Proceedings of ICMMGH 2026 Symposium: Biomedical Imaging and AI Applications in Neurorehabilitation
DOI: 10.54254/2753-8818/2026.AU29346

# *Advances in CRISPR/Cas Gene Editing Technology in Rice Breeding*

**Shuqi Yang**

*College of Agriculture, Shandong Agricultural University, Tai'an, China*
*2111752189@qq.com*

***Abstract.*** CRISPR/Cas gene editing has emerged as a transformative tool in rice (Oryza sativa) breeding, enabling precise and rapid improvement of key traits compared to traditional breeding methods. This review outlines the current state of CRISPR/Cas technology in rice, including its mechanism and recent advancements. We highlight successful applications in developing male sterile lines for hybrid breeding, engineering disease resistance by targeting host susceptibility or immune genes, and optimizing yield-related traits through direct introduction of high-yield allele variants and multiplex editing. Technical challenges such as off-target effects and PAM sequence constraints are discussed alongside strategies to mitigate them, including high-fidelity Cas variants and broader-spectrum nucleases. Regulatory considerations are examined, contrasting policies that exempt DNA-free edited rice from GMO regulation with stricter process-based frameworks, and emphasizing the importance of public communication. Looking forward, we describe future directions such as exploring novel Cas enzymes, high-throughput multiplex editing for complex traits, integration with traditional breeding and epigenetic modulation, and the prospects for commercialization of gene-edited rice. This condensed review maintains technical accuracy and academic style, providing a comprehensive yet concise overview of CRISPR/Cas in rice breeding.

***Keywords:*** CRISPR/Cas9, rice breeding, male sterility, disease resistance, yield improvement

## 1. Introduction

Rice (Oryza sativa) is the staple food for nearly half of the global population and plays a central role in global food security. By 2019, rice production exceeded 755 million tons, yet rising demand, driven by projected population growth of 34% by 2050, will require ~70% more food. Climate change, reduced arable land, and persistent pests and diseases pose additional challenges. Conventional breeding approaches, such as hybridization and mutagenesis, have generated improved cultivars but are time-consuming and inefficient. Genome editing technologies now provide a transformative alternative, with the CRISPR/Cas system (clustered regularly interspaced short palindromic repeats/CRISPR-associated proteins) emerging as the leading tool due to its simplicity, efficiency, and low cost [1]. Unlike traditional transgenics that insert foreign DNA randomly, CRISPR/Cas enables precise modifications of endogenous loci without cross-species gene

© 2025 The Authors. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

DOI: 10.54254/2753-8818/2026.AU29346

integration, thereby improving breeding precision and public acceptance(Figure 1) [2,3]. Since its first application in rice in 2013, CRISPR/Cas9 has revolutionized rice breeding by enabling direct induction of mutations within a single generation, supporting multiplex editing, and accelerating the creation of customized germplasm for yield, stress resistance, and quality improvements [4]. Mechanistically, CRISPR/Cas pairs a guide RNA (gRNA) with a Cas nuclease to recognize a target sequence adjacent to a protospacer adjacent motif (PAM). The widely used Cas9, guided by a single guide RNA (sgRNA), introduces a double-strand break (DSB) upstream of a PAM (5′-NGG-3′), repaired via non-homologous end joining (NHEJ) for knockouts or homology-directed repair (HDR) for precise edits. Derivative tools—such as base editors, prime editors, and transcriptional regulators (CRISPRi/a) , enable nucleotide substitutions or reversible modulation of gene expression without DSBs, expanding applications beyond simple knockouts. These advances have transformed CRISPR/Cas into a versatile platform for rice breeding, from generating stable heritable mutations to fine-tuning gene expression and creating precise, tailored improvements.

