EU Perspectives on New Plant-Breeding Techniques

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In the last two decades, numerous new biotechnological methods have greatly accelerated the efficiency of plant breeding, referred to as new plant-breeding techniques (NPBTs). These techniques are heterogeneous and may or may not involve modification of the plant genome[2]. In the former case, the end product may not possess the genetic modification, *e.g.* fruits and some vegetables. By the European definition, a genetically modified organism (GMO) is one "in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination" (The Council of the European Communities, 1990a, 1990b). These two directives, aimed at covering the legal issues of contained use and intentional release of GMOs, were passed not long after the first field trials with transgenic plants in the late 1980s. The intention was to protect human and animal health and the environment against possible risks from organisms created by recombinant-DNA technology. Both directives have been revised several times, resulting in 2001/18/EC and 2009/41/EC (European Parliament and European Council, 2001; 2009). They list techniques that:

- give rise to genetic modification (annex I, part A of directive 2009/41/EC and annex IA part 1 of directive 2001/18/EC);
- are not considered to result in genetic modification (annex I, part B of directive 2009/41/EC and annex IA, part 2 of directive 2001/18/EC); and
- yield organisms that are excluded from the directive (annex II, part A of directive 2009/41/EC and annex IB of directive 2001/18/EC).

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[2]In the context of this manuscript, genetic modification means that a recombinant DNA sequence has been introduced.

However, the definition of what a GMO constitutes, or the use of which techniques will result in a GMO, still originate from 1990 and, consequently, do not match the development of modern biotechnologies in plant breeding.

Since the vociferous discussions and subsequent formulation of GMO legislation in 1990, numerous new techniques in plant breeding have been developed for which it is not clear if they have to be covered by GMO-legislation or not. Therefore, in 2007, the European Commission and the European National Competent Authorities (NCAs) initiated a review of eight of these plant-breeding techniques defined as NPBTs. At the request of the NCAs, a working group—the new techniques working group (NTWG)—was established with the aim of clarifying the legal status of these new techniques in line with the GMO legislation. The final report of the NTWG was not officially published, but was distributed to the NCAs in 2012 and will be discussed below (New Techniques Working Group, 2012). In 2009, another working group was established on request of the directorate-general for the Environment of the European Commission with the mandate to summarize the state of science for adoption, economic impact and possibility of detection of these new techniques. The study, led by the Joint Research Center (JRC), was finished in 2011 and published as a comprehensive public JRC report and later in summarized form as a peer-reviewed article (Lusser *et al.*, 2011, 2012).

Report of the NTWG to the European Commission

The preamble of the final report includes the statement that the views expressed are those of an expert working group and do not necessarily represent those of the European Commission or the Member States Competent Authorities (New Techniques Working Group, 2012). The NTWG, consisting of two experts per European Member State, at first compiled a list of eight techniques that had to be discussed. This list included: oligo-directed mutagenesis (ODM); zinc finger nuclease (ZFN) technology; cisgenesis; grafting; agro-infiltration; RNA-dependent DNA methylation (RdDM); reverse breeding; and synthetic genomics (SG). Next, the NTWG defined and interpreted terms important for their analyses and then examined the techniques one by one in relation to directives 2001/18/EC and 2009/41/EC.

ODM

Concerning ODM (Beetham *et al.*, 1999) the NTWG came to the conclusion that the oligonucleotides cannot be considered as recombinant nucleic acids. The reasoning for this majority opinion was as follows. Oligonucleotides introduced into the cell by ODM are not capable of replication and are not heritable. Furthermore, the resulting organism itself is captured by annex IB because the technique entails mutagenesis. This view is shared by several competent authorities in Europe [*e.g.* the German Central Biosafety Commission (ZKBS, 2012)]. However, a minority of NTWG experts suggested that ODM leads to a new combination of genetic material resulting in a heritable change in the DNA sequence and that the oligonucleotide has been prepared outside the organism. Both are criteria listed in annex IA, part 1 of directive 2001/18/EC.

