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Functional and Structural Insights from the *Oryza* Genome: Implications for Crop Enhancement

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Abstract The *Oryza* genus holds immense genetic diversity critical for global food security. This study synthesizes recent advances in the structural and functional genomics of *Oryza*, highlighting their implications for crop enhancement. Key insights include the identification of genes and quantitative trait loci (QTLs) associated with yield, stress resistance, and nutritional quality, facilitated by high-throughput sequencing technologies and comprehensive genomic databases. Emerging genomic tools, such as CRISPR/Cas9 and genomic selection, have enabled precise genetic modifications and accelerated breeding programs aimed at developing resilient, high-yielding rice varieties. The integration of genomics with other omics approaches provides a holistic understanding of the biological processes underlying agronomic traits. However, the deployment of these technologies necessitates addressing ethical, regulatory, and social considerations. This study underscores the potential of leveraging *Oryza* genomics to meet future food security challenges and emphasizes the need for continued research and innovation in rice breeding.

Keywords *Oryza* genome; Crop enhancement; Genetic diversity; Structural variations; Functional genomics; CRISPR/Cas9

1 Introduction

Rice (*Oryza sativa* L.) is a fundamental staple food crop, feeding more than half of the global population and playing a crucial role in global food security. The genus *Oryza*, which includes both cultivated and wild rice species, serves as a model system for studying molecular evolution and genetic diversity (Hechanova et al., 2021; Zhang et al., 2022). Understanding the *Oryza* genome is essential for improving rice varieties to meet the challenges posed by biotic and abiotic stresses, climate change, and the increasing food demands of a growing population. The genetic diversity within the *Oryza* genus, including structural variations (SVs) and gene copy number variations (gCNVs), has been pivotal in the evolution, domestication, and improvement of rice (Kong et al., 2019; Chen et al., 2023).

Over the past decade, significant advancements have been made in rice functional genomics, with over 2000 genes controlling important agronomic traits being cloned and partially characterized (Li et al., 2018). High-quality genome assemblies and pan-genome analyses have revealed hidden genomic variations and provided valuable resources for rice breeding and functional genomics research (Zhang et al., 2022). Studies have also focused on the regulatory mechanisms of complex traits, such as salt stress tolerance and grain yield, using advanced genomic tools like CRISPR/Cas9 and miRNA sequencing (Kong et al., 2019; Usman et al., 2020; Chen et al., 2023). Additionally, the development of genome-wide marker sets and introgression libraries has facilitated the efficient use of wild rice species for varietal improvement (Hechanova et al., 2021).

This study aims to synthesize the current knowledge on the functional and structural aspects of *Oryza* genomes, providing a comprehensive overview of key findings in genomic research. It emphasizes the implications of these discoveries for crop improvement and identifies gaps that need to be addressed in ongoing research. By integrating and analyzing existing literature, this study offers valuable insights for researchers and practitioners in the fields of plant genomics and breeding. It is hoped that this study will contribute to the development of more resilient and productive rice varieties, thereby supporting global food security and sustainable agriculture.

2 Structural Genomics of *Oryza*

2.1 Genome sequencing projects

The sequencing of the rice (*Oryza sativa*) genome has been a pivotal milestone in plant genomics, providing a comprehensive resource for genetic studies and crop improvement. The first complete rice genome was sequenced nearly two decades ago, setting a precedent for subsequent genomic projects. Among the major sequenced genomes are *Oryza sativa*, *Oryza rufipogon*, and *Oryza glaberrima*. The sequencing of these genomes has provided a comprehensive view of the genetic makeup of both cultivated and wild rice species, revealing insights into their domestication, adaptation, and potential for crop improvement (Chen et al., 2019).

Recent advancements have led to the development of gap-free reference genomes for two elite *O. sativa* varieties, 'Zhenshan 97 (ZS97)' and 'Minghui 63 (MH63)' (Figure 1), which are instrumental in studying heterosis and yield (Song et al., 2021). These high-quality reference genomes facilitate a deeper understanding of the genetic architecture and functional elements within the rice genome.