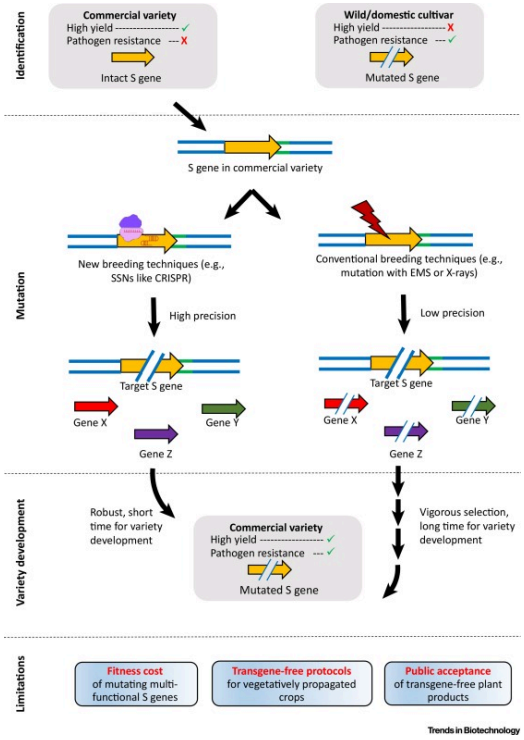


Figure 1. Advantages of CRISPR-mediated gene editing compared with conventional breeding techniques [5]

## 2. Types and mechanism of action of the CRISPR/Cas system

### 2.1. CRISPR/Cas system classification

Naturally occurring CRISPR/Cas systems are divided into two classes and six types (I–VI) [6]. Class 1 systems (Types I, III, IV) employ multi-protein complexes such as Cascade; for example, Type I uses Cas3 with helicase–nuclease activity to degrade DNA, while Type III (Cas10) targets both DNA and RNA. Class 2 systems (Types II, V, VI) rely on a single multidomain nuclease, making them more amenable to engineering. Type II is represented by Cas9, requiring a downstream PAM (e.g. NGG) for cleavage. Type V, typified by Cas12a (Cpf1), recognizes a T-rich 5′ PAM (TTTV), does not require tracrRNA, and generates staggered DNA breaks with 5′ overhangs; it also

DOI: 10.54254/2753-8818/2026.AU29346

processes its own crRNAs, facilitating multiplex editing [7]. Type VI systems, such as Cas13, uniquely target single-stranded RNA via HEPN domains, enabling antiviral strategies or post-transcriptional regulation in plants [6].

Most rice genome editing still uses SpCas9 ( Streptococcus pyogenes Cas9, Type II), but Cas12a, Cas12b, and Cas13 are expanding the toolkit. Cas12a's distinct PAM requirement broadens targetable regions and has been used to optimize rice architecture. Cas13 has shown promise in virus resistance, for example, LshCas13a targeting Southern rice black-streaked dwarf virus (SRBSDV) provided immunity in transgenic rice [8]. Thus, by diversifying Cas effectors, CRISPR offers an increasingly versatile "toolbox" for crop breeding.

### 2.2. Mechanism of CRISPR/Cas9

SpCas9 (~1368 amino acids) contains two nuclease domains, HNH and RuvC. Guided by a single guide RNA (sgRNA), Cas9 scans for target sites using an ~8–12 nt "seed" region and requires an adjacent PAM (NGG). This PAM safeguard prevents cleavage of the CRISPR locus itself. Once bound, the HNH and RuvC domains cleave complementary and non-complementary strands, respectively, producing a double-strand break (DSB).

In rice, DSBs are primarily repaired by non-homologous end joining (NHEJ), creating indels that disrupt gene function, while homology-directed repair (HDR)—less efficient—enables precise insertions when a donor is supplied. Novel platforms overcome HDR's limitations: base editors (Cas9 nickase fused to cytidine deaminase) allow single-base substitutions, e.g. ALS mutation conferring imazapic resistance; prime editors (Cas9–reverse transcriptase plus pegRNA) enable precise modifications, such as editing Xa13 and Xa25 to generate blight-resistant rice. Moreover, dCas9 fused to transcriptional effectors enables CRISPRi (repression) or CRISPRa (activation). For instance, dCas9-HDAC fusions silence gene expression, while dCas9-HAT or dCas9-demethylase can activate transcription [9]. These derivative systems expand CRISPR's utility from simple knockouts to fine-tuned gene regulation, greatly enhancing its value for rice improvement.

