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MEETING REPORT

Separate product from process: framing the debate that surrounds the potential uptake of new breeding technologies

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In July 2017, the Society for Experimental Biology hosted a symposium on new breeding technologies (NBTs) in Plant Sciences at the University of Gothenburg. This report summarises the major outcomes of this meeting. Delegates discussed both the technical and policy aspects of NBTs, with a focus on CRISPR-Cas9 gene editing. While NBTs have the potential to revolutionise the future generation of new crop varieties, a major outcome of the meeting was the acceptance that we are at a critical juncture regarding the policy decisions that will govern the future use of plants generated using these technologies. This meeting report offers insights into how scientists can frame their input into the upcoming debate, as well as a discussion about what is technically possible with NBTs.

The use of the CRISPR-Cas9 system for precision genome editing (GE) has been regularly described as a 'game-changing technology' that allows a more precise targeting of DNA to induce specific nucleotide variations (Belhaj et al. 2015); however, the use of GE in plants for the production of food or feed still faces an uncertain regulatory future. This follows on from a long-standing public distrust of genetically modified organisms (GMOs), an opinion predicated from, amongst other things, controversial yet discredited scientific studies and public miscommunications. Public unease with this technology has guided government policy on the permitted uses of the products of GMOs, such that growth of these crops is now restricted throughout most of the European Union (EU). The plant science community stands at an important crossroads at which the future uses of plants generated by GE technologies will be decided.

This issue was a primary concern for the group of 70 international delegates who met in Gothenburg, Sweden, for the Society for Experimental Biology Plant Section Symposium on New Breeding Technologies in July 2017. This meeting was organised in collaboration with the Global Plant Council, GARNet, the Scandinavian Plant Physiology Society and the Australian Society of Plant Scientists, and brought together experts on both the regulatory and technical aspects of using CRISPR-Cas9, the most popular type of GE technology. This special issue of Physiologia Plantarum includes articles from meeting participants on a variety of topics that were discussed at this meeting.

Outdated regulations and delayed decisions

There have been continued delays with the EU decision that will confirm the Europe-wide regulatory status of crops modified using GE technologies (Nature Editorial 2017). The problems caused by this delay were highlighted by Joachim Schiemann (Julius Kühn-Institut) and Petra Jorasch (European Seed Association) who, speaking during the session entitled 'Policy and Legislative Implications for Use of Gene Editing Technologies', stated that their own interactions with academics and businesses have confirmed that the current uncertainty is inhibiting innovation. Researchers are unable to make long-term plans to develop products from GE plants as they do not know whether the growth of these plants will be permitted in the future.

In the same session, Piet van der Meer (Ghent University, Free University of Brussels) provided an overview of biosafety legislation, including a historical discussion regarding which organisms should be subject to risk assessment. He questioned whether this should include all conventionally produced organisms or only 'novel' organisms, and pondered the follow-on assessment of what exactly defines 'novelty'? Looking to the future, it could be argued that GE crops might be covered by existing regulations on the modification process, given that they are often produced by *Agrobacterium*-mediated gene transfer; however, as the final GE plant is often indistinguishable from those generated by conventional

mutagenesis-driven breeding, should they really be subject to different regulations?

The question remains whether these crops are 'novel' and should be regulated differently. The current EU definition of a GMO states that it is *an organism in which the genetic material has been altered in a way that does not occur naturally*. As this definition relies on material containing a level of novelty resulting from the combination of genetic material from sexually incompatible species, Piet van der Meer concluded that a crop plant containing only a small nucleotide change obtained via GE should not fall within the remit of this legislation. Policymakers around the world are currently battling with these types of technical and semantic descriptions, and an overview of the ways in which different countries have tackled these arguments was discussed at length during the meeting (see section below).

Focus on product not process

The complexity of the future regulatory environment was summarised by Petra Jorasch, who highlighted the different types of GE that might need to be legislated upon (Fig. 1). During his presentation, Joachim Schiemann presented the key questions that will be deliberated by EU decision makers: 'Are gene edits different from those that might occur during natural processes'? and 'Are edits distinguishable from those that occur spontaneously in nature or by conventional cross breeding'? Schiemann reported that, in May 2017, the European Academics Science Advisory Committee (EASAC) recommended that the products of GE technologies that do not contain DNA from an unrelated organism should not fall under the scope of current GMO regulations (EASAC 2017). In addition, the EASAC report states that, where the method of production is fully transparent and no novel product-based risks are identified, the products of GE should be regulated on the basis of the agricultural trait modified, rather than its method of production.

