Research Insight Open Access

Genome Editing and Rice Improvement: The Role of CRISPR/Cas9 in Developing Superior Yield Traits

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Genomics and Applied Biology, 2024, Vol.15, No.4 doi: 10.5376/gab.2024.15.0020
Received: 27 May, 2024
Accepted: 03 Jul., 2024
Published: 19 Jul., 2024

Preferred citation for this article:
Chen Z.F., and Zhang D.P., 2024, Genome editing and rice improvement: the role of CRISPR/Cas9 in developing superior yield traits, Genomics and Applied Biology, 15(4): 182-190 (doi: 10.5376/gab.2024.15.0014)

Abstract The study demonstrated that the CRISPR/Cas9 system is highly efficient in rice, with nearly half of the target genes edited in the first generation of transformed plants (T0). The mutations were found to be heritable, following classic Mendelian inheritance patterns, with no detectable large-scale off-target effects. Additionally, the CRISPR/Cas9 system enabled high-efficiency multiplex genome editing, allowing for the simultaneous targeting of multiple genes, which is crucial for improving complex traits such as yield. The use of CRISPR/Cas9 has also been shown to enhance grain quality and other agronomic traits, making it a versatile tool for rice improvement. The findings underscore the potential of the CRISPR/Cas9 system as a powerful and precise tool for rice genome engineering. By enabling targeted and heritable gene modifications with minimal off-target effects, CRISPR/Cas9 can significantly contribute to the development of rice varieties with superior yield traits. This technology holds promise for addressing global food security challenges by improving rice productivity and quality.
Keywords CRISPR/Cas9; Genome editing; Rice improvement; Yield traits; Heritability; Multiplex genome editing; Grain quality

1 Introduction

Rice (*Oryza sativa* L.) is one of the most important staple food crops globally, providing a primary source of calories for more than half of the world's population. Its adaptability to various environmental conditions and its significant economic and social importance necessitate continuous efforts to improve its agronomic characteristics, such as yield, nutritional value, and stress tolerance (Romero and Gatica-Arias, 2019). Given the increasing global population and the challenges posed by climate change, enhancing rice production is critical for ensuring food security (Haque et al., 2018).

Traditional breeding methods have significantly contributed to crop improvement; however, they are often time-consuming and less precise. Recent advancements in genome editing technologies, such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system, have revolutionized the field of plant breeding (Li and Jiong, 2024). Among these, CRISPR/Cas9 stands out due to its simplicity, efficiency, and precision in editing specific genome sequences (Chen et al., 2019; Liu et al., 2022). This technology allows for targeted modifications, including gene knockouts, precise edits, and multiplex genome engineering, making it a versatile tool for crop improvement (Arora and Narula, 2017).

CRISPR/Cas9 has emerged as a groundbreaking tool in plant biology, enabling the development of new plant varieties with enhanced traits such as increased yield, improved nutritional quality, and greater resilience to biotic and abiotic stresses (Abdelrahman et al., 2018; Zegeye et al., 2022). Its ability to create transgenic-free edited plants without introducing foreign DNA has received regulatory approval in several countries, further facilitating its adoption in agriculture (Zegeye et al., 2022). The technology's rapid evolution and myriad functionalities have made it a preferred choice for researchers aiming to address the challenges of modern agriculture, including the need for sustainable crop production under changing climatic conditions (Ricroch et al., 2017; Haque et al., 2018).

The primary goal of this study is to explore the application of the CRISPR/Cas9 genome editing system in rice to develop superior yield traits. By reviewing recent advancements and applications of CRISPR/Cas9 in rice breeding, and assessing the efficiency, specificity, and heritability of CRISPR/Cas9-induced gene modifications in rice, we hope to highlight its potential in enhancing rice production and ensuring food security.

2 CRISPR/Cas9 Technology: Mechanism and Applications

2.1 Mechanism of CRISPR/Cas9 in gene editing

The CRISPR/Cas9 system, which stands for Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9, is a revolutionary genome editing tool that has transformed genetic research and crop improvement. The mechanism involves an RNA-guided DNA endonuclease, Cas9, which is directed to a specific DNA sequence by a single-guide RNA (sgRNA). The sgRNA base-pairs with the target DNA sequence, allowing Cas9 to introduce double-strand breaks (DSBs) at the specified location. These DSBs trigger the cell's natural repair mechanisms, leading to mutations that can knock out or modify the function of the target gene (Bao et al., 2019; Li et al., 2021).

