs) are synthesized in the following by-products of elongation of a C18 fatty acyl precursor by two carbons originating from malonyl CoA. Each elongation step requires four enzymatic reactions: condensation between an acyl precursor and malonyl-CoA, followed by a reduction, dehydration, and another reduction.

## 6. Conclusion

The reason for the great diversity in plant storage oils is unknown. The special physical or chemical properties of the “unusual” plant fatty acids have been exploited for centuries. Many of the unusual fatty acids have properties that are valuable as renewable feedstocks for the chemical industry. Medium fatty acids (lauric acid) are the ingredients of a soap or shampoo. VLCFAs like erucic acid (C22:1) can be used as a lubricant or participate in the formation of plastic film. Hydroxy fatty acids such as ricinoleic acid could be a source of biodiesel.

These unusual fatty acids synthesized by spontaneous plants are therefore obtained in small quantities. In order to obtain these fatty acids regularly and in large quantities for industrial use, it will either be necessary to domesticate the plant or introduce the specific gene of the nonconventional fatty acid into an oleaginous plant grown to obtain sufficient yields for industrial uses.

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