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Transgenic or not? No simple answer!

New biotechnology-based plant breeding techniques and the regulatory landscape

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The global cultivation area of genetically modified plants (GMPs) including soybean, maize, cotton, canola (oilseed rape) and sugar beet has been increasing consistently since they were first cultivated commercially in 1996, reaching 160 million hectares (ha) in 2011 [1]. By 2011, the global area of planted insect-resistant crops was 66 million ha. The rapid adoption of insect-resistant crops indicates that they have become a primary tool for managing lepidopteran and coleopteran target pest species in cotton and maize [2]. Herbicide-resistant GMPs have changed weed management practices and made an important contribution to the global production of commodity crops [3]. Yet, most of these GMPs were created by using first-generation transgenic technologies: particle bombardment or *Agrobacterium*-mediated genetic engineering techniques. As such, they typically carry recombinant DNA from organisms including bacteria and viruses, as well as other plants, to provide resistance against pests or herbicides.

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In the meantime, plant science has made considerable progress both in identifying genetic factors for traits conferring improved disease resistance, drought tolerance, nutrient use and nutritional value, but also in developing new biotechnology-based plant breeding techniques to alter genetic and epigenetic factors more efficiently [4]. These new techniques enable the transfer of limited amounts of DNA between related genotypes from the 'breeders' gene pool', as well as the introduction of specific modifications to plant genomes through targeted mutagenesis by using zinc-finger nucleases or oligonucleotide-directed mutagenesis. Although in some cases (for example, zinc finger nuclease type 3; see Sidebar A) DNA from outwith the breeders' gene pool can be inserted, the insert is highly targeted within the plant genome, unlike in transgenesis. They also allow breeders to modify traits without making changes to genome sequences—for instance, through epigenetic changes by inducing DNA methylation or by reconstituting a desired plantvariety through reverse breeding (Sidebar A). In addition, these new techniques enable breeders to create so-called 'cisgenic' or 'intragenic' plants by inserting a sequence comparable with that from a sexually related species or by knocking out undesirable genes.

For example, cisgenic scab-resistant apple and herbicide-resistant oilseed rape produced by targeted mutagenesis are close to commercialization, and more new plant products (NPPs) obtained by using these techniques are in the pipeline. These include cisgenic potatoes with a higher content of amylopectin for industrial applications, or with improved resistance to pathogens such as *Phytophthora infestans*, as well as pest-resistant plants grown on a genetically modified (GM) rootstock [4,5].

The main rationales behind the creation of NPPs are to accelerate the breeding process and to address consumer concerns about GMPs—which are partly based on a perceived lack of 'naturalness'—by creating plants that could also have been obtained by conventional breeding [6]. Although naturalness is a controversial concept, plant products produced by conventional breeding are more familiar to consumers. Cisgenic NPPs seek to maintain this familiarity by relying on the existing genetic variation in the breeders' gene pool. A survey about the perception of biotechnology in the European Union (EU) highlighted that 55% of EU citizens support cisgenic products compared with only 22% support for transgenic plants. Overall, cisgenic products are perceived to be more natural, less problematic for the environment and generally safer and more promising [6]. Another major premise for the development of NPPs is the regulation of GMPs. Cisgenic NPPs could fall outside the definitions of GMPs in some jurisdictions, and might not be subjected to regulatory oversight beyond that applied to other conventionally bred plants.

NPPs blur the sharp distinction between GMP and non-GMP, and introduce a new continuum between genetic engineering and conventional breeding

Indeed, new biotechology-based plant breeding techniques and their derived products raise several regulatory challenges, as they do not necessarily fit into known product definitions, regulatory frameworks and risk assessment approaches for GMPs. Regulators and policy-makers will have to decide whether NPPs are actually GMPs as categorized by standard definitions [4,7,8]. If they were to be classified as GMPs, it raises the question of whether product definitions should be modified to take into account these new techniques and any future advances in plant breeding methods. It also raises the question of whether the regulatory frameworks and risk assessment approaches implemented for GMPs provide a sustainable and proportionate approach

