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MOLECULAR GENETICS AND GENOME EDITING IN RICE (ORYZA SATIVA L.): INTEGRATING GENE DISCOVERY, FUNCTIONAL GENOMICS AND CRISPR-BASED PRECISION BREEDING

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ABSTRACT
Rice is a major crop for which productivity and quality are being challenged by climate change, the development of new biotic stressors and pests, and the need for sustainable resource use. Through advances in molecular genetics and genomics, there may be greater opportunities to identify key genes and alleles controlling this large range of important agronomic traits. This review summarizes the potential pathways through which genomic resources, gene discovery, functional genomics and genome editing (based on CRISPR technology) can be integrated to accelerate rice improvement. Using examples, the role of reference genomes, pan-genomes, genome resequencing, genome-wide association studies, quantitative trait loci mapping, and genomic selection in the discovery of candidate loci for yield, plant architecture, stress tolerance, disease resistance, and grain quality are discussed. In addition, principles in using transcriptomics, reverse genetics, gene-regulatory networks and multi-omics to prioritize candidates and infer gene function are considered, as well as the latest approaches in genome-editing: CRISPR/Cas9, Cas12a, base editing, prime editing, multiplex editing, and promoter editing for yield, stress, and quality enhancements. Although most advances have been made, translation into the field is still predicted to be limited by genotype-dependent transformation and regeneration, off-target effects, editing efficiency, background effects, pleiotropy, and validation on a large scale in the field. An integrated gene-to-cultivar framework for linking genomic discovery and multi-omics evidence with functional validation, specific genome modification, molecular characterization, and multi-environmental field evaluation is proposed. Combining such methods with traditional breeding and genomic selection may help to develop productive, resilient, and high-quality rice cultivars adapted to various target environments and farmer and consumer priorities.

KEYWORDS: Rice; Molecular genetics; Functional genomics; CRISPR/Cas genome editing; Multi-omics; Precision breeding; Stress tolerance.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the world's most important cereal crops and a principal source of dietary energy for a substantial proportion of the global population. Sustaining rice productivity is essential for food security, particularly in regions where population growth, limited natural resources and climate variability place increasing pressure on agricultural systems. Conventional rice improvement has contributed substantially to the development of high-yielding, disease-resistant and regionally adapted cultivars. Nevertheless, the complex inheritance of many agronomic traits and interactions between genetic background and environmental conditions continue to complicate crop improvement. Molecular genetics and genomic technologies provide opportunities to address these limitations by connecting genetic variation with biological function and breeding outcomes (Moin *et al*., 2018; Wei *et al*., 2021).

The increasing availability of genomic information has transformed the investigation of genetic diversity and agronomic performance in rice. Genome-wide association studies, quantitative trait locus mapping and large-scale genome resequencing provide complementary strategies for identifying genomic regions associated with yield, plant architecture, flowering time

and other economically important characteristics. Wei *et al*. (2021) developed a quantitative genomics map of rice that integrated genetic association information to provide insights into the genetic basis of agronomic traits and support breeding applications. Such approaches can help identify candidate loci and favourable alleles for crop improvement. However, a statistically significant association does not necessarily establish that a particular variant is responsible for the observed phenotype. Linkage disequilibrium, population structure and interactions among loci can complicate causal-gene identification, making functional validation an essential component of the gene-discovery process (Moin *et al*., 2018; Wei *et al*., 2021).

Functional genomics provides the experimental framework for determining how candidate genes contribute to biological processes and agronomic traits. Transcriptome profiling can identify genes whose expression changes during development or in response to environmental stress, whereas reverse-genetic approaches can investigate their functions through targeted disruption, overexpression or other gene-manipulation strategies. In rice, CRISPR/Cas9-mediated mutagenesis has become an important approach for generating targeted mutations and examining gene function. Moin *et al*. (2018) reviewed CRISPR/Cas9 and other reverse-genetic methods for functional genomic research in rice, highlighting the importance of mutant resources, gene characterization and transformation systems. Nevertheless, changes in gene expression alone cannot establish causality, and mutant phenotypes must be interpreted in the context of genetic background, developmental stage and environmental conditions. Combining molecular evidence with reproducible phenotypic validation therefore provides a stronger basis for identifying genes with genuine breeding value (Moin *et al*., 2018).

The development of CRISPR-associated genome-editing systems has strengthened the connection between functional genomics and crop improvement. Depending on the system and experimental design, genome editing can introduce targeted insertions or deletions, disrupt gene function, alter selected nucleotides or modify regulatory sequences. These approaches offer opportunities to investigate gene function and develop useful alleles without relying exclusively on conventional mutagenesis. Lin *et al*. (2020) demonstrated prime genome editing in rice and wheat, establishing a route for introducing precise sequence changes in cereal genomes. Wang *et al*. (2020); Meena *et al*. (2026) also demonstrated predictable multi-nucleotide deletions in rice and wheat using an APOBEC–Cas9 system. Together, these studies illustrate how genome-editing technologies can expand the range of genetic modifications available for crop improvement, although editing efficiency and the types of changes achievable depend on the biological system and experimental conditions.

Despite these advances, translating genomic discoveries into improved rice cultivars remains a multistage process. Candidate genes must be prioritized using genetic, molecular and physiological evidence; their functions must be experimentally established; and suitable editing strategies must be selected according to the intended biological outcome. For example, gene knockout may be appropriate when loss of function produces a favourable phenotype, whereas precise nucleotide replacement or modification of regulatory sequences may be preferable when complete gene disruption would be undesirable. Prime editing offers possibilities for introducing selected substitutions and small insertions or deletions, although its performance depends on target sequence, editing design and delivery conditions (Lin *et al*., 2020). Furthermore, generating an edited plant does not automatically demonstrate agronomic superiority. Potential off-target changes, genetic background effects, pleiotropy and environmental interactions must be assessed before a modified allele can be considered suitable for breeding.

An integrated approach combining genomics, transcriptomics, functional validation and precision genome editing can provide a stronger foundation for rice improvement than treating these technologies as independent research activities. Genomic studies can identify candidate loci, transcriptomic and other molecular analyses can help prioritize genes and pathways, and functional experiments can test hypotheses about gene activity. Genome editing can then be used to investigate whether specific genetic changes produce the expected biological effects. The resulting material requires molecular characterization and agronomic evaluation before its value for cultivar development can be established. The integration of genome-editing tools with functional-genomics resources is particularly important because it connects gene-level discoveries with experimentally testable breeding objectives (Moin *et al*., 2018; Lin *et al*., 2020).

Accordingly, this review critically examines the integration of molecular genetics, gene discovery, functional genomics and CRISPR-based genome editing in rice. The specific objectives are to: (i) evaluate genomic resources and genetic approaches used to identify genes and alleles controlling important agronomic traits; (ii) discuss functional-genomics strategies for establishing gene function and validating candidate loci; (iii) compare CRISPR/Cas9, Cas12a, base editing, prime editing and regulatory-sequence editing in the context of rice improvement; (iv) synthesize applications of genome editing in yield enhancement, abiotic stress resilience, disease resistance and grain quality; and (v) identify technical, biological and translational challenges that constrain the development of improved cultivars. By organizing these areas within a gene-discovery-to-cultivar framework, the review aims to identify research priorities for more precise, evidence-based and breeding-oriented rice improvement.

2. GENOMIC FOUNDATIONS OF RICE IMPROVEMENT

2.1. Rice Genome Organization and Genetic Diversity

The availability of a high-quality reference genome marked a major milestone in rice genetics and molecular breeding. The International Rice Genome Sequencing Project established a map-based sequence covering approximately 95% of the estimated 389-Mb rice genome, providing an essential foundation for gene identification, genome annotation and comparative genomics. The reference sequence enabled researchers to investigate gene organization, chromosome structure and the genetic basis of important agronomic characteristics. It also strengthened the use of rice as a model cereal for understanding genome evolution and transferring genomic knowledge to other grass species. Nevertheless, a reference genome represents one genetic background and cannot fully capture the sequence diversity present across cultivated rice

varieties and their wild relatives (International Rice Genome Sequencing Project, 2005; Wing *et al*., 2018).

Genetic diversity in rice is an important resource for identifying favourable alleles and developing cultivars adapted to different production environments. Cultivated rice exhibits substantial variation in plant architecture, flowering time, grain characteristics, disease resistance and environmental adaptation. Genome-wide analysis of diverse rice accessions has revealed extensive nucleotide variation, small insertions and deletions, structural variation and differences in gene content. These variants provide opportunities to investigate domestication, local adaptation and the genetic mechanisms underlying agronomic diversity. However, the usefulness of a particular allele depends on its biological effect, genetic background and interaction with other loci, making the characterization of genetic diversity an essential first step rather than a sufficient basis for breeding decisions (Wang *et al*., 2018; Zhao *et al*., 2018).

2.2. Reference Genomes and Rice Pan-Genome Resources

Traditional rice genomic studies have relied heavily on a single reference genome, particularly the *japonica* cultivar *Nipponbare*. Although this approach supports standardized genome annotation and variant detection, it can introduce reference bias when sequencing reads from genetically divergent accessions are aligned to a single genome. Genes or genomic regions absent from the reference may remain undetected, potentially limiting the identification of useful variation in indica varieties, landraces and wild relatives. Pan-genome analysis addresses this limitation by incorporating genomic sequences from multiple accessions, thereby providing a broader representation of shared and accession-specific genetic content. Zhao *et al*. (2018) demonstrated the extent of genomic variation in cultivated and wild rice through pan-genome analysis, illustrating its value for discovering previously unrecognized variants and genes.

The 3,000 Rice Genomes Project further expanded the resources available for investigating genetic diversity and population structure in Asian cultivated rice. Analysis of 3,010 rice accessions identified approximately 29 million single-nucleotide polymorphisms, 2.4 million small insertions and deletions, and more than 90,000 structural variants. These data revealed extensive genetic differentiation among rice populations and provided a foundation for investigating domestication, adaptation and agronomically important alleles. Integrating such large-scale genomic datasets with phenotypic information can improve the identification of candidate genes and support the development of breeding populations with complementary genetic backgrounds. However, genomic variants must still be evaluated for their functional effects and agronomic relevance before they can be confidently incorporated into improvement programmes (Wang *et al*., 2018; Wei *et al*., 2021).

2.3. Genome-Wide Association Studies and Quantitative Trait Locus Mapping

Genome-wide association studies (GWAS) are widely used to identify statistical associations between genetic markers and phenotypic variation in diverse rice populations. These analyses exploit historical recombination and naturally occurring genetic variation to identify genomic regions associated with traits such as flowering time, plant height, grain size and yield components. GWAS can provide relatively high-resolution mapping when suitable diversity panels, reliable phenotyping and appropriate statistical models are available. However, population structure, relatedness among accessions, linkage disequilibrium and environmental variation can influence association signals. Consequently, a significant marker trait association should generally be interpreted as evidence for a candidate genomic region rather than definitive proof that the associated variant is causal (Huang *et al*., 2010; Wei *et al*., 2021).

Quantitative trait locus (QTL) mapping provides a complementary approach by identifying genomic regions associated with phenotypic variation in segregating populations derived from selected parental lines. Unlike GWAS, which commonly examines diversity panels, QTL mapping uses recombination within controlled crosses to investigate the inheritance of traits. This approach is useful for identifying loci contributing to complex characteristics, including grain yield, plant architecture, flowering time and stress responses. Nevertheless, a QTL interval may contain multiple genes, and the estimated effects of individual loci can depend on the parental genotypes, population size and experimental environment. Combining QTL mapping with fine mapping, sequence variation, gene-expression evidence and functional validation can therefore improve the resolution of candidate-gene identification (Huang *et al*., 2010; Wei *et al*., 2021).

2.4. Genome Resequencing and Identification of Functional Alleles

Whole-genome resequencing provides a comprehensive method for identifying nucleotide substitutions, insertions, deletions and other genomic variants among rice accessions. When integrated with high-quality phenotypic data, resequencing can help identify alleles associated with agronomic performance and reveal genetic differences between elite cultivars, traditional landraces and wild relatives. Large-scale rice genome datasets have demonstrated the extensive distribution of genetic variation across cultivated populations and highlighted the value of these resources for understanding domestication and identifying potentially useful alleles. However, detecting a sequence variant does not establish its biological function; additional evidence is required to determine whether the variant influences gene activity or contributes directly to a phenotype (Wang *et al*., 2018; Zhao *et al*., 2018).

