A proposed regulatory framework for genome-edited crops

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Crop breeding is being revolutionized by rapid progress in DNA sequencing and targeted alteration of DNA sequences by genome editing. Here we propose a regulatory framework for precision breeding with 'genome-edited crops' (GECs) so that society can fully benefit from the latest advances in plant genetics and genomics.

Crops provide food, feed and fiber for humankind, with the top 60 species covering 96% of arable land in the world. The survival and well-being of our species critically depend on the output of these crops. The growing human population faces a plethora of challenges, from degradation and loss of arable land and climate change to the sensible demand for more sustainable agriculture practices. These are multifaceted problems, but crop breeding surely has an essential role in meeting the goals of agriculture and food production. To address these challenges, it is essential to fully exploit the latest developments in all scientific disciplines.

Crop genome sequencing

Genomics is beginning to provide a holistic perspective from which to dissect the organization and regulation of biological circuits, and this knowledge is greatly accelerating crop breeding. It has been 16 years since the first reference genome sequence of a plant, the model species *Arabidopsis thaliana*, was finished in 2000 (ref. 1); two years later, the genome sequences of two important crops, the two major types of cultivated rice (*Oryza sativa* ssp. *indica* and *japonica*)[2,3], were published. The advent of next-generation sequencing accelerated crop genome sequencing: the first crop with a short-read genome assembly was cucumber[4], which has since been followed by assemblies for approximately 50 other crops (**Supplementary Table 1**). Among the crops that remain to be sequenced are those with very large genomes such as onion (16.4-Gb haploid genome size) and very complex, polyploid genomes such as cultivated potato, sweet potato and sugarcane, with assemblies for these genomes coming into reach with new long-read technologies[5]. The decreasing cost of next-generation sequencing is also fueling population-scale sequencing, which enables the discovery of agronomically important genetic variants[6–9]. Coupled with rapid advances in high-throughput phenotyping[10], genome sequencing is greatly expanding the potential to identify genes and alleles that control agronomic traits and to understand the interacting mechanisms that weave the genes into functional networks. Together, this and related research serve as the foundation of precise genome editing for crop improvement.

Crop genome editing

Genome editing begins with the introduction of a targeted DNA double-stranded break at a predetermined locus using a sequence-specific nuclease. Three types of sequence-specific nucleases are in general use, namely zinc-finger nucleases (ZFNs), transcription activator–like effector nucleases (TALENs) and clustered, regularly interspaced short palindromic repeat–associated endonucleases (CRISPR/Cas). The tradeoffs between these three systems, in terms of efficiency versus off-target effects, are still being investigated. Sequence targeting using ZFNs and TALENs is mediated by protein-DNA interactions, whereas CRISPR/Cas recruits a guide RNA to direct an endonuclease to a target DNA sequence via base-pairing. The type II CRISPR/Cas9 system from *Streptococcus pyogenes* is currently the most widely used, owing to its high efficiency and simplicity, with alternatives such as the recently described CRISPR-Cpf1 system[11] promising further improvements. By linking Cas9 to other domains, the CRISPR/Cas system can also be exploited for epigenome editing or targeted transcriptional control and other types of genome engineering[12]. In plants, the CRISPR/Cas components are typically introduced through transgenes, although there are alternatives such as delivery by viruses[13] or direct delivery of protein-RNA complexes into plant protoplasts[14]. Transgenes can be removed through self- or backcrossing, which can also be used to remove undesirable off-target mutations.

Geneticists have been quick to adopt genome editing as a powerful tool for crop improvement. It is in principle straightforward to mitigate an unwanted trait or to create a favorable trait by introducing knockout mutations in the causal genes by genome editing. For instance, phytate, an antinutritional compound that limits mineral absorption by farm animals and increases environmental pollution, can be

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eliminated by directed mutations. Using ZFNs, the *IPK1* gene, encoding the key enzyme catalyzing the final step in phytate biosynthesis, was disrupted in maize[15]. In rice, fragrance is controlled primarily by recessive alleles of the gene *OsBADH2*, and fragrant rice varieties have been developed by knocking out *OsBADH2* using TALEN technology[16]. Perhaps the most impressive example comes from hexaploid bread wheat, where the simultaneous editing of three homeologous alleles of *MLO* (*MILDEW RESISTANCE LOCUS*) genes created a novel cultivar that is resistant to powdery mildew, a devastating disease and a threat to food security in several countries[17].

