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Unlocking crop diversity: enhancing variations through genome editing

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The germplasm resource repository harbors an extensive collection of genetic variations, providing a crucial foundation for the survival and sustainable development of humankind. Throughout history, major agricultural breakthroughs have relied on safeguarding, exploring, and harnessing germplasm resources. However, the pursuit of high yields in modern agriculture has led to a continuous reduction in biodiversity, resulting in monocultures and an undesirable homogeneity of breeding materials. As a consequence, germplasm resources are facing the alarming prospect of accelerated loss leading to a decline in crop diversity. Furthermore, modern agricultural varieties encounter formidable challenges in terms of adapting to unfavorable growing conditions, such as environmental heterogeneity and the prevalence of pests and pathogens (Fig. 1a). Enhancing the genetic variability of modern crops becomes paramount for fostering innovation within germplasm resources and ensuring food security.

Spontaneous mutation and recombination are the primary drivers of genetic variations and crop diversity. Nevertheless, the low frequency and time-consuming nature of these processes severely restrict the quantity and quality of crop diversity, rendering them inadequate for the demands of contemporary breeding. Consequently, there is an urgent need to develop effective and targeted strategies to accelerate the generation of genetic variations.

The emergence of genome editing technologies has provided powerful tools for generating genetic diversity by performing effective, targeted and precise manipulation of specific genes. In recent years, genome editing technologies have undergone three major revolutions (Fig. 1b). First, the groundbreaking discovery of clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems, particularly the CRISPR/Cas9 system (in 2013), transformed the genome editing field [1]. The CRISPR/Cas9 system, known for its simplicity, high efficiency, and versatility, has been extensively applied in various crops, primarily for the purpose of gene knockout. Second, CRISPR/Cas-based base editors (BEs) have ushered in a new era of precise editing, serving as highly efficient tools for precisely modifying individual DNA bases in a

programmable manner. Prominent examples of BEs include cytosine base editor (CBE) and adenine base editor (ABE) [2]. BEs have the advantage of inducing point mutations that lead to altered but still functional target genes and so are of use in investigating the function of crop genes and assisting in directed evolution. Third, the advent of the prime editing (PE) system has propelled precise genome editing to unprecedented heights [3, 4]. PE systems facilitate the insertion and deletion of small DNA fragments, as well as all 12 types of base-to-base alterations. In plants, the PE system has undergone multiple optimizations, resulting in a significant enhancement in editing efficiency. Furthermore, a site-specific recombinase has been incorporated into the plant PE system, known as PrimeRoot, enabling precise manipulation of kilobase-sized DNA fragments [5]. The PrimeRoot system substantially expands the range of applications of PE. The emergence and ongoing refinement of these revolutionary genome editing tools have opened up endless possibilities for introducing genetic diversity in crops.

Genome editing has revolutionized the most fundamental source of genetic variations, spontaneous mutation, and brought it into the era of "high-speed rail". The advances achieved include but are not limited to (Fig. 1c): (1) Generating targeted mutations of beneficial trait genes. This most straightforward approach to breeding improvement avoids the extensive genetic crosses required in conventional breeding. Due to its efficiency and specificity, this method is currently being applied in many different crops. Typically, there is often a trade-off between disease resistance and yield. However, a novel approach has been developed using the CRISPR/Cas9 system to engineer *RESISTANCE TO BLAST1* (*RBL1*) in rice, resulting in the acquisition of a new allele named $RBL1^{\Delta 12}$. Notably, $RBL1^{\Delta 12}$ conferred broad-spectrum resistance without compromising yield [6]. Moreover, the advent of multiplex genome editing systems has allowed the stacking of multiple beneficial trait genes, enabling the rapid generation of different combinations of genetic changes. (2) Manipulating gene regulatory elements to generate various types of new alleles. Deep sequencing has revealed single nucleotide polymorphisms (SNPs) and structural variations (SVs) in

