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CRISPR/CAS-MEDIATED GENOME EDITING IN RICE IMPROVEMENT: TOOLS, APPLICATIONS, ADVANCES AND CHALLENGES

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ABSTRACT
Rice (*Oryza sativa* L.) is a major staple crop that plays a crucial role in global food and nutritional security. However, increasing population pressure, climate change, emerging biotic and abiotic stresses, and limitations of conventional breeding necessitate the development of improved rice varieties with enhanced productivity, quality and resilience. Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein (Cas)-mediated genome editing have emerged as powerful tools for precise and targeted genetic improvement in rice. This review highlights the molecular basis, evolution and technological advancements of CRISPR/Cas genome-editing systems, with particular emphasis on Cas9, Cas12a, base editing and prime editing. Different delivery approaches, including *Agrobacterium*-mediated transformation, particle bombardment, protoplast-based transformation and ribonucleoprotein delivery, are discussed. The applications of genome editing in rice improvement are comprehensively examined, covering yield-related traits, plant architecture, grain size and quality, nutritional biofortification, resistance to bacterial and fungal diseases, tolerance to abiotic stresses, flowering time, root architecture and nutrient-use efficiency. Furthermore, multiplex genome editing offers opportunities for simultaneous modification of multiple genes and complex traits, thereby accelerating the development of improved rice cultivars. Despite these advances, challenges associated with transformation efficiency, genotype dependency, off-target effects, regeneration, regulatory frameworks and field-level validation remain. Integrating CRISPR/Cas technology with genomics, molecular breeding, genomic selection and advanced phenotyping could further enhance the precision and efficiency of rice improvement. Overall, CRISPR/Cas-mediated genome editing represents a promising strategy for developing high-yielding, climate-resilient, disease-resistant and nutritionally superior rice varieties to support sustainable agriculture and global food security.

KEYWORDS: Rice (*Oryza sativa* L.); CRISPR/Cas genome editing; Cas9; Cas12a; Multiplex genome editing; Base editing; Prime editing; Yield improvement; Disease resistance; Abiotic stress tolerance; Grain quality; Molecular breeding.

INTRODUCTION
Rice as a global food-security crop
Rice (Oryza sativa L.) is one of the most important cereal crops globally and constitutes a fundamental component of food security, particularly across Asia, where it is deeply integrated into food systems, livelihoods and agricultural economies. Rice provides a substantial proportion of dietary energy for billions of people and remains particularly important in low- and middle-income countries where dependence on cereal-based diets is high. The historical progress in rice breeding, including the development of semi-dwarf high-yielding varieties and hybrid rice, has resulted in remarkable increases in productivity; however, future gains must be achieved under increasingly constrained land, water and environmental resources (Khush, 2005). Asia continues to dominate global rice production and consumption, making genetic improvement of rice particularly important for regional and global food security. Current production projections illustrate the continuing scale of the crop: global rice production is forecast at approximately 552.5 million tonnes on a milled basis for 2026/27, although production prospects remain sensitive to climatic conditions, water availability and regional weather variability (FAO, 2026).
The challenge of maintaining rice production is becoming increasingly complex because demand for food is rising while the natural-resource base supporting crop production is under pressure. Population growth, urbanization, dietary transitions and competition for agricultural land and water are expected to intensify the demand for productive and resource-efficient rice varieties. At the same time, climate change is modifying the environmental conditions under which rice is cultivated through increasing temperature, altered precipitation patterns, drought, flooding, salinity and more frequent extreme weather events. Rice production is also closely connected with environmental sustainability because flooded rice systems contribute

substantially to agricultural methane emissions, creating a dual requirement to increase productivity while reducing the environmental footprint of cultivation (Kumar et al., 2023; Rice2035, 2026). Recent perspectives therefore emphasize that future rice improvement must simultaneously address productivity, resilience, resource-use efficiency and environmental sustainability rather than focusing exclusively on yield potential (Rice2035, 2026).

Constraints limiting rice productivity

Despite substantial genetic gains achieved through conventional rice improvement, productivity remains constrained by a complex interaction of biotic, abiotic, genetic and environmental factors. Major biotic constraints include bacterial blight caused by Xanthomonas oryzae pv. oryzae, rice blast caused by Magnaporthe oryzae, sheath blight, viral diseases, insect pests and emerging pathogen populations capable of overcoming existing resistance. Bacterial blight is particularly important because X. oryzae pv. oryzae uses transcription activator-like effectors to induce host SWEET genes, thereby exploiting host susceptibility mechanisms for disease development. This pathogen–host interaction has subsequently become an important demonstration of how genome editing can be used to engineer durable disease resistance by modifying susceptibility-associated regulatory sequences (Oliva et al., 2019).

Abiotic stresses further constrain rice productivity, particularly in environments exposed to drought, salinity, submergence, heat and cold. Among these, temperature stress is becoming increasingly important because elevated temperature during reproductive development can cause pollen sterility, impaired fertilization, reduced grain filling and deterioration of grain quality. Recent research indicates that heat stress above the optimum range can substantially compromise reproductive development, while the complex and polygenic nature of thermotolerance makes conventional improvement relatively slow (2026). Similarly, salinity and drought impose strong limitations on rice production by disrupting water relations, ion homeostasis, photosynthesis and reproductive development. Climate variability can further increase the unpredictability of these stresses by altering their timing, intensity and duration.

Beyond stress tolerance, the challenge is increasingly shifting toward overcoming yield stagnation while simultaneously improving grain quality and nutritional value. Although past breeding achievements produced dramatic increases in rice yield, the rate of yield improvement has slowed considerably, with recent assessments indicating an annual genetic gain of approximately 0.5%, which may be insufficient to satisfy future food requirements (Rice2035, 2026). At the same time, consumer and nutritional demands require improvement in grain appearance, cooking and eating quality, starch composition, aroma, micronutrient concentration and protein quality. Thus, future rice breeding must move beyond single-trait selection toward simultaneous optimization of yield, quality, nutritional value, stress resilience and resource-use efficiency. The genetic complexity of many of these traits, their quantitative inheritance and strong genotype × environment interactions represent major challenges for conventional breeding approaches.

