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CONTENTS

Stacking of Natural Resistance Genes against Late Blight in Potato Shows Great Promise, but also Presents a Regulatory Challenge

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Potato and late blight

The most devastating disease in potato cultivation throughout the world is late blight caused by *Phytophthora infestans*, a fungus-like micro-organism from the class of oomycetes. Late blight problems result in economic costs that sum to 5.7 billion dollars annually[1]. A large part of these costs are associated with the purchase and application of fungicides. Farmers in humid moderate climates spray on average 10 to 15 times to control the disease, but this can go up to 20 times in wet growing seasons[2].

Many wild potato species (belonging to the genus *Solanum*) contain resistance genes that encode proteins which recognize avirulence proteins from the late blight pathogen and evoke a hypersensitive response to stop the infection[1]. Late blight resistance (LBR) genes have been transferred from wild species into commercial potato varieties through conventional cross breeding activities. This process is complicated due to differences in ploidy and is time-consuming because of the many backcrosses needed to develop a variety suitable for commercial use[1]. The big drawback of introducing single LBR genes by breeding is that the 10–40 years needed to obtain a good resistant variety is often annihilated in a few years due to mutations in the pathogen that cause loss of recognition. The combination of multiple, different LBR genes is a better strategy to protect potato against Phytophthora infection, but this is very difficult to achieve using conventional breeding. In the optimal scenario, different varieties with different LBR genes should either be alternated in time and space or used as so called mixed varieties to ensure a durable resistance.

DuRPh

In recent years various LBR genes that recognize different avirulence proteins have been isolated and characterized[3]. At Wageningen University & Research, focus on the DuRPh (Durable Resistance against Phytophthora) programme was initiated in 2006. A number of these genes have been introduced in different combinations by genetic engineering into the potato variety Desiree[4]. It is indeed possible to join several resistance genes from different wild potato species into one cassette, allowing in a single step the transfer of several genes into a commercial potato variety while maintaining the original variety. In addition, a differential set of ten plants harboring single LBR genes in the same genetic background was developed. This Desiree differential set appears to be very accurate for virulence typing of Phytophthora isolates[5].

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Field trials

To assess the quality of the resistance in the genetically engineered potatoes, field trials were performed in The Netherlands and Belgium in 2011 and 2012[6]. The Desiree potatoes in those trials contained individual or multiple LBR genes from *Solanum bulbocastanum*, *S. stoloniferum* and *S. venturii*. The results show that stacking multiple genes is both functional and necessary to obtain good resistance against late blight. It is estimated that using a combination of three or four different LBR genes can reduce fungicide use with at least 80%.

Cisgenesis is similar to breeding, but beats it in precision and speed

The overarching concept of the DuRPh programme is to develop potato varieties with a durable Phytophthora resistance through a cisgenic approach. In this approach plants receive genes by genetic engineering but in contrast to transgenesis, these genes come only from crossable species and are under control of their natural regulatory elements[7]. Furthermore, foreign genetic material, such as selectable marker genes, is absent. Initially, marker genes are used to be able to quickly assess the performance and resistance level of the LBR genes in DuRPh, but the final objective is to produce cisgenic potatoes containing only LBR genes.

Cisgenesis employs genetic engineering and allows for the rapid transfer of useful genes from wild relatives into domesticated crops avoiding co-transfer of genes with unwanted effects and conserving the characteristics of the commercial variety. Cisgenesis is very similar to breeding because in both cases only genes from the sexually compatible gene pool of the recipient plant are introduced. The European Food Safety Authority (EFSA) Panel on Genetically Modified Organisms concluded that similar hazards can be associated with cisgenic and conventionally bred plants, while novel hazards can be associated with transgenic plants[8].

Cisgenesis differs from breeding by a number of advantages: the absence of linkage drag; combination of allelic forms of the LBR; conservation of the characteristics of the variety; and a shorter development time. Genetic engineering furthermore allows the easy combination of several resistance genes from different wild relatives into one variety, enabling a more durable resistance[4].

