REVIEW

Seeds as oil factories

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Abstract

Key message **Studying seed oil metabolism.**

Abstract The seeds of higher plants represent valuable factories capable of converting photosynthetically derived sugars into a variety of storage compounds, including oils. Oils are the most energy-dense plant reserves and fatty acids composing these oils represent an excellent nutritional source. They supply humans with much of the calories and essential fatty acids required in their diet. These oils are then increasingly being utilized as renewable alternatives to petroleum for the chemical industry and for biofuels. Plant oils therefore represent a highly valuable agricultural commodity, the demand for which is increasing rapidly. Knowledge regarding seed oil production is extensively exploited in the frame of breeding programs and approaches of metabolic engineering for oilseed crop improvement. Complementary aspects of this research include (1) the study of carbon metabolism responsible for the conversion of photosynthetically derived sugars into precursors for fatty acid biosynthesis, (2) the identification and characterization of the enzymatic actors allowing the production of the wide set of fatty acid structures found in seed oils, and (3) the investigation of the complex biosynthetic pathways leading to the production of storage lipids (waxes, triacylglycerols). In this review, we outline the most recent developments in our understanding of the underlying biochemical and molecular mechanisms of seed oil production, focusing on fatty acids and oils that can have a significant impact on the emerging bioeconomy.

Keywords Fatty acid · Metabolism · Oil · Triacylglycerol · Seed

Introduction

In seed plants, production of seeds interrupts the life cycle, linking two sporophytic generations and allowing dispersion and survival of plants. Seeds originate from the fertilized ovule in Angiosperms. Double fertilization of the embryo sac initiates the development of the embryo and the endosperm. These zygotic tissues are protected by a seed coat comprising several cell layers of maternal origin. The coordinated growth of these tissues of distinct origins leads to the formation of seed structures containing all the genetic material and nutrients required to establish the next generation. Seed development comprises two major phases: early embryo morphogenesis is followed by a maturation phase (Vicente-Carbajosa and Carbonero 2005). Storage compounds are accumulated during maturation. Their biosynthesis is developmentally controlled by a complex network of transcription factors termed master regulators of the maturation program that were shown to regulate the transition between vegetative phases of development and seed maturation (Baud and Lepiniec 2010; Devic and Roscoe 2016; Fatihi et al. 2016). Seed reserves play an important role in the acquisition of desiccation tolerance by maturing seeds. They also significantly impact the subsequent seed germination and seedling establishment success by providing the materials required to support growth until the developing seedling becomes autotrophic.

Main seed storage compounds usually consist of storage proteins, carbohydrates (starch or β-glucans), and storage lipids like waxes or triacylglycerols (TAGs). Depending on plant species, the relative proportions of these components vary greatly, but a source of nitrogen (N) and a source of carbon (C) are usually associated. Oils are composed of

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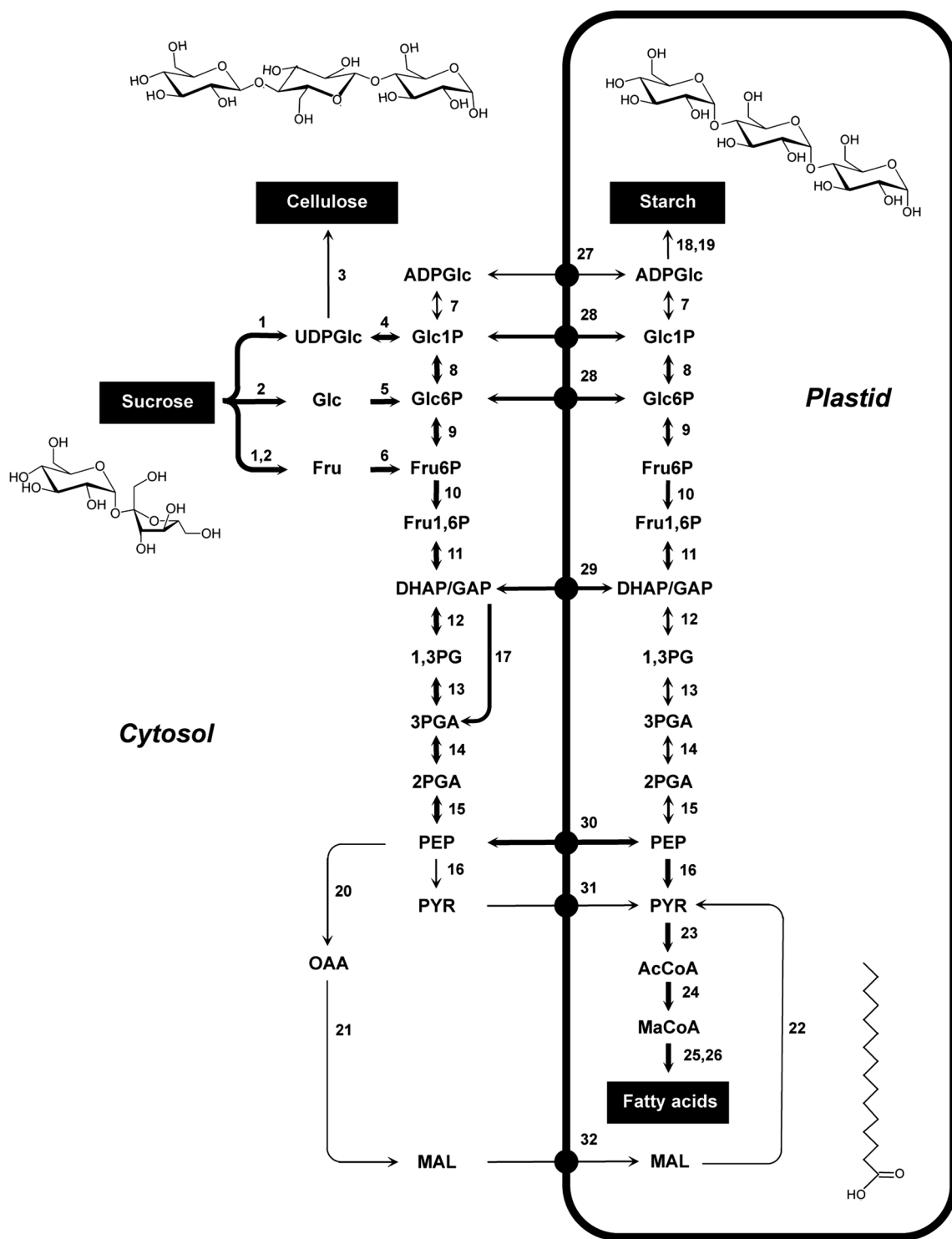
long-chain hydrocarbons that are one of the most highly reduced and energy-dense carbon compounds produced by nature. Oxidation of oils in germinating seeds thus releases more than twice as much energy as the oxidation of storage carbohydrates or proteins on a per g basis (Graham 2008). These molecules are also important sources of calories for human and animal nutrition, as well as petroleum

alternatives for the chemical industry and for biofuels (Aznar-Moreno and Durrett 2017b).

TAGs, the main components of plant oils, are composed of three fatty acids (FAs) esterified to glycerol. They are stored in subcellular structures termed oil bodies or oleosomes that comprise a matrix of TAGs surrounded by a phospholipid monolayer (Miquel et al. 2014; Shimada et al.

◄**Fig. 1** Overview of central metabolism in maturing oilseeds. This simplified scheme of carbon metabolism presents the various biosynthetic pathways using sucrose as a precursor for the production of cellulose, starch, and fatty acids in oilseeds. Arrows represent net fluxes of carbon; the arrow thicknesses are proportional to net fluxes of carbon. For the sake of clarity, amino acid biosynthetic pathways have been omitted. 1, Sucrose synthase; 2, invertase; 3, cellulose synthase; 4, UDP-glucose pyrophosphorylase; 5, hexokinase; 6, fructokinase; 7, ADP-glucose pyrophosphorylase; 8, phosphoglucomutase; 9, phosphoglucose isomerase; 10, phosphofructokinase; 11, aldolase; 12, glyceraldehyde-3-phosphate dehydrogenase; 13, phosphoglycerate kinase; 14, phosphoglycerate mutase; 15, enolase; 16, pyruvate kinase; 17, NADP-dependent glyceraldehyde-3-phosphate dehydrogenase; 18, starch synthase; 19, starch branching enzyme; 20, phosphoenolpyruvate carboxylase; 21, malate dehydrogenase; 22, NADP-dependent malic enzyme; 23, pyruvate dehydrogenase; 24, acetyl-coenzyme A carboxylase; 25, malonyl-coenzyme A:acyl carrier protein S-malonyltransferase; 26, fatty acid synthase; 27, ADP-glucose/ADP transporter; 28, glucose-6-phosphate/phosphate transporter; 29, triose-phosphate/phosphate transporter; 30, phosphoenolpyruvate/phosphate transporter; 31, sodium-dependent pyruvate transporter; 32, malate/α-ketoglutarate transporter. Abbreviations: AcCoA, acetyl-coenzyme A; ADPGlc, adenosine diphosphate glucose; DHAP, dihydroxyacetone-3-phosphate; Fru, fructose; Fri1,6P, fructose-1,6-bisphophate; Fru6P, fructose-6-phosphate; GAP, glyceraldehyde-3-phosphate; Glc, glucose; Glc1P, glucose-1-phosphate; Glc6P, glucose-6-phosphate; MaCoA, malonyl-coenzyme A; MAL, malate; OAA, oxaloacetate; PEP, phosphoenolpyruvate; 1,3PG, 1,3-bisphosphoglycerate; 2PGA, 2-phosphoglycerate; 3PGA, 3-phosphoglycerate; PYR, pyruvate; UDPGlc, uridine diphosphate glucose

2017). Depending on the species considered, the abundance and location of olesomes in seeds vary greatly. Some oilseed species like soybean (*Glycine max*), rapeseed (*Brassica napus*), linseed (*Linum usitatissimum*), and sunflower (*Helianthus annuus*) predominantly store oil in embryo tissues that occupy most of the space available in between the integuments of the seed. On the contrary, oil is mostly accumulated in the large endosperm tissue observed in seeds of castor bean (*Ricinus communis*) for example. Additionally to mechanisms controlling cell proliferation and degeneration within the developing endosperm, and therefore the relative importance of this tissue within the seed (Ingram 2017; Olsen 2004; Sreenivasulu and Wobus 2013), transcription factors specifically induced in the maturing endosperm further control the partitioning of reserves like oil between zygotic tissues (Barthole et al. 2014). Interestingly, recent methodological developments in lipidomics, microscopy, and imaging have provided us with high-resolution spatial maps of lipids in the different tissues of maturing oilseeds. These observations have revealed gradients of oils within zygotic tissues as well as contrasting FA compositions of oils between the different regions of oil-storing tissues (Borisjuk et al. 2013; Horn and Chapman 2014). These observations reveal both the influence of environmental parameters (e.g., light, temperature, oxygen availability) on oil metabolism and the existence of local developmental regulations that are not well understood.

