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REVIEW ARTICLE

Plant genome editing in the European Union—to be or not to be—a GMO

Thorben Sprink[1] · **Janina Metje**[2] · **Joachim Schiemann**[1] · **Frank Hartung**[1]

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Abstract New plant-breeding techniques have been boosting plant breeding, since only a few years but already first promising products are pushing to the market. In contrast to this, in many countries, the current Directives regulating genetically modified organisms have been established more than 25 years ago, especially in the European Union being based on clear differentiation between transgenic plants and conventional breeding. Therefore, the question arises if these Directives are suitable to face the new challenge of genetic engineering or if there is a need for updated regulations.

Keywords Genome editing · GMO regulation · NPBTs · CRISPR/Cas · Side directed nucleases · Regulatory framework

Introduction

For thousands of years, mankind has been using genetic selection to further improve wild plants to crops, but it took centuries until the rules behind have been understood. It was Mendel who discovered the laws of genetic inheritance back in 1866, starting the age of knowledge-based breeding.

During the last decades, further scientific benefits, such as double cross (1933) and single cross hybrids (1964), marker assistant breeding, and gene technology (1986), have been promoting modern plant breeding resulting in a tremendous increase in yield over the years (Fig. 1), but new challenges for farmers and breeders are on the rise. The growing world population shrinking arable land per person (Fig. 2) as well as the globally changing climate and factors accomplished with it like invading pests is the major challenges which have to be addressed in the next decades and yields have to improve further. Research is an ongoing process and the latest scientific achievements are promising candidates to become the newest acquisition in the toolbox of breeders, these—new plant-breeding techniques—(NPBTs)—allow precise modification of the genome by enabling site-specific changes in the DNA (Schaeffer and Nakata 2015; Sprink et al. 2015). This technology is an opportunity to further improve breeding using a tremendous amount of possibilities, such as transfer of naturally occurring mutations from wild relatives of crop plants into elite varieties to provide them with resistances against multiple pests (for review, see Bortesi and Fischer 2015).

However, to be used for breeding and for crop optimization, scientific achievements need legal security and appropriate guidance. Particularly, the genome editing techniques call for an updated legislation regulating the use of plants produced by genome editing as the current legislations which are regulating GMO technologies have been established more than 25 years ago and miss the requirements associated with the use of these techniques. Especially, in the European Union (EU), these legislations are treated to be outworn, because they are based on a clear distinction between transgenic plants (using recombinant nucleic acids) and conventionally bred plants (including hybridization and mutagenesis approaches) but fail to seize the new bridge between transgenesis and conventional breeding. In many countries, such as Argentina and the

✉ Thorben Sprink
Thorben.Sprink@julius-kuehn.de

[1] Institute for Biosafety in Plant Biotechnology, Julius Kuehn Institute, Erwin Baur Strasse 27, 06484 Quedlinburg, Germany

[2] Research Group Autophagy, Max Plank Institute for Biophysical Chemistry, 37077 Gottingen, Germany

