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CRISPR/Cas9 Gene Editing Technology in Crop Quality Improvement: Applications and Challenges

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Abstract. CRISPR/Cas9 gene editing technology has become an important tool for crop quality improvement due to its simplicity, efficiency, and precision. Compared to traditional breeding methods, CRISPR/Cas9 can achieve targeted modification of specific genes in a short period, thereby improving crop yield and quality. This paper reviews the progress of CRISPR/Cas9 technology in quality improvement of food crops, economic crops, and horticultural crops. In food crops, CRISPR/Cas9 has been used to regulate genes related to rice appearance and eating quality; in economic crops, the technology has been successfully applied to improve the quality of cotton and rapeseed oil; in horticultural crops, the nutritional components and taste of tomatoes have been significantly improved. Despite the remarkable achievements of CRISPR/Cas9 in crop quality improvement, technical challenges remain, including plant organelle genome editing, establishment of transgene-free editing systems, and transformation of important crop varieties. With continuous refinement of CRISPR/Cas9 technology, it will provide more possibilities for crop quality improvement and make important contributions to solving food security problems.

Keywords: CRISPR/Cas9, gene editing, crop quality improvement, transgene-free, organelle genome, precision breeding

1. Introduction

With the continuous growth of global population and the intensification of climate change, improving crop yield and quality has become key to ensuring food security [1]. Although traditional breeding methods have achieved significant success, their long cycles, low efficiency, and poor accuracy limit rapid improvement of crop quality [2]. The emergence of genome editing technology has opened new avenues for crop breeding, especially the development of CRISPR/Cas9 systems, which has ushered crop quality improvement into the era of precision editing [3].

The CRISPR/Cas9 system originates from the adaptive immune system of bacteria and archaea, consisting of Cas9 protein and sgRNA [4]. Compared to previous ZFNs and TALENs, CRISPR/Cas9 does not require complex protein engineering; it only needs to design sgRNA complementary to the target sequence to edit specific sites in the genome, greatly reducing operational difficulty [5]. Moreover, the CRISPR/Cas9 system can edit multiple genes simultaneously, a feature particularly important for regulating complex traits [6].

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Currently, CRISPR/Cas9 technology has been widely applied in quality improvement of major crops such as rice, wheat, maize, and soybean. In rice, grain size and shape have been successfully improved by editing genes such as GS3 and GW2 [7,8]; in wheat, gluten content has been reduced by targeting the α-gliadin gene family, laying the foundation for breeding low-allergy wheat varieties [9]; in tomato, genes related to fruit color and size have been edited to improve nutritional value and market competitiveness [10].

Although CRISPR/Cas9 has made significant progress in crop quality improvement, many challenges remain, such as difficulties in plant organelle genome editing, imperfect transgene-free editing technologies, and low transformation efficiency of elite varieties [11]. This paper will review the applications of CRISPR/Cas9 in crop quality improvement and discuss future directions and challenges.

2. Theoretical research

2.1. The mechanism of CRISPR-Cas9 system

In 2002, bioinformatics analysis found a new type of DNA sequence that was exclusively existed in bacteria and archaea and named it CRISPR (Clustered regularly interspaced short palindromic repeats) and named the related gene Cas (CRISPA RNA associated gene). Principles of CRISPR/Cas9 gene editing technology were clarified in 2012. It is a breakthrough technology. CRISPR/Cas9 technology has been widely used in various fields and greatly improved the knock-out efficiency of gene and multi-gene knock-out [12].

CRISPR/Cas9 technology application in plant genome editing process can be divided into 5 steps: selection of target genes, design of specific sgRNA, Cas9 and sgRNA assembly, transformation into target plants and obtain edited plants by regeneration and screening [13].

This technology can realize the precise editing of crop genome by constructing DNA double-strand breaks (DSBs) and taking advantage of the cellular DNA repair method. This method can use non-homologous end junction (NHEJ) or homologous directed repair (HDR) [14]. Many studies have proved that CRISPR/Cas9 can cause different types of DNA mutations such as deletions, insertions, single nucleotide polymorphisms (SNPs) and large fragment substitutions and further change the crop quality traits. In addition, besides CRISPR/Cas9, ZFNs and TALENs are also established genome editing technology. The ZFNs are fusion proteins formed by binding the zinc finger DNA binding domain and FokI restriction enzymes. The TALENs work similarly to ZFNs, but use the DNA binding domain of transcriptional activator like effector proteins (TALEs). Different from ZFNs and TALENs, CRISPR/Cas9 system uses RNA guided DNA cleavage and has higher editing efficiency and simpler design principles. At the same time, it is the most advanced genome editing tool in recent years. The gene editing technology based on the above methods is more suitable for crop breeding research [11].

