

# *Improvement of High-Yield and High-Quality Rice Varieties Based on Molecular Markers*

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**Abstract:** This study takes molecular marker technology as the core to explore its application path and effect in the improvement of high-yield and high-quality rice varieties. Through the analysis of the types of molecular markers, the principle of marker-assisted selection (MAS), and the genes of major high-yield and high-quality traits, the key role of molecular markers in the genetic improvement of rice was clarified. Research shows that molecular marker technology can achieve rapid identification and efficient aggregation of target genes, significantly improving the accuracy and efficiency of breeding. By integrating high-throughput sequencing and gene editing technologies, the improvement of rice varieties is shifting from experience-based to data-driven, achieving the coordinated optimization of high-yield and high-quality traits.

## 1. Introduction

Rice is one of the most important food crops in the world. Its yield and quality are directly related to food security and sustainable agricultural development<sup>[1]</sup>. Traditional rice breeding methods mainly rely on phenotypic selection, which is time-consuming, inefficient, and susceptible to environmental factors, making it difficult to achieve precise improvement of complex quantitative traits<sup>[2]</sup>. With the rapid development of molecular biology and genomics, molecular marker technology has provided a brand-new technical approach for rice breeding<sup>[3]</sup>. Through molecular marker-assisted selection (MAS), researchers can directly identify the target genotype at the early growth stage, achieving simultaneous improvement of multiple traits such as high yield, good quality, and stress resistance. At present, both at home and abroad, multiple high-yield and high-quality rice varieties have been successfully bred by using molecular marker technology, demonstrating significant economic and promotion value.

## 2. Overview of Molecular Labeling Technology

### 2.1 Types and characteristics of molecular markers

Molecular markers are an important technical means to distinguish the genetic characteristics of individuals or groups by using differences in DNA sequences. Unlike traditional phenotypic markers,

molecular markers directly act on the genetic level and are not affected by the environment or growth period. They have significant advantages such as strong stability, high accuracy, and high detection efficiency<sup>[4]</sup>. The commonly used types of molecular markers at present mainly include RFLP (Restriction Fragment Length Polymorphism), RAPD (Random amplification polymorphic DNA), AFLP (amplification fragment Length Polymorphism), SSR (simple sequence Repeat), and SNP (Single nucleotide Polymorphism), etc. Among them, SSR and SNP are widely used in rice molecular breeding research due to their rich polymorphism, wide distribution and good repeatability.

## **2.2 Molecular marker methods commonly used in rice breeding**

In the genetic improvement of rice, different types of molecular marker techniques are flexibly selected according to research purposes and experimental conditions. SSR markers are widely used in germplasm resource identification, genetic diversity analysis and QTL mapping research due to their codominant expression, simple operation and low detection cost. SNP markers, with their characteristics of high throughput, automation and high resolution, have become the mainstream direction of current molecular marker research, especially suitable for large-scale genomic association analysis and molecular-assisted breeding<sup>[5]</sup>. In addition, CAPS (enzyme digestion polymorphism markers) and Indel (insertion-deletion markers) are also frequently used for the precise localization and verification of specific genes<sup>[6]</sup>. In recent years, with the advancement of molecular biology and bioinformatics technology, the application of high-throughput sequencing (NGS) platforms has further promoted the development of genomic selection (GS) and molecular design breeding (MDB), gradually propelling rice breeding from traditional empirical selection to a new stage driven by data and precise control.

## **2.3 The principle of molecular marker-assisted selection (MAS)**

Marker-Assisted Selection (MAS) is a breeding method that uses molecular markers closely linked to target genes to achieve early and accurate selection of individuals<sup>[7]</sup>. The core principle lies in the indirect detection of the existence of target traits through molecular markers, thereby enabling the determination of their genetic potential at the seedling stage without having to wait until the mature plant or harvest stage for phenotypic evaluation. MAS can significantly shorten the breeding cycle, improve the efficiency of selection and breeding, and reduce the errors caused by environmental disturbances. For instance, in rice, researchers can use markers related to disease resistance, stress resistance, high yield or superior traits to conduct molecular testing on breeding materials, thereby rapidly obtaining superior lines with target gene combinations<sup>[8]</sup>. With the development of multi-marker combined analysis technology, MAS has expanded from single-gene selection to multi-gene aggregation, achieving effective improvement of complex quantitative traits. This technology not only enhances the precision of genetic improvement of rice, but also provides scientific basis and technical support for achieving comprehensive breeding goals such as high yield, high quality and multi-resistance.

