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GM foods: is there a way forward?

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There are many quality targets in cereals that could generate step-change improvements in nutritional or food-processing characteristics. For instance, levels of acrylamide, soluble and insoluble fibre, antioxidants, allergens and intolerance factors in food are, to a large extent, determined by the genetics of the raw materials used. However, improvements to these traits pose significant challenges to plant breeders. For some traits, this is because the underlying genetic and biochemical basis of the traits is not fully understood but for others, there is simply a lack of natural genetic variation in commercially useful germplasm. One strategy to overcome the latter hindrance is to use wide crosses with more exotic germplasm; however, this can bring other difficulties such as yield loss and linkage drag of deleterious alleles. As DNA sequencing becomes cheaper and faster, it drives the research fields of reverse genetics and functional genomics which in turn will enable the incorporation of desirable traits into crop varieties via molecular breeding and biotechnology. I will discuss the evolution of these techniques from conventional genetic modification to more recent developments in targeted gene editing and the potential of biotechnology to complement conventional breeding methods. I will also discuss the role of risk assessment and regulation in the commercialisation of GM crops.

Genetically modified organism: Genetic modification: Plant breeding: Gene editing: Crops: Regulation

Fundamental changes in plant breeding

Forward genetics

The earliest forms of agriculture can be traced back more than 10 000 years when foragers first became farmers(1). Gradually, human subjects began making choices about what species to cultivate and what characteristics of individual plants to maintain into future generations. Domesticating wild plants for managed cultivation inevitably resulted in the selection of certain traits and in this way we have slowly and deliberately nudged nature to our advantage. Modern plant breeding depends on the principles of genetics, which has its origins in the work of Gregor Mendel who in the mid-1800s used obvious features of peas and other species to describe how factors for specific characteristics can be transmitted from parents to offspring and inherited through subsequent generations. Our constantly improving knowledge and understanding of plant genetics has led to major changes in plant breeding methods and we are probably now on the cusp of the biggest change so far.

The application of DNA sequencing, bioinformatics and the ability to alter a plants genetic code through transformation and genome editing is driving a huge development in plant research and is set to revolutionise plant breeding through both GM and non-GM approaches. Underpinning this is the rapid reduction in the cost of nucleotide sequencing. In 2001, the cost of sequencing 3·2 billion bp of DNA comprising the human genome was well over $1 billion(2) but the same task can now be done for just $1000(3)! This is fuelling a massive expansion in the completion of whole-genome sequences, including many crop species, and an exponential rise in short sequence submissions to public DNA databases which in turn enables further research in bioinformatics, comparative and functional genomics. The discovery of new, allele-specific, SNP helps to screen breeding populations for the presence of desirable traits

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Abbreviations: GMO, genetically modified organism.
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by marker-assisted selection. This is an example of how DNA sequence information can be used in a non-GM approach to speed-up the selection of desirable characteristics and is becoming increasingly incorporated into modern plant breeding. However, another major hindrance in plant breeding is the availability of useful genetic variation. The use of wide crosses or synthetic polyploids to introduce novel genes from exotic germplasm into crop breeding programmes is one way to generate new variation. If this is done via crossing, or rather illogically, mutation breeding using treatment with radiation or chemical mutagens, the resulting varieties are not covered by the EU genetically modified organism (GMO) regulations. However, wide crossing is limited by the ability to cross-hybridise between taxonomically divergent plant types. In some cases, the breeders' gene pool can further extended by the use of 'bridging species' to progress from incompatible species into commercial crop genomes using a step-wise hybridisation process. This method of introgressing alien genes into crop plants is also not covered by the EU GMO regulations. These are examples of classical or forward genetics where a series of crosses or random mutations are used to generate genetic variation in an inherently unpredictable manner and which requires subsequent repeated and resource-intensive phases of phenotypic selection to identify the desirable effects of specific combinations of alleles in the various breeding populations and to discard the others.

How reverse genetics can help

As we improve our understanding of genes function and interaction, it is possible to apply the concepts of reverse genetics to plant breeding and move specific gene sequences between species using GM techniques with predictable outcomes. In this way, the genome of the host (commercial, elite) crop variety can be enhanced by introducing one or a few specific genes without being compromised by the other, unwanted DNA changes that would be introduced by crossing or mutagenesis. This is particularly important when a desirable trait is in a donor species that also possesses human toxins or allergens. For example, the compound actinidin found in wild relatives of commercial Kiwi fruit is a potent allergen in susceptible individuals and poses challenges for Kiwi breeders. Also, genetic improvement in potato, tomato, pepper and eggplants sometimes uses wide crosses from wild relatives and land races which risk increasing the levels of toxic glycoalkaloids(4). There have been past instances of new commercial potato varieties with unacceptably high levels of glycoalkaloids(5) and testing for these toxins, even in conventional breeding programmes, is now routine. These unintentional breeding outcomes can be avoided by the application of reverse genetics methods such as genetic modification. However, there are also other new techniques (described later) that exploit the concept of reverse genetics and that are being used for plant breeding. It is not yet clear whether new varieties made using these methods will be defined as GMO in the EU.