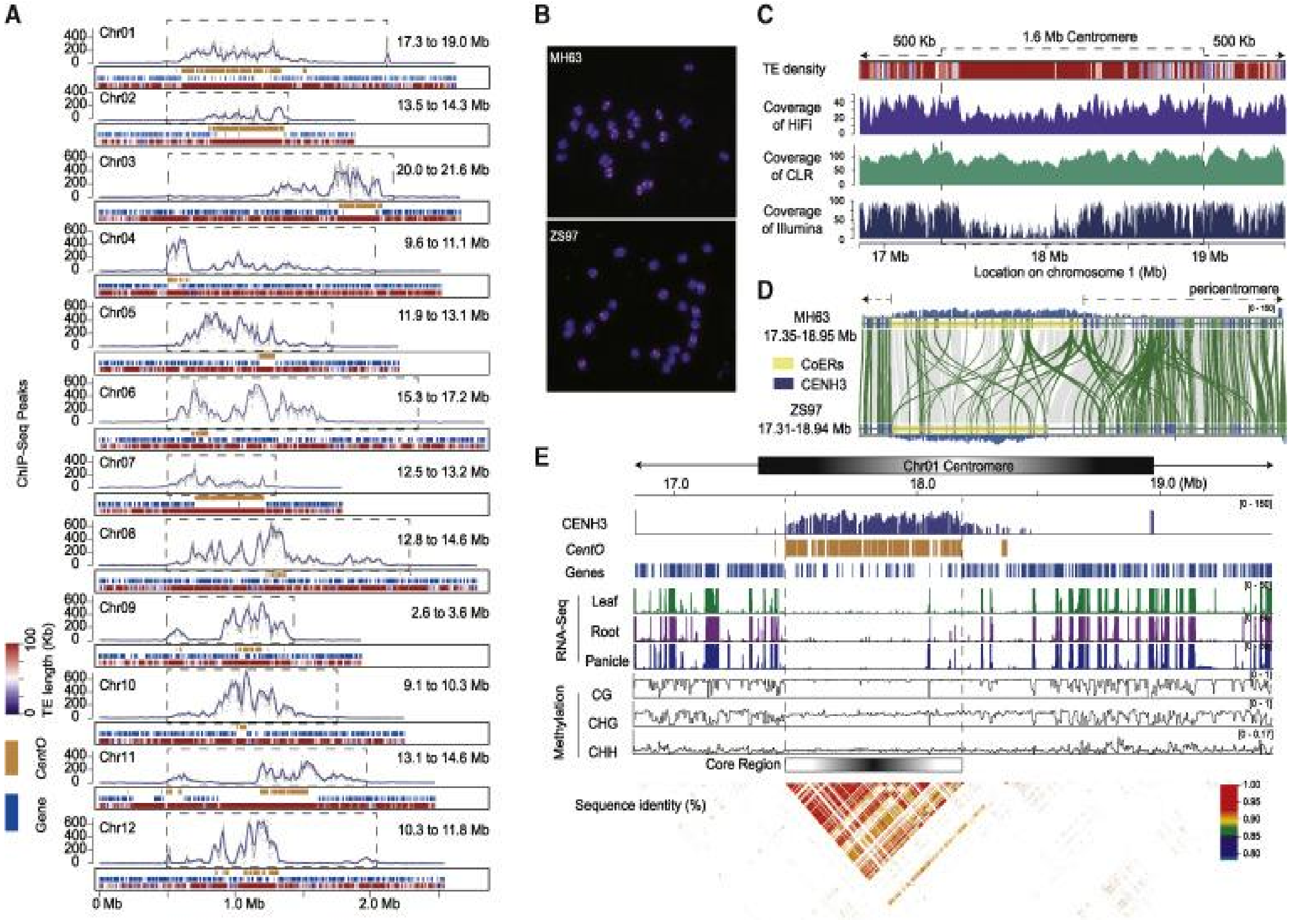


Figure 1 Characterization of complete rice centromeres (Adopted from Song et al., 2021)

Image caption: A: The delimiting of MH63RS3 centromeres. The layers of each chromosome graph indicate (1) the density of read mapping from CENH3 ChIP-seq with sliding windows of 10 kb and 20 kb shown in gray and blue lines, respectively; (2) the CentO satellite distribution; (3) non-TE gene distribution; and (4) TE distribution, respectively. The dotted frame represents the defined centromere region; B: Fluorescence in situ hybridization of mitotic metaphase chromosomes in MH63 and ZS97 using CENH3 ChIP-DNA as probe (red) with chromosomes counterstained with DAPI (blue); C: Coverage of HiFi, CLR, and Illumina reads and distribution of TEs in the centromere on chr1 (extended 500 kb left and right) of MH63RS3; D: The pairwise synteny visualization of chr1 centromere regions between ZS97RS3 and MH63RS3. Green lines link synteny genes between ZS97RS3 and MH63RS3. Yellow blocks are CoERs; E: Characteristics of the centromere on chr1 of MH63RS3. The 10 layers demonstrate the histone CENH3 distribution, CentO satellite distribution, gene distribution, gene expression level (in leaf, root, and panicle), methylation distribution (of CG, CHG, and CHH), and CentO sequence similarity, respectively (Adopted from Song et al., 2021)

Comparative genomics has revealed significant insights into the genetic diversity and evolutionary history of rice. High-confidence assemblies of genomes, such as those of *Oryza sativa* and *Oryza brachyantha*, have enabled detailed comparative analyses, revealing significant chromosomal rearrangements and gene duplications that contribute to phenotypic diversity and adaptability (Liao et al., 2018). Pangenomic studies, which integrate genomic data from multiple rice accessions, have highlighted the extensive structural variations (SVs) that contribute to genetic diversity within the species. These studies enable the identification of candidate SVs associated with important agronomic traits, thereby enhancing the potential for targeted breeding and crop improvement (Zanini et al., 2021). Additionally, the functional impact map of genetic variants (GVs) in rice provides a valuable resource for prioritizing causal variants in mapping populations, further aiding in the functional characterization of genetic diversity (Zhao et al., 2021).

2.2 Chromosomal organization and structure

Karyotyping and chromosome mapping have been essential tools in studying the structural organization of *Oryza* genomes. These techniques have helped identify the number, size, and shape of chromosomes, facilitating the mapping of genes and QTLs associated with important traits. Detailed chromosomal maps have been developed for several *Oryza* species, highlighting regions of structural diversity and evolutionary significance (Stein et al., 2018). In the study of Song et al. (2021), the gap-free reference genomes of 'ZS97' and 'MH63' have provided a global view of centromere architecture, revealing conserved centromere-specific satellite motifs and structural variants that affect gene copy numbers. These insights are crucial for elucidating the mechanisms of chromosomal behavior and stability, which are fundamental to rice breeding and genetic studies.

Structural variations, including inversions, duplications, deletions, and translocations, are prevalent in *Oryza* genomes and have significant implications for genetic diversity and adaptation. Studies have shown that these variations can affect gene expression and contribute to the domestication process. For instance, the identification of large inversions and translocations in indica and japonica rice varieties has provided insights into the evolutionary mechanisms underlying rice domestication (Kou et al., 2020).