Some argue that the insertion of cisgenes in a random genomic position leads to "genomic disruption" and consequently unpredictable side effects. However, genomic disruption is a natural process that occurs as a result of transposon activity and natural DNA duplication or rearrangements of DNA-sequences during breeding and evolution[9].

Regulatory costs, administrative burden and GMO stigma

Cisgenic potatoes are the product of genetic engineering. In the DuRPh project, *Agrobacterium tumefaciens* is being used to introduce the LBR genes, thereby triggering the need for deregulation by USDA-APHIS[10]. Such cisgenic potatoes are so far also considered to trigger the European GMO legislation, and as a consequence, a battery of safety testing, a market authorization, and labeling of the potatoes and derived food and feed are required[11].

The regulatory cost for commercialization of a GMO is estimated to be US$ 1 to 15 million, limiting the use of this technology to large multinational corporations

with deep pockets and the most valuable crops[12]. If cisgenic potatoes are subject to the requirements of the GMO regulation, these high costs will impede their commercialization. And although highly desirable from a resistance management viewpoint, it is very unlikely that several different varieties with various combinations of resistance genes will make it to the market, as each individual transformation event needs to repeat the whole costly regulatory process.

The question however is whether such cisgenic potatoes require a lot of safety testing and regulatory oversight, as they do not differ in a meaningful way from LBR potatoes developed using conventional breeding. The only differences are the presence of T-DNA border base pairs and the chromosomal location of the LBR genes. T-border sequences have been proven to naturally occur in tobacco and sweet potato[13,14], and it is known that the chromosomal location of genes can naturally change following gene duplication events or transposon activity. Additionally, in this particular case the LBR genes belong to a category of general resistance genes (the NBS-LRR genes) of which potato possesses already a few hundred variants that are dispersed all over the genome[3].

A key difference between the EU and US regulation of GM crops is that the US employs deregulation, declaring the new crop as safe as existing crops after thorough evaluation, with no need for further actions. In the EU, GM crops can get an authorization after being declared as safe as the conventional counterpart. This authorization (that is limited in time to ten years) implies further administrative burdens because of obligatory monitoring and labeling, the latter triggering in turn coexistence measures and segregation of GM products. Although the label was created to allow consumer choice between GM or non-GM products, both safe to eat, it has become a stigma. Non-GMO is being used as a marketing tool, sometimes even when genetic engineering is not at stake. An absurd example of the latter is the non-GMO salt that is being advertised on the Amazon website[15]. To avoid negative effects on their image and sales, almost all companies in the EU avoid using GM in their food products, resulting in the absence of choice for consumers. However GM products are abundantly present in animal feed in the EU.

The question is again why LBR potatoes need to be monitored, segregated, and labeled if only obtained by genetic engineering (cisgenesis). This inconsistency in different regulation of plants with the same properties has also been disputed by scientific organizations and government expert panels[16,17].

Can cisgenic potatoes not be a GMO?

Despite the fact that the EFSA Panel on Genetically Modified Organisms concluded that similar hazards can be associated with cisgenic and conventionally bred plants, their conclusion is that the same GMO legislations and procedures can be applied towards transgenic and cisgenic organisms (EFSA, 2012). It remains to be seen if current regulatory discussions will consider that cisgenic plants are not subject to the requirements of the GMO legislation because the same gene combination could in principle also be obtained by conventional breeding. Treating cisgenic plants in the same way as plants obtained by conventional breeding would remove the regulatory burden and the GMO stigma. This would enable Small and Medium Enterprises, representing a large part of the EU's plant breeding sector, to more rapidly develop new varieties, that are, for example, disease resistant, not only in potato but in a whole range of smaller crops such as vegetable and fruit crops.

To determine whether or not a cisgenic crop would be subject to GMO legislation, it should first be recognized that this legislation in most jurisdictions in the world contains both process and product related criteria. This is most apparent in the definitions part of the Cartagena protocol on Biosafety, but also holds true for the European GMO legislation[11]. This has as a consequence that, even though a certain technology may trigger the legislation, whether the organisms obtained through them are covered by this legislation is dependent on the question of whether the organism contains a "novel combination of genetic material." As the same combination of resistance genes in the potato genome can in principle be obtained by conventional breeding, the cisgenic potatoes do not have a novel combination compared to conventionally bred potatoes, and therefore would not be subject to the European GMO legislation.

