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The potential of genome editing for improving seed oil content and fatty acid composition in oilseed crops

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Abstract

A continuous rise in demand for vegetable oils, which comprise mainly the storage lipid triacylglycerol, is fueling a surge in research efforts to increase seed oil content and improve fatty acid composition in oilseed crops. Progress in this area has been achieved using both conventional breeding and transgenic approaches to date. However, further advancements using traditional breeding methods will be complicated by the polyploid nature of many oilseed crops and associated time constraints, while public perception and the prohibitive cost of regulatory processes hinders the commercialization of transgenic oilseed crops. As such, genome editing using CRISPR/Cas is emerging as a breakthrough breeding tool that could provide a platform to keep pace with escalating demand while potentially minimizing regulatory burden. In this review, we discuss the technology itself and progress that has been made thus far with respect to its use in oilseed crops to improve seed oil content and quality. Furthermore, we examine a number of genes that may provide ideal targets for genome editing in this context, as well as new CRISPR-related tools that have the potential to be applied to oilseed plants and may allow additional gains to be made in the future.

Introduction

Triacylglycerol (TAG) is the main component of vegetable oils and acts as the predominant storage compound in the seeds or fruits of most oleaginous plants. Such plant-derived oils have enormous economic importance and are widely used in food and feed applications, as well as a broad range of industrial products, including pharmaceuticals, surfactants, plasticizers, emulsifiers, detergents, lubricants, adhesives, cosmetics and oleochemicals (Rahman and de Jiménez, 2016). Among oil crops, oil palm (*Elaeis guineensis*) provides approximately 40% of global vegetable oil (5). Soybean (*Glycine max*) and rapeseed/canola (*Brassica napus*) together generate another ~40% of vegetable oil globally, while sunflower (*Helianthus annuus*), peanut (*Arachis hypogaea*), cotton (*Gossypium hirsutum*), coconut (*Coco nucifera*) and olive (*Olea europaea*) make up the majority of the remainder (United States Department of Agriculture Foreign Agricultural Service, 2020).

Due to our rapidly increasing global population and commodity consumption rates, food and energy demands are increasing steadily. Indeed, it has been estimated that our current global agricultural output needs to be increased by at least 60-100% by 2050 to meet these demands (Dhanker and Foyer, 2018), with very little, if any, opportunity to increase arable land area (Alexandratos and Bruinsma, 2012). To further exacerbate matters, there is also a growing need for vegetable oils as an alternative to fossil fuels, which are dwindling due to the limited availability of petrochemical reserves (El-Hamidi and Zaher, 2018; Xu et al., 2018a). As such, this projected increase in required production could be underestimated in the case of oilseed crops (El-Hamidi and Zaher, 2018; Villanueva-Mejia and Alvarez, 2017). While meeting this challenge will likely require the remedy of various sociopolitical issues, including excessive levels of food wastage and inequities in terms of global food distribution, the development of new oilseed cultivars with increased seed oil content (Xu et al., 2018b) or altered fatty acid composition optimized for end use (food, feed, energy or industry; Singer et al., 2013), could provide one piece of the puzzle.

In line with this, much research has been directed towards the enhancement of seed oil content and quality over the years using conventional or molecular-assisted breeding approaches, as well as more targeted genetic manipulation. While some success has been achieved using all of these breeding platforms, each approach possesses both advantages and drawbacks. In the case of traditional and molecular-assisted breeding approaches, such as artificial selection, hybridization and induced mutagenesis/Targeting Induced Local Lesions in Genomes (TILLING), they are lengthy processes that are complicated by linkage drag, the polyploid nature of most oilseed species, and the fact that oil content (and in certain cases fatty acid composition) is a quantitative trait (Weselake et al., 2009; Yang et al., 2017a). Furthermore, as is the case with many agronomically important crops, modern oilseed varieties tend to lack genetic diversity, which can impede additional improvements via conventional breeding. While substantial gains have been made in certain areas using these breeding tools, for example the successful development of canola that possesses seed oil lacking erucic acid (22:1(n-9); hereafter 22:1) and low glucosinolate levels in the seed meal (Stefansson and Hougen, 1964), other seed oil traits have been more recalcitrant. In addition, the introduction of

non-native fatty acids with health or industrial benefits into agronomically amenable oilseed species (such as n-3 very long-chain polyunsaturated fatty acids [n-3 VLC-PUFA] or hydroxy fatty acids [HFA]) cannot be achieved using these tools since the genes necessary for their production are not present in these species.

In an attempt to expedite further improvements in seed oil quantity and quality, genetic engineering approaches including the over-expression of native or foreign genes, or the down-regulation of endogenous gene expression, have been employed (Villanueva-Mejia and Alvarez, 2017; Zafar et al., 2019). Despite the promise of such strategies, with the exception of high-lauric acid (12:0) canola (Laurical^{TM}) and 'super high'-oleic acid (18:1(n-9); hereafter 18:1) safflower (*Carthamus tinctorius*; Wood et al., 2018), their commercial implementation in agronomically important crops has been severely hindered by negative public perception, as well as the lengthy and prohibitively costly regulatory processes required for commercialization of genetically modified ('GM') crops. To circumvent these issues while maintaining the pace of improvement, genome editing techniques (including clustered regularly interspaced short palindromic repeats/CRISPR-associated protein [CRISPR/Cas]) have gained considerable attention in the past decade due to their capacity to elicit mutations at highly specific, pre-defined genomic loci (e.g., Bortesi and Fischer, 2015; Mohanta et al., 2017). In their simplest forms, these platforms lead to mutations that are virtually indistinguishable from those occurring either spontaneously or through induced mutagenesis (Ma et al., 2016). In the case that transgenic methods are utilized to introduce the editing machinery, the transgene can be segregated out or removed through programmed death of pollen/embryos containing the transgene (He et al., 2018), resulting in edited, but non-transgenic plants. For this reason, several countries, including the United States, do not regulate genome edited crops as 'GM', which greatly facilitates their commercialization (Scheben and Edwards, 2018).

While there are a growing number of studies in which a wide range of genes have been assessed using CRISPR/Cas in the model oilseed species, *Arabidopsis thaliana* (e.g., Li et al., 2019a; Pyott et al., 2016), there is a paucity of information regarding the improvement of seed oil content or composition using this genome editing technology (Table 1). The use of CRISPR/Cas in a form where a heritable edit can exist without the presence of a transgene tends to elicit loss-of-function or null mutations through the

interruption of a coding sequence (e.g., Bao et al., 2019), and as such, ideal targets for this type of manipulation in the context of seed oil content and quality would need to exhibit negative regulatory functions in the lipid biosynthetic pathway. However, new editing technology is being developed rapidly, and our capacity to effectively alter specific nucleotides, up-regulate gene expression, and replace alleles is steadily improving. In this review, we will discuss CRISPR/Cas technology, as well as current progress in its use to enhance seed oil quantity and composition in oilseed species. We will also examine potential gene targets that have been found previously to elicit improvements in these traits when down-regulated/mutated using other approaches. In addition, we will consider emerging genome editing technologies that could pave the way for further improvements in lipid-related qualities of oilseed crops in the future.

