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Application of Genome Editing Technologies in Crop Breeding and Future Agriculture

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Abstract. Genome editing has become an essential technology for modern crop improvement, allowing for targeted, efficient, and relatively predictable changes to genomic loci related to agronomic traits. Conventional breeding depends on natural variation, numerous crosses, and choosing based on appearance over a lengthy duration; whereas genome editing can add or remove particular sections of DNA (indels) or change certain characters within the genetic code at places chosen by the user, potentially hastening the creation of excellent plant seeds. From a mechanics standpoint, it usually refers to programmable nucleases that cause DSBs which are repaired through NHEJ or HR. Early site-directed platforms such as ZFNs and TALENs were significant advancements, but CRISPR/Cas systems have become the primary tools due to their ease of designing guides, higher efficiency, and ability to handle multiple targets simultaneously. Base editing and prime editing derivatives expand the range of editable regions and enhance the control over the results of edits. This review covers the principles and technical surroundings of crop genome editing, major application strategies for disease resistance, stress tolerance, yield, and quality/nutrition, and advanced breeding concepts including de novo domestication, heterosis fixation, and haploid induction. Major limitations – especially delivery and regeneration constraints, off-target effects, and variable efficiencies – are discussed along with optimization strategies. Lastly, new tools and AI-aided designs, together with changing regulations, are considered important factors influencing how these technologies will be applied in sustainable, precise farming in the future.

Keywords: Genome editing, CRISPR/Cas systems, Crop breeding, Precision agriculture

1. Introduction

Because of the increase in the number of people on Earth, changes in weather conditions, and a lack of farmland and farming supplies, all over the world, farms have trouble adjusting to what farmers want them to do. Meeting food demands for the rapidly growing population has been forecasted to require massive increases in crop yields. However, the available land for farming has been continuously shrinking and the overuse of farming resources has started to threaten the sustainability of farming. As we advanced into the second half of the century, the world population will continue

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grow rapidly. This has been paired with climate change, which has increased the volatility of the environment. This has made growing food to feed the population more difficult.

Many resources and techniques to enhance crop yields and improve crop genetics existed previously. However, the rapid change in the global population and requirements to improve crop yield sustainability has led to the necessity for more advanced techniques in order to change the standard in crop production [1]. These techniques are required to improve the sustainability of crop production as demanded by the urgent food security threats and environmental crises. Meeting these demands has caused a need for crop needs to move away from traditional techniques [2].

Genetic modification to improve crops has met major roadblocks due to the traditional techniques. These techniques typically rely on extensive modification to improve one crop, as they avoid slow natural variations. However, many of these variation and modification techniques have started to become outdated. The initiation of these techniques has allowed for major breakthroughs in crop modification. These include some previously unachievable changes such as more refined changes in genetics with a directed modification as opposed to the previously relied upon random variation.

The new CRISPR/Cas systems have changed the game once again as they have become the new standard in genome editing as they offer simplistic operational methods, low-cost procedures, and high efficiency [3]. Genome editing is now able to be done with precision, as opposed to other methods that offer no or low precision. Genome editing also uses the concept of "natural variation" to improve populations with desired traits in fewer generations. This new technology offers breeders the chance to modify populations with desirable agronomic traits to improve the development of new varieties. It also offers new methods of improving the quality of crops by increasing resistance to diseases and other stresses and by improving the nutritional quality of the crops. All of these factors put together offer immense value for the use of genome editing in breeding and the advancement of agriculture [4].