The integration of genomic variation with association mapping and functional evidence can improve the identification of candidate causal variants. Wei *et al*. (2021) developed a quantitative genomics map using multiple rice GWAS cohorts and investigated quantitative trait nucleotides associated with important agronomic characteristics. Their work demonstrated how information about genetic variants and their estimated effects can inform the selection and combination of favourable alleles. For precision breeding, this approach provides a basis for prioritizing candidate loci before functional investigation or genome editing. Nevertheless, the estimated effect of an allele should be evaluated across relevant genetic backgrounds and environments, particularly when the target trait is quantitative or influenced by multiple interacting genes (Wei *et al*., 2021).

2.5. Genomic Selection and Prediction of Breeding Values

Genomic selection uses genome-wide marker information to estimate the genetic merit of individuals and identify promising breeding candidates. Unlike conventional marker-assisted selection, which generally focuses on a limited number of markers

associated with major-effect loci, genomic selection can incorporate information distributed across the genome. This approach is particularly relevant to complex traits influenced by many loci, including grain yield and other quantitative characteristics. Its predictive performance depends on factors such as the size and composition of the training population, marker density, phenotyping quality, genetic relatedness and the genetic architecture of the target trait. Integrating genomic prediction with conventional breeding can help prioritize individuals for further evaluation and potentially increase the efficiency of selection (Xu *et al*., 2021).

Genomic selection may also complement functional genomics and genome editing by helping breeders evaluate the broader genetic background in which an edited allele will be introduced. For example, an allele that improves a particular yield component may not necessarily produce a corresponding increase in overall yield if it interacts unfavourably with other loci or environmental conditions. Genomic prediction models that incorporate genotype-by-environment interactions, multiple traits or functional marker information may help capture some of this complexity. However, their predictive ability must be assessed using suitable validation populations and independent phenotypic data. Genome editing and genomic selection should therefore be viewed as complementary tools: editing enables targeted modification of selected loci, whereas genomic selection supports the evaluation and advancement of breeding material carrying complex combinations of genetic effects (Xu *et al*., 2021; Wei *et al*., 2021).

2.6. Integrating Genomic Resources for Gene Discovery and Precision Breeding

The most effective use of rice genomic resources requires the integration of complementary evidence rather than reliance on a single mapping method. Reference genomes and pan-genomes provide the sequence framework; resequencing identifies genetic variation; GWAS and QTL mapping identify candidate genomic regions; and genomic prediction supports selection across breeding populations. These resources can be combined with transcriptomics, functional-genomics experiments and targeted genome editing to investigate the biological effects of specific alleles. Such integration is particularly valuable for traits whose inheritance is complex or whose expression varies across developmental stages and environments. The rice quantitative genomics framework developed by Wei *et al*. (2021) illustrates how accumulated genomic knowledge can be connected with breeding decisions, while pan-genome studies demonstrate the importance of representing genetic variation beyond a single reference sequence (Zhao *et al*., 2018; Wei *et al*., 2021).

Despite considerable progress, important limitations remain in translating genomic discoveries into improved rice cultivars. Candidate genes identified through association mapping may lack direct functional evidence, while variants absent from the reference genome can be difficult to characterize using reference-dependent approaches. Furthermore, genetic effects estimated in one population or environment may not transfer consistently to another, especially for complex traits involving gene by environment interactions. Future research should therefore prioritize the integration of diverse genomic resources, robust phenotyping, functional validation and breeding oriented evaluation. This integrated strategy provides the foundation for the next stages of the gene discovery to cultivar framework, in which candidate genes and alleles are investigated through functional genomics and evaluated for their suitability as targets for CRISPR-based precision breeding (Wang *et al*., 2018; Xu *et al*., 2021).

3. GENE DISCOVERY AND GENETIC ARCHITECTURE OF AGRONOMIC TRAITS IN RICE

3.1. Genetic Architecture of Quantitative Traits

The genetic architecture of an agronomic trait describes the number, distribution and effects of genetic loci contributing to phenotypic variation. In rice, traits such as grain yield, plant height, tillering, flowering time and drought tolerance frequently exhibit quantitative inheritance, in which multiple loci interact with environmental conditions to determine the observed phenotype. Some traits may be influenced by major-effect genes, whereas others depend on numerous loci with relatively small individual effects. Genome-wide association studies and quantitative trait locus mapping have helped characterize this variation, but the detection of associated genomic regions does not necessarily identify the causal genes. Understanding genetic architecture is therefore essential for selecting appropriate strategies for gene discovery and subsequent functional validation (Huang *et al*., 2010; Wei *et al*., 2021).

The effects of individual alleles may also depend on genetic background, epistasis and environmental conditions. A favourable allele identified in one rice population may not produce an equivalent response in another population because of differences in interacting loci or genetic linkage. Furthermore, an allele that improves one trait may negatively affect another through pleiotropic effects. These considerations are particularly important when genome editing is used to modify genes associated with yield, plant development or stress responses. Integrating genetic mapping with functional-genomics evidence can help distinguish promising candidate genes from loci whose effects are context-dependent, thereby improving the selection of targets for precision breeding (Li *et al*., 2018; Chen *et al*., 2022).

3.2. Gene Discovery for Yield and Plant Architecture

Grain yield is a complex agronomic trait determined by several interrelated components, including panicle number per plant, spikelet number per panicle, grain-filling percentage and individual grain weight. Genetic variation in these components provides opportunities to improve rice productivity, but simultaneous modification of multiple yield-related traits can be challenging because their effects may be compensatory. For example, increasing grain number does not necessarily improve total yield if grain filling or individual grain weight decreases. Consequently, gene discovery for yield improvement should evaluate the biological functions of candidate genes alongside their effects on multiple yield components and overall plant performance. Large-scale rice genetic studies provide useful resources for identifying loci associated with these characteristics and prioritizing targets for further investigation (Huang *et al*., 2010; Wei *et al*., 2021).

Plant architecture is another important determinant of rice productivity because it influences light interception, canopy

development, resource allocation and lodging resistance. Genes regulating tiller formation, stem elongation, panicle branching and flowering time can have substantial effects on plant form and reproductive performance. Functional genomics has contributed to the identification of regulatory pathways controlling these characteristics, while genomic association studies have expanded the number of candidate loci available for investigation. However, the agronomic value of an architectural modification depends on its effects across the entire plant and under relevant production conditions. Therefore, candidate genes identified through mapping approaches should be evaluated using functional experiments and field-based phenotyping before they are prioritized for genome editing (Li *et al*., 2018; Chen *et al*., 2022).

3.3. Genes Associated with Abiotic Stress Tolerance

Abiotic stresses, including drought, salinity, extreme temperatures and flooding, can disrupt rice growth, reproductive development and grain production. The genetic basis of stress tolerance involves diverse biological processes, such as stress perception, hormonal signalling, transcriptional regulation, osmotic adjustment, ion homeostasis and antioxidant defence. Gene discovery approaches can identify candidate regulators and stress-responsive pathways, while transcriptomic and physiological analyses provide complementary evidence about their potential functions. Nevertheless, changes in gene expression under stress conditions do not necessarily indicate that a gene improves tolerance. Functional validation should establish whether manipulating the candidate gene produces a reproducible improvement in stress response without unacceptable penalties in growth or yield (Li *et al*., 2018; Chen *et al*., 2022).

Drought and salinity tolerance are particularly challenging targets because they involve multiple interacting physiological and developmental processes. Candidate genes may regulate root architecture, water relations, osmoprotectant accumulation, ion transport or signalling pathways that coordinate stress responses. Genome-wide association studies and QTL mapping can identify genomic regions associated with relevant phenotypes, while functional-genomics approaches can investigate the underlying biological mechanisms. CRISPR-based editing may subsequently be used to test whether specific genes or regulatory elements contribute causally to the observed responses. However, a successful modification under controlled stress conditions must still be evaluated under realistic field environments to determine whether it improves yield stability and agronomic performance (Huang *et al*., 2010; Chen *et al*., 2022).

3.4. Gene Discovery for Disease and Pest Resistance

Rice production is affected by several pathogens and insect pests, making genetic resistance an important target for crop improvement. Disease resistance can involve recognition of pathogen-associated signals, activation of defence pathways and restriction of pathogen growth, whereas susceptibility may depend on host factors exploited by pathogens during infection. Identifying genes involved in these processes can support the development of resistant cultivars through conventional breeding or targeted genetic modification. Functional-genomics approaches are particularly valuable for distinguishing genes that contribute directly to resistance from those whose expression changes as a secondary consequence of infection. Combining genetic mapping, molecular characterization and controlled disease assays can therefore strengthen the evidence used to prioritize candidate resistance genes (Li *et al*., 2018; Chen *et al*., 2022).

Genome editing also offers opportunities to investigate host susceptibility genes and modify defence-related pathways. For example, targeted disruption of a susceptibility factor may reduce pathogen infection if the gene is required for successful colonization and its loss does not substantially impair normal plant development. However, resistance mechanisms can be pathogen-specific, and alterations in host defence may introduce undesirable effects on growth or interactions with other pathogens. Consequently, candidate genes should be evaluated for their biological functions, genetic background effects and potential trade-offs before they are selected for breeding applications. This approach connects the discovery of resistance-associated loci with functional evidence and the development of more durable disease-management strategies (Moin *et al*., 2018; Zegeye *et al*., 2022).

3.5. Genetic Regulation of Grain Quality and Nutritional Traits

Rice grain quality encompasses several characteristics, including grain shape, appearance, starch composition, amylose content, aroma, cooking behaviour and nutritional properties. These traits influence consumer preference, market value and the suitability of rice for different food products. Their genetic regulation involves structural genes, transcriptional regulators and metabolic pathways that control endosperm development and grain composition. Genetic mapping and functional studies have helped identify candidate genes associated with these characteristics, creating opportunities to improve grain quality while maintaining agronomic performance. However, the preferred combination of quality traits differs among markets and production systems, so the objectives of genetic improvement should be defined according to the intended end use (Li *et al*., 2018; Zegeye *et al*., 2022).

Genome editing can be used to investigate and modify genes involved in starch biosynthesis, grain development and other quality-related processes. The suitability of a target depends on whether the desired phenotype can be achieved through gene disruption, a precise nucleotide change or modification of gene expression. A complete knockout may be inappropriate if the gene performs several essential functions or if the desired phenotype requires only a moderate change in enzyme activity. Therefore, candidate-gene selection should integrate molecular evidence with grain composition, cooking quality and agronomic evaluation. The ultimate objective is not simply to generate a molecular change, but to develop rice material with a reproducible and commercially relevant improvement in grain quality (Li *et al*., 2018; Zegeye *et al*., 2022).

3.6. From Candidate Loci to Causal Genes

The identification of candidate loci is an intermediate step in gene discovery rather than its final outcome. Association mapping and QTL analysis can narrow a trait to a genomic region, but that region may contain several genes and multiple linked variants. Fine mapping, haplotype analysis, sequence annotation, tissue-specific expression data and comparative genomics can help prioritize candidates for experimental investigation. Nevertheless, computational predictions and

expression patterns provide indirect evidence and cannot independently demonstrate that a candidate gene causes the phenotype. A strong gene-discovery pipeline therefore combines complementary evidence and explicitly distinguishes statistical association, biological plausibility and experimentally established function (Huang *et al*., 2010; Li *et al*., 2018).
Functional validation can involve loss-of-function mutants, overexpression, complementation and targeted genome editing. When independent genetic approaches produce consistent phenotypic effects, confidence in the proposed gene function increases. CRISPR-mediated mutations can be particularly useful for testing candidate genes identified through GWAS, QTL mapping or transcriptomic analysis, provided that the edited plants are appropriately genotyped and phenotyped. Where feasible, complementation or independent alleles can help distinguish the effect of the intended mutation from background variation or unintended changes. These steps are essential before a candidate gene is considered a reliable target for precision breeding (Moin *et al*., 2018; Chen *et al*., 2022).

