Cell
Leading Edge

CellPress

Review

Genome engineering for crop improvement and future agriculture

Caixia Gao[1,2,*]
[1]State Key Laboratory of Plant Cell and Chromosome Engineering, Center for Genome Editing, Institute of Genetics and Developmental Biology, Innovation Academy for Seed Design, Chinese Academy of Sciences, Beijing, China
[2]College of Advanced Agricultural Sciences, University of Chinese Academy of Sciences, Beijing, China
*Correspondence: cxgao@genetics.ac.cn
https://doi.org/10.1016/j.cell.2021.01.005

SUMMARY

Feeding the ever-growing population is a major challenge, especially in light of rapidly changing climate conditions. Genome editing is set to revolutionize plant breeding and could help secure the global food supply. Here, I review the development and application of genome editing tools in plants while highlighting newly developed techniques. I describe new plant breeding strategies based on genome editing and discuss their impact on crop production, with an emphasis on recent advancements in genome editing-based plant improvements that could not be achieved by conventional breeding. I also discuss challenges facing genome editing that must be overcome before realizing the full potential of this technology toward future crops and food production.

INTRODUCTION

The human population is expected to reach 10 billion by 2050 (FAO, 2017). A major challenge of our time is learning how to feed the expanding population and succeed in doing so. Owing largely to the Green Revolution and advances in plant breeding techniques, current crop yields can provide sufficient food for a majority of the human population. However, crop production appears to be plateauing and even declining due to both climate change and the limited availability of arable land; a 60% increase in production yields would be needed to feed a global population of 10 billion people (Springmann et al., 2018). Therefore, improving agricultural productivity and sustainability is essential to the whole world. Scientific breakthroughs and technological innovations in crop production are urgently needed to ensure future global food security.

Genetic variation is the basis of agricultural improvement. The aim of plant breeding is to create and exploit these genetic variations. Over the long history of plant breeding (Hickey et al., 2019), four major techniques have been used: cross-breeding, mutation breeding, transgenic breeding, and breeding by genome editing (Chen et al., 2019; Figure 1). Traditional plant breeding (cross-breeding), which involves targeted crossing of plants to combine desirable traits via sexual recombination, has played an important role in improving agricultural productivity. This strategy is exemplified by the first Green Revolution beginning in the late 1950s, in which "dwarfing" gene mutations were bred into major staple crops, such as wheat (*Triticum aestivum*) and rice (*Oryza sativa*), to obtain high-yielding varieties (Khush, 2001). However, since cross-breeding can only be used to introduce traits that are already present in the parental genomes, the low genetic variability in elite germplasms limits the use of this technique (Figure 1). In mutation breeding, chemically or radiation-induced mutagenesis is used to induce random mutations genome-wide, which greatly expands genetic variation (Holme et al., 2019). However, identifying the rare individuals that harbor a desired trait from a large population of mutagenized plants is labor-intensive and time-consuming (Figure 1). A key breakthrough in plant breeding was the development of transgenic breeding, in which genes or traits from other organisms are introduced into crop plants, leading to higher yields, lower pesticide use, and improved nutrition. Nevertheless, only a few transgenic crops have been utilized thus far (Raman, 2017), as this technique randomly integrates foreign DNA into plant genomes and these genetically modified organisms (GMOs) are subject to strict government regulations (Figure 1). In addition, adverse public opinions regarding the safety of these products limit their potential.

Genome editing techniques have been developed to introduce precise and predictable genome modifications into plants to obtain desired traits, and they are giving rise to precision breeding techniques that are defining the next-generation of plant breeding (Chen et al., 2019) (Figure 1). CRISPR (clustered regularly interspaced short palindromic repeats)-Cas (CRISPR associated) has emerged as one of the most advanced systems for engineering crop genomes (Shan et al., 2013a). This technology has been rapidly expanding and applied to major cereals such as rice, wheat, and maize (*Zea mays*) and to other crops that are important for food security, such as potato (*Solanum tuberosum*) and cassava (*Manihot esculenta*) (Chen et al., 2019; Zhu et al., 2020). In addition, recently developed CRISPR-associated tools such as base editors and prime editors have greatly expanded the scope of genome editing (Anzalone et al., 2020), allowing for the creation of precise nucleotide substitutions and targeted

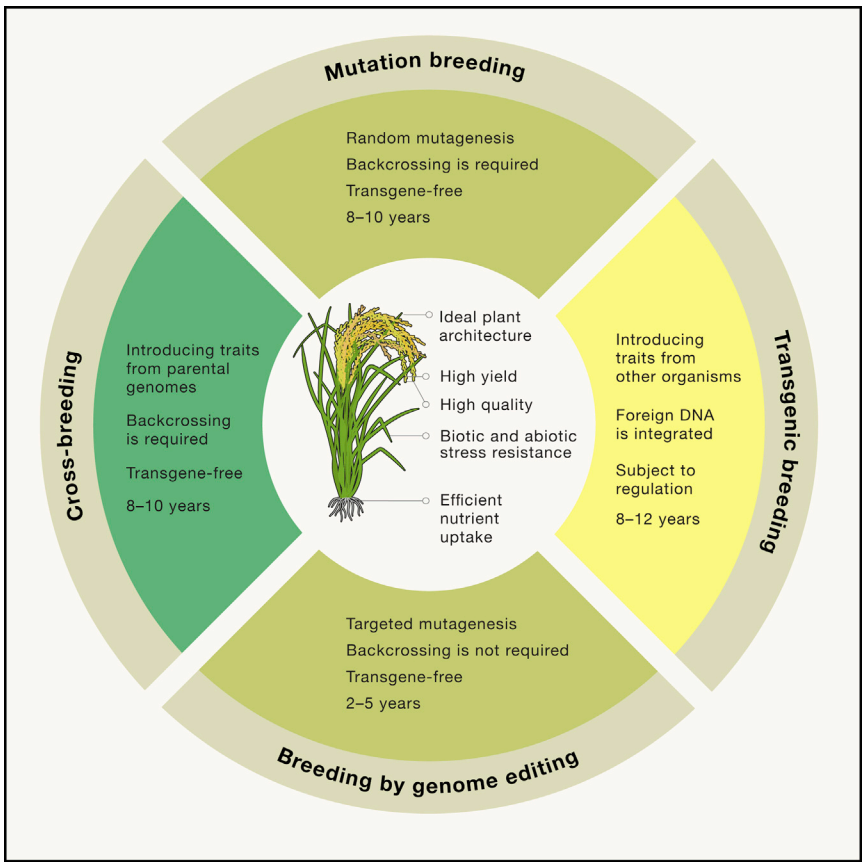

Figure 1. Plant breeding techniques commonly used to introduce new traits into an elite crop variety

Four techniques used during different periods of plant breeding based on biotechnological developments. Cross-breeding based on naturally occurring mutations has been used to introduce various traits into elite recipient lines. Mutation breeding has been used to induce random mutations genome-wide, which greatly expands genetic variation. Cross-breeding and mutation breeding generally require a long time period due to the need for backcrossing. Transgenic breeding can be used to introduce genes or traits from other organisms, but the foreign DNA is randomly integrated into the plant genome. The commercialization of transgenic crops is subject to a long and costly regulatory evaluation process. Genome editing technologies can be used to efficiently modify plant genomes to improve traits without integrating foreign DNA into the genome. These precise breeding techniques are coming to define next-generation plant breeding.

DNA deletions and insertions. CRISPR-Cas technologies, in combination with modern breeding methods, will play an important role in crop improvement programs. In this review, I describe the current status of plant genome editing with an emphasis on the genetic modifications that can be produced using these techniques as well as application of plant genome editing as the next-generation plant breeding technology for crop improvement.

Plant genome editing technologies

Plant genome editing is carried out using programmable sequence-specific nucleases (SSNs). SSNs include engineered homing endonucleases or meganucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the CRISPR-Cas system. These nucleases make DNA double-strand breaks (DSBs) at target sites, and precision genome modifications are achieved via DNA repair pathways (Voytas and Gao, 2014). While meganucleases, ZFNs, and TALENs recognize target sequences via protein-DNA interactions, the CRISPR-Cas system targets DNA sequences through Watson-Crick base pairing, relying on the homology between the target DNA and a programmable "guide" RNA. Because of its low cost, simplicity, and high efficiency, the CRISPR-Cas system has become the most widely used system for plant genome editing (Yin et al., 2017). Here, I introduce the general plant genome editing procedure and describe the many genetic modifications that can be produced through genome editing in plants.

General procedure for plant genome editing

The general procedure for genome editing in plants can be divided into six steps: (1) select the appropriate nuclease based on the target sequence; (2) construct genome editing vectors; (3) validate the activity of these vectors using protoplasts (wall-free plant cells released from enzyme-digested tissues; optional step); (4) deliver genome editing reagents into plant cells; (5) regenerate genome-edited cells into plantlets via tissue culture; and (6) screen and genotype the resulting genome-edited plants (Figure 2A).

Although the same editing tools are used for all organisms, some aspects of the delivery and regeneration are specific to plants. Genome editing reagents can sometimes be transformed directly into protoplasts, but in most cases, they are delivered via particle bombardment (using a gene gun) or *Agrobacterium* into plant cells in the form of calli, embryos, or leaf explants (Figures 2B and 2C). The transformation and regeneration steps still represent bottlenecks for plant genome editing, as these processes must be optimized for each species and each plant variety, which is an arduous task for most elite varieties and wild species (Altpeter et al., 2016).

Three common forms of genome editing reagents include CRISPR-Cas9 DNA, RNA (*in vitro* transcripts of Cas9 and single guide RNA [sgRNA]), and RNP (ribonucleoprotein, composed of Cas9 protein and *in-vitro*-transcribed sgRNA) (Ran et al., 2017). While DNA can be delivered into plant cells by both particle bombardment and *Agrobacterium*-mediated transformation, RNA and RNP can only be delivered into plant cells by particle bombardment (Liang et al., 2017; Svitashev et al., 2016; Zhang et al., 2016; Figures 2B and 2C). When DNA is chosen as the genome editing reagent, two different strategies can be used for the subsequent tissue culture process: the conventional method and the transient DNA expression method (Zhang

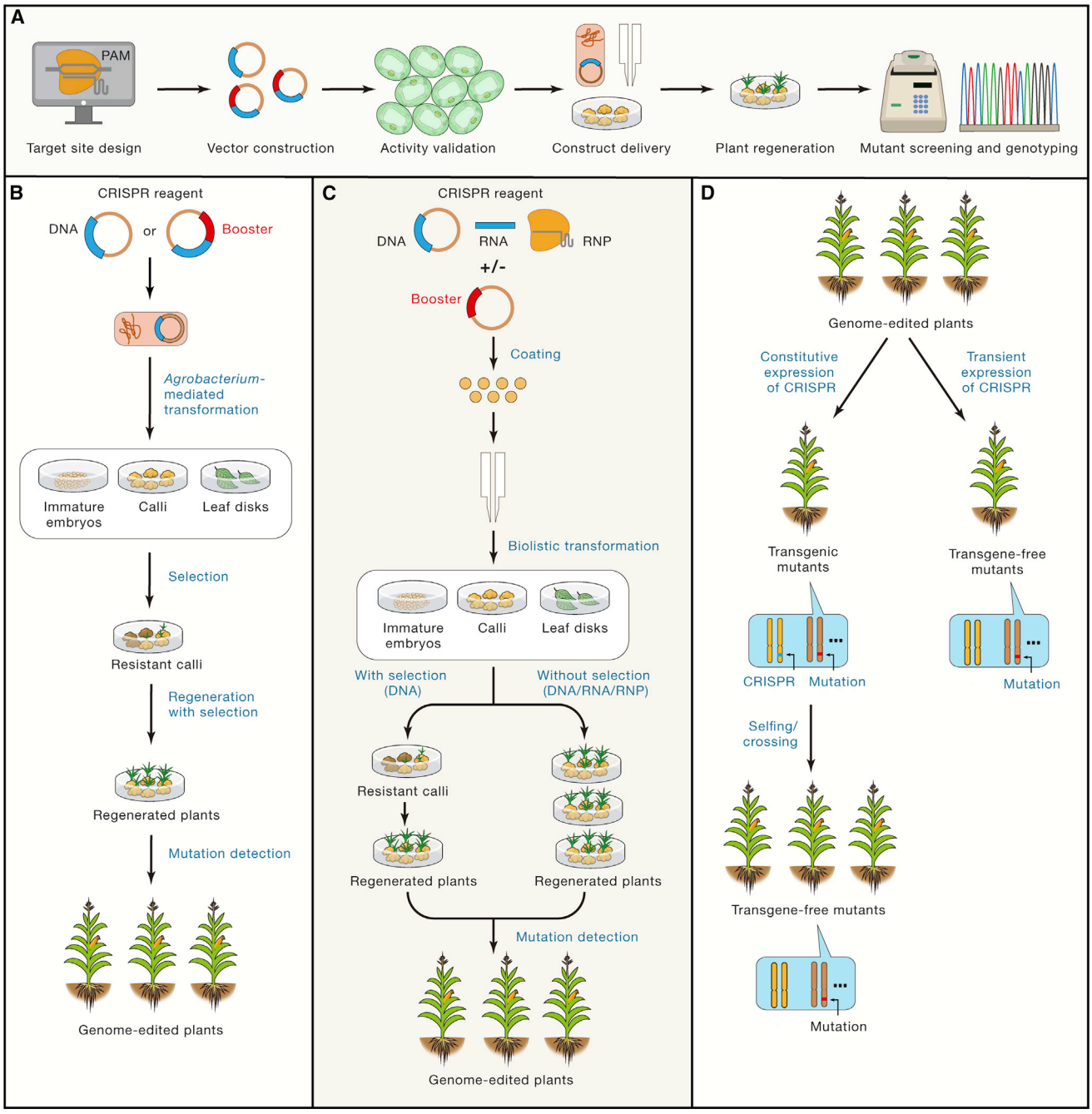

Figure 2. General procedure for plant genome editing

(A) Schematic illustration of the six major steps in plant genome editing.