The genome editing approach

Meganucleases, zinc-finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN) and CRISPR/Cas comprise genome editing tools that can be utilized to induce double-stranded DNA breaks (DSB) at targeted, pre-defined chromosomal regions in many organisms, including plants (e.g., Bortesi and Fischer, 2015; Mohanta et al., 2017). These DSB are subsequently repaired via the plant's own DNA repair mechanisms, which inherently elicit the production of a mutation at the targeted locus. Although the general mechanisms driving all of these genome editing platforms are similar, CRISPR/Cas has become the tool of choice due to its ease of use, relatively low cost, exceptional versatility, and ease with which one can target multiple genes simultaneously (van de Wiel et al., 2017).

The functionality of CRISPR/Cas has been adapted from bacterial or archaeal adaptive immunity systems that protect against invading viruses by integrating small DNA fragments from the virus into their own genome, which are then transcribed into short RNA that act as recognition signals to prevent subsequent attacks through the cleavage of homologous viral DNA by Cas proteins (Bortesi and Fischer, 2015). When applying this system in its simplest form to targeted genome editing, it consists of a Cas nuclease, which is responsible for eliciting the DSB, along with a small, approximately 20-nt non-coding single guide RNA (sgRNA) that guides Cas to the appropriate genomic

locus. This sgRNA typically consists of a chimeric gRNA (complementary to the target region) and *trans*-activating CRISPR-RNA (tracrRNA; required for crRNA maturation). In most Cas systems, the sgRNA must be designed to anneal immediately upstream of a protospacer adjacent motif (PAM), which in the case of Cas9 from *Streptomyces pyogenes* (currently the most widely used Cas protein for genome editing) consists of 5'-NGG-3'. In these instances, the PAM is a requirement for cleavage, which tends to occur approximately 3-nt upstream of this site (Jinek et al., 2012).

As is the case with all genome editing tools, DSB are repaired by the plant itself using either non-homologous end joining (NHEJ) or homology-directed repair (HDR) processes, which are both inherent cell repair mechanisms. The error-prone nature of NHEJ, which is the major DNA repair pathway in higher plants (Yang et al., 2017b) and also the predominant and simplest route in the context of genome editing, typically leads to the production of small insertions or deletions (indels) that disrupt the targeted gene. Since Cas/sgRNAs will precisely target all homologous regions within a genome, CRISPR/Cas-mediated editing is not limited to diploid species, and successful editing of multiple homologous gene copies can also be achieved simultaneously in polyploid species (e.g., Braatz et al., 2017; Wang et al., 2014). Such an attribute makes this technology exceptionally useful for the breeding of the many crop species that are difficult to improve using conventional methods.

In some cases, CRISPR/Cas9 has also led to off-target mutations in plants (e.g., Lawrenson et al., 2015; Zhang et al., 2018a); however, the frequency at which this occurs is still unclear. Indeed, when off-target effects were assessed on a genome-wide scale, non-specific mutations derived from the editing components themselves were found to be very rare, and the vast majority of mutations at loci other than those targeted were the result of background mutations that were incurred during seed amplification or tissue culture (Li et al. 2019b; Tang et al. 2018). These findings are consistent with other recent studies in which CRISPR/Cas editing was shown to be extremely precise in plants (e.g., Feng et al., 2018; Lee et al., 2018; Nekrasov et al., 2017). In any case, off-target mutations can be minimized through the careful selection of target sites, and several freely accessible web-based tools are now available to assist with gRNA design (e.g., Michno et al., 2015; Xie et al., 2014), and to assess specificity and potential off-target

effects (e.g., Bae et al., 2014; Lei et al., 2014; Minkenberg et al., 2019). The use of truncated gRNAs (Fu et al., 2014), paired Cas9 nickases with paired gRNA (Mikami et al., 2016), or the fusion of a catalytically inactive Cas9 to the FokI nuclease (Guilinger et al., 2014) can also provide benefits in this area. In addition, the use of alternative Cas enzymes can also increase specificity and reduce off-target effects (Hahn and Nekrasov, 2019). For example, SaCas9 (derived from *Staphylococcus aureus*) and StCas9 (derived from *Streptococcus thermophilus*) require longer PAM motifs than the commonly used SpCas9 (e.g., Kaya et al., 2016; Wolter et al., 2018), while Cas12a (formerly known as Cpf1) exhibits higher fidelity than SpCas9 due to its particular DNA recognition and cutting properties (Strohkendl et al., 2018).

Since NHEJ-driven site-specific mutagenesis tends to knock-out/knock-down gene function, potential target genes for breeding using this platform are often limited to those that have a negative role with respect to the particular trait chosen for improvement. While the knock-out/knock-down of target genes can elicit desirable traits in certain instances, more precise edits in the form of targeted DNA sequence or nucleotide base replacements are also advantageous in many cases. For example, the use of the HDR mechanism (involving Cas9, a donor DNA template and two sgRNA) has been used for allele replacement in plants (e.g., Li et al., 2018a; de Pater et al., 2018); however, the efficiency of this system is far lower than NHEJ-based editing, which has limited its application. In addition, cytosine and adenine base-editor systems comprising a modified Cas enzyme (either catalytically inactive or bearing nickase activity) fused to a cytidine or adenosine deaminase, respectively (e.g., Li et al., 2018b; Zong et al., 2017), have been developed to attain C-to-T or A-to-G substitutions in plants without the need for HDR. However, base editing can be constrained by the distance between the targeted base and PAM sequences (Shimatani et al., 2017). Prime editing, which is the newest addition to the CRISPR toolbox, may provide an answer to many of these issues. This technology allows specific changes to be introduced at a targeted locus via a single-strand DNA break, which minimizes off-target effects. This is achieved through the action of a catalytically impaired Cas endonuclease fused to an engineered reverse transcriptase, along with a prime editing guide RNA that not only specifies the target site, but also acts as a template for the chosen edit (Anzalone et al., 2019). While this approach has not yet

been successfully utilized in plants, attempts are almost surely underway and its effective use could revolutionize genome editing technology in an agricultural context.

Most commonly, the Cas and sgRNA editing components are introduced into plants via a plant binary vector using *Agrobacterium*-mediated transformation, which results in the stable insertion of a transgenic cassette into the plant's genome. RNA polymerase II promoters driving constitutive (e.g., CaMV 35S promoter; Sun et al., 2015) or tissue-specific (e.g., dividing cell-specific *YAO*, germline-specific *SPOROCYTELESS* and egg cell-specific *EC1.2* promoters; Mao et al., 2016; Wang et al., 2015; Yan et al., 2015) expression are commonly used to control the expression of *Cas*, which is fused to a nuclear localization signal and often codon-optimized for plants. Conversely, RNA polymerase III promoters such as U3 or U6 tend to be used for sgRNA expression (Belhaj et al., 2013). Although this approach initially yields plants bearing foreign DNA, the transgene is unlinked to the edit and can thus be segregated out (or removed via the use of associated 'suicide' genes expressed in pollen or embryos) while maintaining the targeted edit (He et al., 2018; Xu et al., 2018c; Yang et al., 2017b). In oilseed species such as canola, camelina and soybean, CRISPR/Cas-mediated editing frequencies (the proportion of transgenic plants bearing an edit) ranging from 0 to 100% have been reported in the first generation when the editing machinery was introduced as a stable transgenic cassette (e.g., Al Amin et al., 2019; Aznar-Moreno and Durrett, 2017; Braatz et al., 2017; Morineau et al., 2017; Yang et al., 2018). This high level of variation likely results from differences in the target site, nature of the sgRNA, transformation method, promoters used to drive *Cas* and sgRNA expression, and the background vector (Ma et al., 2015; Mikami et al., 2015; Yang et al., 2017b, 2018).