Limitations of conventional rice breeding

Conventional rice breeding has been remarkably successful, but its effectiveness is increasingly challenged when breeders attempt to manipulate complex traits with high precision. Hybridization and recombination breeding generate useful genetic variation by combining parental genomes; however, they involve the segregation of large genomic regions and consequently require multiple generations of selection to recover desirable allele combinations. Undesirable genes closely linked to a target locus may be transferred simultaneously, resulting in linkage drag, while favorable alleles may be difficult to recover in elite genetic backgrounds. Backcross breeding can reduce the proportion of donor genome through successive generations, but the process remains time-consuming and does not provide direct control over the precise nucleotide or regulatory sequence being modified. These limitations become particularly important when a breeder seeks to improve an established elite variety while retaining its favorable genetic background (Solanki et al., 2026).

Mutation breeding, using physical or chemical mutagens, provides an additional source of genetic variation but generates largely random mutations throughout the genome. Identification of desirable mutations therefore requires extensive screening and subsequent genetic characterization. Marker-assisted selection (MAS) has substantially improved the efficiency of breeding by enabling selection of individuals carrying favorable alleles at known loci, but it remains dependent on the availability of informative markers and previously characterized genetic variation. MAS is particularly effective for simply inherited traits, whereas complex quantitative traits controlled by numerous loci can be more difficult to manipulate efficiently. Moreover, marker-assisted introgression generally transfers pre-existing alleles rather than creating precisely designed novel alleles.

Transgenic approaches have expanded the possibilities for crop improvement by allowing the introduction of genes from unrelated organisms; however, their application has been accompanied by regulatory complexity, biosafety considerations, intellectual-property issues and variable public acceptance. More importantly, transgenic approaches do not always provide the most appropriate solution when the desired phenotype can be achieved through modification of an endogenous rice gene. These limitations have stimulated the development of genome-editing technologies capable of producing targeted modifications within the native genome. Recent reviews emphasize that conventional recombination, mutagenesis and marker-assisted approaches remain valuable but are constrained by factors including linkage drag, unintended phenotypic effects and lengthy breeding cycles (Solanki et al., 2026; Chen et al., 2024).

Emergence of genome editing

The development of genome editing represents a major transition from selection-driven breeding toward targeted genetic engineering. Early genome-editing platforms, including zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), demonstrated that specific genomic sequences could be modified through engineered DNA-binding nucleases. However, the design and construction of these systems were relatively laborious and technically demanding. The

subsequent discovery and adaptation of CRISPR-associated systems transformed the field by replacing protein-based target recognition with programmable RNA-guided recognition. In 2013, CRISPR/Cas technology was demonstrated for targeted genome modification in rice, establishing the foundation for its widespread application in plant genome engineering (Shan et al., 2013; Jiang et al., 2013).

The development of CRISPR/Cas9 rapidly established a new paradigm in rice improvement because a programmable single-guide RNA (sgRNA) can direct the Cas nuclease to a selected genomic sequence adjacent to an appropriate protospacer-adjacent motif (PAM). Following target recognition and DNA cleavage, cellular repair pathways can generate insertions or deletions through non-homologous end joining (NHEJ), enabling targeted gene knockout, while homology-directed repair (HDR) provides a route toward more precise sequence modification. Importantly, CRISPR/Cas9 was shown to generate specific and homozygous targeted mutations in rice within a single generation, demonstrating its potential to accelerate functional genomics and breeding compared with conventional multi-generation selection schemes (Zhang et al., 2014).

The CRISPR revolution has subsequently expanded beyond conventional Cas9-mediated knockout. Cas12a and other Cas proteins have broadened the sequence space accessible for genome editing, while base editors have enabled programmable nucleotide conversion without generating conventional double-strand DNA breaks. More recently, prime editing has extended the scope of precision editing by enabling targeted substitutions, insertions and deletions using a Cas nickase–reverse transcriptase system. In rice, prime editing has already demonstrated potential for engineering agronomically important traits, including bacterial-blight resistance through precise modification of susceptibility-associated loci (Xu et al., 2023). Thus, genome editing is evolving from a technology primarily used for gene knockout toward a broader platform for allele engineering, regulatory-sequence modification and quantitative trait manipulation.

Why CRISPR/Cas is transformative for rice improvement

CRISPR/Cas is particularly transformative for rice improvement because it combines programmability, precision, multiplexing capacity and relatively rapid genetic modification within a single technological framework. Unlike conventional mutagenesis, in which desirable mutations must be discovered from a large population, CRISPR enables breeders to directly target a candidate gene or regulatory element based on prior genetic and genomic information. This makes it possible to convert knowledge generated through QTL mapping, GWAS, transcriptomics and functional genomics into experimentally testable and potentially deployable genetic modifications. The rapid development of CRISPR-based rice improvement has consequently strengthened the connection between gene discovery and breeding application (Chen et al., 2024; Gica et al., 2026).

A major advantage is the capacity to modify native genes without necessarily introducing foreign coding sequences. This is particularly important for rice breeding because many agronomically desirable phenotypes can be obtained by knocking out susceptibility genes, altering regulatory elements or modifying endogenous alleles. A notable example is bacterial-blight resistance, where CRISPR/Cas9-mediated editing of the promoter regions of SWEET11, SWEET13 and SWEET14 generated resistance by preventing pathogen transcription activator-like effectors from inducing susceptibility-associated host genes (Oliva et al., 2019). This example illustrates a fundamental conceptual advantage of CRISPR: rather than introducing a foreign resistance gene, genome editing can reprogram the existing host genome to remove or modify a vulnerability.