(B) Genome-edited plants generated by *Agrobacterium*-mediated delivery of CRISPR DNA.

(C) Conventional and transient expression methods for particle bombardment-mediated genome editing by delivery of CRISPR DNA, RNA, or RNP.

(D) Two strategies used to obtain transgene-free mutants.

et al., 2016; Figures 2C and 2D). In the conventional method, a selection agent is used during the tissue culture process to select for resistant calli and transgenic plants (Figures 2B and 2C). Once the transgenic genome-edited mutants are generated, the genome editing vectors can be segregated out from the mutant genomes through selfing or crossing to obtain transgene-free mutant plants (Figure 2D). In the transient DNA expression method, no selection agent is used during the tissue culture process, resulting in the production of transgene-free mutants without the need for a segregation process (Zhang et al., 2016) (Figures 2C and 2D). DNA-free genome editing can be obtained using either RNAs or RNPs. These transient methods do not

result in genomic integration events into the plant genome. Therefore, no selection agent is needed during the subsequent tissue culture processes, and the genome-edited plants created through transiently expressing CRISPR RNA (crRNA) or RNP are DNA-free mutants (Liang et al., 2017; Svitashev et al., 2016; Figures 2C and 2D). DNA-free genome editing is preferable to the conventional method because it involves no foreign DNA and can drastically reduce off-target editing events in plants.

Genetic modifications generated by genome editing in plants

In addition to ZFNs and TALENs, the introduction of the CRISPR-Cas system has accelerated the development of plant genome editing. The most widely used CRISPR-Cas systems are the Cas9 and Cas12a complexes, both of which are single effector proteins that perform nucleic acid cleavage (Chen et al., 2019; Figure 3A). Recently, the Cas12b system was also developed for plant genome editing (Ming et al., 2020). All of these systems rely upon crRNAs to guide the Cas protein to target sequences. The Cas9 protein requires an additional RNA molecule known as a *trans*-acting crRNA (tracrRNA), which can be artificially fused with the corresponding crRNA to form a sgRNA (Jinek et al., 2012). CRISPR-Cas systems can be programmed by simply designing the DNA target protospacer sequence into the crRNAs or sgRNAs. Various Cas orthologs and variants with different PAM (protospacer adjacent motif) specificities have been identified and exploited to maximize the editing scope of these tools (Anzalone et al., 2020). The CRISPR-Cas system and newly developed tools such as base editors (Gaudelli et al., 2017; Komor et al., 2016; Figure 3B) and prime editors (Anzalone et al., 2019; Figure 3C) have greatly expanded its potential applications. To date, genome editing has been used to generate a variety of heritable genome modifications in plants including (1) small random insertions/deletions (indels) (Figure 3D); (2) point mutations or nucleotide substitutions (Figure 3E); (3) DNA fragment insertions (Figure 3F); (4) DNA fragment deletions (Figure 3G); and (5) targeted chromosomal rearrangements (Figure 3H).

Classical genome editing involves the repair of DSBs at target loci. When SSN reagents are delivered into plant cells, they recognize and cleave target DNA and generate DSBs, which are repaired by endogenous DNA repair pathways including non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ is the major pathway used to repair DSBs, and when DSBs are repaired by NHEJ, indels may be introduced at the junctions of the rejoined chromosomes (Chen et al., 2019; Zhu et al., 2020; Figure 3D). The resulting indels are stochastic, varying in length and sequence, and typically result in gene knockouts due to frameshift mutations. Alternatively, HDR can occur if a homologous DNA template is available or provided. Precise gene replacements, point mutations, and DNA insertions and deletions can be produced by HDR-mediated genome editing (Figure 3A), but the efficiency of HDR in plant cells is extremely low.

Beyond DSB-mediated genome editing, CRISPR-Cas-derived base editors have emerged as powerful tools for generating programmable single DNA base changes. There are two main classes of base editors: cytosine base editors (CBEs) and adenine base editors (ABEs). Base editors are fusions of catalytically impaired Cas9 (nCas9 D10A) nucleases with single-stranded DNA (ssDNA)-specific deaminases. These deaminases, such as rAPOBEC1 and PmCDA1 cytidine deaminases in CBEs or laboratory-evolved TadA deoxyadenosine deaminase in ABEs, catalyze C·G to T·A or A·T to G·C transitions in the ssDNA strand of the R-loop induced by CRISPR-Cas at target sites, respectively (Gaudelli et al., 2017; Komor et al., 2016; Nishida et al., 2016) (Figures 3B and 3E). The fusion of uracil DNA glycosylase inhibitor in CBEs helps increase the base-editing efficiency by manipulating endogenous DNA repair machinery (Komor et al., 2016). Both CBEs and ABEs have been optimized for plant genomes (Li et al., 2018a; Zong et al., 2017). Other CBEs using the deaminases PmCDA1, hAID, and hAPOBEC3A have also been successfully used in plants (Ren et al., 2018; Shimatani et al., 2017; Zong et al., 2018). Dual base editors that combine the functional domains of CBEs and ABEs can induce simultaneous C·G to T·A and A·T to G·C changes at the same target site (Li et al., 2020a), further broadening the scope of base editing in plants.

Current base editors are limited to base transitions (C·G to T·A and A·T to G·C), but DNA base transversions and predefined DNA insertions and deletions cannot be produced. However, a recent technological breakthrough, prime editing, allows for the creation of all 12 types of base substitutions and small DNA insertions and deletions in human cells (Anzalone et al., 2019; Figure 3C). Prime editors are composed of two components: an engineered Cas9 nickase (H840A)-reverse transcriptase (RT) fusion protein and a prime editing guide RNA (pegRNA). The pegRNA is a modified sgRNA with 3′-extended bases comprising a primer binding site (PBS) and an RT template encoding the desired edit(s). The Cas9 nickase (H840A) recognizes the target site and nicks the non-target DNA strand, releasing a ssDNA that pairs with the PBS and serves as a primer for RT. Through reverse transcription, the edit encoded on the pegRNA is transferred to the non-target DNA strand. The newly synthesized edited DNA flap is subsequently incorporated into the target site by DNA repair (Anzalone et al., 2019). Prime editing has been rapidly adapted for use in plant cells, and prime-edited rice and maize plants have been successfully regenerated (Zhu et al., 2020; Jiang et al., 2020), albeit the editing efficiency of prime editors is currently much lower than that of base editors at most target sites in plant genomes (Lin et al., 2020). Attempts have been made to improve the efficiency of prime editing by varying the lengths of the PBS and RT template in the pegRNA, using other RTs, processing the pegRNAs with a ribozyme, raising the culture temperature to favor reverse transcription (Lin et al., 2020), using enhanced promoters for pegRNA expression (Jiang et al., 2020), and enriching for transformed cells (Xu et al., 2020a, 2020b). Prime editing efficiency has also been improved by designing the pegRNA sequence based on melting temperature and using dual-pegRNAs. In addition, an automated pegRNA design platform has been developed in rice (Q. Lin, S. Jin, Y. Zong, H. Yu, Z. Zhu, L. Kou, Y. Wang, J. Qiu, J. Li, and C.G., unpublished data).

The precise insertion of DNA has made it possible to manipulate gene functions and stack multiple crop traits. HDR-mediated DNA insertion in plants occurs at relatively low efficiency (Chen et al., 2019; Figure 3F). Alternatively, the NHEJ pathway can be

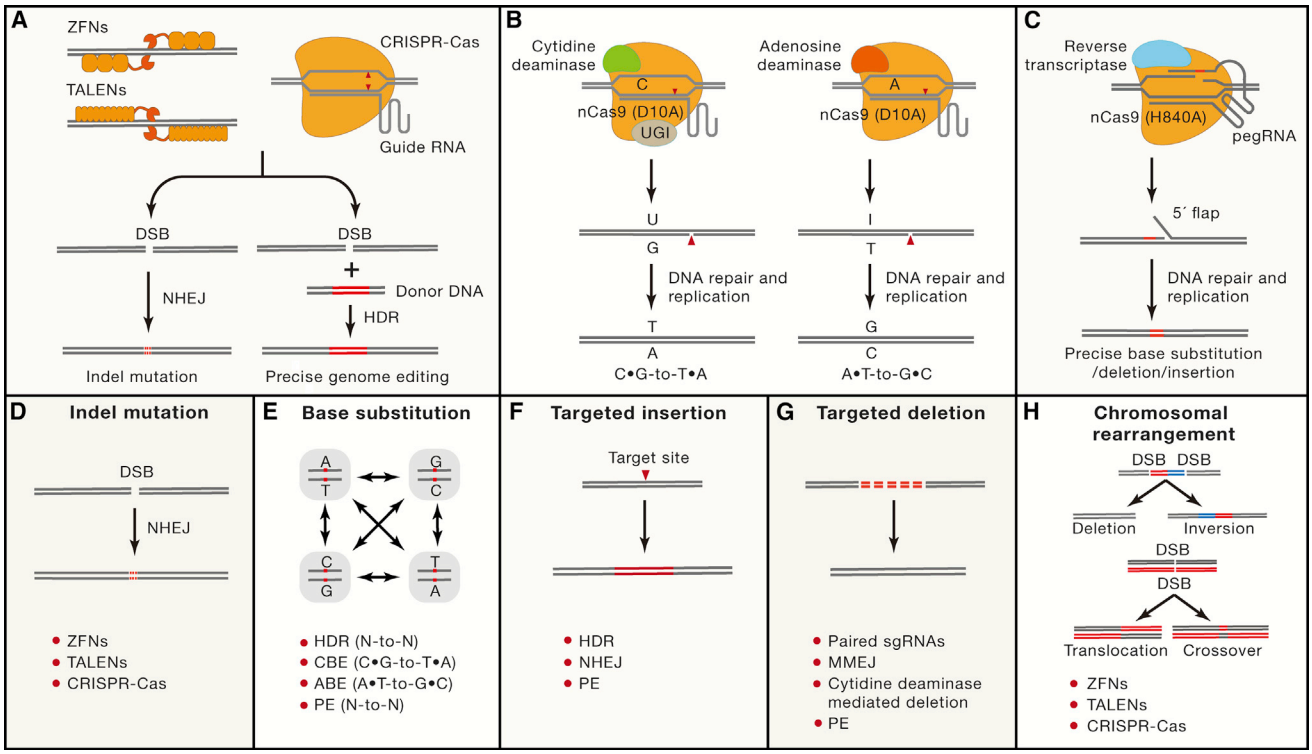

Figure 3. Genetic modifications generated by genome editing in plants

(A) Schematic diagram of the NHEJ and HDR DNA repair pathways when DNA double-strand breaks (DSBs) are produced by sequence-specific nucleases (SSNs).

(B) Base editing technology. Cytidine or adenosine deaminase is fused with Cas9 nickase (nCas9 (D10A)) to generate a cytosine base editor (CBE) or adenine base editor (ABE), respectively. The CBE generates C·G-to-T·A base substitutions, and the ABE generates A·T-to-G·C base substitutions. UGI, uracil DNA glycosylase inhibitor.