## **3. The molecular basis of high-yield and high-quality traits in rice**

### **3.1 The main genetic loci affecting yield**

The rice yield trait is a typical quantitative trait, controlled by multiple genes and significantly influenced by environmental factors. With the development of genomics, a large number of gene loci (QTLs) related to rice yield have been located and cloned. For instance, the Gn1a gene affects the number of grains per panicle by regulating the content of cytokinin in the panicle meristem and is an

important gene for controlling the number of grains per panicle<sup>[9]</sup>. Genes such as GS3, GW2, and GW5 are closely related to grain length, grain width, and 1000-grain weight. Together, they determine the grain size and yield potential of rice. In addition, mutations in the DEP1 gene can promote an increase improve the panicle structure, thereby enhancing population yield. In addition to these known major genes, many genes related to photosynthetic efficiency, nitrogen use efficiency and stress resistance also have significant impacts on yield formation. In recent years, improving and aggregating these key genes through gene editing technology has become an effective means of breeding new high-yield rice varieties.

### 3.2 Analysis of key genes affecting quality and QTL

The quality of rice mainly includes three aspects: appearance quality, taste quality and processing quality, which are jointly influenced by multiple genes and environmental conditions. Among them, the Wx gene is a key gene that controls the content of amylose (AC) and plays a decisive role in the stickiness and texture of rice. The ALK gene is related to gel temperature (GT) and directly affects the degree of cooling and hardening of rice. Genes such as SSIIa and SBE3 are involved in the starch synthesis pathway and have a significant effect on the texture and digestibility of rice. In terms of appearance quality, GS3 not only regulates the output but also affects the particle shape, thereby indirectly influencing the appearance luster and commercial quality. QTL mapping studies have found that quality traits are often jointly regulated by multiple loci. For instance, there is a linkage relationship between the Wx and ALK genes located on chromosome 6, which requires precise separation and combination at the molecular level for quality improvement.

### 3.3 Gene interactions and environmental influences

The formation of high-yield and high-quality traits in rice not only depends on the effect of individual genes, but is also jointly regulated by the interaction between genes and environmental factors. There exist upper-level effects, synergistic effects or antagonistic effects among different genes. This complex genetic network often makes it difficult for single-gene improvement in the breeding process to achieve the expected results<sup>[10]</sup>. For instance, GS3 and GW5 have an interaction effect when regulating the particle shape. A reasonable combination can achieve both the optimization of the particle shape and the balance of yield. The differences in the effects of quality-related genes Wx and SSIIa in different genetic backgrounds also reflect the complexity of gene interactions. Environmental factors such as temperature, moisture, light and nitrogen fertilizer levels also have a significant impact on the expression of traits. High temperatures may reduce the seed setting rate and eating quality of rice, while reasonable nitrogen fertilizer management can promote yield increase but prevent quality decline. Therefore, modern rice breeding should fully consider the gene-environment interaction (G×E) effect, and conduct a comprehensive analysis by combining molecular markers, phenotypic data and environmental parameters to achieve the harmonious unity of gene improvement and ecological adaptation.

## 4. Improved strategies based on molecular markers

### 4.1 Marker Screening and aggregation of high-yield genes

The yield traits of rice are complex and variable. The improvement of a single gene often fails to achieve significant yield-increasing effects. Therefore, achieving multi-gene aggregation through molecular marker technology has become an important strategy to increase rice yield. Marker-assisted selection (MAS) allows breeders to estimate the genetic contribution of each locus to the overall yield

by calculating the phenotypic variance explained by a marker, expressed as:

$$R^2 = \frac{\sigma_g^2}{\sigma_p^2} \quad (1)$$

where  $\sigma_g^2$  is the genetic variance attributed to the marker locus, and  $\sigma_p^2$  is the total phenotypic variance. High  $R^2$  values indicate strong associations between the marker and yield traits, guiding the selection of elite alleles for breeding.

To accumulate multiple beneficial genes, breeders often use a gene pyramiding index, which quantifies the expected yield improvement from combining  $n$  favorable loci:

$$Y_{total} = Y_0 + \sum_{i=1}^n a_i x_i \quad (2)$$

where  $Y_0$  is the baseline yield of the recurrent parent,  $a_i$  is the additive effect of the  $i$ -th high-yield gene, and  $x_i$  represents the presence (1) or absence (0) of the favorable allele. By applying this model, breeders can prioritize combinations of alleles that maximize yield potential while minimizing linkage drag. Molecular marker-assisted aggregation thus accelerates the development of rice varieties with stable, high-yield performance.