2.2. Theoretical basis of gene editing in plant breeding

Application of gene editing technology in plant breeding based on the overall analysis of target gene function and regulatory network.

Analysis of gene function and acquisition of target gene. Through omics analysis and functional verification, we can find out the target gene closely related to the agronomic traits such as disease resistance gene, stress resistance gene and metabolic pathway gene affecting quality and yield, and provide theoretical basis for precise editing.

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Plant trait development is often controlled by polygenic networks and quantitative trait loci (QTLs). Traditional breeding has limitations in integrating and optimizing complex traits. In crop breeding projects, people obtain phenotypic information (such as seed yield) through multi-year and multi-site field trials and multi-site and individual genotyped diversity and then find out the genetic variation (such as QTLs) that is associated with trait modification [15]. This process finds out quantitative variation that is controlled by multiple small effector sites, and usually, the QTLs are localized in the recombinant inbred (RIL) population. The genome-wide association study (GWAS) improves the predictability of breeding by analyzing genome-wide markers, associating loci and phenotypic traits, and combining the genomic marker and phenotypic information for the genomic prediction [16]. Although these techniques can greatly improve the speed and accuracy of breeding, the genes underlying many QTLs have not been found.

Traditional breeding has difficulty in integrating and optimizing complex traits efficiently. With the development of gene editing technology and intelligent breeding strategy, it is possible to make coordinated modifications of multiple loci at the genome level to drive directed improvement of complex quantitative traits. In this study, Christian et al. developed BREEDIT as a pipeline to rapidly generate diverse multiplex gene-edited plants [17]. They targeted up to 12 candidate genes encoding growth-related factors simultaneously with a single SCRIPT construct in a maize line expressing Cas9 (EDITOR). Finally, the edited plants were self-bred and/or crossed to generate strains with improved agronomic phenotypes, such as larger leaves and improved drought tolerance under controlled conditions. They found significantly associations between certain gene edits and improved phenotypes. These results suggested that fewer gene mutations than previously expected might be required to generate superior phenotypes. Therefore, BREEDIT offers a way to rapidly obtain valuable gene edits as reliable candidates for subsequent breeding programs.

As an advanced technology in plant breeding, gene editing should be combined with traditional and new breeding approaches. This integration of multi-technologies can not only speed up the stabilization of excellent genotypes but also greatly reduce the breeding cycle and solve the problems caused by traditional hybridization and selection [18]. For example, researchers obtained maternally derived diploid embryos by expressing BABY BOOM gene and cyclin D-like gene in unfertilized maize egg cells [19]. This method avoids the in vitro tissue culture of traditional maize embryos and the chemical chromosome doubling agent. Combined with gene editing, it can directly produce gene-edited diploid embryos and then mature seeds.

3. Existing problems of the research subject

3.1. Existing technical problems of the research subject

When CRISPR/Cas technology transfers to DNA, it is not very accurate and can tolerate some base mismatches, which means that editing tools can edit unintended DNA that look like a target DNA. Such off-target effects lead to the appearance of unwanted mutations, which reduce the efficiency of a desired trait improvement, but can also introduce harmful mutations, which can affect crop life and safety. Such problems are especially severe for plants with complex genomes, where similar DNA fragments are common, and reliable accuracy of gene editing is hard to achieve.

CRISPR/Cas9 requires specific PAM sequence, which means that the editing can be applied only at the places, where suitable PAM sequences are available. For the gene regions, where no suitable PAM sequences are available, the editing tools cannot be applied, which means, that not the whole genome can be edited using such technology. Many important genes, controlling the target traits are

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still unavailable, because no suitable PAM sequences are available near them. It is a serious limitation for the gene editing [20].