The regulatory landscape surrounding genome edited crops varies widely among countries, and in many cases such crops have not yet been incorporated into existing frameworks. As a result, the regulatory policies of many countries are currently in a transitional state in an attempt to modernize guidelines to encompass crops developed through NHEJ-mediated genome editing, as well as newer editing technologies such as prime editing (for reviews see Metje-Sprink et al., 2020; Parrott et al., 2020; Schulman et al., 2020). However, in countries that presently make use of process-based regulatory platforms where the method utilized to achieve crop improvement is the driving factor

behind regulatory decisions rather than the enhancement itself (as opposed to product-based policies where the new trait determines its regulation), even the initial use of a stably-introduced transgene can trigger 'GM' legislation. As a means of mitigating such challenges, transgene-free edited genotypes have also been achieved through the transient introduction of DNA or RNA encoding Cas/sgRNA into plant cells, followed by plant regeneration (e.g., Andersson et al., 2017; Zhang et al., 2016). However, editing frequencies using this approach are typically lower than with the stable integration of a transgenic cassette, and in the case of DNA introduction, the potential exists for fragments to be incorporated into the genome (Andersson et al., 2017). This can complicate regulatory processes involved in variety release due to the lasting presence of foreign DNA. In an attempt to alleviate these issues, the direct introduction of ribonucleoprotein (RNP) complexes consisting of *in vitro* assembled sgRNA and Cas protein into plant cells, followed by the regeneration of edited genotypes, is gaining momentum as an alternative option (Andersson et al., 2018; Liang et al., 2017; Woo et al., 2015). This strategy avoids the use of exogenous DNA altogether and the RNP themselves are simply degraded within a short period of time, which may also reduce the potential for off-target effects (Kim et al., 2014a). The use of RNP for CRISPR/Cas-based editing has been achieved in oilseed species including soybean, *B. oleracea* and *B. rapa* through their introduction into protoplasts (Kim et al., 2017; Murovec et al., 2018; Park et al., 2019); however, edited plants were only regenerated in the case of *B. oleracea* (Park et al., 2019). In line with this, it seems likely that the feasibility of this method will not be constrained by the technology itself, but will instead be limited by our ability to regenerate whole plants from protoplasts or other tissue types, which remains problematic in many crop species.

Seed oil biosynthesis

Triacylglycerol comprises an esterified derivative of a glycerol molecule and three fatty acids, and is used as a primary storage component in seeds (Singer et al., 2013). Its biosynthesis consists of acyl carrier protein (ACP)-dependent *de novo* fatty acid synthesis, which occurs exclusively in plastids (Ohlrogge et al., 1979), and TAG biosynthesis, which takes place mainly on the endoplasmic reticulum (ER) (Allen et al.,

2015; Weselake et al., 2009; Figure 1). The synthesis of fatty acids (Figure 1) begins with the irreversible carboxylation of acetyl-CoA to malonyl-CoA through the catalytic action of acetyl-CoA carboxylase (ACCase; EC 6.4.1.2) in plastids. The resulting malonyl-CoA is then transferred to ACP to produce malonyl-ACP. An initial condensation reaction between acetyl-CoA and malonyl-ACP to produce 4:0-ACP is catalyzed by 3-ketocyl-ACP synthase III (KASIII; EC 2.3.1.180), while subsequent rounds of condensation are catalyzed by KASI (EC 2.3.1.41) to elongate 4:0-ACP up to 16:0-ACP. Finally, KASII (EC 2.3.1.179), which exhibits a preference for 16:0-ACP, is responsible for its conversion to 18:0-ACP, and stearoyl-ACP Δ9 desaturase (SAD; EC 1.14.19.2) catalyzes the first desaturation reaction to convert 18:0-ACP to 18:1-ACP in plastids. The termination of *de novo* fatty acid synthesis can be catalyzed by plastidial acyl-ACP acyltransferases, which directly use acyl-ACP as an acyl donor to form glycerolipids within the plastid, or acyl-ACP thioesterases (EC 3.1.2.14), which hydrolyze acyl-ACPs to release free fatty acids and ACP. Two major types of thioesterases have been identified in plants thus far (Voelker et al., 1997), including fatty acid thioesterase A (FATA) and FATB. While the FATA class prefers 18:1-ACP as its substrate, the FATB class shows a preference for saturated acyl groups (Voelker et al., 1997; Salas and Ohlrogge, 2002). Free fatty acids released by thioesterases, which mainly comprise palmitic (16:0), stearic (18:0) and oleic (18:1) acids, are then transferred to the outer envelope of the plastid where they are re-esterified to CoA and then enter the cytosolic acyl-CoA pool (Ohlrogge and Browse, 1995; Koo et al., 2004).

In higher plants, 18:1 within the cytoplasmic pool can be further desaturated to linoleic acid (18:2(n-6); hereafter 18:2) and α-linolenic acid (18:3(n-3); hereafter 18:3) on the ER membrane through the activities of fatty acid desaturase 2 (FAD2; EC 1.3.1.35) and FAD3 (EC 1.14.19.25), respectively (Lou et al., 2014). The production of VLC-FA with chain lengths of 20C or longer are synthesized in the ER from C16 and C18 fatty acids by a membrane-bound fatty acid elongation complex comprising four enzymatic reactions. The condensation of long-chain acyl-CoA with malonyl-CoA by β-ketoacyl-CoA synthase (EC 2.3.1.199; encoded by *FATTY ACID ELONGASE 1* [*FAE1*]) is rate-limiting in this process (Huai et al., 2015).

The *de novo* biosynthesis of TAG (also known as the Kennedy pathway) involves three sequential acyl-CoA-dependent acylations of a glycerol backbone derived from glycerol-3-phosphate (Weiss et al., 1960; Figure 1). The first and second acylation reactions are catalyzed by glycerol-3-phosphate acyltransferase (GPAT; EC 2.3.1.15) and lysophosphatidic acid acyltransferase (LPAAT; EC 2.3.1.51), respectively, followed by the phosphatidic acid phosphatase (PAP; EC 3.1.3.4)-mediated dephosphorylation of phosphatidic acid to yield diacylglycerol (DAG). The final acylation reaction is catalyzed by diacylglycerol acyltransferase (DGAT; EC 2.3.1.20) to produce TAG (Hobbs et al., 1999; Lung and Weselake, 2006). In plants, TAG can also be synthesized through an acyl-CoA-independent pathway involving the activity of a phospholipid:DAG acyltransferase (PDAT; EC 2.3.1.158), which uses phosphatidylcholine (PC) as the acyl donor and DAG as the acceptor (Dahlqvist et al., 2000; Pan et al., 2013).