2.3 Transposable elements and repeat sequences

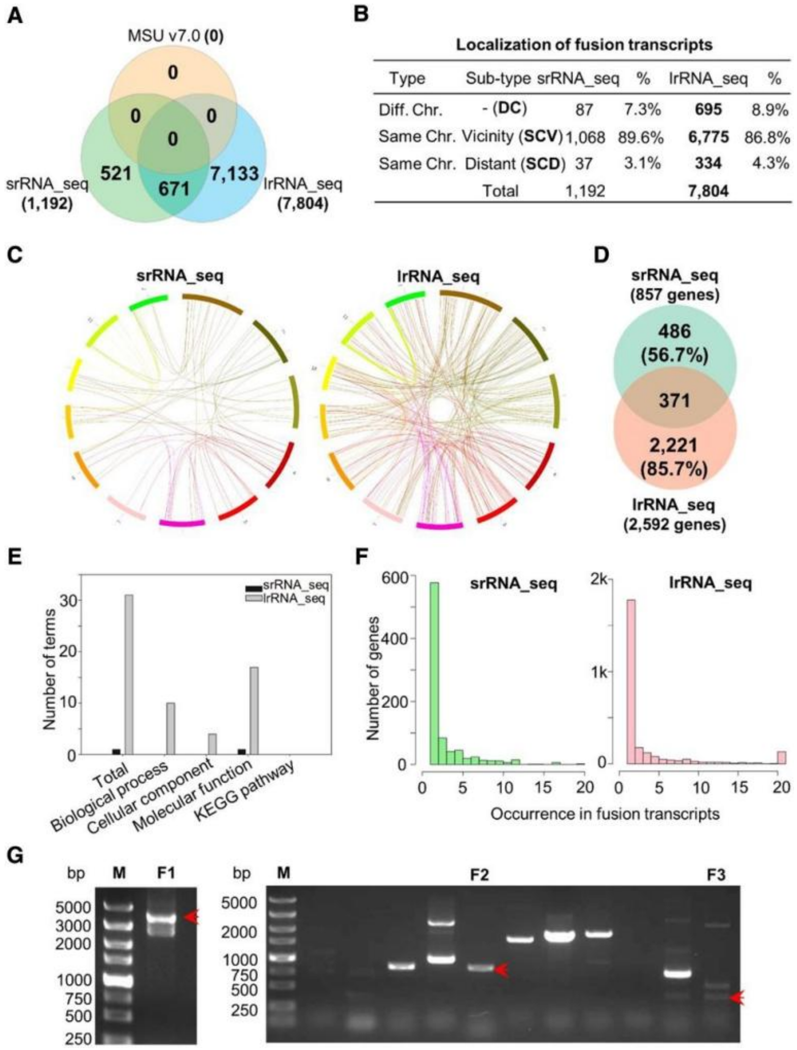
Transposable elements (TEs) and repeat sequences are abundant in the *Oryza* genome and play a pivotal role in shaping genome structure and evolution. TEs are classified into different types based on their mechanism of transposition, with long terminal repeat (LTR) retrotransposons being the most prevalent. The distribution of these elements varies across different *Oryza* species, contributing to genomic diversity and complexity (Dai et al., 2022).

The impact of transposable elements and repeat sequences on genome evolution is profound, influencing gene regulation, genome stability, and adaptation. The functional impact map of genetic variants in rice has demonstrated that large-effect GVs in both coding and regulatory regions are subject to selection pressures, which may be driven by the activity of TEs and repeat sequences (Zhao et al., 2021). These elements contribute to the generation of genetic diversity and the evolution of new traits, underscoring their significance in the ongoing adaptation and improvement of rice varieties. By integrating these structural and functional insights from the *Oryza* genome, researchers can better understand the genetic basis of important traits and develop strategies for crop enhancement, ultimately contributing to global food security.

3 Functional Genomics of *Oryza*

3.1 Gene annotation and functional prediction

Gene annotation and functional prediction are critical steps in understanding the biology of *Oryza* species. Various gene families have been identified in *Oryza*, each with distinct functions contributing to the plant's growth, development, and stress responses. For instance, the Dof transcription factor family regulates various stresses and developmental processes in *Oryza*, with evolutionary analyses revealing significant structural and functional diversity across different species (Tabassum et al., 2022). Similarly, the MIR gene, specific to the *Oryza* genus, plays a crucial role in iron deficiency response, highlighting the importance of specific gene families in nutrient uptake and stress tolerance. Additionally, the use of proteogenomics has revealed the complexity of the rice transcriptome, identifying numerous fusion and intergenic transcripts, as well as a vast array of splicing isoforms, which contribute to the functional diversity of gene families (Figure 2) (Chen et al., 2020; Zanini et al., 2021). The functional impact map of genetic variants (GVs) in rice further elucidates the roles of missense mutations and chromatin accessibility in gene regulation, providing a valuable resource for gene cloning and functional studies (Zhou et al., 2018).



Localization of fusion transcripts

Type	Sub-type	srRNA_seq	%	lrRNA_seq	%
Diff. Chr.	- (**DC**)	87	7.3%	**695**	8.9%
Same Chr.	Vicinity (**SCV**)	1,068	89.6%	**6,775**	86.8%
Same Chr.	Distant (**SCD**)	37	3.1%	**334**	4.3%
	Total	1,192		**7,804**	

Figure 2 Comparative analysis of fusion transcripts (Adopted from Chen et al., 2020)

Image caption: A: Venn diagram showing the overlapping and unique fusion transcripts identified by srRNA_seq and lrRNA_seq; B: Summary of fusion transcript subtypes; C: Circos representations of fusion transcripts consisting of two genes; D: Venn diagram presenting the overlapping and unique genes involved in fusion transcript formation; E: GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses of fusion genes; F: Loci frequency present in fusion transcripts; G: RT-qPCR validation of fusion transcripts; Red arrows indicate the validated fusion transcripts. F1 to F3: Three fusion transcripts; M: marker (Adopted from Chen et al., 2020)

Several tools and databases have been developed to facilitate the functional annotation of rice genes. The eggNOG-mapper v2, for example, offers comprehensive functional annotation, orthology assignments, and domain prediction, optimized for large-scale genomic data sets (Cantalapiedra et al., 2021). The IC4R-2.0 annotation system integrates multiple omics data, including long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs), to enhance the accuracy and completeness of rice genome annotations. These resources are crucial for comparative and functional genomic studies, providing a robust framework for understanding gene functions in rice.