In the US the regulatory trigger used by USDA-APHIS is the use of a plant pest[10]. The use of disarmed *Agrobactrium tumefaciens* has always constituted such a regulatory trigger, even though the disarmed bacterium is no longer able to cause disease. The introduction of

cisgenes using particle bombardment does not trigger this legislation, leading to the conclusion that some cisgenic crops are but others are not covered by it. The regulatory trigger used by the EPA in the US is the incorporation of so-called 'Plant-Incorporated Protectants' (PIPs). Apparently genes that are introduced to produce resistance factors are considered PIPs irrespective of whether such genes already exist in the natural gene pool. This does not seem to be very logical and also leads to the conclusion that some cisgenic organisms are covered and others are not. It is known that the US regulatory authorities are currently reviewing their legislation to propose a revision that would bring more logic to their regulatory approach and set requirements more consistently proportionate to the risk. It is clear that on the basis of what we have presented above, regulatory requirements for cisgenic LBR potatoes, if any, should be very limited.

Conclusion

There is a huge opportunity for breeding companies in Europe and elsewhere to speed up innovation by using so called new breeding technologies that currently trigger the GMO legislation in most cases. A special case is the improvement of potato by the introduction of a durable resistance against late blight through cisgenesis. Our field trials have shown that stacking at least three LBR genes is both functional and necessary to obtain good resistance. Generating different varieties with different wide stacks of resistance genes is essential to fully implement durable resistance to late blight. As potato is clonally propagated, resistance genes introduced by genetic engineering cannot be crossed to develop new varieties or to make new combinations. This means that every variety and every gene combination in that variety would need to go through the whole regulatory process. With the current costs involved in completing this process, generating different varieties and different gene combinations is beyond reach.

The GMO legislation was established at a time in which molecular insights in plant genomes were limited. In the last decade this has changed dramatically. High throughput sequence analysis has revealed that extensive DNA changes occur during breeding and that some plants such as sweet potato and tobacco naturally have T-DNAs from *Agrobacterium tumefaciens*. With this new knowledge, it is vital to reconsider the way in which we regulate biotechnology and to adjust it on the basis of the evolving science. It is sensible to subject novel crop varieties to a case-by-case risk assessment but this should be independent of the method used for their development, as there is no scientific basis for making a distinction between the risks of conventional breeding versus cisgenesis.

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Transforming *Camelina sativa* into an Industrially Useful Oilseed

Jose Aznar-Moreno and Timothy P. Durrett

Introduction

The large quantities of fossil fuel consumed by society have raised concerns about contributions to greenhouse gas emission and the long-term availability of this non-renewable energy resource. In its replacement, plant oils represent a more sustainable source not only of fuel, but also as feedstocks for industrial chemistry. Triacylglycerols (TAG) are the main component of vegetable oil and consist of three fatty acid chains esterified to a molecule of glycerol (**Fig. 1**). The quality and thus uses of plant oils are determined by the fatty acid composition in TAG molecules. In major oilseed crops, just five fatty acids — palmitate (16:0), stearate (18:0), oleate (18:1), linoleate (18:2) and linolenate (18:3) — predominate. However, within the plant kingdom there is a very large repertoire of unusual fatty acids present in seeds. These unusual modifications include medium chain length (8 to 14 carbons), unusual double bond positions, and the addition of functional groups such as hydroxyl or epoxy moieties. Oils containing these unusual fatty acids are therefore valuable industrial chemical feedstocks. For example, ricinoleic acid (12-D-hydroxy-octadeca-cis-9-enoic acid: 18:1-OH) is widely used as industrial feedstock for manufacturing lubricants, nylon, dyes, soaps, inks, and adhesives. However, the plants that produce unusual lipids are typically not well suited for large-scale agricultural production. Continuing with the previous example, ricinoleic acid is a major component of the seed oil of the Castor plant (*Ricinus communis*). However, Castor seeds also contain the toxin ricin and a highly allergenic seed protein, causing health problems for workers involved in the cultivation and harvesting of the plants. A longstanding goal of researchers therefore has been to metabolically engineer oil seed crops to produce unusual lipids. To this end, the isolation of enzymes from different plants and an increased understanding of metabolic fluxes in developing seeds have allowed the production of genetically engineered plants with moderate levels of unusual lipids. Here we summarize recent work to metabolically engineer *Camelina sativa* to produce high levels of another unusual lipid, acetyl-1,2-diacylglycerols (acetyl-TAG).