2. Principles and technical framework of crop genome editing

2.1. Molecular mechanisms of genome editing (DSBs and DNA repair)

One of the key principles of genome editing is the application of engineered nucleases to cause DSBs at a given genomic location with subsequent generation of mutations at or close to the break point by endogenous DNA repair. Some popular nuclease systems are ZFNs, TALENs and CRISPR/Cas systems [5] that identify target sequences and cut double-stranded DNA to form DSBs [6]. The two main repair pathways highlighted in plant cells are NHEJ and HR. NHEJ is usually efficient and prone to errors, frequently leading to small insertions/deletions (indels). However, HR is dependent on a homologous template and can be used to achieve accurate sequence replacement [7]. When applying genome editing, in many cases, when no exogenous repair template is introduced, DSBs are fixed through NHEJ, which creates indels capable of disrupting coding sequences or regulatory elements, allowing knockout of genes or disruption of functionality in a specific way. With the presence of a donor template with engineered sequence changes and flanking homology, HR might facilitate exact insertion or substitution at the locus. Recent findings also indicate that engineerization of the donor side can enhance the efficiency of template-dependent precise editing (Fig. 1). By employing these mechanisms in a purposeful manner, scientists can introduce desired mutations or sequence inserts into crop genomes to allow a delicate control of agronomic properties.

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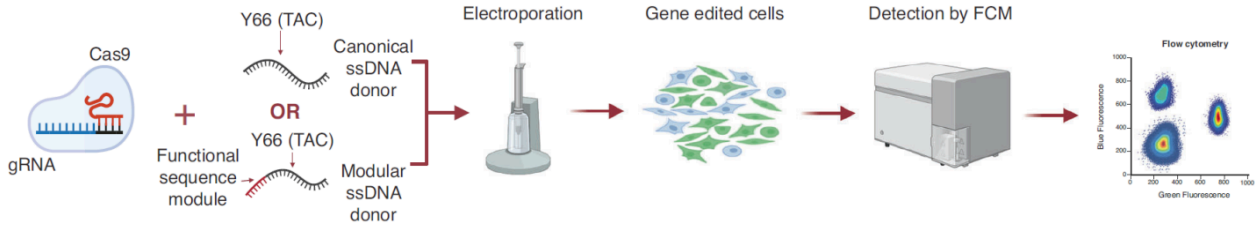


Figure 1. Modular ssDNA donor design can enhance donor recruitment and improve HDR-mediated precise editing efficiency

2.2. CRISPR/Cas systems and derivative platforms

A major benefit to using CRISPR tools is their ease of use, and high efficiency. CRISPR/Cas9 works by utilizing single guided RNA (sgRNA). sgRNA hones in on a specific genomic target and directs the Cas9 enzyme to cut the corresponding DNA. This cut leads to a double strand break. Compared to ZFNs (zinc finger nuclease) and TALENs (transcription activator like effector nucleases), the CRISPR systems only require the redesign of sgRNA to target a different sequence. This makes the process simpler, and decreases costs. CRISPR systems also allow for multiplexing, or the targeting of multiple genomic loci at the same time. This is particularly important for improving multiple traits in a single plant.

Over time and with development, CRISPR systems have branched out to include alternate Cas effectors, such as, Cas12a, Cas12b, and the high-fidelity variants of Cas9. This expands the use and flexibility of CRISPR [8]. For the purpose of this review, CRISPR/Cas systems are the foundation for all plant genome editing, due to their speed and precision.

Genome editing looks to achieve a high level of precision, with strategies.

2.3. Precision genome editing strategies

2.3.1. Base editing

Base editing can make a single nucleotide change at a certain position without causing a double-strand break (DSB). Traditional base editor consists of deaminase enzyme covalently connected with Cas9 (or its variants), allowing for the deamination of a target nucleotide under single-stranded DNA conditions, leading to precise base modifications such as C→T or A→G. It does not require a homologous donor template and reduces the high insertion/deletion (indel) rate caused by DSB repair, making it suitable for improving the accuracy and efficiency of editing. There are many kinds of base editing technology, such as cytosine base editors (CBEs), adenine base editors (ABEs), glycosylase base editors (GBEs), etc. , so there are different ways to change the target bases.