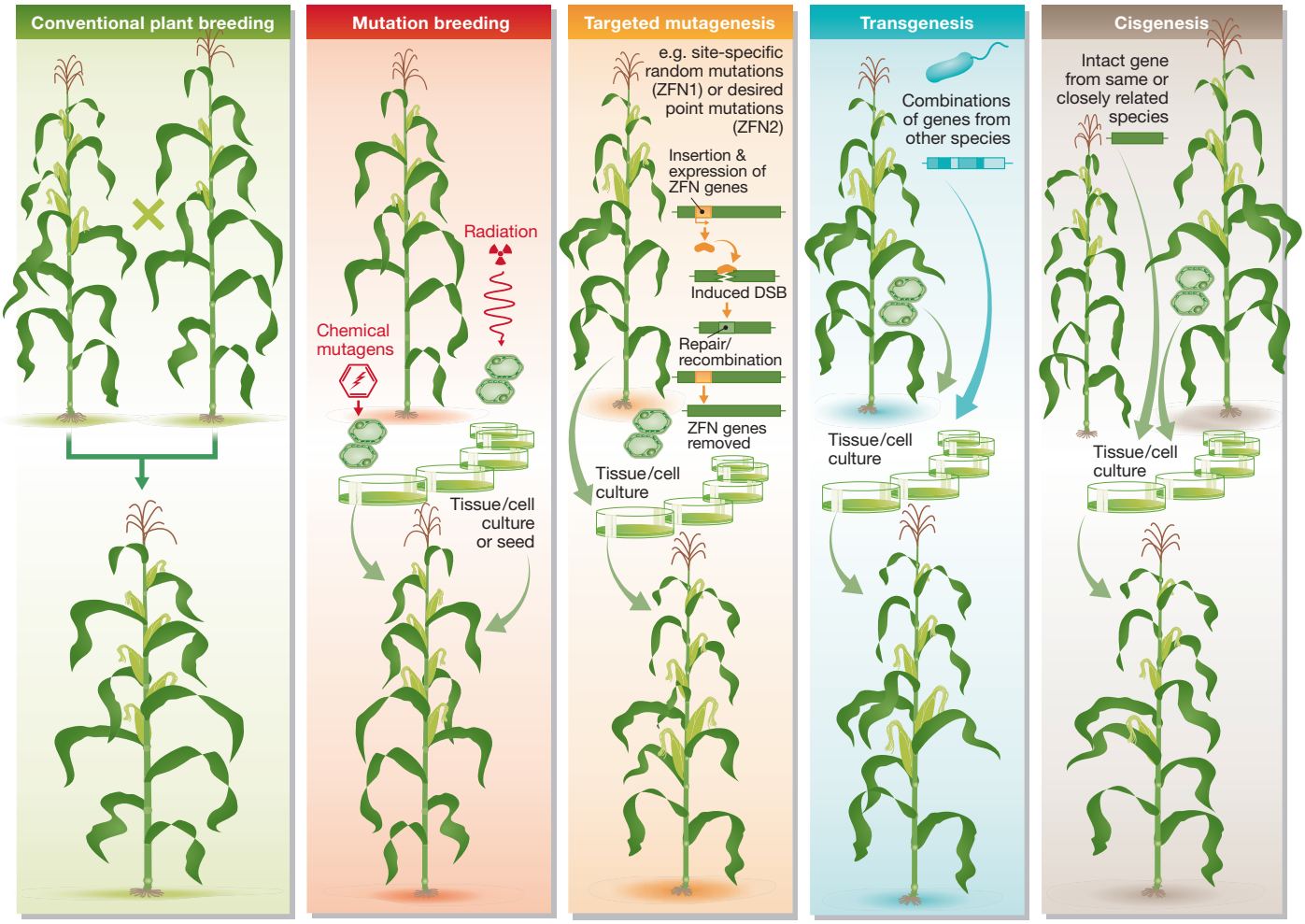

Fig 1 | Techniques that breeders use commonly to create new plant varieties. DSB, double-strand break; ZFN1/2, zinc-finger nuclease 1/2.

for the regulation and safety assessment of NPPs. In addition, regulators and policy-makers have to consider whether the frameworks put in place provide an optimal balance between policy objectives, international harmonization and equal regulatory oversight for different products that raise similar safety concerns.

The first challenge is to make sure that regulatory frameworks remain fit for purpose. However, frameworks that use process-based definitions as a trigger for regulatory oversight might not be functional over time (Sidebar B). Several authors have argued that new biotechnology-based plant breeding techniques might not fit into, or might rapidly outgrow, the established definitions for GMPs [9,10] or other narrowly defined product definitions [8,11,12]. NPPs blur the sharp distinction between GMP and non-GMP, and introduce a new continuum between genetic engineering and conventional breeding. Process-based and narrowly defined product-based legislation therefore run the risk of quickly becoming obsolete given the rate of innovation in the field. Process-based legislation will require not only updates to the lists of new biotechnological plant breeding techniques but also debate on their classification as GMP or non-GMP. However, such flexibility is rarely evident in regulatory frameworks.

