Fashioning designer genes

New technologies, collectively called genome engineering, are unlocking new ways to improve oil crops while avoiding the stigma associated with traditional GMOs. Ile Kauppila explores their potential, limitations and regulatory issues

Genetically modified organisms (GMOs) have been around since the 1970s. During the decades they have existed, they have provided tremendous advantages to agriculture but also sparked debate around their safety and ethics. However, in the last decade, a new player has entered the field of genetic modification and it could change the game for everybody.

Genome engineering (GE) or editing – sometimes also referred to as gene editing – is a relatively recent group of various different technologies that allow genetic material to be added, removed or altered in a particular location in an organism's genome. These genome editing methods enable an organism's DNA to be split at a targeted location and then repaired through either non-homologous end-joining (NHEJ) or homologous recombination (HR), resulting in the desired change.

To put this opaque explanation in plainer language, GE technologies work with what has been called "molecular scissors". While the exact method and enzymes used differ from one technology to another, all of them function in more or less similar ways. By using them, scientists can target single genes in the target genome and cut the DNA at that particular point. Then, they make use of the DNA's own cell repair mechanisms to either delete or add a piece of related genetic material or turn a gene on or off.

Not GMOs

But for something that is supposed to change the field for genetically modified crops, this sounds an awful lot like traditional GMO development. On a superficial glance it might seem so, but looking at the details reveals there is actually a great difference between GE and "old school" GMO methods.

"With GMOs, we introduce a foreign material into the plant," Adrian Percy, global head of R&D at Bayer Crop Science says. "With gene editing, we make changes to the existing genome, rather than introducing foreign genetic material."

As mentioned, there are multiple technologies available for genome engineering. The most popular – and possibly the most easily accessible one – is known as CRISPR/cas9. Its name comes from clustered regularly interspaced short palindromic repeats, which are genetic elements that bacteria store in order to be able to identify viral DNA.

When the bacterium is attacked by a virus, Cas9 enzymes process the stored DNA and cut the viral DNA at the corresponding spot, destroying the virus. Scientists at companies such as DuPont Pioneer and Cellectis have been able to use this mechanism to cut DNA in plant cells, thus either introducing DNA stored in a carrier bacterium into the sequence or deleting/inserting an existing gene (*see Figure 1, p28*).

Another technology that has already been used to produced engineered plants is TALEN (transcription activator-like effector nucleases), which is proprietary to Minnesota-based biotech firm Calyxt. It achieves ultimately the same results as CRISPR, but using restriction enzymes to cut the DNA sequence.

Other common technologies include the use of zinc finger nucleases, meganucleases and oligonucleotide-directed mutagenesis (ODN). All vary in method and efficiency.

The kinds of changes that are being made with GE also happen naturally through a process called mutagenesis. Mutagenesis takes place randomly and over time for a variety of reasons. One very common cause of DNA change is organisms being hit by rays emitted by the sun, the same reason why we should wear sunscreen on the beach.

Mutagenesis has been used by humans to alter crop varieties for ages but, so far, this process has been slow and random, sometimes taking years only to result in unwanted or no changes at all.

GE promises to take control of this process and to speed it up. The time it takes to develop a new crop trait can be cut from years to months, while also significantly reducing the time it takes to get the product to the marketplace. Additionally, GE trumps GMO technology in precision, eliminating the factor of randomness almost entirely.

"One of the differentiators of genetic [engineering] technology is the amount of specificity we can achieve," says Federico Tripodi, CEO of Calyxt. "We identify a genetic sequence only involved with that gene and not the rest of the genome. To get sulfonylurea herbicide resistance in canola, we have made two- to three-letter changes in the codes for that gene."

Tripodi adds that GMO products might take 13 years and US$130M to commercialise but, with GE, that time can be cut by half.

Oilseed applications

Already, agritech companies have jumped on the chance to make use of these new technologies. Calyxt has developed through its TALEN platform a soyabean variety in response to legislation banning *trans* fats in partially hydrogenated soyabean oil.

As a high linoleic oil, soyabean oil goes rancid fast and manufacturers have long been partially hydrogenating the oil to extend shelf life.

According to Calyxt, its GE soyabean variety has a higher oleic acid content and less saturated fatty acids than other soyabean varieties. These properties increase the oil's shelf life by up to five times and fry-life by three times.

Through the technology, the company has been able to decrease *trans* fats, allergens and toxic compounds, while increasing dietary fibres, nutrients, vitamin content and plant proteins, says Calyxt. By April 2018, it had contracted over 6,400ha with 75 farmers in the US Midwest to farm the engineered soyabeans, with more than 90% of them signing to replant and double their hectarage.

