The CRISPR Journal
Volume 4, Number 3, 2021

DOI: 10.1089/crispr.2021.29130.kad

The CRISPR Journal

FIRST CUT

A Field Day for Gene-Edited Brassicas and Crop Improvement

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A new report in this issue from a U.K. plant science group offers a welcome path forward for crop research in the field.

Similar to other branches of life sciences, plant researchers were quick to recognize the transformative nature of CRISPR-Cas9–mediated gene editing, and this technology (along with its ever-expanding repertoire of variants and enhancements) has been gleefully embraced by the international plant sciences community. CRISPR's flexible and user-friendly technology is seen as central to accelerating plant breeding and crop improvement, which in turn will underpin global food security.

In particular, meeting the targets of the United Nations 2015 Sustainable Development Goals—specifically SDG2's headline aim of ''zero hunger'' (or the full goal, ''end hunger, achieve food security and improved nutrition and promote sustainable agriculture'') by 2030—is a daunting task that gets harder every day. So, from one perspective, CRISPR technology could be seen as arriving just in the nick of time. Certainly, it will play a vital role in developing new crops that are better adapted to our changing climates, less impactful on the environment, and deliver improved nutrition.[1]

However, although the adoption of CRISPR tools is now ubiquitous in molecular plant sciences research, propelling advances in our basic understanding of many aspects of crop physiology and stress tolerance, there is still a gulf between these laboratory-based findings and what might be termed ''the real world.'' This is important, because the closeted research environments we grow our pampered modified plants in bear no resemblance to the unpredictable and increasingly variable climates crops routinely face in the field. Ultimately, if we want our plant research to lead to some positive societal outcome, making a contribution to SDG2, then we need to discover whether genetic changes engineered in the laboratory will actually work in the field. Will they be robust enough to continue to deliver benefits, even under a gauntlet of environmental factors?

For those outside the plant sciences sphere, it might seem logical if not mandatory for laboratory discoveries to transition to field evaluation, but this is where wider issues (such as the regulation of transgenic crops) come into play. These are themselves something of a hot (genetically modified [GM]) potato. And although nowadays it seems almost obligatory to conclude the write-up of any new discovery as being a ''game-changer'' in delivering food security, etc., in reality, very few fundamental studies seem prepared to put their money where their (or others) mouths might be and confirm the potential of their research by carrying out field trials. This is crucial—you cannot eat potential!

Now, of course there are many reasons for research not to transition from the glasshouse to the field, including less funding for what is still viewed dismissively as ''applied'' research, as well as regional differences in how straightforward (or not) it is to carry out an environmental release of plants that have been genetically modified. Moreover, there is a lack of consistency, at least from various regulatory agencies, as to whether or not genome editing should be classified as either GM or a form of mutagenesis (or both). This is a particular barrier to translation in the European Union (EU), which has some of the least enabling regulations when it comes to genetic modification. It was surprising and frankly disheartening that

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FIG. 1. Environmental release and field trialing of GE and GM crops in the United Kingdom. (**A**) Plots at the Rothamsted experimental farm of GE, GM, and GE+GM Camelina, June 2020. Note that GE lines are classified as GM and, as such, are grown within one of the dedicated GM trial areas on the farm. Right panel—large plot of fully flowering GM Camelina on the Rothamsted experimental farm, June 2018. The profound difference between controlled glasshouse conditions and those in the field can be exemplified by the variable environmental conditions and, for example, the presence of insects (**B**). It is for these reasons it is vital to validate laboratory results aimed at crop improvement in the field. GE, gene edited; GM, genetically modified.

a review published last year listing all known examples of genome-editing field trials produced a rather short list, the bulk of which were run in China on rice.[2] Clearly there is a bottleneck in the translation of much cutting-edge plant sciences research.

Field Effect

Because of (or perhaps despite) all the mentioned reasons, a new article by Neequaye et al.[3] in Lars Østergaard's group at the John Innes Centre, in Norwich, United Kingdom, in this issue of *The CRISPR Journal* should be applauded, for the authors have opted to take that road less traveled and evaluate their CRISPR-Cas9 edited *Brassica oleracea* plants in the field.