3.2 Transcriptomics and gene expression

RNA sequencing (RNA-Seq) has become a powerful tool for transcriptome analysis in *Oryza*, allowing researchers to identify and quantify RNA molecules in different tissues and under various conditions. Transcriptome analyses have revealed significant insights into gene expression patterns, regulatory networks, and functional genomics of *Oryza* species. For example, transcriptome analysis of *Oryza granulata* identified high-quality transcripts that provide valuable resources for functional and evolutionary studies, including genes associated with stress responses (Yang et al., 2018).

Gene regulation in rice involves intricate mechanisms, including the roles of natural antisense transcripts (NATs) and posttranscriptional events. The high-density genomic arrangement of NAT genes suggests their potential roles in multifaceted gene expression control (Chen et al., 2019). Additionally, the functional impact map of GVs provides insights into tissue-specific gene regulation, emphasizing the importance of chromatin accessibility in regulatory regions (Zhao et al., 2021). These findings underscore the complexity of gene regulation and the diverse expression patterns observed in rice.

Gene regulation and expression patterns in *Oryza* are influenced by various genetic and environmental factors. Studies have shown that differentially expressed genes under stress conditions can provide insights into the plant's adaptive mechanisms. For instance, genome-wide analysis of salinity and submergence stress-responsive genes in *Oryza coarctata* has identified key regulatory genes and pathways involved in stress tolerance (Bansal et al., 2020).

3.3 Proteomics and metabolomics

Proteomics, the large-scale study of proteins, is essential for understanding the functional aspects of the *Oryza* genome. Protein profiling has been used to identify and characterize proteins involved in various biological processes and stress responses. For instance, protein structure modeling in *Magnaporthe oryzae*, a fungal pathogen of rice, has identified numerous effector proteins that interact with the host plant, providing insights into pathogen-host interactions (Seong and Krasileva, 2021). The integration of functional genomics, transcriptomics, proteomics, and metabolomics has provided comprehensive insights into the gene functions, regulatory mechanisms, and metabolic pathways in rice. These advancements have significant implications for crop enhancement and the development of stress-resistant rice varieties.

Metabolomics, the study of metabolites, complements proteomics by providing a comprehensive understanding of metabolic pathways and networks. Studies on metabolic pathways in *Oryza* have revealed critical insights into plant metabolism and stress responses. For example, the identification of metabolic pathways involved in secondary metabolite biosynthesis and hormone signal transduction in *Oryza coarctata* has provided valuable information for improving stress tolerance in rice (Bansal et al., 2020).

4 Insights into Key Agronomic Traits

4.1 Yield-related traits

The identification and mapping of quantitative trait loci (QTLs) associated with yield-related traits have been pivotal in understanding the genetic basis of yield in rice. For instance, a study using F2:3:4 populations derived from two alien introgression lines identified several QTLs linked to yield traits such as plant height, tiller number, and grain weight. Notably, qTGW8.1 was consistently identified across generations, indicating its potential for improving grain weight in rice breeding programs (Beerelli et al., 2022). Similarly, research on interspecific backcross populations of *Oryza sativa* and *Oryza glaberrima* identified 20 QTLs associated with yield-enhancing traits, with qGY-4.1 showing significant phenotypic variance and potential for yield improvement (Bharamappanavara et al., 2023). These findings underscore the importance of wild and relative species in broadening the genetic base and enhancing yield traits in cultivated rice.

Genomic selection (GS) has emerged as a powerful tool for yield improvement in rice. By using high-throughput genotyping and phenotyping data, GS allows breeders to predict the genetic potential of plants for yield traits accurately. For example, a study using GS in rice identified multiple QTLs for root system architecture and agronomic traits, demonstrating the effectiveness of this approach in enhancing yield-related traits (Sharma et al., 2021). Moreover, integrating GS with machine learning models such as Bayesian networks has shown to improve prediction accuracies for complex traits like yield (Sharma et al., 2021).

4.2 Stress resistance and tolerance

Abiotic stress, including drought and salinity, poses significant challenges to rice production. The identification of QTLs and genes associated with abiotic stress tolerance is essential for developing resilient rice varieties. The introgression lines developed from various *Oryza* species have shown traits such as drought resistance and aerobic adaptation, which are critical for improving rice performance under stress conditions (Zhang et al., 2022). These genetic resources provide a foundation for breeding programs aimed at enhancing abiotic stress tolerance in rice. Several genes associated with abiotic stress responses have been identified. For instance, the *Oryza coarctata* genome revealed genes involved in salinity and submergence stress responses (Bansal et al., 2020). Additionally, studies have shown that genetic variations in heterotrimeric G proteins play a significant role in the regulation of key agronomic traits under varying climatic conditions.

Biotic stresses, such as diseases and pests, also impact rice yield and quality. The genetic analysis of interspecific backcross populations has revealed QTLs linked to biotic stress resistance. For instance, *Oryza glaberrima* has been identified as a valuable source of genes for resistance to various biotic stresses, which can be introgressed into *Oryza sativa* cultivars to enhance their resilience (Bharamappanavara et al., 2023). Additionally, the introgression library includes lines with blast resistance, highlighting the potential of wild species in providing durable resistance to biotic stresses (Zhang et al., 2022).

4.3 Nutritional and quality traits

Improving the nutritional quality of rice is a key objective in rice breeding. The genetic diversity present in wild and relative species offers opportunities to enhance nutrient biosynthesis pathways. The introgression lines developed from multiple *Oryza* species include traits related to nutrient biosynthesis, which can be harnessed to improve the nutritional profile of rice (Zhang et al., 2022). These genetic resources are invaluable for breeding programs focused on enhancing the nutritional quality of rice.

Grain quality and processing traits are important determinants of rice marketability and consumer preference. The identification of QTLs associated with grain quality traits, such as grain length and width, has been facilitated by the development of introgression libraries. For example, novel allelic variations related to grain length and width have been identified in the introgression lines, providing valuable resources for improving grain quality (Zhang et al., 2022). These findings highlight the potential of utilizing genetic diversity from wild and relative species to enhance grain quality and processing traits in rice.