Driving incoming carbon into the fatty acid biosynthetic pathway

Photosynthetically assimilated carbon is transported into seeds through the phloem, mostly in the form sucrose. A first important aspect of the regulation of oil metabolism in early-maturing seeds lies in the activation of metabolic pathways driving incoming carbon into the FA biosynthetic network at the expense of competitive pathways using carbon to synthesize starch, amino acids, storage β-glucans, or cellulose. Following sucrose breakdown, hexoses are converted to hexose-phosphates that can be metabolized through any of the above-mentioned pathways (Fig. 1).

Storage β-glucans are polysaccharides that consist of unbranched and unsubstituted chains of β-D-glucosyl residues stored in the endosperm walls of some cereals (Hrmova et al. 2002; Guillon et al. 2011). UDP-glucose appears to be the glucose donor supplying synthesis of these polysaccharides (Marcotuli et al. 2016). *Cellulose synthase-like* genes are believed to be involved in the biosynthesis of these β-glucans, and their controlled transcriptional activation may play a key role in determining β-glucan concentration in developing grains (Houston et al. 2014; Wong et al. 2015). In oilseeds, this pathway is usually inactive. On the contrary, cellulose, a linear polymer of (1 → 4) β-D-glucosyl residues synthesized from UDP-glucose by cellulose synthases and representing the principal structural component of plant cell walls, is produced in every type of seed. Carbon supplied to the seed is thus partially incorporated into cell wall via cellulose biosynthesis, and this biosynthetic pathway competes with the conversion of carbon supplies into reserves like oil (Alonso et al. 2007).

The synthesis of starch begins with the enzyme ADP-glucose pyrophosphorylase (AGPase), which catalyzes the reaction of glucose-1-phosphate with ATP to produce the activated glucosyl donor ADP-glucose (Cross et al. 2004). ADP-glucose is then used as a substrate for the synthesis of starch through the complex interplay between starch synthase (elongation of the glucose polymer), and starch branching and debranching enzymes (Zeeman et al. 2010). The exact molecular processes that control starch metabolism in seeds largely remain to be elucidated (Zhang et al. 2016). Nevertheless, coordinated transcriptional activation of several genes coding for biosynthetic enzymes participating in the pathway has been reported during grain filling in wheat or maize for instance (Cao et al. 2016; Mukherjee et al. 2015; Xiao et al. 2016). The recent discovery and characterization of transcriptional activators of the pathway such as OsbZIP58 (Wang et al. 2013), ZmbZIP91 (Chen et al. 2016a), ZmDof3 (Qi et al.

2017), ZmMYB14 (Xiao et al. 2017), and ZmNAC36 (Zhang et al. 2014a) further reinforce the hypothesis of important transcriptional controls governing starch storage in the endosperm of cereal grains in particular (Zheng and Wang 2015).

In oilseeds, hexose-phosphates are efficiently metabolized through the glycolysis, and pyruvate is the primary precursor for FA synthesis in the plastid. The cytosolic glycolysis pathway is commonly accepted as the major route for the metabolism of hexose-phosphates in many oilseeds. In seeds of the Brassicaceae (*B. napus*, *Arabidopsis thaliana*), a complete glycolytic pathway was also described in the plastid. This plastidic glycolysis pathway is interconnected with the oxidative pentose phosphate pathway (OPPP) and, in some species, with a ribulose-1,5-bisphophate carboxylase/oxygenase (RuBisCO) shunt fixing CO_2 apart from the Calvin cycle (Schwender et al. 2004) (for a complete review, see Baud and Lepiniec 2010). Several transporters of the plastid envelope link the cytosolic and plastidial glycolysis pathways. Hexose-phosphates, triose-phosphates, as well as end-products of the glycolysis like phosphoenolpyruvate or even pyruvate can be actively transported (Furumoto et al. 2011; Lee et al. 2017). However, import of phosphoenolpyruvate from the cytosol to the plastid and its subsequent conversion to pyruvate by plastidial pyruvate kinase is generally thought to be a major route for providing metabolic precursors for FA production. In some plant species, a minor part of the carbon for FA synthesis is attributable to the import of malate and the subsequent conversion of this compound in pyruvate by NADP-dependent malic enzyme.

The oxidative decarboxylation of plastidial pyruvate by the pyruvate dehydrogenase complex (PDC) produces acetyl-CoA that is used in turn by acetyl-CoA carboxylase (ACC) to form malonyl-CoA (Li-Beisson et al. 2010). ACC has long been considered as a key regulatory step for the FA biosynthetic process. Activity of this heteromeric complex is fine-tuned by a complex set of post-translational regulatory mechanisms integrating different parameters (light and redox status, carbon and energy availability, concentration of end-products of the pathway; for detailed reviews on ACC's regulatory network, see Salie and Thelen 2016; Troncoso-Ponce et al. 2016b; Xu and Shanklin 2016). Malonyl-CoA produced by plastidial ACC constitutes the carbon donor for each cycle of the FA biosynthetic process. Before entering the process, the malonyl group is transferred from CoA to a protein cofactor named acyl carrier protein (ACP) by a malonyl-CoA:ACP S-malonyltransferase. Production of saturated FAs (Fig. 2) is performed in a stepwise manner by the FA synthase (FAS) of type II (Fig. 3). This multi-subunit complex consisting of monofunctional enzymes uses acetyl-CoA as a starting unit and malonyl-ACP as the elongator. The malonyl-thioester enters in a series of condensation reactions catalyzed by β-ketoacyl-ACP synthase

(KAS). 3-Ketoacyl-ACP thus obtained is then reduced by a β-ketoacyl-ACP reductase (KAR), yielding 3-hydroxy-acyl-ACP that is subjected to dehydration by the enzyme hydroxyacyl-ACP dehydratase (HAD). The enoyl-ACP obtained is finally reduced by the enzyme enoyl-ACP reductase (EAR). Following their synthesis, acyl groups are hydrolyzed by acyl-ACP thioesterases that release free FAs (Moreno-Pérez et al. 2012). After their transport through the plastid envelope (Li et al. 2015a, 2016), they are activated to CoA esters by long-chain acyl-CoA synthetases (LACS) (Zhao et al. 2010) prior to their transport into the ER (Kim et al. 2013), where they can be used for the assembly of storage lipids.

Extensive transcriptomic analyses have provided detailed expression patterns for lipid biosynthetic genes in various oilseed species (Abdullah et al. 2016; Dussert et al. 2013; Li et al. 2015b; Troncoso-Ponce et al. 2011; Huang et al. 2017; Venglat et al. 2011). In early-maturing seeds, a coordinated transcriptional activation of actors of the glycolytic and FA biosynthetic pathways can be observed. These genes exhibit a bell-shaped pattern of expression during the course of seed maturation (Baud and Lepiniec 2009). Interestingly, rates of FA production in early-maturing seeds increase in a manner proportional to the transcript levels of genes encoding corresponding biosynthetic enzymes, thus highlighting the importance of transcriptional regulations for the control of FA production in these storage organs. Their concerted transcriptional activation is controlled by the WRINKLED1 (WRI1) transcription factors that bind AW boxes present in their promoter sequences (Maeo et al. 2009). These transcription factors of the APETALA2/ethylene-responsive element binding (AP2/EREBP) family have been characterized in a wide range of oilseeds and their function appears highly conserved (Guerin et al. 2016; Liu et al. 2009; Ma et al. 2013; Qu et al. 2012; Shen et al. 2010; To et al. 2012). Upon binding to DNA, transcription factors recruit the Mediator complex and, in turn, the Pol II complex to initiate transcription. In *A. thaliana*, WRI1 was shown to interact with the MED15 subunit of the Mediator complex that mediates activation of FA biosynthetic genes (Kim et al. 2016). Considering that *wri1* knockout mutants maintain a basal level of glycolytic and FA biosynthetic gene transcriptional activity, the transcriptional machinery governing these pathways certainly includes additional transcription factors able to activate the pathways in the absence of WRI1. A direct transactivation of certain targets of WRI1 by master regulators of seed maturation was proposed (Wang et al. 2007; Mu et al. 2008). However, complementary studies would be required to elucidate the molecular mechanisms underpinning these transcriptional regulations.