2.2 Comparison with other genome editing tools

CRISPR/Cas9 has several advantages over earlier genome editing technologies such as Zinc Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs). Unlike ZFNs and TALENs, which require the design and assembly of protein-DNA binding domains for each target site, CRISPR/Cas9 uses a simple RNA molecule for targeting, making it easier to design and implement. Additionally, CRISPR/Cas9 is more versatile and cost-effective, allowing for the simultaneous editing of multiple genes (multiplexing) (Zeb et al., 2022; Zegeye et al., 2022). Recent advancements have also led to the development of Cas variants like ScCas9++ and Cas9-NG, which expand the targeting scope and improve editing efficiency in plants (Ren et al., 2019; Liu et al., 2021a).

2.3 Success Stories of CRISPR/Cas9 in plant research

CRISPR/Cas9 has been successfully applied in various plant research projects, particularly in rice, to improve yield, quality, and stress resistance. For instance, the CRISPR/Cas9-mediated knockout of the *Os8N3* gene in rice has conferred enhanced resistance to the bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* without affecting other agronomic traits (Kim et al., 2019). Another notable success is the editing of the *OsSAP* gene to improve drought tolerance, which is crucial for maintaining yield under adverse climatic conditions (Park et al., 2022). Additionally, CRISPR/Cas9 has been used to improve rice grain quality by targeting genes involved in grain size, shape, and nutritional content (Fiaz et al., 2019; Zeb et al., 2022) (Figure 1). These examples highlight the potential of CRISPR/Cas9 to address key challenges in crop production and food security.

3 Yield Traits in Rice: Key Targets for Improvement

3.1 Understanding yield components in rice

Yield in rice is a complex trait influenced by various components, including the number of grains per panicle, grain size, and panicle length. These components are critical as they directly impact the overall productivity of rice plants. For instance, the number of grains per panicle and the size of each grain are primary determinants of yield, while panicle length can influence the number of grains that can be supported (Jaganathan et al., 2018; Liu et al., 2021b). Understanding these components is essential for targeted genetic improvements aimed at enhancing rice yield.

3.2 Genetic basis of yield traits

The genetic basis of yield traits in rice involves multiple genes that regulate various aspects of plant growth and development. Advances in genome sequencing have facilitated the identification of these genes, enabling precise genetic modifications. For example, genes such as *OsPIN5b*, *GS3*, and *OsMYB30* have been identified as key regulators of panicle length, grain size, and stress tolerance, respectively (Zeng et al., 2018). The genetic control of these traits is often complex, involving interactions between multiple genes and environmental factors. Therefore, a comprehensive understanding of the genetic architecture is crucial for effective yield improvement strategies.