5 Genomic Tools and Technologies for *Oryza* Improvement

5.1 Genome editing and CRISPR/Cas systems

The advent of CRISPR/Cas systems has revolutionized the field of functional genomics in rice (*Oryza sativa* L.). One notable tool is the CRISPR applicable functional redundancy inspector (CAFRI-Rice), which addresses the challenge of functional redundancy in the rice genome. This tool utilizes a phylogenetic heatmap to estimate the similarity between protein sequences and expression patterns, thereby identifying genes with potential functional redundancy. CAFRI-Rice has successfully predicted functional redundancy in 7 075 genes and demonstrated its utility in overcoming redundancy in root-preferred genes through loss-of-function analyses (Figure 3) (Hong et al., 2020). This approach not only accelerates functional genomic studies in rice but also holds potential for application in other plant species.

The research of Hong et al. (2020) validated functional redundancy and dominance in rice using the CAFRI-Rice model. Figure 3 (a) shows the functional dominance of *RUPO* and *MATL* genes in anther/pollen, with the uniqueness of the *MTD1* gene also being confirmed. Figure 3 (b) demonstrates the high functional redundancy of

MIKC*-type MADS-box transcription factors and *RopGEF* genes, with a correlation coefficient (PCC) of gene expression higher than 0.85, indicating strong functional redundancy among these genes. Figure 3 (c) illustrates an example of overcoming functional redundancy in the RSL gene family within the *bHLH* gene family, by comparing root hair length among wild-type, single mutants, and double knockout mutants, particularly noting significant root hair shortening in the double knockout mutants. These findings provide crucial insights into understanding gene functions in organisms and how gene editing technologies can be leveraged for agricultural biotechnology improvements (Figure 3).

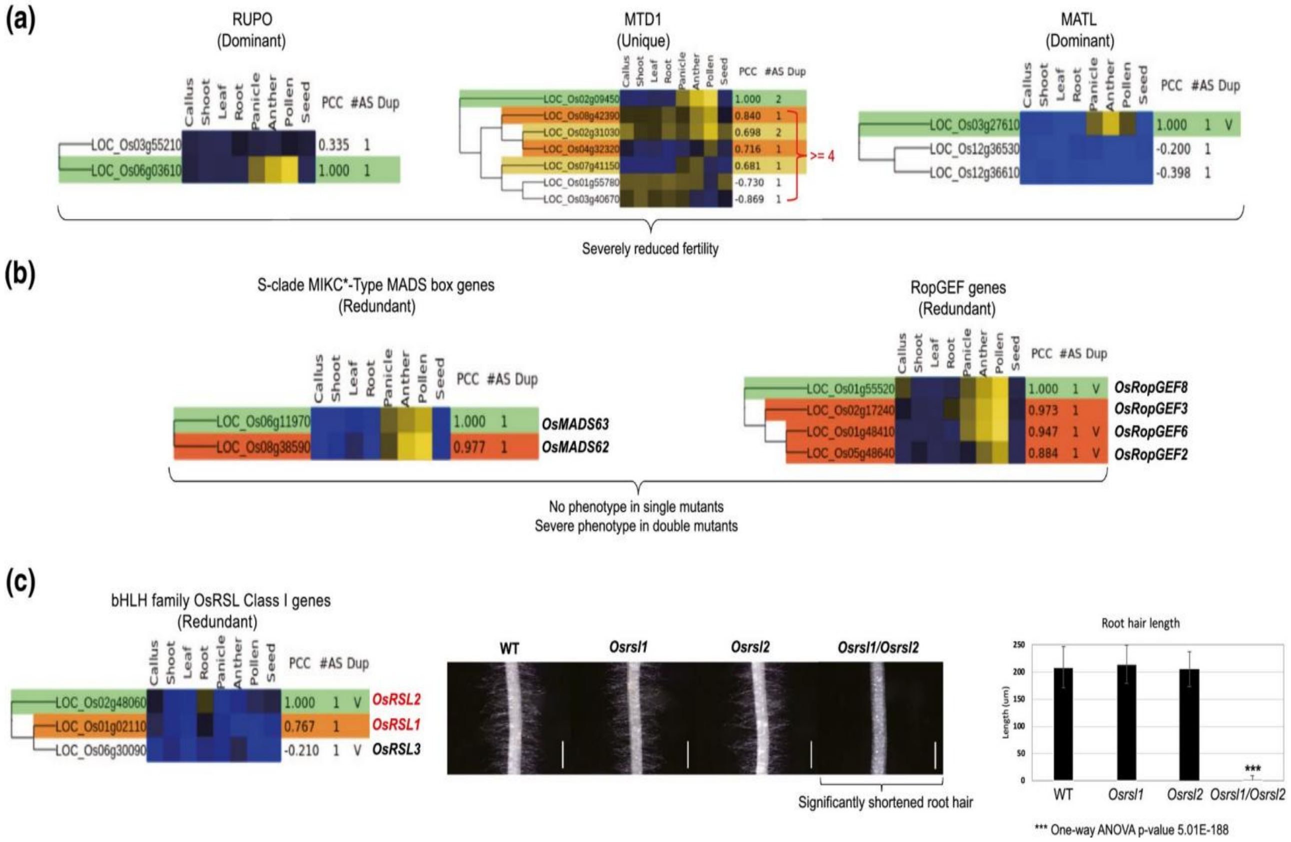


Figure 3 Validation and case studies of functional redundancy using CAFRI-Rice (Adopted from Hong et al., 2020)

Image caption: a: Functional dominance of three characterized anther/pollen genes predicted by CAFRI-Rice The left and right phylogenetic heatmaps indicate the functional dominance of the *RUPO* and *MATL* genes, respectively. In the nearest gene clade, there are one and two sister nodes, or genes which show different expression patterns than the two queried genes. The phylogenetic heatmap of MTD1, which is located in the center, represents the functional dominance regardless of the nearest gene clade as 'unique' clade gene; b: Functional redundancy of two characterized anther/pollen genes predicted by CAFRI-Rice. The phylogenetic heatmap on the left indicates the functional redundancy of two S-clade MIKC*-type MADS box transcription factors. The phylogenetic heatmap on the right presents the functional redundancy among four *RopGEF* genes. Both analyses yielded high PCC values (above 0.85), implying strong redundancy; c: Example of overcoming the functional redundancy among the RSL class I genes within the bHLH gene family. The two RSL class I genes that exhibited a predicted functional redundancy by CAFRI-Rice are highlighted in red. The middle panel provides root hair photographs from wild-type plants, single mutants, and double knockout mutants. The white bar in the picture represents 500 μm (Adopted from Hong et al., 2020)