## 3. Applications of CRISPR/Cas in rice breeding

CRISPR/Cas technology has been extensively applied to improve key agronomic traits in rice, including male sterility for hybrid breeding, resistance to diseases and stresses, and yield and quality enhancement(Figure 2) [10]. Unlike traditional breeding, which requires years of backcrossing, gene editing can generate targeted mutants in much shorter timeframes [11]. Numerous studies have confirmed that CRISPR-mediated knockouts or precise mutations can efficiently produce hybrid male sterile lines, disease-resistant cultivars, and high-yield, high-quality materials.

DOI: 10.54254/2753-8818/2026.AU29346

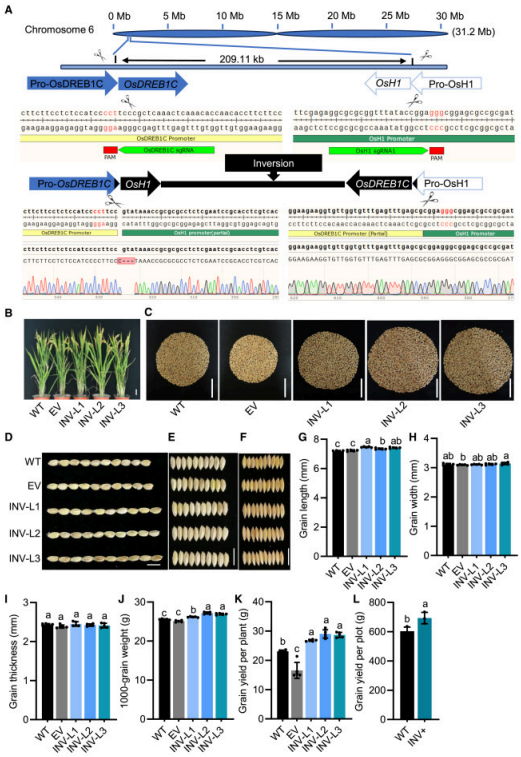


Figure 2. CRISPR-Cas9-mediated OsDREB1C promoter swap to enhance rice grain characteristics and yield [12]

## 3.1. Application in developing male sterile lines in rice

Hybrid rice relies on stable male sterile lines, particularly photoperiod- and thermo-sensitive genic male sterility (PGMS/TGMS). Conventional breeding of such lines required random mutagenesis or wide crosses, making the process lengthy and inefficient [13]. CRISPR/Cas now enables precise and rapid creation of sterile lines by targeting fertility genes.

One major TGMS gene, TMS5, encodes a fatty-acid esterase; its loss of function causes pollen sterility at high temperatures but fertility recovery at lower ones. The cultivar Annong S-1 carries a natural tms5 allele. In 2016, CRISPR/Cas9 disruption of TMS5 in japonica backgrounds yielded new TGMS lines. Barman et al. further produced stable tms5 mutants free of T-DNA, which exhibited complete sterility at high temperature and restored fertility at low temperature [11]. Field tests of the CRISPR-derived line YK17S showed >10% yield advantage when crossed with various restorers.

Another key gene is CSA (Carbon Starved Anther), where knockout mutations confer PGMS. Using CRISPR/Cas9, CSA was disrupted in a single step to create a photoperiod-sensitive sterile line, reducing a process that once required years to a single generation. Overall, CRISPR/Cas enables efficient manipulation of fertility genes such as TMS5 and CSA directly in elite cultivars, offering an effective strategy for breeding hybrid rice parents and producing environmentally controllable sterile lines to enhance yield stability.