ZFN

The ZFN technique (Bibikowa *et al.*, 2001) was divided into subcategories named ZFN-1, -2 and -3. In the case of ZFN-1, the nuclease is applied without integration of the respective gene and no additional template DNA is added. ZFN-2 resembles ZFN-1 but, in addition, an added template DNA for guided DNA-repair, which is not integrated into the genome of the host, is explored. Considering the non-replicative template DNA and a not-integrated ZFN construct, the experts agreed by majority that ZFN-1 and -2 are captured by annex IB (directive 2001/18/EC) or annex IIA (directive 2009/41/EC). In the view of the experts, ZFN-1 and -2 result in a GMO that should be excluded from GMO regulation because the provoked genetic change is a mutation that can also be introduced by other forms of mutagenesis and cannot be distinguished from a mutation introduced by other techniques. ZFN-3 is characterized by the addition of a larger stretch of homologous DNA as a repair template for homologous recombination, aiming for a site-specific integration of transgenes (gene targeting). ZFN-3 was considered by all experts of the NTWG to fall within the scope of directive 2001/18/EC and to be covered by annex IA, part 1 as the resulting plants would be transgenic. However, some cases may meet the criterion of self-cloning and might be considered to fall outside of the scope of annex IIB of directive 2009/41/EC. The opinions expressed by the experts of the NTWG concerning ZFN-1 to -3 are shared by the German ZKBS and, concerning ZFN-3, also by the European Food Safety Authority (EFSA).

Cisgenesis

In the case of cisgenesis/intragenesis, recombinant DNA is introduced into the genome of the recipient plant (Belfanti *et al.*, 2004). The difference from transgenesis is that the recombinant DNA originates from a crossable plant (*i.e.* the same or a closely related species). All experts came to the conclusion that this technique falls under the scope of directive 2001/18/EC and is sufficiently covered by annex IA, part 1. This conclusion is shared by the ZKBS and EFSA. In addition, experts of the different working groups expressed the opinion that, in some cases, cisgenesis may be equivalent to self-cloning (EFSA, 2012a; New Techniques Working Group, 2012).

Grafting

Applying a non-GM scion to a GM rootstock (MacKenzie *et al.*, 1991) results in a chimeric plant that falls within the scope of directive 2001/18/EC, whereas the fruits, seeds or progeny should not be regulated as GMOs. For the converse (*i.e.* non-GM rootstock and GM scion), the chimera, as well as the fruits, seeds or progeny from the scion, are transgenic and, therefore, fall under the scope of directive 2001/18/EC (New Techniques Working Group, 2012). This view is agreed with by other expert bodies (*e.g.* ZKBS).

Agro-Infiltration

Agro-infiltration (Lee *et al.*, 2001) was divided into two subcategories by the NTWG experts. In the case of agro-infiltration *sensu stricto*, only non-germline tissue is infiltrated and the T-DNA of the *Agrobacterium* accumulates but does not replicate in the cells. The aim of this method is not to produce offspring in which the T-DNA is integrated into

the genome, which would result in a transgenic plant. As recombinant *Agrobacterium* is a genetically modified microorganism, the infiltrated plants containing recombinant *Agrobacterium* would fall within the scope of directive 2009/41/EC. In rare cases, the T-DNA might integrate into the genome, but as there is no selection on these integration events, a transfer of the recombinant DNA to the progeny, albeit highly unlikely, has to be controlled. The majority of the experts concluded that progenies of these plants, in which it is proven that no recombinant DNA is integrated into the genome, should be considered to fall outside the scope of directive 2001/18/EC. The second category is agro-infiltration of germline tissues, called "floral dip." This method is usually employed to generate offspring that do contain recombinant DNA in the genome. Therefore, all experts agreed that offspring that do harbor a stable integration event fall within annex IA, part 1 of directive 2001/18/EC. The German ZKBS agreed with this view, but expressed the opinion that multicellular organisms (including plants) that do contain some cells with a recombinant genome should not be defined as GMO. This point was not explicitly discussed by the NTWG experts.

RdDM

Regarding RdDM (Mette *et al.*, 2000), all experts agreed that, in cases where any DNA that encodes the effector RNA is integrated into the genome, the resulting plants are GMOs. In cases where the DNA is only transiently present and not stably integrated, the intermediate plant is a GMO but not the fruit, seeds or other progeny. In cases in which the RNA is directly delivered into the cell without being able to replicate, the experts agreed that such a plant should not be defined as GMO. Summarizing the different scenarios that are possible using RdDM, the conclusion of the NTWG was that all plants containing RNA-dependent DNA methylations only are not genetically modified. Therefore, such plants would not fall under the scope of directive 2001/18/EC (New Techniques Working Group, 2012). This view is shared by the German ZKBS.

Reverse Breeding

During reverse breeding (Dirks *et al.*, 2009), a number of steps are taken that transiently might involve genetic modification. In line with conclusions drawn for other NPBTs such intermediate plants would fall under the scope of directive 2001/18/EC, but not their fruits, seeds or other progeny if they do not contain recombinant DNA. As the goal of RB is to produce parental genomes of superior F1-hybrids in a controlled manner, the screening procedure is on genomes containing no genetic modification at all. Therefore, the NTWG experts agreed that plants resulting from RB do not fall under the scope of directive 2001/18/EC. This view is in agreement with the opinion of the German ZKBS.