4. FUNCTIONAL GENOMICS: ESTABLISHING GENE FUNCTION IN RICE

4.1. Transcriptomics and Differential Gene Expression

Transcriptomics is an important component of functional genomics because it provides information about gene-expression patterns across tissues, developmental stages and environmental conditions. In rice, transcriptome profiling can identify genes associated with reproductive development, grain formation, nutrient utilization and responses to abiotic and biotic stresses. RNA sequencing (RNA-seq) has substantially expanded the capacity to investigate these processes by enabling genome-wide quantification of transcript abundance and identification of previously uncharacterized transcripts. However, differential expression indicates an association between transcriptional activity and a biological condition rather than definitive proof that a gene controls the observed phenotype. Consequently, transcriptomic findings should be interpreted alongside genetic evidence, physiological measurements and functional experiments (Wang *et al*., 2009; Li *et al*., 2018; Meena *et al*. 2026)).
Comparative transcriptomic analysis can help identify molecular responses associated with contrasting rice genotypes, including stress-tolerant and stress-sensitive accessions. Under drought conditions, for example, expression profiling may reveal candidate genes involved in abscisic acid signalling, transcriptional regulation, osmotic adjustment, antioxidant defence and root development. Nevertheless, observed expression differences may reflect differences in stress severity, tissue composition, developmental stage or sampling time. Reliable interpretation therefore requires appropriate biological replication, carefully controlled experimental conditions and statistical correction for multiple testing. Integrating RNA-seq results with QTL mapping, genome-wide association studies and gene-function databases can further improve candidate prioritization and reduce reliance on expression patterns alone (Chen *et al*., 2022; Li *et al*., 2018).

4.2. Reverse Genetics and Functional Characterization

Reverse genetics investigates gene function by deliberately modifying a candidate gene and examining the resulting phenotype. In rice, approaches such as targeted mutagenesis, insertional mutagenesis, RNA interference and gene overexpression have been used to investigate the roles of genes involved in plant development, hormone signalling, stress responses and agronomic traits. These methods complement forward-genetic approaches, which begin with a phenotype and seek to identify its genetic basis. Reverse genetics is particularly useful when genomic studies have identified a candidate gene but its biological contribution remains uncertain. However, the interpretation of mutant phenotypes requires consideration of genetic background, possible off-target effects, gene redundancy and environmental variation (Jung *et al*., 2008; Moin *et al*., 2018).
Loss-of-function and gain-of-function experiments provide complementary evidence for determining gene function. A loss-of-function mutation can reveal whether a gene is necessary for a particular biological process, whereas overexpression may indicate whether increased gene activity is sufficient to alter the phenotype under the tested conditions. Complementation experiments, in which the functional gene is reintroduced into a mutant background, can provide additional evidence that the observed phenotype results from the intended genetic alteration. These approaches are especially valuable when a candidate gene has been identified through transcriptomics or association mapping but its causal role has not been established. Combining independent experimental approaches generally provides stronger evidence than relying on a single mutant or an expression correlation (Jung *et al*., 2008; Chen *et al*., 2022).

4.3. CRISPR-Based Functional Validation

CRISPR/Cas9 has expanded the possibilities for reverse-genetic analysis by enabling targeted modifications of selected genomic regions. In rice, researchers can use CRISPR-based mutagenesis to disrupt candidate genes, investigate gene-family redundancy and examine whether specific loci influence agronomic traits. Compared with some conventional mutant-generation approaches, targeted editing allows researchers to design experiments around a defined genetic hypothesis. Nevertheless, successful editing does not automatically establish gene function: the resulting plants must be genotyped, their phenotypes must be characterized, and potential unintended changes must be considered. Independent edited lines and, where appropriate, complementation experiments can help distinguish genuine gene effects from background variation or experimental artefacts Figure 2, (Moin *et al*., 2018; Chen *et al*., 2022).
A rigorous CRISPR-based validation pipeline begins with candidate selection based on genetic, molecular and biological evidence. Appropriate guide RNAs are designed, edited plants are generated and regenerated, and the target region is characterized using suitable molecular methods. The resulting lines are then evaluated for the phenotype relevant to the original hypothesis. For example, editing a candidate drought-response gene should be followed by measurements of plant performance under well-defined water-deficit conditions, alongside appropriate control treatments. Where a candidate is proposed to influence yield, measurements should extend beyond an isolated morphological change to include relevant yield

components and, eventually, field performance. Such a pipeline connects molecular modification with biological interpretation and establishes a more reliable basis for subsequent breeding applications (Li *et al*., 2018; Chen *et al*., 2022).

4.4. Gene-Regulatory Networks and Transcription Factors

Agronomically important traits are often controlled by networks of interacting genes rather than by isolated loci. Gene-regulatory networks describe relationships among transcription factors, regulatory DNA sequences, signalling components and downstream genes. In rice, these networks contribute to developmental processes, flowering, reproductive development and responses to environmental stresses. Transcriptomic data can identify coordinated expression patterns, while genetic studies and molecular experiments can help determine which components regulate others. However, co-expression alone does not establish a direct regulatory relationship. Stronger evidence may require promoter-binding assays, reporter-gene experiments, chromatin immunoprecipitation or targeted genetic perturbation (Chen *et al*., 2022; Li *et al*., 2018).

Transcription factors are particularly important functional-genomics targets because individual regulators may influence multiple downstream genes and biological pathways. Modifying a transcription factor can therefore produce broad phenotypic effects, potentially improve stress tolerance while also affect growth, flowering or grain production. This complexity makes regulatory genes attractive but potentially challenging targets for precision breeding. Understanding their tissue specificity, developmental timing and downstream targets can help determine whether complete gene disruption or a more moderate alteration of expression is appropriate. Combining regulatory-network analysis with targeted editing may ultimately allow researchers to investigate and modify selected components of these networks while minimizing undesirable pleiotropic effects (Chen *et al*., 2022; Li *et al*., 2018).

4.5. Epigenomics and Chromatin-Mediated Gene Regulation

Gene function is influenced not only by DNA sequence but also by epigenetic mechanisms that affect chromatin structure and transcription. DNA methylation, histone modifications, chromatin remodelling and non-coding RNAs contribute to the regulation of gene activity during plant development and in response to environmental conditions. Rice is a valuable system for investigating these mechanisms because extensive genomic resources can be integrated with genome-wide epigenetic profiles. Research on the rice epigenome has demonstrated that epigenetic regulation is relevant to developmental processes, transcriptional activity and the control of transposable elements. These findings broaden functional genomics beyond the identification of coding sequences and emphasize the importance of regulatory mechanisms in determining phenotype (Deng *et al*., 2016; Chen and Zhou, 2013).

Comparative epigenomic studies can also reveal differences in DNA methylation, histone modifications and other chromatin features among rice varieties. Such variation may be associated with differences in gene expression, development or environmental responses, although the causal relationships require careful investigation. Zhao *et al*. (2020) integrated reference epigenomic information from multiple rice varieties, providing a resource for examining variation in epigenetic regulation across genetic backgrounds. These data can complement transcriptomic and genomic analyses by helping identify candidate regulatory regions and potential epigenetic contributions to trait variation. Nevertheless, epigenetic associations should be distinguished from stable, heritable causal effects, and their relevance to breeding must be assessed through appropriate molecular and phenotypic validation (Zhao *et al*., 2020; Deng *et al*., 2016).

4.6. Integrating Multi-Omics Data for Functional Validation

The integration of multiple molecular datasets can improve the prioritization of candidate genes and clarify the biological mechanisms underlying complex traits. Genomics identifies sequence variation, transcriptomics characterizes gene-expression patterns, proteomics investigates protein abundance and modification, and metabolomics measures changes in small-molecule profiles. Combining these layers can help researchers connect genetic variation with molecular activity and physiological outcomes. For example, a candidate locus associated with drought tolerance may be investigated through sequence variation, stress-responsive expression, protein abundance and changes in metabolites associated with osmotic adjustment. However, differences among datasets in resolution, sampling design and statistical power can complicate integration, so conclusions should reflect the strength and limitations of the available evidence (Chen *et al*., 2022; Li *et al*., 2018).

A practical multi-omics workflow begins with a clearly defined biological question and a suitable experimental design. Candidate loci are identified using genetic evidence, followed by molecular profiling to investigate their potential roles and regulatory relationships. Selected genes are then tested through reverse genetics or targeted genome editing, and the resulting plants are evaluated using molecular, physiological and agronomic measurements. This process can help distinguish genes that respond to a condition from those that actively contribute to the phenotype. Importantly, computational integration should guide experimental priorities rather than replace experimental validation. Linking multi-omics evidence with independent genetic experiments can provide a stronger foundation for identifying breeding-relevant genes and selecting targets for precision editing (Wang *et al*., 2009; Chen *et al*., 2022).

4.7. From Functional Validation to Breeding-Relevant Evidence

A candidate gene becomes a stronger breeding target when its biological function is supported by reproducible experimental evidence and its modification produces a desirable phenotype. The validation process should consider the magnitude and consistency of the effect, the possibility of pleiotropy, the genetic background and the environmental conditions under which the phenotype was observed. For complex traits such as grain yield and drought tolerance, controlled-environment experiments are useful for testing initial hypotheses, but they cannot fully represent the variability of field conditions. Subsequent evaluation should therefore include relevant agronomic traits, suitable controls and, where appropriate, independent genetic backgrounds and multiple environments, Figure 2 (Li *et al*., 2018; Chen *et al*., 2022).

Functional genomics ultimately provides the bridge between gene discovery and precision breeding. A robust evidence chain

connects the identification of a candidate locus with its molecular characterization, experimental validation and evaluation of its agronomic effects. This framework also helps distinguish targets suitable for gene knockout from those that may require precise nucleotide substitutions or regulatory modifications. By integrating genetic mapping, molecular profiling, targeted perturbation and field-based assessment, researchers can make more informed decisions about which genes should advance into breeding programmes. The next stage is to examine how different CRISPR-associated systems can be selected and applied to achieve these objectives in rice (Moin *et al*., 2018; Chen *et al*., 2022).

5. CRISPR-BASED GENOME-EDITING TECHNOLOGIES IN RICE

5.1. Development and Applications of CRISPR/Cas Systems

CRISPR-associated genome-editing technologies have transformed plant molecular genetics by enabling targeted modifications of selected genomic sequences. The CRISPR/Cas9 system uses a guide RNA to direct the Cas9 nuclease to a complementary DNA sequence adjacent to an appropriate protospacer-adjacent motif (PAM). Following target recognition and DNA cleavage, cellular repair processes can introduce insertions or deletions that disrupt gene function. Shan *et al*. (2013) demonstrated targeted genome modification in crop plants, including rice and wheat, establishing an important foundation for the application of CRISPR technology in cereal improvement. Subsequent developments have expanded its applications from gene-function studies to the modification of agronomically important characteristics.

In rice, CRISPR/Cas9 has become an important tool for investigating genes associated with plant development, reproductive biology, disease resistance and grain quality. Miao *et al*. (2013) demonstrated targeted mutagenesis in rice using a CRISPR/Cas system, while Ma *et al*. (2015) developed a robust multiplex genome-editing platform applicable to monocot and dicot plants. These studies demonstrated the value of programmable nucleases for investigating multiple genes and generating targeted genetic variation. Nevertheless, editing performance depends on guide-RNA design, target accessibility, nuclease activity and the genetic background of the recipient plant. Molecular confirmation and phenotypic evaluation are therefore necessary to establish whether an editing event produces the intended biological outcome.

5.2. CRISPR/Cas9-Mediated Targeted Mutagenesis

CRISPR/Cas9-mediated mutagenesis is widely used to investigate gene function and generate loss-of-function alleles. DNA cleavage followed by cellular repair can introduce insertions or deletions that disrupt coding sequences or alter gene activity. This approach is particularly useful when candidate genes have been identified through genetic mapping, transcriptomics or comparative genomic analysis but their functional roles remain uncertain. In rice, targeted mutagenesis provides a direct method for testing whether a candidate locus contributes to a specific phenotype. Early studies established the feasibility of this approach and demonstrated its usefulness for functional genomics and genetic improvement (Miao *et al*., 2013; Ma *et al*., 2015).

The suitability of gene knockout depends on the biological function of the target. Disruption of a susceptibility gene may improve disease resistance, whereas disruption of a gene required for reproductive development could reduce fertility or yield. Gene redundancy may also complicate interpretation because related genes can compensate for the loss of a single gene. Consequently, experiments should include appropriate controls, sequence confirmation and phenotyping of independent edited lines. Complementation studies, when feasible, can provide additional evidence that the observed phenotype results from the intended mutation rather than background variation or unintended genetic changes (Jung *et al*., 2008; Moin *et al*., 2018).