GM varieties for improved nutritional quality

Of the more than 181 million hectares planted globally with GM crops in 2014 (up from 175 million in 2013), most were crops with agronomic traits such as herbicide tolerance and insect resistance. However, there are now several new varieties that are either close commercialisation or actually being cultivated that possess end-use quality or nutritional enhancements. For example, GM varieties with altered oil profiles are currently cultivated outside the EU. Two competing soya varieties (Monsanto's Vistive Gold and DuPont-Pioneer's Plenish™) have high oleic/low linolenic oil giving better heat stability for frying, longer fry life and improved flavour of fried products. Both have received positive risk-assessment opinions from European Food Safety Authority and after a long delay, the European Commission granted approval for import into the EU for Food and Feed uses (but not cultivation) in April 2015. Monsanto has also developed a GM soyabean variety producing higher than normal levels of the *n*-3 fatty acid stearidonic acid. Tissue-specific expression of two enzymes creates a shift in the fatty acid metabolic pathways, yielding significant levels of stearidonic acid in the seeds. Commercial cultivation in North America, using close stewardship protocols for identity preservation is expected in the next few years. Other GM crops with enhanced nutritional qualities, either close to commercialisation or already available, include Phytaseed™, a rapeseed with increased phosphorous availability (BASF), Laurical™ a high lauric acid rapeseed (Monsanto) and Maveral™, a high lysine maize variety (Renessen).

Crops not normally subjects of biotechnology breeding are also close to market. Arctic apples (Okanagan Specialty Fruits) have less of the enzymes that cause browning when apples are cut(6) and in February 2015 received approval from APHIS, one of the US regulatory bodies for commercialisation in 2016. In another significant development, the J.R. Simplot Company received a determination of non-regulated status from USDA in November 2014(7) and recently publicised its commercial rollout plan for the Innate™ potato(8) that is engineered for low-acrylamide potential and reduced black spot bruising. The genetic modification results in potatoes with reduced free asparagine, a lower content of reducing sugars and with a non-browning phenotype resulting in tubers with reduced black spot bruising.

Future plant breeding methods

Gene stacking

Stacking (also known as pyramiding) multiple transgenes by conventional crossing is a common strategy to combine several GM traits into a single new crop variety and is the fastest growing class of cultivated GM seed type. Once individual single-gene traits have been authorised and proved commercially successful, it is relatively facile for breeders to bring these together in a new plant variety by conventional crossing. An example is the eight-gene maize stack (SmartStax™

made by Monsanto) which combines two herbicide tolerance genes with six insect-resistance Bt genes. The resulting stack features dual modes of control for weeds, as well as resistance to lepidopteran insects and coleopteran pests. Some regulatory authorities (e.g. USA and Canada) do not necessarily require the stack to be re-evaluated, however others, such as the EU and Japan, do require an automatic fresh risk assessment of potential 'interactions' between the single events in each new stack. Stacking-by-crossing is particularly suitable for independent, single-gene traits but the approach has a drawback. As the number of transgenic loci increase, even larger breeding populations are needed to find individuals where random assortment and independent segregation of all the transgenic loci plus the desired assortment of non-transgenic elite traits come together effectively. For more complex traits that require precise and simultaneous engineering of several proteins in a metabolite pathway, stacking-by-crossing is not practicable. Instead, molecular stacking is adopted where of all the gene components need for pathway engineering are first stitched together and inserted into the plant as a single DNA molecule. This ensures all the genes are predictably inherited as a single genetic locus and remain together in future generations.

Gene silencing

Conventional genetic modification alters crop traits by adding new genes that express a novel protein and hence alter the plant's phenotype. However, there are an increasing number of new GM varieties where the goal is to silence (switch off) a gene, either in the crop plant itself, or more innovatively, in a pest insect or pathogenic fungus. The Vistive Gold soya described earlier achieves the changes in fatty acid profile partly through a silencing effect. The Arctic non-browning apples also function by genetic silencing of the oxidation reaction that produces the brown phenolic compounds when apples are cut and exposed to air. These within-plant silencing effects are becoming part of the normal commercial landscape for biotechnology crops. However, we will soon see a new suite of self-protecting crops in the USA that use a novel variation to the silencing mechanism. These plants still generate a silencing signal but rather that targeting a gene within the plant itself, the silencing effect acts on a gene in an attacking pests or pathogen. If successful, and if the potential risks associated with 'off-target' gene silencing can be addressed, this cross-species or host-induced gene silencing could largely replace chemical insecticides and fungicides in the control of major crop pests.