Possible routes to modify fatty acid composition using genome editing

Triacylglycerol derives its character from the types of fatty acids attached to the glycerol backbone since they impart distinct properties such as melting point, oxidative stability, sensory quality and chemical functionalities. Fatty acids are classified by the length of the hydrocarbon chain, as well as the number and positional distribution of double bonds within the chain (Dolowy and Pyka, 2015). For example, saturated fatty acids (SFA) such as 16:0 and 18:0 contain no double bonds, while monounsaturated fatty acids (MUFA) such as 18:1 and 22:1 contain a single double bond and polyunsaturated fatty acids (PUFA) such as 18:2 and 18:3 carry two or more double bonds. The five most abundant fatty acids in plant oils are 16:0, 18:0, 18:1, 18:2 and 18:3, whose proportions vary depending on the oilseed crop and cultivar, and their relative amounts largely determine the oil's end use (McVetty and Scarth, 2002; Villanueva-Mejia and Alvarez, 2017). Therefore, altering the fatty acid composition of seed oils has long been a top priority for oilseed breeders.

Reducing saturated fatty acids

From a health perspective, there tends to be a preference for oils with reduced amounts of SFA, which are linked to elevated total cholesterol and low-density-lipoprotein (LPL)

cholesterol levels, as well as associated coronary heart disease and type-II diabetes risks. Such decreases in SFA tend to occur alongside concomitant increases in MUFA and PUFA such as 18:1, 18:2 and 18:3, which are desirable due to the propensity of these fatty acids to lower the risk of heart disease (Belide et al., 2012). Since FATB hydrolyzes 16:0-ACP to release free 16:0, the modulation of genes encoding this enzyme can influence 16:0 accumulation, and thus the level of SFA in seed oil. In line with this, the disruption or down-regulation of *FATB* through random transfer-DNA (T-DNA) insertion (Bonaventure et al., 2003) and seed-specific artificial microRNA (Belide et al., 2012), respectively, was found to lead to a substantial reduction in 16:0 and total SFA in Arabidopsis seed oil. Unfortunately, the knock-out of this gene in Arabidopsis also led to growth retardation (Bonaventure et al., 2003). Such a consequence could limit the potential use of this strategy for reducing SFA content via a genome editing approach since tissue-specificity of a heritable edit is not possible in the absence of an accompanying transgene.

An alternative approach to lower SFA in seed oils could conceivably involve the alteration of SAD activity in an attempt to increase the desaturation of 18:0-ACP to 18:1-ACP. While such a strategy would not likely be possible using knock-out/knock-down-based editing, the directed evolution of a cyanobacterial Δ9 desaturase with a single amino acid alteration has been found to lead to a 25-fold increase in its desaturation capacity in yeast (Bai et al., 2016). If similar alterations could also elicit the same effect in a plant SAD enzyme, it is feasible that such a change could be achieved using base- or prime-editing to reduce the amount of SFA in plants, although effects on vegetative tissues would need to be assessed.

Increasing monounsaturated fatty acids

While oils with high levels of PUFA, such as those from flax and camelina, have many health benefits, they are more susceptible to oxidation and rancidity than oils with a higher proportion of MUFA (Belide et al., 2012). Therefore, vegetable oils that are high in MUFA and low in PUFA can be desirable for many food applications. To date, 18:1-enriched seed oils from various oilseed species such as rapeseed, sunflower, olive, soybean, camelina, and flax have been obtained using conventional breeding (Singer et

al., 2014; Velasco and Fernández-Martínez, 2002) and transgenic approaches (Chen et al., 2015). Small but significant increases in 18:1 have been achieved in canola through the RNAi-mediated down-regulation of *PHOSPHATIDYLCHOLINE: DIACYLGLYCEROL CHOLINEPHOSPHOTRANSFERASE* (*PDCT*) homologs, which are responsible for a proportion of PUFA flux into TAG via the interconversion of PC and DAG (Bai et al., 2020). However, the vast majority of studies with this aim have typically involved the down-regulation/knock-out of *FAD2*, which encodes an enzyme that is responsible for the desaturation of 18:1 to 18:2 and results in an accumulation of 18:1 at the expense of 18:2 and 18:3. 'Super high'-18:1 safflower, which bears up to 94% 18:1 in its seed oil compared to the approximately 80% found in other high oleic lines, has also been generated through the simultaneous seed-specific RNAi-mediated silencing of *FAD2.2* and *FATB* genes (Wood et al., 2018).

Recently, genome editing has similarly been used to precisely mutate *FAD2* genes as a means of achieving increased levels of 18:1 in seed oils, and 18:1-enriched soybean oil derived from TALEN-based genome editing is already on the market in the United States (Calyno^{TM}, Calyxt Inc.). CRISPR/Cas9- or TALEN-mediated editing of *FAD2* genes has also been achieved in soybean (Al Amin et al., 2019; Demorest et al., 2016; Do et al., 2019; Haun et al., 2014) and camelina (Jiang et al., 2017), resulting in up to 50-83% 18:1 in seed oil compared to the 10-25% present in wild-type seeds, along with concomitant decreases in 18:2 and 18:3. These results suggest that such an approach has the potential to be used for the development of high-18:1 germplasm in species or cultivars for which this trait has not yet been achieved. However, care will need to be taken to achieve edits in desirable dosages since no increases in 18:1 were noted in *B. napus* with CRISPR/Cas-mediated edits in a single *FAD2* gene (Okuzaki et al., 2018) and a pronounced developmental phenotype was observed in homozygous camelina mutants with edits in all three *FAD2* gene copies (Morineau et al. 2017).

Lowering long-chain unsaturated fatty acids

Unlike the relatively short fatty acid chain of 18:1, the monounsaturated 22:1 is a long-chain fatty acid that is typically present in *B. napus* and crambe (*Crambe abyssinica*) seed oils. This fatty acid has been found to lead to myocardial lipidosis or erucic acid

accumulation in the heart (Chien et al., 1983), and is therefore detrimental in terms of human and animal health. Reducing the elongation of 18:1 into longer-chain fatty acids can lead to higher levels of 18:1, while concomitantly decreasing 22:1 content in seed oil. This has been achieved through the down-regulation/mutation of *FAE1* (James et al., 1995; Ozseyhan et al., 2018), which catalyzes this elongation reaction. This approach has also been successful using CRISPR/Cas9 in camelina, leading to a reduction in VLC-FA, including 22:1, as well as an increase in shorter-chain fatty acids such as 18:1, 18:2 and 18:3 without any deleterious effects on seed physiology or plant growth (Ozseyhan et al., 2018). While T_1 plants possessed deletions or substitutions in only one or two of the three *FAE1* gene copies, homozygous mutants were obtained in the T_2 generation. As one might expect, T_2 lines with mutations in all three *FAE1* genes had the greatest alterations in fatty acid composition, with 22:1 reduced to 1.5% in some lines compared to 22% in wild-type lines (Ozseyhan et al., 2018). It is also possible that targeting both *FAE1* and *FAD2* simultaneously through genome editing may provide even greater changes in fatty acid composition since the concurrent RNAi-mediated silencing of *FAE1* and *FAD2* was more effective at lowering 22:1 and elevating 18:1 in seed oil due to the additional reduction in desaturation of 18:1 to 18:2 (Li et al., 2016; Peng et al., 2010). However, the homozygous disruption of the *FAE1* homolog in diploid pennycress (*Thlaspi arvense*) using CRISPR/Cas9 has been found to abolish 22:1 production to undetectable levels, which indicates that the modulation of *FAE1* alone can elicit this effect, at least in certain species. Intriguingly, the resulting genome edited pennycress lines produced seed oil with a comparable composition to that of canola, providing one step towards the possible domestication of this species as an alternative oilseed crop (McGinn et al., 2019).