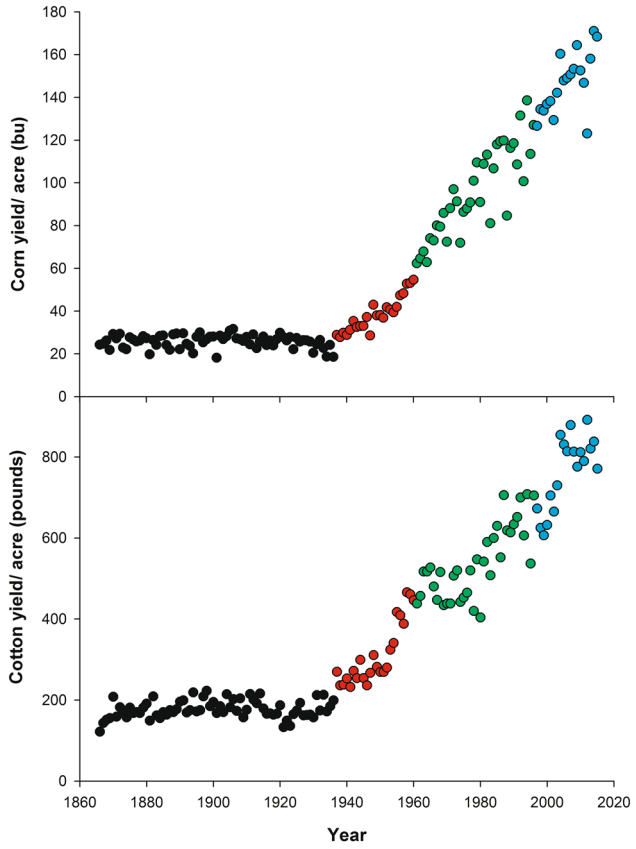

Fig. 1 Yield development of corn and cotton in the US from 1866 to 2015. Data source: USDA-NASS (http://www.nass.usda.gov). *Black circles* represent time frame of open pollination, *red circle* represents introduction of double cross hybrids, *green circles* represent introduction of single cross hybrids, and *blue circles* represent introduction of GMO lines into the cropping system of the US

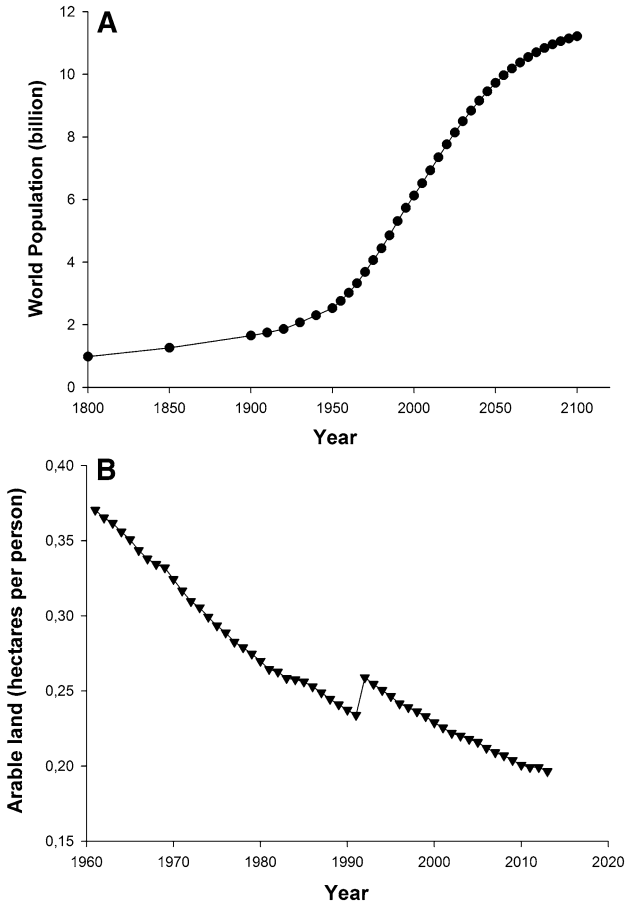

Fig. 2 Predicted world population growth until 2100 using a medium growth model (**a**) and development of arable land/person from 1960 to 2013 (**b**). Data source: the United Nations (http://faostat.fao.org/beta/en/#home)

United States, this problem has been recognized and the regulatory frameworks are under examination or have been revised already (Memorandum 2015; Whelan and Lema 2015).

The regulatory framework of the European Union

The regulatory framework of the EU was established back in 1990; 2 years before, the first GMO crops have been applied for release, resulting in the Council Directives 90/219/EEC and 90/220/EEC (The Council of the European Communities 1990a, b). These Directives have been revised resulting in two Directives which are in force today. Directive 2001/18/EC on the deliberated release of genetically modified organisms (GMOs) into the environment which has been amended by the Regulations (EC) Nos. 1829 and 1830/2003, and Directive 2009/41/EC on the contained use of genetically modified micro-organisms (GMMs) (European Parliament and European Council 2001, 2003, 2009) Directive 2009/41/EC also regulates activities involving cell cultures of higher organisms, i.e., protoplasts, under conditions of containment. These Directives and the included definitions on genetically modified organisms are in line with the Cartagena protocol on biosafety and define several techniques of genetic modifications, such as (1): "recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation"; (2) "techniques involving the direct introduction into an organism of heritable material prepared outside the organism including micro-injection, macro-injection and micro-encapsulation", and (3) "cell fusion (including protoplast fusion) or hybridization techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally."