Base editing has also been used to improve signature agronomic traits in crops. For example, creating point mutation lines that provide herbicide resistance through targeted substitution within a herbicide-resistance gene such as rice OsALS, or introducing point mutations into resistance or signaling pathway genes to produce disease-resistant and stress-tolerant varieties. In this review, base editing is seen as a powerful molecular breeding method that allows for the exact and directed addition of beneficial mutations into target genes [9].

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2.3.2. Prime editing

In addition to multi-base substitutions and small insertions or deletions, Prime editing is touted to have more flexibility to install a wider range of sequence modifications. Typically, a Cas9 (D10A) nickase is used with a fusion to a reverse transcriptase, along with a specially designed prime editing guide RNA (pegRNA) that specifies a template for reverse transcription. Following target recognition and nicking, reverse transcriptase uses the pegRNA template to install the desired edit at the specified locus in the genome. While more complex editing outcomes are possible with prime editing, more widespread use is limited by the fact that it is lower in efficiency with greater editing outcome complexity and more sophisticated pegRNA design requirements.

Prime editing was reportedly first applied to plant somatic cells in 2020 and multiple directed modifications were possible, including an engineered mutation to a glyphosate-resistance target (e.g., EPSPS). Based on the described tradeoffs, prime editing is a useful complementary technique that is more suitable for less achievable multi-base replacement or small insertion/deletion patterns compared to standard base editors. In contrast, base editing continues to be the more common technique for single-nucleotide conversion due to its higher efficiency. Recent strategies derived from PE include dual prime editing (DualPE), which furthers the scope of prime editing to include large deletions, replacements, and inversions of DNA (Fig. 2).

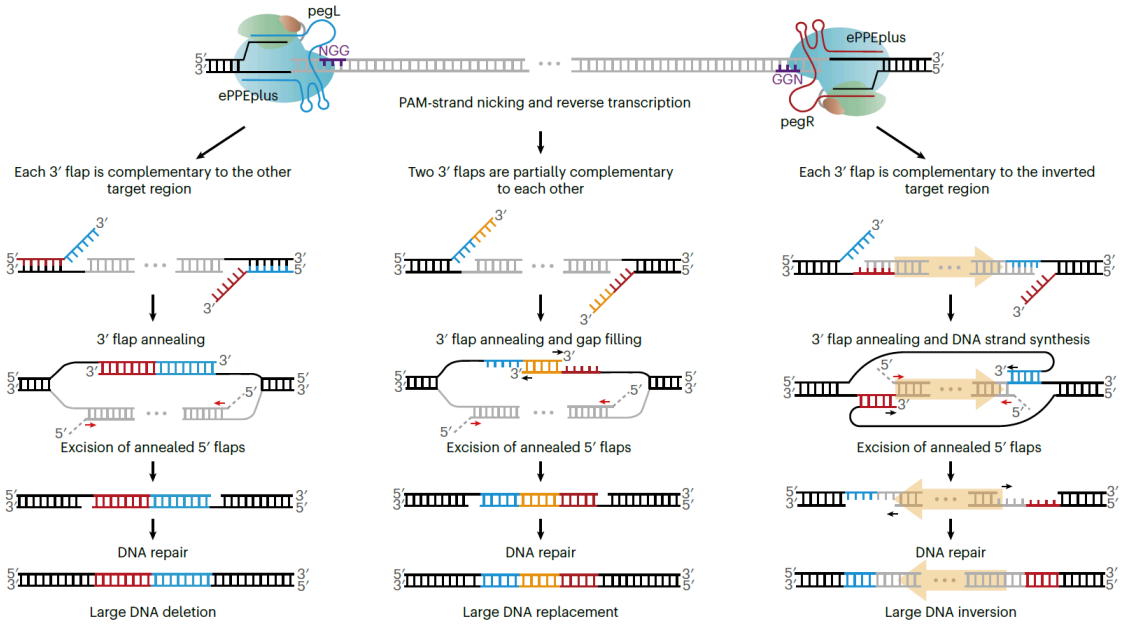


Figure 2. DualPE uses paired pegRNAs to achieve large-fragment deletion, replacement, and inversion without donor DNA