Recent results regarding the regulation of WRI1 in *A. thaliana* suggest that this actor integrates a variety of information that are essential for the successful accumulation of
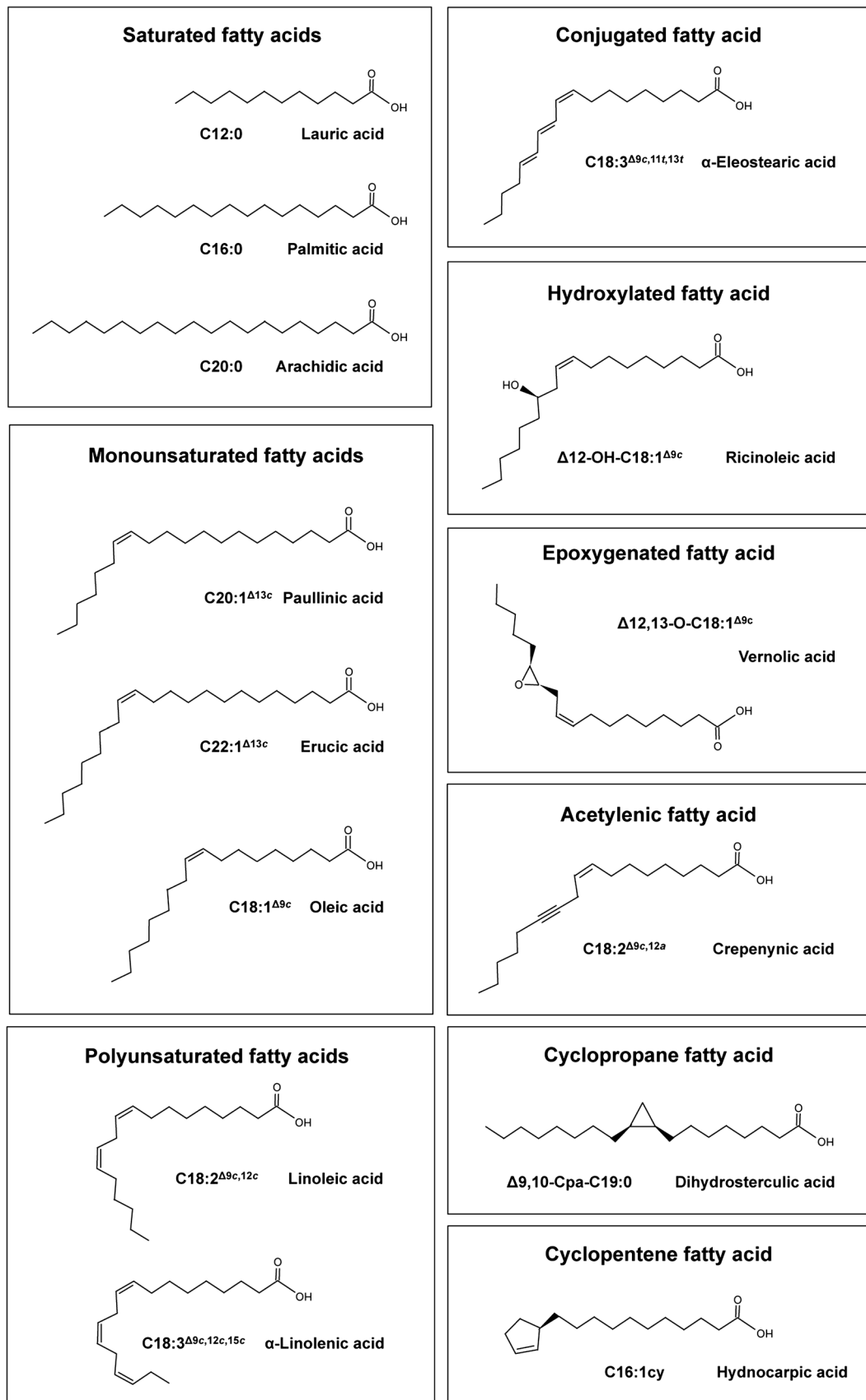
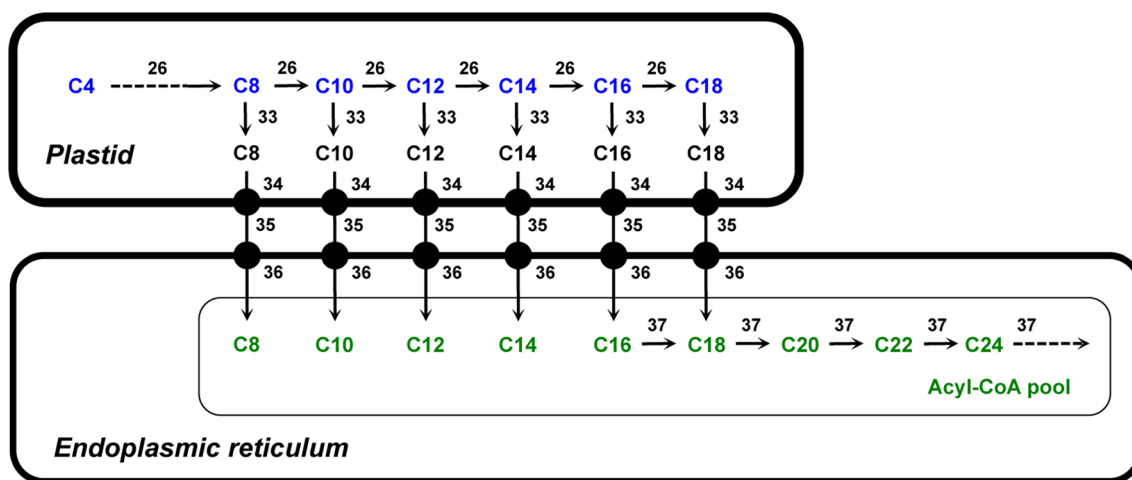**Fig. 2** Examples of structural diversity among fatty acids

Fig. 3 Fatty acid elongation. The scheme depicts the different pathways involved in the synthesis and elongation of fatty acids, in the plastids and in the endoplasmic reticulum. Growing fatty acid chains bound to acyl carrier proteins in the plastids are presented in blue, free fatty acids released by thioesterases in black, and acyl-coenzyme A in green. 26, Fatty acid synthase; 33, thioesterase; 34, fatty acid transporter; 35, long-chain acyl-coenzyme A synthetase; 36, acyl-coenzyme A transporter; 37, acyl-CoA elongase

storage lipids in developing seeds (Marchive et al. 2014). Transcriptional activation of *WRI1* by master regulators of the seed maturation program like LEAFY COTYLEDON1 (LEC1) and LEC2 first ensures that FA biosynthesis is triggered in a coordinated manner with other maturation-related programs. Post-translational regulations of WRI1 by various molecular mechanisms involving 14-3-3 proteins (Ma et al. 2016), kinases (Zhai et al. 2017), and the ubiquitine proteasome pathway (Chen et al. 2013) may then contribute to integrate signal inputs like sugar and energy availability.

Interestingly, transient expression in leaves of *Nicotiana benthamiana* of five different WRI1 homologs originating from different species followed by transcriptome sequencing not only confirmed the ability of these transcription factors to activate the FA biosynthetic pathway in this context but also revealed a concomitant repression of the starch biosynthetic pathway and an activation of starch degrading enzymes (Grimberg et al. 2015). Whether this negative regulation of starch accumulation was directly orchestrated by WRI1 is not known. In contrast with the results of the above-mentioned study, gene co-expression network analysis carried out in the mesocarp of oil palm revealed positive correlations between the actors of FA biosynthesis and the actors of starch biosynthesis and degradation (Guerin et al. 2016). These apparent discrepancies suggest that the complex interplay between starch and FA metabolism might well vary between tissues and/or between species. In several oilseeds of the Brassicaceae, transient starch deposition was described in early-maturing embryos (Baud et al. 2008). The role of this transient accumulation of starch at the onset of oil storage remains a matter of debate. Enzymatic and expression data have established that the changes in starch content throughout embryo development reflect the net balance between synthetic and degradative capacity rather than a synthetic phase followed by a degradative phase (da Silva et al. 1997; White et al. 2000). Further analyses will be required to better understand the interactions between these pathways.

The negative correlation between oil and protein contents observed by breeders in seeds of soybean and Brassica (Eskandari et al. 2013; Mao et al. 2013; Mahmood et al. 2006) suggests that corresponding biosynthetic pathways interfere too. This negative correlation reflects a competition for space to store end-products of these pathways since most embryo cells in mature seeds are filled with storage compounds. To a certain extent, these observations also illustrate how the two pathways compete for carbon resources (Kanai et al. 2015). Biosynthesis of seed storage proteins massively uses amino acids imported from the mother plant or derived from these amino acids through transamination reactions. However, approaches of metabolic flux analyses carried out in various oilseeds have unraveled that a minor part of 3-carbon sugars, pyruvate, and acetyl-CoA derived from imported sucrose could serve as precursors for de novo amino acid biosynthesis in maturing seeds (Schwender et al. 2006; Iyer et al. 2008; Lonien and Schwender 2009). The mechanisms regulating carbon partitioning between these different pathways remain poorly understood.

Seeds as reservoirs for a variety of fatty acids

A huge variety of fatty acid structures

Plant oil production is dominated by a few established species and the majority of these food oils are comprised of only five FAs: palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1$^{\Delta 9c}$), linoleic acid (C18:2$^{\Delta 9c,12c}$), and α-linolenic acid (C18:3$^{\Delta 9c,12c,15c}$). In striking contrast, seed plants collectively display enormous variation in the FAs they synthesize and store into seeds. More than 300 different FA structures have been reported to occur in plant seeds (https://plantfadb.org) and more structures might yet be discovered in plant families never analyzed so far for FA composition. These unusual FAs present differences in chain length (shorter than 16 carbons or longer than 18), position or number of double bonds, or due to the addition of hydroxyl, epoxyl, ketone, acetylenic, cyclopropyl, and other functional groups (Napier 2007) (Fig. 2). The biological function of most of these unusual FAs is unknown, even though a role in defense against pests and pathogens has been put forward (Schultz et al. 1996; Cahoon et al. 2003). Many of these unusual FAs have a great potential in the chemical industry due to the interesting physicochemical properties inherent in their original structure. Although some of these FAs can be present in very high amount in the seed oils from wild species, the low yields and poor agronomical performance of these species usually preclude their large-scale commercial production. Attempts made to alter oil composition of oilseed crops through bioengineering to produce these unusual FAs and enable the development of new and productive crops that can serve as renewable sources of industrial feedstock were extensively described in excellent reviews (Aznar-Moreno and Durrett 2017b; Beaudoin et al. 2014; Carlsson et al. 2011; Dyer et al. 2008; Napier 2007; Vanhercke et al. 2013).

Variation in chain length

FA structures present variation in chain length. Elongation of acyl chains by the FAS complex involves three different KAS. The initial condensation reaction of acetyl-CoA and malonyl-ACP is catalyzed by KAS isoform III, yielding a four-carbon product. Subsequent condensations (up to C16:0-ACP) require a second KAS isoform, KASI, whereas the final elongation of C16:0-ACP to C18:0-ACP is catalyzed by a third condensing enzyme, KASII. In most species, only C16 and C18 long-chain FAs are released from the FAS complex, so that C16 and C18 FAs are the predominant FAs found in most seed oils.