Although it is more complicated, genome editing can also be used for targeted homologous recombination and, thus, the introduction of change-of-function mutations. For example, glyphosate is a widely used broad-spectrum, post-emergent herbicide, which inhibits the activity of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) by competing with the natural phosphoenolpyruvate substrate[18]. Amino acid substitutions in EPSPS that confer tolerance to glyphosate are well known and are typically deployed on transgenes to confer herbicide resistance in a broad range of crops. Using the genome editing approach, targeted modification of the endogenous *EPSPS* genes in crops such as soybean, rapeseed and rice, among others, could confer herbicide tolerance without the introduction of transgenes[19], similarly to what has been done for acetolactate synthase (ALS), another herbicide target[20]. Genome editing has also been used for the discovery of novel favorable alleles. For example, by optimizing the balance of flowering and antiflowering signals, plant architecture together with flower production and yield can be optimized, using combinations of alleles with subtly different activities[21]. Finally, the targeted mutagenesis of multiple genes is essential for modifying functionally redundant homologs in polyploid crops[17], and this is also an important practical application of the CRISPR/Cas9 system[22]. Overall, we expect that such 'genome surgery' will be applied extensively, as crop genetics and genomics increase the knowledge of the genes and alleles controlling useful traits.

Current regulation of crops developed via conventional breeding and genetic modification

Modern conventional plant breeding, drawing on the insights of Darwin and Mendel, has made enormous contributions to increased global food production. It encompasses a broad range of techniques that go beyond the simple crossfertilization of existing cultivars, involving, for example, wide crosses between related species, *in vitro* fertilization, induction of polyploidy, protoplast fusion and mutagenesis with chemicals or radiation. Sensibly, the products of sexual crosses, mutagenesis and tissue culture–based plant breeding are free of government regulation other than registration of varieties.

However, conventional breeding is limited by the ability to introduce novel traits not present in the domesticated or wild germplasm; this restriction has been overcome by genetic modification (GM) techniques using transgenes introduced by several different methods. GM methods were initially used to insert DNA sequences from other species, such as selected genes for anti-insect proteins from *Bacillus thuringiensis*, which were previously in wide use as externally applied pesticides. There is broad scientific consensus that GM food and feed pose no greater risk to the consumer than conventional products; however, GM crops remain heavily regulated in many countries, including China and several European nations. Similarly, often poorly justified criticism has been leveled against so-called 'cisgenesis', in which genes from the same or a closely related species are introduced by DNA transformation, even though the European Food Safety Authority (EFSA) has concluded that "similar hazards can be associated with cisgenic and conventionally bred plants" (ref. 23).

A proposal for the regulation of genome-edited crops

As discussed above, genome editing offers unique opportunities to improve and increase the success of crop breeding. Humans have been manipulating crop genomes for more than 10,000 years, albeit in a random and non-targeted manner and, for most of this time, using only simple trial-and-error approaches. Conventional breeding changes crop genomes by direct selection of observable traits conditioned by natural variants or induced mutations or by using molecular markers linked to advantageous genes and alleles. Importantly, even with molecular markers and extensive backcrossing, genetic crosses introduce myriads of nucleotide variants, often creating undesirable effects as a result of genotype × genotype interactions. For two decades, these conventional methods have been complemented by genetic modification using transgenes. Although the insertion of transgenes into the host genome is random, the breeder knows exactly which sequences are introduced, and the effects are therefore much more predictable than in conventional breeding. Genome editing is in many ways even more precise and predictable than transgenesis. It is by nature similar to the use of spontaneous variants or induced mutations in conventional breeding, with the advantage that only the desired change is introduced. We hereby define crops bred by genome editing as genome-edited crops (GECs) (**Fig. 1**).