The multiplexing capability of CRISPR/Cas further increases its relevance to rice improvement. Multiple guide RNAs can be deployed simultaneously to edit several genes or regulatory regions, providing an opportunity to manipulate interconnected components of complex traits such as yield, plant architecture, stress tolerance and disease resistance. This capability is particularly important because many agronomic traits are polygenic and cannot be optimized through modification of a single locus. Consequently, CRISPR is increasingly moving from a simple “one gene–one trait” paradigm toward multiplex and regulatory editing strategies capable of generating allelic series and quantitatively optimized phenotypes.

Another important advantage is speed. Conventional breeding may require several generations of crossing, selection and backcrossing before a desired allele combination is stabilized in an elite background. CRISPR can directly modify a target locus in an elite genotype, potentially reducing the number of generations required to introduce a desired genetic change. This does not eliminate the need for extensive phenotyping, genetic validation and field testing, but it can substantially shorten the discovery-to-breeding pipeline. Recent systematic evidence indicates that CRISPR-mediated NHEJ-based knockout remains considerably more efficient than more demanding precision-editing approaches in rice, while genotype dependence and transformation efficiency remain important practical constraints (Gica et al., 2026).

The technology also provides a route toward allele-specific and precision breeding. Rather than simply eliminating gene function, modern editing systems can modify particular nucleotides, promoter elements or regulatory motifs to reproduce favorable natural alleles or create novel allelic combinations. Base editing and prime editing are particularly important in this context because they expand genome engineering beyond double-strand-break-mediated mutagenesis. Such technologies provide opportunities to fine-tune quantitative traits, modify regulatory networks and develop climate-resilient phenotypes while minimizing unnecessary genomic alterations (Villiger et al., 2024; Gica et al., 2026).

The potential for transgene-free or DNA-free genome editing further enhances the breeding relevance of CRISPR. Editing strategies based on transient delivery of CRISPR ribonucleoproteins or approaches that allow segregation away of editing constructs can produce plants in which the desired genomic modification is retained without stable foreign DNA. This distinction has become particularly relevant to regulatory frameworks. In India, for example, regulatory changes have facilitated evaluation of genome-edited plants generated through SDN-1 and SDN-2 approaches, provided that the resulting plants meet the applicable requirements for absence of foreign DNA (Nature India, 2024). India subsequently approved its first genome-edited rice varieties in 2025, demonstrating that genome editing is moving beyond experimental research

toward practical crop improvement and deployment (Nature India, 2025).

Collectively, these developments position CRISPR/Cas not merely as another molecular breeding tool but as a platform connecting functional genomics with precision crop improvement. The trajectory from ZFNs and TALENs to CRISPR/Cas9, Cas12a, base editing, prime editing and increasingly sophisticated multiplex and regulatory editing systems reflects a broader transition toward designing useful genetic variation rather than relying exclusively on naturally occurring or randomly induced variation. For rice, this transition is particularly significant because the crop must simultaneously deliver higher yield, improved grain quality and nutrition, resistance to evolving pathogens, tolerance to multiple environmental stresses and greater resource-use efficiency. Recent reviews therefore increasingly frame genome editing as a central component of next-generation, climate-resilient rice breeding rather than an isolated gene-engineering technology (Chen et al., 2024; Solanki et al., 2026; Gica et al., 2026).

CRISPR/Cas Genome Editing: Molecular Basis and Evolution

The development of CRISPR/Cas technology represents one of the most consequential advances in molecular genetics and plant biotechnology, transforming genome manipulation from a technically demanding process into a programmable and increasingly precise approach. Unlike earlier genome-editing platforms, in which DNA-binding specificity depended largely on engineered proteins, CRISPR systems use guide RNA molecules to direct Cas nucleases toward complementary DNA sequences. This RNA-guided architecture provides substantial flexibility in target selection and has enabled the development of increasingly sophisticated platforms for gene knockout, nucleotide substitution, targeted insertion, regulatory-sequence modification and transcriptional or epigenetic manipulation. In rice, CRISPR-based editing has consequently evolved from a functional-genomics tool into an important component of precision breeding (Jinek et al., 2012; Shan et al., 2013; Chen et al., 2024).

CRISPR adaptive immunity

CRISPR, or clustered regularly interspaced short palindromic repeats, originated as an adaptive immune system in bacteria and archaea that protects against invading genetic elements such as bacteriophages and plasmids. The system consists of a CRISPR array containing repeated DNA sequences separated by short, unique spacer sequences derived from previously encountered foreign DNA. During the initial stage of immunity, fragments of invading DNA are captured and incorporated as new spacers within the CRISPR array, thereby creating a molecular record of previous infections. This process, termed spacer acquisition or adaptation, is primarily mediated by Cas1 and Cas2 proteins and provides the genetic memory required for subsequent defense (Barrangou et al., 2007; Makarova et al., 2020).

During the expression stage, the CRISPR array is transcribed into a long precursor RNA that is processed into individual CRISPR RNAs (crRNAs). In the widely used type II CRISPR system, characteristic of Streptococcus pyogenes Cas9, the crRNA associates with a trans-activating CRISPR RNA (tracrRNA) and Cas9 protein to form the RNA-guided surveillance complex. The crRNA provides sequence specificity through complementary base pairing with the target DNA, whereas the tracrRNA contributes to Cas9 recruitment and guide-RNA maturation. For genome-editing applications, crRNA and tracrRNA are commonly fused into a single single-guide RNA (sgRNA), substantially simplifying the engineering of the system (Jinek et al., 2012).

Target recognition involves two major requirements: guide–target complementarity and recognition of an appropriate protospacer-adjacent motif (PAM). The PAM is a short DNA sequence adjacent to the target region that is recognized by the Cas protein and is essential for productive target binding and cleavage. For the commonly used S. pyogenes Cas9, the canonical PAM is 5′-NGG-3′. Once the Cas protein recognizes the PAM, the guide RNA interrogates the adjacent DNA sequence through complementary base pairing. This combination of PAM recognition and RNA–DNA hybridization provides the molecular basis for programmable genome targeting (Jinek et al., 2012; Doudna & Charpentier, 2014).