(C) Prime editing technology. The prime editor (PE) is composed of a fusion of nCas9 (H840A) with reverse transcriptase and a prime editing guide RNA (pegRNA).

(D) ZFNs, TALENs, and the CRISPR-Cas system induce small random indels mutation via the DNA non-homologous end joining (NHEJ) repair pathway.

(E) Base substitutions can be created by HDR, CBE, ABE, and PE.

(F) Targeted insertion editing by HDR, NHEJ, and PE.

(G) Targeted deletion editing by paired sgRNAs, cytidine deaminase-mediated deletion, MMEJ, and PE.

(H) Pairs of DSBs are introduced simultaneously into chromosomes, inducing chromosome deletions, inversions, translocations, and crossover.

harnessed for efficient DNA insertion at DSB sites when a donor DNA template is provided (Wang et al., 2014). One successful example of this is the CRISPR-Cas9-mediated gene replacement and insertion achieved by targeting introns through the NHEJ pathway (Li et al., 2016; Figure 3F). To increase the frequency of the targeted insertions by NHEJ, short homologous chromosomal segments are added to the ends of the donor DNA to produce compatible ends or microhomology with the DSB surrounding sequence (Dong et al., 2020). Targeted insertion via NHEJ can also be stimulated using chemically stabilized double-stranded oligodeoxynucleotides (dsODNs) donors with 5′-phosphorylated ends (Lu et al., 2020).

Target DNA deletion is especially important for editing regulatory and non-coding DNAs for which small indels are unlikely to cause loss of function. Targeted DNA deletions can be obtained by inducing two separate DSBs using SSNs (Shan et al., 2013b; Figure 3G). For example, co-expressing Cas9 with a pair of sgRNAs can result in a >100 kb deletion of the region between the two target sites (Zhou et al., 2014). Alternatively, by fusing Cas9 or Cas12a with T5 exonuclease or by co-expressing a SSN with exonucleases, it is possible to generate targeted deletions with a single gRNA, but the length of such deletions is limited (Zhang et al., 2020a). The deleted DNA sequences obtained using these strategies are not predictable or precise because the repair occurs via the NHEJ pathway.

Precise DNA deletions can be generated by microhomology-mediated end joining (MMEJ), which uses microhomologous sequences to align the ends of DSBs prior to their joining (Tan et al., 2020; Figure 3G). However, this strategy can only generate deletions between two microhomologous sequences. Multinucleotide deletions can be generated by the newly developed APOBEC-Cas9 fusion-induced deletion systems (AFIDs) (Wang et al., 2020; Figure 3G). In these systems, Cas9 generates a DSB at the target DNA sequences, while simultaneously APOBEC deaminates cytidines on the non-target strand to uridines, which is then excised by uracil DNA glycosylase to generate an abasic (AP) site. The removal of the AP site by AP lyase results in a predictable and precise deletion extending from the deaminated cytidine to the DSBs (Wang et al., 2020).

Targeted chromosomal rearrangements, which are useful for breaking or fixing genetic linkages, can also be achieved when SSNs are used to induce DSBs (Schmidt et al., 2019b;

Figure 3H). When pairs of DSBs are introduced simultaneously into the same chromosome, deletions and inversions could be generated between the two breaks (Schmidt et al., 2019a; Shan et al., 2013b). These rearrangements are primarily due to NHEJ processes and sometimes by MMEJ (Schmidt et al., 2019b). It was recently shown that megabase pair (Mbp)-targeted chromosome inversions can be achieved in maize (Schwartz et al., 2020) and *Arabidopsis thaliana* (Schmidt et al., 2020). Furthermore, the latter demonstrated that the restoration of genetic crossovers is indeed achievable through this approach. Interchromosomal rearrangements, such as crossovers, translocations, and sequence exchanges, are also triggered when two or more DSBs are generated on different chromosomes (Schmidt et al., 2019b; Figure 3H). Reciprocal translocations between heterologous chromosomes were recently produced in *A. thaliana* using the CRISPR-Cas9 system (Beying et al., 2020). Importantly, these translocations were in the Mbp range and were heritable. Still, more effective tools must be developed to realize the tremendous potential of targeted chromosomal rearrangements for plant breeding.

Next-generation plant breeding techniques involving genome editing

Conventional plant breeding has reached its limits on feeding the ever-growing global population (Hickey et al., 2019). Technical advances, such as marker-assisted selection and genomics-assisted breeding, are pushing the limit further. Genome editing opens a new toolkit for plant breeding to be performed at an unprecedented pace and in an efficient and cost-effective way, which will propel plant breeding to go beyond its current limit and move to the next generation.

Directed mutagenesis and precision breeding

Programmable targeted mutagenesis facilitates the transfer of desired traits to crops and greatly reduces the need for extensive genetic crossing and large-scale progeny genotyping (Lassoued et al., 2019). Directed knockout of *BETAINE ALDEHYDE DEHYDROGENASE 2* (*BADH2*) blocks the biosynthesis of 2-acetyl-1-pyrroline, the major fragrance compound in fragrant rice, resulting in the creation of a fragrant rice variety (Shan et al., 2015). *ACETOLACTATE SYNTHASE* (*ALS*) encodes a key enzyme in the biosynthesis of branched-chain amino acids in plants and is the target protein of various herbicides, and mutations at certain residues confer herbicide tolerance. Rice plants with broad-spectrum tolerance to ALS-inhibiting herbicides were generated by targeting the P171 and/or G628 codons of *OsALS* with a CBE, providing the opportunity for better weed management for rice farmers (Zhang et al., 2020b). The major contribution of genome editing to plant breeding is the elimination of "deleterious" genes. For example, CRISPR-Cas9 was successfully used to knock out *OsERF922*, a negative regulator of fungal blast resistance in plants, leading to the production of blast-resistant rice (Wang et al., 2016). Since some of the "deleterious" load is polygenic and is caused by many minor-effect mutations, efficient multiplex genome editing would be a promising approach to remove all the deleterious alleles (Johnsson et al., 2019).

Barriers to plant crossing prohibit trait sharing across species by conventional breeding. Efficient plant genome editing is based on advanced basic research in genome function and genomics, since the molecular mechanisms underpinning major traits such as flowering time, resistance, plant height, and seed size are often conserved in different plant species (Eshed and Lippman, 2019). Complex traits in various crop species could be directly improved using genome editing technologies based on genetic and biological information obtained from model plant research (Figure 4A). Therefore, in principle, important traits could be shared across species. Such cross-species trait "sharing" has been demonstrated by editing *MILDEW RESISTANCE LOCUS* (*MLO*), a recessive gene first identified in barley whose inactivation leads to the durable, broad-spectrum resistance to powdery mildew (Jørgensen, 1992). Powdery mildew resistance has been achieved through editing *MLO* in various plant species, including wheat (Wang et al., 2014), tomato (*S. lycopersicum*) (Nekrasov et al., 2017), and grapevine (*Vitis vinifera*) (Wan et al., 2020). *OsNP1* and *ZmIPE1* encode a putative glucose-methanol-choline oxidoreductase and are required for male sterility. CRISPR-Cas9 was used to edit the orthologs of these genes in wheat (*TaNP1*) to result in complete male sterility (Li et al., 2020b).

Cross-breeding, which relies on genetic recombination, is challenging when tightly linked loci must be separated, especially when one locus is beneficial and the other deleterious. Breaking such a genetic linkage requires extensive backcrossing, which is time-consuming and may even be impossible in some cases (Lee and Wang, 2020). In such instances, editing can be used in two ways to alter the deleterious allele. One way is to induce chromosomal rearrangements to increase recombination events, since SSNs have the capacity to create reciprocal chromosomal translocations and intrachromosomal inversions in plants (Beying et al., 2020; Shan et al., 2013b; Figure 4A). The alternate approach is to directly edit or delete the unwanted allele, thereby bypassing traditional introgression (Figure 4A). Editing has been used to break linkage drag through knocking out an undesired allele in tomato (Roldan et al., 2017), and more recently, to combine two closely linked genetic alleles in maize (Gao et al., 2020). A novel RecQ helicase gene in wheat controls genome-wide gene conversions and represents an endogenous "linkage breaking mechanism" that coverts one allele to another during DSB repair (Gardiner et al., 2019). Harnessing the same mechanism by genome editing could provide a new way to break genetic linkage in other species.

Multiplex genome editing and trait stacking

A major advantage of CRISPR over other SSNs is its capability for editing multiple target sites simultaneously. Several sgRNAs can be expressed in the same cell when using transfer RNA processing, ribozyme self-cleavage, arrays of crRNAs, or Csy4 ribonuclease cleavage (Minkenberg et al., 2017). Multiplex gene editing will drastically accelerate gene stacking for important traits. To further expedite cycles of editing and trait stacking, lab-free approaches could open the door for integration with rapid cycling systems such as speed breeding technology (Hickey et al., 2019).

Approximately one-quarter of vascular plants are polyploid, and many of these plants are agriculturally important, such as hexaploid bread wheat, tetraploid *Brassica*, cotton (*Gossypium* spp.), and potato (*S. tuberosum*) (Abe et al., 2019). Most genes

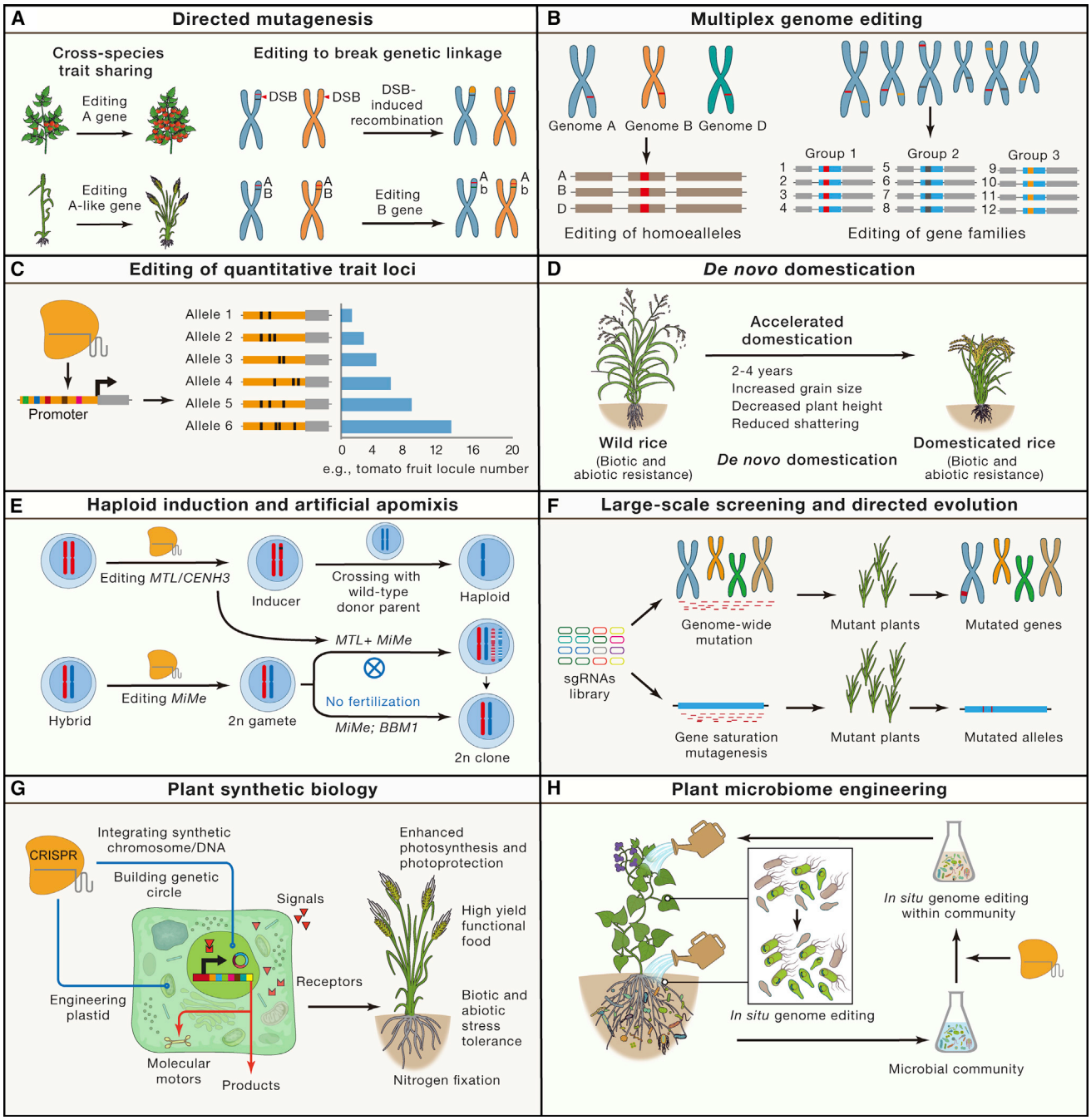

Figure 4. Crop improvement strategies based on genome editing

(A) Cross-species trait sharing and genetic linkage breaking by genome editing-directed mutagenesis.
(B) Multiplex genome editing of homoealleles and gene families.
(C) Editing of quantitative trait loci to produce new alleles and traits.
(D) Schematic diagram of accelerated domestication of wild rice through genome editing.
(E) Haploid induction and artificial apomixis via genome modification of endogenous genes.
(F) Large-scale screening and directed evolution for trait discovery via CRISPR.
(G) CRISPR-mediated plant synthetic biology in which plant cell behavior is altered to enhance plant growth and product generation.
(H) Modifying the plant microbiome to improve crop growth and pathogen resistance.

in polyploids are present in multiple copies (homeologs) that perform the same functions to control specific plant traits. These homeologs must be simultaneously mutated to generate recessive changes. Genome editing is ideally suited for this purpose (Figure 4B). This approach was first demonstrated in bread wheat, where simultaneous editing of the three homeoalleles of

MLO conferred resistance to powdery mildew, a major fungal disease (Wang et al., 2014). Since then, genome editing has been deployed to generate valuable agronomic traits in other polyploid crops (Zaman et al., 2019; Zhang et al., 2019a). Gene dosage, which is important for dosage-dependent phenotypes, can also be altered by gene editing in polyploid crops (Zhang et al., 2019a).