2.3.3. Large DNA fragment editing

With the maturation of genome editing platforms, the review outlines a burgeoning enthusiasm in the careful manipulation of large DNA fragments and complex structural variation, such as targeted insertion, replacement, inversion, and translocation. The methods discussed are: use of HR with long homology arms and multiplex sgRNA to add or replace sequences of thousands of bases to specific genomic sites, as well as incorporation of larger DNA fragments through engineered transposases or retrotransposon-mediated systems (Fig. 3). The authors state that these advances provide fresh

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avenues of engineering complex structural variation and investigating genetic diversity, which might be applicable to the enhancement of complex traits like stress resistance, grain-based characteristics, or secondary metabolite levels [10].

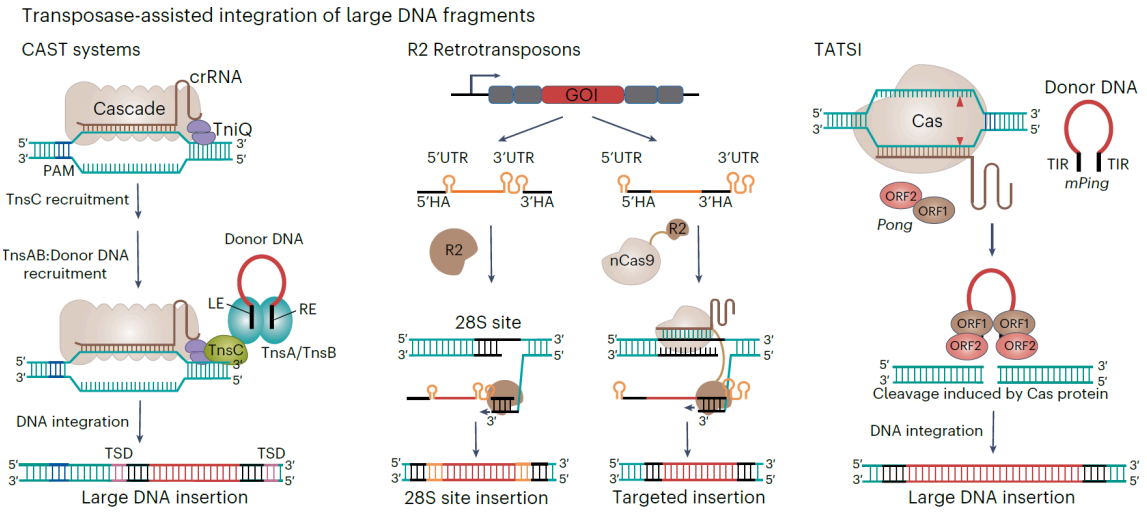


Figure 3. Transposase-assisted and retrotransposon-based systems provide targeted routes for large DNA insertion in plants

Meanwhile, the review also highlights that the accurate large-fragment editing is still limited by efficiency and controllability issues, however, it is seen as one of the key factors that will extend the utility of genome editing in breeding.