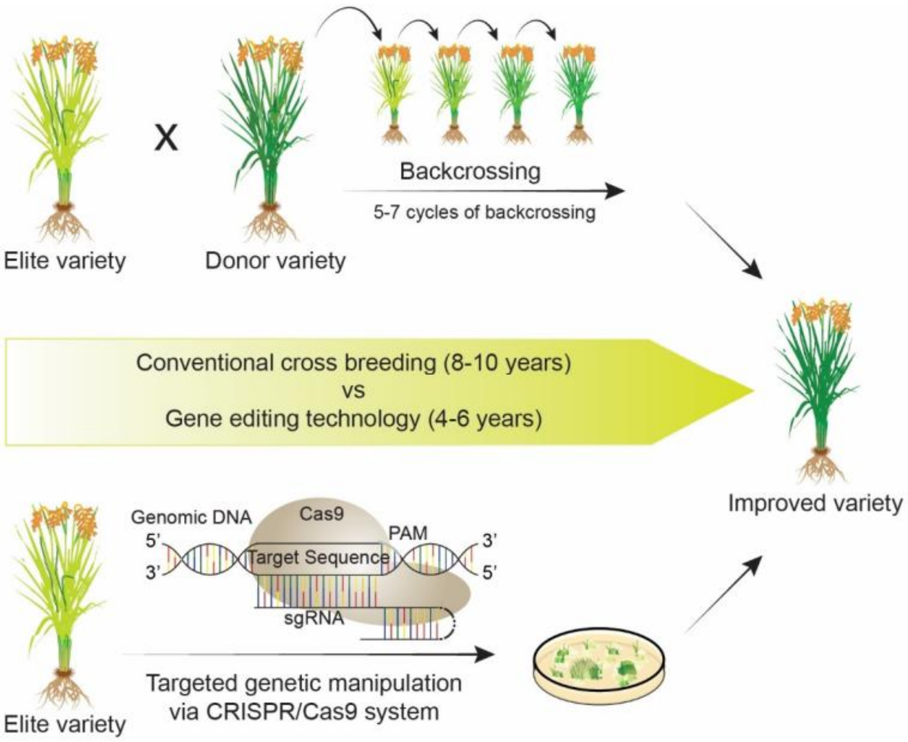

Figure 1 Application of CRISPR/Cas9 system for developing elite variety with desirable improved traits in rice and its comparison with conventional crop breeding technique (Adopted from Zeb et al., 2022)

3.3 Key genes targeted by CRISPR/Cas9 for yield enhancement

CRISPR/Cas9 technology has revolutionized the field of plant breeding by enabling precise and targeted modifications of the genome (Wang, 2024). Several key genes have been targeted using this technology to enhance yield traits in rice. For instance, the editing of *OsPIN5b* and *GS3* genes has resulted in increased panicle length and grain size, respectively, leading to higher yields (Zeng et al., 2018). Additionally, the *CLE* genes in maize, which are involved in meristem size regulation, have been edited to create quantitative variations in yield-related traits, demonstrating the potential of similar approaches in rice (Liu et al., 2021b). The *OsSAP* gene, associated with drought stress response, has also been targeted to improve yield under adverse environmental conditions (Park et al., 2022) (Figure 2). These examples highlight the effectiveness of CRISPR/Cas9 in developing rice varieties with superior yield traits by precisely targeting and modifying specific genes.

4 Case Studies: CRISPR/Cas9-Mediated Improvements in Rice Yield

4.1 Increasing grain size and weight

CRISPR/Cas9 technology has been effectively utilized to enhance grain size and weight in rice. A notable example is the editing of the Grain Size 3 (GS3) gene, which resulted in significant increases in grain length and weight. Specifically, the grain length and 1000-grain weight of the mutants were increased by 31.39% and 27.15%, respectively, compared to the wild type. This was achieved by targeting specific sites within the *GS3* gene, leading to stable long-grain rice mutants with improved yield traits (Usman et al., 2021). Additionally, simultaneous editing of the *GS3* gene along with other yield-related genes such as *OsPIN5b* has shown promising results in increasing panicle length and grain size, further contributing to higher yields (Zeng et al., 2020) (Figure 3).

4.2 Enhancing photosynthetic efficiency

Improving photosynthetic efficiency is another critical area where CRISPR/Cas9 has shown significant potential. By knocking out the hexokinase gene *OsHXK1*, researchers have developed rice varieties with enhanced photosynthetic efficiency. The edited plants exhibited increased light saturation points, stomatal conductance, light tolerance, and overall photosynthetic products, leading to higher rice yields. Transcriptome analysis revealed that the expression of photosynthesis-related genes was significantly upregulated in the OsHXK1-CRISPR/Cas9 lines, underscoring the gene's role in regulating photosynthesis and yield (Zheng et al., 2021).