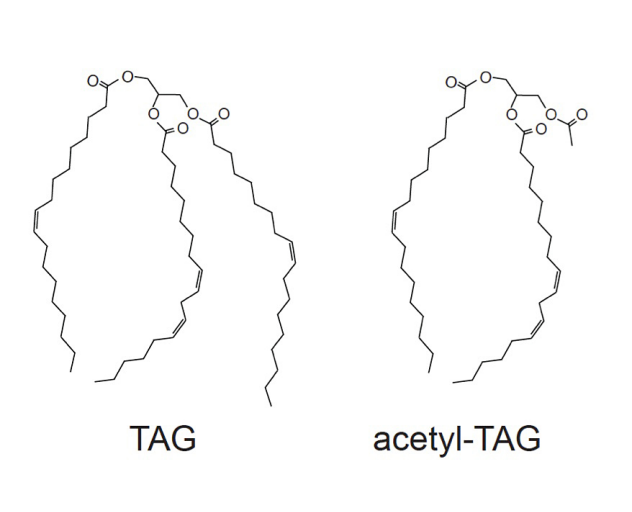

Figure 1. Acetyl-TAGs possess an *sn*-3 acetate group Typical triacylglycerol (TAG) molecules contain three long fatty acids esterified to a glycerol backbone whereas acety. l-TAGs contain an *sn*-3 acetate group. The presence of the much shorter acetyl group means that acetyl-TAGs possess very different physical and chemical properties compared to regular TAGs.

Using Camelina to produce industrially useful seed oil

The oil seed crop *Camelina sativa* (Camelina) has emerged as an ideal platform for the production of such industrially useful seed oils[1]. It possesses many useful agronomic properties such as being drought and cold tolerant and requires lower fertilizer and pesticide inputs than other oil seed crops. The quick growing time of Camelina also means that it can be grown in rotation with other crops. Moreover, it is not currently a source of human feed, reducing the risk of competition for agricultural resources between food and fuel/chemicals. Importantly, besides its useful agronomic characteristics, Camelina can also be rapidly and easily genetically engineered using a floral-dip transformation method. Further, the Camelina genome has been recently sequenced and assembled, providing the framework for targeted genetic manipulation. Because it is related

to the model plant *Arabidopsis thaliana*, it is often possible to leverage the considerable amount of existing knowledge of lipid biosynthesis in Arabidopsis and apply it to Camelina.

Acetyl-TAGs: unusual TAGs with useful properties

The seed oil composition of *Euonymus alatus* (Burning Bush) contains very high levels of 3-acetyl-1,2-diacylglycerols (acetyl-TAG), an unusual TAG in which the *sn*-3 position is esterified with an acetyl group instead of the long chain fatty acid found in typical TAG (**Fig. 1**). The presence of a short two-carbon acetyl group at the *sn*-3 position of acetyl-TAG confers different physical and chemical properties to these molecules. For example, the viscosity of acetyl-TAG (20.3 mm^2s^{-1} at 40 °C) is lower than that of regular TAG[2] and is within the range of the Diesel #4 specification (5-24 mm^2s^{-1} at 40 °C). Diesel #4 fuel (a heavier grade diesel than the more common Diesel #2) is typically used in locomotives, ships, generators, and other engines run at lower rpm. Thus, acetyl-TAG oil can potentially be used as an improved direct use biofuel that does not require further modification, such as conversion via transmethylation to biodiesel. Additionally, acetyl-TAGs possess superior cold temperature properties, remaining liquid at temperatures that cause other oils to solidify[3,4]. These properties also influence the use of this unusual oil as a biodegradable lubricant, hydraulic fluid, or transformer oil or fuel.