"Strong interest from farmers and food companies for our first product to market is a reflection that Calyxt's gene editing platform with its unique business model is a disrupter in the ag and food space," says Manoj Sahoo, Calyxt chief commercial officer.

Another company that has successfully developed GE oilseeds is US firm Cibus, which has developed the SU Canola variety. The canola – developed using the Rapid Trait Development System (RTDS) – is resistant against sulfonylurea (SU) herbicide, which, according to Cibus, makes it a good fit for crop rotation with glyphosate tolerant soyabeans, thus reducing weed pressure caused by glyphosate tolerant canola in soyabean fields.

"SU Canola offers many opportunities to farmers, including high yields and an economical and easy-to-use weed control system," says Dave Voss, Cibus vice president of commercial development.

"Canola growers are really responding to Cibus canola because of the new options and flexibility this system brings them."

Due to its development through GE, the canola is non-transgenic, meaning it does not contain genetic material from unrelated organisms. There is a growing market for non-transgenic canola oil, according to Cibus, and the firm is expecting to launch SU Canola outside North America in the coming years.

The firm has also developed a glyphosate resistant flax, with support from the Canadian government. It is projected to launch in the USA in 2019, when it will be the world's first non-transgenic crop with glyphosate tolerance.

Not a silver bullet

But, for all its potential, GE is not the be-all and end-all of agricultural technology. Among its limitations are the need to map the genome of the target organism and the sometimes questionable accuracy of the edits, despite their touted high precision.

Needing to know the genome – the complete genetic sequence – of the crop to be edited is a primary requirement for GE, alongside having the know how and physical equipment to perform the edits. Without the genome, scientist will not know what the genes they're trying to turn off or on are, nor where they are located.

Many oil plant genomes have already been mapped out, mostly major ones such as soyabean, and the list is constantly expanding. For example, the sunflower's genome was unlocked in May 2017 (*see p29*).

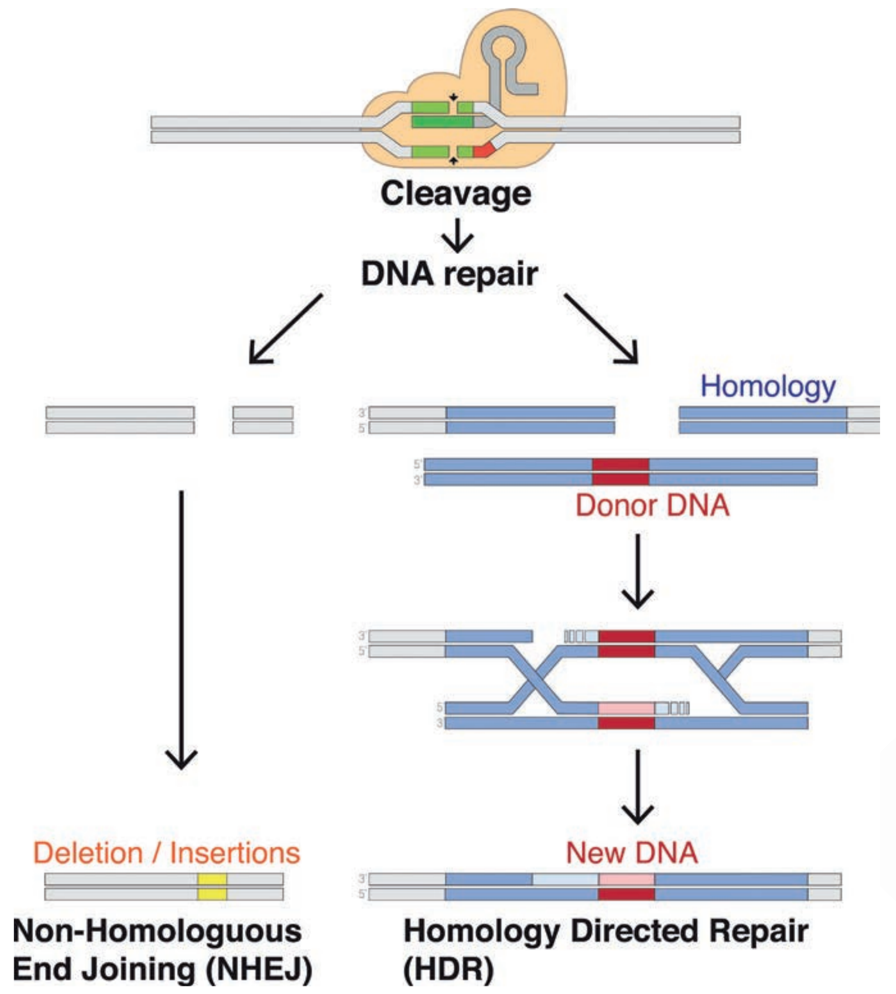
Having a mapped out genome is not enough, though, says Dean Bushey, global regulatory manager for research at Bayer Crop Science. "We have to know what genes do before we can edit them. We may know the sequence of the genes, but we have to know how they work," he says.