2.4. Comparative assessment and suitability of editing technologies

The original manuscript has an application-based comparison of the platforms. Targeting flexibility: ZFNs and TALENs use engineered proteins that have to be re-designed with every target site, whereas CRISPR/Cas9 can be re-targeted through sgRNA sequence alteration, which greatly lowers the design and operating costs. Regarding efficiency and multiplexing, CRISPR systems are portrayed as very compatible with multi-locus engineering to enable the development of polygenic or composite traits. The high levels of accuracy in base editing and prime editing are highlighted in the context of nucleotide substitution or minor sequence change. Base editing is typically seen as more efficient than prime editing but restricted to limited types of base conversions; prime editing is more versatile but relatively inefficient. Large-fragment insertion or structural variation engineering special strategies (e.g., large donor templates and specialized vectors) are also available but still require further refinement. The final synthesis of all the facts is that crop genome editing technology system consists of the diversified ZFN/TALEN, CRISPR/Cas9, and precision derivatives. ZFNs/TALENs are said to be mostly replaced by CRISPR due to the simplicity and effectiveness of CRISPR. Breeding tasks requiring precise point mutations are described as being better suited to base editing [11]; breeding tasks needing complex changes or multi-base insertions/deletions are better suited to prime editing; and breeding tasks involving structural variation or large sequence exchange are best met with large-fragment editing strategies. Proper breeding results rely upon choosing and integrating proper tools depending on the target trait structure and biological requirements specific to the particular crop.

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3. Application strategies of genome editing in crop breeding

3.1. Trait-oriented improvement

3.1.1. Disease resistance and stress tolerance

Genome editing enables targeted modification of genes associated with resistance to pathogens/pests and tolerance to abiotic stresses, supporting development of more robust crop varieties. An example is described in which CRISPR/Cas9 targets and knocks out a wheat gene such as TaMLO to obtain broad-spectrum powdery mildew resistance, explicitly noted as a hypothetical illustrative example in the source manuscript (i.e., not presented as a confirmed report in this text). In another example, a point mutation introduced through base editing in a gene such as rice OsALS is described as conferring tolerance to glyphosate. Also, it says that base editing enables targeted point mutations in resistance genes or signaling pathway genes to enhance disease resistance and stress tolerance. And there are other things that genome editing could do as well – maybe it could help plants cope with various types of environmental stress better by altering their reactions to hormones, antioxidants, ion channels, etc., making them more resistant to droughts, salty soil, and extreme heat waves. Through such specific modifications, we can produce plant seeds that have improved resistance and the ability to withstand stress, which will assist in maintaining a good harvest and increasing agricultural production.

3.1.2. Yield-related traits

Improving the yield is considered as the main goal. Many studies show that by changing some things that affect how much grain you get, you can get either more grain or better quality grain. For example, if we make the promoter region of rice OsSPL14 similar to the beneficial variation found in wild-type lines, then the rice yield will increase greatly. Similarly, it was said that changing certain parts of wheat TaGW2 would result in larger grains. All these examples suggest that if we alter particular sections of the plant's gene sequence, there could be increased growth and more food. And genome editing is thought to assist with enhancing other complicated features connected to production such as photosynthetic efficiency, tillering capability, and nourishment supply to seeds. Conventional breeding needs lots of generations for selecting yield-related genes; genome editing can change many yield-related genes all at once, making the breeding process shorter. Also mentioned in the review is that if several yield component genes are changed together, it may be possible to develop breeding materials with a higher chance of increasing yield.

3.1.3. Quality and nutrition

In addition to resilience and yield, quality and nutritional traits of crops can be enhanced through selective editing of the relevant quality-determining genes. This review illustrates the targeted modification of pathways that impact starch, oil, amino acid profiles, and anti-nutritional factors. Base editing is, for example, described as having been used to improve the starch composition of rice and the protein content of maize grains to make them more suitable for processing. It is described that frontier research demonstrates the application of genome editing to boost the functional metabolites like γ-aminobutyric acid (GABA), oleic acid, and anthocyanins [11]. Moreover, genome editing has the potential to eliminate anti-nutritional factors by, for example, reducing glycoalkaloid toxins in potatoes or lowering the phytic acid-related constituents in legumes, thereby enhancing the safety of the food and the bioavailability of nutrients. The review

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establishes precision genome editing as a desirable approach to satisfy the rising need for high-quality, nutrition-dense crops in modern agriculture.