Genome editing

There are several experimental methods for directing the improvement of plant genomes in a more predictable way then via conventional crossing and subsequent selection. Genome editing is attracting significant attention and has already been used to make a new herbicide rapeseed variety recently authorised for cultivation in Canada (described later). Genome editing collectively describes the various site-directed nucleases that can be programmed to recognise and cleave specific DNA sequences. Examples of site-directed nucleases include zinc-finger nucleases, transcription activator-like effector nucleases, meganucleases and clustered regularly interspaced short palindromic repeats. A variant on these methods is oligonucleotide-directed mutagenesis which does not incorporate a nuclease to cleave DNA but also results in a predetermined gene edit such as site-directed nucleases. These methods do not in themselves insert whole new genes like conventional genetic modification but instead make small mutations of a few nucleotides in the existing genes(9,10). These new methods are hugely significant for two reasons. Firstly, making directed changes to specific alleles with predictable consequences is genuine disruptive technology for breeding. Secondly, the resulting genomic changes are generated by the cells own DNA repair mechanisms and are indistinguishable from natural mutations or those generated by chemical or radiation mutagenesis, all of which are excluded from the EU GMO legislations(11).

The first commercial application of genome editing was developed by Cibus Global, a San Diego based company who describe themselves as a precision gene editing firm. In March 2014, they received regulatory approval from Canadian Food Inspection Agency and Health Canada to commercialise a novel sulfonylurea tolerant rapeseed generated using their proprietary Genome Repair Oligonucleotide Technology(12). It is expected to be launched for cultivation in Canada in 2016(13).

Regulations, politics and consumer perceptions

It is clear that many EU consumers are, at best, sceptical and at worst, completely opposed to consuming foods composed of, or containing, GM ingredients. This is despite the fact that plant varieties made using biotechnology are the most heavily regulated and risk assessed of any cultivated crop. In the EU, the cultivation and marketing of GM plants are covered by three main legal instruments. The Contained Use Directive (90/219/EEC) describes the legislation surrounding the generation, storage and growth of GM plants in laboratories and glasshouses. The Deliberate Release Directive (2001/18/EC) governs the release of GMO to the environment including research trials. The GM Food and Feed Regulation (1829/2003/EC) authorises the marketing of food and feed containing GMO. As of 1 January 2014, a total of 50 GM events were authorised in the EU; one for current cultivation and the rest for import and processing, mostly for animal feed. The EU is not self-sufficient in producing animal feed and imports large quantities of soyabeans, an important source of inexpensive protein and oil for incorporation into feed for cows, pigs and chickens. Although GM animal feed, like GM foods, must be labelled, the end products of animal production like milk, eggs and meat do not require labelling. Applications for import of GM materials into the EU are expensive and laborious but at least the process functions largely as expected. Conversely, the approval process for cultivation of GM crops on EU soil is broken. One insect-resistance maize variety (MON 810) was approved for EU cultivation in 1998 and is popular with growers in specific regions of five member states (Spain, Portugal, Czech Republic, Romania and Slovakia) which suffer from infestations of the European maize borer (*Ostrinia nubilalis*). In 2013, MON 810 maize was cultivated on almost 150 000 ha of which most (137 000 ha), were in Spain. Although the EU risk assessors at European Food Safety Authority found no reasons to block further adoptions, the European Commission has not found a way to follow its own legal processes and there are currently no other GM varieties authorised for cultivation within the EU. However, the adoption in January 2015 of a controversial, compromise ‘opt-out clause’, giving individual member states the ability to block the cultivation of risk-assessed GM varieties in their own countries (for reasons other than safety), may serve to break the current deadlock.

While in the past it has been the smooth implementation of the GM approvals process that has been problematic, it could be the fundamental definitions within the regulations themselves that pose challenges in the future. Unlike the Canadian ‘novel traits’ system of regulating new crop varieties or to a certain extent, the US system which also has aspects of being trait-based, the EU approvals framework is squarely defined by the breeding process. For example, if a herbicide tolerant crop is made using particular recombinant DNA methods (e.g. the insertion of a defined piece of DNA via genetic transformation), it is deemed to be a GMO and must undergo a full risk assessment estimated to cost the breeder about $10 million(14,15). If however, a herbicide tolerant crop is made using other methods to recombine DNA (such as mutation breeding), then it is not required to undergo GM risk-assessment procedures and of course there is no requirement to label the product either. Because they are not GMO, herbicide tolerant crops made via mutation breeding are freely available and cultivated by EU farmers today. While this is clearly illogical, at least the breeders know the legislative landscape and can work within it. However, there are new breeding techniques that were simply not foreseen by the EU regulators 15 years ago and for which the definitions of genetic modification in 2001/18/EC are not well-suited. These uncertainties are stifling innovation and could lead to molecular plant breeding and EU agricultural practices falling behind those in the rest of the world.

The way forward

For the EU to embrace the revolution in genome sequencing, molecular genetics and synthetic biology and take full advantage of the new techniques of modern plant breeding, there are three big challenges. Firstly, to move away from the current position of regulating new varieties based on process and adopt an appropriate regulatory framework for biotechnology that can adapt to changes in breeding methods and future agricultural practices in a logical and predictable manner. Secondly, to apply a proportionate, transparent risk/benefit analysis to novel crop types on a case-by-case basis using conventional varieties and farming practices as the baseline comparator and also taking into account the risks of not adopting change. Thirdly, to enable informed choice in all sections of the agricultural and food/feed supply chain by openly communicating the benefits, and also taking into account the risks and longer-term sustainability of different agricultural systems.

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Conflicts of Interest

None.

Authorship

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