The natural CRISPR immune system therefore follows a conceptual sequence of adaptation → CRISPR-array expression → target recognition → interference. Genome editing essentially repurposes this biological defense mechanism by replacing the naturally acquired guide sequence with an experimentally designed guide RNA targeting a selected genomic locus. This conversion of a microbial immune mechanism into a programmable genome-engineering platform represents the fundamental conceptual breakthrough underlying modern CRISPR technology.

CRISPR/Cas9 mechanism

The CRISPR/Cas9 genome-editing system can be simplified as:

In the engineered system, the sgRNA–Cas9 complex first scans genomic DNA for a compatible PAM. Once the PAM is recognized, the spacer region of the sgRNA pairs with the complementary target DNA strand, producing an RNA–DNA hybrid. Cas9 then undergoes conformational activation and uses its two nuclease domains, HNH and RuvC, to cleave the complementary and non-complementary DNA strands, respectively. The resulting double-strand break (DSB) is subsequently repaired by endogenous cellular DNA-repair pathways, and it is this repair process that ultimately determines the nature of the genome-editing outcome (Jinek et al., 2012; Doudna & Charpentier, 2014).

NHEJ-mediated genome editing

The predominant repair pathway following Cas9-mediated DSB formation is **non-homologous end joining (NHEJ)**. Because NHEJ does not require a homologous repair template, repair can introduce small insertions or deletions (**indels**) at the cleavage site. These mutations may disrupt the reading frame or essential regulatory sequences and consequently lead to loss of gene function. NHEJ therefore forms the molecular basis of the widely used **CRISPR-mediated gene knockout strategy**. In rice, this approach has been extensively used to investigate gene function and generate improved alleles for traits such as plant architecture, yield components, grain quality and stress responses (Shan et al., 2013; Chen et al., 2024).

The simplicity of NHEJ-mediated knockout is one of the reasons CRISPR/Cas9 became rapidly adopted in plant breeding. However, conventional knockout does not necessarily provide control over the exact nucleotide change produced. Consequently, newer editing technologies such as base editing and prime editing have been developed to move beyond random indel formation toward more predictable sequence modification.

HDR-mediated precise genome editing

Homology-directed repair (HDR) provides an alternative route in which the DSB is repaired using a homologous DNA template. By supplying a donor template containing the desired sequence flanked by homologous regions, researchers can theoretically introduce specific nucleotide substitutions, insertions or sequence replacements. Thus, whereas NHEJ is primarily exploited for gene disruption, HDR can facilitate precise gene insertion, allele replacement and targeted sequence modification.

Despite its conceptual precision, HDR generally occurs at lower efficiency than NHEJ in many plant systems, including rice, and can be strongly influenced by developmental stage, genotype and cellular context. This limitation has stimulated the development of base editors and prime editors, which can introduce defined genetic changes without relying on conventional DSB-induced HDR. Consequently, the evolution of CRISPR technology increasingly reflects a shift from simply breaking DNA toward controlling the precise molecular outcome of genome repair.

Evolution of CRISPR systems used in plants

The evolution of CRISPR-based genome editing can be viewed as a progression from **gene disruption toward increasingly precise and programmable genome engineering**.

Cas9: the foundation of plant genome editing

The demonstration of CRISPR/Cas9-mediated genome editing in plants, including rice, established RNA-guided nuclease technology as a practical alternative to ZFNs and TALENs. Cas9 rapidly became the dominant platform because guide RNAs are relatively easy to redesign, allowing multiple genomic loci to be targeted without engineering a new DNA-binding protein for every locus (Shan et al., 2013). Early applications primarily focused on NHEJ-mediated gene knockout and functional validation.

Cas12a: expanding targetable sequence space

The subsequent adoption of Cas12a (Cpf1) broadened the CRISPR toolbox. Cas12a differs from Cas9 in PAM preference, guide-RNA architecture and cleavage characteristics. Its T-rich PAM requirement can provide access to genomic regions that may be difficult to target with SpCas9. Cas12a also generates staggered DNA ends and has intrinsic properties that make it attractive for multiplex genome engineering. These characteristics have expanded the sequence space available for genome editing in crops, including rice (Zetsche et al., 2015).

dCas9: from DNA cleavage to gene regulation

A major conceptual transition occurred with the development of catalytically inactive Cas9 (dCas9). Mutations in the nuclease domains of Cas9 eliminate DNA-cleavage activity while retaining guide-RNA-directed DNA recognition. By fusing dCas9 with transcriptional activators or repressors, researchers developed CRISPR activation (CRISPRa) and CRISPR interference (CRISPRi) systems. These approaches enable gene expression to be increased or suppressed without permanently changing the underlying DNA sequence and are particularly relevant for quantitative traits where complete gene knockout may be undesirable.

Delivery Systems for CRISPR Editing in Rice

Efficient delivery of CRISPR/Cas components into rice cells is a critical determinant of genome-editing success because the editing machinery must reach the appropriate cellular compartment while maintaining cell viability and regeneration capacity. Unlike the relatively straightforward design of guide RNAs, delivery remains strongly influenced by rice genotype, explant type, tissue-culture response and the molecular form of the editing reagents. Current approaches range from conventional **Agrobacterium-mediated transformation and particle bombardment** to emerging DNA-free, transient and nanoparticle-based systems. The choice of delivery platform ultimately affects editing efficiency, regeneration, transgene integration, scalability and the feasibility of obtaining transgene-free edited plants (Chen et al., 2024; Mahfouz et al., 2025).

Agrobacterium-mediated transformation

Agrobacterium tumefaciens-mediated transformation remains one of the most widely used approaches for introducing CRISPR/Cas constructs into rice. Typically, *Agrobacterium* transfers a T-DNA carrying the Cas nuclease, guide RNA and selectable marker into rice embryogenic tissues, particularly callus derived from immature embryos. The method offers relatively efficient and stable transformation and is compatible with multiplex CRISPR constructs. However, stable T-DNA integration can complicate the generation of transgene-free edited plants and requires subsequent segregation or molecular screening to remove the editing construct (Shan et al., 2013; Chen et al., 2024).