The second challenge is to ensure that regulatory frameworks and risk assessment approaches for NPPs remain proportional to the level of risk that these plants might pose to human and animal health and the environment. Despite the fact that genetic engineering techniques have been used to insert recombinant DNA transiently or stably during the development of NPPs, the genomic changes are often similar to those obtained by conventional breeding, such that the end products are indistinguishable from conventionally bred plants (Fig 1; Sidebar A). Therefore, from a product-based perspective, the risk profile of certain NPPs resembles conventionally bred plants more closely than GMPs [13]. This questions the proportionality inherent in regulatory frameworks and risk assessment practices.

As many of the changes introduced in NPPs could also be obtained through conventional breeding, it is important to consider whether any unintended changes arising from these techniques are specific to NPPs and differ from those caused by conventional breeding. The *in vitro* procedures (for example, cell and tissue culture) used to

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obtain NPPs are also used in conventional plant breeding, so unintended changes owing to somaclonal variation will be similar in both cases [13]. Only unintended changes attributed to the stable or transient presence of recombinant DNA in NPPs are new compared with conventional breeding, and therefore merit further investigation. In the case of NPPs obtained after transient expression of recombinant DNA or after the use of a GM intermediate, it will be essential to verify that the recombinant DNA is no longer present in the genome of the selected plant if such NPPs were to be excluded from legislation for GMPs.

In 2012, the European Food Safety Authority (EFSA) published a scientific opinion to address the safety assessment of plants developed through cisgenesis and intragenesis [13]. The EFSA Panel on Genetically Modified Organisms (GMO) concluded that cisgenic and conventionally bred plants represent similar hazards, whereas intragenic and transgenic plants could raise new hazards. Whether or not identified hazards would translate into risks to human and animal health and the environment depends on exposure; for instance, the extent to which the plant is cultivated or its derived products are consumed. The Panel's conclusion is consistent with similar reports by the National Academy of Sciences [14] and other risk assessment bodies [9]. However, if cisgenic plants were to be considered GMPs in the EU, the EFSA GMO Panel considered that its existing risk assessment guidelines for plants and products developed through transgenesis would generally apply to cisgenic and intragenic plants, but that it would require less event-specific data, depending on the specific case.

Sidebar A | Categorization of new biotechnology-based plant breeding techniques

Depending on the level of integration of the recombinant DNA into the plant genome, new biotechnology-based plant breeding techniques can be divided into three categories.

Category 1—Transient introduction of recombinant DNA

Techniques that introduce recombinant DNA molecules transiently to plants are zinc-finger nucleases (ZFNs) introduced into the cell with or without a repair template (ZFN1 and ZFN2), oligonucleotide-directed mutagenesis (ODM) and agro-infiltration. These processes resemble transgenesis—*in vitro* synthesized nucleic acids and DNA delivery methods—but the end products are similar to, and indistinguishable from, plants obtained through conventional plant breeding. Therefore, the new plant products (NPPs) are in most cases undetectable [7].

Definitions according to the EU working group on new techniques:

- ODM uses oligonucleotides for targeted (site-specific) induction of point mutations;
- ZFN1 generates site-specific random mutations by non-homologous end joining;
- ZFN2 generates site-specific desired point mutations by DNA repair processes through homologous recombination;
- Agro-infiltration aims to use *Agrobacterium* to inject several foreign DNA molecules into the plant cells.

Category 2—Stable introduction of recombinant DNA during an intermediate step in the development of NPPs

Techniques that use stable genetically modified intermediates include: ZFN1 and ZFN2, RNA-dependent DNA methylation (RdDM) and reverse breeding. Intermediate plants are genetically modified plants, but the end products are similar to and indistinguishable from plants obtained through conventional plant breeding. Therefore, the NPP is in most cases undetectable [7].

Definitions according to the EU working group on new techniques:

- ZFN1 and ZFN2 have been defined above;
- RdDM is a technique that uses the effect of small RNA sequences to alter gene expression through methylation of specific DNA sequences without changing the nucleotide sequence itself (epigenetic change);
- Reverse breeding is able to reconstitute parental lines starting with an elite F1 hybrid whose genetic material is unknown. Reverse breeding combines several other techniques such as RNAi to suppress meiotic recombination, tissue culture to regenerate plants from cells and the double haploidization technique to create double haploid plants, which are used as the respective parental lines to produce new elite F1 hybrids.