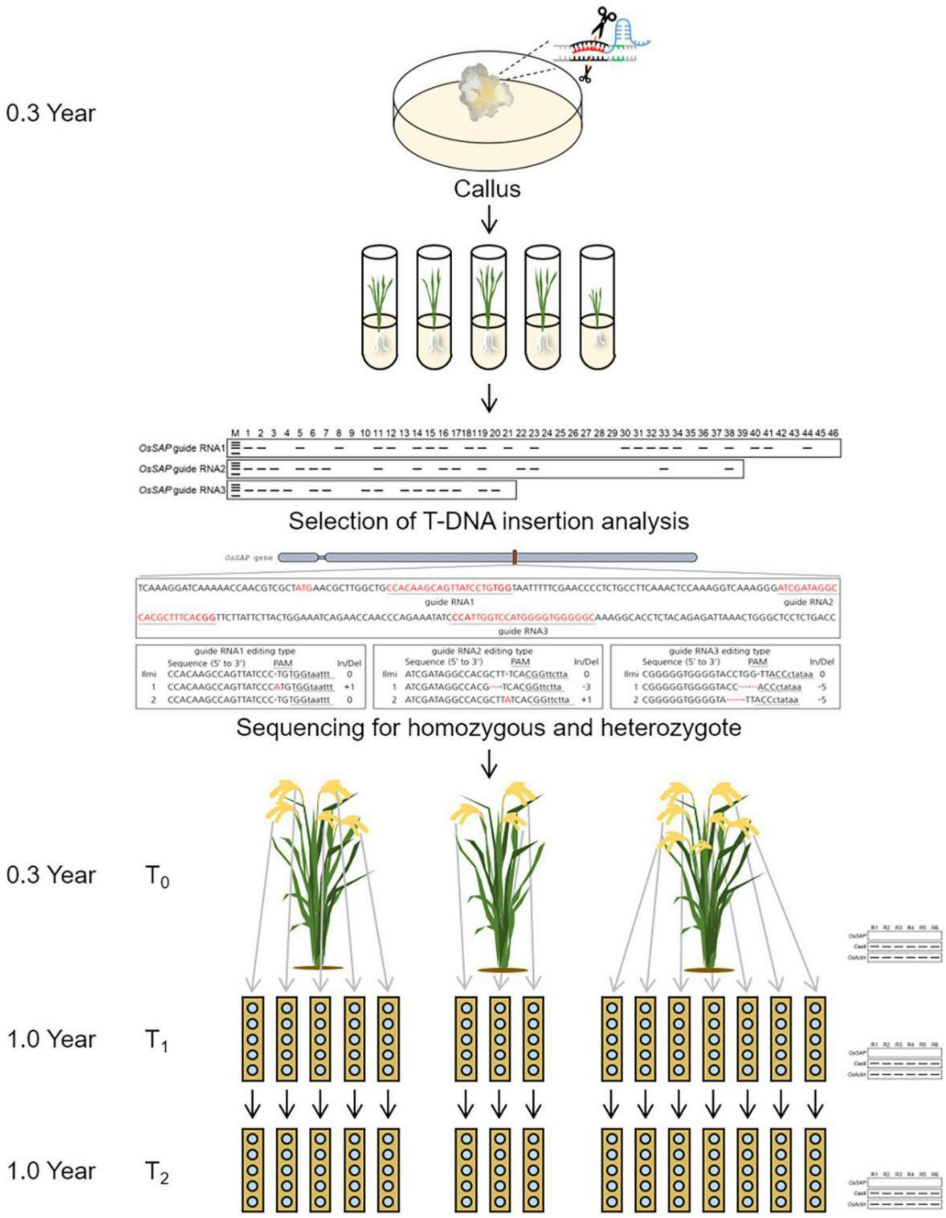

Figure 2 Scheme of short breeding cycle using CRISPR/Cas9 in rice. *Agrobacterium*-mediated transformation used for genome editing with CRISPR/Cas9. In regenerated plants, a gene was selected and edited by T-DNA insertion and sequencing. On the T_0 plant, each spike is assigned a different accession number. T_1 plants are transplanted to one row for each line. T_2 seeds are harvested in bulk (Adopted from Park et al., 2022)

4.3 Reducing susceptibility to environmental stress

CRISPR/Cas9 has also been instrumental in developing rice varieties with reduced susceptibility to environmental stress. For instance, editing the *OsMYB30* gene, which is associated with cold tolerance, has resulted in rice mutants with enhanced resilience to cold stress. These mutants, when combined with edits in other yield-related genes like *OsPIN5b* and *GS3*, not only showed improved stress resistance but also maintained high yield traits. This dual improvement in yield and stress tolerance demonstrates the versatility and effectiveness of CRISPR/Cas9 in addressing multiple breeding goals simultaneously (Zeng et al., 2020). Furthermore, the technology has been applied to develop rice varieties that can sustain high yields under various biotic and abiotic stresses, ensuring food security in the face of climate change and other environmental challenges (Abdelrahman et al., 2018; Haque et al., 2018).