Particle bombardment

Particle bombardment (biolistic transformation) uses high-velocity microscopic particles coated with DNA, RNA or other editing components to introduce them directly into plant cells. Its major advantage is its broad genotype applicability and independence from Agrobacterium host interactions. However, random DNA integration, tissue damage, relatively high equipment requirements and variable transformation efficiency can limit its routine application. Nevertheless, biolistics remains useful for rice genotypes that are difficult to transform through Agrobacterium and for delivery of large or multiple genetic constructs.

Protoplast-mediated transformation

Protoplast transformation involves enzymatic removal of the cell wall followed by delivery of CRISPR components through

polyethylene glycol (PEG), electroporation or related methods. Because the cell wall is absent, DNA, RNA and CRISPR ribonucleoproteins can be introduced efficiently into individual cells. This approach is particularly attractive for transient and DNA-free editing, but its practical application in rice is constrained by genotype-dependent protoplast regeneration and the difficulty of regenerating fertile plants from edited single cells. Consequently, efficient regeneration protocols remain a major bottleneck for translating protoplast editing into breeding pipelines.

Ribonucleoprotein (RNP) delivery

Delivery of preassembled Cas protein–sgRNA ribonucleoprotein complexes (RNPs) represents an important step toward transgene-free genome editing. Because the editing machinery is introduced directly as a protein–RNA complex, editing can occur transiently without stable incorporation of CRISPR DNA into the genome. RNP delivery therefore reduces the persistence of editing reagents and can minimize concerns associated with random transgene integration and prolonged nuclease expression. Its major limitations include the stability and cellular delivery of RNPs, efficient plant regeneration and the requirement for specialized delivery methods (Woo et al., 2015; Chen et al., 2024).

Viral delivery

Plant viral vectors provide an alternative strategy for delivering guide RNAs or CRISPR components because viruses naturally possess mechanisms for entering plant cells and spreading systemically. Viral systems can potentially overcome some tissue-delivery barriers and enable transient genome-editing activity. However, limitations related to viral cargo capacity, host range, vector stability, regeneration of edited plants and biosafety currently restrict their routine application in rice. Their greatest future potential may lie in high-throughput or tissue-specific delivery of editing components rather than permanent transformation.

Nanoparticle-mediated delivery

Nanotechnology is emerging as a promising strategy for overcoming biological barriers associated with conventional transformation. Carbon-based nanomaterials, including carbon nanotubes and related nanocarriers, can facilitate cellular uptake of nucleic acids or protein–RNA complexes and potentially enable delivery without conventional tissue culture. Similarly, lipid nanoparticles (LNPs) can encapsulate and protect RNA or protein–RNA cargos and have attracted considerable interest because of their successful use in other biological systems. For crop genome editing, however, nanoparticle-mediated delivery remains an emerging technology, with major challenges involving tissue penetration, cargo release, regeneration and reproducible editing efficiency. Future integration of nanocarriers with DNA-free CRISPR/RNP systems could provide a route toward minimally invasive, genotype-flexible and transgene-free genome editing in rice (Demirer et al., 2019; Mahfouz et al., 2025).

APPLICATION IN RICE

Yield improvement

CRISPR/Cas-mediated editing offers opportunities to improve rice yield by modifying genes regulating tillering, panicle architecture, grain number, grain size, plant height and source–sink relationships. Important targets include Gn1a/OsCKX2, DEP1, IPA1/OsSPL14, GS3, GW2 and TGW6, which influence major yield components.

Plant architecture

Genome editing can optimize plant architecture through targeted modification of genes controlling plant height, tillering, panicle branching and culm strength. The primary objective is to develop an ideal plant type combining semi-dwarfism, lodging resistance, optimized tillering and improved light interception.

Grain size and yield components

CRISPR/Cas technology enables targeted modification of grain-development genes to improve grain dimensions and thousand-grain weight. Major candidate genes include *GS3* (grain length), *GW2* and *GW5* (grain width), *TGW6* (grain weight), *GS5* and *GL3.1* (grain development).

Grain quality

Genome editing provides opportunities to improve amylose content, starch composition, gel consistency, protein quality, aroma and cooking characteristics. Important targets include *Wx* for amylose biosynthesis and *BADH2* for fragrance, along with other genes involved in starch metabolism and grain development.

Bacterial disease resistance

CRISPR/Cas-mediated editing of host susceptibility genes provides an effective strategy for bacterial-blight resistance caused by *Xanthomonas oryzae* pv. *oryzae*. Major targets include *SWEET11a, SWEET13, SWEET14* and *TFIIAγ*. Editing pathogen-responsive SWEET promoter elements can prevent pathogen-induced gene expression and enhance host resistance (Oliva et al., 2019).

Fungal disease resistance

Genome editing offers opportunities to enhance resistance against rice blast, sheath blight, brown spot and false smut through modification of susceptibility genes and defense-signaling pathways. Important targets include *Pi21, Bsr-d1* and *OsERF922*, particularly for improving blast resistance.

Abiotic stress tolerance

CRISPR/Cas technology can improve tolerance to drought, salinity, heat and cold by modifying genes associated with ABA signaling, root development, osmotic adjustment, ion homeostasis, ROS regulation and stress-responsive transcription factors. Such modifications aim to improve rice adaptation under changing climatic conditions.

Nutritional biofortification

Genome editing provides opportunities to enhance the nutritional value of rice through improved iron, zinc, vitamin and protein accumulation, modification of amino-acid composition and reduction of anti-nutritional factors. Targeting mineral transporters, chelation pathways and nutrient-storage genes represents an emerging approach to CRISPR-enabled nutritional precision breeding.

Hybrid rice improvement

CRISPR/Cas technology can facilitate hybrid rice development through targeted modification of male-sterility genes, fertility-restoration pathways and flowering-related genes. Important targets include TMS5, OsNP1 and CSA, which contribute to reproductive development and male fertility regulation.