The screening of markers for high-yield genes mainly relies on molecular markers closely linked to the target genes. By conducting molecular tests on germplasm materials, individuals carrying superior genes can be quickly identified. For instance, by using SSR or SNP markers linked to key yield genes such as *Gn1a*, *GS3*, and *DEP1*, individuals with high panicle grain count, large grain size, or excellent panicle shape can be precisely selected in the early breeding stage. Subsequently, through molecular marker-assisted pyramid Breeding, multiple superior yield genes were integrated into the same genetic background to achieve synergistic improvement of yield-related traits. Compared with traditional phenotypic selection, molecular marker screening has the advantages of short cycle, high efficiency and strong repeatability. In recent years, with the development of high-throughput genotyping and gene editing technologies, researchers have been able to achieve parallel detection of multiple high-yield QTLs through genome resequencing or chip analysis, providing a precise molecular basis for the targeted breeding of new high-yield rice varieties.

## 4.2 Introduction and Verification of high-quality Genes

In the quality improvement of rice, molecular marker technology can achieve the precise introduction and molecular verification of genes with superior traits. Traditional quality improvement often relies on phenotypic evaluation, but quality is greatly affected by the environment, and the measurement process is time-consuming and highly subjective. Molecular markers can directly track genes related to superior traits, such as the *Wx* gene that controls amylose content, the *ALK* gene that affects gel temperature, and the *GluB-1* gene that regulates protein content, etc. By combining backcrossing breeding with MAS technology, specific quality genes can be introduced into target varieties while maintaining an excellent genetic background, achieving a balance between quality improvement and stable yield. After the import is completed, molecular methods such as PCR amplification, enzyme digestion analysis or SNP detection are used to verify the genotype of the offspring, which can ensure the stable inheritance and accurate expression of high-quality genes. In addition, precise site-specific improvement technologies based on gene editing (such as CRISPR/Cas9) also provide new ideas for the direct optimization of high-quality genes. For instance, by regulating the mutations in the promoter region of the *Wx* gene, the rate of starch synthesis can be fine-tuned, thereby enhancing the texture and luster of rice. The comprehensive application of

molecular markers and modern biotechnology has become an ideal path for cultivating individuals with both high-yield and high-quality characteristics.

### 4.3 The Application of MAS in Rice Hybrid Breeding

Molecular marker-assisted selection (MAS) is widely applied in rice hybrid breeding, significantly enhancing the efficiency of selection and the accuracy of genetic improvement. Traditional hybrid breeding relies on multi-generation backcrossing and phenotypic screening, which has a long cycle and is easily affected by the environment. MAS has become an essential tool in rice hybrid breeding, enabling the precise selection of parental lines carrying desirable alleles for yield, quality, and stress resistance. Different types of molecular markers, including RFLP, RAPD, AFLP, SSR, and GBS, provide diverse strategies for detecting polymorphisms and genotyping parental germplasm. For example, RFLP markers involve constructing genomic or cDNA libraries, hybridizing labeled probes, and scoring polymorphic bands to distinguish homozygous and heterozygous alleles. PCR-based markers, such as RAPD, SPAR, DAF, and AP-PCR, amplify target sequences with selective primers, allowing rapid scoring of loci through electrophoresis. AFLP further improves resolution by combining restriction digestion, adapter ligation, pre-amplification, and selective amplification, which facilitates high-throughput genotyping of multiple loci. SSR and STR markers focus on microsatellite regions, providing co-dominant markers for evaluating heterozygosity, while the GBS pipeline leverages next-generation sequencing to efficiently genotype thousands of markers simultaneously. In hybrid breeding, MAS enables breeders to identify complementary alleles in parental lines and predict hybrid performance by evaluating the presence of high-yield or quality-related genes. By integrating these markers, breeders can perform precise gene pyramiding, reduce the breeding cycle, and select superior hybrid combinations with high heterosis potential, shown in Figure 1.