Analysis of 16 fully sequenced plant genomes revealed that 72% of protein-coding genes could be classified into paralogous gene families (Hyams et al., 2018). Members of a gene family normally have similar structures and overlapping functions. Such functional redundancy provides genetic robustness (Kafri et al., 2006). Therefore, knocking out one paralog may not be sufficient, and it is often necessary to mutate two or more paralogs to result in a phenotypic effect. Multiplex editing can be used for this purpose (Figure 4B). For example, the gluten gene family in wheat includes genes encoding for at least 29 α-gliadins, 18 γ-gliadins, and 10 ω-gliadins, along with 16 low molecular weight and 6 high molecular weight glutenins (Jouanin et al., 2020). Immunogenic epitopes in the α-, γ-, and ω-gliadins and (to a lesser extent) in the low molecular weight glutenins trigger the autoimmune disorder celiac disease in 1%–2% of the human population (Jouanin et al., 2020). Gluten-free wheat is difficult to obtain by conventional breeding due to the underlying genetic complexity. CRISPR-Cas9 was successfully used to simultaneously edit multiple α- and γ-gliadin genes in hexaploid bread wheat. Impressively, 35 genes were successfully mutated in a single line and immunoreactivity was reduced by 85% (Sánchez-León et al., 2018). A more efficient and precise approach would be to use base editors and prime editors to specifically modify amino acids in the immunogenic epitopes.

Editing of QTLs

Quantitative traits are of great agronomic importance. These traits are polygenic and controlled by quantitative trait loci (QTLs), with each QTL contributing only a minor effect directly on the phenotype while interacting with one another. QTLs are not inherited in a simple Mendelian manner and are therefore exceedingly difficult to study and manipulate. The main reason to investigate genetic variation underlying QTLs in plants is for crop improvement. QTLs must be identified using statistical methods such as QTL mapping and genome-wide association studies (GWASs) rather than classical genetic analysis (Cooper et al., 2009). QTL mapping, which relies on measurable phenotypes, generally works well for major QTLs (Nadeau and Frankel, 2000). Deep sequencing-based GWAS and pan-genome technologies have revealed that a large number of single-nucleotide polymorphisms (SNPs) and structural variants (SVs) are linked to quantitative trait variation in plants (Huang and Han, 2014). Many of these SNPs and SVs are located in non-coding or regulatory regions of genes, which complicates molecular characterization and confirmation. Genome editing shows great potential for overcoming these constraints by providing tools to link genetic polymorphisms with phenotypic differences (Figure 4C). QTL editing can be used to introduce multiple desired quantitative alleles directly into elite crop varieties, thus avoiding the need for intensive crossing (Shen et al., 2018); Gao et al., 2020). This technique would be especially suitable for editing QTLs in low-recombination regions.

CRISPR-Cas9 was used to generate hundreds of targeted mutations to facilitate a systematic analysis of the association of *cis*-regulatory regions with phenotypic variation in tomato (Rodríguez-Leal et al., 2017); genome editing was also used to identify QTLs via a high-throughput editing screen of candidate QTLs (Liu et al., 2020a). Using CRISPR-Cas9 and base editing to edit the upstream open reading frame (uORF) of genes has been used to fine-tune target protein expression levels in plants (Zhang et al., 2018; Xing et al., 2020), facilitating a balance between plant productivity, food quality, and adaptations to stress. Moreover, the CRISPR multiplex strategy could be used to modify a combination of candidate QTLs or all genes in a defined QTL region to result in changes to measurable phenotypes.

De novo domestication

All major crops today were domesticated from wild progenitors over many millennia. Domestication enriches for traits that increase crop productivity, such as an ideal plant architecture, high yield, and easy harvest; however, over time this leads to a genetic bottleneck that results in a decrease in genetic diversity and a loss of stress resistance. To improve cultivated crops, beneficial traits from wild relatives have since been crossed into them. Unfortunately, this type of crossing is only possible for monogenic traits and many useful traits in wild species, such as abiotic stress tolerance, are polygenic and difficult to fix by segregation during crossing and backcrossing (Kushwah et al., 2020). The *de novo* domestication of wild species by genome editing provides a promising alternative breeding strategy (López-Marqués et al., 2020; Figure 4D). As a proof of concept, multiplex editing of domestication genes was successfully performed to partially domesticate wild tomato (*S. pimpinellifolium*), while retaining the stress tolerance of the wild strain (Li et al., 2018b; Zsögön et al., 2018). The domestication of wild rice is also an attractive goal. The allotetraploid wild rice species *Oryza alta* has a large biomass and is resistant to biotic and abiotic stresses, making it a promising crop for the future. A desirable *O. alta* ecotype and created mutant lines with improved agricultural traits using CRISPR has recently been identified (Yu et al., 2021). These studies have laid the foundation for the accelerated domestication of wild plant species.

The *de novo* domestication also has the great potential to increase yields and nutrient contents of orphan crops to suit local needs. The yields of these wild crops are much lower than those of cultivated crops, but they are resilient to environmental stresses and can be grown on marginal lands (López-Marqués et al., 2020; Figure 4D). Lemmon et al. (2018) showed that ground cherry (*Physalis pruinosa*), an orphan crop distantly related to tomato, could be rapidly improved through accelerated domestication. Other orphan crops, such as sorghum (*Sorghum bicolor*), millet (*Setaria viridis*), cowpea (*Vigna unguiculata*), quinoa (*Chenopodium quinoa*), cassava, and teff (*Eragrostis tef*), are good starting materials for *de novo* domestication. The creation of fully domesticated new cultivated crops would likely require iterated editing events in addition to classic breeding and other technologies (Van Tassel et al., 2020). Leveraging *de novo* domestication to convert orphan crops to new super crops might be an effective way to secure global food supply. It is worth recalling that the introduction of a new staple food, namely potato, to

Europe played a key role in European population growth and urbanization during the 18th and 19th centuries (Nunn and Qian, 2011).

Haploid induction and artificial apomixis

Traditional plant breeding requires six to seven generations of self-pollination to deliver highly homozygous, stable cultivars. The production of doubled haploids effectively fixes recombinant haploid genomes within two generations, thereby dramatically accelerating the breeding process and reducing costs compared with traditional lengthy breeding procedures. The direct editing of endogenous plant genes is an efficient approach to produce haploid inducer lines. Knockout of *MTL/PLA1/NLD*, encoding a sperm-cell-specific phospholipase, led to the generation of defective male gametophytes and a maternal haploid induction phenotype in maize, rice, and wheat (Zhu et al., 2020). Similar results were obtained in maize by manipulating *DMP* via CRISPR-mediated mutagenesis (Zhong et al., 2019; Figure 4E).

CRISPR-Cas9-mediated deletion of the N-terminal α-helix of CENH3 resulted in the production of a haploid inducer line in *A. thaliana* (Kuppu et al., 2020). Genome editing of *TaCENH3*α in wheat led to a haploid induction rate of ~7%; editing restored frameshift alleles for heterozygous genotypes triggered higher paternal haploid induction rates than homozygous combinations (Lv et al., 2020). Importantly, several transformation-recalcitrant crop varieties were successfully modified by haploid induction editing (HI-Edit) (Kelliher et al., 2019) and haploid inducer-mediated genome editing (IMGE) (Wang et al., 2019a). HI-Edit/IMGE enables direct genomic modifications into any elite commercial background and produces transgene-free edited crops when pollinated by a haploid inducer line carrying a CRISPR-Cas cassette targeting for a desired agronomic trait.

Seed development in flowering plants (angiosperms) is triggered by double fertilization, in which the haploid egg cell and the diploid central cell each fuse with a sperm cell, resulting in a diploid embryo and triploid endosperm (Berger et al., 2008). In some species, seeds can be propagated asexually via a process known as apomixes. This process leads to the production of genetically identical seeds, which serve many applications in plant breeding (Sailer et al., 2016). Apomixis occurs naturally in >400 species. However, this process does not occur in most major crops and is very difficult to engineer by conventional breeding (Willmann, 2019). Three major steps are required for apomixis: the formation of unreduced female gametophytes (apomeiosis), embryo development from gametophytes without fertilization of the egg cell (parthenogenesis), and fertilization of the endosperm. Apomeiosis, or "mitosis instead of meiosis" (MiMe), can be induced in rice by knocking out the meiotic genes *REC8*, *PAIR1*, and *OSD1* via CRISPR-Cas9 (Khanday et al., 2019; Wang et al., 2019b; Figure 4E). One approach to creating apomictic plants is genome elimination. Wang et al. (2019b) demonstrated that Cas9-induced knockout of rice *MATRILINEAL* (*MTL*) led to haploid induction, and the simultaneous editing of *OSD1*, *PAIR1*, *REC8*, and *MTL* yielded plants that produced clonal seeds. Another approach is to trigger the embryonic development of female gametes without fertilization (Figure 4E). The misexpression of *BBM1* in unfertilized egg cells triggered embryogenesis in rice. Combining this process with *MiMe* mutations generated by editing resulted in synthetic apomixis (Khanday et al., 2019). Since the genes used in both of these approaches are conserved in other plants, these methods should be applicable to other major crops (Willmann, 2019). Despite the success of clonal seeds in rice, more improvements are needed before realizing the practical use of synthetic apomixis in modern agriculture, such as toward the fixation of hybrid vigor. Manipulating endosperm fertilization could facilitate the generation of synthetic apomixis in crops.

Large-scale screening for trait discovery

It is essential to understand the genetic regulation of beneficial traits in order to apply genome editing to plant breeding. CRISPR-Cas9 screens can be used as a forward genetic screening tool for the genome-wide characterization of the relationship between genotypes and phenotypes (Gaillochet et al., 2020). For this type of screening, Cas9 is expressed together with a library of sgRNAs that target many or all genes in a plant. Following plant transformation, edited plants are regenerated and their progeny screened for a trait of interest. The genes or mutants of interest are identified through sequencing the sgRNA of enriched variants. CRISPR-Cas9 has been successfully used for genome-wide screening in rice (Lu et al., 2017; Meng et al., 2017) and to generate mutant populations in tomato (Jacobs et al., 2017), soybean (*Glycine max*) (Bai et al., 2020), and maize (Liu et al., 2020a; Figure 4F). This approach is more effective for generating genome-wide mutations in plants than chemical, physical, or transposon mutagenesis. However, current CRISPR screens in plants require laborious plant tissue culture procedures. In the future, perhaps single-cell or protoplast-based screening methods that take advantage of robust phenotypic readouts and single-cell sequencing could speed the future of trait discovery.