Category 3—Stable integration of recombinant DNA

Integration-based plant breeding techniques include: cisgenesis, intragenesis, grafting and ZFNs (ZFN3). The process of generating cisgenic plants resembles transgenesis (random DNA insertion), but the product is similar to plants obtained through conventional breeding. Detection might be challenging [7,13].

Definitions according to the EU working group on new techniques:

- Cisgenesis is genetic modification of a recipient organism with a gene (cisgene) from a crossable—sexually compatible—organism;
- Intragenesis is genetic modification of a recipient organism that involves the insertion of a reorganized, full or partial coding region of a gene combined frequently with a promoter and/or terminator from another gene of the same species or a crossable species;
- ZFN3 technique targets delivery of transgenes (insertions) by homologous recombination;
- Grafting a non-genetically modified scion onto a genetically modified rootstock results in a fruit that does not contain the insert.

The third challenge is to develop regulatory frameworks and risk assessment practices that not only prevent harm, but also stimulate the innovation required to meet other policy objectives such as food security, economic development and building consumer trust [15,16]. It can be argued that extensive GMO legislation has actually stimulated the development of some of these new biotechnological plant breeding techniques to circumvent the regulatory burden and improve consumer attitudes [7]. NPPs also provide new opportunities for small companies and research institutes to develop innovative consumer-oriented products that might not be subjected to extensive and expensive regulatory requirements and procedures. However, as NPPs are already being designed, developed and tested in the field, plant breeders need clarity on the regulatory implications and on whether or not specific NPPs are covered by legislation [12].

To ensure that NPPs and their development are accepted by citizens and consumers, it will be essential to clarify and accommodate factual and normative premises about the governance of new biotechnology-based plant breeding techniques. This will help to increase accountability and improve inclusivity through the involvement of diverse stakeholders [17]. Kuzma and Kokotovich [8] argue that proactive engagement involving stakeholders and the public might help to create a shared responsibility for the governance of new biotechonological plant breeding techniques. This would also reduce the risk of market failures and mistrust among citizens [8]. Although there are significant challenges in addressing the wide range of societal concerns, European

Sidebar B | Process-based compared with product-based regulatory frameworks

Process-based regulatory frameworks

Argentina, Brazil, the EU and many other countries have put new process-based regulatory systems in place to regulate the use of genetically modified organisms (GMOs), as the techniques used for their production were thought to raise specific safety concerns. In these jurisdictions, a GMO is mainly characterized by the transformation techniques used in its production. The definitions of GMOs used by these countries are often partly or fully based on those put forward by international organizations such as the United Nations Food and Agricultural Organization (FAO) and international treaties such as the Cartagena protocol.

FAO: GMOs and derived products are produced by using techniques that alter the genetic material of an organism in a way that does not occur naturally by mating and/or natural recombination. Techniques of genetic engineering include, but are not limited to: recombinant DNA, cell fusion, micro- and macro-injection, encapsulation, gene deletion and doubling. GMOs do not include organisms resulting from techniques such as conjugation, transduction and hybridization (http://www.fao.org/DOCREP/005/Y2772E/y2772e04.htm).

Cartagena protocol (also adopted by Codex Alimentarius): A living modified organism is defined as any living organism that has a combination of genetic material obtained through the use of modern biotechnology, namely: (i) in vitro nucleic acid techniques, including recombinant DNA and direct injection of nucleic acid into cells or organelles, or (ii) fusion of cells beyond the taxonomic family that overcomes natural, physiological reproductive or recombination barriers, and that are not techniques used in conventional breeding and selection (http://bch.cbd.int/protocol).

Product-based regulatory frameworks

Canada and the USA opted to regulate all plants or products with new traits developed either through genetic engineering or any other plant breeding techniques under the same, yet existing, regulatory system [26,27]. The transformation techniques were not considered inherently risky. Therefore, the focus of product-based regulatory systems is on the risks of products and new traits or attributes introduced into a plant, rather than the method of production.

parliamentarians have already taken steps in this direction, including a public hearing on cisgenesis (http://ecrgroup.eu/?p=4521). In addition, the European Commission (EC) has commissioned several reports on specific aspects of new biotechnological plant breeding techniques. In the USA, the Environmental Protection Agency has opened up a debate on a draft rule that would exempt certain cisgenic organisms from registration as GMOs [18].

