Are plants engineered with CRISPR technology genetically modified organisms?

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We are only two or three breeding cycles from 9 billion people and an altered climate. This future population will rightly expect to access a nutritious, balanced and affordable diet, and to expect that the animals and plants it comes from are produced sustainably. However, during the same period there will be changes to the global climate resulting in local weather patterns with more frequent extreme events. To ensure medium-term food security will be challenging; to ensure it over the longer-term without permanently degrading the natural resources of our planet will need step-changes in agricultural systems. Breeding new varieties of plants will be a necessity to meet these future needs, but achieving this through conventional methods is likely to prove problematic. The novel technique of genome editing, with its ability to turn off or improve existing genes, may well be the answer to this problem.

The world's population is projected to increase from 7.4 billion today to 8.5 billion by 2030, 9.7 billion by 2050 and to 11.2 billion by 2100[1]. Balancing the expectations of the future population against changes in global climate could prove difficult. Overall, 15 of the 16 hottest years on record have occurred since 2000, with 2015 being significantly warmer than the previous record-level temperatures seen in 2014[2]. However, average global temperatures mask the frequent and extreme local climate anomalies that, along with other abiotic and biotic stressors, have a negative effect on agricultural production. For example, the 2010 spike in European wheat prices from €120/tonne in April 2010 to €220/tonne in August the same year was a result of a collapse of the Russian wheat harvest and the consequent temporary export ban after a prolonged period of extreme hot and dry weather[3]. Many commentators have also connected the rise in wheat costs to riots and social unrest in Bahrain, Yemen, Jordan, Egypt and Morocco[4]. In Egypt local food prices rose 37% in 2008–2010. "The food-price spike was the final nail in the coffin for regimes that were failing to deliver on their side of the social contract," says Jane Harrigan of London's School of Oriental and African Studies[4].

By 2050, overall agricultural productivity will need to increase by at least 70%[5]. At the same time, agricultural production will need to become more resilient to biotic and may inadvertently generate varieties ill-equipped to cope with the uncertain biotic and abiotic stressors of the future. Clearly the response to these significant challenges must be wide ranging and multifaceted but plant breeding must be one key element to produce new varieties better suited to meet the challenges articulated above. As an example, researchers are making progress in genetically re-engineering aspects of plant photosynthesis to better cope with rising CO2 and temperature[6]. A drawback to this concept is that plant breeding has long timescales of, depending on the crop species, 10 to 20 years from initial crosses to the marketing of new commercial varieties. Thus, plant breeders have the challenging task of making crosses and initial selections today that largely delimit the allele diversity in the breeding populations and will generate future varieties for a time when many of the biotic and abiotic stressors are still uncertain. Technologies such as marker-assisted selection

that allow early identification of desirable allele combinations are crucial, but only applicable when the desirable combinations already exist within the segregating breeding populations or within gene pools that can be accessed by conventional methods. A fundamentally more powerful technology is one like genome editing that does not rely on making novel crosses but instead can alter less good alleles into better ones directly in current commercial elite varieties or in the later stages of the breeding cycle.

Genome editing – a tool to savour

The words 'genome editing' define the use of a suite of Site-Directed Nucleases (SDN) capable of cutting or otherwise modifying predetermined DNA sequences in the genome. Examples of SDNs are: Zinc-Finger Nucleases (ZFN), Transcription Activator-Like Effector Nuclease (TALEN) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) (Figure 1). Others such as Meganucleases (MN) and Oligonucleotide-Directed Mutagenesis (ODM) also exist along with as yet unpublished molecules with genome-editing potential. Regardless of the specific tool, they have one thing in common: to cut or otherwise modify a predetermined sequence in the target genome and generate novel phenotypes in animals and plants.

The end products of genome editing are organisms with edits to existing genes, targeted insertions, deletions or other changes for genetic improvement and, except for one specific subset (type 3, see below), contain no pre-recombined DNA from another species (Figure 2). Arguably the most powerful and adaptable tool for genome editing is the most recent one to be developed; CRISPR-Cas9. Although the intellectual property landscape of CRISPR-Cas9 is yet to be finally clarified (discussed below), and there are no commercial crop varieties currently marketed using CRISPR-Cas9, this particular technology is proving to be the most adaptable and has the potential to drive rapid advancements in plant breeding. In only a few years, CRISPR-Cas9 has been rapidly adopted by the research community as a routine method to knock-in and knock-out DNA sequences in animals and plants, including foreign genes into predetermined 'safe harbour' sites in a host genome. Recent refinements of the basic technology are being reported, including the use of Cas9 variants that generate single-stranded 'nicks' as well as deactivated forms of Cas9 that modify expression of a targeted native gene by recruiting enhancers or repressors to specific locations within the regulatory regions of genes. Cas9 nickase can be used to efficiently mutate