Furthermore, techniques which are not considered to result in genetic modification, on condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms made by techniques/methods other than those excluded by Annex I B are defined in these Directives: (1) in vitro fertilization; (2) natural processes, such as conjugation, transduction, and transformation; and (3) polyploidy induction. The Annexes of these Directives also list techniques/methods of genetic modification yielding organisms which should be excluded from the Directive, on the condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms (GMO) other than those produced by one or more of the techniques/methods listed below those are in the Directive 2001/18/EC listed in Annex IB: (1) mutagenesis and (2) "cell fusion (including protoplast fusion) of plant cells of organisms which can exchange genetic material through traditional breeding methods." In the Directive 2009/41/EC for the contained use of GMMs, these techniques are listed in the Annex II Part A and in addition to mutagenesis the following three techniques are excluded: (1) cell fusion (including protoplast fusion) of prokaryotic species that exchange genetic material by known physiological processes; (ii) cell fusion (including protoplast fusion) of cells of any eukaryotic species, including production of hybridomas and plant cell fusions; and (iii) self-cloning consisting in the removal of nucleic acid sequences from a cell of an organism which may or may not be followed by reinsertion of all or part of that nucleic acid (or a synthetic equivalent), with or without prior enzymic or mechanical steps, into cells of the same species or into cells of phylogenetically closely related species which can exchange genetic material by natural physiological processes, where the resulting micro-organism is unlikely to cause disease to humans, animals, or plants (self-cloning may include the use of recombinant vectors with an extended history of safe use in the particular micro-organisms).

Interpretations of the legal framework

Regardless the intention of the Directives and the facts that the Directives 2001/18/EC and 2009/41/EC both contain process- and product-related terms, they are frequently being interpreted as strictly process-based legislations. In the view of several NPBTS, which are closer to conventional breeding than to common genetic engineering, the interpretation is important for the further status of the resulting plants. Dependent on the emphasis, such plants could either be regarded as GMO or non-GMO. If the emphasis is on the techniques used, the resulting organism is a GMO, even if an identical organism containing the same modification(s) exists or could be produced by conventional breeding or techniques listed in the Annexes of Directives 2001/18/EC or 2009/41/EC. If the emphasis is on the product/the resulting organism, such an organism would be regarded as non-GMO and would, therefore, be considered out of the scope of the Directives. In the common sense of science, as those two organisms, the one created via mutagenesis and the one created via GE, are indistinguishable from each other, the Directives should be actually interpreted facing more on the resulting product. As first commercial plants have been developed using NPBTs and are going to be addressed for commercial release also in the European Union, it is urgent to provide legal guidance; otherwise, it could happen that plants enter the market in legal uncertainty for both, the consumer and the producer.

The changed ways of breeding

Regardless of the used technique, the aims of breeding are always the same—enhancing the genetic variation to get crops with improved characteristics—such as higher yield or pest resistance. To get there, nowadays, three major techniques are available: (1) conventional breeding through crossing, (2) genetic engineering; whereas conventional breeding depends on random and undirected genome alterations by mutagenesis or natural occurring recombination events, i.e., crossing over, genetic engineering allows specific but still undirected genome alterations by integration of recombinant DNA; and (3) genome editing allows specific and directed genome alterations using one of the different nuclease techniques by introducing desired mutations (SDN-1 and -2) or by intended and specific cisgenic, intragenic, or transgenic DNA integration (SDN-3) (Lusser et al. 2012).

Legal guidance on how to define plants produced by the NPBTs in relation to the decade old legislation is needed as products arising from such techniques have been developed already and are going to be requested for release also in the EU soon. In a recently published paper (Sprink et al. 2016), we outlined the process in the EU to develop a legislation that properly matches the scientific progress. As the process is facing several hurdles, we also compared it with existing frameworks in other countries and discussed ideas for an alternative regulatory system.