Another important application of large-scale screening by CRISPR-Cas involves the introduction of saturated mutations in a gene or its functional domain followed by a directed-evolution screening for trait engineering and new trait discovery (Figure 4F). This approach requires the production of a variety of mutants using an sgRNA library as well as efficient methods for screening and selecting plants with desired properties (Gionfriddo et al., 2019). CRISPR-Cas9 has been successfully exploited for directed protein evolution in the native plant environment by coupling Cas9 with a library of sgRNAs tilling all potential sites on both strands of the relevant coding sequence (Butt et al., 2019). Nevertheless, the predominant mutations induced by Cas9 are indel-associated frameshifts, making it difficult to generate all the amino acid substitutions needed for the generation of SNPs, the most common sources of genetic variation in nature (Capdeville et al., 2020). Base editors are tools ideally suited for overcoming this difficulty. For example, a library of sgRNAs was delivered into plant cells together with either CBE or ABE to promote the evolution of herbicide resistance by generating functional variants of OsACC in rice (Liu et al., 2020b). Moreover, dual cytosine and adenine base editors were developed to generate simultaneous C·G to T·A and A·T to G·C conversions and have been used for near-saturation mutagenesis of the chosen target domain of OsACC (Li et al., 2020a). Both known and novel variants were recovered in these CRISPR-directed evolution campaigns. However, only a limited

number of plant proteins can be engineered in this manner. Methods for iterative mutation and selection using single cells or protoplasts could expand the utility of approach in the future.

Challenges and future perspectives

Increasing precise genome editing efficiency

Despite the latest technical advances in plant genome editing, it is still not possible to generate all desired changes in a genome. Precise genome editing, such as the generation of targeted base substitutions, gene insertions/deletions, and gene replacements, is urgently needed for trait improvement in crops. In principle, HDR-mediated genome editing could be used to precisely re-write any genome and produce a specified edit. Various strategies have been used to improve HDR efficiency in plant cells, such as the use of geminivirus constructs (Baltes et al., 2014), an *in planta* gene targeting (GT) system (Fauser et al., 2012), or chemical modification to stabilize donor templates (Lu et al., 2020). HDR efficiency can also be enhanced by bringing the donor DNA template close to the DSBs (Ali et al., 2020), manipulating DNA repair pathways (Christian et al., 2013), taking advantage of specific cell-cycle phases and cell types (Wolter et al., 2018), or using different SSNs (Merker et al., 2020; Wolter and Puchta, 2019). Although precise HDR-mediated genome editing has been reported in many plants based on the above-mentioned strategies, the process itself is still extremely inefficient in somatic plant cells (Steinert et al., 2016).

To overcome these limitations, the newly developed DNA base editing systems (CBE and ABE) provide efficient and simple ways to convert a specific DNA base into another base at a targeted genomic locus; however, this is currently limited to C·G to T·A and A·T to G·C substitutions (Chen et al., 2019). Therefore, other base editing systems, such as C·G to G·C (Kurt et al., 2021; Zhao et al., 2020), must be developed through engineering deaminases, manipulating the DNA repair pathway, or protein engineering. In addition to base editing, prime editing could be used to generate any base substitutions, but they currently exhibit rather low editing efficiencies. Since the activity of a prime editor is determined by many factors, such as the activity of the RT, the length of the PBS in the pegRNA, and the RT template, more strategies are needed to improve prime editor activity in plant cells. Although prime editing cannot generate large gene insertions, recently discovered CRISPR-associated transposases can integrate DNA into bacterial genomes at high efficiency (Klompe et al., 2019; Strecker et al., 2019), opening the door to a future possibility of large DNA insertions into plant genomes.

Improving the specificity of genome editing

Off-target effects are one of the major concerns in genome editing. CRISPR technologies generate two types of off-target edits: sgRNA-dependent and sgRNA-independent off-target edits. sgRNA-dependent off-target edits are induced by editing off-target sites that contain mismatches to the on-target sgRNA sequence. Whole-genome sequencing suggested that CRISPR-Cas systems do not induce sgRNA-independent off-target effects in rice or cotton (Li et al., 2019; Tang et al., 2018). However, some CBEs induce genome-wide sgRNA-independent off-target mutations in rice (Jin et al., 2019; Jin et al., 2020b), which are triggered by the cytidine deaminase activity in ssDNA regions genome wide. Compared with conventional mutation breeding, which introduces many unintended mutations into the plant genome, plant genome editing is highly specific. Moreover, a limited number of off-target mutations can be eliminated by backcrossing. The specificity of CRISPR-Cas can be enhanced by transiently expressing the editing reagents, as demonstrated in wheat and maize (Liang et al., 2017; Svitashev et al., 2016), by employing rationally designed guide RNAs (Bae et al., 2014), or by using engineered precise variants of Cas9, Cas12a and deaminases (Zhang et al., 2019b; Jin et al., 2020a). However, further research is needed to address the propensity for off-target editing, especially in developing more sensitive methods to detect genome-wide off-target mutations in plants, and in identifying improved or new editors with higher specificity. I believe that off-target effects of plant genome editing will not be an issue as the technology advances.

Optimizing plant cell delivery and regeneration systems

The ideal plant genome editing reagent delivery system is genotype independent, tissue culture free, and directly applied to specific tissues, such as meristems, leaves, seeds, or hypocotyls. The current delivery methods include particle bombardment, transformation mediated by *Agrobacterium*, polyethylene glycol (PEG), viral vectors, and nanoparticles. The fusion of an sgRNA and an endogenous mobile RNA sequence expressed by an RNA virus was recently shown to migrate to meristematic regions and generate heritable mutations (Ellison et al., 2020). Nanoparticles and other new materials might serve as useful vehicles for editing reagents (Cunningham et al., 2018). For example, carbon nanotubes could be used to deliver DNA into plant leaves, leading to successful protein expression (Demirer et al., 2019). If this system could deliver genome editing reagents into the shoot apical meristem, tissue culture-free editing could be achieved.

Once genome editing reagents are delivered into somatic cells, subsequent tissue culture and plant regeneration are required to obtain edited plants (Atkins and Voytas, 2020); however, this regeneration step is extremely challenging in most crops (Altpeter et al., 2016). Plant regeneration is based on the totipotency of somatic cells, which distinguishes plant cells from most other eukaryotic cells (Vasil and Vasil, 1972). Developmental regulators (designated boosters) that promote somatic embryogenesis have been used to boost plant regeneration (Figures 2B and 2C). Overexpressing two developmental regulators, *WUSCHEL* (*WUS*) and *BABY BOOM* (*BBM*), improves regeneration frequencies in various transformation-recalcitrant genotypes and species (Lowe et al., 2016). Although stable transformation of *WUS* and *BBM* causes morphologic defects and sterility in maize, the use of suitable promoters to control for tissue- and timing-specific expression of *WUS* and *BBM* alleviates their pleiotropic effects (Lowe et al., 2018). *GROWTH-REGULATING FACTORs* (*GRFs*), *GRF-INTERACTING FACTORs* (*GIFs*), and GRF-GIF chimeras have also been used to improve the regeneration efficiency of various monocot and dicot plants (Debernardi et al., 2020; Kong et al., 2020). In contrast to *WUS* and *BBM*, *GRFs*, *GIFs*, and GRF-GIF chimeras have no apparent side effects when they are constitutively expressed, bypassing the laborious and time-intensive steps of excising them after transformation but before regeneration process. Developmental

regulators can also be used to generate genome-edited dicots through *de novo* meristem induction, which sidesteps the need for tissue culture (Maher et al., 2020). Despite these promising techniques, plant genetic transformation and regeneration still require the use of specialized facilities, making it necessary to simplify these two processes to enable routine plant genome editing in most laboratories.

Plant synthetic biology

Synthetic biology is a new strategy used to accelerate the development of novel agronomic traits. The CRISPR-Cas system has great potential for improving plant design and synthetic biology (Figure 4G). By editing endogenous genes or introducing foreign genes encoding various enzymes or signaling pathway components, researchers have been able to redirect inherent metabolic networks or establish new pathways in plants in order to produce foods enriched in the desired natural or artificial compounds (Chen et al., 2019; Figure 4G). CRISPR-Cas-mediated multiplex gene editing and regulation could be used to accomplish this synthetic biology task. For example, photosynthesis systems in plants are far from perfect, as Rubisco, the core enzyme that functions in the photosynthetic pathway, is inefficient for CO_2 fixation and poisoned through photorespiration, leading to massive losses of carbon, nitrogen, and energy. By introducing components to artificially bypass photorespiration (South et al., 2019) or by redesigning Rubisco (Gunn et al., 2020) via CRISPR-mediated DNA insertion, the photosynthetic efficiency and biomass of plants could be increased. Beyond these prospects, genome editing could also facilitate other aspects of plant synthetic biology, such as building plant biosensors to monitor intracellular signals or plant biorecorders to detect environmental stimulation.

Plant microbiome engineering

In nature, plants are exposed to trillions of microbes, including bacteria, fungi, protozoa, archaea, and viruses (Mueller and Sachs, 2015). Beneficial plant-microbiome interactions can improve plant growth or control pathogens. Inoculating microbiomes within a consortium of plant growth-promoting rhizobacteria can enhance plant development and help protect plants from both pathogens and abiotic stress (Arif et al., 2020). Hence, the microbiome is considered to represent a ''second genome'' in plants. The plant microbiome influences plant growth by altering nutrient absorption and gene expression and by acting as a biocontrol pathogen. Microbiome engineering has already had a significant effect on agricultural production (Mueller and Sachs, 2015). Recent advances in high-throughput sequencing and CRISPR-mediated genome editing have provided insights into the roles of bacterial genes in microbial communities, and these new approaches to modify the microbiome may improve future crop growth and pathogen resistance (Figure 4H).

The CRISPR system could be used to precisely edit the genomes of specific organisms within a complex microbial community (Rubin et al., 2020). These edited organisms could then be returned to the laboratory environment to study whether a specific modification results in new characteristics in the host plant, such as improved nutrient absorption or pathogen resistance. Such information would reveal the exact function of a specific organism within a microbial community. The CRISPR system could first be transformed into a microbial community, followed by delivery into a natural plant growth environment, such as soil and leaves. The CRISPR system could also be transferred from modified microbes to other microbial community members via interactions and conjugation, leading to *in situ* genome editing of the plant microbiomes (Figure 4H). Such precise genome editing of plant microbiomes would enable new approach for improving crop production.

Contributing to a science-based regulatory framework for genome-edited crops

The regulation and social acceptance of genome-edited plants are crucial for the development of new breeding technologies and their derived crops and products, but these steps remain problematic (Lassoued et al., 2019). Currently, process-based or product-based regulatory approaches are employed when regulating genome-edited crops. The European Union uses process-based regulations, while Canada, the United States, and Argentina are proponents of the product-based approach, but most other countries have not yet established their regulatory frameworks (Lassoued et al., 2020; Scheben and Edwards, 2018). To date, several edited plants have been fully approved using the product-based approach. Most of the genome-edited plants currently waiting for approval in the regulatory pipeline have been filed by public research institutions and small- or medium-sized companies (Friedrichs et al., 2019). However, if a restrictive regulatory approach is adopted and treats edited plants as GMOs, it would create huge financial burdens that only large multinational companies could tolerate. Regulations must be reasonable and easy to navigate for transgene-free edited products (Zhang et al., 2020c). A science-based regulatory framework for genome-edited crops has been proposed (Huang et al., 2016). Since genome editing is not a single technology but rather a molecular toolbox, a comprehensive, one-size-fits-all regulatory approach may be unsuitable. Instead, a tiered regulatory system should be used to accommodate both existing and future technologies (Chen and Gao, 2020; Lassoued et al., 2020; Macnaghten and Habets, 2020). More effort is needed to ensure regulatory transparency and open dialog. Public communication should be fact-based and science-based (Friedrichs et al., 2019). We should open the conversation and engage with different voices, including those from developing and underdeveloped countries where increased food production is most needed. Nonetheless, reconciling the conflicting interests of different stakeholders is bound to pose a major challenge (Lassoued et al., 2020).

Conclusions

The development of genome editing technologies in plants enables a breadth of opportunities for plant breeding. Efficient, precise, and targeted mutagenesis via genome editing has laid the foundation for many next-generation breeding strategies that will revolutionize the future of agriculture. To exploit the full potential of plant genome editing, all approaches must be explored. Genome editing allows for a combination of genetic traits to be rationally designed into crops. These precise and efficient techniques when used for rapid plant breeding results in outcomes similar to those of classical breeding. However, it is unlikely that genome editing-based next-generation breeding will completely displace conventional approaches; only when

combined with other technologies, such as high-throughput phenotyping, genomic selection and speed breeding, can we guarantee the widespread implementation of genome editing in agriculture. This multidisciplinary approach will advance plant breeding to help secure a second Green Revolution in order to meet the increasing food demands of a rapidly growing global population under ever-changing climate conditions.