## 3.2. Application in rice disease resistance breeding

CRISPR/Cas can enhance disease resistance by disrupting host susceptibility genes or modifying their regulatory elements. Rice bacterial blight, caused by Xanthomonas oryzae pv. oryzae, relies on

DOI: 10.54254/2753-8818/2026.AU29346

TAL effectors that activate host OsSWEET transporters (OsSWEET11, OsSWEET13, OsSWEET14). Oliva et al introduced promoter mutations in OsSWEET11/13/14 using CRISPR/Cas9, blocking TAL binding sites [14]. Edited indica cultivars (e.g. IR64) displayed broad-spectrum resistance across Asian and African pathogen strains, exemplifying how targeted disruption of susceptibility elements confers durable resistance.

CRISPR has also been applied to fungal pathogens such as rice blast (Magnaporthe oryzae). Knockout of the negative regulator ERF922 reduced lesion severity without yield penalties, demonstrating the utility of editing multiple susceptibility loci to generate broad and durable resistance.

Beyond susceptibility knockouts, CRISPR can enhance or rewire innate immunity. For example, editing the Pi-d2 gene produced gain-of-function alleles with stronger blast resistance and minimal growth cost. Similarly, promoter editing of OsPBS1 enabled synthetic resistance by triggering cell death in response to pathogen proteases. Collectively, CRISPR enables both subtractive (loss of susceptibility) and additive (enhanced immunity) strategies, offering versatile tools for engineering disease-resistant rice.

### 3.3. Application in improving rice yield-related traits

Rice yield involves complex traits such as plant height, panicle architecture, and flowering time. CRISPR allows direct modification of key yield-related genes, expediting improvement. The classic dwarfing gene sd1 (OsGA20ox2), encoding GA20 oxidase, reduces plant height while improving lodging resistance and fertilizer efficiency. Researchers used CRISPR/Cas9 to edit OsGA20ox2 in Basmati rice, producing semi-dwarf lines with enhanced lodging resistance and no yield penalty, replicating the “Green Revolution” trait in an elite background [15].

Multiplex CRISPR editing further enables pyramiding of favorable alleles. For instance, simultaneous edits of DEP1, Gn1a, and GS3 produced lines with improved panicle structure and grain size, boosting yield. Knockouts of Ghd7 or PRR37 modified photoperiod sensitivity, allowing earlier flowering under long-day conditions and enabling multi-cropping at higher latitudes. Editing OsEPT1 increased panicle branching and grain number, further contributing to yield gains.

In summary, CRISPR facilitates “tailor-made” yield improvements by directly introducing elite alleles such as IPA1, Gn1a, and Ghd7 into desired cultivars. With improved QTL dissection and multiplex efficiency, breeders can combine traits such as plant height, flowering time, and panicle architecture within a single line, opening new possibilities for substantial yield enhancement.

## 4. Challenges of CRISPR in rice breeding

### 4.1. Off-target effects

Although CRISPR/Cas9 is highly specific, sgRNAs may guide Cas9 to off-target sites with high sequence similarity, causing unintended mutations that may alter phenotypes or raise safety concerns [16]. Two main strategies reduce this risk: (1) optimized sgRNA design, avoiding targets with near-identical genomic sequences and using computational prediction tools; and (2) engineered high-fidelity nucleases, such as eSpCas9 and SpCas9-HF1, which incorporate amino acid substitutions to reduce non-specific binding. In addition, genetic mutations may also alter potential binding sites, leading to off-target effects (Figure 3). These variants, along with newer versions like HypaCas9 and HiFi Cas9, show markedly reduced off-target activity while maintaining high efficiency. Some

DOI: 10.54254/2753-8818/2026.AU29346

natural nucleases, such as Bacillus-derived Cas12b with stricter PAM requirements, also exhibit inherently high specificity in rice.