Down-regulation of KASII in seeds by biotechnological means can significantly increase C16 levels at the expense of C18 (Liu et al. 2017).

The synthesis of medium-chain FAs (MCFAs), including caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0) (Fig. 2), and myristic acid (C14:0) is a variation on typical de novo FA synthesis that occurs in seeds that accumulate primarily C16 and C18 FAs. Whereas hydrolysis of long-chain acyl-ACP is catalyzed primarily by FatA (C18-ACP) and FatB thioesterases (C16-ACP), divergent FatB enzymes with substrate specificities for saturated FAs with chain length less than C16 are responsible for the synthesis of MCFAs (Kim et al. 2015b). Once the free MCFA is formed, it is exported from the plastid and acylated to glycerol, yielding medium-chain TAGs. These MCFAs are stored in seeds of members of the genera *Litsea* and *Cuphea* for example. MCFAs are important for industrial production of detergents, soaps, cosmetics, surfactants, and lubricants (Dyer et al. 2008). Laurate is currently derived from plants of the Arecaceae family (*Cocos nucifera* and *Elaeis guineensis*). Introduction of thioesterase genes with specificities toward medium-chain acyl-ACP from different *Cuphea* species or from California bay (*Umbellularia californica*) was successfully achieved in several Brassicaceae species (*A. thaliana*, *B. napus*, *Camelina sativa*) (Hu et al. 2017). One of the earliest successes in engineering unusual FA composition in oilseeds consisted in the production of lauric acid (Dyer et al. 2008; Wiberg et al. 2000).

Very long-chain FAs (VLCFAs) contain more than 18 carbon atoms. They are synthesized from long-chain acyl-CoA by the endoplamic reticulum (ER)-associated FA elongase (FAE) complex (Fig. 3) (Haslam and Kunst 2013). Each cycle of elongation adds two carbon units to the acyl chain and involves four reactions. Malonyl-CoA and long-chain acyl-CoA are first condensed by a β-ketoacyl-CoA synthase (KCS) (Chen et al. 2011; Jasinski et al. 2012; Mietkiewska et al. 2007). The resulting 3-ketoacyl-CoA is then reduced by the action of a β-ketoacyl-CoA reductase (KCR) (Beaudoin et al. 2009), yielding 3-hydroxyacyl-CoA that is subsequently dehydrated to 2-enoyl-CoA by a β-hydroxyacyl-CoA dehydratase (HCD). A second reduction reaction performed by an enoyl-CoA reductase (ECR) forms the elongated acyl-CoA (Salas et al. 2005). KCS is the rate-limiting enzyme for seed VLCFA production. The enzyme plays a key role in determining the chain length of FAs. VLCFAs are common components of plant seed oils in a number of plant families including Brassicaceae, Limnanthaceae and Tropaeolaceae. Erucic acid (C22:1$^{\Delta 13c}$) (Fig. 2), obtained by elongation of oleic acid by the FAE complex, is the major VLCFA in the seed oil from *Crambe abyssinica* and HEAR (high erucic acid rapeseed) *B. napus* cultivars. A major derivative of erucic acid is erucamide, which is used as a surface-active additive in coatings and in the production of plastic films as

an antiblock or slip agent (Mietkiewska et al. 2007). High concentrations of erucic acid being nutritionally undesirable, LEAR (low erucid acid rapeseed) *B. napus* cultivars have also been produced for food purposes, the low erucic acid phenotype of these varieties being due to variations in the sequence or expression level of the KCS-coding gene *FAE1* (Roscoe et al. 2001; Yan et al. 2015).

Monounsaturated fatty acids

If the FAS complex preferentially produces C18:0-ACP, only limited amounts of stearic acid (C18:0) are exported from the plastids, so that this FA seldom accumulates in seed oils. Molecules of C18:0-ACP are efficiently desaturated by a stromal $\Delta 9$ stearoyl-ACP desaturase (SAD) of the acyl-ACP desaturase (AAD) family, so that oleic acid (C18:1$^{\Delta 9cis}$) (Fig. 2) is preferentially exported to the ER (Fig. 4). In most plant oils, monounsaturated FAs (MUFAs) usually consist of oleic acid and its elongated derivatives gondoic acid (C20:1$^{\Delta 11c}$) and erucic acid (C22:1$^{\Delta 13c}$) (Fig. 2). Oleic acid, one of the most widely distributed FA in seed oils, represents an important component of human diet. Aside from food and

feed uses, MUFA also have significant industrial potential. They are relatively resistant to oxidation and impart better stability for direct use of the oil in products like biolubricants or biodiesel (Durrett et al. 2008). Cleavage of their double bonds by chemical processing then yields precursors highly demanded by the chemical industry for the production of various polyamides (nylons) (Vanhercke et al. 2013). High-oleic compositions have been obtained in various oilseed crops using either conventional breeding or bioengineering. A particularly successful approach of metabolic engineering aimed at increasing the proportion of oleic acid in seed oil consisted in the concomitant inhibition, by seed-specific RNAi-mediated gene silencing, of C16:0 release from the FAS complex by *FatB* and of further desaturation of oleic acid by *FAD2* (see below) (Vanhercke et al. 2013).

In some plant species, variant AAD isoforms have been characterized that vary from the archetypal $\Delta 9$ SAD in their substrate specificity or regioselectivity (Guy et al. 2011). For example, $\Delta 9$ palmitoyl-ACP desaturases (PAD) prefer C16:0-ACP as a substrate and produce palmitoleic acid (C16:1$^{\Delta 9c}$), that can be further elongated to form vaccenic acid (C18:1$^{\Delta 11c}$) and paullinic acid (C20:1$^{\Delta 13c}$) (Fig. 2)
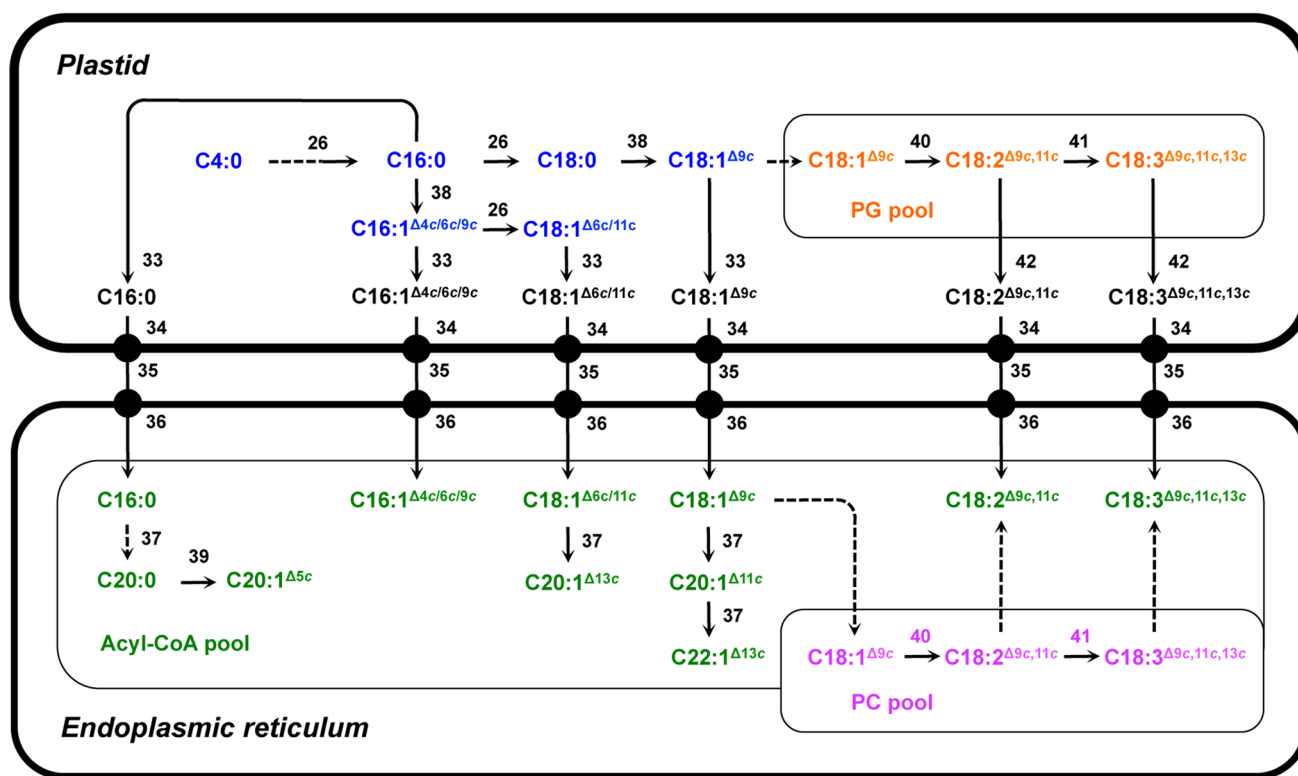

Fig. 4 Fatty acid desaturation. The scheme depicts the different pathways involved in the biosynthesis of unsaturated fatty acids in seeds. Fatty acid chains bound to acyl carrier proteins in the plastids are presented in blue, free fatty acids released by thioesterases in black, acyl-coenzyme A in green, fatty acids bound to phosphatidylglycerol in orange, and fatty acids bound to phosphatidylcholine in purple. 26, Fatty acid synthase; 33, thioesterase; 34, fatty acid transporter; 35, long-chain acyl-coenzyme A synthetase; 36, acyl-coenzyme A transporter; 37, acyl-CoA elongase; 38, acyl–acyl carrier protein desaturase; 39, acyl-coenzyme A desaturase; 40, $\Delta 12$ fatty acid desaturase; 41, $\Delta 15$ fatty acid desaturase; 42, phosphatidylglycerol lipase. Abbreviations: PC, phosphatidylcholine; PG, phosphatidylglycerol

(Bryant et al. 2016; Cahoon et al. 1998; Troncoso-Ponce et al. 2016a, b). These omega-7 MUFAs are abundant in seed oils of milkweed (*Asclepias syriaca*), macadamia (*Macadamia integrifolia*), and cat's claw vine (*Doxantha unguis-cati*). Transcriptional activation of PAD-coding genes by the MYB115 and MYB118 transcription factors in the maturing endosperm of *A. thaliana* seeds was recently shown to be responsible for the tissue-specific accumulation of omega-7 MUFAs in these seeds (Troncoso-Ponce et al. 2016a). Omega-7 MUFAs sourced from sea buckthorn (*Hippophae rhamnoides*) berries are increasingly exploited for their positive effects on health (Wu et al. 2012). These particular MUFAs also have great industrial potential. Olefin metathesis constitutes a powerful tool for polymer chemistry and ethenolytic metathesis of omega-7 FAs from plant oils could potentially provide a competitive source of 1-octene to make linear low-density polyethylene. Due to the high economic value of plant omega-7, there is an increasing interest in either finding new natural plant sources with potentially better agronomical performances or in improving existing oilseed crops for better omega-7 production. Approaches of metabolic engineering relying on the overexpression of *PAD* genes have been successfully achieved in *A. thaliana* and in crops like *C. sativa*, *B. napus*, and soybean (*Glycine max*) (Bondaruk et al. 2007; Nguyen et al. 2010, 2015; Ettaki et al. 2018).