ACKNOWLEDGMENTS

The author is grateful to Jin-Long Qiu for critical reading of the manuscript and Yanpeng Wang for assistance in figure drawing. This work was supported by grants from the Strategic Priority Research Program of the Chinese Academy of Sciences (Precision Seed Design and Breeding, XDA24020102), the National Natural Science Foundation of China (31788103), the National Key Research and Development Program of China (2016YFD0101804), and the Chinese Academy of Sciences (QYZDY-SSW-SMC030). I apologize to those whose work is not cited due to the space limitation.

DECLARATION OF INTERESTS

C. Gao is an advisory board member at *Cell*.

REFERENCES

Abe, F., Haque, E., Hisano, H., Tanaka, T., Kamiya, Y., Mikami, M., Kawaura, K., Endo, M., Onishi, K., Hayashi, T., et al. (2019). Genome-Edited Triple-Recessive Mutation Alters Seed Dormancy in Wheat. Cell Rep. *28*, 1362–1369.e1364.

Ali, Z., Shami, A., Sedeek, K., Kamel, R., Alhabsi, A., Tehseen, M., Hassan, N., Butt, H., Kababji, A., Hamdan, S.M., and Mahfouz, M.M. (2020). Fusion of the Cas9 endonuclease and the VirD2 relaxase facilitates homology-directed repair for precise genome engineering in rice. Commun. Biol. *3*, 44.

Altpeter, F., Springer, N.M., Bartley, L.E., Blechl, A.E., Brutnell, T.P., Citovsky, V., Conrad, L.J., Gelvin, S.B., Jackson, D.P., Kausch, A.P., et al. (2016). Advancing Crop Transformation in the Era of Genome Editing. Plant Cell *28*, 1510–1520.

Anzalone, A.V., Koblan, L.W., and Liu, D.R. (2020). Genome editing with CRISPR-Cas nucleases, base editors, transposases and prime editor. Nat. Biotechnol. *38*, 824–844.

Anzalone, A.V., Randolph, P.B., Davis, J.R., Sousa, A.A., Koblan, L.W., Levy, J.M., Chen, P.J., Wilson, C., Newby, G.A., Raguram, A., and Liu, D.R. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. Nature *576*, 149–157.

Arif, I., Batool, M., and Schenk, P.M. (2020). Plant Microbiome Engineering: Expected Benefits for Improved Crop Growth and Resilience. Trends Biotechnol. *38*, 1385–1396.

Atkins, P.A., and Voytas, D.F. (2020). Overcoming bottlenecks in plant gene editing. Curr. Opin. Plant Biol. *54*, 79–84.

Bae, S., Kweon, J., Kim, H.S., and Kim, J.-S. (2014). Microhomology-based choice of Cas9 nuclease target sites. Nat. Methods *11*, 705–706.

Bai, M., Yuan, J., Kuang, H., Gong, P., Li, S., Zhang, Z., Liu, B., Sun, J., Yang, M., Yang, L., et al. (2020). Generation of a multiplex mutagenesis population via pooled CRISPR-Cas9 in soya bean. Plant Biotechnol. J. *18*, 721–731.

Baltes, N.J., Gil-Humanes, J., Cermak, T., Atkins, P.A., and Voytas, D.F. (2014). DNA replicons for plant genome engineering. Plant Cell *26*, 151–163.

Berger, F., Hamamura, Y., Ingouff, M., and Higashiyama, T. (2008). Double fertilization - caught in the act. Trends Plant Sci. *13*, 437–443.

Beying, N., Schmidt, C., Pacher, M., Houben, A., and Puchta, H. (2020). CRISPR-Cas9-mediated induction of heritable chromosomal translocations in *Arabidopsis*. Nat. Plants *6*, 638–645.

Butt, H., Piatek, A., Li, L., S N Reddy, A., and M Mahfouz, M. (2019). Multiplex CRISPR Mutagenesis of the Serine/Arginine-Rich (SR) Gene Family in Rice. Genes (Basel) *10*, 596.

Capdeville, N., Schindele, P., and Puchta, H. (2020). Application of CRISPR/Cas-mediated base editing for directed protein evolution in plants. Sci. China Life Sci. *63*, 613–616.

Chen, K., and Gao, C. (2020). Genome-edited crops: how to move them from laboratory to market. Front. Agric. Sci. Eng. *7*, 181–187.

Chen, K., Wang, Y., Zhang, R., Zhang, H., and Gao, C. (2019). CRISPR/Cas Genome Editing and Precision Plant Breeding in Agriculture. Annu. Rev. Plant Biol. *70*, 667–697.

Christian, M., Qi, Y., Zhang, Y., and Voytas, D.F. (2013). Targeted mutagenesis of *Arabidopsis thaliana* using engineered TAL effector nucleases. G3 (Bethesda) *3*, 1697–1705.

Cooper, M., van Eeuwijk, F.A., Hammer, G.L., Podlich, D.W., and Messina, C. (2009). Modeling QTL for complex traits: detection and context for plant breeding. Curr. Opin. Plant Biol. *12*, 231–240.

Cunningham, F.J., Goh, N.S., Demirer, G.S., Matos, J.L., and Landry, M.P. (2018). Nanoparticle-Mediated Delivery towards Advancing Plant Genetic Engineering. Trends Biotechnol. *36*, 882–897.

Debernardi, J.M., Tricoli, D.M., Ercoli, M.F., Hayta, S., Ronald, P., Palatnik, J.F., and Dubcovsky, J. (2020). A GRF-GIF chimeric protein improves the regeneration efficiency of transgenic plants. Nat. Biotechnol. *38*, 1274–1279.

Demirer, G.S., Zhang, H., Matos, J.L., Goh, N.S., Cunningham, F.J., Sung, Y., Chang, R., Aditham, A.J., Chio, L., Cho, M.J., et al. (2019). High aspect ratio nanomaterials enable delivery of functional genetic material without DNA integration in mature plants. Nat. Nanotechnol. *14*, 456–464.

Dong, O.X., Yu, S., Jain, R., Zhang, N., Duong, P.Q., Butler, C., Li, Y., Lipzen, A., Martin, J.A., Barry, K.W., et al. (2020). Marker-free carotenoid-enriched rice generated through targeted gene insertion using CRISPR-Cas9. Nat. Commun. *11*, 1178.

Ellison, E.E., Nagalakshmi, U., Gamo, M.E., Huang, P.J., Dinesh-Kumar, S., and Voytas, D.F. (2020). Multiplexed heritable gene editing using RNA viruses and mobile single guide RNAs. Nat. Plants *6*, 620–624.

Eshed, Y., and Lippman, Z.B. (2019). Revolutions in agriculture chart a courseresee for targeted breeding of old and new crops. Science *366*, eaax0025.

FAO (2017). The future of food and agriculture: Trends and challenges (FAO).

Fauser, F., Roth, N., Pacher, M., Ilg, G., Sánchez-Fernández, R., Biesgen, C., and Puchta, H. (2012). In planta gene targeting. Proc. Natl. Acad. Sci. USA *109*, 7535–7540.

Friedrichs, S., Takasu, Y., Kearns, P., Dagallier, B., Oshima, R., Schofield, J., and Moreddu, C. (2019). Policy Considerations Regarding Genome Editing. Trends Biotechnol. *37*, 1029–1032.

Gaillochet, C., Develtere, W., and Jacobs, T.B. (2020). CRISPR screens in plants: approaches, guidelines, and future prospects. Plant Cell. https://doi.org/10.1105/tpc.20.00463.

Gao, H., Gadlage, M.J., Lafitte, H.R., Lenderts, B., Yang, M., Schroder, M., Farrell, J., Snopek, K., Peterson, D., Feigenbutz, L., et al. (2020). Superior field performance of waxy corn engineered using CRISPR-Cas9. Nat. Biotechnol. *38*, 579–581.

Gardiner, L.J., Wingen, L.U., Bailey, P., Joynson, R., Brabbs, T., Wright, J., Higgins, J.D., Hall, N., Griffiths, S., Clavijo, B.J., and Hall, A. (2019). Analysis of the recombination landscape of hexaploid bread wheat reveals genes controlling recombination and gene conversion frequency. Genome Biol. *20*, 69.

Gaudelli, N.M., Komor, A.C., Rees, H.A., Packer, M.S., Badran, A.H., Bryson, D.I., and Liu, D.R. (2017). Programmable base editing of A·T to G·C in genomic DNA without DNA cleavage. Nature *551*, 464–471.

Gionfriddo, M., De Gara, L., and Loreto, F. (2019). Directed Evolution of Plant Processes: Towards a Green (r)Evolution? Trends Plant Sci. *24*, 999–1007.

Gunn, L.H., Martin Avila, E., Birch, R., and Whitney, S.M. (2020). The dependency of red Rubisco on its cognate activase for enhancing plant photosynthesis and growth. Proc. Natl. Acad. Sci. USA *117*, 25890–25896.

Hickey, L.T., N Hafeez, A., Robinson, H., Jackson, S.A., Leal-Bertioli, S.C.M., Tester, M., Gao, C., Godwin, I.D., Hayes, B.J., and Wulff, B.B.H. (2019). Breeding crops to feed 10 billion. Nat. Biotechnol. *37*, 744–754.

Holme, I.B., Gregersen, P.L., and Brinch-Pedersen, H. (2019). Induced Genetic Variation in Crop Plants by Random or Targeted Mutagenesis: Convergence and Differences. Front. Plant Sci. *10*, 1468.

Huang, X., and Han, B. (2014). Natural variations and genome-wide association studies in crop plants. Annu. Rev. Plant Biol. *65*, 531–551.

Huang, S., Weigel, D., Beachy, R.N., and Li, J. (2016). A proposed regulatory framework for genome-edited crops. Nat. Genet. *48*, 109–111.

Hyams, G., Abadi, S., Lahav, S., Avni, A., Halperin, E., Shani, E., and Mayrose, I. (2018). CRISPys: Optimal sgRNA Design for Editing Multiple Members of a Gene Family Using the CRISPR System. J. Mol. Biol. *430*, 2184–2195.

Jacobs, T.B., Zhang, N., Patel, D., and Martin, G.B. (2017). Generation of a Collection of Mutant Tomato Lines Using Pooled CRISPR Libraries. Plant Physiol. *174*, 2023–2037.

Jiang, Y.Y., Chai, Y.P., Lu, M.H., Han, X.L., Lin, Q., Zhang, Y., Zhang, Q., Zhou, Y., Wang, X.C., Gao, C., and Chen, Q.J. (2020). Prime editing efficiently generates W542L and S621I double mutations in two *ALS* genes in maize. Genome Biol. *21*, 257.

Jin, S., Zong, Y., Gao, Q., Zhu, Z., Wang, Y., Qin, P., Liang, C., Wang, D., Qiu, J.-L., Zhang, F., and Gao, C. (2019). Cytosine, but not adenine, base editors induce genome-wide off-target mutations in rice. Science *364*, 292–295.

Jin, S., Fei, H., Zhu, Z., Luo, Y., Liu, J., Gao, S., Zhang, F., Chen, Y.H., Wang, Y., and Gao, C. (2020a). Rationally Designed APOBEC3B Cytosine Base Editors with Improved Specificity. Mol. Cell *79*, 728–740.e6.

Jin, S., Gao, Q., and Gao, C. (2020b). An unbiased method for evaluating the genome-wide specificity of base editors in rice. Nat. Protoc. *16*, 431–457.

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A., and Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science *337*, 816–821.

Johnsson, M., Gaynor, R.C., Jenko, J., Gorjanc, G., de Koning, D.J., and Hickey, J.M. (2019). Removal of alleles by genome editing (RAGE) against deleterious load. Genet. Sel. Evol. *51*, 14.

Jørgensen, I.H. (1992). Discovery, characterization and exploitation of *Mlo* powdery mildew resistance in barley. Euphytica *63*, 141–152.

Jouanin, A., Gilissen, L.J.W.J., Schaart, J.G., Leigh, F.J., Cockram, J., Wallington, E.J., Boyd, L.A., van den Broeck, H.C., van der Meer, I.M., America, A.H.P., et al. (2020). CRISPR/Cas9 Gene Editing of Gluten in Wheat to Reduce Gluten Content and Exposure-Reviewing Methods to Screen for Coeliac Safety. Front. Nutr. *7*, 51.

