Plant genome editing made easy: Sophien Kamoun on the CRISPR/Cas system

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Breeding plants to meet the demands of a population has driven agricultural progress throughout history. However classical methods of plant breeding can be time-consuming, whilst genetically modified crops have been mired in controversy with regards to the potential environmental and health implications of transgenics. New techniques are now under development that are faster than traditional breeding methods but notably do not involve the introduction of foreign DNA. The latest in this group of new plant breeding techniques (NPBTs) is the CRISPR/Cas system for genome editing. Khaoula Belhaj, Angela Chaparro-Garcia, Sophien Kamoun and Vladimir Nekrasov from The Sainsbury Laboratory, UK, discuss this technique and its applications in their recent review in *Plant Methods*. Here they tell us more about the advances this new technique heralds and its potential agricultural impact.

What is CRISPR/Cas?

CRISPR/Cas is a part of the adaptive immune system in bacteria that protects them against invading foreign DNA, such as bacteriophage DNA. There are three types of the CRISPR/Cas system. In the type II system, the Cas9 DNA nuclease and two non-coding RNAs, crRNA and tracrRNA, mediate DNA target recognition and cleavage. It is the crRNA that determines the DNA target specificity of Cas9 as it carries a

so-called guide sequence at its 5' end. The crRNA guide sequence pairs up with a matching DNA target, and once pairing occurs, the Cas9 nuclease cuts the DNA.

In August 2012, Jinek and co-authors published a paper in Science where they unraveled the molecular mechanism of the CRISPR/Cas system and proposed its application as a new tool for genome engineering. Just a few months later, a series of publications demonstrated the application of the CRISPR/Cas technology as a genome editing tool in various organisms, including human, zebrafish, yeast, Drosophila, bacteria and plants.

How much of a step-forward is this technology over previously available methods for genome editing in plants?

Genome editing technologies based on zinc finger nucleases (ZFNs) and TAL effector nucleases (TALENs) have been available for plant genome editing. The key advantage of the CRISPR/Cas system over these technologies is the ease of design and recombinant DNA assembly, and, eventually, low cost. For example, the design of ZFNs for a specific DNA locus may not be straightforward due to the context dependency of zinc fingers' capacity to recognize DNA nucleotides. At the same time, the assembly of both ZFNs and TALENs is relatively laborious and time consuming. In the case of CRISPR, a 20 base pair guide sequence is sufficient to direct the Cas9 nuclease to a DNA site of choice. At the moment, the only constraint for the CRISPR target selection is the NGG motif that must be present right next to the target at its 3' end. This motif is, however, common.

In conclusion, the key advantage of the CRISPR technology is its simplicity and robustness. This is why we titled the article 'plant genome editing made easy'. We believe that CRSPR will enable a broad community of plant researchers to access genome-editing technology.

How have you been using the technology in your own research?

We initially used the CRISPR technology as a proof of concept to generate gene deletions in planta and introduce indels in the phytodesaturase (PDS) locus in the model plant *Nicotiana benthamiana*. However, we are now moving to the second phase of implementing this technology to edit genes encoding for agronomically important traits in crops, such as tomato and potato.

How do you see the future for the CRISPR technology? How widely do you think it will be adopted?

The CRISPR technology has been already adopted by a number of labs in animal and plant fields. Given that the assembly of the targeting construct is relatively easy and cost effective, the technology has great potential to become a routine method applied to various organisms for making targeted single and multiple gene knockouts, introducing SNPs into a gene of interest, expressing proteins tagged with affinity or

fluorescent tags at their native genomic loci and much more.

Crop varieties produced using CRISPR are non-GM. Why is this?

Similar to ZFNs and TALENs, the CRISPR technology is one of a number of new plant breeding techniques (NPBTs). All three technologies can be used to manipulate plant genomes without introducing any foreign DNA into the final product. Therefore, these technologies make possible the introduction of precise modifications into a plant genome, which are indistinguishable from those introduced by conventional breeding or chemical or physical mutagenesis. Introduction of mutations that can occur naturally using the above-mentioned techniques is useful for speeding up the process of plant breeding.