5.2 Marker-assisted selection and genomic selection

Marker-assisted selection (MAS) and genomic selection (GS) are pivotal in modern rice breeding programs. The development of molecular markers is essential for these techniques, enabling the identification of desirable traits at the genetic level. Advances in high-throughput sequencing and genotyping technologies have facilitated the discovery of numerous molecular markers linked to important agronomic traits. These markers are instrumental in accelerating the breeding process by allowing for the early selection of superior genotypes. For example, the development of introgression lines using molecular markers has enabled the incorporation of favorable alleles from wild *Oryza* species into cultivated rice, enhancing traits such as grain size and drought resistance (Zhang et al., 2022). Additionally, retrotransposon-based markers have been used to study genetic diversity and stress-induced genomic instabilities in rice (Chadha, 2021).

Integrating genomic selection into breeding programs involves the use of genome-wide markers to predict the performance of breeding lines. This approach enhances the efficiency and accuracy of selecting high-yielding and stress-resistant rice varieties. By leveraging genomic selection, breeders can make more informed decisions, ultimately leading to the development of improved rice cultivars with enhanced agronomic traits. Studies have shown that integrating GS with other breeding technologies can significantly enhance the efficiency of selecting high-yielding and stress-tolerant rice varieties. For instance, a study demonstrated the use of GS in predicting root system architecture and above-ground agronomic traits, showing the potential of GS to accelerate breeding cycles (Sharma et al., 2021). Another research highlighted the effectiveness of GS models incorporating functional markers and genotype-by-environment interactions in improving predictive abilities for rice breeding (Xu et al., 2021).

5.3 High-throughput phenotyping and genotyping

High-throughput phenotyping technologies have greatly advanced the study of rice genetics and breeding. These technologies allow for the rapid and accurate measurement of plant traits under various environmental conditions, facilitating the identification of phenotypic variations linked to genetic markers. Advances in imaging and sensor technologies have enabled detailed phenotyping of traits such as plant height, biomass, and root architecture (Chen et al., 2019). These technologies include imaging systems, remote sensing, and automated phenotyping platforms, which enable the rapid assessment of large populations of rice plants. Such advancements are crucial for understanding the phenotypic variation and its genetic basis, thereby facilitating the selection of superior genotypes.

Genotyping platforms have evolved to offer high-throughput and cost-effective solutions for analyzing genetic variation in rice. Techniques such as next-generation sequencing (NGS) and single nucleotide polymorphism (SNP) arrays are widely used to genotype large populations. These platforms provide comprehensive data on genetic diversity, which is essential for mapping quantitative trait loci (QTL) and conducting genome-wide association studies (GWAS). The integration of high-throughput genotyping with phenotyping data accelerates the identification of genes associated with important traits, thereby enhancing the efficiency of rice breeding programs. The integration of advanced genomic tools and technologies, including CRISPR/Cas systems, marker-assisted selection, genomic selection, and high-throughput phenotyping and genotyping, is transforming rice breeding. These innovations are paving the way for the development of superior rice varieties with improved yield, stress resistance, and other desirable traits, ultimately contributing to global food security.

6 Case Studies of Genomic Applications in Crop Enhancement

6.1 Disease resistance breeding

Disease resistance in rice has been significantly enhanced through genomic insights and technologies. The identification of resistance genes and QTLs has been pivotal. For instance, the study by Kim et al. (2019) focused on utilizing the CRISPR/Cas9 system to target mutations in the rice *Os8N3* gene to enhance resistance against *Xanthomonas oryza*e pv. *oryza*e (Xoo), the pathogen responsible for bacterial blight in rice (Figure 4). By editing the *Os8N3* gene, researchers successfully produced mutants with enhanced disease resistance in the Japanese rice variety Kitaake. These mutants exhibited significant resistance to Xoo in the T1, T2, and T3 generations and did not contain transgenic DNA (T-DNA). Additionally, the study demonstrated that these mutants showed no significant differences in agronomic traits, such as pollen development, compared to non-transgenic control plants. This research not only confirmed the effectiveness of the CRISPR/Cas9 system in precise gene editing but also provided a successful example of using gene editing technology to improve crop disease resistance.

6.2 Drought and salinity tolerance

Understanding the genomic basis of stress tolerance has been critical for developing drought- and salinity-tolerant rice varieties. Studies have identified QTLs associated with these traits, such as those found in the *Oryza sativa* IR64×*Oryza glaberrima* interspecific backcross populations, which revealed significant genetic variability and QTLs linked to yield-enhancing traits under stress conditions (Bharamappanavara et al., 2023).

Breeding programs have leveraged genomic insights to develop rice varieties with enhanced stress tolerance. For instance, the introduction of genes from wild rice species through genetic transformation and genome editing has led to varieties with improved drought and salinity tolerance. The Agrobacterium-mediated transformation system has been successfully used to introduce stress-tolerance traits from wild rice into cultivated varieties, demonstrating the potential for neodomestication (Xiang et al., 2022).