In addition to applications as improved biofuels or as chemical feedstocks, acetyl-TAG modified oil can also be used in the food industry. Indeed, synthetic acetylated mono- and di-glycerides (ACETEM) which have related structures to acetyl-TAG have been widely utilized as food coatings, foam stabilizers, emulsifiers, and as plasticizers for food packaging[5]. The ability to obtain such compounds directly from seeds that synthesize these molecules offers an opportunity to obtain these products more cheaply and without the complications that can arise from chemical processing.

Engineering Camelina to produce acetyl-TAGs

Because the Burning Bush is not suitable for development as an oilseed crop, the acyltransferase enzyme responsible for the synthesis of acetyl-TAGs was identified by analysis of RNA-Seq data from developing seeds of *Euonymus alatus*. The enzyme, *Euonymus alatus* diacylglycerol:acetyl-CoA transferase (*Ea*DAcT) is a member of the membrane bound O-acyltransferase (MBOAT) family. In vitro experiments demonstrated that *Ea*DAcT acetylates diacylglycerol (DAG) using acetyl-CoA to form acetyl-TAGs[2]. Transformation of the *Ea*DAcT gene under the control of a strong seed-specific promoter in wild-type Arabidopsis and Camelina resulted in seed oil containing up to 45 mol% and 62 mol% of acetyl-TAG respectively[3]. Similar results were achieved when *Ea*DAcT was expressed in soybean. In these plants, acetyl-TAG accumulation might be limited due to competition between endogenous TAG biosynthetic enzymes and *Ea*DAcT for their common DAG substrate (**Fig. 2**). Two such types of enzymes are the diacylglycerol acyltransferases (DGAT) and the phospholipid:diacylglycerol acyltransferases (PDAT). DGAT1 is responsible for the synthesis of the majority of TAG in Arabidopsis[6]. Supporting this idea of competition for substrate, the expression of *Ea*DAcT in an Arabidopsis *dgat1* mutant increased acetyl-TAG levels from 45 mol% to 65 mol% of total TAG. Similarly, when *Ea*DAcT was co-expressed in Camelina along with RNAi-mediated suppression of DGAT1, the analysis of the oil composition resulted in up to 85 mol% of acetyl-

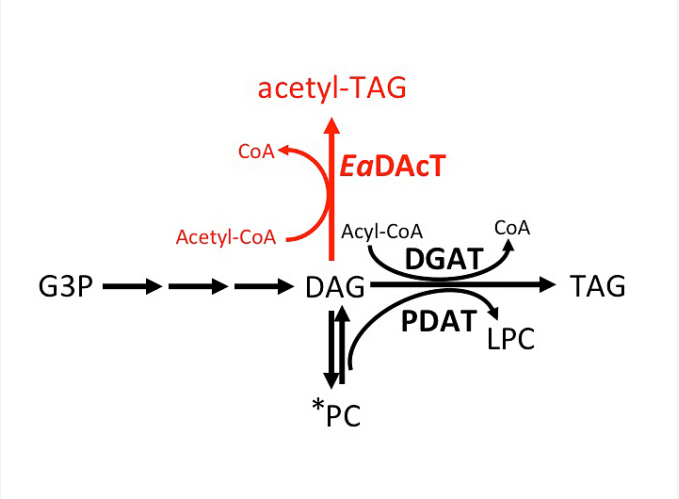

Figure 2. A simplified pathway for the synthesis of triacylglycerol (TAG). For simplicity, many of the reactions involved in the synthesis of TAG are not shown. Diacylglycerol (DAG) is substrate for diacylglycerol acyltransferase (DGAT) and phospholipid:diacylglycerol acyltransferase (PDAT), both of which synthesize TAG using different acyl donors. Both enzymes compete with Euonymus alatus diacylglycerol:acetyl-CoA transferase (EaDAcT) which catalyzes the last step (red color) in the synthesis of acetyl-TAG. Phosphatidylcholine (PC) is the site of synthesis of unusual fatty acids (indicated with an asterisk). G3P, glycerol-3-phosphate (G-3P); LPC, lysophosphatidylcholine.