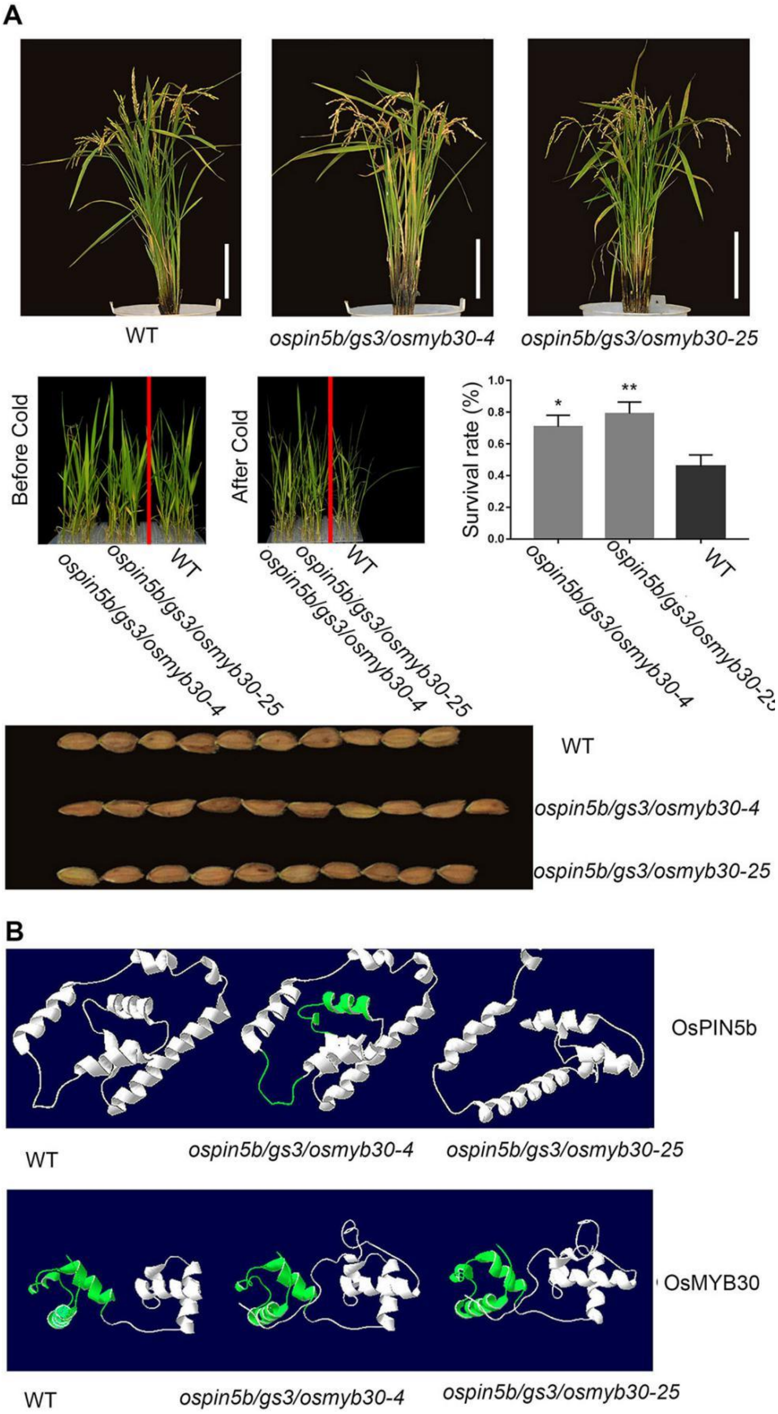

Figure 3 The phenotypes of *ospin5b/gs3/osmyb30-4*, *ospin5b/gs3/osmyb30-25* and the predicted protein structure of OsPIN5b and OsMYB30 in *ospin5b/gs3/osmyb30-4*, *ospin5b/gs3/osmyb30-25* (Adopted from Zeng et al., 2020)

Image caption: (A) Whole plant morphology, survival rate after cold treatment and grain size in WT, *ospin5b/gs3/osmyb30-4*, *ospin5b/gs3/osmyb30-25*. Bar = 20 cm. (B) The OsPIN5b and OsMYB30 protein structures of WT, *ospin5b/gs3/osmyb30-4* and *ospin5b/gs3/osmyb30-25* were predicted by SWISS-MODEL. The observed differential regions were highlighted in green (Adopted from Zeng et al., 2020)