Elevating saturated fatty acids

Although oils high in MUFA and/or PUFA may be desirable from a health perspective, seed oil containing elevated levels of SFA are useful for many industrial applications, as well as for the manufacture of margarine, shortening and confectionary products without the need for hydrogenation processes and the resulting *trans* fatty acids (Liu et al., 2016). An increased relative abundance of total SFA (at the expense of 18:3) has been achieved in camelina via the CRISPR/Cas-mediated knock-out of three homologs encoding the

seed storage protein, cruciferin C (CRUC) (Lyzenga et al., 2019). While no significant alterations in seed oil or protein content were noted in these lines, alterations in amino acid composition were also present, which may suggest that there is a link between the seed proteome and lipidome through the sharing of metabolites. Alternatively, the heterozygous T-DNA knock-out of *KASII*, which is responsible for the elongation of 16:0-ACP to 18:0-ACP, in Arabidopsis resulted in up to 53% 16:0 in seed oil compared to the 8% present in wild-type lines (Pidkowich et al., 2007). This resulted from an enhanced flux of 16:0-ACP towards the competing FATB enzyme, leading to an increase in the export of 16:0 from the plastid. Similarly, reducing KASII activity using an ethyl methanesulfonate-derived partial loss-of-function mutation in Arabidopsis, or the mutation of one of two *KASII* gene copies in soybean, also increased 16:0 accumulation at the expense of 18-carbon fatty acids in seeds (Aghoram et al., 2006; James and Dooner, 1990; Wu et al., 1994). In addition, the seed-specific down-regulation of *KASII* via RNAi also substantially increases the proportion of 16:0 in the seed oil of cotton and camelina (Hu et al. 2017; Liu et al. 2016). However, homozygous Arabidopsis T-DNA *KASII* knock-out lines were found to be embryo-lethal (Pidkowich et al., 2007), and increases in 16:0 within leaf tissue in even partial loss-of-function mutants lead to increased chilling sensitivity in the form of decreased growth under low temperatures. This is at least partially due to an elevation in the melting-point of phosphatidylglycerol, and possibly also sulfoquinovosyldiacylglycerol, as a result of the increased saturation level of incorporated fatty acids (Wu et al., 1994, 1997).

Due to the negative health effects associated with oils high in 16:0 (Cox et al., 1995), there has also been an interest in alternatively increasing the levels of 18:0 since it is a less deleterious SFA in terms of thrombogenic and atherogenic risk (e.g., Kelly et al., 2001). Increased proportions of 18:0 (mainly at the expense of 18-carbon unsaturated fatty acids) up to approximately 30-40% in seed oil (compared to 1-3% in wild-type seeds) were achieved by down-regulating *SAD* expression using seed-specific antisense (Knutzon et al., 1992) or RNAi technology (Liu et al., 2002). However, seed germination and seedling establishment were compromised in at least some high-18:0 oilseed lines in which *SAD* was down-regulated in a seed-specific manner (Knutzon et al., 1992; Liu et al., 2002), and full knock-out of *SAD* would likely be detrimental due to an inability to

synthesize any C18 unsaturated fatty acids, which would impair membrane fluidity (Lightner et al., 1994) and reduce defense signaling (Kachroo et al., 2001). Since plant genomes typically contain multiple genes that encode SAD (Ohlrogge and Jaworski, 1997), genome editing may be effective in targeting a subset of these homologues to elicit the desired high 18:0 phenotype without associated growth impairments. In line with this, soybean lines bearing mutations in one of three known *SAD* genes, which appears to be preferentially expressed in seed tissues, produced modest increases in 18:0 (up to approximately 13-15% compared to 2% in wild-type lines), and this modification was not associated with substantial growth or yield penalties (Pantalone et al., 2002; Zhang et al., 2008).

Possible routes to boost seed oil content using genome editing

While individual plant seed yield capacity plays a substantial role in seed oil yield, the overall oil yield per unit of land mass can also be enhanced by increasing seed oil content. Achieving such a feat could provide a contribution towards meeting the ever increasing demand for vegetable oils, and would also maximize economic profit for producers without the use of additional land or agricultural inputs (Weselake et al., 2009). For example, a 2% absolute increase in seed oil content by weight would raise the profit margin of canola by 8.7 CAD per metric ton (@ 986.38 CAD/MT of canola oil in 2018; data extracted from the Canola Council of Canada, 2018). In Canada alone, this would increase the value of this crop by approximately 176.6 million CAD. As such, it is not surprising that improving this trait in oilseed crops has been the focus of much research over recent years (Savadi et al., 2017; Zafar et al., 2019).

A common strategy to enhance seed oil yield involves modulating the expression of various genes directly involved in fatty acid and TAG biosynthesis. In addition, the manipulation of many other genes, including those encoding master transcriptional regulators or enzymes with roles in carbon flux or TAG breakdown, has also led to seed oil content increases. For example, relative increases in seed oil content up to approximately 20-40% have been achieved through the heterologous expression of genes encoding the upstream master transcriptional regulator leafy cotyledon 1 (LEC1; Zhu et al., 2018), the fatty acid biosynthetic transcription factor wrinkled 1 (WRI1; Liu et al.,

2010), or the Kennedy pathway DGAT1 (Jako et al., 2001), to name a few. These studies have mainly relied upon the over- or heterologous expression of genes, and as of yet, genome editing-based methods have not been used successfully for this purpose. However, the enhancement of enzymatic activities through directed evolution of amino acid sequences or the use of natural variants is also beginning to emerge as a promising avenue for increasing seed oil content (Chen et al., 2017; Zheng et al., 2008). For example, a natural variant of DGAT1 from maize with a phenylalanine insertion has been found to be a key determinant of oil and 18:1 contents in maize embryos (Zheng et al., 2008). Similarly, the directed evolution of DGAT1 has been utilized to generate variants that are capable of increasing oil content in tobacco leaves to a greater extent than wild-type enzyme (Chen et al., 2017). These findings could provide a fascinating basis for the future improvement of seed oil content using CRISPR-derived tools such as prime editing to precisely modify particular nucleotides in a variety of lipid biosynthetic genes.