TAG[3]. Therefore, the ability to downregulate the activity of an endogenous acyltransferase (DGAT1) and replace it with a novel activity (*Ea*DAcT) successfully allowed the synthesis of unusual lipids at a level higher than those obtained in previous efforts to engineer oilseeds.

Acetyl-TAG synthesis does not affect field growth or production

Typically, synthesis of unusual lipids in transgenic seeds is associated with negative effects, including smaller seeds, reduced oil content, and an inability to germinate. In contrast, high acetyl-TAG containing seeds germinate at a rate similar to that of wild-type plants. Analysis of the lipid content reveals that germinating seeds metabolize acetyl-TAGs, suggesting that these unusually structured storage lipids can still be utilized as a source of carbon precursors or energy by the seedling[3]. Further, the accumulation of high levels of acetyl-TAG had no major impact on key traits when these transgenic Camelina lines were grown in the field. For example, there were no visible differences in morphology or reductions in seed weight or between the genetically engineered plants and the wild type controls. However, the oil content of seeds containing over 80 mol% acetyl-TAG was reduced by 7.5-11%[3]. This trait was also observed in high-oleic acetyl-TAG plants[4]. This reduced oil phenotype is probably caused by the RNAi-mediated suppression of DGAT1 in these Camelina lines; similar results have been observed in Arabidopsis where expression of *Ea*DAcT in a *dgat1* mutant also leads to a high accumulation of acetyl-TAG but fails to fully complement the reduced oil phenotype[3]. The inability of *Ea*DAcT to fully compensate for the loss of DGAT1 could be caused by an insufficient supply of acetyl-TAG precursors, such as acetyl-CoA or glycerol-3-phosphate (**Fig. 2**). Metabolically engineering higher levels of these compounds represents one potential strategy to address the reduced oil content.

Whereas many attempts to engineer unusual plants oils have only achieved low levels of accumulation of the desired product, the genetic engineering of acetyl-TAG production in Camelina represents the highest accumulation of unusual oil achieved so far in plants. This high accumulation is the result of a number of complementary effects. First, while the *sn*-3 acetate can be considered an unusually short acyl group, the acetyl-CoA substrate required for its addition is ubiquitously available in plants. Second, this addition of the acetate group occurs at the end of the biosynthetic pathway (**Fig. 2**). This distinguishes the synthesis of acetyl-TAGs from attempts to synthesize other unusual lipids, where bottlenecks in flux of unusual fatty acids from the site of their synthesis to their incorporation into TAG often limit their accumulation[7].

The acetyl-TAG levels achieved Camelina resulted in up to 85 mol% in the best lines. Can these levels be improved to the levels seen in *E. alatus* oil, which contains over 95 mol% acetyl-TAGs? Reducing the activity of other enzymes that compete with *Ea*DAcT for their common DAG substrate has been an effective way to increase acetyl-TAG levels. In this regard, it is useful to note that in developing *E. alatus* seeds, *Ea*DAcT is the highest expressed acyltransferase, whereas DGAT1 and PDAT transcripts are only present at very low levels[2]. However, the RNAi-mediated suppression of Camelina PDAT failed to achieve statistically higher levels of acetyl-TAGs when combined with the expression of *Ea*DAcT and the silencing of Camelina DGAT1[3,4]. One possibility is that sufficient suppression of PDAT1 transcript levels may not have been achieved. Additionally, because both DGAT1 and PDAT are required for pollen and seed development in Arabidopsis[6], it is possible that suppression of both enzyme activities is lethal. The emergence of a number of different genome editing technologies such as CRISPR-Cas9 will provide additional strategies with which to generate mutants in a target gene and offer opportunities to carefully titrate the activity levels of these different enzymes to maximize acetyl-TAG production.

