Levering på bestillingen ”Vurderingen af konsekvenserne for opbygning af viden og kompetencer inden for planteforædling og bioteknologi samt erhvervsmæssige konsekvenser af EU-dommen om nye planteforædlings- teknikker”


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Besvarelsen er udarbejdet som led i ”Rammeaftale mellem Miljø- og Fødevareministeriet og Aarhus Universitet om forskningsbaseret myndighedsbetjening af Miljø- og Fødevareministeriet med underliggende styrelser 2018-2021”.

Venlig hilsen

Ulla Sonne Bertelsen
An assessment of the consequences for R&D and plant biotechnology in Denmark, following the EU Court of Justice decision on New Breeding Technologies

Report from DCA - National Centre for Food and Agriculture

Date: 15 March 2019

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Background

This assessment addresses the consequences in Denmark of the EU Court of Justice (CoJ) decision for the building of knowledge and competence for teaching and research within plant breeding and biotechnology, and the consequences for the recruitment of skilled employees to the private sector. For the general effects of the court decision on Danish agriculture and plant breeding, this assessment builds on a report in Danish “Vidensyntese om nye planteforædlingsteknikker og deres effekt på dansk landbrug” (New Breeding Techniques (NBTs) and their effects on Danish agriculture, DCA report #127, (Brinch-Pedersen et al. 2018).

Summary

In contrast to the situation in many non-EU countries, the EU CoJ decision implies that new crop varieties generated by NBT’s are regarded as GM plants in the EU. Given the potential of NBT demonstrated so far, the share of NBT crops developed and grown outside EU is expected to develop fast in the coming years. In the current situation these crops have to be regarded as GMO’s when entering the EU, and in principle they will impossible to distinguish from plants with natural or induced mutations. This has a number of implications for handling, breeding, R&D and plant biotechnology in Denmark. Over the last 20 years, the R&D activities of private companies within the area of plant biotechnology/GM plants have decreased considerably, due to reluctance of companies to embark on projects associated with GM plants. Because the EU CoJ decision implies that new crop varieties generated by NBT’s must be regarded as GM plants, this reluctance will probably persist in the coming years, and private companies will probably not build their own competences on NBT’s. This will make it difficult to keep high research activities on NBT in crop plants in the public sector, which can strongly influence the building of knowledge and competences in the area. This could

again have negative effects on the teaching/training of young scientists in the area, and could also in general impede the development of research and collaborative R&D activities with the private sector. A “third way”, in which NBT’s are used in basic research to define mutation targets in combination with classical mutation strategies to utilized the mutations, might be a temporary, but not optimal, strategy that could secure research and building of competences within the NBT area.

Introduction

New Breeding Techniques (NBT’s) for targeted mutagenesis has successfully been achieved in many important crops (Brinch-Pedersen et al. 2018). Targeted mutagenesis relies on the ability of sequence directed nucleases (SDNs) to recognize and cleave a specific site within a genome and thereby create a double strand break (DSB) at the targeted site. Mutations are then induced during the repair of the DSB through the non-homologous end-joining (NHEJ) pathway of the cell. Mutations derived by NHEJ as described above are referred to as SDN1 mutations. SDN2 and 3 mutagenesis operate with different degrees of homologous recombination after DSB but is not of prime focus for current plant breeding. Currently, the most widely used SDNs for targeted mutagenesis in plants are TALENs and CRISPR/Cas9 as molecular constructs for both tools are easy to assemble and can be designed to target almost any location in a genome.

After the EU CoJ decision on NBT one overall law regulates the use of gene technology and mutagenesis by NBT in Denmark: Law on environment and gene technology. This law corresponds to the Danish implementation of the EU Directive 2001/18/EC on deliberate release of GMOs and the EU Directive 2009/41/EC on contained use of GM microorganisms. The Law on environment and gene technology is attached to a range of instructions, such as approval on production with GM plants and animals and on relevant fees.