Editing efficiency varies greatly for different plants and gene regions inside plants. It depends on many factors, like genome complexity, chromatin structure, gene characteristics and others. Some plants have very complex genomes, which are hard to modify, and some plants have dense packed chromatin, which makes it hard for editing tools to reach and cut target DNA. Different plants react differently to the same editing tools, which also leads to varying editing efficiency. Low editing efficiency makes the experiments harder to reproduce, make breeding cycles longer and increase costs, which are serious limitations for the gene editing technology [12].

Many valuable agricultural traits, like disease resistance, stress tolerance and higher yields are controlled by multiple genes, which work in a collective manner and means, that multiple genetic targets should be edited simultaneously and multiple genes should be cut at multiple places. However, when trying to edit multiple places, researchers often face the problems with efficiency, when cutting multiple places, get inconsistent results and edited genes can rarely be stably inherited. Even when multiple genes are edited successfully, the edited plants cannot transfer these changes reliably to their offspring. The ability to edit multiple genes and stably inherit these changes across multiple generations is a serious limitation for the gene editing technology, which makes it hard to apply the technology broadly [21].

3.2. Application and regulatory issues

While gene editing makes plant breeding faster, it also raised concerns and controversies over its application and regulation [21].

In theory, gene editing can bring targeted improvements by generating specific mutations in the genome, without introducing foreign genes, which means that it is different from GMO technology. However, in practice, it is still possible that fragments of editing vectors or other external sequences are present after editing, which means, that are these plants "GMO" or "non-GMO". It not only raises concerns and controversies over public acceptance, but also creates uncertainty over commercialization and market access.

We can’t overlook the possible biosafety concerns. Gene-edited crops that are released into fields may spread their edited genes to their wild relatives through crossbreeding and cause ecological issues. New traits may result in new allergens and toxic substances that may harm human health. Edits may also upset the balance of ecosystems, for example insect-resistant crops may impact insect populations or natural predator communities indirectly. ERM analysis requires careful risk assessment before gene-edited crops are widely released.

Interestingly, despite demonstrating ecological advantages and being reasonably accepted by the public, gene editing technology is still faced with many challenges in its promotion and use. Insufficient and inconsistent clear terms usage causes various stakeholders to interpret gene editing in different ways, which hinders policy development and public acceptance. Current regulations have not caught up with the current fast-paced gene editing technology development. Therefore, there are currently no clear standards and inconsistent guidelines across countries and regions. Meanwhile, patent monopolies also cause more social inequality and technological barriers. Finally, limited public participation and transparency cause society to be unfamiliar with both the advantages and risks of gene editing, which hinders social acceptance and perceived legitimacy [22].

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4. Perspectives on the problems and future directions for improvement

Given the existing issues surrounding the efficiency, precision, stability, and regulatory compliance of gene editing in plant breeding, the researchers and industrial experts put forward different improvement strategies and future development directions.

Firstly, the editing efficiency should be further enhanced for its wide application and acceptance. By generating more Cas9 variants (such as Cas12a, Cas12b etc.) with the ability to recognize different PAM sequences, and refining the promoter expression and gRNA regulation, the editing efficiency in different species and target loci could be significantly improved [23].

The off-target effects should be minimized to ensure the safety and reliability of gene editing technologies. Recently, dual nickase systems, shorter sgRNA designs, and high-fidelity Cas9 variants with reduced unintended mutations at non-target DNA sequences have been reported, which exhibited improved editing precision [24]. For cytosine base editors (CBEs), the authors optimized the selection of cytidine deaminase, engineered the Cas proteins and guide RNA sequences, and introduced uracil DNA glycosylase inhibitors (UGIs) to significantly reduce Cas9-independent off-target edits. The improved CBE systems not only enhance the editing efficiency and purity but also cause markedly fewer off-target edits [25]. These advances further enhance the precision and safety of gene editing technologies.

For the polygenic and complex traits, scientists developed the multi-target editing vector designs and BREEDIT strategies. These methods could systematically optimize quantitative traits at the genomic level, and exhibited promising applications on complex agronomic traits, including stress resistance, yield and quality [26].

Kumar et al. surveyed different technical risks (off-target effects, mosaicism, chromosomal rearrangements and transformation problems) and their emerging solutions, including compact/engineered nucleases, RNA-processing arrays, morphogenic regulators and AI-driven sgRNA design integrated with multi-omics approaches. They argued that, given the complexity of polygenic trait stacking strategies, multiplex tools are essential for accelerating precision breeding and climate-resilient agriculture.