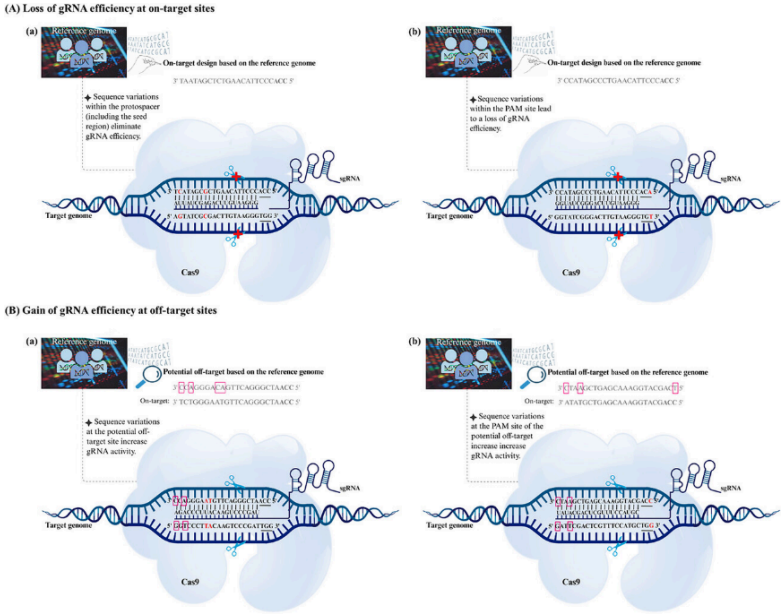


Figure 3. Impact of Genetic Variation (GV) on CRISPR-based targeting [17]

Off-target detection employs both in silico prediction and genome-wide assays. GUIDE-seq tags double-strand breaks in vivo, while CIRCLE-seq detects cleavage sites in vitro using genomic DNA. Though delivery efficiency limits GUIDE-seq in plants, these methods provide valuable tools to identify rare edits. In practice, combining high-fidelity Cas enzymes, stringent sgRNA design, and screening has already reduced off-target rates to very low levels. Future advances, including AI-assisted design and novel Cas variants, are expected to further minimize or eliminate off-target events. Continuous monitoring, however, remains essential for ensuring editing safety and predictability in rice breeding.

## 4.2. PAM sequence limitations

SpCas9 editing requires a 5′-NGG-3′ PAM adjacent to the target, restricting accessible sites and creating “blind spots,” especially in AT-rich or regulatory regions. Precision applications such as base and prime editing impose even stricter PAM positioning, further limiting options. Two strategies have been developed to overcome this: (1) Discovery of alternative Cas proteins with different PAM requirements. For example, SaCas9 recognizes 5′-NNGRRT-3′, while Cas12a (Cpf1) recognizes 5′-TTTV-3′ and has been applied successfully in rice to expand target coverage. (2) Engineering SpCas9 variants with relaxed PAM recognition. xCas9 recognizes multiple PAMs (NG, GAA, GAT) but shows limited plant activity. SpCas9-NG, however, efficiently edits NGN sites in rice, greatly expanding the editable genome fraction. More advanced variants include XNG-Cas9 (combining xCas9 and NG mutations) and SpRY, which enables near-PAMless editing (NRN, NYN) and has been validated in rice and wheat. Although broader PAM compatibility may sometimes reduce activity, ongoing protein engineering aims to balance efficiency and flexibility. Achieving highly active, PAM-relaxed nucleases will allow editing of virtually any site in rice genomes, enabling more precise and versatile trait improvement.

DOI: 10.54254/2753-8818/2026.AU29346

### 4.3. Transgenic vs. non-transgenic classification

The classification of CRISPR-derived crops as GMOs depends on whether foreign DNA is introduced and retained. Traditional transgenic rice contains exogenous genes and is subject to strict biosafety regulations. In contrast, DNA-free CRISPR edits (SDN1/SDN2) produce only small mutations without foreign DNA, making resulting lines indistinguishable from those obtained by natural mutation or conventional breeding.