The new technology working group

The European Commission has recognized the need for an updated legislation and established, already back in October 2007, an expert working group with the mandate to

examine a non-exhaustive list of NPBTs in the context of the GMO legislation. The final report of the working group was provided in February 2012 and distributed among the Competent Authorities of the EC Member States but was never published formally. In this report, the expert working group examined the following eight techniques: (a) Oligonucleotide-Directed Mutagenesis (ODM); (b) Zinc Finger Nuclease Technology (ZFN) (comprising ZFN-1, ZFN-2, and ZFN-3), nowadays, formally known as SDN (site directed nuclease) or SSN (site-specific nuclease) (for a better understanding and uniformity, the authors will refer to SDN1, -2, and -3 whenever ZFN or SSN is written in the original texts); (c) cisgenesis (comprising cisgenesis and intragenesis); (d) grafting (on GMO rootstock, or with GMO scion); (e) agro-infiltration; (f) RNA-dependent DNA methylation (RdDM); (g) reverse breeding; and (h) synthetic genomics.

In parallel, the joint research centre (JRC) of the EC also had a look on these techniques, but focussing on the adaption of them in Science and breeding; to get detailed information on the techniques, the authors suggest to read the final report of the JRC (Lusser et al. 2011).

The report of the expert group examining NPBTs in the context of the GMO legislation provided the following suggestions for techniques to be excluded from GMO legislation in regard to the Directives 2001/18/EC and 2009/41/EC:

(a) ODM;
(b) SDN-1 and -2 (without recombinant DNA) [according to present knowledge, this would be valid for all site directed nucleases (SDN)];
(c) offspring and fruits from grafting with non-GM scion;
(d) offspring of plants subjected to agro-infiltration "sensu stricto";
(e) RdDM-subjected plants without heritable change of their DNA (methylation alone is not a heritable genetic change);
(f) offspring from reverse breeding.

Especially, in the cases of ODM and SDN-1 and -2, there has been general agreement that organisms resulting from SDN1 and -2 and ODM are similar to organisms resulting from mutagenesis and it is expected that fewer unintentional changes or effects are generated compared to irradiation or chemical mutagenesis. Therefore, ODM is captured by the Annexes IB (Directive 2001/18/EC) and II Part A (Directive 2009/41/EC). For SDN-1-and -2, there has been a general agreement that those techniques should be excluded from the Directives. The working group also defined techniques which should be within the scope of GMO legislation:

(a) SDN-3;
(b) cis- and intragenesis;
(c) grafting with GM scion;
(d) agro-infiltration "sensu stricto" (subjected plants) or floral dip (also offspring);
(e) RdDM-subjected plants with integrated foreign DNA.
(f) All intermediate organisms containing recombinant DNA.

For SDN-3, there was a general agreement that the technique is generally in the scope of the Directives, but in some cases, the criteria of self-cloning (Directive 2009/41/EC Annex II Part A) might be met.

The EFSA mandate

In addition, also a mandate has been given to the European Food Safety Authority (EFSA) to examine if the current guidance for risk assessment of food and feed from genetically modified plants (EFSA 2011) and the guidance on the environmental risk assessment of genetically modified plants (EFSA 2010) are appropriate and if there are new or additional hazards related to the use of the NPBTs compared to conventional breeding or to current GMOs. The EFSA examined cisgenesis and intragenesis approaches and SDN-3 approaches (EFSA 2012a, b). The EFSA stated that: "… with respect to the trait the products developed using cisgenes are the same as those that could be produced using conventional breeding approaches. This is not necessarily the case for intragenesis and transgenesis…" The panel concluded that: "… that similar hazards can be associated with cisgenic and conventionally bred plants, while novel hazards can be associated with intragenic and transgenic plants." Nonetheless, the Panel considers that the current guidance: "are applicable for the evaluation of food and feed products derived from cisgenic and intragenic plants and for performing an environmental risk assessment and do not need to be developed further."

For SDN-3 approaches the panel stated: "The main difference between the SDN-3 technique and transgenesis is that the insertion of DNA is targeted to a predefined region of the genome. Therefore, the SDN-3 technique can optimise the genomic environment for gene expression and minimise hazards associated with the disruption of genes and/or regulatory elements in the recipient genome. The SDN-3 technique can induce off-target changes but these would be fewer than those occurring with most mutagenesis techniques. Where they do occur, the changes would be the same types as those produced by conventional breeding techniques."