Synthetic Genomics

Synthetic genomics (Smith *et al.*, 2003) is a rapidly evolving field within synthetic biology, which may include techniques of genetic modification. Because of the breadth of SG, the NTWG did not discuss it in general, but only some aspects of it (New Techniques Working Group, 2012). The view of the experts is that, in most cases, SG would fall under the scope of directive 2001/18/EC and/or 2009/41/EC if a living (micro-)organ-

ism is the recipient of the synthetic genome. As most of the work done so far has been basic research in microorganisms, this falls under the scope of directive 2009/41/EC. The NTWG offers two possible interpretations of how this technique might be covered in future, either with emphasis on the recipient, which is not considered as a (micro-)organism, or with emphasis on the resulting entity which is considered to be a (micro-)organism (New Techniques Working Group, 2012). In the first case, the technique falls outside of GMO legislation, in the latter case it falls under the scope of the respective directive 2001/18/EC or 2009/41/EC.

Report of the Joint Research Center Working Group

The JRC working group—established in 2009 on request of the Directorate-General for the Environment—analyzed various aspects of the NPBTs which were the state of science for adoption, the economic impact and the possibility of detection of these new techniques. The main conclusions have been published and are as follows:

- a significant part of R&D on NPBTs was done in public research institutes in Europe, but most of the patents are held by US-based companies;
- some of the NPBTs are already in the late stages of commercial breeding programs and will appear on the market in the near future; and
- many regulatory jurisdictions around the world will make decisions on the legislation of NPBTs (Lusser *et al.*, 2011; 2012).

The last point has implications for the adoption of these techniques; regulation of them as GMOs will hamper the adoption of new crops. Furthermore, differences in the regulation of new crops in different parts of the world will cause severe asynchrony in the approval of such crops. Consequently, Lusser *et al.* (2012) have demanded that global discussion concerning governance of the NPBTs is necessary to achieve synchronized and evidence-based governance.

Opinion of the EFSA Concerning the Safety Assessment of Cisgenesis and ZFN-3

The EFSA expressed its scientific opinion in 2012 upon request by the European Commission to address the question of whether the existing guidelines to assess the safety of GMOs can be applied also to NPBTs. To perform an analysis of selected NPBTs and their potential risks, the EFSA established two working groups consisting of GMO panel members and external experts. At first, cisgenesis and intragenesis were evaluated and the results published in 2012 (EFSA, 2012a). The working group came to the conclusion that the existing guidance documents for GMO-risk assessment are applicable to NPBTs and do not need to be developed further (EFSA, 2012a). Furthermore, the experts expressed the opinion that, in some cases of cisgenic plants, fewer event-specific data may be needed to perform the risk assessment.

In the case of ZFN-3, the EFSA working group at first changed the term ZFN to SDN (site-directed nuclease) as in recent years in total four different nuclease systems have been developed that are applicable for the introduction of sequence-specific DNA-

strand breaks and the specific incorporation or exchange of genetic material. The expert group came to the conclusion that the aim of the SDN-3 technique is to integrate or exchange recombinant DNA and, therefore, it is comparable to transgenesis but more precise (EFSA, 2012b). Therefore, the existing guidance documents for transgenic plants are applicable also for plants derived from SDN-3 but, again, in some cases (*e.g.* SDN-3 combined with cisgenesis) fewer event-specific data might be needed for the risk assessment (EFSA, 2012b).

Report of the European Academies Science Advisory Council

The EASAC has provided an extensive report on the risks and benefits of so-called "crop genetic improvement technologies," a term that includes NPBTs, gene technology and techniques that will evolve in the future (European Academies Science Advisory Council, 2013). The report did not find evidence of intrinsic higher risk of GM technology in comparison to conventional breeding. This finding is based on solid science conducted in several thousand research projects and published in the last 20 years. Therefore, EASAC came to the following key conclusion and recommendation (European Academies Science Advisory Council, 2013):

> *The trait and product not the technology in agriculture should be regulated, and the regulatory framework should be evidence-based.*

This request for a trait/product-based regulation reflects the scientific evidence that is very solidly based on GMO-safety research and risk analyses accumulated in the last two decades (Heap, 2012; Swiss National Science Foundation, 2012; Hartung and Schiemann, 2014). The EASAC report was recently endorsed by Anne Glover, chief scientific adviser to the president of the European Commission. Besides the EASAC statement mentioned above that intrinsic risks of gene technologies do not exist, concerning NPBTs, she stated that "… we shouldn´t make the mistake of regulating them to death as we have done with GM" (Glover, 2013).