Regulatory policies vary internationally. The USDA (2018) ruled that transgene-free gene-edited crops are not GMOs, allowing their commercialization without special oversight. The first example was a non-browning mushroom developed at Penn State in 2016. Argentina, Brazil, Canada, and Japan have similarly exempted or simplified regulation for such crops; Japan announced in 2019 that gene-edited products without foreign DNA would not be classified as GM. Conversely, the EU applies a process-based approach: any organism created by nucleic acid techniques is considered GMO, regardless of transgene presence, subjecting CRISPR crops to strict approval procedures.

China has recently adopted a more supportive stance. In 2022, the Ministry of Agriculture and Rural Affairs issued interim measures allowing simplified safety evaluations for gene-edited plants lacking foreign DNA, enabling faster entry into variety trials. However, the lack of international harmonization creates barriers for global trade and adoption. Broad public communication that "gene editing ≠ transgenic" is essential, alongside traceability and detection methods to ensure transparency and consumer confidence.

### 4.4. Regulatory oversight and public acceptance

The success of gene-edited crops relies not only on technological advances but also on regulation and public perception. Due to past GMO controversies, many consumers conflate CRISPR with traditional GMOs, raising concerns about safety, environmental risks, and ethics. Effective science communication is needed to emphasize that CRISPR can improve crops without foreign DNA, producing changes similar to natural mutations. Governments and research institutions should also increase transparency through traceability systems and open regulatory processes to strengthen public trust.

Divergent national policies currently create uncertainty for trade and commercialization. Ideally, regulatory frameworks should focus on product characteristics rather than breeding processes, distinguishing transgene-free edits (SDN1/SDN2) from GMOs. Developing dedicated standards and detection methods would facilitate efficient risk assessment. International cooperation among regulators, researchers, and industry is critical to balance biosafety with innovation.

Large-scale commercialization will ultimately require both supportive policies and public acceptance. As more CRISPR-derived products (e.g., high-GABA tomatoes, non-browning mushrooms) enter markets, consumers are likely to judge them based on tangible benefits, fostering greater acceptance and encouraging favorable policy directions.

## 5. Conclusions and outlook

CRISPR/Cas gene editing is being rapidly integrated into rice breeding, marking the advent of precision agriculture. This review summarized system types and mechanisms, applications in male sterility, disease resistance, and yield improvement, and discussed challenges such as off-target effects, PAM constraints, and regulation. Looking ahead, several directions deserve emphasis:

DOI: 10.54254/2753-8818/2026.AU29346

(1) New Cas systems: Beyond Cas9, nucleases such as Cas12a, Cas12b, and Cas13 expand editing options. Cas12a recognizes TTTV PAMs and supports multiplex editing, while Cas12b offers compact size and high specificity. Ultra-compact enzymes (e.g., CasΦ, Cas14) may overcome delivery limitations. Mining natural Cas diversity and applying directed evolution may yield nucleases with high efficiency, minimal PAM dependence, and reduced off-target activity.

(2) Multigene editing with high-throughput screening: Complex traits like yield and stress tolerance require simultaneous gene modifications. CRISPR enables stacked edits; for example, multi-sgRNA constructs have produced rice resistant to multiple pathogens. Coupled with mutant libraries, sequencing, and phenotyping, this "edit + select" strategy accelerates identification of optimal allele combinations, integrating forward genetics with targeted breeding.

(3) Integration with traditional breeding and epigenetic regulation: CRISPR complements GWAS and QTL mapping by validating candidate genes and can be combined with marker-assisted selection to accelerate introgression of edits. Epigenome editing with dCas9 fusions (e.g., dCas9-HDAC) allows reversible regulation of traits such as flowering, offering on-demand control without permanent DNA changes.

(4) Commercial prospects: Gene-edited rice is advancing from research to practice. In China, companies such as Longping High-Tech are developing herbicide-resistant and hybrid-parent lines. In the US, Japan, and elsewhere, CRISPR-derived crops (e.g., high-GABA rice, shelf-stable tomatoes) are entering markets. The first commercialized gene-edited rice varieties are expected within 5–10 years, though success requires optimized pipelines for screening, field evaluation, and variety registration, as well as clear regulatory frameworks.