Other unusual MUFAs synthesized by divergent AAD isoforms have been described in seed oils. For example, petroselinic acid (C18:1$^{\Delta 6c}$) is abundant in seeds of *Daucus carota* and *Coriandrum sativum*. A Δ4 PAD identified in *C. sativum* produces C16:1$^{\Delta 4c}$ that is further elongated to C18:1$^{\Delta 6c}$ by a specialized KAS isoform (Cahoon et al. 1992; Mekhedov et al. 2001). In seeds of *Thunbergia alata*, a Δ6 PAD produces sapienic acid (C16:1$^{\Delta 6c}$) (Cahoon et al. 1994). Petroselenic and sapienic acid can be split by ozonolysis to yield adipic acid and lauric acid (C12:0) or capric acid (C10:0). Adipic acid is one of the building blocks in 6,6-nylon. A high-yielding crop with high amounts of these unusual MUFAs in seed oil would be an economically favorable alternative feedstock for the production of adipic acid (Carlsson et al. 2011). Despite the identification of the desaturases involved in the biosynthesis of these MUFAs, classical approaches of metabolic engineering by overexpression of the corresponding genes yielded low amounts of these FAs of interest in seed oils. A set of enzymes with particular specificities may be required to efficiently channel these unusual MUFAs into TAGs.

In seeds of meadowfoam (*Limnanthes alba*), a different mechanism of unusual MUFA biosynthesis involves a presumptive ER-bound fatty acyl-CoA desaturase. Arachidic acid (C20:0) serves as a substrate for Δ5 desaturation by this enzyme that produces C20:1$^{\Delta 5c}$ (Fig. 4) (Cahoon et al. 2000). The close position of the double bond to the carboxy

terminus results in original chemical and physical properties of this very long-chain MUFA. Seed oil of *Limnanthes* is therefore desirable for use in cosmetics, surfactants, and lubricants. Metabolic engineering of soybean by co-expression of specialized KCS and acyl-CoA desaturase from *Limnanthes* resulted in the accumulation of C20:1$^{\Delta 5c}$ in the seed oil of transgenic lines (Jadhav et al. 2005).

Polyunsaturated fatty acids

Linoleic acid (C18:2$^{\Delta 9c,12c}$) and α-linolenic acid (C18:3$^{\Delta 9c,12c,15c}$) (Fig. 2) are the most common polyunsaturated FAs (PUFAs) found in seed oils. These important dietary PUFAs are synthesized by membrane-bound FA desaturases that sequentially desaturate oleic acid (Fig. 4). The main pathway leading to the production of linoleic and α-linolenic acid in oilseeds starts in the ER with the incorporation of oleoyl-CoA into membrane phosphatidylcholine (PC) where it is desaturated to linoleic acid by the Δ12 FA desaturase 2 (FAD2) (Kang et al. 2011; Zeng et al. 2017). Linoleoyl-PC can be further desaturated by the Δ15 FA desaturase FAD3, yielding α-linolenic acid (O'Neill et al. 2011). Plastidial membrane-bound desaturases were long known to be major contributors to PUFA biosynthesis in leaves: After incorporation into plastidial glycerolipids, oleic acid can be desaturated to linoleic acid by the Δ12 FA desaturase FAD6, yielding linoleic acid that can be further desaturated by the Δ15 FA desaturases FAD7/8 (Román et al. 2015). Interestingly, a plastid phosphatidylglycerol (PG) lipase was recently shown to hydrolyze polyunsaturated acyl groups from PG in seeds of *A. thaliana* (Wang et al. 2017). The acyl groups thus released are then exported from the chloroplast to be used for the biosynthesis of storage lipids in the ER.

Omega-6 and omega-3 PUFAs found in edible oils are essential for normal human growth and development. Due to increasing world population, there is an increasing interest in searching for plant oils rich in long-chain omega-3 PUFAs such as α-linolenic acid, that is abundant in linseed oil for example (Rao et al. 2008). A further interesting long-chain omega-3 PUFA is stearidonic acid (C18:4$^{\Delta 6c,9c,12c,15c}$) present only in species of few plant families (mainly the Boraginaceae and the Primulaceae) (Guil-Guerrero et al. 2017; Kuhnt et al. 2012; Surette 2013). Stearidonic acid is synthesized from α-linolenic acid by a Δ6 FA desaturase that contains an N-terminal cytochrome b5 domain. Overexpression of the corresponding cDNA in seeds of *A. thaliana* or linseed was sufficient to trigger accumulation of stearidonic acid in transgenic seeds (Ruiz-Lopez et al. 2009).

Finally, dietary very long-chain omega-3 PUFAs that mostly originate from fish oils have a proven role in reducing the risk of cardiovascular disease (Mozaffarian 2008). Decreasing availability of fish oils due to overfishing led

plant lipid biotechnologists to develop seed oils enriched in very long-chain omega-3 PUFAs. For example, efficient metabolic engineering of transgenic *C. sativa* producing the nutritionally important eicosapentaenoic acid (EPA; C20:5$^{\Delta5c,8c,11c,14c,17c}$) and docosahexaenoic acid (DHA; C22:6$^{\Delta4c,7c,10c,13c,16c,19c}$) has been reported (Ruiz-Lopez et al. 2015; Usher et al. 2017). A variety of strategies have been used to introduce the very long-chain omega-3 PUFA metabolic pathways in oil crops, mainly by expressing desaturase and elongase genes involved in different biosynthetic routes for EPA and DHA accumulation (Haslam et al. 2013).

Non-methylene-interrupted fatty acids, conjugated fatty acids

While common PUFAs of seed oils contain *cis*-double bonds that are separated by a single methylene group, some plant species produce unusual PUFAs with non-methylene-interrupted double bonds. A first category of non-methylene-interrupted PUFAs (NMI-PUFAs) consists of Δ^{5c} polymethylene interrupted FAs. These PUFAs bear the first double bond on C-5 (Δ^{5c} unsaturation) that is separated by several methylene units from the next double bond (Hammann et al. 2015). For example, the $\Delta5$ desaturase from *L. douglasii* catalyzing the synthesis of C20:1$^{\Delta5c}$ was shown to also use C22:1$^{\Delta13c}$ as a substrate to form C22:2$^{\Delta5c,13c}$ (Jadhav et al. 2005). Other representatives of Δ^{5c} NMI-PUFAs are taxoleic acid (C18:2$^{\Delta5c,9c}$), pinolenic acid (C18:3$^{\Delta5c,9c,12c}$), sciadonic acid (C20:3$^{\Delta5c,11c,14c}$), and juniperonic acid (C20:4$^{\Delta5c,11c,14c,17c}$) that are present in seed oils of a great variety of gymnosperm species (Wolff et al. 2001; Wolff and Christie 2002; Hammann et al. 2015) and in some species of the Ranunculaceae family (Aitzetmuller 1995). Two 'front end' $\Delta5$ desaturases that use acyl-CoA substrates to form Δ^{5c} NMI-PUFAs were identified in *Anemone leveillei* (Sayanova et al. 2007). Co-expression of one of these enzymes with an elongase in transgenic *A. thaliana* seeds yielded production of sciadonic acid and juniperonic acid, albeit at low levels (Sayanova et al. 2007). Biomedical benefits of Δ^{5c} NMI-PUFA-containing oils including triglyceride-lowering effect and anti-inflammatory properties have been established by several studies (Asset et al. 1999; Chen et al. 2012).

Conjugated FAs are NMI-PUFAs in which at least one pair of double bonds are separated by only one single bond (lack of methylene interruption). Conjugated linolenic acid (CLN) isomers are found in the seed oils of various plant species belonging to the Cucurbitaceae, Punicaceae, Bignoniaceae, Rosaceae, Chrysobalanaceae, Lythraceae, Balasaminaceae, and Euphorbiaceae as either C18 trienes or C18 tetraenes (Rawat et al. 2012). Examples of these conjugated FAs include α-eleostearic acid (C18:3$^{\Delta9c,11t,13t}$) (Fig. 2) found in bitter gourd (*Momordica charantia*), white mahlab (*Prunus mahaleb*) and tung tree

(*Aleurites fordii*), β-eleostearic acid (C18:3$^{\Delta9t,11t,13t}$) in pomegranate (*Punica granatum*) and bitter gourd, punicic acid (C18:3$^{\Delta9c,11t,13c}$) in pomegranate, snakeground (*Trichosanthes kirilowii*), and *Cayaponia africana*, calendic acid (C18:3$^{\Delta8t,10t,12c}$) in pot marigold (*Calendula officinalis*), jacaric acid (C18:3$^{\Delta8c,10t,12c}$) in *Jacaranda mimosifolia*, catalpic acid (C18:3$^{\Delta9t,11t,13c}$) in *Catalpa bignonioidesi* and *Catalpa ovata*, and α-parinaric acid (C18:4$^{\Delta9c,11t,13t,15c}$) in *Parinarum laurinum* and *Impatiens balsamina* (Dulf et al. 2013; Sbihi et al. 2014). These conjugated FAs are synthesized from linoleic acid (C18:2$^{\Delta9c,12c}$) and α-linolenic acid (C18:3$^{\Delta9c,12c,15c}$) by enzymes termed conjugases (FADX) that are divergent forms of $\Delta12$ oleate desaturase FAD2 (Cahoon et al. 2001; Qiu et al. 2001; Rawat et al. 2012). Most of the FADX are bifunctional and possess both desaturase and conjugase activities. Depending on the isoform considered, production of conjugated double bonds results from the modification of preexisting $\Delta9$- (to produce calendic acid from linoleic acid for example) or $\Delta12$-double bonds (to produce punicic acid from linoleic acid for example). Exploration of structure–function relationships in this class of enzymes by domain swapping and site-directed mutagenesis has shown that a small number of residues at key positions in the vicinity of the active site can have a profound impact on catalytic outcome of the enzymes (Rawat et al. 2012).