In Annex 1B of 2001/18/EC, techniques/methods of genetic modification yielding organisms excluded from the Directive are mentioned. They are: (1) mutagenesis, (2) cell fusion (including protoplast fusion) of plant cells of organisms which can exchange genetic material through traditional breeding methods. Importantly, the classical mutation breeding methods applying radiation and chemicals to induce mutations in crop plants (Brinch-Pedersen et al. 2018) fall under the category of mutagenesis, and the mutated plants are, thus, exempted from regulation.

In addition, Denmark was the first of the Scandinavian countries to make (1) coexistence rules for GM crops in relation to conventional and organic crops and (2) a law on coexistence that came into force in 2005 (Tolstrup et al. 2003, 2007). The law contains two executive orders, one on cultivation of GM crops (defining isolation distances, cropping intervals, control of volunteer plants, cleaning of machinery and transportation measures) and the other, which is unique to Denmark, on compensation to neighboring farmers in case of economic loss due to GM admixture. Also, GM farmers and GM handlers need a license for certain education requirements within the field of coexistence in order to perform the growing, handling and transport of GM crops. Additionally, the law describes the rules for providing information to neighbors and the public about the location of the GM fields. If an unintended spread of GM material to conventional or organic neighboring fields should happen, the farmers will get compensation, which is financed by a fund into which farmers pays 100 DKK per hectare grown with GM crops.
Building knowledge and competences for the use of NBT in plant breeding

The building up of competences, i.e. education of young scientists, in this area is mainly taking place by the plant science educations at the Danish Universities, primarily University of Copenhagen and University of Aarhus. The introduction to the basic NBT techniques, e.g. CRISPR/Cas9, is and will in the future be an integrated part of the basic teaching of biological educations. There is no specialized education in plant breeding in Denmark, and students interested in this area need to specialize during their Master’s projects or in post-graduate PhD projects/education. Thus, for development of high expertise competences needed in R&D activities of private companies and in public research in the area of plant biotechnology involving NBT techniques, research activities encompassing education of Master and PhD students are required. These activities are mainly based on external funding to public research institutions. For basic plant research on model plant systems these activities will probably continue as previously. For research on crop plants, where projects are usually run in collaboration with private companies, the activities will probably come under pressure following the decision by the EU court, since the private sector will be reluctant to embark on projects associated with GM plants and, thus, also refrain from building their own competences within the NBT area. This has in fact been the situation with respect to projects involving GM plants over the previous 15-20 years. A recent Swedish survey concludes the same for Swedish research (Swedish-Board-of-Agriculture 2018).

Historically, 20-25 years ago private Danish breeding companies had considerable R&D initiatives directed towards GM/biotechnology solutions in their plant breeding strategies. This is exemplified by the establishment of a biotechnology section by DLF Trifolium at Risø National Laboratory in the late 1990’s (Risø 1999). The first GM crops in Denmark, namely glyphosate tolerant sugar- and fodder beet, were developed in the late 1980s and early 1990s by Maribo Seed, Danisco Seed and DLF-Trifolium A/S. These crops were tested in field trials in several different locations in Denmark between 1990-1999. Moreover, field trials were also conducted with glyphosate-tolerant oilseed rape developed by Aventis (now Bayer A/S) and potatoes with altered carbohydrate composition in the tubers or with resistance to virus. A total of 38 notifications for field trials were approved during this period (Eriksson et al. 2018). However, in 2001, the EU placed a ‘de facto moratorium’ on approvals of GMOs. Some Member States, including Denmark, therefore agreed to vote against any new GM approvals until an improved regulation was implemented in EU. However, regulation has still not been modernized and rests today still on 2001 technology levels (defined by EU Directive 2001/18/EC), and accentuated by the EU CoJ decision on NBT.