In summary, with efficiency, precision, and versatility, CRISPR/Cas is fast-tracking rice breeding. Despite ongoing technical and regulatory challenges, emerging tools and integrated strategies promise "on-demand customized" rice varieties that combine high yield, quality, and stress resilience, contributing substantially to food security and sustainable agriculture.

## References

[1] Song J, Tang L, Fan H, et al. Enhancing Yield and Improving Grain Quality in Japonica Rice: Targeted EHD1 Editing via CRISPR-Cas9 in Low-Latitude Adaptation. Current Issues in Molecular Biology 2024.
[2] Ahmad A, Jamil A, Munawar N. GMOs or non-GMOs? The CRISPR Conundrum. Frontiers in Plant Science 2023.
[3] Shaheen N, Ahmad S, Alghamdi SS, et al. CRISPR-Cas System, a Possible "Savior" of Rice Threatened by Climate Change: An Updated Review. Rice 2023.
[4] Chen F, Chen L, Yan Z, et al. Recent advances of CRISPR-based genome editing for enhancing staple crops. Frontiers in Plant Science 2024.
[5] Zaidi SS-e-A, Mukhtar MS, Mansoor S. Genome Editing: Targeting Susceptibility Genes for Plant Disease Resistance. Trends in Biotechnology 2018.
[6] Hillary VE, Ceasar SA. A Review on the Mechanism and Applications of CRISPR/Cas9/Cas12/Cas13/Cas14 Proteins Utilized for Genome Engineering. Molecular Biotechnology 2022.
[7] Bandyopadhyay A, Kancharla N, Javalkote VS, Dasgupta S, Brutnell TP. CRISPR-Cas12a (Cpf1): A Versatile Tool in the Plant Genome Editing Tool Box for Agricultural Advancement. Frontiers in Plant Science 2020.
[8] Freije CA, Myhrvold C, Boehm CK, et al. Programmable Inhibition and Detection of RNA Viruses Using Cas13. Molecular Cell 2019.
[9] Zhang H, Zhu J-K. Epigenetic gene regulation in plants and its potential applications in crop improvement. Nature Reviews Molecular Cell Biology 2024.
[10] Le VT, Kim M-S, Jung Y-J, Kang K-K, Cho Y-G. Research Trends and Challenges of Using CRISPR/Cas9 for Improving Rice Productivity. Agronomy 2022.
[11] Barman HN, Sheng Z, Fiaz S, et al. Generation of a new thermo-sensitive genic male sterile rice line by targeted mutagenesis of TMS5 gene through CRISPR/Cas9 system. BMC Plant Biology 2019.
[12] Luo Y, Zhan X, Zhang Y, et al. CRISPR-Cas9-mediated knockup of OsDREB1C enhances rice yield without compromising grain quality. Plant Communications 2025.

DOI: 10.54254/2753-8818/2026.AU29346

[13] Gu W, Zhang D, Qi Y, Yuan Z. Generating Photoperiod-Sensitive Genic Male Sterile Rice Lines with CRISPR/Cas9. Methods in Molecular Biology 2019.
[14] Oliva R, Ji C, Atienza-Grande G, et al. Broad-spectrum resistance to bacterial blight in rice using genome editing. Nature Biotechnology 2019.
[15] Nawaz G, Usman B, Zhao N, et al. CRISPR/Cas9 Directed Mutagenesis of OsGA20ox2 in High Yielding Basmati Rice (Oryza sativa L.) Line and Comparative Proteome Profiling of Unveiled Changes Triggered by Mutations. International Journal of Molecular Sciences 2020.
[16] Guo C, Ma X, Gao F, Guo Y. Off-target effects in CRISPR/Cas9 gene editing. Frontiers in Bioengineering and Biotechnology 2023.
[17] Alariqi M, Ramadan M, Yu L, et al. Enhancing Specificity, Precision, Accessibility, Flexibility, and Safety to Overcome Traditional CRISPR/Cas Editing Challenges and Shape Future Innovations. Advanced Science 2025.