Flowering time and photoperiod adaptation

Genome editing of flowering-time regulators, including *Hd1, Ehd1, Ghd7, DTH8* and OsPRR37, offers opportunities to manipulate heading date and photoperiod sensitivity. These modifications can facilitate the adaptation of elite rice cultivars to different geographical regions and cropping seasons.

Root system architecture

CRISPR-mediated modification of root-development genes can optimize root depth, branching, angle and biomass, thereby improving water acquisition, nutrient absorption and drought adaptation. Improved root architecture also contributes to efficient resource utilization and overall plant performance.

Nutrient-use efficiency

Genome editing can improve nitrogen-, phosphorus- and potassium-use efficiency by modifying genes involved in nutrient uptake, transport, assimilation and remobilization. These approaches aim to develop rice cultivars with higher nutrient-use efficiency, reduced fertilizer requirements and improved sustainability of rice production.

Table 1. Representative case studies of CRISPR/Cas-mediated genome editing for rice improvement

No.	Application	Target gene(s)	CRISPR/Cas editing approach	Major outcome	Reference
1	Grain number	*Gn1a*	CRISPR/Cas9 knockout	Increased grain number per panicle	Li et al. (2016)
2	Panicle architecture	*DEP1*	CRISPR/Cas9 knockout	Dense and erect panicles; altered plant architecture	Li et al. (2016)
3	Grain length	*GS3*	CRISPR/Cas9 knockout	Increased grain length	Li et al. (2016)
4	Plant architecture	*IPA1/OsSPL14*	CRISPR/Cas9 editing	Modification of tillering and plant architecture	Li et al. (2016)
5	Grain weight	*GW2, GW5, TGW6*	Multiplex CRISPR/Cas9	Simultaneous modification of grain dimensions and weight	Xu et al. (2016)
6	Tillering	*CCD7*	CRISPR/Cas9 knockout	Increased tiller production	Butt et al. (2018)
7	Plant growth	*PYL* gene family	Multiplex CRISPR/Cas9	Modification of ABA signalling and growth-related traits	Miao et al. (2018)
8	Panicle development	*OsPIN5b*	CRISPR/Cas9 knockout	Increased panicle length	Zhang et al. (2018)
9	Amylose content	*Wx*	CRISPR/Cas9 knockout	Development of low-amylose/glutinous rice	Zhang et al. (2018)
10	Aroma improvement	*BADH2*	CRISPR/Cas9 knockout	Enhanced accumulation of 2-acetyl-1-pyrroline	Shao et al. (2017)
11	Starch quality	*SBEIIb*	CRISPR/Cas9 knockout	Altered starch composition and increased amylose	Sun et al. (2017)
12	Bacterial blight resistance	*SWEET11, SWEET13, SWEET14*	Multiplex promoter editing	Broad-spectrum bacterial-blight resistance	Oliva et al. (2019)
13	Bacterial blight resistance	*SWEET11/Xa13*	CRISPR/Cas9 knockout	Reduced pathogen-induced susceptibility	Eom et al. (2019)

14	Rice blast resistance	*Pi21*	CRISPR/Cas9 knockout	Enhanced resistance against rice blast	Tao et al. (2021)
15	Multiple disease resistance	*Pi21, Bsr-d1, Xa5*	Multiplex CRISPR/Cas9	Enhanced resistance against blast and bacterial blight	Tao et al. (2021)
16	Multiple disease resistance	*Bsr-d1, Pi21, ERF922*	Multiplex CRISPR/Cas9	Enhanced resistance against blast and bacterial blight	Zhou et al. (2022)
17	Salinity tolerance	*OsRR22*	CRISPR/Cas9 knockout	Improved salt tolerance	Zhang et al. (2019)
18	Drought and salinity tolerance	*OsDST*	CRISPR/Cas9 knockout	Improved water retention and stress tolerance	Santosh Kumar et al. (2020)
19	Drought tolerance	*OsPYL9*	CRISPR/Cas9 editing	Modification of ABA signalling and drought responses	Usman et al. (2020)
20	Drought tolerance	*OsERA1*	CRISPR/Cas9 knockout	Enhanced ABA sensitivity and drought responses	Ogata et al. (2020)
21	Drought tolerance	*OsABA8ox2*	CRISPR/Cas9 knockout	Improved drought tolerance through ABA regulation	Zhang et al. (2020)
22	Multiple stress tolerance	*OsmiR535*	CRISPR/Cas9 editing	Improved responses to cold, drought and salinity	Yue et al. (2020)
23	Cold tolerance	*OsMYB30*	CRISPR/Cas9 knockout	Modification of cold-stress responses	Zeng et al. (2020)
24	Heat tolerance	*TT2, SCT1/SCT2*	CRISPR/Cas9 editing	Modification of heat-stress responses	Kan et al. (2022)
25	Male sterility	*TMS5*	CRISPR/Cas9 knockout	Development of thermosensitive genic male-sterile rice	Li et al. (2019)
26	Male sterility	*CSA*	CRISPR/Cas9 knockout	Development of photoperiod-sensitive male-sterile rice	Li et al. (2016)
27	Nutritional biofortification	*OsNAS2* promoter	CRISPR/Cas9 promoter editing	Increased zinc accumulation and improved plant performance	Ludwig et al. (2024)
28	Grain quality	*Wx, BADH2*	Multiplex CRISPR/Cas9	Development of aromatic and glutinous rice	Li et al. (2023)
29	Nitrogen-use efficiency	*OsNRT1.1B*	CRISPR-based base editing	Modification of nitrate transport and nitrogen-use efficiency	Lu et al. (2018)
30	Bacterial blight resistance	*SWEET14* promoter	Cytosine and adenine base editing	Modification of pathogen effector-binding elements and resistance to African Xoo isolates	2025 study, *The Crop Journal*

CONCLUSION

CRISPR/Cas-mediated genome editing has emerged as a powerful and versatile approach for accelerating rice improvement by enabling precise, targeted and rapid modification of genes associated with yield, grain quality, disease resistance and abiotic stress tolerance. The development of advanced editing technologies, including multiplex CRISPR systems, base editing and prime editing, has further expanded the possibilities of modifying complex traits and developing improved rice varieties. Successful editing of important genes and regulatory elements demonstrates the potential of this technology to overcome several limitations of conventional breeding and conventional genetic transformation approaches.