5 Challenges and Limitations of CRISPR/Cas9 in Rice Yield Improvement

5.1 Off-target effects and genetic stability

One of the primary challenges associated with the use of CRISPR/Cas9 in rice yield improvement is the potential for off-target effects. Although CRISPR/Cas9 is known for its precision, unintended mutations can occur at sites with similar sequences to the target, leading to genetic instability. Studies have shown that while the

CRISPR/Cas9 system is highly efficient in inducing targeted gene editing, off-target mutations, although rare, can still occur and may affect the stability of the edited genes across generations. This necessitates extensive screening and validation to ensure that the desired traits are stably inherited without unintended consequences (Zhang et al., 2014; Li et al., 2016).

5.2 Regulatory and ethical considerations

The regulatory landscape for genome-edited crops, including those developed using CRISPR/Cas9, varies significantly across different countries. Some nations have stringent regulations that classify genome-edited crops similarly to genetically modified organisms (GMOs), which can hinder the adoption and commercialization of CRISPR/Cas9-edited rice varieties (Abdelrahman et al., 2018; Zegeye et al., 2022). Additionally, ethical concerns regarding the manipulation of plant genomes and the potential long-term ecological impacts pose significant challenges. Public perception and acceptance of genome-edited crops also play a crucial role in the regulatory approval process, making it essential to address these concerns through transparent communication and robust regulatory frameworks (Abdelrahman et al., 2018; Chen et al., 2019).

5.3 Integration into breeding programs

Integrating CRISPR/Cas9 technology into traditional rice breeding programs presents several challenges. Conventional breeding methods rely on the selection of naturally occurring mutations, which can be a slow and labor-intensive process. While CRISPR/Cas9 offers a faster and more precise alternative, its integration requires significant changes in breeding strategies and infrastructure. Breeders need to develop expertise in genome editing techniques and establish protocols for the efficient delivery of CRISPR/Cas9 components into rice cells. Additionally, the development of high-throughput screening methods to identify and select desirable traits is essential for the successful integration of CRISPR/Cas9 into breeding programs (Arora and Narula, 2017; Chen et al., 2019).

5.4 Technical limitations

Despite its advantages, CRISPR/Cas9 technology has technical limitations that can affect its efficiency and effectiveness in rice yield improvement. For instance, the delivery of CRISPR/Cas9 components into rice cells can be challenging, and the efficiency of gene editing can vary depending on the target gene and the rice variety (Lu and Zhu, 2017; Fiaz et al., 2019). Moreover, the repair of CRISPR/Cas9-induced double-strand breaks (DSBs) through non-homologous end joining (NHEJ) can result in random insertions and deletions (indels), which may not always produce the desired genetic modifications (Lu and Zhu, 2017). Developing more efficient delivery systems and improving the precision of gene editing are critical areas of ongoing research (Lu and Zhu, 2017; Fiaz et al., 2019).

5.5 Socio-economic impacts

The adoption of CRISPR/Cas9 technology for rice yield improvement also has socio-economic implications. Smallholder farmers, who constitute a significant portion of rice producers in many developing countries, may face challenges in accessing and adopting this technology due to high costs and lack of technical expertise. Ensuring that the benefits of CRISPR/Cas9-edited rice varieties are accessible to all farmers, regardless of their economic status, is crucial for achieving equitable agricultural development. Additionally, the potential displacement of traditional rice varieties with genome-edited ones could impact biodiversity and cultural heritage, necessitating careful consideration of socio-economic factors in the deployment of CRISPR/Cas9 technology (Abdelrahman et al., 2018; Bandyopadhyay et al., 2018).

6 Future Perspectives and Opportunities

6.1 Potential for CRISPR/Cas9 in precision agriculture

The CRISPR/Cas9 system holds immense potential for precision agriculture by enabling targeted modifications in the rice genome to enhance yield and stress resistance. This technology allows for precise editing of specific genes, which can lead to the development of rice varieties with improved agronomic traits such as increased yield, enhanced nutritional quality, and better resistance to biotic and abiotic stresses (Arora and Narula, 2017; Li et al., 2021; Zegeye et al., 2022). The ability to create transgenic-free edited plants without introducing foreign DNA has also received regulatory approval in several countries, making it a safer and more acceptable option for crop improvement (Zegeye et al., 2022). As the efficiency and precision of CRISPR/Cas9 delivery systems improve, its application in precision agriculture is expected to expand, offering new opportunities for sustainable rice production (Chen et al., 2019; Li et al., 2021).

