The future of CRISPR technologies in agriculture

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Conventional plant breeding is unlikely to meet increasing food demands and other environmental challenges. By contrast, CRISPR technology is erasing barriers to genome editing and could revolutionize plant breeding. However, to fully benefit from the CRISPR revolution, we should focus on resolving its technical and regulatory uncertainties.

Modern agriculture exemplifies how research and technology can come together to improve crop yield and quality. Although conventional breeding is now much faster than it was 50 years ago, it is likely unable to keep up with the increasing demand for food and with the global environmental challenges that we face. As long as plant breeding remains fully dependent on finding plant populations with sufficient variation and on conventional crossing approaches to introduce traits into target crops, time and resource limitations to crop improvement will persist. CRISPR technologies could surmount these limitations and accelerate plant breeding beyond what was previously imaginable. Although sometimes subjected to exaggerated headlines, the use of CRISPR in agriculture should be best considered as simply 'a new breeding method' that can produce identical results to conventional methods in a much more predictable, faster and even cheaper manner.

CRISPR technology has already revolutionized research in the life sciences since it was first applied in 2012. CRISPR–Cas9 and CRISPR–Cpf1 are the best studied and most widely used CRISPR systems in plants[1,2]. CRISPR reagents are delivered into plant cells as DNA, RNA or protein–RNA that assemble into an active site-directed nuclease (SDN) and cleave targeted DNA sequences to generate double-strand breaks (DSBs)[3]. Plant cells can repair these DSBs either by untemplated annealing of the DNA ends (known as SDN-1 editing), which often causes small sequence changes and generates gene knockouts, or by integrating a different piece of DNA at the DSB to generate short sequence replacements of less than 20 nucleotides (SDN-2 editing) or longer sequence replacements or insertions (SDN-3 editing).

A tool for crop improvement

Promising uses of CRISPR tools in agriculture have already been shown in crop plants such as wheat, corn and tomato. SDN-1 in wheat is being used to provide resistance against the devastating powdery mildew fungus, whereas more challenging, complex traits have been altered in corn and tomato. In maize, application of SDN-3 to the *Argos8* (also known as *Zar8*) gene promoter conferred constitutive expression of the endogenous gene and resulted in improved maize yield during drought stress[3]. SDN-1 was used to generate mutations in the regulatory regions of tomato yield genes, which increased their genetic variation and boosted yield in a fraction of the time it took to achieve a similar result through conventional breeding approaches[4].

The next generation of CRISPR tools being developed for agriculture goes beyond DSB-based editing in leveraging the ability of CRISPR systems to specifically target DNA sequences. Following deactivation of the Cas9 and Cpf1 nuclease domains, which are distinct from the DNA recognition domains, these DNA-targeting proteins can be fused with various enzymatic activities. For example, the fusion of a deaminase to deactivated Cas9 enables direct conversion of a single DNA nucleotide into another independently of DSB formation[5]. Current versions of such base editing are limited to C-to-T or A-to-G conversions and to narrow sequence-editing windows. These limitations are likely to be overcome within a year as research on base editing intensifies. We can expect an even broader suite of CRISPR tools in the near future as deactivated Cas9 and Cpf1 are further utilized to visualize specific genomic loci, to directly regulate gene transcription and to induce targeted epigenetic modifications.

Breakthroughs still needed

Base editing and SDN-1 boost plant breeding by producing extensive and precise point mutations in an existing plant population. Another crucial source of variation in plant populations comes from inversion and translocation of genomic sequences, which can result in protein domain swaps, altered gene regulation and even new gene functions. This can be achieved in different scales with SDN-2 or SDN-3, although the low frequency of homology-directed repair (HDR) in plants presents a

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doi:10.1038/nrm.2018.2
Published online 31 Jan 2018

considerable technical hurdle for its precise and efficient application. In many plant cell types, non-homology end joining (NHEJ) is the dominant DSB repair mechanism and results in imprecise genome alteration or in rapid DSB repair. HDR currently requires delivery of repair templates in large amounts to outcompete NHEJ, which has always been a challenge. The delivery of DNA repair templates for HDR has been improved by using DNA replicons (deconstructed geminiviruses), but there is no clear indication of a more notable forthcoming breakthrough. A broader effort is required for improving SDN-2 and SDN-3, for example, using HDR boosters or NHEJ inhibitors, which are currently being explored in mammalian cells.

Another substantial bottleneck to the implementation of CRISPR tools in agriculture is effective delivery of CRISPR machinery to the right plant cells and subsequent regeneration of viable plants. Traditional tissue culture approaches remain the preferred method for delivery, but these are lengthy, labour-intensive and prone to producing random somatic mutations, which together reduce the efficiency gain provided by CRISPR tools. Moreover, many crop species are recalcitrant to regeneration through tissue culture. Novel delivery methods need to be established in order to achieve high efficiency genome editing in plants. These could include use of regeneration boosters to enable tissue culture in recalcitrant species or even direct delivery to plant apical meristems or pollen grains to obtain edited plants without the use of tissue culture.

Changes on the horizon

Exciting practical applications of CRISPR tools for sustainable agriculture can now be envisioned. The relatively low cost and ease of use of CRISPR tools are spurring innovative research in academia and in companies of all sizes, essentially democratizing crop-trait development. It is now feasible to consider performing research dedicated to niche crops that have typically been neglected. Moreover, instead of expanding the environmental and disease tolerance of already domesticated crops, plant species that are already well adapted to different environments could be domesticated with high-value traits. For example, the start-up Arvegenix is using CRISPR tools to improve oil and meal quality in pennycress. The goal is to make pennycress both a cover crop that is used between typical growing seasons and a product similar to canola for the oil and feed markets. If successful, farmers would benefit from increased income while still reaping the benefits of using sustainable cover crops. Other examples will likely come from using CRISPR tools with synthetic biology for targeted evolution of endogenous genes to generate new beneficial functions, such as the ability to fix atmospheric nitrogen, which currently is found only in legumes.

Avoiding past mistakes

The biggest potential pitfall for the use of CRISPR technologies in agriculture is not scientific but public acceptance and government regulation. The majority of expected uses would produce 'nature-identical' traits, that is, traits that could also be derived by conventional plant breeding. Although it is true that CRISPR technologies could be used for different purposes, for example, to introduce exogenous genes, I foresee this would be quite limited. I also believe such uses can easily be distinguished so that nature-identical CRISPR applications would not need to be equated with genetically modified organisms. Despite this, confidence in applying CRISPR tools in agriculture remains limited owing to the uncertain global regulatory environment. Overcoming this will require a political willingness to establish a clear position on CRISPR technologies and striving for some form of consistency among countries.

The next step together

As scientists, we should not discount the challenge of providing transparency to CRISPR breeding methods, which would be crucial for gaining public trust and influencing regulatory policies that are evolving to govern the use of CRISPR technologies in agriculture. What has been achieved so far with CRISPR technologies is just the tip of the iceberg. A sustainable future for agriculture can now be imagined using this new powerful plant breeding tool. With that comes a responsibility to continue to resolve both the scientific and public concerns regarding its usage.

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Acknowledgements

The author is supported by grants from the National Natural Science Foundation of China (31788103) and the National Key Research and Development Program of China (2016YFD0101804).

Competing interests

The author declares no competing interests.