A common theme of discussion during the meeting considered how regulations might keep pace with future technological changes. Barry Pogson (Australian National University) highlighted that many current GMO regulations are 20 years old and are no longer fit for purpose given the technological advances that have occurred over that time period. Ruth Bastow (Global Plant Council) asked the group to not only consider how regulations can be retrofitted to new technologies, but also how scientists can set the agenda regarding the ways in which their future research might be regulated. Piet van der Meer challenged researchers to go to regulators with fully documented descriptions of the crop varieties they have generated and ask them to make a de novo judgement on what has been done. This would put the onus on regulators to keep pace with the science and not, as for the current system for GMOs, limit researchers by guidelines that largely legislate on the method of production rather than the final outcome. Attila Molnar (University of Edinburgh) provided an outstanding example of this approach; he contacted 'Science and Advice for Scottish Agriculture' and asked them to consider a CRISPR-Cas9 technology developed in his laboratory that has the potential to generate virus-resistant crops.

Science communicator Craig Cormick (ThinkOutsideThe) led an extended discussion regarding the importance of scientists engaging with the court of public opinion to discuss the use of GE technology. As the public can have significant influence on future political policy, he posited that these interactions do not just have prosaic value; therefore, persuading the public that a particular technology is safe might be an important avenue for influencing politicians. The early framing of the debate is key when influencing public and political will, and scientists need to learn from mistakes that were made in early discussions concerning the use of genetic modification for crop improvement. Cormick's own research showed that there are outliers in any general population who will either always support or oppose the technology; however, there is also crucially an enormous middle ground of people who can be convinced to accept the use of technology, if the argument is properly framed. On the debate surrounding the regulation of GE crops, Cormick suggested that focusing on *product not process* might be a useful strategy. Much of the anti-GM attitude results from an opposition to a process that is considered 'unnatural'; therefore, in this case it might be a useful strategy to provide the explanation that GE is simply an evolution of techniques that have been used over millennia to apply changes to genomes, and focus on what can be achieved.

The global regulation of GE crops

Plants generated by GE are providing countries with many challenging decisions regarding their legislation. The many types of modifications that can be achieved using GE have led researchers to call for the legislation of the final product, which may be indistinguishable from conventionally bred cultivars, not the process used to develop it. In this special issue of Physiologia Plantarum, Barry Pogson introduces the Global Plant Council statement on the role of GE in plant science and agriculture, which was developed during and after the meeting by an international team of experts. Staffan

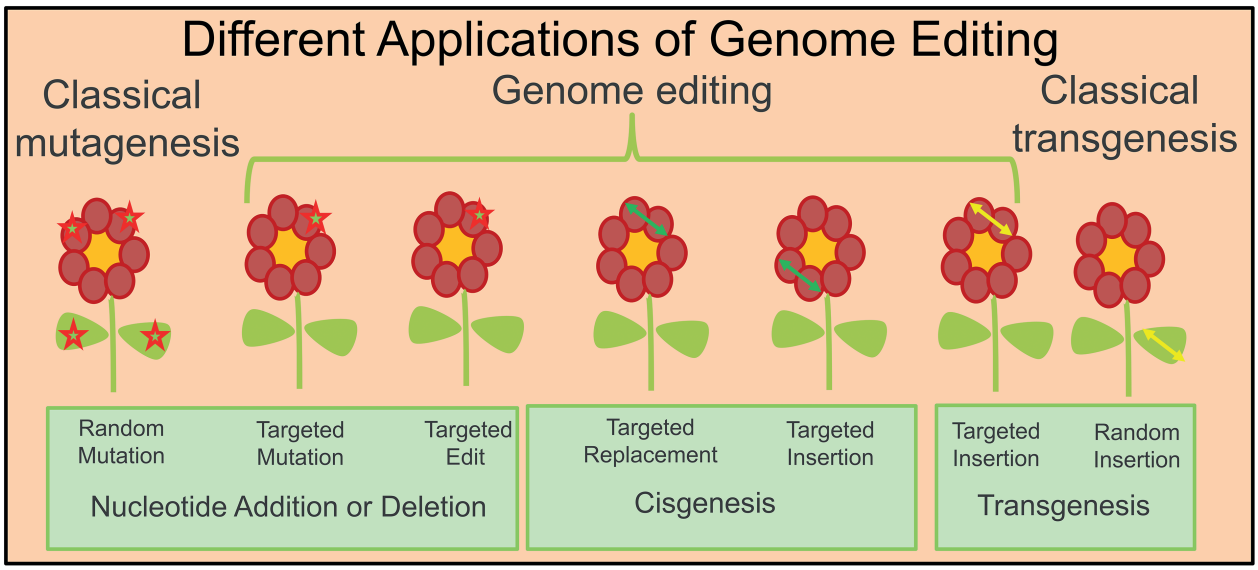

Fig. 1. Types of genome manipulation used in plant breeding and cultivar development. Image modified from that kindly supplied by Petra Jorasch, European Seed Association (www.euroseeds.eu).