Because of the precision of genetic changes introduced in GECs, we strongly advocate product-based rather than technology-based regulation. In Executive Order 13563 (ref. 24),

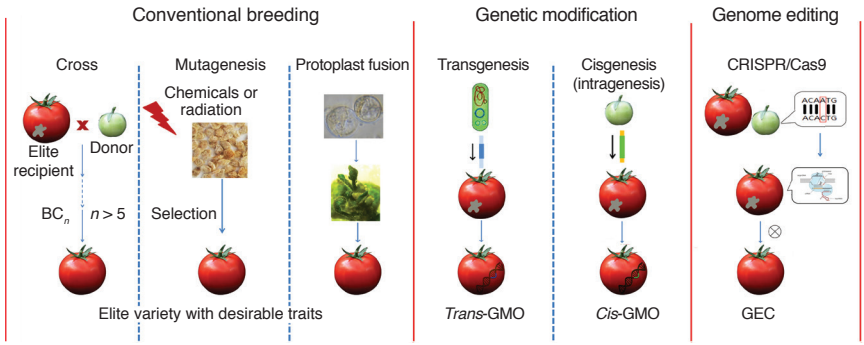

Figure 1 Comparison of three breeding methodologies. Conventional breeding mainly includes sexual crosses, mutagenesis and tissue culture–based techniques. Crosses rely on intra- or interspecific hybridization between a donor and an elite recipient line. The progeny of the cross are selected for the desired characteristic. To remove unwanted traits (shown as a star on the tomato) inherited from the donor plant, the best line of the progeny is obtained by backcrossing with the elite recipient line. Mutagenesis with chemicals or radiation is the process of exposing seeds to mutagens to generate mutants with desirable traits. Protoplast fusion, also called somatic fusion, is a technique where cells from two related species (or two different varieties of the same species) are induced to fuse, to form a new hybrid plant that ideally has characteristics from both parents. Transgenesis is the genetic modification of a recipient line with genes from other species that are sexually incompatible with the recipient plant. Cisgenesis (sometimes called intragenesis) is the genetic modification of a recipient plant transformed with a natural gene for a crossable plant. In genome editing, DNA is directly inserted, replaced or removed from a genome using engineered nucleases, colloquially called 'molecular scissors', to effect a desirable trait. BC*n*, backcross *n*th generation; GMO, genetically modified organism; GEC, genome-edited crop; ⊗, self-pollination.

President Obama reaffirmed that regulatory agencies shall "propose or adopt a regulation only upon a reasoned determination that its benefits justify its costs." In agreement with these principles, we argue that there is no reason to regulate GECs with gene knockouts or nucleotide variants that either have been documented to exist within crop species or closely related wild species or that can reasonably be expected to arise by spontaneous mutation. Because such genetic stocks could in principle —although generally not in praxis—be generated by conventional breeding or random mutagenesis, they should be considered the same as those used in conventional breeding, which are not regulated. Importantly, whole-genome sequencing allows excellent documentation of the variation introduced by genome editing.

We recommend five steps as the primary guiding principles when considering the generation and regulation of GECs.

1. Minimize the risk of escape of GECs from laboratories and fields during the research and development phase.

2. Demonstrate the absence of foreign sequences, if genome engineering proteins were introduced as DNA constructs.

3. Document DNA sequence changes at the target sites. If new sequences were introduced by homologous recombination, identify the phylogenetic relationship between the donor and recipient, as a proxy for the likelihood of new interactions with genetic background. Sequences from distantly related species introduced into GECs by homologous recombination may have to be considered on a case-by-case basis.

4. Ensure that the primarily targeted site did not suffer unintended secondary editing events and consider the consequences of potential off-target events on the basis of available reference genome information and whole-genome resequencing technologies.

5. Include documentation of the above four points for cultivar registration. Beyond these four points, GECs should only be subject to rules and regulations that apply to products of conventional breeding before commercial release.

The opportunities that GECs offer for ensuring global food and nutrition security are at least on the same order as those from GM crops and in many cases are more promising than those from conventional breeding. The world cannot afford to miss the opportunity of using the most relevant technologies to achieve the lofty targets stated in the recently released United Nations Sustainable Development Goals. The US Department of Agriculture does not consider GECs to be GM organisms[25] as long as GECs do not contain DNA from plant pests. Similarly, German authorities recently confirmed that genome-edited canola generated with an older oligonucleotide method does not constitute a GM organism, as it is not distinguishable from the products of conventional mutagenesis. We urge other countries to follow suit.

URLs. The website of the Federal Office of Consumer Protection and Food Safety (BVL) of the German government, http://www.bvl.bund.de/DE/06_Gentechnik/04_Fachmeldungen/2015/2015_06_03_Fa_CIBUS.html.

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