Oils rich in conjugated FAs are important medicinally as a source of nutraceuticals (Chen et al. 2016b; Dulf et al. 2013; Shabbir et al. 2017). Then, conjugated double bonds increases the rate of oxidation of conjugated FAs relative to PUFAs with methylene-interrupted double bonds, making seed oils rich in conjugated FAs well suited for use as drying agents in paints and inks. If the tung tree is commercially cultivated for production of its seed oil, the other plant species known to produce conjugated FAs do not have agronomic traits allowing large-scale production. Biotechnological efforts have therefore been directed toward the production of conjugated FAs in seeds of transgenic A. *thaliana*, as proof of concept, then in transgenic oilseed crops. The transfer of conjugases thus realized allowed producing α-eleostearic acid, calendic acid, or punicic acid in host seeds (Cahoon et al. 2006; Mietkiewska et al. 2014; Song et al. 2017). The amounts of conjugated FAs thus produced were much lower than what is found in seeds of plants that naturally produce conjugated FAs though. What is more, the physiology of seeds was dramatically altered as a consequence of the accumulation of likely disruptive conjugated FAs in phospholipids (Cahoon et al. 2006).

Hydroxylated fatty acids

Another important class of unusual FAs found in seed oils are hydroxylated FAs (HFAs). Castor (*Ricinus communis*) produces ricinoleic acid

($\Delta12$–OH–C18:1$^{\Delta9c}$) (Fig. 2), and members of the *Physaria* and *Paysonia* genera accumulate oils with high amounts of lesquerolic acid ($\Delta14$–OH–C20:1$^{\Delta11c}$), densipolic acid ($\Delta12$–OH–C18:2$^{\Delta9c,15c}$), ricinoleic acid, and auricolic acid ($\Delta14$–OH–C20:2$^{\Delta11c,17c}$) (Horn et al. 2016; Kim and Chen 2015; Yamamoto et al. 2008). Hydroxylated FAs are synthesized in the ER membrane by the addition of a hydroxyl group to the $\Delta12$ position of oleic acid (C18:1$^{\Delta9c}$) esterified to PC (Smith et al. 2003). The $\Delta12$-hydroxylases catalyzing this reaction are homolog to FAD2. Some $\Delta12$ hydroxylases are bifunctional and possess both desaturase and hydroxylase activities. Site-directed mutagenesis experiments have demonstrated that subtle changes near the active site of the enzyme play a key role in influencing desaturation and hydroxylation product partitioning (Broun et al. 1998; Broadwater et al. 2002). Hydroxylated products of the $\Delta12$ hydroxylase can be further desaturated and elongated in the ER (Lee et al. 2015; Horn et al. 2016; Smith et al. 2003).

Hydroxylated FAs are high-value, widely used feedstocks for industrial products, including greases, polymers, paints, coatings, and high-quality lubricants. Despite the commercial importance of castor oil, castor is considered to be unsuitable for large-scale agricultural production due to the presence of a potent toxin (ricin) and highly allergenic 2S albumins in its seeds. Improvement of castor seed for elimination of the toxic components and breeding efforts and research to improve the agronomic performance of other plant species of the *Physaria* and *Paysonia* genera producing hydroxylated FAs have been proposed. Considering that cultivation of the above-mentioned species will be limited to hot regions, development of transgenic oilseed crops for production of ricinoleic acid in high amounts in the temperate climates is desirable. Numerous attempts for expressing various $\Delta12$ hydroxylases in *A. thaliana* and other Brassicaceae plants have therefore been reported (Lee et al. 2015; Snapp et al. 2014). Collectively, the accumulation of hydroxylated FAs in transgenic plants has been relatively disappointing. Research efforts are now concentrating on the elucidation of the metabolism of hydroxylated FAs in the gene-donor species to overcome metabolic bottlenecks limiting hydroxylated FAs accumulation in host seeds (Bates 2016; Horn et al. 2016; Kim et al. 2011; Kim and Chen 2015; van Erp et al. 2011).

Epoxygenated fatty acids

The most studied epoxygenated FA is vernolic acid ($\Delta12,13$–O–C18:1$^{\Delta9c}$) (Fig. 2). This unusual FA is enriched in the seed oils of several Asteraceae genera, including *Crepis*, *Vernonia*, and *Stokesia*. Important amounts of vernolic acid were also detected in seeds of certain Euphorbiaceae species like *Euphorbia lagascae* and *Bernardia pulchella*. Coronaric acid ($\Delta9,10$–O–C18:1$^{\Delta12c}$) was found

in several *Acacia* species, in *Lactuta sativa* and *Xeranthemum coronarium*. Alchornoic acid ($\Delta14,15$–O–C20:1$^{\Delta11c}$) and $\Delta9,10$–O–C18:0 were observed in *Alchornea cordifolia* and *Tragopogon porrifolius*, respectively. The epoxy group of vernolic acid results from the insertion of an oxygen atom at the $\Delta12$ desaturation of PC-bound linoleic acid (C18:2$^{\Delta9c,12c}$) (Cahoon et al. 2002). Divergent classes of enzymes can catalyze this reaction. In *Crespis palestina* and *Vernonia galamensis*, the $\Delta12$ epoxygenase is a member of the di-iron dioxygenase family of plant membrane-bound FAD2 desaturases (Rezzonico et al. 2004). In *E. lagascae*, a cytochrome P450-type epoxidase is involved in the formation of the epoxy group (Cahoon et al. 2002). Thus, two distinct reaction centers carry out the same reaction but within different protein environments (Billault et al. 2012).

Because of the unique chemical properties associated with the $\Delta12$-epoxy group, vernolic acid-enriched seed oils have a number of potential industrial applications. They can be used as a plasticizer of polyvinyl chloride, as a component of adhesives and paints or as a precursor in the manufacture of nylon. Transgenic production of epoxygenated FAs by heterologous expression of single epoxygenase genes in *A. thaliana*, *Nicotiana tabacum*, and *G. max* can result in accumulation of these FAs of interest in seed oils (Li et al. 2010; Rezzonico et al. 2004). However, in contrast to the native plants, the majority of the transgenic plants accumulate only low amounts of these FAs (Cahoon et al. 2002). Transgenic production of epoxygenated FAs could be improved by increasing the availability of the substrate of epoxygenases (by co-expression of the epoxygenase with a typical FAD2 enzyme in a *fad3 fae1* mutant background) (Rezzonico et al. 2004) and by favoring the channeling of the epoxygenated FAs synthesized into storage lipids (Li et al. 2010, 2012).

Acetylenic fatty acids

Acetylenic bonds are present in various compounds, including FAs found in some seeds. Crepenynic acid (C18:2$^{\Delta9c,12a}$) (Fig. 2) is found in seed oils of plant species from Compositae, Caesalpinioideae, and Rubiaceae. Ximenynic (C18:2$^{\Delta9a,11t}$) and stearolic acids (C18:1$^{\Delta9a}$) were described in members of the Santalaceae family (Okada et al. 2013). A variant FAD2 desaturase termed acetylenase was shown to catalyze the insertion of a triple bond at the $\Delta12$ position of linoleic acid (C18:2$^{\Delta9c,12c}$), forming crepenynic acid in *Crepis alpina* (Carlsson et al. 2004; Nam and Kappock 2007). In addition to producing crepenynic acid, the acetylenase was shown to produce both *cis* and *trans* isomers of C18:2 (Carlsson et al. 2004). Many different plants contain an acetylenase gene (Cahoon et al. 2003), but these acetylenases do not necessarily participate to the production of acetylenic FAs stored in oil. These acetylenic FAs and their

biologically active polyacetylenic derivatives having potent antimicrobial, insecticidal, and antifungal properties, they are indeed synthesized in various plant tissues (Cao et al. 2013). Acetylenic FAs like crepenynic acid also represent interesting raw material for the production of high-quality coatings or cold weather ester-type lubricant for example (Lee et al. 1998).

Cyclopropane and cyclopropene fatty acids

Cyclopropane (CPA) FAs contain 3-membered carbocyclic ring. The carbocyclic group is introduced by the action of a cyclopropane FA synthase (CPS). The enzyme adds a methylene group derived from *S*-adenosylmethionine across the double bond of a monounsaturated FA esterified to PC (Bao et al. 2002; Yu et al. 2011). CPA FAs can be desaturated by a cyclopropane desaturase to form cyclopropene (CPE) FAs. These infrequent carbocyclic FAs are found in Malvaceae, Sterculiaceae, Bonbaceae, Tilaceae, Gnetaceae, and Sapindaceae (Yu et al. 2011). Sterculic acid (Δ9,10-cpe-C19:1) is usually the prevalent CPE FA, but malvalic acid (Δ8,9-cpe-C18:1), present in cotton (*Gossypium hirsutum*) seeds, can be a significant component. In *Litchi chinensis* seed oil, high amounts of the CPA FA dihydrosterculic acid (Δ9,10-cpa-C19:0) (Fig. 2) are accumulated. Carbocyclic FA are desirable for numerous industrial applications. For example, hydrogenation of CPA FAs such as dihydrosterculic acid results in ring opening and produces methyl-branched FAs ideally suited for use in lubricants. Oils with high levels of CPE FAs self-polymerize at elevated temperatures and therefore have potential applications as feedstocks for the production of coatings and polymers (Yu et al. 2011). For these reasons, approaches of metabolic engineering were implemented to produce carbocyclic FAs of interest in *A. thaliana* and *C. sativa* (Yu et al. 2011, 2017).