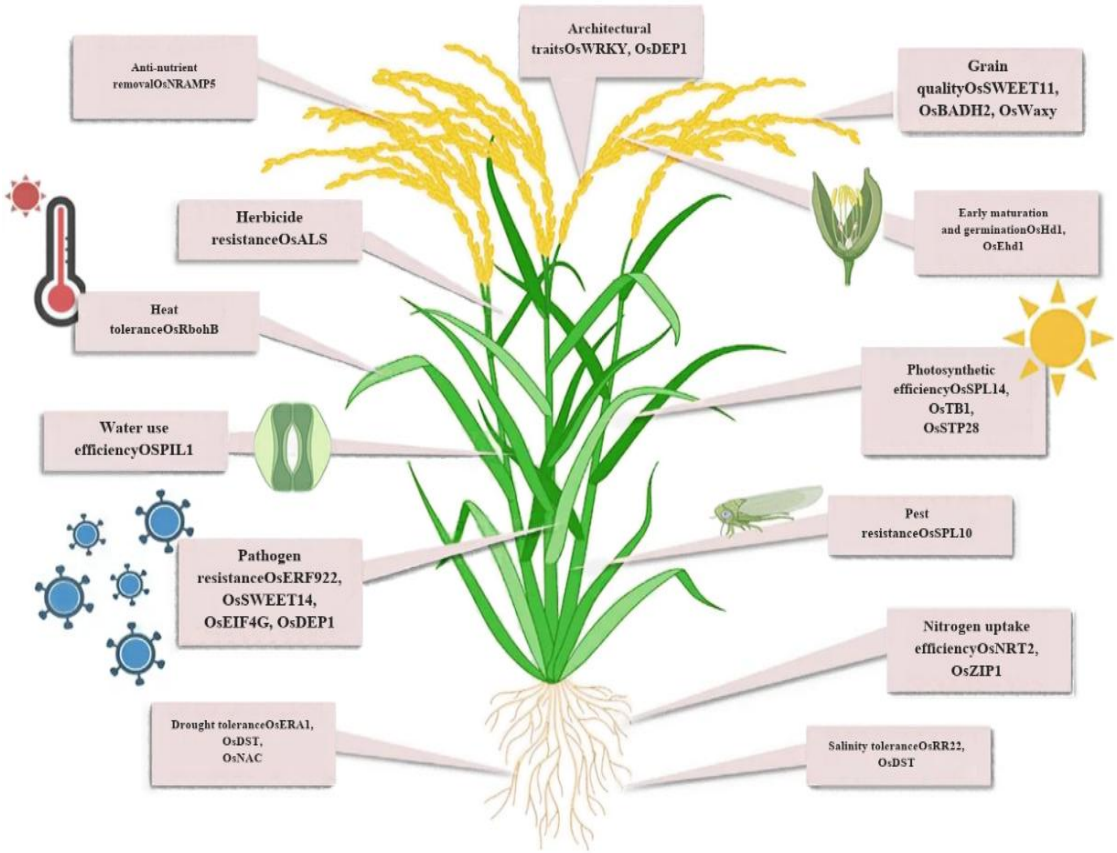


Figure 1: Key genes associated with agronomic traits, biotic and abiotic stress tolerance, nutrient-use efficiency, and grain quality in rice. These genes are involved in controlling important characteristics related to plant growth, development, productivity and grain quality. Among them, *OsWRKY* and *OsDEP1*, for example, are thought to be related to plant architecture and panicle development, and *OsSWEET11*, *OsBADH2* and *OsWaxy* to grain quality and grain nutrition. Genes such as *OsHdl*, *OsEhd1* and others are suggested to be related to flowering, early maturation and development, and *OsSPL14*, *OsTB1* and *OsSTP28* to photosynthesis. OsPIL1 has a role in water-use efficiency, while *OsRbohB*, *OsERA1*, *OsDST*, *OsNAC* and *OsRR22* are thought to play a role in heat, drought and salt stress, respectively. Genes such as *OsERF922*, *OsSWEET14*, *OsEIF4G* and *OsDEP1* are involved in disease resistance, and *OsSPL10* in pest resistance. The genes *OsNRT2* and *OsZIP1* are related to efficient absorption and use of nutrients, especially nitrogen and zinc. Finally, *OsALS* and *OsNRAMP5* are believed to play a role in herbicide resistance and mineral accumulation regulation. These genes are a substantial genetic resource and may lead to a range of benefits for developing high-yielding, superior-quality, resilient and nutrient-use-efficient rice varieties by the use of molecular breeding, marker-assisted breeding, and genome editing technologies.

5.3. CRISPR/Cas12a and Alternative Cas Nucleases

CRISPR/Cas12a provides an alternative to conventional Cas9-based editing. The two systems differ in PAM recognition, guide-RNA architecture and DNA-cleavage characteristics, allowing Cas12a to target some genomic regions that may be inaccessible to particular Cas9 configurations. Li *et al*. (2018) demonstrated the expansion of CRISPR/Cpf1-mediated genome editing in rice, and Li *et al*. (2019) subsequently reported efficient biallelic gene targeting using CRISPR/Cas12a. These studies established Cas12a as a useful alternative for targeted gene modification and functional investigation in rice.

The development of additional Cas proteins has further expanded the available editing strategies. Illa-Berenguer *et al*. (2023) investigated the effects of Cas9 orthologues, Cas12a endonucleases and temperature on editing efficiency in rice, illustrating that editing performance depends on both the nuclease and experimental conditions. Lv *et al*. (2024) demonstrated genome editing in rice using CRISPR/Cas12i3 and developed a multiplex editing system based on this nuclease. Such developments increase the range of possible target sites and editing configurations. However, the choice of nuclease should be based on target compatibility, editing efficiency, specificity and experimental feasibility rather than the assumption that one system is universally superior.

5.4. Base Editing for Precise Nucleotide Substitution

Base editing enables the conversion of selected nucleotides without requiring the conventional double-strand DNA break associated with standard CRISPR/Cas9 mutagenesis. Cytosine base editors can facilitate C-to-T substitutions, whereas adenine base editors can facilitate A-to-G substitutions, depending on the editor architecture and target sequence. Zong *et al*. (2017) demonstrated precise base editing in rice, wheat and maize using a Cas9–cytidine deaminase fusion. Li *et al*. (2018) subsequently expanded the range of base-editing applications through an adenine deaminase-based system for rice and wheat. These advances established base editing as an important strategy for introducing selected nucleotide changes into crop

genomes.

Base editing is particularly relevant when a desirable natural allele differs from an alternative allele by a specific nucleotide rather than a complete gene disruption. However, editing efficiency and product purity depend on the target sequence, editing window, guide-RNA design and editor configuration. Unwanted substitutions or bystander edits may occur when additional editable nucleotides are present within the activity window. Hua *et al*. (2019) expanded the base-editing scope in rice by using engineered Cas9 variants, improving the potential to target genomic regions with different PAM requirements. Molecular characterization is still essential to distinguish the intended substitution from other sequence changes and to determine whether the resulting allele produces the desired phenotype.

5.5. Base Editing for Trait Improvement and Targeted Evolution

Beyond introducing a predetermined nucleotide substitution, base editing can generate allelic variation for investigating the relationship between sequence and phenotype. Targeted evolution uses controlled sequence diversification to produce variants that can subsequently be screened for desirable properties. This approach may be useful when the functional consequences of individual nucleotide changes are uncertain or when a range of alleles is needed to identify an optimal phenotype. Wang *et al*. (2022) demonstrated base-editing-mediated targeted evolution of the acetyl-CoA carboxylase gene in rice to generate herbicide-resistant mutants. Their study illustrates how targeted nucleotide modification can produce useful variation in a gene associated with an agronomically relevant trait.

The value of targeted evolution depends on the quality of subsequent molecular and phenotypic screening. A sequence change that improves herbicide resistance, for example, must also be assessed for possible effects on growth, development and productivity. Candidate alleles should be characterized to establish the intended sequence modification, and promising lines should be evaluated under relevant experimental conditions. Base editing can therefore complement conventional breeding by generating specific genetic variants, but the identification of a useful edit remains an evidence-based selection process rather than an automatic consequence of editing (Wang *et al*., 2022).

5.6. Prime Editing for Programmable Sequence Modification

Prime editing combines a Cas9 nickase with a reverse-transcriptase domain and a prime-editing guide RNA (pegRNA). Depending on the design, this system can introduce selected nucleotide substitutions and small insertions or deletions without requiring a conventional double-strand break or a separate donor-DNA template. Lin *et al*. (2020) demonstrated prime genome editing in rice and wheat, establishing its feasibility in cereal crops. Xu *et al*. (2020) subsequently developed plant prime-editing systems designed to improve the application of this technology to plant genomes. These studies expanded the potential for introducing precise sequence modifications that may be difficult to obtain through conventional knockout or base-editing approaches.

Prime editing has potential applications in correcting selected alleles, generating novel variants and investigating the functional consequences of specific sequence changes. Butt *et al*. (2020) demonstrated prime editing for engineering herbicide resistance in rice, providing an example of the use of targeted sequence modification for an agronomic objective. Nevertheless, editing efficiency can vary substantially with the target site, pegRNA design, editor expression and plant regeneration conditions. Prime editing should therefore be selected when the intended genetic change is compatible with the method and when its performance can be validated experimentally. It should not be assumed to be more efficient than Cas9 mutagenesis or base editing at every locus.

5.7. Multiplex Genome Editing for Complex Traits

Multiplex genome editing enables simultaneous modification of multiple genomic targets. This strategy is particularly relevant to rice because important agronomic traits, including yield, stress tolerance and grain quality, often depend on multiple genes and interacting biological pathways. Ma *et al*. (2015) developed a multiplex editing system that enabled several genomic targets to be addressed within a coordinated experimental framework. Multiplex approaches can help investigate gene redundancy, test interactions among candidate loci and generate combinations of potentially favourable alleles. However, editing efficiency may differ among targets, and the resulting plants require confirmation of the genotype at every relevant locus.

Recent advances have extended multiplex editing beyond conventional gene knockout to precise nucleotide modification. Cheng *et al*. (2023) demonstrated efficient multiplexed base editing in rice using CRISPR-Cas12a base editors. Lv *et al*. (2024) also reported a Cas12i3-based multiplex editing system in rice. These approaches broaden the opportunities for investigating multiple genes and regulatory regions within a single experimental programme. Nevertheless, the combined effects of several edits may differ from the effects observed when each target is modified independently. Multiplex editing therefore requires evaluation of individual alleles, their combined phenotypic effects and potential unintended consequences before the resulting material is considered suitable for breeding.

5.8. Promoter Editing and Cis-Regulatory Variation

Genome editing can be directed towards promoters and other cis-regulatory elements rather than exclusively towards protein-coding regions. These sequences influence the timing, location and magnitude of gene expression and may contain natural variants associated with agronomic differences. Editing regulatory sequences can therefore create alternative alleles that modify gene activity without completely eliminating the encoded protein's function. This strategy is especially relevant when gene knockout causes undesirable developmental effects or when an intermediate level of gene expression is preferable. Wu *et al*. (2024) reviewed promoter-editing approaches in rice and described their potential for modifying yield-related traits, heading date, grain quality and disease resistance.

Promoter editing requires careful identification of the regulatory sequence responsible for the intended expression pattern. Comparative genomics, expression profiling and functional assays can help identify candidate regions, while targeted editing

can generate sequence variants for subsequent evaluation. However, promoters may contain overlapping transcription-factor binding sites, and modifications can produce effects that differ among tissues, developmental stages and environments. Therefore, promoter-edited plants should be evaluated for gene expression, relevant molecular phenotypes and overall agronomic performance. This strategy offers a potential route to fine-tune quantitative traits, but its success depends on demonstrating a reproducible relationship between the edited regulatory sequence and the intended phenotype (Wu *et al*., 2024).

5.9. Transformation, Delivery and Plant Regeneration

Genome-editing efficiency depends not only on the activity of the editing system but also on the successful delivery of editing components into plant cells and the regeneration of whole plants. Agrobacterium-mediated transformation and particle bombardment are established approaches for introducing editing constructs into rice tissues. However, transformation and regeneration efficiencies can vary among genotypes, explant types and tissue-culture conditions. These differences may limit the application of editing systems to elite breeding material even when the molecular editing strategy performs well in a model genotype. Consequently, improvements in delivery and regeneration remain important for translating laboratory-level editing into practical rice improvement.