6.2 Expanding CRISPR/Cas9 applications to other rice traits

Beyond yield improvement, CRISPR/Cas9 technology can be applied to enhance other important rice traits such as grain quality, disease resistance, and stress tolerance. For instance, researchers have successfully used CRISPR/Cas9 to edit genes related to rice grain quality, leading to improvements in appearance, palatability, and nutritional content (Fiaz et al., 2019; Liu et al., 2021c). Additionally, CRISPR/Cas9 has been employed to develop rice varieties with increased resistance to pests and diseases, as well as enhanced tolerance to environmental stresses such as drought and salinity (Arora and Narula, 2017; Haque et al., 2018; Rao and Wang, 2021). The versatility of CRISPR/Cas9 makes it a powerful tool for addressing a wide range of challenges in rice cultivation, thereby contributing to the overall improvement of rice crops.

6.3 Synergy with other genomic tools and technologies

The integration of CRISPR/Cas9 with other genomic tools and technologies presents exciting opportunities for advancing rice improvement. Combining CRISPR/Cas9 with high-throughput sequencing, bioinformatics, and other genome editing technologies such as base editors and prime editors can enhance the precision and efficiency of genetic modifications (Chen et al., 2019; Li et al., 2021). Moreover, the use of CRISPR/Cas9 in conjunction with traditional breeding methods and modern biotechnological approaches, such as marker-assisted selection and genomic selection, can accelerate the development of superior rice varieties (Arora and Narula, 2017; Zegeye et al., 2022). The synergy between CRISPR/Cas9 and other genomic tools will likely lead to breakthroughs in understanding complex genetic traits and developing innovative solutions for sustainable rice production.

7 Concluding Remarks

The application of CRISPR/Cas9 in rice improvement has shown significant promise in enhancing yield traits and addressing global food security challenges. The technology's simplicity, robustness, and high efficiency make it a preferred choice for genetic manipulation in rice. CRISPR/Cas9 has been instrumental in developing rice varieties that can withstand biotic and abiotic stresses, thereby ensuring sustainable crop production under varying environmental conditions. The ability to produce transgene-free edited plants has also facilitated regulatory approvals in several countries, further accelerating the adoption of this technology. Additionally, advancements in CRISPR/Cas9, including the development of base editors and prime editing, have expanded the potential for precise and efficient genome editing in rice.

The future of genome editing in rice improvement looks promising with continuous advancements in CRISPR/Cas9 technology. Emerging tools such as CRISPR/Cpf1 and base editors offer more efficient and accurate genome editing capabilities, which could further accelerate the pace of crop improvement. The integration of CRISPR/Cas9 with other innovative breeding technologies, such as speed breeding, is expected to enhance the accuracy and speed of developing superior rice varieties. Moreover, the development of DNA-free delivery systems and high-throughput mutant libraries will likely play a crucial role in fine-tuning gene regulation and trait improvement. As researchers continue to explore the full potential of CRISPR/Cas9, it is anticipated that new strategies for breeding virus resistance and improving nutritional quality will emerge, contributing to the overall goal of global food security.

CRISPR/Cas9 has revolutionized the field of plant science and agriculture, offering a powerful tool for genome engineering that can significantly contribute to global food security. By enabling the development of rice varieties with enhanced yield, stress tolerance, and nutritional quality, CRISPR/Cas9 addresses the urgent need to produce more food for a growing population. The technology's ability to produce specific and homozygous targeted gene edits in a single generation further underscores its potential to transform rice breeding practices. As the global community continues to face challenges such as climate change and population growth, the role of CRISPR/Cas9 in ensuring a stable and sufficient food supply cannot be overstated. Continued research and innovation in this field will be essential to harnessing the full potential of genome editing for the benefit of global food security.

Acknowledgments

The authors are grateful to the reviewers for their insightful comments and suggestions during the research process.

Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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