gene regulatory regions that play key roles in regulating gene expression. By editing these gene regulatory elements, researchers can create superior allele combinations. For instance, the high-throughput CRISPR technology was employed to systematically examine multiple non-lethal variants in the promoter region of the WUSCHEL HOMEOBOX9 (*WOX9*) gene in tomato and noted a striking variety of effects on gene regulation [7]. (3) Performing high-throughput genome editing to create, in a single step, libraries of genetic variants that cover all possible genes. Pooled CRISPR mutant collections have already been generated in rice, maize, tomato, soybean and cabbage [8]. These collections are essential for gene function studies and for exploring novel elite variants. Moreover, saturating mutagenesis of a crucial functional gene allows one to identify useful allelic variants. Thus, by combining sgRNA library with CRISPR-mediated PE system, it is possible to generate a large number of point mutations *in vivo*. This method has been successfully applied to identify key amino acid sites and drive the directed evolution of rice (*Oryza sativa*) acetyl-coenzyme A carboxylase 1 (OsACC1) [9]. (4) Achieving rapid *de novo* domestication of wild germplasm resources. Wild germplasms are highly diverse genetically and can withstand a variety of biotic and abiotic stresses. However, transitioning from wild germplasms to cultivated crops typically requires thousands of years of domestication, leading to a loss of genetic diversity. A *de novo* domestication strategy has been successfully applied to wild-tomato and tetraploid wild rice using genome editing [10, 11]. The use of this strategy can shorten the breeding cycle and expand our ability to exploit genetic variations in wild species, ultimately enhancing food production and improving adaptability to environmental changes.

Spontaneous recombination serves as another highly effective source of genetic variations by exchanging DNA segments between homologous chromosomes and shuffling parental genes among the offspring. However, recombination occurs at a low frequency and only during meiosis. Typically, only 1–2 recombination events take place per pair of homologs at each meiosis. The extremely low-frequency occurrence of recombination results in the phenomenon of linkage drag, which restricts the

integration of elite alleles in the breeding process, and leads to a significant slowing of the breeding cycle. To address this issue, genome editing can be used to manipulate the recombination process by altering the distribution of recombination events and reducing linkage drag. This strategy not only accelerates the generation of variations by creating different gene combinations but also shortens the breeding cycle.

Currently, there are two main strategies for manipulating meiotic recombination through genome editing (Fig. 1d): (1) Recombination frequency and the position of recombination sites are both controlled by multiple suppressors. Therefore, the most direct way to promote recombination is to disable these suppressors. The helicase Fanconi anemia complementation group M (FANCM) was the first crossover suppressor to be identified in plants. When the *FANCM* gene was knocked out in wheat, the recombination frequency increased by 31% [12]. Another helicase, RecQ like helicase 4 (RECQ4), which is a homolog of Bloom syndrome protein (BLM), has also been identified as a crossover antagonist. Inactivating *RECQ4* in hybrid rice, pea, and tomato resulted in a striking three-fold increase in crossover frequency [13]. In addition, the loss-of-function of ZEP1 (the rice homolog of *Arabidopsis thaliana* ZYP1), a protein involved in the formation of the synaptonemal complex, simultaneously eliminated genetic interference and increased recombination frequency in hybrid rice [14]. (2) The RING finger E3 ubiquitin ligase, Human Enhancer of Invasion 10 (HEI10), has a dose-dependent effect on the regulation of recombination. By manipulating the expression level of *HEI10*, it is possible to fine-tune the recombination frequency. Saturation mutagenesis of the promoter and 5'-UTR regions of *HEI10* was conducted in rice using the CRISPR/Cas9 system. They discovered that the expression level of *HEI10* was significantly higher in some mutants in the 5'-UTR regions, resulting in various extents of improvement in recombination frequency [15].

Contrary to the effect of increasing recombination frequency, suppressing recombination can facilitate the rapid stability of desirable trait genes. A novel strategy called synthetic apomixis has been developed in rice using genome editing with the primary goal of fixing hybrid vigor [16, 17]. This strategy enables seed

production without any homologous recombination, and thus has the extraordinary ability to fix any heterozygous genotype. For example, during the process of crop breeding, numerous heterozygous genetic variations that display desirable traits are generated. However, due to the genetic recombination and segregation during sexual reproduction, the elite phenotype of those plants will be totally lost in subsequent generations. By employing synthetic apomixis through genome editing, the heterozygous genotype of those plants can be permanently fixed (Fig. 1e). Hence, synthetic apomixis serves as another robust strategy for enhancing crop genetic diversity by allowing one to exploit any genotype, and so provides valuable technical support for crops genetic improvement and the conservation of germplasm resources. Currently, synthetic apomixis is still in the developmental phase and requires simultaneous improvement in both plant fertility and the induction rate of clonal seeds before it can be effectively applied.