In this study, Neequaye et al. used gene editing to generate loss-of-function mutant alleles of the transcription factor MYB28, which is a key regulator of aliphatic glucosinolate biosynthesis. In doing so, the authors hoped to change the wider balance of the metabolites present within the plant, to better understand how to modulate these health-beneficial compounds without impacting crop growth. Gene editing in plants can be made more complicated by both polyploidy and neofunctionalization, meaning that the authors had to seek the plants with useful (disruptive) edits in the different MYB28 paralogues present in *B. oleracea*. Having identified mutations in two of the three MYB28 paralogues, the stage was set for further investigation: at this point, the study diverges from the majority of CRISPR-mediated mutagenesis stories by opting to take the evaluation of the plants into the field.

As noted earlier, environmental release of gene-edited plants is subject to regulation, the complexity of which is dependent on jurisdiction defined by location (national and regional). In the United Kingdom, a previous field release in 2018 of CRISPR-edited Camelina was classified by the U.K. regulator as being outside the scope of the European legal definition of a genetically modified organism, as such, these plants did not need approval under the mechanism used to approve GM field trials. However, during that field release in 2018, the European Court of Justice (ECJ) ruled that gene editing was a form of genetic modification, at least in the context of the definition first used by the EU in 2001, eliciting strong reactions from many quarters.[4]

The ECJ ruling of July 25, 2018, trumped the earlier U.K. ruling as to the status of gene editing, meaning that these edited Camelina metaphorically transformed into GM plants overnight.[5] Thus, Neequaye and colleagues would need to apply for ''Ministerial consent'' to grow their edited *B. oleracea* plants in the field, since they were now considered to be GM. This was even though the authors clearly demonstrated that the transgene carrying the Cas9, gRNAs, and selectable marker was absent from their plants. All that remained was the tiny edits in the MYB28 genes that they had engineered.

For many, obtaining such consent would be viewed as too cumbersome—the process takes months and requires mandatory public disclosure of the intention to run a GM field trial, which can then generate a response from anti-GM campaign groups. Similarly, there is the obvious requirement of not only having access to a suitable experimental farm (or similar) but also a commitment to actually perform experiments in the field, which is quite different from the pristine laboratory environment (see Fig. 1 for visual example).

It is to the immense credit of the John Innes group that they actively embraced the transition from laboratory to field, validating their hypothesis on the role of MYB28 on the regulation of glucosinate content in Brassicas, and also obtaining a comprehensive RNAseq data sets for field-grown WT and *myb28* plants. Perhaps most importantly, it confirmed the commitment of U.K. plant scientists to continue to deliver research outcomes that are both academically exciting and of wider relevance to society.

Although the United Kingdom is currently adhering to the 2018 ECJ ruling, exit from membership of the EU in January 2021 presented an opportunity to diverge from the assertion that gene editing is a form of GM. To that end, the U.K. government recently concluded a 10-week public consultation on the future regulation of the new genetic technologies, with the outcome eagerly awaited this summer.

In the future, studies such as that highlighted here will hopefully not have so many impediments in the translation of their fundamental research. By reducing that burden, we might break the bottleneck and see many more field trials as researchers get to test their innovations in the real world. This can only be a good thing, especially if we want to use the phenomenal power of gene editing to help meet our global challenges.

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DOI: 10.1089/crispr.2021.29128.sma

CRISPR and Chromothripsis: Proceed with Caution

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A new study in* Nature Genetics *is a timely reminder of the need to address on- and off-target genome rearrangements in CRISPR gene editing.

CRISPR-Cas9 has revolutionized all aspects of biology, making it easier and faster to generate authentic models of disease through its precise programmable nuclease activity. Recently, attention has turned to the clinic, where the technology is tackling several diseases such as β thalassemia and sickle cell disease (SCD).[1] As with all therapies, specificity and safety are paramount, and are areas of intense research.[2] To date, most efforts have focused on spurious editing at unintended sites in the genome. A wealth of literature is dedicated to identifying[3,4] and mitigating[5–10] these off-target edits. Recent observations have extended spurious editing events to the target site itself,[11] again prompting careful examination of editing outcome.

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