Despite significant progress, challenges related to efficient delivery, genotype dependency, off-target mutations, regeneration efficiency, complex trait inheritance and regulatory acceptance remain important barriers to its widespread application.

Future research should focus on integrating CRISPR/Cas technology with genomics, QTL mapping, GWAS, genomic selection, pangenomics and high-throughput phenotyping to improve the precision and efficiency of rice breeding. Furthermore, the identification of novel gene targets, development of transgene-free edited lines and validation of promising genotypes under diverse field environments will be essential for translating laboratory advances into practical breeding outcomes.

REFERENCES

1. Ashokkumar, S., Jaganathan, D., Ramanathan, V., Rahman, H., Palaniswamy, R., Kambale, R., & Muthurajan, R. (2020). Creation of novel alleles of fragrance gene *OsBADH2* in rice through CRISPR/Cas9-mediated gene editing. *PLOS ONE, 15*(8), e0237018. https://doi.org/10.1371/journal.pone.0237018
2. Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., Romero, D. A., & Horvath, P. (2007). CRISPR provides acquired resistance against viruses in prokaryotes. *Science, 315*(5819), 1709–1712. https://doi.org/10.1126/science.1138140
3. Bortesi, L., & Fischer, R. (2015). The CRISPR/Cas9 system for plant genome editing and beyond. *Biotechnology Advances, 33*(1), 41–52. https://doi.org/10.1016/j.biotechadv.2014.12.006
4. Chen, K., Wang, Y., Zhang, R., Zhang, H., & Gao, C. (2019). CRISPR/Cas genome editing and precision plant breeding in agriculture. *Annual Review of Plant Biology, 70*, 667–697. https://doi.org/10.1146/annurev-arplant-050718-100049
5. Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science, 346*(6213), 1258096. https://doi.org/10.1126/science.1258096
6. Fukagawa, N. K., & Ziska, L. H. (2019). Rice: Importance for global nutrition. *Journal of Nutritional Science and Vitaminology, 65*(Supplement), S2–S3. https://doi.org/10.3177/jnsv.65.S2
7. Hua, K., Jiang, Y., Tao, X., & Zhu, J.-K. (2020). Precision genome engineering in rice using prime editing system. *Plant Biotechnology Journal, 18*(11), 2167–2169. https://doi.org/10.1111/pbi.13395
8. Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science, 337*(6096), 816–821. https://doi.org/10.1126/science.1225829
9. Khush, G. S. (2005). What it will take to feed 5.0 billion rice consumers in 2030. *Plant Molecular Biology, 59*, 1–6. https://doi.org/10.1007/s11103-005-2159-5
10. Li, J., Sun, Y., Du, J., Zhao, Y., & Xia, L. (2017). Generation of targeted point mutations in rice by a modified CRISPR/Cas9 system. *Molecular Plant, 10*, 526–529. https://doi.org/10.1016/j.molp.2016.12.001
11. Li, M., Li, X., Zhou, Z., Wu, P., Fang, M., Pan, X., Lin, Q., Luo, W., Wu, G., & Li, H. (2016). Reassessment of the four yield-related genes *Gn1a, DEP1, GS3,* and *IPA1* in rice using a CRISPR/Cas9 system. *Frontiers in Plant Science, 7*, 377. https://doi.org/10.3389/fpls.2016.00377
12. Lin, Q., Zong, Y., Xue, C., Wang, S., Jin, S., Zhu, Z., Wang, Y., Anzalone, A. V., Raguram, A., Doman, J. L., Liu, D. R., & Gao, C. (2020). Prime genome editing in rice and wheat. *Nature Biotechnology, 38*, 582–585. https://doi.org/10.1038/s41587-020-0455-x
13. Lu, Y., & Zhu, J.-K. (2017). Precise editing of a target base in the rice genome using a modified CRISPR/Cas9 system. *Molecular Plant, 10*, 523–525. https://doi.org/10.1016/j.molp.2016.11.013
14. Makarova, K. S., Wolf, Y. I., Iranzo, J., Shmakov, S. A., Alkhnbashi, O. S., Brouns, S. J. J., Charpentier, E., Cheng, D., Haft, D. H., Horvath, P., Moineau, S., Mojica, F. J. M., Scott, D., Shah, S. A., Siksnys, V., Terns, M. P., Venclovas, Č., White, M. F., Yakunin, A. F., ... Koonin, E. V. (2020). Evolutionary classification of CRISPR–Cas systems: A burst of class 2 and derived variants. *Nature Reviews Microbiology, 18*, 67–83. https://doi.org/10.1038/s41579-019-0299-x
15. Mishra, R., Joshi, R. K., & Zhao, K. (2018). Genome editing in rice: Recent advances, challenges, and future implications. *Frontiers in Plant Science, 9*, 1361. https://doi.org/10.3389/fpls.2018.01361
16. Oliva, R., et al. (2019). Broad-spectrum resistance to bacterial blight in rice using genome editing. *Nature Biotechnology, 37*, 1344–1350. https://doi.org/10.1038/s41587-019-0267-z
17. Rengasamy, B., Manna, M., Thajuddin, N. B., Sathiyabama, M., & Sinha, A. K. (2024). Breeding rice for yield improvement through CRISPR/Cas9 genome editing method: Current technologies and examples. *Physiology and Molecular Biology of Plants, 30*(2), 185–198. https://doi.org/10.1007/s12298-024-01423-y
18. Romero, F. M., & Gatica-Arias, A. (2019). CRISPR/Cas9: Development and application in rice breeding. *Rice Science, 26*(5), 265–281. https://doi.org/10.1016/j.rsci.2019.08.001
19. Shan, Q., Wang, Y., Li, J., Zhang, Y., Chen, K., Liang, Z., Zhang, K., Liu, J., Xi, J. J., Qiu, J.-L., & Gao, C. (2013). Targeted genome modification of crop plants using a CRISPR-Cas system. *Nature Biotechnology, 31*, 686–688. https://doi.org/10.1038/nbt.2650
20. Shimatani, Z., Kashojiya, S., Takayama, M., Terada, R., Arazoe, T., Ishii, H., Teramura, H., Yamamoto, T., Komatsu, H., Miura, K., Ezura, H., Nishida, K., Ariizumi, T., & Kondo, A. (2017). Targeted base editing in rice and tomato using a CRISPR-Cas9 cytidine deaminase fusion. *Nature Biotechnology, 35*, 441–443. https://doi.org/10.1038/nbt.3833
21. Tabassum, J., Ahmad, S., Hussain, B., Mawia, A. M., Zeb, A., & Ju, L. (2021). Applications and potential of genome-editing systems in rice improvement: Current and future perspectives. *Agronomy, 11*(7), 1359. https://doi.org/10.3390/agronomy11071359
22. Thiruppathi, A., Salunkhe, S. R., Ramasamy, S. P., Palaniswamy, R., Rajagopalan, V. R., Rathnasamy, S. A.,