Increasing the functionality of acetyl-TAGs

Because the PUFAs typically found in Camelina seed oil are at least 10-fold more susceptible to oxidation than monounsaturates, expressing *Ea*DAcT in a high-oleic Camelina background provided an opportunity to improve oxidative stability of the resulting acetyl-TAGs[4]. Thus high-oleic acetyl-TAG is expected to be an important characteristic for potential uses at high temperatures and in other oxidative environments. Interestingly, the viscosity of high-oleic acetyl-TAG was slightly increased (26.3 mm^2s^{-1} at 40 °C). High-oleic

acetyl-TAG oil also demonstrated a major crystallization exotherm at minus 64 °C compared to minus 34 °C for high-oleic TAG[4]. Thus, this structure may provide improved low temperature performance for acetyl-TAG oils as lubricants and fuel.

This approach can be extended to increase functionality of acetyl-TAGs by combining their synthesis with that of other unusual fatty acids. The ability to synthesize medium chain fatty acids in Camelina[8] suggests it should be possible to introduce these shorter fatty acids to the *sn*-1 and -2 positions of acetyl-TAGs. As viscosity is related to molecular mass[9], this approach has the potential to further reduce the viscosity of acetyl-TAGs, possibly to that of the more widely used Diesel #2. The potential to incorporate unusual fatty acids will therefore allow a combinatorial approach to generate a wider range of structurally different TAG molecules with higher value properties.

Conclusion

Developing plant oils as replacements for fuels and oleochemicals represents a renewable option for reducing fossil fuel use. The ability to produce very high levels of acetyl-TAGs in transgenic Camelina plants is but one example of how a better understanding of seed lipid metabolism has allowed the development of modified oilseeds with higher levels of industrially useful lipids. Increasing the levels of other unusual fatty acids, particularly those that require multiple specialized enzymes to be incorporated into TAG, will be one of the most important challenges for the future. In addition to altering the oil composition of Camelina, improvements in seed and/or oil yield per hectare and increasing tolerance to abiotic stresses, particularly heat, will also need to be considered. Such developments will be important in order to providing sufficient oil for feedstock needs without diverting land from other uses.

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Opportunity and Challenge of Genome Editing for Crop Improvement

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Biotechnology crops expressing transgenic traits were planted on over 450 million acres in 2014. This rapid worldwide adoption should stand as a testament to the promise of modern biotechnology, but after nearly two decades of worldwide use these crops are an unending source of public controversy. Public questioning of transgenic crops has meant increased regulatory burdens and delays. Development costs and timelines restrict commercial transgenic products to relatively few crops and limited traits developed by large multinational concerns. The initial promise of modern biotechnology to provide public sector scientists opportunities to develop small market crops as a public good is largely unrealized.

The rapid pace of innovation in bioengineering is leading to a host of new breeding technologies that may offer renewed opportunities for biotechnology crop development. The emergence of tools for genome editing and their successful use in plant science is especially remarkable (see Box 1). Genome editing utilizes knowledge of biological mechanisms for DNA targeting and repair to allow highly specific changes at any location within the plant genome. Specifically targeted double stand breaks in DNA are repaired by nucleases to cause changes ranging from single point mutations to whole gene additions or deletions. In conjunction with rational design strategies the specificity of genome editing procedures can increase the efficiency of transformations and limit off-target effects elsewhere in the genome. Initial scientific interest has focused on using these tools to discover gene function by mutation or knockouts of specific genes. But scientists have quickly realized gene editing can generate useful plant traits, and proof-of-concept of traits in crops has been rapidly achieved (see Box 2). The tools for genome editing are readily accessible to the individual researcher and are proving quite simple to use, so there are clear opportunities to use genome editing to improve crops that are too expensive to develop using current transgenic techniques.