In addition to over-expression studies, several efforts have also been made to enhance seed oil content through the down-regulation or mutation of genes that would provide ideal candidates for knock-out using CRISPR/Cas. Plastidial ACCase, which catalyzes the first committed step in fatty acid biosynthesis (Ohlrogge and Jaworski, 1997), comprises a heteromeric complex composed of four subunits in most plants: a biotin carboxyl carrier protein (BCCP), biotin carboxylase (BC), and the α- and β-subunits of carboxyltransferase (CT) (Sasaki and Nagano, 2004). Biotin/lipoyl attachment domain containing (BADC) proteins resemble BCCP but are not biotinylated (Salie et al., 2016). Since ACCase requires the attachment of biotin to its BCCP subunit for successful activity, the incorporation of BADC into ACCase inhibits activity by displacing active (biotin-containing) BCCP subunits and therefore functions as a negative regulator of ACCase. Correspondingly, the seed-specific RNAi-mediated down-regulation of *BADC1* in Arabidopsis led to an approximately 10% relative increase in seed oil content as compared to wild-type plants (Salie et al., 2016). Similarly, mutation of *badc1* in Arabidopsis either alone or together with *badc2* or *badc3* resulted in 15% to 30% relative increases in total seed fatty acid levels and 18 to 30% relative increases in seed TAG compared to wild-type plants with no major physiological abnormalities (Keereetaweep et al., 2018).

An alternative approach to enhance seed oil content is to increase carbon flux towards seed oil biosynthesis. In line with this, 8% relative increases in seed oil content were achieved in Arabidopsis through the mutation of *GLABRA2* (*GL2*), which encodes a transcription factor that is required for the differentiation of several epidermal cell types and is involved in seed coat mucilage biosynthesis (Shen et al., 2006; Shi et al., 2012). The high seed oil phenotype in *gl2* mutants has been proposed to result from the re-allocation of carbon away from seed coat mucilage production and toward seed oil biosynthesis, since seed mucilage and oil act as competing sinks for limited amounts of photosynthate in the seed (Shi et al., 2012). Arabidopsis *gl2* mutants also exhibited abnormal trichomes, an elevation in root hair density and a lack of seed coat mucilage extrusion (Shi et al., 2012), but the agronomic consequences of these effects are not known. Arabidopsis bearing mutations in the mucilage biosynthesis-related genes *TRANSPARENT TESTA GLABRA 1* (*TTG1*), *MUCILAGE MODIFIED 4* (*MUM4*), *MYB5*, and *TRANSPARENT TESTA 8* (*TT8*), also lead to increases in seed oil content (Shi et al., 2012). While *gl2* and *mum4* Arabidopsis mutants did not exhibit obvious deleterious effects in terms of plant growth or development, at least some mutants deficient in mucilage display delayed germination under conditions where water is limiting (Arsovski et al., 2009). The fact that certain Brassicaceae species lack seed mucilage altogether, and at least a proportion of *B. napus* cultivars extrude little mucilage when imbibed, suggests that mucilage is not critical for plant performance. However, this also implies that this strategy may not be applicable to all oilseed species/cultivars.

Another target for enhancing carbon flux into seed oil synthesis is the mitochondrial pyruvate dehydrogenase kinase (PDHK). The oxidative conversion of pyruvate to acetyl-CoA, which is the primary substrate for fatty acid synthesis, is catalyzed through the action of the pyruvate dehydrogenase complex (PDC), which consists of three primary components: pyruvate dehydrogenase (PDH), dihydrolipoamide transacetylase and dihydrolipoamide dehydrogenase. Plants contain two PDC isoforms, known as plastidic and mitochondrial PDC (mtPDC), and the latter is regulated by reversible phosphorylation (Budde et al., 1988). PDHK inactivates PDH through its phosphorylation, functioning as a negative regulator of mtPDC. Correspondingly, the seed-specific down-regulation of *PDHK* in Arabidopsis has been found to result in

elevated mtPDC activity and up to 50% relative increases in seed oil content without any alteration in fatty acid composition or obvious negative impacts on plant morphology or seed yield (Marillia et al., 2003). It has been proposed that this occurs due to the promotion of mtPDC-mediated pyruvate oxidation in the mitochondria, which provides additional acetyl-CoA moieties that can be transported into plastids for fatty acid synthesis (Marillia et al., 2003; Zou et al., 1999). As is the case with the mutation of many other genes, constitutively down-regulated lines exhibited altered growth phenotypes, including a reduction in vegetative biomass and earlier flowering, compared to wild-type lines (Marillia et al., 2003; Zou et al., 1999). However, due to the increase in harvest index (proportion of plant biomass that is partitioned into harvested fractions) in these lines, seed yield was not adversely affected (Marillia et al., 2003).

Similarly, Arabidopsis lines with simultaneous mutations in two cytosolic *GLUCOSE-6-PHOSPHATE DEHYDROGENASE* genes (*G6PD5* and *6*), which are NADPH-generating enzymes that function in the oxidative pentose phosphate pathway (OPPP), also exhibited increased relative seed oil content (approximately 9%) and seed weight (approximately 11%) compared to wild-type. This was achieved without any concomitant reduction in protein levels or alteration in fatty acid composition (Wakao et al., 2008). This has been suggested to result from an impairment in the OPPP, which could increase the amount of carbon substrates available for glycolysis and thus precursors for storage lipid biosynthesis (Andre et al., 2007). While the agronomic performance of lines generated using this strategy remains uncertain at this point, there is evidence that cytosolic G6PDH plays a role in plant defense, and thus the down-regulation of this enzyme may compromise disease resistance (e.g., Scharte et al., 2009).

The flux of carbon into seed oil biosynthesis can also be enhanced by reducing the production of other storage compounds due to the propensity for their levels to be inversely related (e.g., Gunasekera et al., 2006). For example, the T-DNA mutation of *AMINO ACID PERMEASE 2* (*AAP2*), which is involved in the phloem-mediated transfer of amino acids from source leaves into seeds, has been shown to elicit reductions in total seed N and storage proteins. These mutants also displayed concomitant increases in photosynthetic rates, leaf area, seed yield and total seed fatty acid levels (Zhang et al., 2010), which insinuates that the targeting of this gene via CRISPR/Cas9 could provide an

excellent approach for improving seed oil content. However, in certain cases, a reduction in the protein content of the seed meal would be undesirable due to its value as a feed source for non-ruminants (e.g., Gacek et al., 2018), and as such, this strategy may be limited to those crops that would be grown solely for oil purposes.

Seed oil content can also be increased by suppressing the activity of enzymes involved in TAG breakdown. For example, *SUGAR-DEPENDENT 1 (SDP1)* encodes a TAG lipase with a patatin-like acyl hydrolase domain that is involved in the degradation of TAG during seed development and post-germination stages (Kelly et al., 2011, 2013; Kim et al., 2014b). Arabidopsis mutants deficient in *SDP1* have been found to accumulate approximately 10% more total fatty acids in mature seeds than wild-type plants, and seeds were also incrementally larger in terms of both size and weight, with increased proportions of unsaturated fatty acids (Kim et al., 2014b). Along the same line, seed-specific and constitutive RNAi-mediated down-regulation of orthologous genes in Jatropha (*Jatropha curcas*), *B. napus* and soybean displayed relative seed oil increases between 7-30% compared to wild-type controls (Kanai et al., 2019; Kelly et al., 2013; Kim et al., 2014b). Similarly, TAG breakdown can also be repressed, and hence TAG accumulation increased, via disruption of the *PEROXISOMAL ABC-TRANSPORTER 1* (*PXA1*) gene, which is responsible for the transfer of a variety of substrates, including fatty acids, into peroxisomes for their degradation (Slocombe et al., 2009). However, the inhibition of TAG degradation has the potential to reduce seed vigor resulting in low germination rates and retarded seedling growth, and could thus adversely affect crop yield (Kelly et al., 2013; Kim et al., 2014b). These issues have been successfully mitigated by germinating mutant seeds in a sucrose-supplemented medium or by expressing the RNAi in a developmental stage-specific manner to suppress the target gene during seed maturation, but not following germination. However, given our inability to achieve heritable gene edits in a tissue-specific manner, this approach could be complicated by similar challenges.