3.2. Breeding strategy innovation

3.2.1. De novo domestication

"De novo domestication" is explained as a method to rapidly transform wild and semi domesticated resources into novel crops. The wild progenitors are a starting point for selection having positive natural traits(increased biomass or potential for stress tolerance), and then in combination with genome editing to add domestication traits (e.g. reduced seed shattering, modified inflorescence architecture, increased fruit size) without losing the positive traits [12]. Research is outlined in the review that describes the potential to edit wild rice and wild tomato that are highly tolerant to salinity/alkalinity or drought in order to create new crop lines that retain the valued characteristics of the diversity that are the characteristics of de novo domestication. This is expanded to include the potential to create new sources of genetic variability needed to support crop improvement and to make more diverse genotypes available for future agriculture to use for the management of more complex growing environments.

3.2.2. Fixation of heterosis

Fixing desirable hybrid traits across generations is a breeding objective of high importance. The review describes the possibility of using a combination of genome editing and artificial hybridization as a means to fix heterosis, including the possibility to create systems for artificial clonal propagation through seeds. Engineered systems referred to include MiMe (mitosis instead of meiosis) and the introduction of the BABY BOOM gene, which is suggested to enable a form of asexual reproduction that can be heterotic, and is compatible with the purity of the heterotic genotypes [13]. This review refers to several examples from the literature that have recently been accomplished in various crops such as rice, which are referred to as synthetic apomictic lines, or in other words are synthetic, clonal seeds, and have been produced with a high level of efficiency, and therefore clearly support the concept that heterosis fixation using genome editing technologies is likely to become a practical breeding method.

Scaled up, this strategy has the potential to transform the economics of agricultural production. Farmers could preserve the benefits of heterosis without the annual expense of hybrid seed purchases.

3.2.3. Haploid induction

It is posited that haploid breeding in conjunction with genome editing will allow the rapid generation of homozygous lines and the significant reduction of breeding cycle time. Through editing specific genes that facilitate haploid induction, like ZmMTL in maize and TaMTL in wheat, it is possible to create high-efficiency haploid induction systems that produce fully homozygous doubled haploid parental lines within a single generation post-chromosome doubling. Figure 4 shows a typical example of pinned haploid induction via genome editing. Different experimental methods have been created for different crops so that problems concerning disease resistance and nutrition can be swiftly resolved by swiftly altering many traits and hastening the next phase of breeding. Generally speaking, according to what the authors of this review state, using genome editing along with haploid induction requires fewer backcrosses, making breeding more efficient.

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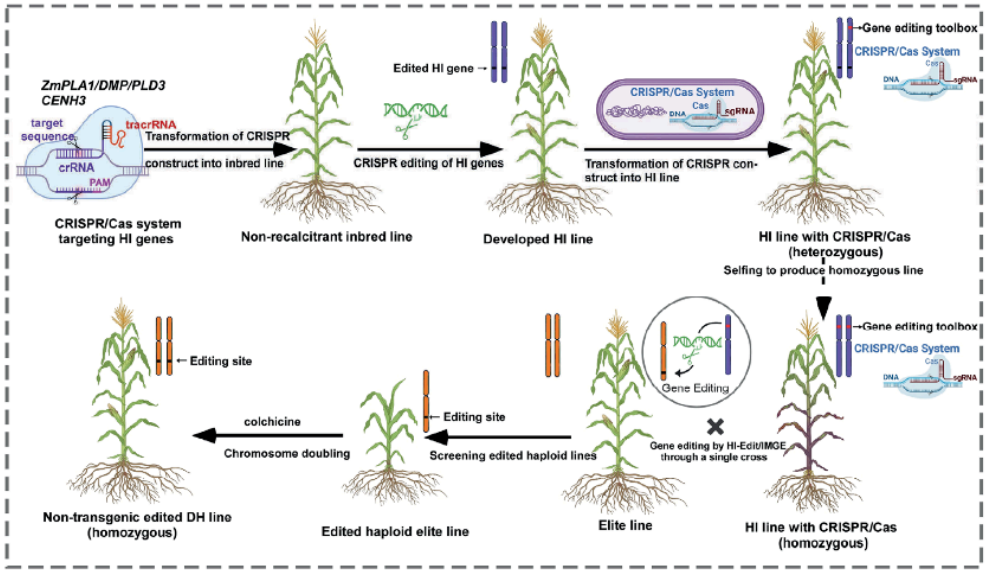