Eklöf, an administrative officer at the Swedish Board of Agriculture, told the conference how he and his team had worked with scientists to interpret whether GE plants are regulated by the current EU legislation on GMOs, determining that plants carrying foreign DNA are regulated, whereas those containing mutations that could have occurred 'naturally' are not. This promising outcome in Sweden has been met with interest by policymakers and scientists alike, both in Europe and around the world (for a more in-depth view of the regulatory landscape in Europe and Sweden, see associated articles in this special issue of Physiologia Plantarum). Evidence of the permissive Swedish policy on GE crops was for all to taste at the conference dinner, where a meal was served that included gene-edited cabbage, supplied by the meeting's local host Stefan Jansson (Fig. 2).

Legislative decisions around GE technologies are still in flux. Pogson described the complex story in New Zealand, where a government-owned research institute, Scion, asked the Environmental Protection Authority whether GMOs created using NBTs *Zinc-finger nucleases* (ZFN-1) and transcription activator-like effector nucleases (TALENs) were regulated by the current GMO legislation. The EPA determined that these technologies were similar to other techniques excluded from the GMO regulations, and were therefore not regulated by GMO legislation; however, an appeal taken to the New Zealand High Court led to the overturn of this decision based on a different interpretation of the exclusion in question. Pogson also gave an overview

Fig. 2. Gene-edited cabbage was on the menu at the conference dinner (Source: @GARNetweets).

of the situation in Australia, where the Food Standards Australia and New Zealand recently consulted a wide range of people about whether the Gene Technology Act 2001 is still appropriate for use, or whether this 16-year-old legislation should be updated in the light of more recent technologies. Submissions were received from over 600 institutions and individuals,

and the Australian Government is currently consulting a range of stakeholders regarding making amendments to their GMO legislation to incorporate GE technologies (OGTR 2017).

Perhaps, the most complicated regulatory system for GE crops of those covered at the meeting is that of the United States. Certain new GE crops are not subject to the previous regulations that have been applied to GMOs, including a waxy maize cultivar and a mushroom that resists browning (USDA 2016a, 2016b). However, as the biotechnology field is regulated through a mix of policies administered by three different bodies; the US Department of Agriculture (USDA), the Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA), it is challenging to precisely define which agency will provide decisions on new crop species generated by different methods. Wayne Parrott (University of Georgia) explained how all of these agencies can regulate GE crops in particular circumstances. Whereas the FDA is currently formulating new policies that will affect the future regulation of gene editing, the USDA has, since the Gothenburg meeting, dropped proposed rule changes that would have given it regulatory powers over GE crops (USDA 2017). This news fits with Wayne Parrott's optimistic conclusion that, as genome changes in GE crops could have occurred naturally, they are unlikely to be overly affected by negative regulatory decisions, at least for now.

Decisions on GE legislation are still largely in flux in many countries. As outlined above, the EU and many individual countries are yet to make a definitive statement about the regulation of technologies such as CRISPR-Cas9, although Argentina and Canada have said that they will regulate crops on a case-by-case basis. These decisions are complex, involving the competing interests of many interested parties, the views of scientists, anti-GMO lobbying groups, farmers and the general public, and potential international trade partners must all be considered, posing huge challenges for policymakers around the world.

Tricks from the bench: technical aspects for preparing GE plants

The workshop was also planned to provide information to delegates regarding the technical challenges that exist in the preparation of GE plants, specifically when using CRISPR-Cas9 technology. Wayne Parrott highlighted that a major technical advantage of using CRISPR-Cas9 was in the targeting of entire gene families, which has proven extremely challenging and/or laborious when using RNA interference technology or insertional mutants. Interestingly, it emerged that, although Arabidopsis might remain a favoured research organism, it is probably not an ideal plant model when using CRISPR-Cas9 technology. Wendy Harwood (John Innes Centre) reported that her research group has often found GE in monocots to be more straightforward than in dicots, particularly during attempts to generate non-mosaic plants with germline edits. She discussed their successes using CRISPR-Cas9 to generate edited plants in barley, wheat, brassicas and tomato, highlighted that the technology is working very well in their hands. They found that the main bottleneck when generating GE plants arises from the poor efficiency of plant transformation, which remains a significant challenge (Altpeter et al. 2016).