In recent years, genome editing has expanded the scope of potential variation space at a pace many orders of magnitude faster than natural evolution. This breakthrough technology not only creates dramatic new variants but also holds great promise of helping us cope with the food demands of future population growth, environmental challenges and evolving agricultural models. To further harness the power of genome editing, future efforts could profitably focus on several key areas (Fig. 1f), as follows: (1) It is crucial to enhance the efficiency and specificity of precise editing for advanced breeding. Although current technologies have successfully enabled the introduction of various mutations into multiple crops, challenges still remain. Raising the low frequency of inserting large DNA fragments, and reducing off-target effects, are essential. Improving existing editors and developing new editors will also increase the precision and efficiency of genome editing. (2) While genome editing has accelerated the generation of beneficial mutations, there is a need to improve the efficiency with which such mutations are exploited. Targeted introduction of beneficial genes into different modern varieties through genome editing can enhance crop traits, streamline breeding processes, and bolster efficiency. (3) The complex nature of meiotic recombination, along with the

precise timing and regulation of the process and the intricate genetic interactions involved, creates a need to manipulate this process more effectively. Strategies such as simultaneous editing of key recombination genes in various combinations should achieve more effective exploitation of recombination. Introducing genetic exchanges into regions with low recombination frequencies (recombination cold-spots) or regions where recombination does not naturally occur (such as centromeres) through genome editing could accelerate the aggregation of elite traits, shorten breeding cycles and increase crop diversity. (4) The rapid development of new technologies, such as plant synthetic biology and artificial intelligence (AI), offers additional possibilities for genome editing. Plant synthetic biology can aid in the synthesis *of de novo* gene sequences, it could enhance editing efficiency and precision. AI technology has been employed in the development of novel genome editing tools. AI-assisted large-scale protein structure prediction has enabled the elucidation of deaminase structure and the establishment of a groundbreaking type of BE tool [18]. In the future, AI technology can process and analyze vast amounts of genetic data and assist scientists in formulating more promising genome editing strategies for expediting the crop improvement process [19].

Overall, the unlocking of crop diversity through genome editing holds tremendous promise for addressing current and future agricultural challenges, fostering sustainable farming practices, and ultimately providing a more secure and resilient food supply for generations to come.