Alternative delivery strategies, including approaches designed to avoid stable integration of editing constructs, may be useful when the objective is to obtain edited plants without retaining the editing machinery in the final line. However, the feasibility of such approaches depends on the delivery system, tissue type and regeneration protocol. Regardless of the delivery method, regenerated plants require molecular confirmation of the intended edit, assessment of genetic stability and evaluation of relevant phenotypes. Ma *et al*. (2015) demonstrated the value of robust editing-system design for plant genome modification, while Lin *et al*. (2020) showed that prime-editing systems could be adapted to generate edited cereal plants. These studies illustrate that the editing mechanism and the plant transformation process must be considered together.

5.10. Molecular Validation, Off-Target Effects and Genetic Stability

Molecular validation is essential for establishing whether an editing experiment produced the intended sequence modification. Target-site sequencing can identify the alleles present in edited plants, while additional genotyping can determine whether the edit is heterozygous, homozygous or associated with mosaicism. If editing constructs have been integrated into the genome, segregation analysis may be necessary to determine whether they can be separated from the desired mutation in subsequent generations. The validation strategy should be appropriate to the editing system and the intended application, with particular attention to unintended changes at the target locus and elsewhere in the genome.

Potential off-target effects can be investigated by identifying genomic regions with sequence similarity to the guide RNA and applying suitable molecular analyses. Computational predictions can help prioritize candidate regions, but they cannot independently establish whether unintended editing has occurred. Furthermore, confirmation of the intended genotype does not establish that an edited plant has improved agronomic performance. Independent edited lines, suitable controls and reproducible phenotyping are important for separating the effects of the intended modification from background variation. The biological significance of an edit must ultimately be established through the combined evaluation of molecular evidence and plant performance (Illa-Berenguer *et al*., 2023; Lin *et al*., 2020).

5.11. Selecting an Appropriate Editing Strategy for Precision Breeding

The choice of genome editing technology should be guided by the desired biological outcome. Conventional CRISPR/Cas9 mutagenesis may be appropriate when a loss of function allele is expected to produce a beneficial phenotype, whereas base editing can be used for selected nucleotide substitutions. Prime editing offers additional flexibility for introducing specific sequence changes, while *Cas12a* and *Cas12i3* provide alternative targeting and multiplexing options. Promoter editing may be particularly useful when the objective is to adjust gene expression rather than eliminate gene function. Each technology has distinct limitations related to target compatibility, editing efficiency, delivery and validation, so the choice should be made according to the target gene and experimental objective rather than technological novelty alone.

A breeding-oriented genome-editing pipeline begins with the identification of a candidate gene or allele using genomic, genetic and functional evidence. The intended biological change is then defined, an appropriate editing system is selected, and the resulting plants are molecularly characterized. Phenotypic evaluation should determine whether the edit produces the expected effect and whether it introduces undesirable changes in growth, fertility, yield or quality. Promising material can subsequently be evaluated in relevant genetic backgrounds and across suitable environments. This integrated approach connects gene discovery with precise genetic modification and provides the evidence required to determine whether an edited allele is appropriate for rice improvement.

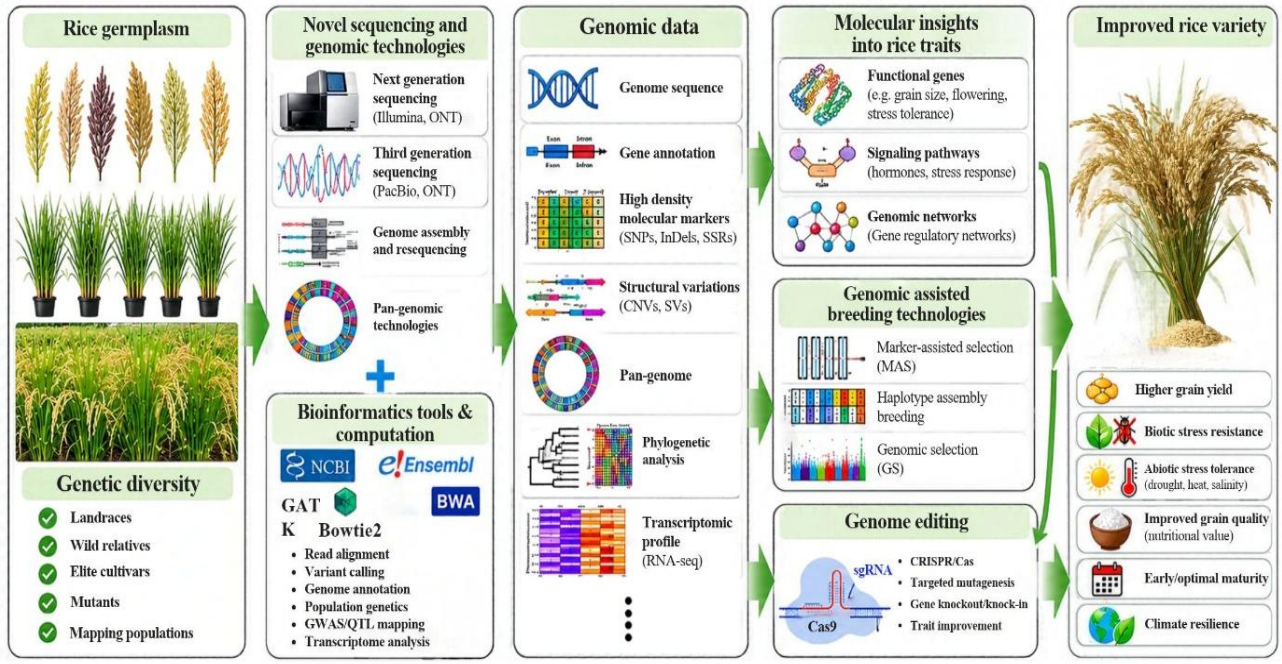


Figure 2: Integrating Genomic Technologies, Bioinformatics, and Precision Breeding for Rice Improvement.

6. APPLICATIONS OF GENOME EDITING IN RICE PRECISION BREEDING

6.1. Genome Editing for Yield Improvement and Plant Architecture

Grain yield is a complex quantitative trait influenced by panicle architecture, grain number, grain size, tillering, flowering time and the efficiency of assimilate partitioning. Genome editing provides a means of testing the contribution of individual genes to these yield components and generating targeted genetic variation. Li *et al*. (2016) used CRISPR/Cas9 to modify four yield-related genes in rice: *Gn1a*, *DEP1*, *GS3* and *IPA1*. The resulting mutants exhibited changes in grain number, panicle architecture, grain size and tillering, demonstrating the potential of targeted editing for investigating multiple components of rice productivity. However, the study also illustrated that the phenotypic consequences of editing depend on the specific mutation and target gene, emphasizing the importance of evaluating yield components together rather than interpreting a single trait as a direct measure of overall productivity, Table 1.

Plant architecture is an important target because it affects canopy structure, light interception, lodging resistance and resource allocation. The *IPA1* gene, which encodes a SQUAMOSA promoter-binding protein-like transcription factor, is involved in the regulation of tiller number and plant architecture. Its activity is influenced by regulatory mechanisms that affect the balance between vegetative growth and reproductive development. Jiao *et al*. (2010) demonstrated the importance of *IPA1* in rice plant architecture, while Li *et al*. (2016) showed that targeted modifications of this gene could produce contrasting tillering phenotypes. These findings indicate that the outcome of editing depends on the nature of the mutation and the regulatory context of the gene. Consequently, precision breeding should seek favourable allelic changes that improve plant architecture without compromising panicle formation, grain filling or total yield.

Improvement of grain size and grain number requires particular attention to the balance among yield components. The *GS3* locus is associated with grain length, while *Gn1a* influences grain number per panicle through cytokinin-related regulation. Manipulating these genes can change individual yield components, but the final agronomic outcome depends on how the changes interact with grain filling, plant density and environmental conditions. Li *et al*. (2016) demonstrated that CRISPR/Cas9 could generate mutations affecting these traits in rice, providing an experimental framework for evaluating the consequences of specific genetic changes. For breeding applications, such findings should be followed by multi-generation evaluation and field trials to determine whether the edited lines achieve stable improvements in grain yield rather than isolated changes in morphology.

6.2. Genome Editing for Drought and Salinity Tolerance

Drought tolerance in rice involves several interacting physiological processes, including stomatal regulation, root development, osmotic adjustment, hormonal signalling and maintenance of cellular function under water deficit. Because these processes are controlled by interconnected gene networks, the modification of a single gene may improve one aspect of stress response without necessarily producing a consistent increase in grain yield. Genome editing can help investigate these relationships by introducing targeted mutations into candidate stress-response genes. Ogata *et al*. (2020) used CRISPR/Cas9 to modify *OsERA1*, a component associated with abscisic acid signalling, and reported enhanced responses to abscisic acid and drought stress alongside increased primary root growth. Their findings illustrate the potential of targeted editing to investigate mechanisms underlying drought responses, although the agronomic value of the resulting changes requires further evaluation under relevant production environments.

DRR Dhan 100 (Kamala) is India's first-ever genome-edited rice variety developed by ICAR-Indian Institute of Rice Research (ICAR-IIRR), Hyderabad by using Crispr Cas technology in genetic background of Samba Mahsuri (BPT 5204). It

is developed by genome editing of the gene *CKX2* (cytokinin oxidase/dehydrogenase 2) based on evidence that this may lead to improved reproductive development and an increase in grain number per panicle. Field trials at multiple locations revealed an average increase of about 19% in grain yield compared to Samba Mahsuri with an average grain yield of 5.37 t ha-1, Maturity period of about 130 days. It also exhibits moderate drought tolerance, higher lodging resistance, and better performance with nitrogen and phosphorus use reduction (ICAR-Indian Institute of Rice Research, 2025; Sadhukhan, 2025; Solanki *et al*., 2026).

Salinity tolerance is another important target because excessive sodium accumulation and ionic imbalance can interfere with rice growth and reproductive development. The *OsRR22* gene encodes a response regulator involved in cytokinin-related signalling, and its targeted modification has been investigated as a strategy for improving salt tolerance. Zhang *et al*. (2019) reported enhanced rice salinity tolerance following CRISPR/Cas9-mediated mutagenesis of *OsRR22*. This study demonstrated that targeted gene disruption can provide a route for developing rice material with improved responses to saline conditions. However, the value of such modifications depends on the severity and duration of salt exposure, developmental stage and genetic background. Future evaluations should determine whether the observed tolerance translates into improved survival, reproductive performance and grain yield under field-relevant salinity conditions.

The *OsDST* gene has also been investigated as a potential target for improving responses to drought and salinity. Kumar *et al*. (2020) used CRISPR/Cas9-mediated editing of *OsDST* in the indica rice cultivar MTU1010, demonstrating the application of genome editing in an agronomically relevant genetic background. Such work is important because editing results obtained in a model genotype cannot automatically be transferred to elite indica varieties or other breeding populations. The effects of a mutation may vary with genetic background, plant developmental stage and environmental conditions. Therefore, stress-related genome editing should combine controlled physiological screening with measurements of biomass, reproductive performance and yield under multiple water and salinity regimes.

6.3. Genome Editing for Disease Resistance

Disease resistance is an important objective in rice improvement because pathogens can substantially reduce yield and grain quality. Genome editing offers two complementary strategies: modifying genes that positively regulate defence responses and modifying host susceptibility genes that pathogens exploit during infection. The latter approach is particularly useful when the targeted host gene is required for pathogen compatibility but is not essential for normal plant performance. Wang *et al*. (2016) demonstrated that CRISPR/Cas9-mediated disruption of *OsERF922*, an ethylene-response-factor transcription-factor gene, could improve rice blast resistance. This study established an important example of using targeted mutagenesis to investigate host defence mechanisms and generate disease-resistant rice material, Table 1.

Bacterial blight, caused by *Xanthomonas oryzae* pv. *oryzae*, is another major constraint on rice production. Certain bacterial transcription activator-like effectors induce host *SWEET* sugar-transporter genes by binding to specific promoter sequences, thereby contributing to disease susceptibility. Oliva *et al*. (2019) used genome editing to modify *SWEET* gene promoters and developed rice lines with broad-spectrum resistance to bacterial blight. Their study demonstrated that editing pathogen-responsive promoter regions can interfere with disease susceptibility while retaining the broader gene function. This strategy is particularly relevant to precision breeding because regulatory modification may preserve essential biological activities that could be disrupted by complete gene knockout.