Some researchers are also questioning the precision of GE. Some of the technologies – CRISPR among them – are known for making unintended edits, although Maywa Montenegro, a food systems researcher at UC Berkeley, says in *Ensia* that such 'off-target edits' are getting less frequent as the technologies develop.

But even if the correct gene is properly activated or silenced, there may be unexpected effects. Genes are not solitary, self-contained entities and making

SOURCE: MARIUS WALTER

FIGURE 1: CRISPR OPERATING PRINCIPLE



In genome engineering, scientists cut a plant's DNA at a particular point. Due to the cut, the DNA begins to naturally repair itself, either without the removed snippet of DNA or by incorporating an introduced piece of related genetic material into itself

changes to one may cause other ones to react in unforeseen ways, possibly producing side effects or traits that were not intended.

Or there is the possibility that the single edited gene fails to produce the sought-after result. As Montenegro points out, many of the traits agritech companies are looking for – such as drought or pesticide resistance – are not dependent on one particular gene but on the combined workings of a multitude of them.

Environmental interactions throw yet another spanner in the works. Gene function can be affected by factors such as heat, moisture or nutrients available in the soil. An engineered crop in the wrong environment might not display the traits it was designed to do.

Each crop's genetic background also has a role to play. Therefore, at best, an engineered crop should not be expected to work as intended outside the particular environment and conditions it was designed for.

Finally, GE can only express or suppress a trait if it is an inherent quality already present in the crop. This means that if a crop does not naturally contain the genetic features necessary to, say, resist glyphosate pesticides, GE cannot make that resistance manifest out of nowhere. Because of this shortcoming, some experts do not expect that genome engineering will be able to completely replace traditional GM technologies.

The regulatory question

Another possible stumbling block for GE, with its peculiarities in the field of genetic modification, is the regulatory ambiguity currently surrounding it. It seems GE has left legislators around the world scratching their heads. Most of the regulatory obscurity stems from one question – do GE crops count as GMOs?

Using brief pedestrian logic, the answer would of course be 'yes'. After all, doesn't tinkering with a plant's genes count as "genetic modification"? But current laws governing GMOs – written decades before GE was developed – are not so simple, with many of the issues related to the defintion of GMOs.

In the USA, the US Department of Agriculture (USDA) defines GMOs as organisms or products developed through "the genetic modification of organisms by recombinant DNA techniques".

In plainer terms, to count as a GMO under the US definition, a crop or product requires DNA from multiple sources. This definition does not apply to GE crops, which do not contain foreign DNA.

The USDA has already decided in several cases that GE products will not be regulated as GMOs. In April 2016, it determined that a white button mushroom engineered to resist browning did not need to be regulated as a GMO, making it the first CRISPR-edited crop to receive such a decision.

Before that, crops such as Calyxt's TALEN engineered soyabeans had received similar decisions. The determining factor in both cases was the lack of foreign DNA.

However, developers of GE crops would like clear wording in the law in order to be able to say their crops do not count as genetically modified for the purposes of foreign trade and GMO labelling laws.

To their disappointment, the draft proposal for USDA's new GMO regulation rules, published on 3 May, did not mention GE technologies.

With a comment period scheduled to run until 3 July, biotech companies engaged in GE are sure to put their demands forward to the USDA. The agency is set to miss its mandated deadline of 29 July to publish its final determination.

In the EU, GMO regulation is not concerned with the end product, but the production process used to create it. The law states that a GMO is "an organism whose genetic material has been altered by genetic engineering to include genes that it would normally contain".

Once again, GE muddles the waters. Does it alter a plant's genetic material? Yes. Does it introduce genes a plant would not normally contain? No. Do crops produced through GE then count as GMOs? Under the current legislation, it's anyone's guess.

However, it seems that the EU's attitude towards GE crops may not be as hostile as it has traditionally been towards GMOs. In January 2018, the European Court of Justice (ECJ) advocate general Michal Bobik released a legal opinion stating that GE plants should be exempt from the EU's GMO rules, provided no foreign DNA is introduced into them. The ECJ is expected to give a final determination later in 2018.