Will this influence current debate about the safety of GM crops?

The field of NPBTs is rapidly evolving. These techniques have now been widely adopted by industry and breeders as they offer technical and economical advantages for targeted mutagenesis and genome editing. NPBTs are currently debated by advisory and regulatory authorities in Europe and elsewhere in relation to GMO legislation. A scientific study was already performed under the aegis of the European Union's Joint Research Center (JRC) to address the research and the state of adoption and commercial development of NPBTs in EU and in non-EU countries, especially the USA and Japan. The first crop varieties developed using these technologies are about to be commercialized and re-evaluation of GMO regulations is urgently needed. Another key issue in the debate is traceability. Detection of genomic changes introduced by site-specific nucleases into the final crop product is difficult by standard methods used for GMO detection, and the fact that the edited mutations can be identical to natural variants further complicates detection. Ultimately this will influence the legislation of genome edited crops.

Why did you choose to publish in the Open Access journal *Plant Methods*?

In August 2013, our laboratory published a paper in Nature Biotechnology by Nekrasov et al. that, together with two other reports in the same issue by Shan et al. and Li et al., described the first application of the CRISPR system as a tool for genome editing in plants. Other articles in Cell Research and Molecular Plant shortly followed these initial reports. We immediately realized that it would be useful to the community to have a summary of all these papers. We, therefore, decided to put together a review article for Plant Methods that includes a table that summarizes the eight papers. Plant Methods was the ideal choice because of the open access format and attractive web portal. We wanted to maximize visibility and ensure broad dissemination. Even readers who could not access the original paywall articles would be able to get an overview through the open access review article.

What difference do you think Open Access publishing makes?

Plant biology is not different from any other area of biology. We are rapidly moving toward open access publishing with more emphasis on the intrinsic quality of an article, rather than the proxy of the journal reputation or worse its 'impact factor'. In addition, much crop breeding research takes place in developing countries. Paywall publishing disproportionately hurts students and scientists in such countries. Open access publishing of plant and agricultural research should be part of the scientific community response to the global food crisis.

More about the author(s)

Sophien Kamoun, Head of The Sainsbury Laboratory and Professor of biology at the University of East Anglia, UK.

Sophien Kamoun obtained his PhD in genetics at the University of California, Davis, USA, where he went on to pursue his postdoctoral training in molecular plant pathology, later moving to Wageningen University, the Netherlands. He returned to the USA to establish a laboratory in the department of plant pathology at Ohio State University, leading to his professorship in 2006. Kamoun next moved to the UK to continue his research at The Sainsbury Laboratory and the University of East Anglia. He is currently head of The Sainsbury Laboratory and Professor of Biology at the University of East Anglia. His research interests in the field of plant pathology centre on understanding how pathogens successfully colonise and reproduce in their host plants, specifically investigating the biochemical activities of pathogen effectors, mainly in the *Phytophthora infestans-Solanaceae* pathosystem.

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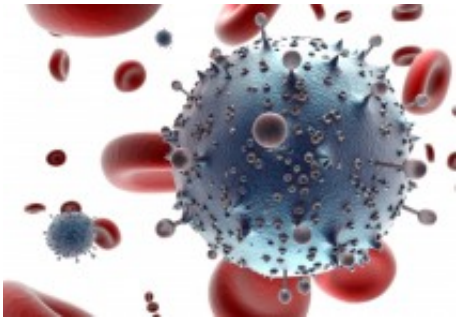

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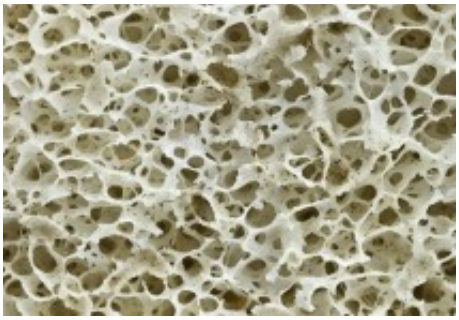

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