The possibility of combining resistance to multiple diseases has further expanded the potential of genome editing. Zhou *et al*. (2022) used CRISPR/Cas9 to modify three genes associated with broad-spectrum blast resistance *Bsr-d1*, *Pi21* and *ERF922* in an indica thermosensitive genic male-sterile rice line. The edited material showed improved blast resistance, demonstrating the value of targeted modification of susceptibility-related pathways. Tao *et al*. (2021) also reported enhanced resistance to both rice blast and bacterial blight through editing multiple susceptibility genes. These studies support the use of multiplex editing for disease management, although the durability of resistance and its effects on plant development must be evaluated across pathogen populations and production environments.

6.4. Genome Editing for Grain Quality and Nutritional Improvement

Grain quality is determined by multiple characteristics, including starch composition, amylose content, grain appearance, aroma, cooking properties and nutritional composition. These traits influence consumer preference and the market value of rice varieties. Genome editing can be used to modify genes controlling grain composition and investigate the relationship between specific alleles and quality characteristics. The *Waxy* (*Wx*) gene is a major determinant of amylose content, while *BADH2* is associated with the production of the aroma compound 2-acetyl-1-pyrroline. Zhang *et al*. (2023) simultaneously edited *Wx* and *BADH2* in parental lines of a two-line hybrid rice, generating material with altered starch characteristics and aroma. This study illustrates how genome editing can combine modifications of different grain-quality traits within a breeding framework.

The application of genome editing to coloured rice varieties provides further opportunities to modify sensory and compositional characteristics. Wu *et al*. (2025) used CRISPR/Cas9 to edit *BADH2* and *Wx* in black and red rice, aiming to improve fragrance and texture. Such studies demonstrate that genome editing can be used not only to improve productivity but also to tailor rice quality to specific consumer and market requirements. However, modifications in starch composition may affect gelatinization, cooking properties and grain texture in different ways. Therefore, edited material should be assessed using biochemical measurements, grain-quality analysis and sensory or processing evaluations where appropriate, rather than relying on the presence of a particular mutation alone.

Nutritional improvement provides another potential application, although the relationship between individual genes and nutritional outcomes can be complex. Rice breeding programmes may seek to modify grain mineral composition, protein characteristics, starch digestibility or other nutritional attributes. Genome editing can facilitate the investigation of candidate

genes involved in nutrient transport, storage and metabolism, but the resulting phenotype must be evaluated for nutritional bioavailability and potential unintended changes in grain composition. The suitability of an edited allele also depends on the target population and intended use of the rice product. Accordingly, quality-oriented genome editing should integrate molecular characterization with biochemical analysis, agronomic evaluation and appropriate food-quality assessment.

6.5. Genome Editing for Nitrogen-Use Efficiency

Nitrogen-use efficiency (NUE) is an important target for sustainable rice production because nitrogen fertilizer inputs contribute to production costs and environmental pollution. NUE depends on nitrogen acquisition, transport, assimilation, internal remobilization and the conversion of absorbed nitrogen into grain yield. Genetic modification of regulatory pathways controlling nitrogen uptake and utilization may therefore provide opportunities to improve productivity while reducing excessive fertilizer requirements. Liu *et al*. (2023) used CRISPR/Cas9 to eliminate *OsHHO3*, a transcriptional repressor of three ammonium transporter genes, and reported improved nitrogen-use efficiency in rice. Their findings demonstrate how targeted editing of a regulatory gene can influence nutrient acquisition and provide a potential route for developing more resource-efficient cultivars, Table 1.

Further work has investigated targeted mutagenesis as a strategy for improving nitrogen-use efficiency in japonica rice. Tao *et al*. (2025) examined CRISPR-mediated targeted mutagenesis for this purpose, expanding the evidence base for genome editing in nutrient management. Such approaches are relevant because improved nitrogen uptake does not necessarily translate into greater grain yield or lower environmental impact under every fertilizer regime. A useful edited line should be evaluated across nitrogen supply levels and should maintain acceptable yield, grain quality and nitrogen utilization under realistic production conditions. Integration of genome editing with conventional breeding and multi-environment trials is therefore necessary to determine the practical value of NUE-related genetic modifications.

6.6. Genome Editing for Complex and Quantitative Traits

Complex agronomic traits are influenced by multiple genes, regulatory networks and environmental factors. Consequently, modifying a single locus may produce only a partial improvement or may introduce trade-offs with other traits. Multiplex genome editing offers an opportunity to investigate combinations of candidate genes and generate favourable allelic combinations within a single genetic background. Ma *et al*. (2015) established a multiplex genome-editing system for plants, while Li *et al*. (2016) demonstrated the simultaneous investigation of several rice yield-related genes. These approaches provide a basis for examining how multiple genetic modifications interact and whether combinations of edits can produce more useful phenotypes than individual mutations, Table 1.

The application of multiplex editing to complex traits requires careful prioritization of targets and rigorous evaluation of combined effects. For example, improving drought tolerance while maintaining high yield may require modifications to water-use regulation, root development and reproductive performance rather than changes to a single stress-response gene. Similarly, improving grain number without compromising grain filling may require coordinated adjustment of developmental pathways. Candidate targets should therefore be selected using genetic evidence, functional validation and physiological data. The resulting lines should be evaluated for the intended trait, potential pleiotropic effects and performance under relevant environmental conditions before they are advanced into breeding programmes.

6.7. From Edited Plants to Breeding-Ready Cultivars

The generation of a genome-edited plant is an intermediate step in crop improvement rather than the final outcome. After editing, plants require confirmation of the intended mutation, evaluation of genetic stability and assessment of the target phenotype. When editing constructs are integrated into the genome, segregation analysis may be required to identify lines that retain the desired edit without the editing construct, where this is feasible and relevant to the breeding strategy. Subsequent evaluation should examine agronomic performance, grain quality, disease response and stress tolerance under conditions relevant to the intended production environment. The success of genome editing must therefore be judged by the performance of the resulting material rather than by editing efficiency alone.

A breeding-oriented pipeline should connect candidate-gene discovery, target selection, genome editing, molecular characterization and field evaluation. Promising lines can then be incorporated into conventional breeding programmes, where their performance is assessed across genetic backgrounds and environments. This is particularly important for traits such as yield, drought tolerance and nitrogen-use efficiency, whose expression depends strongly on environmental conditions. By integrating genome editing with established breeding and evaluation procedures, researchers can improve the probability that molecular modifications translate into useful and stable cultivar improvements.

7. INTEGRATING MULTI-OMICS WITH CRISPR-BASED PRECISION BREEDING IN RICE

7.1. Integrating Genomics and Transcriptomics for Candidate-Gene Discovery

The integration of genomics and transcriptomics provides a framework for connecting genetic variation with gene activity and agronomic phenotypes. Genomic approaches, including genome-wide association studies (GWAS), quantitative trait locus mapping and whole-genome resequencing, identify candidate loci associated with variation in important traits. Transcriptomics complements these approaches by revealing differences in gene expression across tissues, developmental stages and environmental conditions. Combining these datasets can help prioritize genes that are both genetically associated with a trait and biologically responsive under relevant conditions. Nevertheless, an overlap between a genetic association and differential expression does not establish causality; additional functional experiments are needed to determine whether the candidate gene contributes directly to the phenotype (Wang *et al*., 2018; Chen *et al*., 2022).

In rice, the integration of genomic and transcriptomic evidence can be particularly useful for identifying genes associated with drought tolerance, disease resistance, grain development and nutrient-use efficiency. For example, a drought-associated

genomic region can be investigated alongside expression profiles from drought-treated and well-watered plants to identify genes whose expression patterns are consistent with the proposed biological function. The candidate genes can then be prioritized using sequence variation, expression specificity, known biological pathways and available mutant information. Such integration can reduce the number of candidates requiring experimental validation, although the reliability of the prioritization depends on the quality of the genomic annotation, phenotypic data and experimental design (Huang *et al*., 2010; Chen *et al*., 2022).

7.2. Integrating Proteomics and Metabolomics

Transcriptomic data provide information about RNA abundance, but transcript levels do not always correspond directly to protein abundance or biological activity. Proteomics helps address this limitation by examining protein abundance, post-translational modifications and other features that influence cellular function. In rice, proteomic analysis can provide evidence about enzymes, signalling components and stress-response proteins associated with developmental or environmental responses. Combining proteomic information with genomic and transcriptomic datasets can help distinguish changes that are reflected at the protein level from those that occur primarily at the transcriptional level. However, protein abundance alone does not necessarily indicate enzymatic activity or establish a causal relationship with the phenotype (Agrawal *et al*., 2009; Hasin *et al*., 2017).

Metabolomics provides a complementary perspective by measuring changes in metabolites associated with primary and secondary metabolism. In rice, metabolite profiles can help investigate biochemical responses associated with stress adaptation, grain composition, nutrient utilization and plant development. Integration with transcriptomics and proteomics can connect candidate genes with the biochemical pathways in which they may function. For instance, changes in genes associated with amino acid metabolism can be examined alongside relevant protein abundance and metabolite concentrations to develop a more complete hypothesis about the underlying mechanism. Nevertheless, metabolite accumulation can be influenced by multiple pathways, developmental stages and environmental conditions, so these patterns should be interpreted as supporting evidence rather than definitive proof of gene function (Fukushima *et al*., 2009; Hasin *et al*., 2017).

7.3. Multi Omics Integration for Understanding Abiotic Stress Responses

Abiotic stress tolerance involves coordinated changes in gene expression, protein activity, metabolism and physiological processes. Drought, salinity and temperature stress can affect water relations, membrane stability, photosynthesis, hormonal signalling and the accumulation of protective metabolites. A multi-omics approach can investigate these responses at several biological levels, allowing researchers to identify pathways that are consistently associated with stress exposure or tolerance. However, differences between tolerant and sensitive genotypes may reflect developmental variation, stress severity or differences in the timing of sampling. A rigorous analysis therefore requires appropriate biological replication, standardized stress treatments and integration with physiological measurements (Fukushima *et al*., 2009; Chen *et al*., 2022).

In rice, multi-omics analysis can help prioritize genes that participate in several components of a stress-response pathway. A candidate transcription factor, for example, may be associated with drought tolerance through its expression pattern and its predicted regulatory relationships, while proteomic and metabolomic data may provide additional evidence about the downstream processes involved. Such a candidate can then be evaluated using targeted mutagenesis or another suitable functional-genomics approach. This process allows molecular profiling to guide experimental design, while the resulting genetic perturbation tests whether the proposed regulatory relationship is biologically meaningful. The integration of multi-omics evidence with functional validation is therefore more informative than relying on a single molecular dataset (Hasin *et al*., 2017; Chen *et al*., 2022).

7.4. Gene-Regulatory Networks and Systems Biology

Gene-regulatory networks provide a framework for understanding how genes, transcription factors and signalling pathways interact to control biological processes. In rice, these networks are relevant to development, flowering, grain formation and responses to environmental stress. Network-based analysis can help identify candidate regulatory genes that influence multiple downstream components rather than individual traits in isolation. RiceNet v2, developed by Lee *et al*. (2015), provides an integrated functional association network for investigating relationships among rice genes. Such resources can help prioritize candidate genes, generate hypotheses about biological pathways and identify potential interactions that warrant experimental investigation.

Network analysis can also support the selection of genome-editing targets by identifying genes positioned at important points in a regulatory pathway. However, network centrality or the number of predicted interactions does not necessarily mean that a gene is the best editing target. A highly connected regulator may have broad effects on development, potentially creating undesirable pleiotropic consequences when modified. Candidate targets should therefore be evaluated using genetic evidence, tissue-specific expression, known biological function and the predicted consequences of perturbation. Combining network analysis with targeted gene editing can help test specific regulatory hypotheses, provided that the resulting molecular and phenotypic effects are independently validated (Lee *et al*., 2015; Chen *et al*., 2022).

7.5. Using Multi Omics to Prioritize CRISPR Editing Targets

The selection of an appropriate genome-editing target requires more than identifying a gene associated with a desirable trait. A strong candidate should be supported by genetic evidence, have a plausible biological function and be suitable for the type of modification intended. Multi-omics integration can contribute to this decision by combining sequence variation, gene expression, protein abundance, metabolic profiles and regulatory-network information. Such evidence can help distinguish genes that are merely responsive to a condition from those that are plausible contributors to the phenotype. Nevertheless, the strength of the evidence varies among datasets, and computational prioritization should be treated as a means of ranking hypotheses rather than confirming gene function (Hasin *et al*., 2017; Chen *et al*., 2022).