But even if regulators decide that GE crops and foods are not GMOs, the public might not agree. In both the USA and the EU, anti-GMO consumer groups have opposed decisions stating that GE crops will not be regulated.

In Europe, for example, the organic trade body IFOAM EU says the ECJ advocate general's opinion ignores the "precautionary principle".

"There are no legal or scientific reasons to exempt from risk assessment, traceability and labelling, recently developed GE. Exempting these new GE [products] from a risk assessment would be a blatant denial of the precautionary principle and of the citizens' right to know how their food is produced," says IFOAM EU policy manager Eric Gall.

GE technologies hold an undeniable power and they are becoming more and more efficient and accurate, with the latest ones – such as MAGESTIC – able to deliver DNA more accurately and without the use of bacterial carriers.

While the regulatory environment is unclear, it seems there is a lenient undercurrent that could pave the way for increased commercialisation of GE crops that are more resilient and provide higher yields. Perhaps the next goal for their developers, then, should be editing the minds of consumers to welcome these crops. ●

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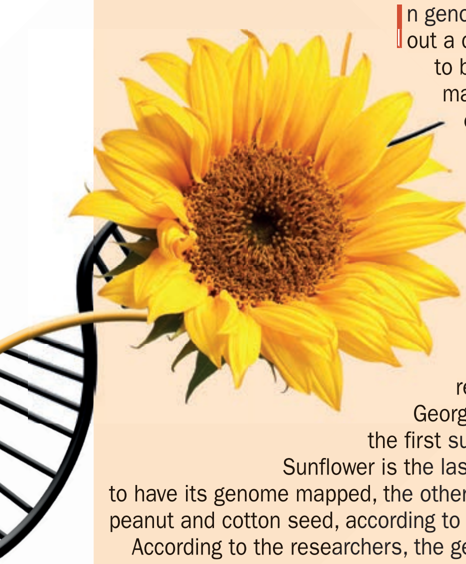
First sunflower genome sequence mapped out



In genome engineering (GE), mapping out a crop's genome is the first step to being able to edit it. Genome mapping allows scientists to know exactly what genes there are in a crop and where they are located.

This, in turn, enables them to discover how these genes function and how they could be changed to achieve traits such as herbicide and drought resistance.

In May 2017, a team of researchers from the University of Georgia (UoG) in the USA published the first sunflower genome sequence.

Sunflower is the last of the five major oil crops to have its genome mapped, the others being soyabean, rapeseed, peanut and cotton seed, according to *The Sunflower*.

According to the researchers, the genome will help future research in increasing sunflower's oil production and to improve crop resilience.

A difficult task

Discovering what makes the sunflower tick was not an easy task, and the sunflower genome ranks amongst one of the most challenging genomes published to date.

The problem so far has been that the sunflower genome consists of highly similar, related sequences.

"As the first sequence of the sunflower genome, it's quite the accomplishment. The sunflower genome is over 40% larger that the corn genome and roughly 20% larger than the human genome. Its highly repetitive nature made it a unique challenge for assembly," says the study's co-author John M Burke, professor of plant biology at the UoG.

"Like many plant genomes, the sunflower genome is highly repetitive, though in this case the situation is a bit worse. The repetitive elements within the genome arose relatively recently, meaning that they haven't had time to differentiate. It's therefore like putting together a massive puzzle wherein many pieces look exactly the same, or nearly so," he adds.

With the first sequence of the sunflower genome now known, it could lead to the development of higher yielding and more resilient sunflower varieties through technologies such as GE.

The USDA, for example, is looking to compare the mapped out parts of the sunflower genome to that of crops that have been more closely researched.

This study could reveal which genes in sunflower are responsible for certain traits, which would in turn enable genetic engineering to improve those traits.

One of the traits the UoG researchers set out to discover was seed oil content and quality and the genes that affect it. The study captured all 40 genes that have already been described as affecting oil production in sunflowers.

In total, the UoG team found 429 genes, corresponding to 12 metabolic pathways involved in oil synthesis.

"The availability of this reference genome and companion resources will not only strengthen interest in the sunflower as a model for ecological and evolutionary studies, but will also accelerate breeding programmes.

"This genome represents a cornerstone for future research programmes aiming to exploit genetic diversity to improve biotic and abiotic stress resistance and oil production, while also considering agricultural constraints and human nutritional needs," the study concludes. ●