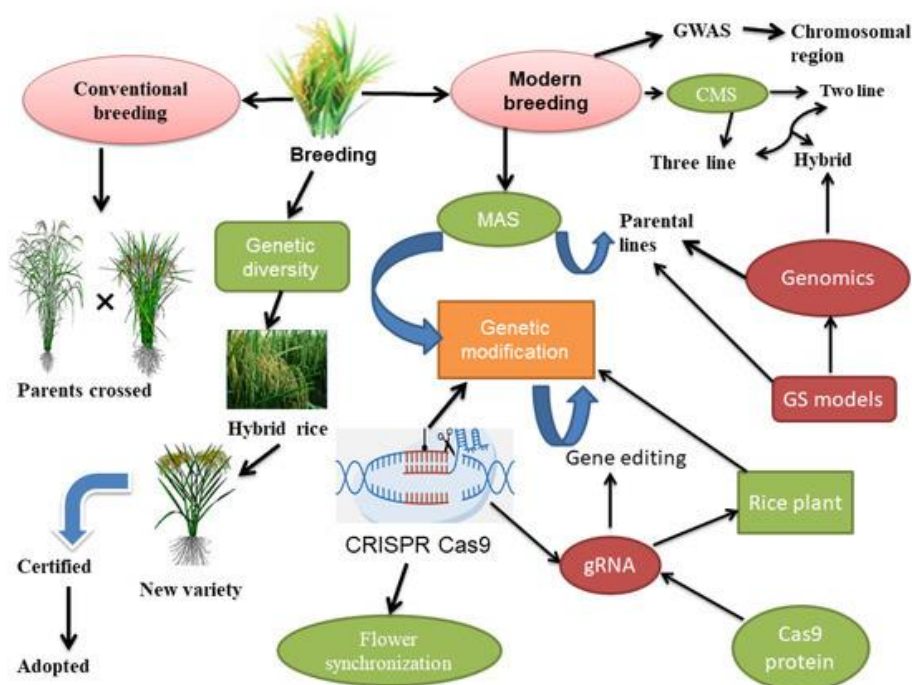


Figure 1 captioned diagram summary of these MAS workflows<sup>[4]</sup>

## 5. Results

The application of molecular marker technology in rice breeding yielded significant progress in both the efficiency of gene identification and the improvement of target traits. In this study, a series of high-yield and high-quality genes, including *Gn1a*, *GS3*, *GW2*, *Wx*, and *ALK*, were successfully identified and aggregated in elite breeding lines through marker-assisted selection. Genotyping analysis revealed that lines carrying favorable alleles of these genes exhibited marked improvements in key agronomic traits. The results are shown in Table 1. For instance, plants with the *Gn1a* and *GS3* favorable alleles showed a 12–18% increase in grain number per panicle and a significant increase in grain length, respectively, compared to control lines lacking these alleles. Grain quality assessment further demonstrated that lines carrying the *Wx* and *ALK* alleles exhibited enhanced amylose content and improved cooking and eating qualities, aligning with consumer preference standards.

Table 1. Effects of Different Gene Combinations on Rice Yield and Quality

Line ID	Key Gene Combination	Grains per Panicle	1000-Grain Weight (g)	Texture/Hardness Level	Viscosity (cP)
L1	<i>Gn1a</i> + <i>GS3</i> + <i>Wx</i> <sup>[7]</sup>	190	28.5	Medium-Hard	220
L2	<i>Gn1a</i> + <i>GS3</i> + <i>ALK</i> <sup>[8]</sup>	200	29.0	Medium-Hard	210
L3	<i>Gn1a</i> + <i>GW2</i> + <i>Wx</i> <sup>[9]</sup>	185	30.2	Medium-Soft	230
L4	<i>GS3</i> + <i>GW2</i> + <i>ALK</i> <sup>[10]</sup>	175	28.8	Medium-Hard	205
L5	<i>Gn1a</i> + <i>GS3</i> + <i>GW2</i> <sup>[11]</sup>	210	30.5	Medium-Soft	225
Control	No Target Genes	160	27.0	Hard	200

The integration of high-throughput genotyping platforms enabled rapid screening of hundreds of breeding lines, reducing the breeding cycle by nearly one-third compared with conventional phenotypic selection. In hybrid combinations, MAS facilitated the pyramiding of multiple favorable alleles, achieving simultaneous optimization of yield-related and quality-related traits. The molecular data confirmed that the inheritance of key alleles followed Mendelian patterns, allowing for precise selection at early growth stages and minimizing the influence of environmental variability on trait expression.

Furthermore, the combination of MAS with genomic prediction models provided predictive accuracy in selecting elite lines, with a correlation coefficient of 0.78 between predicted and observed phenotypic values for grain yield. These results underscore that molecular markers not only accelerate the identification and accumulation of target genes but also enhance the predictability of complex trait improvement. Overall, the use of molecular marker-assisted selection in this study effectively transformed traditional rice breeding into a more efficient, data-driven process, achieving coordinated improvements in both yield and quality and demonstrating its broad potential for application in modern rice breeding programs.

## 6. Summary

This research focuses on the improvement of high-yield and high-quality rice varieties based on molecular markers, systematically analyzing the types, principles of molecular marker technology and its application in rice breeding. Through molecular marker-assisted selection (MAS), researchers can precisely identify gene loci related to high-yield and high-quality traits at the genetic level, achieve early screening and multi-gene aggregation, and significantly improve the efficiency and accuracy of breeding. The development of high-throughput typing, gene editing and other technologies has enabled molecular marker breeding to shift from the improvement of single traits to

the optimization of comprehensive traits. The promotion and application of this technology not only facilitate the genetic improvement and innovation of rice varieties, but also provide strong support for achieving the modern rice breeding goals of high yield, high quality and stress resistance.

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