Additional European Activities to Discuss the Legal Status of NPBTs

In Europe, NPBTs continue to be discussed vociferously. In 2012, in Alnarp, Sweden, Mistra-Biotech organized an international workshop,—*Future of Plant Biotechnology in Europe*—involving various stakeholders, to discuss the governance of NPBTs (Lehrman and Alexandersson, 2012). Experts from EPSO, EFSA, the Swedish Gene Technology Advisory Board, and other competent authorities as well as the Federation of Swedish Farmers and journalists discussed the implications of the currently unclear regulatory status of NPBTs.

In June, 2014, several meetings dealing with the legal uncertainty of NPBTs took place. A symposium in Quedlinburg, Germany, was dedicated to explaining these techniques to stakeholders from the national government, national NGOs and to farmers and representatives of the national press. The urgent need to clarify the legal status of NPBTs in Europe and the negative effects of the current legal uncertainty were expressed (Julius-Kühn-Symposium, 2014).

A similar workshop was organized in London by the Biotechnology and Biological Science Research Council (BBSRC). Members of the European Commission, NCAs, farmers and scientists from all over Europe discussed the opportunities of NPBTs, their uncertain legal status and, especially, what may be done to ameliorate this unsatisfactory situation. As a result of the workshop, a position statement of the BBSRC—to be disseminated and discussed in the UK and Europe—will be published later in 2014 (BBSRC, 2014).

The Position of the European Technology Platform "Plants for the Future"

The ETP published a statement on NPBTs—"Plants for the Future"—in which it welcomed the conclusions described above that the legal definition of a GMO does not apply to most of the NPBTs (European Technology Platform, 2012). To provide the plant and agricultural sectors with legal certainty concerning NPBTs, the ETP requested a move of the existing GMO legislation towards a more suitable science-based and transparent regulation system (European Technology Platform, 2012):

> *It is thus crucial for companies to be certain now that their investments will not be in vain and that their future products will not be subject to the uncertain outcome of politicized regulatory procedures, as is the case with GMOs.*

To provide European Member State representatives with an overview on the technical and legal interpretations of the private and public plant-breeding sectors as regards possible regulatory requirements for the individual techniques as well to show the socio-economic importance of these techniques for industry and society at large, the ETP "Plants for the Future" and New Breeding Technologies (NBTs) platforms jointly hosted an informational meeting on NPBTs, "The Future of Plant Breeding Techniques in the European Union," in June, 2014, attended by representatives from Member State national governments, the European Commission, industry, academia, and the farming community. A key message was that the European Commission's delays in clarifying the legal status of the NPBTs weakens the competitiveness of, and hinders job creation in, the EU agro-food sector. It is important that the European Commission creates favorable regulatory conditions for European plant breeders to maintain leadership in research and innovation. European policymakers are facing difficulties in modifying the existing legislation, due to the absence of consensus amongst the main political EU actors, and the strong divergence in views amongst Member States and stakeholders. This situation mainly reflects broad hostility to GMOs amongst EU citizens.

Summarizing the discussions described above, one can conclude that there is general agreement amongst experts to define a GMO on the presence of foreign recombinant DNA. When an organism does not contain recombinant DNA, it should not be risk assessed and regulated as a GMO. This view also includes techniques that involve creation of GMOs as intermediate steps, but in which the end product does not contain recombinant DNA (*e.g.* ZFN-1, -2, agro-infiltration *sensu stricto*, and reverse breeding). Furthermore, this would include other, so far not extensively discussed, techniques like fast breeding, in which an intermediate plant contains a transgene to accelerate the breed-

ing process, but which is subsequently crossed out and only the null-segregants are used for further breeding. Such an approach concerning GMO legislation would support the further development and adoption of NPBTs in Europe, but it can only be the first step towards a more flexible evidence-based and transparent regulatory system for crop genetic improvement technologies. The future regulatory framework to allow international harmonization and to avoid trade disruptions between countries and continents should take the new trait/product into account and not the technology to generate it.

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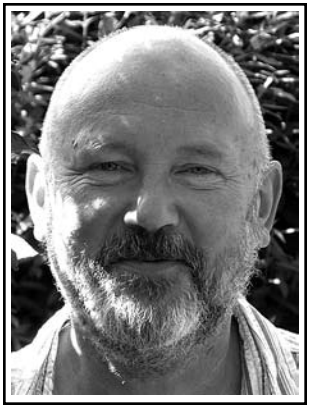

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