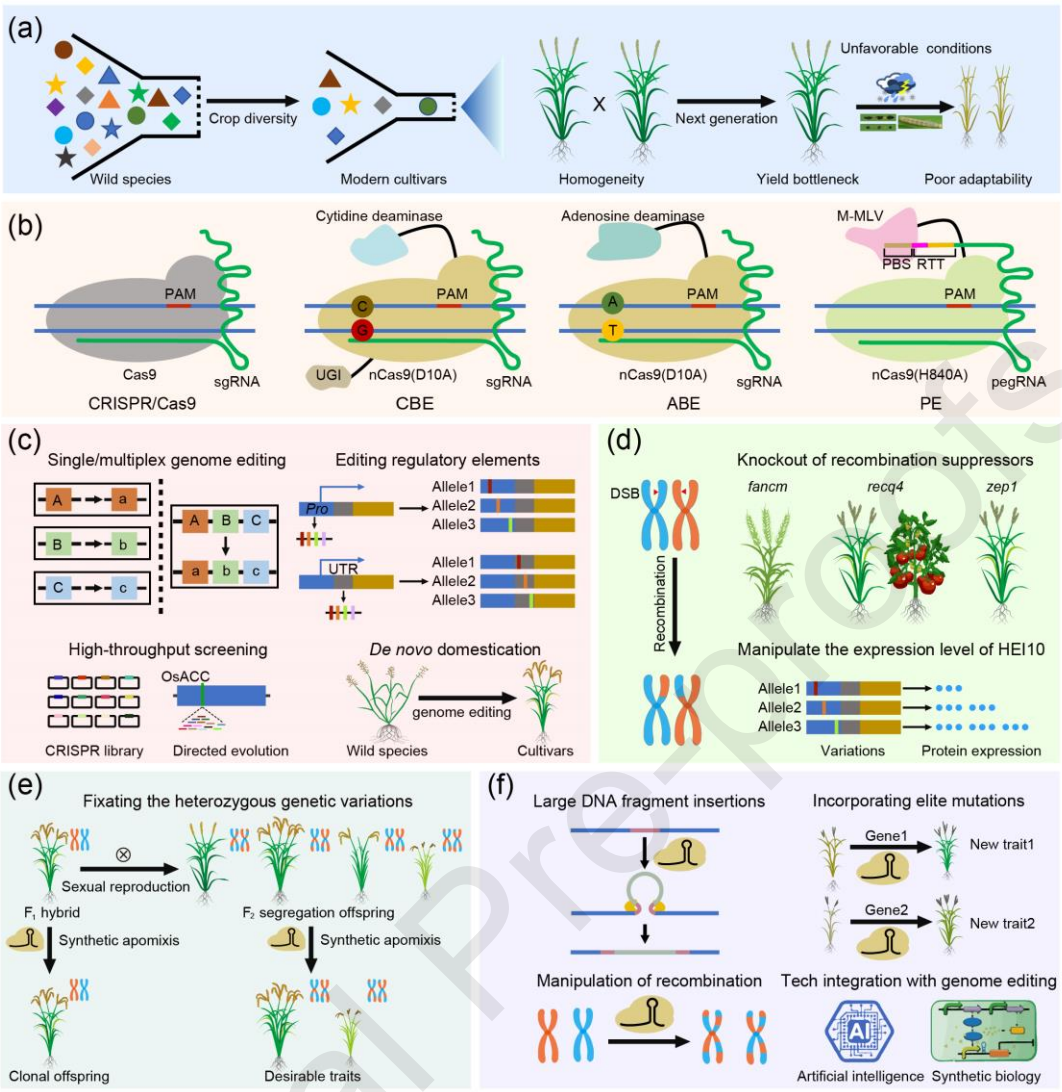

Fig. 1. The boom in available genetic variations created through genome editing. (a) The pursuit of high yields in modern breeding has led to a reduction in genetic diversity along with yield bottlenecks and poor adaptability to unfavorable conditions. (b) Three major revolutions in genome editing technology. The CRISPR/Cas9 system consists of a fusion of Cas9 with a single guide RNA (sgRNA). The Cytosine base editor (CBE) and adenine base editor (ABE) are fusions of Cas9 nickase (D10A), a sgRNA, and cytidine and adenosine deaminases, respectively. The prime editing (PE) system comprises a fusion of Cas9 nickase (H840A) with Moloney murine leukaemia virus (MMLV) reverse transcriptase and a PE guide RNA (pegRNA). PAM, Protospacer Adjacent Motif. UGI, uracil DNA glycosylase inhibitor. PBS, Primer binding site. RTT, Reverse transcriptase template. (c) Four strategies for creating mutations through genome editing. These encompass single and multiplex editing of

important trait genes, manipulating gene regulatory elements to generate novel alleles, high-throughput screening for trait identification and rapid *de novo* domestication of untapped wild germplasms. *Pro, Promoter* region. UTR, Untranslated Regions. (d) Two strategies for manipulating meiotic recombination by genome editing: directly targeting and disabling recombination suppressors, and modifying the (dose-dependent) effect of *HEI10*. DSB: Double-strand breaks. (e) Using the synthetic apomixis strategy to permanently fix the heterozygous genetic variations that display desirable traits. (f) Future prospects for better use of genome editing. These include increasing the efficiency of inserting large DNA fragments, optimizing the use of beneficial genes, manipulating genetic recombination and integrating genome editing with emerging technologies such as AI and plant synthetic biology.

Conflict of interest

The authors declare that they have no conflicts of interest.

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