Just like for Cisgenesis and Intragenesis, the panel stated that the current guidance is applicable for the evaluation

and for performing environmental risk assessments. Furthermore: "…on a case-by-case basis lesser amounts of event-specific data may be needed for the risk assessment of plants developed using the SDN-3 technique. There is therefore a need for flexibility in the data requirements for risk assessments".

In an official inquiry, the Directorate General for Health and Food Safety (DG SANTE) of the EC asked EFSA for technical assistances concerning ODM, SDN-1, −2, and RdDM, in addition to the report of the new technology working group (EFSA 2015). DG SANTE inquired: "Does EFSA consider ODM, ZFN1 and -2 (and similar site directed nuclease techniques) as a form of mutagenesis? Could EFSA provide the rationale behind its opinion in this regard?"

The answer provided by the EFSA is comparable with the findings of the new technology working group as EFSA also stated: "The EFSA GMO unit considers that the currently available ODM, ZFN-1 and -2 and similar SDN techniques create point mutations similar to those introduced via natural or induced mutagenesis, and can thus be considered a form of mutagenesis." As the rationale behind this opinion EFSA states: "A point mutation results in a change of one (or few) base pairs…The EFSA GMO unit considers that the modifications that can be obtained with ODM, ZFN-1, -2 and similar SDN are comparable in type and extent to point mutations which can be obtained via natural or induced mutagenesis… Point mutations introduced with ODM o.a. are indistinguishable from point mutations introduced by natural or induced mutagenesis."

Recent developments in the member states

In June 2015, the European Commission stated in a letter to the Member States' Competent Authorities: "Being aware that the current legal uncertainty is unsatisfactory, the Commission services are committed to present their legal analysis to the Competent Authorities and stakeholders before final adoption by the Commission foreseen before the end of this year."

Even though it was promised, more than a year passed and the European Commission still failed to provide a legal interpretation of the NPBTs, including genome editing until today (November 2016). In a Statement on Crop genetic improvement technologies for a sustainable and productive agriculture addressing food and nutritional security, climate change, and human health, the European Plant Science Organisation (EPSO) is urgently requesting the EC to provide a guideline document that follows these recommendations to provide legal certainty for science and industry [sic!] (EPSO 2015). In addition, the committee of Agriculture and rural development of the European Parliament considered in June 2016 a resolution on technological solutions for sustainable agriculture in the EU (European Parliament 2016):

"Recognises the importance of marker-assisted selection (MAS) and SMART breeding, which are now well integrated into many breeding programmes [sic!], but also the potential offered by precision breeding for crop improvement, such as the use of zinc finger nucleases (ZFNs) and clustered regularly interspaced short palindromic repeats (CRISPR) in genome editing, oligonucleotide-directed mutagenesis (ODM) and the use of cytoplasmic male sterility (CMS) hybrids in protoplast fusion or tissue culture based methods".

- Considers it important to ensure sustained support for development and use of future technological tools which may allow breeding to successfully address the societal challenges ahead.
- "Emphasises that it is crucial not to hamper the application of, and experiments involving, high-precision breeding techniques—without sound scientific reason, and that legislation should be fit-for-purpose in order to keep pace with developments without being burdensome".
- "Considers it timely for the Commission to publish the final report of the 'New Techniques' working group and to use its scientific findings as a basis for, inter alia, clarifying the legal status of the breeding techniques currently under scrutiny and to use sound legal analysis in its deliberations".

However, not only on the European level the discussion concerning NPBTs is ongoing also in the member states the discussions are expanding. In an earlier publication, we covered the case of rape seed produced by CIBUS using ODM and the differing legal interpretations in Germany (see Sprink et al. 2016). Since that time, the federal office for consumer protection and food safety has revised its legal opinion and has added a passage on CRISPR/Cas9 (BVL 2016). Nonetheless, the legal opinion has become a court case, as several institutions have filed a lawsuit against it. The case has not been opened yet and it is unsure whether or when a formal decision is made. In addition, also in France, the NPBTs and mutagenesis in general are a court case, and lately, the European court of justice (ECJ) has also been addressed with questions (USDA 2016). The circumstance that France has involved the ECJ could delay decisions since commonly it takes on average between 18–24 months before questions of the member states are going to be answered by the ECJ. It could happen that responsibilities are displaced by the member states to the ECJ and nothing will be decided resulting in two additional unsatisfying years of uncertainty for all. Nonetheless, some member states are still working on concepts how to