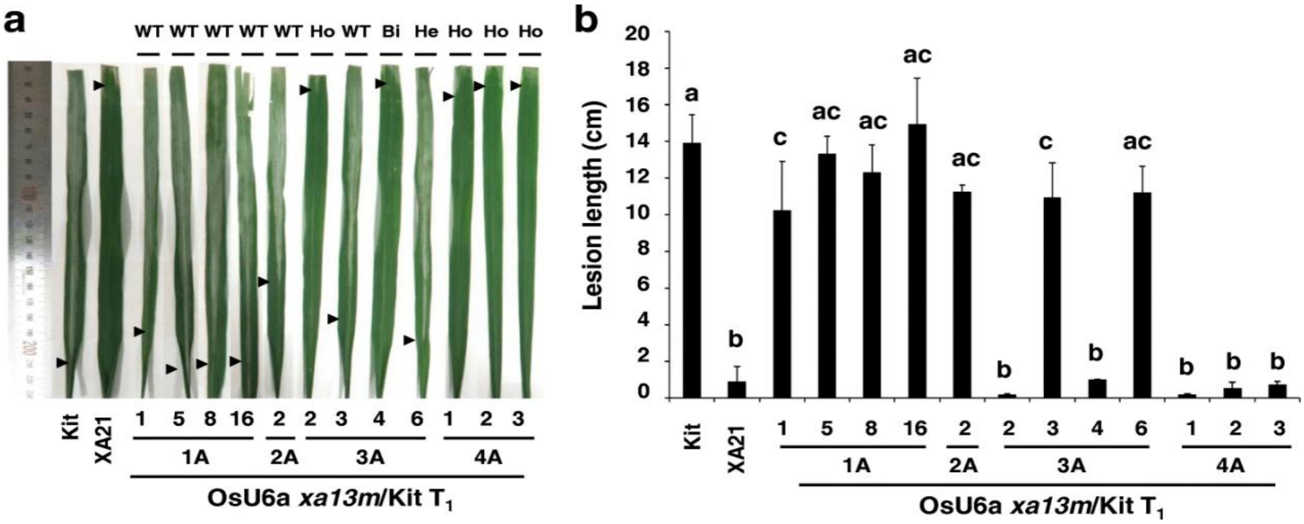


Figure 4 CRISPR/Cas9-targeted mutagenesis of *Os8N3* in rice to confer resistance to *Xanthomonas oryzae* pv. *oryzae* (Adopted from Kim et al., 2019)

Image caption: a: Bacterial blight resistance phenotypes of the xa13 mutant rice lines (T1). Rice plants 12 DAI with Xoo. From left to right: Kitaake (Kit), transgenic line (XA21, 7A-8) carrying Xa21 driven by the ubiquitin promoter, and transgenic lines (OsU6a xa13m/Kit, T1) carrying the OsU6a::xa13m-sgRNA/pHAtC construct. Arrowheads indicated the end of the lesion. WT: wild type: Ho: homozygous; Bi: bi-allelic: He; Heterozygous. b: Lesion lengths measured 12 DAI in Kitaake, XA21, and OsU6a xa13m/Kit T1. Error bars in the graph represent standard error of at least three leaves from each plant. Letters indicate a significant difference at $P < 0.050$ by Tukey's HSD test (Adopted from Kim et al., 2019)

The study by Xiang et al. (2022) successfully established an efficient gene transformation and editing system for Chaling Common Wild Rice (CLCWR). Using thin-layer tissue of mature seed endosperm and Agrobacterium-mediated transformation, the researchers achieved genetic modification of this wild rice with an AA genome (Figure 5). The study showed that CLCWR callus tissue is easily inducible and regenerable, with transformation efficiencies ranging from 87% to 94%. The efficiency for single-gene and multi-gene editing was 60%~70% and 20%~40%, respectively. Additionally, compared to Nipponbare (Nip), CLCWR exhibited a higher frequency of hygromycin-resistant callus and transformation efficiency. Through CRISPR/Cas9-mediated gene editing, the system successfully introduced specific gene variations, demonstrating the potential for efficient gene function studies and molecular breeding applications. This research not only showcased the application of the Agrobacterium-mediated transformation system in wild rice but also provided a valuable foundation for future crop domestication using gene editing technologies.

6.3 Biofortification and quality improvement

Biofortification aims to enhance the nutritional value of rice by increasing the content of essential nutrients. Genetic approaches have identified key genes involved in nutrient biosynthesis pathways. For example, the

QTL-seq approach has been used to identify novel QTLs controlling grain weight and nutrient content, which can be exploited for biofortification (Bommisetty et al., 2020).

Genomic tools have also been employed to improve grain quality traits, such as texture, flavor, and processing quality. Studies have utilized next-generation sequencing and GWAS to uncover genetic loci associated with these traits. The integration of high-throughput SNP analysis and genomic selection has further refined the breeding of rice varieties with superior grain quality (Xu et al., 2021).

7 Future Prospects and Challenges

7.1 Emerging genomic technologies

The field of *Oryza* genomics is rapidly evolving with the advent of new genomic technologies. Emerging technologies such as CRISPR/Cas9 and its variants (e.g., CRISPR/Cpf1 and base editors) have revolutionized the way we can edit the rice genome, enabling precise and efficient modifications for crop improvement. These technologies hold great promise for accelerating breeding programs by introducing desirable traits such as disease resistance, stress tolerance, and improved nutritional quality (Mishra et al., 2018). Another significant development is the construction of high-quality reference genomes and pan-genomes, which provide a comprehensive understanding of genetic diversity and facilitate the identification of novel genes for crop improvement (Huang et al., 2021).

