New plant breeding techniques: prospects for the future

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Science never stands still and the skill-set of plant breeders through the centuries proves the point. Especially during the last decade, several new plant breeding techniques (NPBTs) have been developed which now make it possible to perform genome modifications with an even greater degree of precision than was previously thought possible following earlier break-throughs in producing genetically modified (GM) plants[1]. One effect is that the distinction between NPBTs and the previous genetic modification technologies, which led to GM plants by transferring genes (transgenesis), has led to some confusion about whether NPBT-produced plants should

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be classified as GM plants or not according to the existing nomenclature. First, let us look at the technologies in question.

NPBTs (Box 1) are of special interest because they allow for precise genome modifications and do not necessarily involve transferring entire genes from one organism to another. Two of them, site-directed nuclease mutagenesis (SDN) and oligonucleotide-directed mutagenesis (ODM), introduce genetic

Box 1. Creating genetically modified organisms with new plant breeding techniques[2]

Transgenesis (GM): transfer of a gene (DNA coding region) from another organism.

Cisgenesis: transfer of a gene to a plant of the same or closely-related species (inter-fertile).

Intragenesis: insertion of a reorganised, full or partial gene derived from the same species (usually combined with a promoter or terminator from another gene of the same species).

Targeted mutagenesis: a specific mutation produced by an SDN technology that uses, for example, a zinc-finger nuclease or a transcription activator-like effector nuclease.

Transient introduction of recombinant DNA: mutations directed by oligonucleotides or infiltration techniques, giving rise to end products that can be similar to, and indistinguishable from, plants derived through conventional plant breeding.

Other techniques: RNA-induced DNA methylation (gene silencing) and reverse breeding, where intermediate products are genetically modified but end products are indistinguishable from plants obtained through conventional breeding. Grafting a non-genetically modified scion onto a genetically modified rootstock results in a chimeric plant where only the lower part carries the genetic transformation.

modifications at specific sites in the genome. Another, RNA-dependent DNA methylation (RdDM), introduces a genetic modification in chemical molecules associated with DNA to produce what are called epigenetic modifications.

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All three techniques modify the plant DNA sequence in different ways, either by mutation, insertion or deletion of a different sequence, by gene replacement or by stable silencing of a gene or its promoter (or other regulatory elements). Exploring these new genome-editing techniques allows not only even more precise plant breeding but also a remarkable range of new opportunities for future crop improvement and production.

Following these techniques further, when molecular biologists want to produce a mutation in the genome using SDN, they design proteins that recognise and target a specific DNA sequence. They use a single protein chain which recognises, binds and cuts a specific sequence in the DNA, or use two proteins artificially connected by a peptide linker. In the latter case, the protein responsible for DNA recognition and binding can be designed in various ways for different specific DNA sequences, whereas the single protein cuts non-specifically any DNA sequence. Using SDNs, a mutation in the genome is induced by editing, deleting, inserting or replacing genes. SDN is also very useful because it can also be a way of introducing multiple genes with different functions, which is known as molecular trait stacking. In the past two years, a new kind of SDN has emerged using a protein called CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats) nuclease. In the first potential applications, this nuclease was guided to a genomic sequence by a specific

guide-RNA, which defined by its sequence the part of the genome to which it would bind specifically.

The basis of the second technique, ODM, is the application of a modified DNA or DNA/RNA molecule (oligonucleotide), which has from 20 to 100 nucleotides and is delivered into plant cells in tissue culture by standard methods that have been exhaustively tested. The sequence of the oligonucleotide resembles a (homologous) sequence in the plant's genome but is designed to differ in one or a few nucleotides. After the homologous sequence binds to the DNA a mismatch pairing occurs which will be corrected by the repair system of the host cell, and this leads to new and specific mutations. The sequence of the oligonucleotide can be used as a template for new DNA synthesis during the repair process. In this way ODM can be used to target the editing of the genome (targeted editing), as is required for the introduction of herbicide resistance into plants by specific point mutations.

The third method, RdDM, enables gene expression to be modified by switching off genes (gene silencing) or enhancing their function without bringing about any change in the genomic sequence itself. This can be achieved by altering the methylation patterns of molecules associated with DNA by the introduction of double-stranded RNAs. These latter molecules are processed by different host enzymes of the RdDM machinery and lead to epigenetic changes in gene expression which can be stably inherited for at least a few generations. A feature of this method is that RdDM can be used to modify the expression of one or more genes.

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These spectacular advances in the different ways that genes can be controlled in plant (and bacteria and animal) cells mean that the plant products derived by

NPBTs may be indistinguishable from wild-type crops using available diagnostic tests. This raises the key question about future prospects – do NPBTs really require testing under existing rules for making genetically modified organisms (GMOs)?

In its recent report *Planting the future: opportunities and challenges for using crop genetic improvement technologies for sustainable agriculture*[2], the European Academies Science Advisory Council (EASAC) used the general term "crop genetic improvement technologies". The term covered NPBTs as defined by the European expert group in 2007 referred to above (and those developed subsequently)[3], and the better known GM techniques defined in the Cartagena Protocol on Biosafety. The Protocol came into force in 2003, had been signed by 166 countries by 2013, and covers the measures that relate to the intentional release of GMOs into the environment and the regulations that apply to the transboundary movement of GMOs for food, feed and production.

Technology-specific GMO regulations have been developed in several countries and have proved to be especially restrictive in the European Union (EU). Since the first field releases, a vast amount of safety research has been performed on GM plants. This research was both sound and necessary for scientific reasons, as only limited data concerning the potential impact of GM plants in the environment existed previously. Also, they addressed public concerns and fears at an early stage. By now, a huge amount of data on the safety of GM crops for humans has been reviewed repeatedly. It has led to the conclusion that there is no evidence that GM plants possess a greater adverse impact on health and the environment than any other crop developed by conventional plant-breeding technologies.

Thus, from a scientific point of view the products of GM crop technology that have been reviewed are safe and there is no evidence of a general risk related to this technology *per se*. The recent EASAC report[2] came to the conclusion that

the regulatory framework of GM crops is "expensive, time-consuming and inappropriately focused on the technology rather than the product", and that there was common agreement in the scientific community that an alternative regulatory system should focus on the risk assessment and regulation of the trait and/or the product rather than the technology used to produce it. This would mean taking the risk-benefit analysis into account rather than focusing on risk alone.

All this bears on the future of NPBTs and the ongoing debate about whether the resulting plants and their products have to be regulated as GMOs[3,4]. NPBTs do not necessarily involve the transfer of entire genes from one organism to another. The products of NPBTs may be indistinguishable from wild-type crops using standard available diagnostic tests. Therefore NPBTs would not qualify as GM crops. Obviously, coverage by GMO legislation would hamper severely the use of NPBTs because GM plants have to pass approval procedures which are costly and time consuming, especially in the EU.

The OECD programme on the Harmonisation of Regulatory Oversight in Biotechnology initiated an international discussion on NPTBs, aiming to ensure that the information used in risk-safety assessment of GM crops and other organisms of commercial interest, as well as the methods used to collect this information, are as similar as possible between different national regulatory authorities. It could be that the list of NPBTs defined in 2007 from a European perspective might be shortened (or extended) as a result of this international discussion process.

Recently, Professor Anne Glover, Chief Scientific Adviser to the President of the European Commission, provided the following commentary: "Our obligation as citizens is to look at the evidence presented and have the courage to

reposition our views as that evidence accumulates. All of us, scientists and non-scientists alike, must guard against confirmation bias where we choose to look at only that evidence that fits our opinions." (www.epsoweb.org/ file/1226)

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