regulate NPBTs in accordance with their current laws, i.e., the Swedish Board of Agriculture announced that the application of CRISPR/Cas9-induced mutations in plants should be considered as equivalent to mutagenesis and, therefore, exempted of GMO regulation. Due to that Swedish scientists have been allowed to cultivate CRISPR-edited vegetables in August 2016, the first CRISPR meal ever has been served (ScienceINSIDER 2016). In addition, in Germany, a draft has been prepared to change the genetic engineering act in accordance with the NPBTs (BMEL 2016). In several other member states, the discussion concerning the NPBTs and their legal interpretation is also ongoing and official statements are expected.

Conclusion

Since the legislation regulating the development and commercialisation of GMOs in the EU has been put in place to handle issues of safety and uncertainty, more than 25 years passed by. During this time, scientific progress concerning biosafety of GMOs and genetics has been made, providing us with tremendous knowledge about genomes, genome structure, and the genetic interaction between various organisms. During the last 15 years, more than 50 plant genomes have been sequenced and due to decreasing sequencing costs and the hunger of genetic information for breeding approaches several more are still in progress (Michael and Jackson 2013). By sequencing different varieties of crops genetic variability even in the same species has been unveiled and it has been shown that spontaneous mutations are a common phenomenon in nature and that these mutations occur even in self-pollinating populations as they pass from generation to generation and are part of the molecular evolution (Schultz et al. 1999; Ossowski et al. 2010). Therefore, in respect to hazards, plants produced via the NPBTs should be discussed in the context and the baseline of natural occurring genetic variation and the variation using classical breeding approaches. Recent discoveries widened our knowledge on genetic variation and horizontal gene transfer and showed that horizontal gene transfer or "natural transgenes" seem to be common phenomenon (Kyndt et al. 2015). This is all part of the natural biological evolution and similar events might also happen with GMO or GE crops. Therefore, estimation or assumption of risks by GE plants should be in the same order of magnitude as for those alterations involved in natural genetic variation or in conventional breeding methods (Arber 2010). From a scientific point of view, it is illegitimate to propose that genetic alterations caused by NPBTs per se pose a higher risk than natural occurring or alterations caused by conventional breeding.

If a breeder uses the NPBTs to mimic a mutation of a crops wild relative in elite breeding material in just one or two generations, it is from a scientific point of view illogical to propose that this mutation poses a higher risk compared to a plant with the desired mutation achieved by crosses of the wild relative with the elite material followed by a number of backcrosses. The genetic difference between the plant produced using the NPBTs and the elite line would even be less compared to the backcross line. In addition, the breeder would save time and money as he would receive his desired trait much easier and faster. As explained in a previous publication the intention of the legislators was to include aspects of both the process as well as the product in the current Directives in the EU (Sprink et al. 2016). Therefore, it would be wrong to focus now just on one aspect. Several models have been proposed lately offering different procedures to handle products resulting from NPBTs focusing either on the product, the process or both (Sprink et al. 2016; Huang et al. 2016; Araki and Ishii 2015). In Germany, the federal ministry of food and agriculture (BMEL) made a first step as an authority to propose a model in a draft bill to change the genetic engineering act in Germany by having a look on both the process and the final product and only if both are clearly indicated as GMO the resulting product should be regarded as a GMO (BMEL 2016). This would exclude mutations which are indistinguishable from natural occurring ones or those produced by conventional breeding. We think that this is a step in the right direction. The fact that the ECJ has been put in charge could thwart decisions on European level, but it is important that the member states and other countries all around the world do not stop the development of regulatory frameworks to provide input for the EC and also to provide legal guidance and security for producers and consumers as we are in a risk that products enter the markets without legal guidance; this includes imports from countries (i.e., Argentina) in which a decision on the NPBTs has already been made. Now, it is up to the EC and other countries worldwide to use these models as an inspiration for the development of fair and flexible regulatory systems that are able to provide legal guidance for the existing technologies but also accommodate upcoming novel plant-breeding techniques.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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