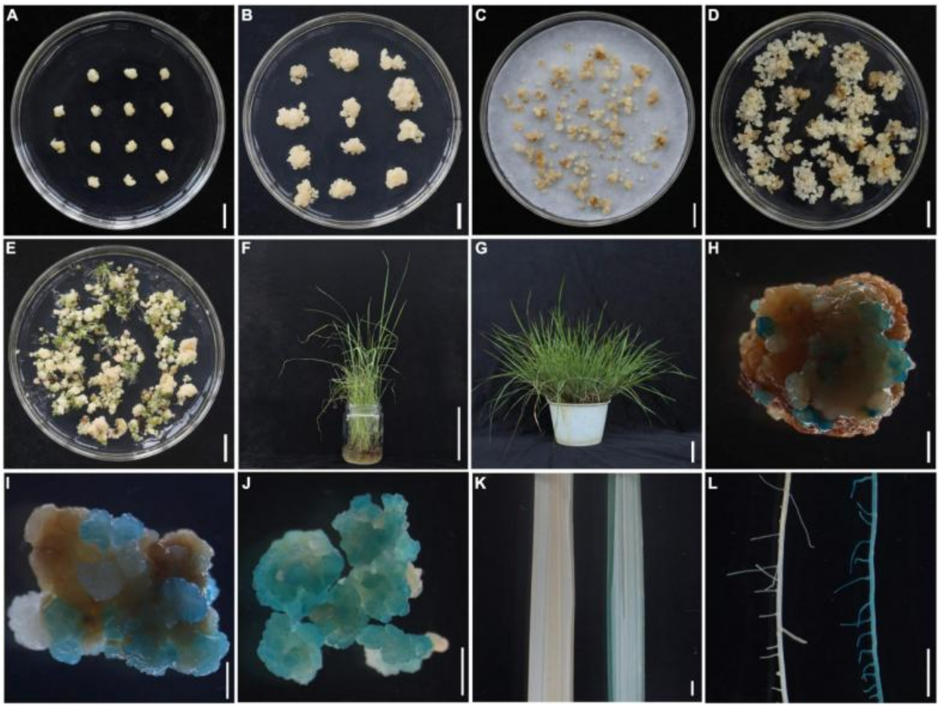


Figure 5 *Agrobacterium-mediated* transformation of CLCWR by using scutellum tissue of embryos in mature seeds (Adopted from Xiang et al., 2022)

Image caption: A: Embryogenic calli induced on callus induction medium containing 2.5 mg/L 2,4-D for 10 days. (B) Calli subcultured on callus induction medium containing 2.5 mg/L 2,4-D for 12 days; C: Subcultured calli co-cultivated with Agrobacterium on co-cultivation medium for 3 days; D: Infected calli were screened by *Hygromycin*. The co-cultured calli were transferred to the selection medium supplemented with 2.5 mg/L 2,4-D, 30 mg/L *Hygromycin* B, and 400 mg/L carbenicillin for 3 rounds of 15 days each; E: Shoot regeneration from transformed calli. The *Hygromycin-resistant* calli were regenerated on the regeneration medium containing 2 mg/L ZT and 0.1 mg/L NAA for 30 days; F: Roots were induced on the root-induction medium containing 0.135 mg/L NAA for 14 days; G: Transgenic plant after transplanting to the soil for 3 months. (H-L) GUS staining of transgenic calli and plant. The calli co-cultivated with *Agrobacterium*; H: selected by *Hygromycin* B (I) and regenerated (J), also the leaves (K) and roots (L) of the transgenic plant (left was non-transgenic and right was transgenic) were stained using GUS staining solution and observed under a stereomicroscope. Scale bars, 1 cm (A-E), 5 cm (F,G), and 2 mm (H-L) (Adopted from Xiang et al., 2022)

7.2 Integrating genomics with other omics

Integrating genomics with other omics approaches, such as transcriptomics, proteomics, and metabolomics, offers a holistic view of the biological processes governing rice development and stress responses. Transcriptomic analyses, for instance, have been instrumental in identifying genes involved in abiotic stress tolerance and understanding the regulatory networks at play (Ahmad, 2022). Additionally, integrating proteomics and metabolomics data can reveal the functional proteins and metabolic pathways that contribute to important agronomic traits, further enhancing our ability to develop resilient rice varieties (Seong and Krasileva, 2021).

7.3 Ethical, regulatory, and social considerations

As genomic technologies advance, it is crucial to address the ethical, regulatory, and social considerations associated with their use. The deployment of genome-editing technologies such as CRISPR/Cas9 raises questions about biosafety, potential off-target effects, and the long-term impacts on ecosystems and human health. Regulatory frameworks need to be updated to ensure the safe and responsible use of these technologies. Moreover, public perception and acceptance of genetically modified organisms (GMOs) and genome-edited crops play a significant role in the adoption of these technologies. Transparent communication and engagement with stakeholders, including farmers, consumers, and policymakers, are essential to address concerns and promote the benefits of genomic innovations in rice breeding (Chen et al., 2019). The future of rice breeding and crop enhancement lies in the continued advancement and integration of genomic technologies with other omics approaches. Addressing the ethical, regulatory, and social challenges will be key to realizing the full potential of these innovations for sustainable agriculture and food security.

8 Concluding Remarks

The study of the *Oryza* genome has yielded significant insights into both its structural and functional aspects, providing a strong foundation for crop enhancement. Key discoveries include the identification of major genes and QTLs associated with yield, stress resistance, and nutritional quality. The advancement of genomic technologies such as CRISPR/Cas9 has enabled precise genetic modifications, leading to improved disease resistance and stress tolerance (Kim et al., 2019). Additionally, the development of comprehensive genomic databases and high-quality reference genomes has facilitated the identification and utilization of genetic diversity in both cultivated and wild rice species (Huang et al., 2021).

Future research should focus on leveraging emerging genomic technologies and integrating multi-omics approaches to further understand and exploit the genetic potential of the *Oryza* genus. The construction of pan-genomes and the application of genomic selection will play a crucial role in identifying and incorporating beneficial traits into rice breeding programs (Huang et al., 2021). Additionally, there is a need to address the ethical, regulatory, and social implications of genomic modifications to ensure the responsible deployment of these technologies (Chen et al., 2019).

The advancements in *Oryza* genomics have set the stage for significant improvements in rice cultivation, with the potential to enhance yield, stress resistance, and nutritional quality. Continued research and the application of cutting-edge genomic tools will be essential in meeting the global food security challenges of the future. By integrating genomic insights with traditional breeding practices, it is possible to develop resilient and high-yielding rice varieties that can thrive in diverse environmental conditions. The ongoing efforts to map and utilize the genetic diversity within the *Oryza* genus will undoubtedly contribute to sustainable agricultural practices and global food security.

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Conflict of Interest Disclosure

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

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