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# Genomic Insights into Grain Size and Weight: The *GS2* Gene's Role in Rice Yield Improvement

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**Abstract** Grain type and weight are key factors determining rice yield and quality, affecting agricultural productivity and market value. The genetic basis of these traits is very complex, and the *GS2* gene is considered an important contributor. This study aims to explore the role of *GS2* gene in improving rice yield, identify and characterize *GS2* gene, elucidate its mechanism of action in rice development, and study its evolutionary perspective in different rice varieties. This study includes the genetic regulation of grain type and weight by *GS2*, phenotypic variations caused by *GS2* mutations, and interactions between *GS2* and other yield related genes. Through case studies, *GS2* gene modification experiments were analyzed, highlighting successful cases in field applications and comparing them with non *GS2* improved rice varieties. We also reviewed the latest technological advancements in genetic engineering, CRISPR, genome sequencing, and bioinformatics tools related to GS2 research. In addition, this study discussed breeding strategies that combine *GS2* research, including traditional breeding programs and molecular marker assisted selection, evaluated the impact of *GS2* research on rice agriculture, and emphasized its significance for yield improvement and global food security. Finally, the future directions of rice genetic research were outlined, emphasizing the potential for new discoveries, collaborative efforts, and emerging technologies. This study emphasizes the importance of the *GS2* gene in improving rice yield and provides recommendations for future research and application.

**Keywords** *GS2* gene; Rice yield; Grain size; Genetic regulation; CRISPR technology

## 1 Introduction

Grain size and weight are critical determinants of rice yield and quality, influencing both the economic value and nutritional content of the crop. Larger grains generally contribute to higher yield, which is a primary goal in rice breeding programs aimed at meeting the food demands of a growing global population (Hu et al., 2015). Additionally, grain size affects the milling quality and market preferences, with different regions favoring specific grain shapes and sizes (Duan et al., 2017). Therefore, understanding the genetic factors that control these traits is essential for developing high-yield, high-quality rice varieties.

The genetic basis of grain size and weight in rice involves multiple quantitative trait loci (QTLs) and genes, among which the *GS2* gene has emerged as a significant player. *GS2*, located on chromosome (Li et al., 2011), encodes the Growth-Regulating Factor 4 (OsGRF4), a transcriptional regulator that influences cell size and number, thereby affecting grain weight and yield1. A rare allele of *GS2*, which results in elevated expression due to a mutation in the microRNA binding site, has been shown to enhance grain size and yield significantly1. Other genes such as *GS3*, *GS5*, and *GW6* also contribute to grain size regulation, but *GS2*'s role as a dominant QTL makes it a focal point for genetic studies and breeding programs (Fan et al., 2006).

This study aims to consolidate current knowledge on the *GS2* gene and its impact on rice grain size and yield. This study elucidates the molecular mechanisms by which *GS2* regulates grain size and weight; compares the effects of *GS2* with other known grain size-related genes such as *GS3*, *GS5*, and *GW6*; explores the potential applications of *GS2* in rice breeding programs aimed at yield improvement. By achieving these objectives, this study will provide a comprehensive understanding of *GS2*'s role in rice yield enhancement, offering valuable insights for future research and breeding strategies.

## 2 Understanding the *GS2* Gene

### 2.1 Identification and characterization of the *GS2* gene

The *GS2* gene has been identified as a significant regulator of grain size and weight in rice. In a study involving 204 diverse rice germplasms, *GS2* was found to be associated with grain length (GL), grain width (GW), and grain thickness (GT). The gene's role in these traits was confirmed through the use of InDel markers, which successfully identified multiple alleles contributing to these phenotypic variations. Additionally, the *GS2* gene was one of the nine major genes studied for its impact on grain size and weight, highlighting its importance in rice breeding programs aimed at improving yield and grain appearance (Gull et al., 2019).

### 2.2 *GS2*'s mechanisms of action in rice development

The mechanisms by which *GS2* influences rice development are multifaceted. *GS2* is a positive regulator downstream of *GSK2* in response to brassinosteroid (BR) signaling, which is crucial for cell expansion in spikelet hulls (Huang et al., 2022). The interaction between *GSK2* and *GS2*, where *GSK2* phosphorylates *GS2*, enhances the accumulation of *GS2* in the nucleus, thereby promoting cell expansion and increasing grain size. This interaction underscores the importance of the BR signaling pathway in regulating grain size through *GS2*. Furthermore, the *GS2* gene's role in regulating grain size was also supported by its association with other genes like *GS3*, *GS7*, and *GW8*, which collectively influence grain length, width, and thickness.

### 2.3 Evolutionary perspective of *GS2* across rice varieties

The evolutionary significance of the *GS2* gene across different rice varieties has been explored through genetic and phenotypic analyses. Studies have shown that *GS2*, along with other grain size-related genes, exhibits considerable genetic variation among different rice germplasms. This variation is crucial for the adaptability and selection of rice varieties with desirable traits. For instance, the *GS2* gene was found to be a part of a complex network of genes that regulate grain size, with different alleles contributing to variations in grain length, width, and thickness (Ngangkham et al., 2018). The evolutionary perspective of *GS2* is further highlighted by its interaction with other genes like *GS3* and *GW2*, which are also key regulators of grain size and have been studied extensively in various rice landraces. These interactions and variations suggest that *GS2* has played a significant role in the domestication and breeding of rice varieties with improved yield and grain quality.