Kafri, R., Levy, M., and Pilpel, Y. (2006). The regulatory utilization of genetic redundancy through responsive backup circuits. Proc. Natl. Acad. Sci. USA *103*, 11653–11658.

Kelliher, T., Starr, D., Su, X., Tang, G., Chen, Z., Carter, J., Wittich, P.E., Dong, S., Green, J., Burch, E., et al. (2019). One-step genome editing of elite crop germplasm during haploid induction. Nat. Biotechnol. *37*, 287–292.

Khanday, I., Skinner, D., Yang, B., Mercier, R., and Sundaresan, V. (2019). A male-expressed rice embryogenic trigger redirected for asexual propagation through seeds. Nature *565*, 91–95.

Khush, G.S. (2001). Green revolution: the way forward. Nat. Rev. Genet. *2*, 815–822.

Klompe, S.E., Vo, P.L.H., Halpin-Healy, T.S., and Sternberg, S.H. (2019). Transposon-encoded CRISPR-Cas systems direct RNA-guided DNA integration. Nature *571*, 219–225.

Komor, A.C., Kim, Y.B., Packer, M.S., Zuris, J.A., and Liu, D.R. (2016). Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature *533*, 420–424.

Kong, J., Martin-Ortigosa, S., Finer, J., Orchard, N., Gunadi, A., Batts, L.A., Thakare, D., Rush, B., Schmitz, O., Stuiver, M., et al. (2020). Overexpression of the Transcription Factor *GROWTH-REGULATING FACTOR5* Improves Transformation of Dicot and Monocot Species. Front. Plant Sci. *11*, 572319.

Kuppu, S., Ron, M., Marimuthu, M.P.A., Li, G., Huddleson, A., Siddeek, M.H., Terry, J., Buchner, R., Shabek, N., Comai, L., and Britt, A.B. (2020). A variety of changes, including CRISPR/Cas9-mediated deletions, in CENH3 lead to haploid induction on outcrossing. Plant Biotechnol. J. *18*, 2068–2080.

Kurt, I.C., Zhou, R., Iyer, S., Garcia, S.P., Miller, B.R., Langner, L.M., Grünewald, J., and Joung, J.K. (2021). CRISPR C-to-G base editors for inducing targeted DNA transversions in human cells. Nat. Biotechnol. *39*, 41–46.

Kushwah, A., Gupta, S., Bindra, S., Johal, N., Singh, I., Bharadwaj, C., Dixit, G.P., Gaur, P.M., Nayyar, H., and Singh, S. (2020). Gene pyramiding and multiple character breeding. In Chickpea: Crop wild relatives for enhancing genetic gains, M. Singh, ed. (Academic Press), pp. 131–165.

Lassoued, R., Macall, D.M., Hesseln, H., Phillips, P.W.B., and Smyth, S.J. (2019). Benefits of genome-edited crops: expert opinion. Transgenic Res. *28*, 247–256.

Lassoued, R., Macall, D.M., Smyth, S.J., Phillips, P.W.B., and Hesseln, H. (2020). How should we regulate products of new breeding techniques? Opinion of surveyed experts in plant biotechnology. Biotechnol. Rep. (Amst.) *26*, e00460.

Lee, K., and Wang, K. (2020). Level up to chromosome restructuring. Nat. Plants *6*, 600–601.

Lemmon, Z.H., Reem, N.T., Dalrymple, J., Soyk, S., Swartwood, K.E., Rodriguez-Leal, D., Van Eck, J., and Lippman, Z.B. (2018). Rapid improvement of domestication traits in an orphan crop by genome editing. Nat. Plants *4*, 766–770.

Li, J., Meng, X., Zong, Y., Chen, K., Zhang, H., Liu, J., Li, J., and Gao, C. (2016). Gene replacements and insertions in rice by intron targeting using CRISPR-Cas9. Nat. Plants *2*, 16139.

Li, C., Zong, Y., Wang, Y., Jin, S., Zhang, D., Song, Q., Zhang, R., and Gao, C. (2018a). Expanded base editing in rice and wheat using a Cas9-adenosine deaminase fusion. Genome Biol. *19*, 59.

Li, T., Yang, X., Yu, Y., Si, X., Zhai, X., Zhang, H., Dong, W., Gao, C., and Xu, C. (2018b). Domestication of wild tomato is accelerated by genome editing. Nat. Biotechnol. *36*, 1160–1163.

Li, J., Manghwar, H., Sun, L., Wang, P., Wang, G., Sheng, H., Zhang, J., Liu, H., Qin, L., Rui, H., et al. (2019). Whole genome sequencing reveals rare off-target mutations and considerable inherent genetic or/and somaclonal variations in CRISPR/Cas9-edited cotton plants. Plant Biotechnol. J. *17*, 858–868.

Li, C., Zhang, R., Meng, X., Chen, S., Zong, Y., Lu, C., Qiu, J.-L., Chen, Y.H., Li, J., and Gao, C. (2020a). Targeted, random mutagenesis of plant genes with dual cytosine and adenine base. Nat. Biotechnol. *38*, 875–882.

Li, J., Wang, Z., He, G., Ma, L., and Deng, X.W. (2020b). CRISPR/Cas9-mediated disruption of *TaNP1* genes results in complete male sterility in bread wheat. J. Genet. Genomics *47*, 263–272.

Liang, Z., Chen, K., Li, T., Zhang, Y., Wang, Y., Zhao, Q., Liu, J., Zhang, H., Liu, C., Ran, Y., and Gao, C. (2017). Efficient DNA-free genome editing of bread wheat using CRISPR/Cas9 ribonucleoprotein complexes. Nat. Commun. *8*, 14261.

Lin, Q., Zong, Y., Xue, C., Wang, S., Jin, S., Zhu, Z., Wang, Y., Anzalone, A.V., Raguram, A., Doman, J.L., et al. (2020). Prime genome editing in rice and wheat. Nat. Biotechnol. *38*, 582–585.

Liu, H.J., Jian, L., Xu, J., Zhang, Q., Zhang, M., Jin, M., Peng, Y., Yan, J., Han, B., Liu, J., et al. (2020a). High-Throughput CRISPR/Cas9 Mutagenesis Streamlines Trait Gene Identification in Maize. Plant Cell *32*, 1397–1413.

Liu, X., Qin, R., Li, J., Liao, S., Shan, T., Xu, R., Wu, D., and Wei, P. (2020b). A CRISPR-Cas9-mediated domain-specific base-editing screen enables functional assessment of ACCase variants in rice. Plant Biotechnol. J. *18*, 1845–1847.

López-Marqués, R.L., Nørrevang, A.F., Ache, P., Moog, M., Visintainer, D., Wendt, T., Østerberg, J.T., Dockter, C., Jørgensen, M.E., Salvador, A.T., et al. (2020). Prospects for the accelerated improvement of the resilient crop quinoa. J. Exp. Bot. *71*, 5333–5347.

Lowe, K., Wu, E., Wang, N., Hoerster, G., Hastings, C., Cho, M.J., Scelonge, C., Lenderts, B., Chamberlin, M., Cushatt, J., et al. (2016). Morphogenic

Regulators *Baby boom* and *Wuschel* Improve Monocot Transformation. Plant Cell *28*, 1998–2015.

Lowe, K., La Rota, M., Hoerster, G., Hastings, C., Wang, N., Chamberlin, M., Wu, E., Jones, T., and Gordon-Kamm, W. (2018). Rapid genotype "independent" *Zea mays* L. (maize) transformation via direct somatic embryogenesis. In Vitro Cell. Dev. Biol. Plant *54*, 240–252.

Lu, Y., Ye, X., Guo, R., Huang, J., Wang, W., Tang, J., Tan, L., Zhu, J.K., Chu, C., and Qian, Y. (2017). Genome-wide Targeted Mutagenesis in Rice Using the CRISPR/Cas9 System. Mol. Plant *10*, 1242–1245.

Lu, Y., Tian, Y., Shen, R., Yao, Q., Wang, M., Chen, M., Dong, J., Zhang, T., Li, F., Lei, M., and Zhu, J.K. (2020). Targeted, efficient sequence insertion and replacement in rice. Nat. Biotechnol. *38*, 1402–1407.

Lv, J., Yu, K., Wei, J., Gui, H., Liu, C., Liang, D., Wang, Y., Zhou, H., Carlin, R., Rich, R., et al. (2020). Generation of paternal haploids in wheat by genome editing of the centromeric histone CENH3. Nat. Biotechnol. *38*, 1397–1401.

Macnaghten, P., and Habets, M.G.J.L. (2020). Breaking the impasse: Towards a forward-looking governance framework for gene editing with plants. PLANTS. People Planet *2*, 353–365.

Maher, M.F., Nasti, R.A., Vollbrecht, M., Starker, C.G., Clark, M.D., and Voytas, D.F. (2020). Plant gene editing through *de novo* induction of meristems. Nat. Biotechnol. *38*, 84–89.

Meng, X., Yu, H., Zhang, Y., Zhuang, F., Song, X., Gao, S., Gao, C., and Li, J. (2017). Construction of a Genome-Wide Mutant Library in Rice Using CRISPR/Cas9. Mol. Plant *10*, 1238–1241.

Merker, L., Schindele, P., Huang, T.K., Wolter, F., and Puchta, H. (2020). Enhancing in planta gene targeting efficiencies in *Arabidopsis* using temperature-tolerant CRISPR/LbCas12a. Plant Biotechnol. J. *18*, 2382–2384.

Ming, M., Ren, Q., Pan, C., He, Y., Zhang, Y., Liu, S., Zhong, Z., Wang, J., Malzahn, A.A., Wu, J., et al. (2020). CRISPR-Cas12b enables efficient plant genome engineering. Nat. Plants *6*, 202–208.

Minkenberg, B., Wheatley, M., and Yang, Y. (2017). CRISPR/Cas9-Enabled Multiplex Genome Editing and Its Application. In Progress in molecular biology and translational science, D.P. Weeks and B. Yang, eds. (Academic Press), pp. 111–132.

Mueller, U.G., and Sachs, J.L. (2015). Engineering Microbiomes to Improve Plant and Animal Health. Trends Microbiol. *23*, 606–617.

Nadeau, J.H., and Frankel, W.N. (2000). The roads from phenotypic variation to gene discovery: mutagenesis versus QTLs. Nat. Genet. *25*, 381–384.

Nekrasov, V., Wang, C., Win, J., Lanz, C., Weigel, D., and Kamoun, S. (2017). Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion. Sci. Rep. *7*, 482.

Nishida, K., Arazoe, T., Yachie, N., Banno, S., Kakimoto, M., Tabata, M., Mochizuki, M., Miyabe, A., Araki, M., Hara, K.Y., et al. (2016). Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems. Science *353*, aaf8729.

Nunn, N., and Qian, N. (2011). The potato's contribution to population and urbanization: evidence from a historical experiment. Q. J. Econ. *126*, 593–650.

Raman, R. (2017). The impact of Genetically Modified (GM) crops in modern agriculture: A review. GM Crops Food *8*, 195–208.

Ran, Y., Liang, Z., and Gao, C. (2017). Current and future editing reagent delivery systems for plant genome editing. Sci. China Life Sci. *60*, 490–505.

Ren, B., Yan, F., Kuang, Y., Li, N., Zhang, D., Zhou, X., Lin, H., and Zhou, H. (2018). Improved Base Editor for Efficiently Inducing Genetic Variations in Rice with CRISPR/Cas9-Guided Hyperactive hAID Mutant. Mol. Plant *11*, 623–626.

Rodríguez-Leal, D., Lemmon, Z.H., Man, J., Bartlett, M.E., and Lippman, Z.B. (2017). Engineering Quantitative Trait Variation for Crop Improvement by Genome Editing. Cell *171*, 470–480.e8.

Roldan, M.V.G., Périlleux, C., Morin, H., Huerga-Fernandez, S., Latrasse, D., Benhamed, M., and Bendahmane, A. (2017). Natural and induced loss of function mutations in *SlMBP21* MADS-box gene led to *jointless-2* phenotype in tomato. Sci. Rep. *7*, 4402.

Rubin, B.E., Diamond, S., Cress, B.F., Crits-Christoph, A., He, C., Xu, M., Zhou, Z., Smock, D.C., Tang, K., Owens, T.K., et al. (2020). Targeted Genome Editing of Bacteria Within Microbial Communities. bioRxiv. https://doi.org/10.1101/2020.07.17.209189.

Sailer, C., Schmid, B., and Grossniklaus, U. (2016). Apomixis Allows the Transgenerational Fixation of Phenotypes in Hybrid Plants. Curr. Biol. *26*, 331–337.