The fourth challenge is the development of regulatory frameworks for NPPs that allow international harmonization. So far, there is a lack of consensus on detailed product definitions for NPPs. At an international workshop organized by the EC's Joint Research Centre, it became clear that the definition of a GMO differs between jurisdictions and that this determines whether or not NPPs are classified as GMO [19]. Products generated through targeted mutation, for example, will probably be considered as non-GMO in many countries [12,19], although they might be defined as a GMO in other jurisdictions.

The issues of definition and regulatory approach have been discussed extensively by the international scientific community, but no consensus has yet been reached. Various open questions for cisgenic plants illustrate the challenge of harmonizing definitions. These include: can genes be used from primary, secondary and tertiary gene pools (Sidebar C) available to conventional breeders? Should the gene(s) being transferred be assessed for potential allergenic or toxic effects of the expressed proteins? Does the introgressed sequence need to be 100% identical to the sequence from the donor plant? Are small extra sequences allowed and what should be the maximum size of these? Can all transformation methods be used to produce a cisgenic plant? Last, do the expression levels of the introgressed gene need to be within a certain range?

> **…regulatory systems for NPPs need to be dynamically scalable to the rate of innovation and advances in the field [and] remain proportionate to the level of risk…**

As the scientific community has not yet developed a consensus, national regulatory authorities have put forward their own definitions.The US Department of Agriculture, for example, announced that it does not have the authority to oversee cisgenic plants created without the help of a plant pathogen [11,12], whereas the Australian Office of the Gene Technology Regulator considered that certain cisgenic plants might not be regulated [19]. EU member states and the EC are also considering developments in plant breeding and discussing whether new biotechnology-based plant breeding techniques would be captured by, or excluded from, the existing definition of GMOs. In the case of cisgenic plants, the experts have indicated that they fall under GMO legislation, as the definition of a GMO in the EU is mainly based on the technique used to produce it (Sidebar B; http://ec.europa.eu/food/food/biotechnology/index_en.htm).

As NPPs might be defined and regulated differently in different jurisdictions, the existing problem of asynchronous market approvals could be further amplified [20]. The outcome of continuing discussions will have far-reaching consequences for the development of future NPPs, international trade and requirements for labelling and detection. Differences in regulatory systems, and the fact that many jurisdictions still have to establish a consistent regulatory framework for existing GMPs, suggest this is not a straightforward process [4,21].

The fifth and final challenge is the need to avoid disparities in risk assessment practices between products with equal potential to cause harm. Several authors have argued that process-based regulatory approaches lack consistency because conventionally bred products can raise safety concerns similar to those for their transgenic counterparts [22–24]. According to these authors, there are no convincing arguments for applying more stringent regulatory requirements for one particular technique if another one might result in similar adverse impacts. The European Policy Evaluation Consortium, which was commissioned by the EC to evaluate the EU GMO legislation, came to a similar conclusion [25]. Only the Canadian legislative approach enables consistent evaluation of plants with similar new traits, irrespective of the techniques used. To ensure consistent and proportionate risk assessment practices, it is therefore important that regulatory requirements for NPPs are based on risk assessments associated with the plant species, traits, receiving environments and intended uses, and the combination of these characteristics, rather than the production method itself.

Sidebar C | The 'breeders' gene pool'

The 'breeders' gene pool' is the total of all genes, or genetic information, in any population that can be used by conventional breeders to improve their crops. Breeders might need to overcome barriers to gene transfer depending on the source of the genes used. The primary gene pool comprises species that interbreed freely with the plant of interest. The secondary gene pool includes species that cross-breed only but with difficulty with the plant of interest, and that produce at least some fertile hybrids. The tertiary gene pool comprises species that are more distantly related with the plant of interest, but such species cross-breed by using advanced techniques such as embryo rescue, induced polyploidy and bridge crosses.

In conclusion, the NPPs being developed raise various regulatory challenges that need to be addressed urgently. It requires broad international consensus on whether, and how, NPPs will be regulated to avoid potential adverse effects on human and animal health and the environment, whilst stimulating innovation to meet other policy objectives. However, initiatives for ensuring international agreement on the governance of NPPs are lacking.

In addition, to build and maintain public and consumer trust in NPPs, new regulations must be built on factual and normative premises for governance. Importantly, the regulatory environment needs to support innovation, wider policy objectives and consumer acceptance of NPPs. Therefore, regulatory systems for NPPs need to be dynamically scalable to the rate of innovation and advances in the field, remain proportionate to the level of risk that the use of the technology might pose, prevent harm without jeopardizing other policy objectives, support international harmonization and avoid disparities in risk assessment practices. This in itself is a major challenge, and there is no time to further delay discussion among experts in the scientific community with the public and stakeholders.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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