Once a candidate gene has been prioritized, the intended biological change should determine the editing strategy. If loss of function is expected to produce the desired phenotype, CRISPR/Cas9-mediated mutagenesis may be appropriate. If a favourable allele requires a particular nucleotide substitution, base editing may offer a more suitable approach, whereas prime editing can provide additional flexibility for selected sequence alterations. Lin *et al*. (2020) demonstrated prime editing in rice and wheat, illustrating how precise sequence modification can complement the identification of useful alleles. The final selection should consider the target sequence, expected biological effect, editing feasibility and potential consequences for other traits.

7.6. Computational Prediction and AI-Assisted Target Selection

Computational methods can support genome editing by prioritizing candidate genes, predicting guide-RNA activity and identifying potential off-target sites. Machine-learning models trained on experimental data can estimate guide-RNA performance and help researchers select candidate guides for laboratory testing. Doench *et al*. (2016) developed an optimized framework for designing highly active single-guide RNAs for CRISPR/Cas9-mediated gene inactivation, demonstrating the value of sequence-based prediction in editing design. Haeussler *et al*. (2016) developed CRISPOR, a guide-RNA selection tool that integrates on-target and off-target scoring approaches. These resources provide useful computational support, although their predictions do not guarantee editing efficiency or specificity in every rice genotype or experimental system.

Artificial intelligence can also be used to integrate genomic, transcriptomic and phenotypic information to prioritize candidate genes for breeding applications. In principle, models trained on sufficiently diverse and well-annotated datasets could help identify relationships among sequence variation, gene regulation and agronomic traits. However, model reliability depends on the representativeness of the training data, the quality of phenotypic measurements and the extent to which predictions transfer across genetic backgrounds and environments. Predictions should therefore be validated experimentally before they are used to justify a genome-editing intervention. AI-assisted analysis is best regarded as a decision-support component within a broader experimental framework, rather than a replacement for functional genomics and field evaluation (Doench *et al*., 2016; Haeussler *et al*., 2016).

7.7. Integrating Genome Editing with Genomic Selection

Genomic selection estimates the genetic merit of breeding candidates using genome-wide marker information and statistical prediction models. Genome editing, in contrast, enables targeted modification of selected genomic loci. Integrating these approaches may allow breeders to modify a promising candidate allele while using genomic prediction to evaluate the broader genetic background of the resulting material. This distinction is important because an edit that improves one component of a complex trait may not necessarily improve total yield or performance across environments. Xu *et al*. (2021) reviewed the role of genomic selection in rice breeding, highlighting its potential to improve the efficiency of selection for quantitative traits.

A practical integrated strategy could begin with genomic prediction to identify elite breeding lines carrying favourable combinations of alleles, followed by functional validation of a candidate locus and targeted editing where justified. The edited material could then be assessed using molecular characterization, phenotyping and genomic information to determine whether the intended modification produces the expected outcome. Where necessary, edited lines could be incorporated into crossing programmes to combine the targ*et al*lele with other desirable characteristics. However, this approach requires careful management of genetic background, possible linkage effects and genotype-by-environment interactions. Genomic selection and genome editing are therefore complementary approaches whose value depends on the quality of the breeding population and the evidence supporting the targ*et al*lele (Xu *et al*., 2021; Wei *et al*., 2021).

7.8. An Integrated Multi-Omics-to-Cultivar Framework

A practical framework for precision breeding in rice can be organized into a sequence of connected stages: genetic discovery, multi-omics integration, candidate-gene prioritization, functional validation, genome editing, molecular characterization and agronomic evaluation. Genomic resources identify sequence variation and candidate loci, while transcriptomics, proteomics and metabolomics provide complementary evidence about their potential biological functions. Regulatory-network analysis can help develop hypotheses about gene interactions, and computational prediction can assist in selecting editing targets and guide RNAs. Nevertheless, each stage should retain explicit validation criteria so that associations and predictions are not mistaken for experimentally established causal relationships (Hasin *et al*., 2017; Chen *et al*., 2022).

Following candidate validation, the appropriate editing system should be selected according to the intended sequence modification and expected biological effect. Edited plants should undergo molecular characterization and evaluation of relevant phenotypes, including potential unintended effects and changes in other agronomic traits. Promising material should then be tested in suitable genetic backgrounds and environments to determine whether the modification improves field performance and breeding value. This framework connects molecular discovery with cultivar development while recognizing that genome editing is one component of a broader crop-improvement process. Its ultimate effectiveness depends on the quality of the evidence at each stage and the reproducibility of the resulting agronomic benefits.

8. TECHNICAL, BIOLOGICAL AND TRANSLATIONAL CHALLENGES IN RICE GENOME EDITING

8.1. Genotype-Dependent Transformation and Regeneration

Although CRISPR-based genome editing has expanded the possibilities for rice improvement, efficient delivery of editing reagents and regeneration of edited plants remain important bottlenecks. Rice transformation efficiency is strongly influenced by genotype, explant type, tissue-culture conditions, and regeneration capacity. Protocols developed for a model cultivar may not transfer directly to elite breeding lines, landraces, or locally adapted varieties. This limitation is particularly important for precision breeding because an effective edit has limited practical value if the target cultivar cannot be transformed efficiently

or regenerated without undesirable variation (Altpeter *et al*., 2016; Hiei and Komari, 2008).
Tissue culture can also introduce somaclonal variation, including genetic and epigenetic changes unrelated to the intended edit. Consequently, regenerated plants should be evaluated for both the target modification and unintended variation, especially when the edited material is intended for advanced breeding or cultivar development. Improvements in transformation protocols, genotype-independent regeneration, and DNA-free delivery approaches may help reduce these constraints, although their effectiveness depends on the genotype and editing system employed (Altpeter *et al*., 2016; Barampuram and Zhang, 2011).

8.2. Off-Target Mutations and Genome Integrity

A central technical challenge is distinguishing the intended genetic modification from unintended mutations elsewhere in the genome. CRISPR-associated nucleases may cleave sequences with partial similarity to the guide RNA target, depending on sequence context, mismatch position, nuclease type, and experimental conditions. Although computational tools can predict potential off-target sites, prediction alone cannot establish that an edited plant is free from unintended changes. Appropriate validation therefore combines guide-RNA design, molecular screening of likely off-target sites, and broader genomic assessment when justified by the application (Tsai and Joung, 2016; Zischewski *et al*., 2017).
The consequences of editing extend beyond small insertions and deletions. DNA double-strand breaks can occasionally generate large deletions, complex rearrangements, or other structural changes that may not be detected by routine PCR-based screening. These findings highlight the importance of evaluating genome integrity rather than relying exclusively on confirmation of the intended edit. For rice breeding, the intensity of validation should be proportionate to the editing strategy, the biological significance of the target gene, and the requirements for the intended use of the resulting line (Kosicki *et al*., 2018; Zischewski *et al*., 2017).

8.3. Editing Efficiency, Mosaicism and Inheritance

Editing outcomes vary according to the nuclease system, guide-RNA sequence, chromatin accessibility, target-locus characteristics, and the repair processes operating in the transformed cell. Consequently, an editing construct may generate a mixture of alleles rather than a uniform genetic modification. Mosaicism can arise when editing occurs after cell division has begun, complicating the identification of plants carrying a stable, heritable edit. Molecular screening of regenerated plants and their subsequent generations is therefore necessary to establish the genotype and inheritance pattern of the target modification (Tsai and Joung, 2016; Zischewski *et al*., 2017).
The distinction between detecting an edit in a regenerated plant and demonstrating stable transmission through seed is particularly important in rice breeding. Segregation analysis can help identify progeny carrying the desired allele while lacking the editing construct, where construct segregation is applicable. However, inheritance patterns depend on the editing event, ploidy, reproductive biology, and breeding design. A robust validation pipeline should therefore confirm sequence-level changes, assess relevant phenotypes, and establish genetic stability across generations before edited lines are advanced for field evaluation (Altpeter *et al*., 2016; Kosicki *et al*., 2018).

8.4. Pleiotropy, Genetic Background and Trait Trade-Offs

A further challenge is that genes associated with desirable agronomic traits may influence several biological processes simultaneously. For example, modifying a regulator of plant architecture may alter tillering, panicle development, biomass allocation, and grain yield. Therefore, a change that improves one trait under a particular experimental condition may produce undesirable effects on other traits or under different growing environments. Gene editing should consequently be guided by knowledge of gene function, allelic variation, genetic background, and the physiological relationships among target traits rather than by the magnitude of a single observed response (Jiao *et al*., 2010; Li *et al*., 2016).
This issue is particularly relevant to complex traits such as drought tolerance, nitrogen-use efficiency, and yield stability. These traits are controlled by multiple genes and are strongly affected by environmental conditions and developmental stage. Editing a major-effect gene may produce a useful improvement, but it does not necessarily reproduce the performance of a naturally adapted genotype. Combining functional validation with quantitative genetics, multi-environment trials, and appropriate breeding strategies can help identify edits that deliver consistent benefits without compromising yield potential or grain quality (Huang *et al*., 2010; Wei *et al*., 2021).

8.5. Phenotyping and Multi-Environment Validation

Molecular confirmation of an edited allele is not sufficient to establish its agronomic value. Edited rice lines must also be evaluated for plant growth, yield components, grain quality, and relevant stress responses under controlled and field conditions. Traits such as drought tolerance and nutrient-use efficiency are particularly challenging because their expression depends on stress intensity, soil properties, crop developmental stage, and management practices. Replicated experiments across relevant environments are therefore needed to distinguish genuine genetic improvements from environmental variation and experimental noise (Huang *et al*., 2010; Wei *et al*., 2021).
Field validation should also examine the stability of the target phenotype and its effects on associated traits. An edit that improves performance under one stress regime may not provide the same advantage under another, and benefits observed in controlled environments may be reduced under variable field conditions. Integrating molecular genotyping with standardized phenotyping, multi-location testing, and suitable statistical models can strengthen the evidence required to advance promising edited lines into breeding programmes. This approach is essential for ensuring that genome editing contributes to practical cultivar improvement rather than remaining limited to proof-of-concept experiments (Altpeter *et al*., 2016; Wei *et al*., 2021).

8.6. Regulatory Requirements, Biosafety and Market Acceptance

The commercialization of genome-edited rice depends not only on technical performance but also on regulatory requirements, biosafety assessment, intellectual-property considerations, and market acceptance. Regulatory treatment differs

among countries and may depend on the type of genetic modification, whether foreign DNA remains in the final plant, and the applicable national definitions of genome editing and genetically modified organisms. Consequently, researchers should determine the relevant regulatory pathway early in product development rather than assuming that all CRISPR-edited plants will receive the same classification (National Academies of Sciences, Engineering, and Medicine, 2016; OECD, 2021).

Risk assessment should be proportionate to the characteristics of the edited plant and its intended use. Relevant considerations may include the biological function of the altered gene, potential effects on plant performance and ecological interactions, the presence of unintended genetic changes, and the characteristics of the receiving environment. Transparent documentation of the editing process, molecular characterization, and phenotypic evaluation can support regulatory review and communication with stakeholders. At the same time, public communication should distinguish genome editing from other breeding approaches accurately and avoid presenting the technology as inherently risk-free or inherently hazardous (National Academies of Sciences, Engineering, and Medicine, 2016; OECD, 2021).

8.7. Cost, Infrastructure and Access for Breeding Programmes

Advanced genome-editing approaches require access to molecular laboratories, transformation and tissue-culture facilities, sequencing services, computational resources, and trained personnel. These requirements may limit the adoption of the technology by smaller breeding programmes and institutions with constrained research budgets. The cost of developing an edited line also extends beyond generating the mutation: molecular validation, seed multiplication, field evaluation, quality testing, and regulatory compliance may require substantial additional investment. Thus, the practical value of a genome-editing strategy should be assessed in relation to its expected breeding benefit and the resources needed to deliver a usable cultivar (Altpeter *et al*., 2016; National Academies of Sciences, Engineering, and Medicine, 2016).

For rice-growing regions where climate resilience and productivity are major priorities, collaborative research networks may help distribute these costs and expand access to suitable technologies. Shared transformation platforms, validated molecular protocols, public genomic resources, and partnerships between universities, research institutes, and breeding organizations can improve efficiency. Prioritizing traits with clear farmer and consumer benefits—such as stable yield under drought, improved disease resistance, grain-quality enhancement, or more efficient nutrient use—can also help ensure that genome-editing research addresses practical agricultural needs (Altpeter *et al*., 2016; OECD, 2021).