The 2001 moratorium reduced the number of field trial notifications in Denmark to zero in 2000-2004. From 2005–2011 there were a total of 16 GM field trial notifications, some of which were tested in multiple locations. From 2012–2016 there has only been one notification; a 50 m² field trial with cisgenic barley having increased phytase activity in the mature grain, conducted by Aarhus University in 2012-2013 (Holme et al. 2012). There have been no notifications of field trials from 2013 until now and there have been no GM field trials in Denmark since 2014. A similar process has taken place in other European countries (e.g. Brookes 2018).
Even though collaborative university projects directed at development of GM solutions for plant breeding have been considerably reduced, crop plant science in the area of biotechnology has been and still is pursued, in a non-GM context. This involves basic studies on crop plant quality, marker assisted breeding, and genomics based research. New research projects using the NBT techniques have already been initiated on crop plants, but similarly to the historic development of GM R&D, reluctance among private companies to enter this area will probably occur in the future, when considering the uncertainties around the future regulation of NBT crop plants in the EU. In order for the Danish research communities on crop plant science to keep up with developments within the area of NBT’s, it is essential that funding options for research activities are available. Both to ensure training of young researchers, but also to sustain and develop competences and knowledge among senior researchers, which is needed for proper supervision of young researchers.

Future challenges

It could be argued that the situation is in fact unchanged, and that the Danish and European plant breeding companies could go on with non-GM R&D activities as before – after all these companies have successfully managed over the last 20 years to develop new and improved crop varieties for the European market, even without the use of GM traits. However, the development of the NBT’s, in particular the CRISPR/Cas9 based genome editing techniques, is qualitatively different from the GM techniques developed 25 years ago. Firstly, NBT’s are relatively simple, but still powerful techniques to generate mutations, which are indistinguishable from mutations generated by classical mutation breeding (Brinch-Pedersen et al. 2018). As mentioned above, the latter are exempted from GM EU regulation. This will undoubtedly lead to massive regulatory issues, since it is in principle impossible to determine the origin of a mutation affecting one or few DNA nucleotides, at least without prior knowledge on the history of the mutation (Brinch-Pedersen et al. 2018). Secondly, it must be anticipated that the NBT’s will be implemented to a much larger extent than the classical GM techniques in other parts of the world, in particular in China and the US. The regulation on NBT generated crop plants is already now less stringent in these countries than in the EU when it comes to NBT events without the insertion of foreign DNA (i.e. SDN1 based) (Taylor 2019). This will lead to high pressure on the EU area with respect to control of imported seeds from these countries, again due to the problems in unambiguous detection of NBT events. In particular, it will cause problems for the Danish/European plant breeders, who could encounter a regulatory mess with respect to the danger of introducing, by accident, NBT generated traits in their breeding material.

These issues stress the need for building knowledge and competences on NBT’s in Denmark and the EU, in order to deal with detection issues and in general with NBT generated traits.

“The third way”

Currently, the use of NBT’s lead to generation of de facto GM traits, according to EU regulation. Hence, the reluctance by private industry to embark on projects implementing these techniques in
generation of new varieties will probably persist. However, the techniques could still be used with success in research projects that address the molecular genetics of crop traits. Hand-in-hand with the use of classical mutation techniques through screening of TILLING populations (Brinch-Pedersen et al. 2018), NBT’s could be utilized indirectly, although not optimally, in the modulation of crop traits. First, the precision and specificity of the NBT’s could be used to clearly define strong mutation targets, without the genetic noise that would be present in classical mutation strategies. Subsequently, the defined efficient mutations could be re-constructed/re-gained by the use of classical mutation techniques. This is a cumbersome process, but still applicable, in particular due to the development of efficient methods to build mutant (TILLING) populations and efficient methods to screen them for mutations of specific target genes (Jankowicz-Cieslak et al. 2017).

For two of the major Danish crops, barley and wheat, good TILLING population resources are already available in Europe (Krasileva et al. 2017; Szurman-Zubrzycka et al. 2018), and for most seed propagated species TILLING populations can in principle be generated, if not available. In addition, new generation sequencing techniques have made it easier than previously to screen the populations for the correct mutations. Furthermore, the use of efficient molecular markers has made the transfer of mutations into elite varieties more efficient.

This “third way” is probably the only feasible way to go for the coming years in the EU, since a possible change in the EU regulation, which would make the SDN1 techniques, i.e. NBT without insertion of foreign DNA, exempted from GM regulation, in any case must be expected to take several years. Evidently, in order to be efficient, this strategy requires funding support. Both to build the capacities to directly support private R&D and for the development of competences and skills through training of young PhD students and researchers, who will be available both for public research activities and for R&D activities in the private sector.

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