Sánchez-León, S., Gil-Humanes, J., Ozuna, C.V., Giménez, M.J., Sousa, C., Voytas, D.F., and Barro, F. (2018). Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9. Plant Biotechnol. J. *16*, 902–910.

Scheben, A., and Edwards, D. (2018). Bottlenecks for genome-edited crops on the road from lab to farm. Genome Biol. *19*, 178.

Schmidt, C., Pacher, M., and Puchta, H. (2019a). Efficient induction of heritable inversions in plant genomes using the CRISPR/Cas system. Plant J. *98*, 577–589.

Schmidt, C., Schindele, P., and Puchta, H. (2019b). From gene editing to genome engineering: restructuring plant chromosomes via CRISPR/Cas. aBIOTECH *1*, 21–31.

Schmidt, C., Fransz, P., Rönspies, M., Dreissig, S., Fuchs, J., Heckmann, S., Houben, A., and Puchta, H. (2020). Changing local recombination patterns in *Arabidopsis* by CRISPR/Cas mediated chromosome engineering. Nat. Commun. *11*, 4418.

Schwartz, C., Lenderts, B., Feigenbutz, L., Barone, P., Llaca, V., Fengler, K., and Svitashev, S. (2020). CRISPR-Cas9-mediated 75.5-Mb inversion in maize. Nat. Plants *6*, 1427–1431.

Shan, Q., Wang, Y., Li, J., Zhang, Y., Chen, K., Liang, Z., Zhang, K., Liu, J., Xi, J.J., Qiu, J-L., et al. (2013a). Targeted genome modification of crop plants using a CRISPR-Cas system. Nat. Biotechnol. *31*, 686–688.

Shan, Q., Wang, Y., Chen, K., Liang, Z., Li, J., Zhang, Y., Zhang, K., Liu, J., Voytas, D.F., Zheng, X., et al. (2013b). Rapid and efficient gene modification in rice and *Brachypodium* using TALENs. Mol. Plant *6*, 1365–1368.

Shan, Q., Zhang, Y., Chen, K., Zhang, K., and Gao, C. (2015). Creation of fragrant rice by targeted knockout of the *OsBADH2* gene using TALEN technology. Plant Biotechnol. J. *13*, 791–800.

Shen, L., Wang, C., Fu, Y., Wang, J., Liu, Q., Zhang, X., Yan, C., Qian, Q., and Wang, K. (2018). QTL editing confers opposing yield performance in different rice varieties. J. Integr. Plant Biol. *60*, 89–93.

Shimatani, Z., Kashojiya, S., Takayama, M., Terada, R., Arazoe, T., Ishii, H., Teramura, H., Yamamoto, T., Komatsu, H., Miura, K., et al. (2017). Targeted base editing in rice and tomato using a CRISPR-Cas9 cytidine deaminase fusion. Nat. Biotechnol. *35*, 441–443.

South, P.F., Cavanagh, A.P., Liu, H.W., and Ort, D.R. (2019). Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field. Science *363*, eaat9077.

Springmann, M., Clark, M., Mason-D'Croz, D., Wiebe, K., Bodirsky, B.L., Lassaletta, L., de Vries, W., Vermeulen, S.J., Herrero, M., Carlson, K.M., et al. (2018). Options for keeping the food system within environmental limits. Nature *562*, 519–525.

Steinert, J., Schiml, S., and Puchta, H. (2016). Homology-based double-strand break-induced genome engineering in plants. Plant Cell Rep. *35*, 1429–1438.

Strecker, J., Ladha, A., Gardner, Z., Schmid-Burgk, J.L., Makarova, K.S., Koonin, E.V., and Zhang, F. (2019). RNA-guided DNA insertion with CRISPR-associated transposases. Science *365*, 48–53.

Svitashev, S., Schwartz, C., Lenderts, B., Young, J.K., and Mark Cigan, A. (2016). Genome editing in maize directed by CRISPR-Cas9 ribonucleoprotein complexes. Nat. Commun. *7*, 13274.

Tan, J., Zhao, Y., Wang, B., Hao, Y., Wang, Y., Li, Y., Luo, W., Zong, W., Li, G., Chen, S., et al. (2020). Efficient CRISPR/Cas9-based plant genomic fragment deletions by microhomology-mediated end joining. Plant Biotechnol. J. *18*, 2161–2163.

Tang, X., Liu, G., Zhou, J., Ren, Q., You, Q., Tian, L., Xin, X., Zhong, Z., Liu, B., Zheng, X., et al. (2018). A large-scale whole-genome sequencing analysis reveals highly specific genome editing by both Cas9 and Cpf1 (Cas12a) nucleases in rice. Genome Biol. *19*, 84.

Van Tassel, D.L., Tesdell, O., Schlautman, B., Rubin, M.J., DeHaan, L.R., Crews, T.E., and Streit Krug, A. (2020). New Food Crop Domestication in the Age of Gene Editing: Genetic, Agronomic and Cultural Change Remain Co-evolutionarily Entangled. Front. Plant Sci. *11*, 789.

Vasil, I.K., and Vasil, V. (1972). Totipotency and embryogenesis in plant cell and tissue cultures. In Vitro *8*, 117–127.

Voytas, D.F., and Gao, C. (2014). Precision genome engineering and agriculture: opportunities and regulatory challenges. PLoS Biol. *12*, e1001877.

Wan, D.Y., Guo, Y., Cheng, Y., Hu, Y., Xiao, S., Wang, Y., and Wen, Y.Q. (2020). CRISPR/Cas9-mediated mutagenesis of *VvMLO3* results in enhanced resistance to powdery mildew in grapevine (*Vitis vinifera*). Hortic. Res. *7*, 116.

Wang, F., Wang, C., Liu, P., Lei, C., Hao, W., Gao, Y., Liu, Y.G., and Zhao, K. (2016). Enhanced Rice Blast Resistance by CRISPR/Cas9-Targeted Mutagenesis of the ERF Transcription Factor Gene *OsERF922*. PLoS ONE *11*, e0154027.

Wang, B., Zhu, L., Zhao, B., Zhao, Y., Xie, Y., Zheng, Z., Li, Y., Sun, J., and Wang, H. (2019a). Development of a Haploid-Inducer Mediated Genome Editing System for Accelerating Maize Breeding. Mol. Plant *12*, 597–602.

Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C., and Qiu, J-L. (2014). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat. Biotechnol. *32*, 947–951.

Wang, C., Liu, Q., Shen, Y., Hua, Y., Wang, J., Lin, J., Wu, M., Sun, T., Cheng, Z., Mercier, R., and Wang, K. (2019b). Clonal seeds from hybrid rice by simultaneous genome engineering of meiosis and fertilization genes. Nat. Biotechnol. *37*, 283–286.

Wang, S., Zong, Y., Lin, Q., Zhang, H., Chai, Z., Zhang, D., Chen, K., Qiu, J.-L., and Gao, C. (2020). Precise, predictable multi-nucleotide deletions in rice and wheat using APOBEC-Cas9. Nat. Biotechnol. *38*, 1460–1465.

Willmann, M.R. (2019). Seeds Without Sex: Clonal Seed Production in Rice. CRISPR J. *2*, 11–14.

Wolter, F., and Puchta, H. (2019). In planta gene targeting can be enhanced by the use of CRISPR/Cas12a. Plant J. *100*, 1083–1094.

Wolter, F., Klemm, J., and Puchta, H. (2018). Efficient in planta gene targeting in *Arabidopsis* using egg cell-specific expression of the Cas9 nuclease of *Staphylococcus aureus*. Plant J. *94*, 735–746.

Xing, S., Chen, K., Zhu, H., Zhang, R., Zhang, H., Li, B., and Gao, C. (2020). Fine-tuning sugar content in strawberry. Genome Biol. *21*, 230.

Xu, R., Li, J., Liu, X., Shan, T., Qin, R., and Wei, P. (2020a). Development of Plant Prime-Editing Systems for Precise Genome Editing. Plant Commun. *1*, 100043.

Xu, W., Zhang, C., Yang, Y., Zhao, S., Kang, G., He, X., Song, J., and Yang, J. (2020b). Versatile Nucleotides Substitution in Plant Using an Improved Prime Editing System. Mol. Plant *13*, 675–678.

Yin, K., Gao, C., and Qiu, J.-L. (2017). Progress and prospects in plant genome editing. Nat. Plants *3*, 17107.

Yu, H., Lin, T., Meng, X., Du, H., Zhang, J., Liu, G., Chen, M., Jing, Y., Kou, L., Li, X., et al. (2021). A route to *de novo* domestication of wild allotetraploid rice. Cell. https://doi.org/10.1016/j.cell.2021.01.013.

Zaman, Q.U., Li, C., Cheng, H.T., and Hu, Q. (2019). Genome editing opens a new era of genetic improvement in polyploid crops. Crop J. *7*, 141–150.

Zhang, Y., Liang, Z., Zong, Y., Wang, Y., Liu, J., Chen, K., Qiu, J.L., and Gao, C. (2016). Efficient and transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA. Nat. Commun. *7*, 12617.

Zhang, H., Si, X., Ji, X., Fan, R., Liu, J., Chen, K., Wang, D., and Gao, C. (2018). Genome editing of upstream open reading frames enables translational control in plants. Nat. Biotechnol. *36*, 894–898.

Zhang, R., Liu, J., Chai, Z., Chen, S., Bai, Y., Zong, Y., Chen, K., Li, J., Jiang, L., and Gao, C. (2019a). Generation of herbicide tolerance traits and a new selectable marker in wheat using base editing. Nat. Plants *5*, 480–485.

Zhang, Y., Malzahn, A.A., Sretenovic, S., and Qi, Y. (2019b). The emerging and uncultivated potential of CRISPR technology in plant science. Nat. Plants *5*, 778–794.

Zhang, Q., Yin, K., Liu, G., Li, S., Li, M., and Qiu, J.-L. (2020a). Fusing T5 exonuclease with Cas9 and Cas12a increases the frequency and size of deletion at target sites. Sci. China Life Sci. *63*, 1918–1927.

Zhang, R., Chen, S., Meng, X., Chai, Z., Wang, D., Yuan, Y., Chen, K., Jiang, L., Li, J., and Gao, C. (2020b). Generating broad-spectrum tolerance to ALS-inhibiting herbicides in rice by base editing. Sci. China Life Sci. https://doi.org/10.1007/s11427-020-1800-5.

Zhang, Y., Pribil, M., Palmgren, M., and Gao, C. (2020c). A CRISPR way for accelerating improvement of food crops. Nat. Food *1*, 200–205.

Zhao, D., Li, J., Li, S., Xin, X., Hu, M., Price, M.A., Rosser, S.J., Bi, C., and Zhang, X. (2020). Glycosylase base editors enable C-to-A and C-to-G base changes. Nat. Biotechnol. https://doi.org/10.1038/s41587-020-0592-2.

Zhong, Y., Liu, C., Qi, X., Jiao, Y., Wang, D., Wang, Y., Liu, Z., Chen, C., Chen, B., Tian, X., et al. (2019). Mutation of *ZmDMP* enhances haploid induction in maize. Nat. Plants *5*, 575–580.

Zhou, H., Liu, B., Weeks, D.P., Spalding, M.H., and Yang, B. (2014). Large chromosomal deletions and heritable small genetic changes induced by CRISPR/Cas9 in rice. Nucleic Acids Res. *42*, 10903–10914.

Zhu, H., Li, C., and Gao, C. (2020). Applications of CRISPR-Cas in agriculture and plant biotechnology. Nat. Rev. Mol. Cell Biol. *21*, 661–677.

Zong, Y., Wang, Y., Li, C., Zhang, R., Chen, K., Ran, Y., Qiu, J.-L., Wang, D., and Gao, C. (2017). Precise base editing in rice, wheat and maize with a Cas9-cytidine deaminase fusion. Nat. Biotechnol. *35*, 438–440.

Zong, Y., Song, Q., Li, C., Jin, S., Zhang, D., Wang, Y., Qiu, J.-L., and Gao, C. (2018). Efficient C-to-T base editing in plants using a fusion of nCas9 and human APOBEC3A. Nat. Biotechnol. *36*, 950–953.

Zsögön, A., Čermák, T., Naves, E.R., Notini, M.M., Edel, K.H., Weinl, S., Freschi, L., Voytas, D.F., Kudla, J., and Peres, L.E.P. (2018). *De novo* domestication of wild tomato using genome editing. Nat. Biotechnol. *36*, 1211–1216.