9. FUTURE PERSPECTIVES AND RESEARCH PRIORITIES

9.1. From Gene Discovery to Predictive Rice Breeding

The next phase of rice improvement will require a transition from identifying genes associated with agronomic traits to predicting how specific genetic modifications influence whole-plant performance. Genome-wide association studies, quantitative trait locus mapping, and functional genomics have identified numerous candidate genes and genomic regions associated with yield, grain quality, and stress responses. However, the practical value of these discoveries depends on establishing causal relationships and determining whether the resulting alleles perform consistently across genetic backgrounds. Integrating population genomics with functional validation and precision editing can help bridge the gap between gene discovery and cultivar development (Huang *et al*., 2010; Wang *et al*., 2018; Wei *et al*., 2021).

Future research should prioritize candidate genes supported by multiple independent evidence sources, including genetic association, expression patterns, functional experiments, and physiological relevance. Rather than focusing exclusively on single genes with large effects, researchers should also investigate regulatory elements and combinations of alleles that contribute to complex traits. This strategy could help identify modifications that improve yield stability and stress adaptation while minimizing undesirable effects on plant architecture, grain quality, or maturity (Zhao *et al*., 2018; Wei *et al*., 2021).

9.2. Editing Promoters and Other Regulatory Regions

An important direction for future rice breeding is the targeted modification of cis-regulatory elements rather than the complete disruption of protein-coding genes. Promoters, enhancers, and other regulatory sequences influence the timing, location, and magnitude of gene expression. Editing these regions may allow breeders to adjust the expression of genes involved in plant architecture, stress responses, and disease resistance while retaining essential biological functions. This approach is particularly promising where complete gene knockout produces undesirable pleiotropic effects or reduces agronomic performance (Rodríguez-Leal *et al*., 2017; Li *et al*., 2017).

Regulatory-region editing could also facilitate the development of new allelic series, in which different edits produce graded changes in gene expression. Such variation may be more useful for quantitative traits than a binary knockout strategy because optimal expression levels can depend on developmental stage, environment, and genetic background. Future studies should therefore combine regulatory-sequence analysis, expression profiling, and systematic phenotyping to determine which edits produce useful and stable changes in rice performance (Rodríguez-Leal *et al*., 2017; Li *et al*., 2017).

9.3. Multiplex Editing for Complex Agronomic Traits

Many important rice traits are controlled by multiple genes, making multiplex genome editing a promising strategy for modifying several targets within a single experimental programme. By directing editing reagents to multiple genomic sites, researchers can investigate gene interactions, combine beneficial alleles, and modify related biological pathways more efficiently than through sequential single-gene editing alone. Nevertheless, multiplex editing requires careful guide-RNA design and independent confirmation of each target because editing efficiencies may differ among loci and combinations of edits can produce unexpected phenotypes (Ahmar *et al*., 2024; Rengasamy *et al*., 2024).

Future research should distinguish between multiplex editing for functional genomics and multiplex editing intended for cultivar development. In functional studies, simultaneous disruption of several genes can reveal redundancy and interactions among members of gene families. In breeding applications, however, each edit must be assessed for its contribution to the

target phenotype and for possible trade-offs involving yield, grain quality, development, or stress adaptation. A systematic prioritization framework that considers biological evidence, technical feasibility, expected benefit, and validation requirements can help focus resources on the most promising combinations (Ahmar *et al*., 2024; Thiruppathi *et al*., 2024).

9.4. Improving Editing Delivery and Precision

Improved delivery systems will be essential for extending genome editing to a broader range of rice germplasm. Conventional transformation can be limited by genotype dependence, tissue-culture requirements, and the time needed to regenerate plants. Transient delivery of editing components, ribonucleoprotein-based approaches, and other methods that avoid stable integration of editing constructs may offer alternatives for particular applications. However, the feasibility of these approaches depends on the delivery system, target tissue, regeneration method, and the type of edit required (Altpeter *et al*., 2016; Ahmar *et al*., 2024).

Base editing and prime editing are also expected to expand the range of attainable genetic modifications. Base editors can introduce selected nucleotide substitutions without relying on conventional double-strand-break-mediated editing, whereas prime editors can support targeted substitutions and certain insertions or deletions. Their application in rice will depend on improving editing efficiency, reducing unwanted by-products, and validating the resulting alleles across relevant genetic backgrounds. Continued optimization should be guided by the specific biological objective rather than by the assumption that one editing platform will be optimal for every target (Lin *et al*., 2020; Rengasamy *et al*., 2024).

9.5. Integrating Genome Editing with Genomic Selection and Artificial Intelligence

Genome editing is likely to become more useful when integrated with genomic selection and predictive breeding rather than being used as a stand-alone technique. Genomic selection estimates breeding values using genome-wide marker information, helping breeders identify promising individuals for complex traits. Genome editing, by contrast, enables targeted modification of selected genomic regions. Combining these approaches could support the prioritization of candidate alleles, the development of edited parental lines, and the evaluation of resulting material within established breeding pipelines. The benefits will depend on the quality of training populations, the reliability of trait prediction, and the extent to which candidate edits have been functionally validated (Xu *et al*., 2021; Wei *et al*., 2021).

Artificial intelligence and machine learning may further assist in guide-RNA selection, prediction of editing outcomes, analysis of multi-omics datasets, and identification of gene networks associated with agronomic performance. However, computational predictions are only as reliable as the data and assumptions on which they are trained. Models developed using a limited number of cultivars or controlled environments may not generalize to diverse rice germplasm or field conditions. Future applications should therefore combine computational prioritization with experimental validation, transparent reporting, and independent evaluation of predictive performance (Doench *et al*., 2016; Haeussler *et al*., 2016).

9.6. Developing Locally Adapted and Accessible Edited Rice Varieties

An important future priority is to apply genome editing to locally adapted rice varieties rather than concentrating exclusively on a small number of laboratory or transformation-friendly genotypes. Local cultivars may contain useful adaptations to regional climate, soil conditions, grain preferences, and farming systems. Editing selected alleles directly in these backgrounds could help preserve their existing characteristics while addressing specific limitations, although transformation and regeneration challenges must still be overcome. Large-scale rice genomic resources provide a foundation for identifying useful natural variation and choosing appropriate genetic backgrounds for improvement (Wang *et al*., 2018; Zhao *et al*., 2018).

Successful deployment will also require coordination among molecular biologists, plant breeders, crop physiologists, seed systems, and regulatory authorities. Breeding targets should reflect the needs of farmers and consumers, including yield stability, resistance to locally important diseases, grain quality, resource-use efficiency, and adaptation to changing environmental conditions. Participatory evaluation, transparent communication, and reliable seed production will be important for translating promising laboratory results into varieties that deliver measurable benefits under real farming conditions (Ahmar *et al*., 2024; Rengasamy *et al*., 2024).

10. CONCLUSIONS

The molecular genetics and genome-editing approaches have opened up new possibilities to explore, identify, and improve the genetic architecture of complex traits. Using an integrated approach of genomics resources, gene discovery, functional genomics, and CRISPR-based genome editing, we can now target candidate genes associated with agronomic traits such as grain yield, plant architecture, tolerance to abiotic stress, resistance to biotic stress, grain quality, and nutrient-use efficiency in rice (Oryza sativa L). The progress made in genome-wide association studies (GWAS), quantitative trait locus (QTL) mapping, whole-genome resequencing, and pan-genomes has enabled the discovery of candidate genes and favorable alleles. Transcriptomics, proteomics, and other functional-genomics techniques have helped reveal the molecular functions and networks of these candidate genes.

Further developments in the field of genome editing, such as, CRISPR/Cas9, Cas12a, base editing, prime editing, multiplex genome editing, and promoter editing, have opened up additional possibilities for the precise modification of target sites for new allele creation, gene function analysis, or improvement of complex agronomic traits. Yet, there are many factors to consider for application of genome editing – including target gene identification, editing efficiency, transformation and regeneration efficiency, genetic background, off-target/pleiotropic effects, time and labor resources for transformant regeneration, and time and resources for genotype evaluation. Moreover, molecular validation of a desired targeted change may not result in improved agronomic trait performance; comprehensive phenotype testing at the controlled and field levels is important in demonstrating the consistency and value of altered materials.

Finally, future research should be on bringing multi-omics, functional validation, genomic selection, AI, and precision genome editing to rice breeding to make it more efficient and effective. In this realm, a particular focus on generating genotype-independent transformation systems, improving editing specificity, deciphering gene-gene and genotype-environment interactions, and identifying regulatory variants to tune complex traits is anticipated. The research community should treat genome editing as an additional instrument to serve crop breeding as it does conventional breeding and other crop-improvement tools. Combining molecular discovery with traditional breeding, participatory evaluation, and farmer-oriented selection will be essential to turn molecular knowledge into scalable solutions. Ultimately, a gene-discovery-to-cultivar pipeline will drive the development of high-yielding, climate-resilient, disease-free, nutrient-efficient, and high-quality rice varieties for the long-term future of food security.

Table 1. Applications of Genome Editing and Genetic Transformation for Yield Improvement, Stress Tolerance, Disease Resistance, Grain Quality, and Nutrient-Use Efficiency in Rice.

No.	Gene / genetic component	Technology	Target trait	Major finding / application	Reference
1	*CKX2*	CRISPR/Cas9	Grain yield	DRR Dhan 100 (Kamala), developed from Samba Mahsuri; reported 19% higher mean yield.	ICAR-IIRR (2025)
2	*DST*	CRISPR/Cas9	Drought and salinity tolerance	Pusa DST Rice 1, developed from MTU 1010; improved performance under stress conditions.	ICAR-IARI (2025)
3	*Gn1a, DEP1, GS3, IPA1*	CRISPR/Cas9	Yield and plant architecture	Targeted mutations altered grain number, panicle architecture, grain size and tillering.	Li *et al*. (2016)
4	*OsRR22*	CRISPR/Cas9	Salinity tolerance	Targeted mutations improved salt tolerance in experimental rice lines.	Zhang *et al*. (2019)
5	*OsDST*	CRISPR/Cas9	Drought and salinity responses	Editing was investigated in the indica cultivar MTU 1010.	Kumar *et al*. (2020)
6	*OsERF922*	CRISPR/Cas9	Blast resistance	Targeted mutagenesis enhanced resistance to rice blast.	Wang *et al*. (2016)
7	*SWEET11, SWEET13, SWEET14 promoters*	CRISPR/Cas9	Bacterial blight resistance	Promoter modifications interfered with pathogen-mediated susceptibility and improved resistance.	Oliva *et al*. (2019)
8	*OsSWEET13*	CRISPR/Cas9	Bacterial blight resistance	Targeted editing was investigated in Indian rice genotype CO51.	Ancydiana *et al*. (2022)
9	*OsPDS*	CRISPR/Cas9	Functional genomics	Disruption generated a pigment-deficient phenotype, demonstrating targeted editing.	Zhang *et al*. (2014)
10	*BADH2*	CRISPR/Cas9	Grain aroma	Gene disruption was used to modify fragrance-related traits.	Shao *et al*. (2017)
11	*TMS5*	CRISPR/Cas9	Hybrid seed production	Targeted mutations generated male-sterile lines for hybrid breeding.	Zhou *et al*. (2016)
12	*OsMADS26, OsBsr-d1, OsELF3-2, OsERF922*	Multiplex CRISPR/Cas9	Disease resistance, abiotic stress and yield	A 2026 study reported improvements in multiple traits in experimental edited lines.	Luo *et al*. (2026)
13	*Sub1A*	Genetic transformation; subsequent marker-assisted breeding	Submergence tolerance	Functional validation established the role of Sub1A in submergence tolerance; the gene was subsequently introgressed into popular rice varieties.	Xu *et al*. (2006)
14	*Xa21*	Genetic transformation	Bacterial blight resistance	Functional characterization established Xa21 as a rice disease-resistance gene.	Song *et al*. (1995)
15	*psy, crtI*	Genetic transformation	Provitamin A biofortification	Carotenoid biosynthesis was introduced into rice endosperm, forming the basis of Golden Rice.	Ye *et al*. (2000)

16	*DREB1A*	Genetic transformation	Drought, salinity and cold responses	Transgenic expression was used to investigate abiotic-stress tolerance.	Dubouzet *et al*. (2003)
17	*SNAC1*	Genetic transformation / overexpression	Drought tolerance	Overexpression enhanced drought resistance in experimental rice plants.	Hu *et al*. (2006)
18	*cry genes*	Genetic transformation	Insect resistance	Insecticidal genes from Bacillus thuringiensis were introduced to investigate resistance to target insect pests.	Ye *et al*. (2001)

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