When the potential for gene-editing technologies to facilitate development of novel traits was first recognized, regulators and scientists saw opportunities for crop improvement which avoided the controversies of genetically modified (GM) crops. This was especially true for those applications which caused simple point mutations at specifically targeted sites on genes, since these 'site-directed mutations' are not considered as regulated throughout most of the world. Early interest in Europe however has started to cool because of emerging questioning by civil society groups. The current state of public and regulatory opinion regarding gene-edited crops therefore is adhering to the process versus product arguments that have dogged the development of GM crops. Thus far both US and Canadian regulators have found ways to accommodate the technology, as are regulators in Argentina. In the US this has been largely accomplished by moving gene-edited crops from the greenhouse to the field as null segregant (NS) selections, since regulators with USDA-APHIS have determined they have no statutory remit for regulation of plants devoid of transgenic elements. Conversely, authorities in the EU are increasingly coming toward a position that if recombinant DNA has been used to affect editing, the derived plants will be subject to regulation. The applications of genome-editing are rapidly evolving as is worldwide regulatory opinion; therefore, the regulatory status of crops being developed by genome editing remains uncertain. In the US the recent move toward reevaluation of the coordinated framework for biotechnology has come about in part because of concerns that NS selections for edited crops is an unsound basis for guiding the regulatory process.

The ease by which civil society groups and anti-GMO activists will miscommunicate genome-editing technology as simply another GMO may derail efforts to employ genome-editing without the controversy that accompanies GM crops. Genome-editing techniques are highly nuanced and therefore are difficult to clearly communicate to the broader public. Those applications that involve simple point mutations are indistinguishable from native biological processes and would seem to have no basis for regulatory scrutiny. Other applications, however, lead to purposeful introduction of transgenes

into plant genomes and as such are subject to those same regulatory statutes as exist for GMOs. The ability for genome-editing to provide useful traits in crops may be less encumbered by advances in the laboratory than it will be by public understanding and acceptance.

This summary is abstracted from the recently published review "The Regulatory Status of Genome-Edited Crops," prepared by scientists with the Crop Bioengineering Consortium at Iowa State University, which provides a more comprehensive discussion of this topic. The complete article can be downloaded from http://onlinelibrary.wiley.com/doi/10.1111/pbi.12444/epdf

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Box 1. Current genome editing techniques used for trait discovery and development (after Wolt et al., 2015)

CRSPR	Clustered Regularly-Interspaced Short Paloindromic Repeats	Programmable nucleases comprised of bacterially derived endonuclease (Cas9) and a single-guide RNA (sgRNA).
EMN	Engineered Mega Nuclease	Microbially- derived meganucleases that are modified, fused, or rationally designed to cause site-directed DSB. Also referred to as LAGLIDADG endonucleases or homing nucleases.
OMM	Oligonucleotide Mediated Mutagenesis	Site-specific mutation with chemically-synthesized oligonucleotide with homology to the target site (other than for the intended nucleotide modification).
TALEN	Transcriptional Activator-Like Effector Nuclease	Programmable nucleases comprised of the DNA binding domain of Xanthomonas-derived TAL effectors fused with FokI restriction endonuclease.
ZFN	Zinc Finger Nuclease	Programmable nucleases comprised of the DNA binding domain of a zinc-finger protein and the DNA-cleaving nuclease domain of the FokI restriction endonuclease.

Box 2. Early examples of genome editing for development of useful traits in important crops (Wolt et al., 2015)

Crop	Trait	Technique	Citation
Barley	Phytase reduction	TALEN	Wendt et al., 2013, Plant Mol. Biol. 83, 279–285
Cotton	Herbicide tolerance and insect resistance stacking	EMN	D'Halluin et al., 2013, Plant Biotechnol. J. 11, 933–941.
Maize	Herbicide tolerance	OMM	Zhu et al., 1999, Proc. Nat. Acad. Sci. USA, 96, 8768–8773.
Maize	Herbicide tolerance and phytase reduction	ZFN	Shukla et al., 2009, Nature, 459, 437–441.
Maize	Male sterility	EMN	Djukanovic et al., 2013, Plant J. 76, 888–899.
Rice	Herbicide tolerance	OMM	Okuzaki and Toriyama, 2003
Rice	Bacterial blight resistance	TALEN	Li et al., 2012, Nat. Biotechnol. 30, 390–392.
Rice	Bacterial blight resistance	sgRNA/Cas9	Jiang et al., 2013, Nucleic Acids Res. 41, e188. doi:10.1093/nar/gkt780.