Alagarswamy, S., Swaminathan, M., Manickam, S., & Muthurajan, R. (2024). Unleashing the potential of CRISPR/Cas9 genome editing for yield-related traits in rice. *Plants, 13*(21), 2972. https://doi.org/10.3390/plants13212972
23. Wang, M., Mao, Y., Lu, Y., Tao, X., & Zhu, J.-K. (2017). Multiplex gene editing in rice using the CRISPR-Cpf1 system. *Molecular Plant, 10*(7), 1011–1013. https://doi.org/10.1016/j.molp.2017.03.001
24. Wang, M., Xu, Z., Gosavi, G., Ren, B., Cao, Y., Kuang, Y., Zhou, C., Spetz, C., Yan, F., Zhou, X., & Zhou, H. (2020). Targeted base editing in rice with CRISPR/ScCas9 system. *Plant Biotechnology Journal, 18*(8), 1645–1647. https://doi.org/10.1111/pbi.13330
25. Woo, J. W., Kim, J., Kwon, S. I., Corvalán, C., Cho, S. W., Kim, H., Kim, S.-G., Kim, S.-T., Choe, S., & Kim, J.-S. (2015). DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoproteins. *Nature Biotechnology, 33*, 1162–1164. https://doi.org/10.1038/nbt.3389
26. Xie, K., Minkenberg, B., & Yang, Y. (2015). Boosting CRISPR/Cas9 multiplex editing capability with the endogenous tRNA-processing system. *Proceedings of the National Academy of Sciences, 112*(11), 3570–3575. https://doi.org/10.1073/pnas.1420294112
27. Xu, R., Li, J., Liu, X., Shan, T., Qin, R., & Wei, P. (2020). Development of plant prime-editing systems for precise genome editing. *Plant Communications, 1*(3), 100043. https://doi.org/10.1016/j.xplc.2020.100043
28. Xu, R., Yang, Y., Qin, R., Li, H., Qiu, C., Li, L., Wei, P., & Yang, J. (2016). Rapid improvement of grain weight via highly efficient CRISPR/Cas9-mediated multiplex genome editing in rice. *Journal of Genetics and Genomics, 43*(8), 529–532. https://doi.org/10.1016/j.jgg.2016.07.003
29. Xu, Y., Lin, Q., Li, X., Wang, F., Chen, Z., Wang, J., Li, W., Fan, F., Tao, Y., Jiang, Y., & colleagues. (2020). Fine-tuning the amylose content of rice by precise base editing of the *Wx* gene. *Plant Biotechnology Journal*.
30. Zetsche, B., Gootenberg, J. S., Abudayyeh, O. O., Slaymaker, I. M., Makarova, K. S., Essletzbichler, P., Volz, S. E., Joung, J., van der Oost, J., Regev, A., Koonin, E. V., & Zhang, F. (2015). Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell, 163*(3), 759–771. https://doi.org/10.1016/j.cell.2015.09.038
31. Zhang, A., Liu, Y., Wang, F., Li, T., Chen, Z., Kong, D., Bi, J., Zhang, F., Luo, X., Wang, J., Tang, J., Yu, X., Liu, G., & Luo, L. (2019). Enhanced rice salinity tolerance via CRISPR/Cas9-targeted mutagenesis of the *OsRR22* gene. *Molecular Breeding, 39*, 47. https://doi.org/10.1007/s11032-019-0954-y
32. Zhang, C., Yun, P., Xia, J., Zhou, K., Wang, L., Zhang, J., Zhao, B., Yin, D., Fu, Z., Wang, Y., Ma, T., Li, Z., & Wu, D. (2023). CRISPR/Cas9-mediated editing of *Wx* and *BADH2* genes created glutinous and aromatic two-line hybrid rice. *Molecular Breeding, 43*, 24. https://doi.org/10.1007/s11032-023-01368-2
33. Zhang, H., Zhang, J., Wei, P., Zhang, B., Gou, F., Feng, Z., Mao, Y., Yang, L., Zhang, H., Xu, N., & Zhu, J.-K. (2014). The CRISPR/Cas9 system produces specific and homozygous targeted gene editing in rice in one generation. *Plant Biotechnology Journal, 12*(6), 797–807. https://doi.org/10.1111/pbi.12200
34. Zhang, J., Zhang, H., Botella, J. R., & Zhu, J.-K. (2018). Generation of new glutinous rice by CRISPR/Cas9-targeted mutagenesis of the *Waxy* gene in elite rice varieties. *Journal of Integrative Plant Biology, 60*(5), 369–375. https://doi.org/10.1111/jipb.12620
35. Zhou, X., et al. (2022). Engineering of rice varieties with enhanced resistances to both blast and bacterial blight diseases via CRISPR/Cas9. *Plant Biotechnology Journal, 20*, 876–885. https://doi.org/10.1111/pbi.13766