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EDITORIAL

Next-generation precision genome engineering and plant biotechnology

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In recent history, mutagenesis, selection, and breeding of crop varieties have significantly improved agricultural traits and increased yields, for example, the renowned Green Revolution work on control of plant stature. Early in the 1990s, transgenic technologies were transformative to commercial crop agriculture by adding foreign DNA to improve plant traits. The transgenic methods traditionally used for crop improvement have many limitations, including random insertions, potential silencing, and varied gene expression. Transgenic methods also do not exploit the full potential of the genetic repertoire of the plant species targeted for improvement. Moreover, there remain dogged public concerns on consuming food from transgenic plants, "GMOs," especially those with genes from distantly related organisms. Nonetheless, crop improvement and basic research have greatly benefitted from targeted modification of plant genomes. In this special issue (SI), which includes eight reviews, two opinion papers, and four original articles, the development of targeted genome editing approaches in higher plants is discussed from various perspectives, including research, intellectual property, regulatory affairs, and consumer acceptance issues.

- Efforts to alter plant genomes in a site-specific manner use: (a) homologous recombination-dependent gene targeting, (b) recombinase-mediated site-specific gene integration, (c) oligonucleotide-directed mutagenesis, and (d) nuclease-mediated site-specific genome modifications (Cardi and Stewart 2016). Very recently, and at an incredible pace, the CRISPR/Cas9 system has been harnessed for genome engineering in diverse eukaryotes, including plants (Luo et al. 2016; Paul and Qi 2016; Schaeffer and Nakata 2016; Steinert et al. 2016). As thoroughly discussed in the SI articles, CRISPR/Cas9 is powerful inasmuch as it is facile and inexpensive to engineer single or multiple target genes, or for interrogating gene functions at the genome-wide scale using libraries of single guide RNAs to target genes with Cas9. The site-specific double-strand breaks generated by Cas9 are repaired by the imprecise non-homologous end-joining machinery or the precise homology-dependent repair system. Harnessing the cell's repair machinery enables the researcher to control the genetic information at the single-base level to generate functional gene knock-outs, knock-ins and create landing sites for genes. Most of the genome engineering applications in plants use the *Streptomyces pyogenes* Cas9 endonuclease, SpCas9, but recent work has identified other Cas9 nucleases with lower off-target activities or which generate staggered cuts for subsequent manipulations of the genome.

CRISPR/Cas9 is emerging as a plant genomics and biotechnology research juggernaut. However, key advances

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are still required to maximize the potential of CRISPR/Cas9 for functional genomics and targeted crop improvement. Such advances include development of: (1) efficient gene editing systems that enable gene replacement, fusions, and stacking, (2) Cas9/sgRNA ribonucleoprotein complex (RNP) systems for efficient, DNA-free genome engineering (Kanchiswamy 2016), (3) methods for transient delivery of these RNPs to obviate the need for stable transformation and regeneration, and (4) CRISPR/Cas9-based trait discovery platforms in target crop species. Furthermore, catalytically inactive Cas9 fused with activation or repression domains are useful for transcriptional regulation, modification of epigenetic status, or for imaging purposes, thereby, opening new possibilities for genome-wide functional interrogation of genes. We anticipate that as CRISPR/Cas9 approaches mature, they will be rapidly integrated into practical plant breeding schemes. We anticipate feedforward synergies whereby genomics and plant breeding facilitate more precise characterization of crop genetic resources as well as accelerate the development of new cultivars (Nogue et al. 2016).

The extent of true innovation and adoption of genome editing will depend on a clear and accessible patent landscape and a known and navigable regulatory framework. To avoid another GMO-like thicket, it is important that small and medium-sized enterprises be able to commercialize products in a transparent fashion. Furthermore, scientists and developers should not shy away from discussions among stakeholders and the concerned public. Genome editing patents have taken several different licensing routes—from broad to exclusive licenses. The CRISPR intellectual property path is, at the date of publication, unknown; and therefore, commercialization appears lagging behind research (Schinkel and Schillberg 2016).

GMO regulations are expensive, slow, and asynchronous approvals among countries have resulted in only a few major global companies being able to commercialize products. Genome edited crops should, in at least some cases, be treated as conventional bred, especially if the traits have positive regulatory precedence. Currently, two models of regulation exist, product-based (mainly in North America), and process-based (in Europe and most of the rest of the world). However, CRISPR/Cas9 is not a single technology or platform but rather a molecular toolbox capable of making an expanded range of genomic manipulations. Therefore, the need for regulation depends on the use and ultimate goal, which are largely determined by the nature of the mutation, the target plant species and trait, ecosystem, and the societal impact. If gene-edited plants will be evaluated as a product, we will have the means to alter the genome of the plant species and produce improved varieties that carry no foreign DNA, and more importantly, are indistinguishable from conventional varieties. A comparison of regulatory frameworks in various countries and a possible alternative regulatory system in the EU incorporating a science-based, neutral and experience-based decision making are discussed by Sprink et al. (Sprink et al. 2016). Finally, regulation issues are somehow mutually connected to consumer acceptance. After considering the bottlenecks for consumer acceptance of genome edited crops, Ishii and Araki stress that people should be informed of the benefits of various plant breeding techniques (Ishii and Araki 2016). Furthermore, trust in the relevant regulatory authorities, transparency and dialogues are vital to implementation. Finally, the SI includes four original papers reporting latest developments for delivery and use of CRISPR/Cas9 in plant cells—both in plant models and crops (Zhang et al. 2015; Eid et al. 2016; Subburaj et al. 2016; Zheng et al. 2016).

The transformative technology of CRISPR/Cas9-based genome engineering will reshape the future of agriculture by increasing our ability to use the genetic repertoire of the targeted crop species and boost its resistance to pests, improve yields and consistency of productivity, increase tolerance to abiotic and biotic stresses, and increase nutritional value of food. However, how to regulate and implement this powerful technology to make advances for the common good of humanity remains to be determined. Future agricultural efficiency depends as much on policy, patents, and regulation as it does on science and innovation.

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Compliance with ethical standards

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

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