Laurence Tomlinson works with Jonathan Jones at The Sainsbury Lab, Norwich, and provided an overview of the troubleshooting that their laboratory has undertaken in order to optimise the use of CRISPR-Cas9, particularly in Arabidopsis. The highlights of her presentation are listed in Box A, and Laurence has made her entire talk available online (http://globalplantcouncil.org/initiatives/new-breeding-technologies).

Box A
Laurence Tomlinson, The Sainsbury Laboratory, Norwich

(1) They target Cas9 to a locus of interest using two guide RNAs. Ideally, both of these RNAs should be targeted to 20 nucleotide sequences near the 5′ region of a gene (Cermak et al. 2017).
(2) They find that including the dinucleotide 'GG' sequence at the 3′ end of the guide RNA means it is more effective at inducing sequence-specific mutations.
(3) For GE in dicots, they found that using the PolIII promoter provides an optimal expression of guide RNAs from a single transcript.
(4) They use the RPS5 promoter to express *Cas9* in Arabidopsis (Tsutsui and Higashiyama 2017). Importantly, they found that the commonly used constitutive 35S promoter is not appropriate for effective germline editing, as it has a low activity in the Arabidopsis embryo sac.
(5) Although Laurence did not recommend any particular software tools for designing guide RNAs, she obtained the best results using a mixture of automated and manual guide RNA design.

(6) She strongly advised testing the function of the guide RNAs before undertaking time-consuming plant transformations. Their laboratory's favoured method for pre-testing guide RNAs is the T7 endonuclease assay.
(7) The laboratory uses the FAST marker system to check for successful transformations (Shimada et al. 2010). This removed the need for one generation during the process of identifying homozygous edited plants.

Attila Molnar and Mariette Anderson (Swedish University of Agricultural Sciences, SLU) provided updates on each of their laboratory's work, in which they used CRISPR-Cas9 to investigate different aspects of plant biology. Mariette Anderson has worked with an industrial partner, Lyckeby, to develop a potato variety that has reduced levels of amylose. This was achieved by successfully editing four copies of the *GBSS* gene (Box B; Anderson et al. 2017). Attila Molnars' group used CRISPR-Cas9 to introduce a single base deletion into the Arabidopsis *eLF(iso)4E* gene, which subsequently confers viral resistance (Box C; Pyott et al. 2016).

BOX B
Mariette Anderson, Swedish University of Agricultural Sciences, SLU.

(1) As potato breeding can be very complicated and time-consuming, CRISPR-Cas9 is a promising tool for generating homozygous tetraploids.
(2) They used two guide RNAs to target the *GBSS* gene, which is involved in starch synthesis.
(3) They found an improved mutation rate when they used the potato U6 promoter to drive guide RNA expression.
(4) Protoplast transformation allowed the regeneration of transgene-free plantlets.
(5) Only successful editing of all four copies of *GBSS* resulted in the amylose-free phenotype.
(6) They identified plants with knockouts in all four alleles in 2% of regenerated plants.
(7) Surprisingly, they found a high proportion of full plasmid insertions at the GE target sites. Plantlets containing this insertion were discarded.

Box C
Attila Molnar, University of Edinburgh.

(1) They targeted elongation factor *eLF(iso)4E* in Arabidopsis, which is necessary for Potyvirus infection.
(2) They used the T7 endonuclease assay to identify plants that had been successfully edited.
(3) Growing the Arabidopsis plants at higher temperatures sped up the time between generations.
(4) In the T2 generation, they found a 59% mutation frequency in non-transgenic plants.
(5) Homozygous GE mutants are resistant to Turnip Mosaic Virus and importantly featured no growth or yield penalties.

Johannes Stuttmann (Martin Luther University of Halle-Wittenberg) concluded the meeting with a description of his work using GE to investigate the defence response in Arabidopsis. In addition, he described a set of dicot GE vectors developed by his group and made available to the community (Ordon et al. 2016). These plasmids were generated using the golden gate cloning system and allowed for the facile multiplexing of four guide RNAs. When used successfully, these guide RNAs allow for the targeting of a broader range of sequences, thus increasing the chances of obtaining a successful deletion.

Summary

The NBT symposium focused on meeting the technical and regulatory challenges associated with new GE technologies. Progress is being made more rapidly in the successful application of techniques such as CRISPR-Cas9 than in making the legislative decisions regarding their use. An important piece of advice that emerged from this meeting is that scientists need to take the lead. They should provide guidance for policymakers and initiate discussions that result in the development of informed and appropriate legislation regarding the use of these revolutionary technologies, which have the potential to significantly enhance sustainable food, fibre and energy production in the future.

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