Cyclopentene fatty acids

Cyclopentene FAs like hydnocarpic (C16:1cy) (Fig. 2), chaulmoogric (C18:1cy), and gorlic acids (C18:2cy$^{\Delta 6c}$) were described in seed oils of two tribes of the Flacourtiaceae family, Oncobeae and Pangieae. Due to their anti-inflammatory properties, some of these oils have been used in medicine for the treatment of leprosy until 1940 (Lima et al. 2005). A biosynthetic pathway involving cyclopentenylglycine transamination and oxidative decarboxylation to form aleprolic acid, which then serves as a starter molecule for cyclopentene FA synthesis by chain lengthening has been proposed (Cramer and Spener 1977). However, the actors participating in this pathway have not been identified so far in plants.

Different types of storage lipids

Wax esters

Whatever the structure of reserve lipids found in seeds, they all derive from fatty acyl-CoA thioesters and are synthesized by ER-localized enzymes. Wax esters are a class of highly hydrophobic neutral lipids that serve numerous functions in plants including carbon storage in seeds (Iven et al. 2013). The desert shrub *Simmondsia chinensis* (jojoba) is known to accumulate wax esters as seed storage lipids. These esters of long-chain FAs and long-chain fatty alcohols are synthesized by two enzymatic reactions (Fig. 5). Alcohol-forming fatty acyl-CoA reductase (FAR) firstly reduces acyl-CoA to corresponding alcohols and a wax synthase (WS) then catalyzes the esterification reaction of a fatty acyl-CoA with fatty alcohol (Miklaszewska and Banas 2016).

Wax esters have favorable properties for industrial applications and serve as high-pressure lubricants in all sorts of machineries (Biermaan et al. 2011; Carlsson et al. 2011). Traditionally obtained from the whaling industry, then synthesized from petrochemicals, wax esters have been produced so far from natural resources that are now diminishing. There is an increasing interest in the renewable production of wax esters in plant seed oils. However, species naturally accumulating wax esters in their seeds like jojoba are not suitable for large-scale cultivation. What is more the very long-chain wax esters produced in jojoba seeds are not suitable for technical applications such as high-pressure lubrication. Metabolic engineering of established oilseed crops has therefore been proposed for the renewable production of wax esters with tailored species composition. Different Brassicaceae species (*A. thaliana*, *C. sativa*, *Crambe abyssinica*, *Brassica carinata*) were successfully engineered for the production of wax esters (Heilmann et al. 2012; Ruiz-Lopez et al. 2017; Zhu et al. 2016). First attempts relied on the expression of FAR and WS from jojoba in seeds of the host and yielded jojoba-like wax esters in which the majority of acyl chains are longer than 20 carbons (Lardizabal et al. 2000; Zhu et al. 2016). More recently, expression of FAR and WS from other species together with approaches of systematic metabolic engineering resulted in the production of wax esters with reduced chain lengths more suitable for high-pressure and liquid lubrication purposes (Heilmann et al. 2012; Ruiz-Lopez et al. 2017).

Triacylglycerols

Triacylglycerol (TAG) is composed of three fatty acyl groups esterified to a glycerol backbone at the *sn*-1, *sn*-2
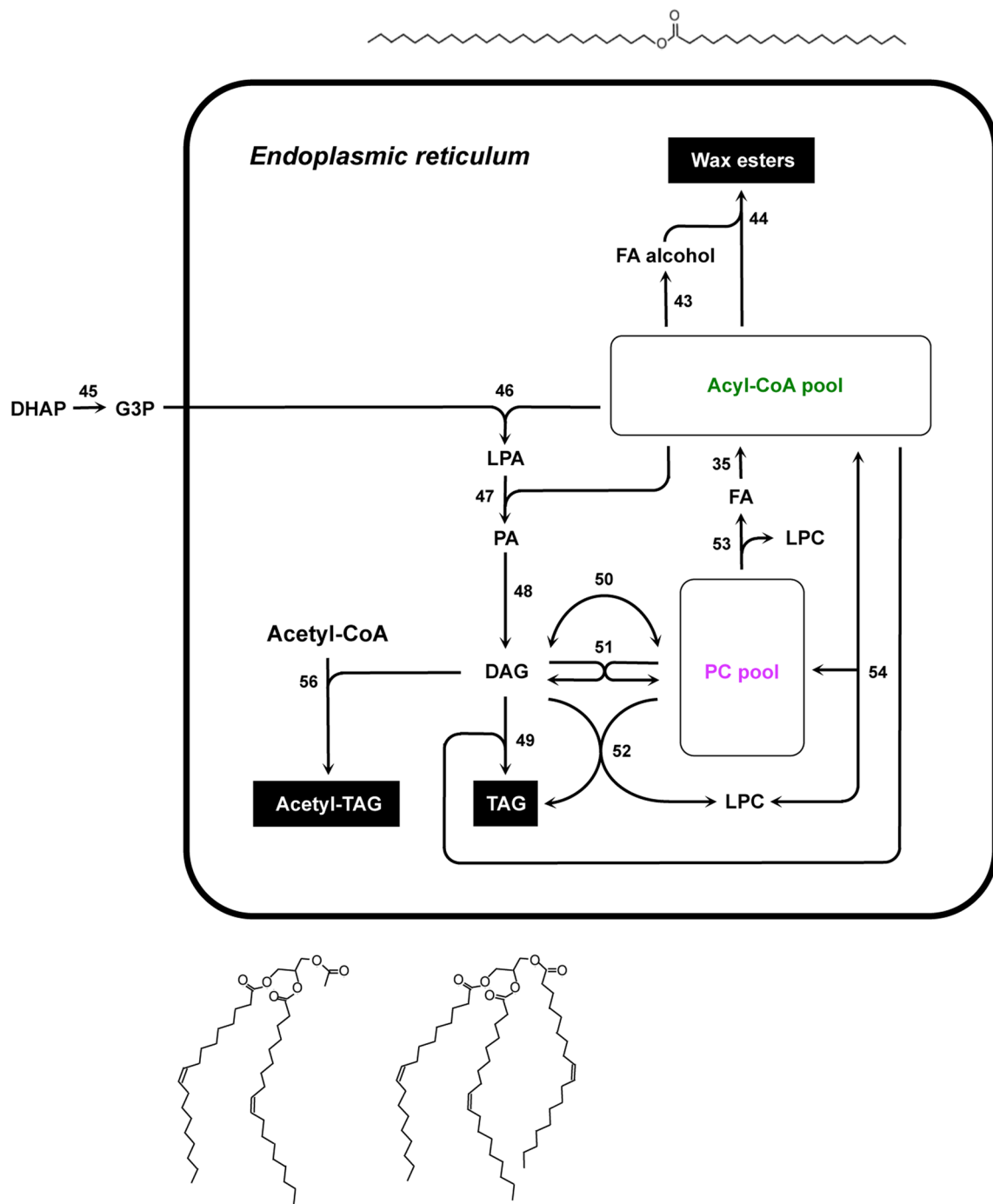
Fig. 5 Storage lipid biosynthesis. The scheme depicts the different pathways involved in the biosynthesis of the different storage lipids found in oilseeds. Examples of wax ester (tetracosanoyl arachidate), acetyl-TAG (1,2-di-oleoyl-3-acetyl-*sn*-glycerol), and TAG structures (1,2,3-tri-oleoyl-*sn*-glycerol) are presented. 35, Long-chain acyl-coenzyme A synthetase; 43, fatty acyl-coenzyme A reductase; 44, wax synthase; 45, glycerol-3-phosphate dehydrogenase; 46, acyl-coenzyme A:*sn*-glycerol-3-phosphate acyltransferase; 47, acyl-coenzyme A:lysophosphatidic acid acyltransferase; 48, phosphatidic acid phosphohydrolase; 49, acyl-coenzyme A:1,2-diacyl-*sn*-glycerol acyltransferase; 50, CDP-choline:1,2-diacyl-*sn*-glycerol choline phosphotransferase; 51, phosphatidylcholine:1,2-diacyl-*sn*-glycerol choline phosphotransferase; 52, phospholipid:1,2-diacyl-*sn*-glycerol acyltransferase; 53, phospholipase A_2; 54, acyl-coenzyme A:lysophosphatidylcholine acyltransferase. Abbreviations: DHAP, dihydroxyacetone phosphate; DAG, diacylglycerol; FA, fatty acid; G3P, glycerol-3-phosphate; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; PA, phosphatidic acid; TAG, triacylglycerol

and *sn*-3 positions. The conversion of dihydroxyacetone phosphate (DHAP) to glycerol-3-phosphate (G3P) by glycerol-3-phosphate dehydrogenase (G3PDH) provides glycerol backbones (Fig. 5). Two acyl-CoA molecules are then used for the sequential *sn*-1 and *sn*-2 acylations of G3P. The first acylation reaction, catalyzed by acyl-CoA:*sn*-glycerol-3-phosphate acyltransferase (GPAT), produces lysophosphatidic acid (LPA) (Shockey et al. 2016). The acylation of LPA by acyl-CoA:lysophosphatidic acid acyltransferase (LPAAT) yields in turn phosphatidic acid (PA) (Maisonneuve et al. 2010). GPAT and LPAAT are stereospecific with regard to acylation of the glycerol backbone and selective in terms of acyl-CoA donors (Kim et al. 2015a). PA is converted to 1,2-diacyl-*sn*-glycerol (DAG) by the action of phosphatidic acid phosphatase (PAP) (Eastmond et al. 2010). In the straightforward Kennedy pathway, the final acyl-CoA-dependent acylation is catalyzed by acyl-CoA:1,2-diacyl-*sn*-glycerol acyltransferase (DGAT), the only enzymatic reaction of the pathway exclusively committed to TAG biosynthesis. Two families of membrane-bound DGAT, DGAT1 and DGAT2, have been described (Aymé et al. 2014, 2015; Aznar-Moreno et al. 2015; Chen et al. 2017; Yu et al. 2008). They both utilize acyl-CoAs but share essentially no homology. DGAT2 were shown to have a role in catalyzing the acylation of unusual FAs such as hydroxylated FAs, epoxygenated FAs, or conjugated FAs into TAGs (Burgal et al. 2008; Li et al. 2010).