Conclusions and future directions

Existing data on seed oil improvements via gene knock-out using CRISPR/Cas-mediated genome editing suggests that altering fatty acid composition is highly feasible due to the

relatively simple genetic basis of these traits (Table 1). On the other hand, the augmentation of seed oil content using this technology will likely be complicated due to the complexity of lipid metabolism (Singer et al., 2013), as well as gaps in our understanding of the biosynthetic pathways involved (e.g., Singer et al., 2016). Indeed, only modest increases in seed oil content have been achieved to date even when using multigene and/or highly tissue-specific transgenic approaches. However, several promising gene targets exist that have been shown to boost seed oil content when down-regulated or mutated and have yet to be explored using genome editing, which will almost certainly lead to progress in this area in the future.

Furthermore, studies involving the directed evolution of various genes involved in lipid biosynthesis are beginning to shed light on a number of small and specific mutations that elicit increased enzymatic activity (Chen et al., 2017; Roesler et al., 2016), which could provide the means to allow researchers the ability to increase the function of a particular gene using base- or prime-editing rather than being limited to knock-outs. Similarly, up-regulation of gene expression has been shown to be possible using CRISPR/Cas through the disruption of a repressor element within a target gene promoter in tomato (Rodríguez-Leal et al., 2017), while CRISPR/Cas-mediated disruption of upstream open reading frames (uORF) within the 5' untranslated region (UTR) of an mRNA has been shown to enhance translation of the associated mRNA (Zhang et al., 2018b). Both of these methods could also greatly facilitate the enhancement of seed oil content via the manipulation of lipid biosynthetic genes such as *DGAT*. In any case, care will need to be taken with the knock-out or up-regulation of any gene due to our inability to limit heritable mutations to particular tissue types when using genome editing, since pleiotropic effects may also be apparent in vegetative tissues that could have a negative impact on agronomic performance. Such effects have been minimized in other plant species through the generation of a range of alleles with varying levels of expression as a means of fine-tuning gene dosage through the targeting of *cis*-elements using CRISPR/Cas (reviewed by Wolter et al., 2019); a technique that could also prove exceptionally useful when improving lipid-related traits.

In addition to increasing oil content and modulating the levels of existing fatty acids, there is also increasing interest in engineering agronomically important oilseeds to

produce non-native fatty acids with nutritional or industrial importance, such as n-3 VLC-PUFA, HFA and epoxy fatty acids. While certain plants possess the biosynthetic pathways to naturally produce high amounts of these unusual fatty acids, most are not suitable for large-scale agronomic production, which has limited the availability of their seed oils. Therefore, a large amount of research is currently being directed towards attempts to produce these novel fatty acids in species in which they are not normally present. For example, Arabidopsis and camelina have been successfully engineered to produce fish oil-like quantities (>12%) of the health-promoting VLC-PUFA docosapentaenoic acid (DHA; 22:6(n-3)), which is typically sourced from marine fish and is primarily produced in microalgae, through the heterologous expression of 7-8 genes involved in the microalgal Δ6 desaturase pathway (Petrie et al., 2012, 2014). Unfortunately, such engineering feats typically involve the introduction of entire metabolic pathways from foreign species into the oilseed of choice, which requires a transgenic approach. With a better understanding of the particular amino acid changes required in the enzymes responsible for the generation of these unusual fatty acids compared to their homologues in species in which they are not produced, a prime-editing approach may be a possibility in the future. Although this approach would be technically challenging due to the number of genes that would require modification in order to elicit sufficiently high levels of the desired fatty acid, the pace at which the CRISPR/Cas toolbox is currently expanding could pave the way for such endeavours. An entirely different approach can also be envisioned, whereby the plant species that produce these valuable fatty acids are edited to enable their large-scale agronomic production. Along these lines, interest has been increasing recently with respect to the possibility of using CRISPR/Cas to elicit the *de novo* domestication of wild/ancestral species exhibiting traits of interest. This has been achieved in wild relatives of tomato that exhibit a high degree of stress-tolerance (Li et al., 2018c; Zsögön et al., 2018) by targeting multiple known domestication genes involved in plant architecture, yield and/or nutritional quality using CRISPR/Cas. Such findings point to the feasibility of a similar approach in oilseed species that are amenable to genetic transformation, which could revolutionize oilseed production in the future.

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Conflict of interest

The authors declare that they have no conflict of interest.

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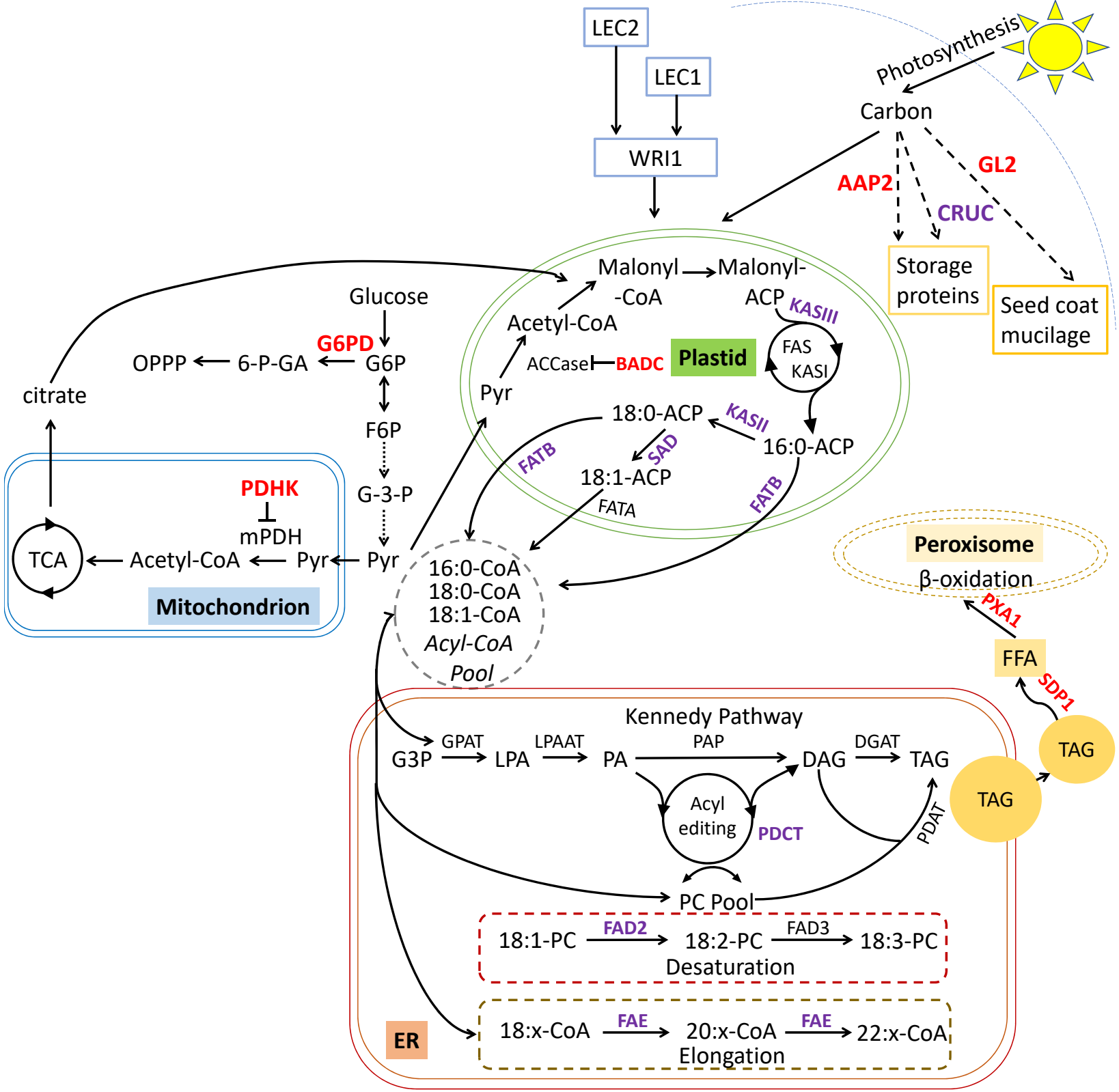
Table 1. Examples of genome editing technology used for seed oil content and fatty acid compositional changes in oilseed species