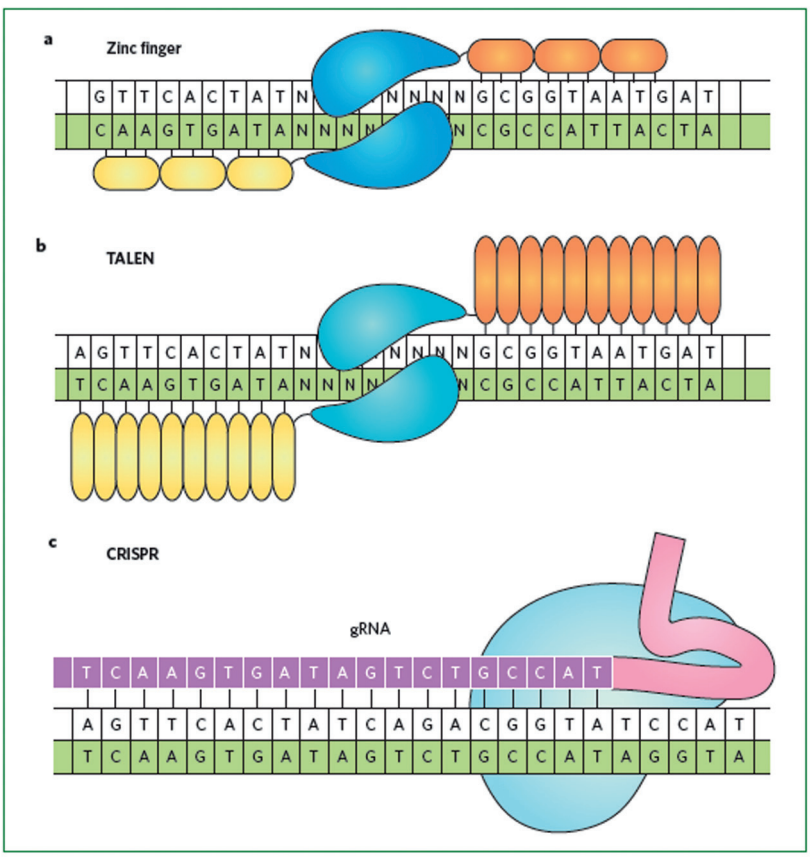

Figure 1. Site-directed nucleases. a–c, DNA nucleases bind to and cut DNA at specific locations. Each nuclease comprises a DNA-cutting domain (depicted in blue) and a DNA-targeting domain. Zinc-Finger Nucleases (ZFN) (a) and Transcription Activator-Like Effector Nucleases (TALENs), (b) possess protein-based DNA recognition domains (depicted by yellow and orange ovals). Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) nucleases (c) rely on single-guide RNAs (sgRNAs) to locate the target DNA. The DNA recognition domain of all three nucleases can be engineered to target predetermined sites in the genome for the purposes of genome editing7. [Reprinted from Jones, H.D. (2015) Regulatory uncertainty over genome editing. Nature Plants 1, pp. 14011. Drawings courtesy of www.addgene.org.]

genes without detectable damage at known off-target sites. This method is applicable for genome editing of any model organism and minimizes confounding problems of off-target mutations. In a different approach, two-component transcriptional activator systems have been demonstrated in animal and plant cells consisting of a deactivated Cas9 fused with a transcriptional activation domain and single-guide RNAs (sgRNAs) with a complementary sequence to gene promoters. This represents a novel way to modulate gene expression, either indirectly by localizing enhancers and repressors to receptive promoter domains or directly by recruiting, for example, acetyltransferases to perform epi-genome editing while leaving the primary DNA sequence unaltered.