Construction of exogenous DNA-free editing technology opens a new way for “non-GMO” classification. If delivering RNP complexes or using viral vector system to avoid foreign sequence integration, it will reduce the restrictive regulations and marketing restrictions. For example, Kuwabara et al. developed a new genome editing technology for soybean – in planta bombardment-ribonucleoprotein (iPB-RNP) and delivered CRISPR/Cas9 into soybean shoot apical meristem (SAM). This method did not introduce foreign DNA into plant and avoids tissue culture [27]. Stably transformed target genes were obtained and all the edited traits were transmitted to the next generation. This is the first report of the development of DNA-free editing technology for target genes using in planta genome editing in dicotyledonous plants.

Similarly, researchers constructed a CRISPR-Cas9 DNA-free delivery system using plant negative-strand RNA viruses and used it to mediate high frequency single and multiplex gene mutations and chromosomal deletions in tobacco [28]. Over 90% of regenerated plants carried target mutations and 57% of them carried inheritable tetra-allelic mutations. This method is efficient, stable, cost-effective and has limited off-target effects. It provides a new method for plant genome editing.

Integration of gene editing and speed breeding technologies provides a new way to fix desirable genotypes rapidly, which is expected to accelerate the breeding of new varieties with improved traits.

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For example, Cas9 protein was fused with viral replication protein Rep to tether donor DNA in vivo and improved the efficiency of precise HDR-based gene knock-in in rice significantly [29]. The knock-in rates were improved 4-7 folds and the inheritable patterns were stable and no off-target effects were detected. This method also decreased the by-products from non-homologous repair mechanisms and is an effective method to improve HDR-mediated genome editing in plant systems.

Transgene residuals have many impacts on genetics analysis, increase off-target effects, and bring certain risks to regulation. For these problems, people designed two strategies to solve them: directly edit genome with DNA free reagents; use DNA method of transgenic technology and improve screening strategy to get transgene free plants [30]. Many methods based on fluorescence, pigments, and chemicals to mark transgenic and transgene free plants separately rapidly. And suicide gene could make transgenic plants die out by themselves and improve screening efficiency directly. Besides, site-specific recombination, transposition, and gene-editing nucleases could remove transgene effectively. This method is suitable for asexuals propagated plants. If combining with haploid induction technology, it could edit recalcitrant plants effectively. With the gradually popularization of gene edited crops in the commercial market, the development of regulations and risk assessment protocol is becoming more and more important. And international recognized safety evaluation standard could reduce the differences between countries and regions, and make the gene edited crop have international recognition. It also could provide institutions support for gene edited crop widely used and popularized in global.

5. Conclusion

In the last decade, CRISPR-Cas9 has revolutionized plant breeding through its precision and ease of use. Researchers have edited genes associated with disease resistance, stress tolerance, and nutrition traits, which were difficult to achieve through traditional breeding. With approaches such as targeted mutagenesis, gene knockouts, and base editing, plant scientists have advanced the theoretical and practical limits of their work.

We are seeing the practical applications of CRISPR breeding. We are developing crops that are resistant to drought or diseases that can ensure our food security in the face of climate change. We have developed crops with improved nutritional quality that can achieve breeding goals of agricultural productivity as well as public health. These examples demonstrate the potential of CRISPR breeding approaches in addressing real-world challenges of modern agriculture.

Several challenges remain in the use of CRISPR breeding approaches. At the technical level, challenges include off-target editing, limited PAM sequences, and editing efficiency variability across species and genomic regions that impact result reliability. Complex traits that require multiple gene edits present a particular challenge. These challenges also exist at the practical level, but are influenced by international regulations and public opinion to a greater degree. Different sets of international standards for classifying edited plants as GMO or non-GMO lead to trade barriers and slow down commercialization. The future of plant breeding lies in blending together different technologies and cross-disciplinary approaches. Integrating CRISPR with epigenetic editing, genomic selection, and computational breeding should accelerate the process of developing new breeding lines with higher yields and greater resilience to stress. When combined with precision agriculture technologies, digital phenotyping, and decision models based on data, the breeding process should become more efficient. As risk assessment frameworks and international regulations mature, we will likely see these technologies move out of the research laboratory setting into commercial use to ensure food security and sustainable agriculture in the decades to come.

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