Figure 4. One-cross production of genome-edited, non-transgenic doubled haploid lines using haploid inducer lines

4. Technical limitations and optimization strategies

4.1. Bottlenecks in delivery systems and regeneration

Main problem is how to efficiently introduce the editing components into the corresponding plant cells and regenerate whole plants from these edited cells. Conventional plasmid transformation or Agrobacterium-mediated transformation varies greatly among different crops and genotypes; some economically important crops are relatively difficult to handle, usually requiring arduous and time-consuming tissue culture procedures such as callus induction. New vectors and methods including biolistic delivery, nanoparticle carriers, viral vectors, etc. , have been developed, but according to the review, there is still no universally applicable solution for all crops. After adding the editing components, selecting the right ones and growing them into adult plants takes a long time, so not much can be done at once and the total time needed increases [10].

To solve these problems, the review gives some answers: no-tissue-culture-or-reduced-tissue-culture-editing-strategies (such as directly giving Cas9/sgRNA ribonucleoprotein complexes, RNPs, to zygotes); improving regeneration by using developmental regulators such as GRF4-GIF fusion proteins; and "DNA-free" fast-editing strategies (such as ExpressEDIT) to avoid foreign DNA lingering too long and speed up the process while covering more genes.

4.2. Off-target effects and control

Off-target effects are seen as a regular problem on different editing platforms. Even if the off-target rate is less than the natural mutation frequency, the editing enzyme may still stick to and change other parts of the genome that are not its intended target area, leading to unexpected changes in DNA [14]. Base editing also has off-target risks: it avoids DSBs so there are fewer indels, but the deaminase part might deaminate non-target DNA or RNA, causing unwanted mutations.

Control strategies for off-target effects include improving sgRNA design, using high-fidelity Cas9 variants, modifying deaminases to improve their specificity, and limiting the expression period

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of editors. In the review, an AI-designed Cas9 variant (e. g. , Cas9-AI-8. 3) is noted to have improved editing efficiency without a significant increase in off-target events; however, general verification is still needed. Some validation methods are given, such as sequencing candidate off-target sites and using in vivo fluorescence reporter systems to find real mutants, which helps keep the genomic stability of the last breeding material.

4.3. Improving editing efficiency

Improving the efficiency of editing is seen as an important field for investigation. Promoter optimization, nuclear localization signals, finding or creating highly active enzyme variants by directed evolution or design-based engineering, and using high-throughput screening platforms to select preferred mutants [15]. It says that if we use genome editing together with other breeding methods such as haploid breeding, which can quickly fix mutants, then we could speed up and improve the efficiency of breeding. In short, this means we have to keep making our editors better and give them to plants in new ways so that we can make nice changes to lots of different kinds of plants and all their different versions.

5. Conclusion and outlook

Genome editing has become an important way to improve crops because it allows us to change some genes that affect how plants grow. It improves disease resistance, stress tolerance, yield, nutritional value, and offers new possibilities for breeding techniques such as de novo domestication, heterosis fixation, and haploid induction. But its wider use is limited by issues such as inefficient delivery, genotype-dependent regeneration, inconsistent editing outcomes, and off-target effects. The future development relies on improving the accuracy and efficiency of editing, creating better delivery and regeneration systems, and establishing clearer criteria for various types of edited crops. And also, advances in computational design can help choose targets and make editors. Generally speaking, genome editing would be a more useful tool for crop breeding, especially when combined with actual-world breeding programs focused on enhancing agricultural output and sustainability.

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