Recent studies have shown that incorporation of newly synthesized FAs into TAGs in seeds is much more complicated than simply the linear Kennedy pathway (Wang et al. 2012). DAG is an important branch point between storage and membrane lipid synthesis. The de novo DAG pool originating from G3P through the Kennedy pathway is rapidly converted to PC and then reconverted back to DAG. This second, PC-derived DAG pool is eventually channeled to TAGs (Bates et al. 2009; Bates and Browse 2011, 2013; Bates 2016). Acyl flux through PC allows accumulating PC-modified FAs in TAGs. Molecules of DAG can be converted into PC by CDP-choline:1,2-diacyl-*sn*-glycerol choline phosphotransferase (CPT). Although the CPT reaction is reversible, the importance of CPT in PC-derived DAG production in oilseeds remains unclear. More recently, a second enzyme was shown to participate in the interconversion of DAG and PC. Phosphatidylcholine:1,2-diacyl-*sn*-glycerol choline phosphotransferase (PDCT) efficiently catalyzes the transfer of phosphocholine head group between DAG and PC. PDCT activity provides a major route for desaturated acyl chains to be returned to the DAG pool (Lu et al. 2009). Finally, an acyl-CoA independent mechanism of TAG synthesis provides another way to move FAs from PC to TAG. Phospholipid:1,2-diacyl-*sn*-glycerol acyltransferase (PDAT) catalyzes the production of TAG through transfer of an acyl

moiety from PC to DAG (Marmon et al. 2017; Pan et al. 2013; Zhang et al. 2009). This reaction may transfer unusual FAs from PC into TAG in some oleaginous species (Kim et al. 2011). The relative flux of FAs into TAG through either the DGAT or PDAT activities remains unclear in most oilseed species though.

Aside from the utilization of PC-derived DAG as the substrate for TAG synthesis and the direct transfer of FAs from PC to DAG producing TAG, acyl-editing mechanisms represent another possible route for moving unusual or polyunsaturated FAs from PC into the acyl-CoA pool for eventual TAG synthesis. Acyl editing is a PC-deacylation and lysophosphatidylcholine (LPC)–reacylation cycle allowing exchanges of FAs between PC and the acyl-CoA pool without net PC synthesis or degradation (Bates et al. 2013). This acyl-editing cycle called Lands cycle firstly involves phospholipase A_2 (PLA_2) that hydrolyzes acyl groups at the *sn*-2 position of PC, thus producing LPC and liberated FAs available for subsequent activation by LACS. Acyl-CoA thus obtained can in turn serve as substrate for the acyl-CoA-dependent acyltransferases of the Kennedy pathway. Reacylation of LPC is mediated by acyl-CoA:lysophosphatidylcholine acyltransferase (LPCAT), producing a new PC molecule different in fatty acyl chains (Wang et al. 2012). The reverse LPCAT reaction, though less thermodynamically favorable, is likely to occur *in planta* (Xu et al. 2012; Lager et al. 2013), but the relative amount of acyl editing by Lands cycle versus reversible LPCAT remains unclear (Bates 2016).

Vegetable oils, composed mainly of TAGs, are important nutritional and industrial commodities. To overcome ever-growing demands for these oils, increasing seed oil content and the yield of oil per unit area of land is of paramount importance. Genetic improvement of oil yields has shown fairly steady progress in oilseed crops (Weselake et al. 2009). After decades of genetic improvement driven by classical breeding programs, elucidation of the molecular bases of oil biosynthesis has provided knowledge and molecular tools useful for further increasing oil yields through marker-assisted selection or metabolic engineering. Plant lipid biotechnologists have firstly demonstrated that significant increase in seed oil content could be achieved through the manipulation of single genes (Chen et al. 2015; Hatanaka et al. 2016). Recent progress in molecular and synthetic biology has then led to the emergence of a second generation of constructs allowing multigene engineering (Aznar-Moreno and Durrett 2017a; Chhikara et al. 2017; Liu et al. 2015b; van Erp et al. 2014). Enzymes catalyzing the acyl-CoA-dependent acylation of the glycerol backbone have constituted privileged targets for biotechnological approaches (Weselake et al. 2009). Increasing the production of building blocks for TAG assembly, including G3P and FAs, was also considered. For this purpose, overexpression

of G3PDH (Vigeolas et al. 2007) or FA biosynthetic genes (Roesler et al. 1997; Marchive et al. 2014) was implemented. Finally, protecting TAGs from degradation during the course of seed maturation through the seed-specific suppression of the SUGAR-DEPENDENT1 (SDP1) TAG lipase also contributed to enhance seed oil yields (Kelly et al. 2013).

While most oils accumulated in oilseed crops contain just five basic FA structures, there is a riche diversity of FA structures present in seed oils of non-crop species, many of which have potential usage in industry (Dyer et al. 2008). Many attempts have been made to modify the FA composition of seed oils so as to produce unusual FAs at commercially viable levels in oilseed crops, with varying degrees of success (Napier and Graham 2010). These approaches have underlined the importance of substrate preference of acyltransferases in the utilization of unusual FAs in transgenic oilseeds (Aznar-Moreno and Durrett 2017b; Snyder et al. 2009). For example, accumulation of medium-chain, saturated FAs in seed oils of transgenic Brassicaceae was significantly improved when co-expressing specialized acyltransferases together with medium-chain acyl-ACP thioesterases (Iskandarov et al. 2017; Knutzon et al. 1999; Wiberg et al. 2000). Specialized acyltransferases are not the only limitation to the successful engineering of crops producing desired seed oils though. Given the importance of mechanisms of acyl editing via phospholipids in FA substrate trafficking and utilization, understanding the control of acyl flux through various branches of the lipid metabolic network (Bates 2016), the substrate specificity and regulations of the enzymatic actors involved will be critical to develop more efficient engineering strategies.

Acetyl-triacylglycerols

3-Acetyl-1,2-diacyl-*sn*-glycerols (acetyl-TAGs) are unusual TAGs with an acetate group at the *sn*-3 position instead of a FA (Bansal and Durrett 2016). Acetyl-TAG molecules are abundant in the seeds of Celastraceae, Balsaminaceae, Lardizabalaceae, Ranunculaceae and Rosaceae species. A diacylglycerol acetyltransferase (DAcT) uses acetyl-CoA to acetylate DAG to form acetyl-TAG (Fig. 5) (Durrett et al. 2010). DAcT belongs to a small, plant-specific subfamily of the membrane-bound O-acyltransferases (MBOAT) that acylate different lipid substrates (Tran et al. 2017).

The presence of the *sn*-3 acetyl group confers useful physical, chemical, and nutritional properties to acetyl-TAGs. For example, reduced viscosity and lower melting point compared to regular TAGs make acetyl-TAGs attractive for biofuel and lubricant production (Aznar-Moreno and Durrett 2017b). Transgenic lines overexpressing the *Euonymus alatus* DAcT in their seeds produced important amounts of acetyl-TAGs (Durrett et al. 2010; Liu et al. 2015a). Inhibition of pathways competing for DAG substrates thanks to

the RNAi suppression of *DGAT1*, for example, led to further increases in the amount of acetyl-TAGs accumulated.

Triacylglycerol-estolides

Acylglycerols containing more than three FAs have been found in seeds of a number of plant species like *Mallotus philippensis*, *Trewia nudiflora*, *Cardamine impatiens*, *Sapium sebiferum*, *Lesquerella lyrata* (Smith et al. 2013; Zhang et al. 2012). They are usually associated with the synthesis of FAs containing a hydroxyl group (Lin et al. 2006). The hydroxyl moiety of these FAs enables the attachment by ester linkage of additional FAs. The resulting molecule is usually referred to as FA-estolide. When these ester linked FAs are attached to a glycerol backbone, the molecule is termed a TAG-estolide. The pathways of biosynthesis of natural TAG-estolides are unknown. Considering the attractive potential of these molecules for industrial applications (e.g., as a source of biodegradable lubricants) and the high cost of production of synthetic TAG-estolides, there is a considerable interest in finding an abundant, natural source of these products. Alternatively, the elucidation of the biosynthetic pathways for the production of these TAG-estolides would pave the way for the metabolic engineering of estolide production in existing oilseed crops (Zhang et al. 2014b).

Conclusions and perspectives

Seed oils produced by domesticated oilseed crops, mostly in the form of TAGs, are primarily and increasingly used for nutritional applications as a consequence of population growth and changing diets. The demand for seed oils for production of biofuels and chemical feedstocks is increasing too in the light of rising environmental concerns. The use of unusual FAs of interest may represent an important step in the transition from a crude oil-based society to a more sustainable economy (Dyer and Mullen 2005). Considering that agricultural lands are finite, increasing seed oil productivity represents a first important challenge. Plant breeders and plant lipid biotechnologists already take advantage of the knowledge acquired regarding storage lipid biosynthetic pathways to increase yields of oil per unit area of land in crop species (Barthole et al. 2012; Marchive et al. 2014; Snyder et al. 2009; Weselake et al. 2009). Although it is very unlikely that seed oils will significantly contribute to replace fossil oil for energy, the prospects of doing so for some material and chemicals for the industry are much more favorable (Carlsson et al. 2011). This implies to produce entirely novel oils so that their compositions meet end-use requirements. Tailoring plant oils to have high purity of single desirable FA will rely on approaches of plant biotechnology. Despite the cloning of many genes involved in the biosynthetic

pathways for unusual FAs of interest, attempts at engineering economic levels of specialty industrial FAs by transferring these genes in oilseed crops have so far met with only limited success (Vanhercke et al. 2013). This illustrates our incomplete understanding of the FA biosynthesis and accumulation pathways as well as our inability to anticipate bottlenecks to acyl flux within engineered oilseeds. The emergence of new technologies allowing the ready availability of transcript profiles, the monitoring of carbon and acyl fluxes within maturing seeds, the high-resolution spatial mapping of lipid populations within seed tissues, and the modular cloning of genes of interest will certainly allow us to deepen our knowledge of oil metabolism and to develop new engineering strategies to produce the designer oilseeds able to replace petrochemicals. Ideally, these novel non-food technical oil crops should be able to grow with minimal inputs on marginal lands so as not to compete with food crops.

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