Oilseed crop	Targeted gene	Editing technique	Delivery method	Trait altered	Reference
Rapeseed	*KASII*	ZFN	*Agrobacterium*	↓ 16:0 and SFA, ↑ total C18 fatty acids	Gupta et al., 2012
	BnaFAD2_Aa	CRISPR/Cas9	*Agrobacterium*	↑ 18:1, ↓ 18:2	Okuzaki et al., 2018
	LPAT2, LPAT5	CRISPR/Cas9	*Agrobacterium*	↓ seed oil content	Zhang et al., 2019
Camelina	*FAD2*	CRISPR/Cas9	*Agrobacterium*	↓ PUFA, ↑ 18:1	Morineau et al., 2017
	FAD2	CRISPR/Cas9	*Agrobacterium*	↓ 18:2 and 18:3, ↑ 18:1	Jiang et al., 2017
	FAE1	CRISPR/Cas9	*Agrobacterium*	↓ 22:1, ↑18:1, 18:2 and 18:3	Ozseyhan et al., 2018
	CRUC	CRISPR/Cas9	*Agrobacterium*	↑ SFA, altered amino acid profile	Lyzenga et al., 2019
	DGAT1 or *PDAT1*	CRISPR/Cas9	*Agrobacterium*	↓ seed oil content	Aznar-Moreno and Durrett, 2017
Soybean	*FAD2*	TALEN	*Agrobacterium*	↑ 18:1, ↓ 18:2	Haun et al., 2014
	FAD2-1A, FAD2-1B, and *FAD3A*	TALEN	*Agrobacterium* and biolistic	↑ 18:1, ↓ 18:2 and 18:3	Demorest et al., 2016
	FAD2	CRISPR/Cpf1	PEG-mediated RNP	ND[a]	Kim et al., 2017
	FAD2-2	CRISPR/Cas9	*Agrobacterium*	↑ 18:1, ↓ 18:2	Al Amin et al., 2019
	FAD2-1A and *FAD2-1B*	CRISPR/Cas9	*Agrobacterium*	↑ 18:1, ↓ 18:2	Do et al. 2019
Peanut	*FAD2*	TALEN	*Agrobacterium*	↑ 18:1	Wen et al., 2018
	FAD2	CRISPR/Cas9	*Agrobacterium*	ND[a]	Yaun et al. 2019
Pennycress	*FAE1*	CRISPR/Cas9	*Agrobacterium*	↓ 22:1 and 20:1(n-9), ↑ 18:1, 18:2 and 18:3	McGinn et al. 2019

[a] Not determined

Figure legends:

Fig. 1 Enzymes and pathways contributing to fatty acid and triacylglycerol biosynthesis in plants. Previously reported enzymatic reactions that have been found to lead to increased seed oil content (red) or altered fatty acid composition (purple) when they are partially or completely inhibited by knock-down or knock-out of the corresponding genes are indicated. Abbreviations: AAP2, amino acid permease 2; ACCase, acetyl-CoA carboxylase; ACP, acyl carrier protein; BADC, biotin/lipoyl attachment domain containing protein; CoA, Coenzyme A; CRUC, cruciferin C; DAG, diacylglycerol; DGAT, diacylglycerol acyltransferase; F6P; fructose-6-phosphate; 6-P-GA, 6-phosphogluconic acid; FAD2, fatty acid desaturase 2; FAD3, fatty acid desaturase 3; FAE, fatty acid elongase complex; FAS, fatty acid synthase; FFA, free fatty acid; FATA, fatty acid thioesterase A; FATB, fatty acid thioesterase B; G3P, glyceraldehyde-3-phosphate; G6P, glucose-6-phosphate; G6PD, glucose-6-phosphate dehydrogenase; GL2, glabra 2; GPAT, glyceraldehyde-3-phosphate acyltransferase; KAS, ketoacyl-ACP synthase; LPA, lysophosphatidic acid; LPAAT, lysophosphatidic acid acyltransferase; mPDC, mitochondrial pyruvate dehydrogenase complex; OPPP, oxidative pentose phosphate pathway; PA, phosphatidic acid; PAP, phosphatidate phosphohydrolase; PC, phosphatidylcholine; PDAT, phospholipid:diacylglycerol acyltransferase; PDCT, phosphatidylcholine diacylglycerol cholinephosphotransferase; PDHK, pyruvate dehydrogenase kinase; PXA1, peroxisomal ABC-transporter 1; Pyr, pyruvate; SAD, stearoyl ACP desaturase; SDP1, sugar-dependent 1; TAG, triacylglycerol; TCA, tricarboxylic acid cycle


LEC2
LEC1
WRI1
Photosynthesis
Carbon
AAP2
GL2
CRUC
Storage proteins
Seed coat mucilage
Malonyl -CoA
Malonyl-ACP
KASIII
FAS KASI
Acetyl-CoA
ACCase
BADC
Plastid
Pyr
18:0-ACP
KASII
16:0-ACP
SAD
18:1-ACP
FATB
FATA
FATB
Glucose
G6PD
OPPP
6-P-GA
G6P
F6P
G-3-P
citrate
PDHK
mPDH
TCA
Acetyl-CoA
Pyr
Pyr
Mitochondrion
16:0-CoA
18:0-CoA
18:1-CoA
Acyl-CoA Pool
Peroxisome
β-oxidation
PXA1
FFA
SDP1
TAG
TAG
Kennedy Pathway
G3P
GPAT
LPA
LPAAT
PA
PAP
DAG
DGAT
TAG
Acyl editing
PDCT
PDAT
PC Pool
18:1-PC
FAD2
18:2-PC
FAD3
18:3-PC
Desaturation
18:x-CoA
FAE
20:x-CoA
FAE
22:x-CoA
Elongation
ER