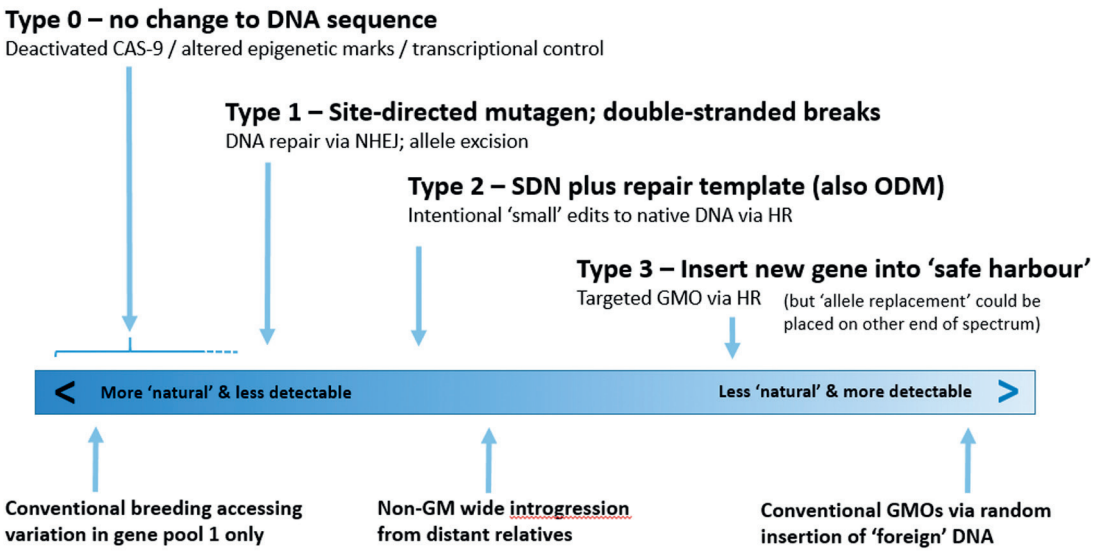

Figure 2. Various practical applications of genome editing placed on a spectrum of how 'natural' or detectable the genome edits would be. NHEJ: non-homologous end joining; HR: homologous recombination

Go, edit—what's stopping you?

Research in model plants and animals, as well as some commercially important crops and livestock, has demonstrated that techniques such as CRISPR-Cas9 can generate targeted changes in the intended DNA sequence and lead to an altered phenotype. More research is needed to understand the optimal editing methodologies for various cell types and the generic principles determining promiscuous annealing of the guide RNA. However, a risk assessment of possible off-target effects in final marketable products can be dealt with on a case-by-case basis. While some technical challenges still remain to be ironed out, the most significant hurdles to using genome editing for crop improvement are the uncertainties over licensing and regulation.

Although many researchers contributed to the development of a CRISPR-Cas9 bacterial immune defence system into a powerful genome-editing technology, it was anticipated that the three main scientists: Jennifer Doudna, Emmanuelle Charpentier and Feng Zhang, may have been awarded a Nobel Prize in 2015. They were not, and it is widely thought that the Nobel committee's deliberations were probably frustrated by public disagreements over the relative contributions of the respective researchers and the ongoing US Patent and Trademark Office's (USPTO) patent interference proceeding to determine which team was the first to invent the technique[8] (See 'Who owns gene editing? Patents in the time of CRISPR' p 26)

In the meantime, the biotech company DuPont Pioneer negotiated a strategic alliance with Caribou Biosciences founded in 2011 as a spin-out from the laboratory at UC Berkeley, led by Jennifer Doudna. Depending on the outcome of the USPTO's patent interference proceeding, DuPont could end up with freedom to operate with CRISPR-Cas9 in major staple crops like maize, canola and soybean, and Caribou in smaller market crops like fruits and vegetables[9]. I hope that DuPont take a fair and open licensing approach to make accessing this technology straightforward and affordable. This approach would also help to regain public trust in the biotechnology industry and help avoid the accusation of tight patentable control over seed supply.

The other, and more immediate hurdle to commercializing products of genome editing is the lack of clarity over how products of the various new plant-breeding technologies will be regulated in different territories around the world[7,10]. To date, the US FDA has stated that at least five products generated using genome editing are not Regulated Products in the US (a low-phytate maize, a herbicide-tolerant (HT) canola, a mildew-resistant wheat and two more in April 2016; a non-browning mushroom[11] and of particular significance, the first 'CRISPR crop', a waxy maize[12]. When asked by US gene-editing firm Cibus, some of the EU member states independently stated that a HT canola made using genome editing was not a GMO[13]. However, this decision was criticized, both by the EC which is yet to rule on the issue and also by non-governmental

organizations (NGOs) including TestBiotech, Greenpeace and Friends of the Earth who published an open letter to the European Commission (EC) urging it to ensure (among other things) "that organisms produced by these new techniques will be regulated as genetically modified organisms under existing EU regulations (Directive 2001/18)". The EC was widely expected to give guidance on what products of modern breeding methods (including genome editing) were classed as GMOs by the end of 2015; however, this date passed with no news. Then the EC announced they would publish during the first quarter of 2016[14]; but, this date also passed with no news. In March 2016 an EC spokesperson said "we are currently working on a legal analysis to give guidance on how to interpret the definition of GMOs in relation to organisms produced by new plant-breeding techniques," and added that both the outcome and the timeline "cannot be pre-empted for the time being"[15]. This situation creates uncertainty for researchers and plant breeders, it stifles innovation and it makes business planning impossible for practical applications of gene editing. If genome-edited organisms are classified as GMOs it will restrict use of the technology to multinational biotech companies working on profitable traits in the main commodity crops. However, for gene-edited plants with no foreign DNA, a regulatory framework that is proportionate to the risks will allow smaller plant breeders and research organizations to use these benign methods for public good breeding projects in locally important crops. Removing products incorporating type 0, 1 and 2 gene edits (Figure 2) from the GMO regulatory system would not only be logical but stimulate more democratic focus on less-profitable traits for sustainable agriculture. A proportionate regulatory framework using existing novel food laws or varietal registration processes could be an alternative approach. ■

The Institute of Biological, Environmental & Rural Sciences (IBERS) receives strategic funding from the Biotechnology and Biological Sciences Research Council (BBSRC).

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