## 3 *GS2* and Its Impact on Grain Size and Weight

### 3.1 Genetic regulation of grain size and weight by *GS2*

The *GS2* gene plays a crucial role in the genetic regulation of grain size and weight in rice. *GS2* is part of a complex network of genes that interact to control these traits. For instance, the *GS2* gene is known to interact with the brassinosteroid (BR) signaling pathway, which is essential for cell expansion in spikelet hulls, thereby influencing grain size (Lyu et al., 2020). Additionally, *GS2* is a downstream component of the *GSK2*-related BR signaling response, which further underscores its role in grain size regulation. The interaction between *GS2* and other genes, such as *OsOFP19*, also highlights its importance in the genetic regulation of grain size and weight (Huang et al., 2022).

### 3.2 Phenotypic variations resulting from *GS2* mutations

Mutations in the *GS2* gene can lead to significant phenotypic variations in rice grain size and weight (Dash, 2020). For example, the *SMALL GRAIN2* (*SG2*) mutant, which harbors a mutation in the *GS2* gene, exhibits smaller grains and a semi-dwarf phenotype due to repressed cell expansion in the spikelet hulls. This mutation causes insensitivity to exogenous BR treatment, further affecting grain size. Additionally, the overexpression of *GS2* can lead to larger grains, while its loss of function results in smaller grains, demonstrating the gene's critical role in determining grain size and weight (Tian et al., 2019).

### 3.3 Interaction of *GS2* with other yield-related genes

*GS2* interacts with several other yield-related genes to regulate grain size and weight. For instance, *GS2* interacts with *OsOFP19*, a negative regulator of grain shape, to control gene expression and grain size (Ngangkham et al., 2018). This interaction plays an antagonistic role, with *GS2* promoting and *OsOFP19* inhibiting grain size. Furthermore, *GS2* is part of a broader network involving other genes such as *GW5*, which also regulates grain width and weight through the BR signaling pathway (Tian et al., 2019). The interaction between *GS2* and these genes highlights the complex genetic network that controls grain size and weight in rice.

## 4 Case Studies: *GS2* Gene Manipulation in Rice

### 4.1 Detailed analysis of *GS2* gene modification experiments

The *GS2* gene has been identified as a significant regulator of grain size and weight in rice. Various studies have explored the genetic and molecular mechanisms underlying its function. For instance, the study by Huang et al. (2022) demonstrated that the *SG2* gene, which encodes a protein with a Ribonuclease H-like domain, is a positive regulator downstream of *GSK2* in response to brassinosteroids (BR) signaling. The mutation in *SG2* resulted in smaller grain size due to repressed cell expansion in spikelet hulls. This indicates that *SG2*, and by extension *GS2*, plays a crucial role in the BR signaling pathway, affecting grain size. Another study Lyu et al. (2020) highlighted the role of the GSK2-OML4 pathway in regulating grain size and weight (Figure 1). The *LARGE1* gene, encoding the OML4 protein, is phosphorylated by GSK2 and negatively controls grain size (Lyu et al., 2020). Loss of function of OML4 leads to larger and heavier grains, suggesting that manipulating the GSK2-OML4 pathway, which includes *GS2*, can significantly impact grain size and weight.

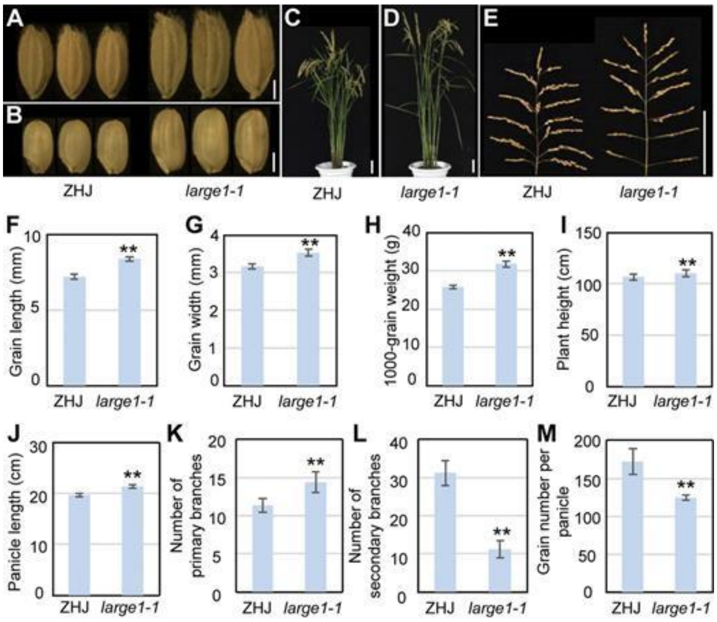

Figure 1 Large1 influences grain size and plant morphology (Adopted from Lyu et al., 2020)

Image caption: (A) Mature paddy grains of ZHJ and large1-1, (B) Brown rice grains of ZHJ and large1-1, (C) and (D) ZHJ (C) and large1-1, (D) plants at mature stage, (E) ZHJ (left) and large1-1 (right) panicles, (F) and (G) Grain length (F) and width (G) of ZHJ and large1-1, (H) The 1000-grain weight of ZHJ and large1-1, (I) Plant height of ZHJ and large1-1, (J) Panicle length of ZHJ and large1-1, (K) Number of ZHJ and large1-1 primary panicle branches, (L) Number of ZHJ and large1-1 secondary panicle branches, (M) Grain number per panicle of ZHJ and large1-1. Values ( [F] to [H]) are given as mean ± SD ($n \geq 50$). Values ( [I] to [M]) are given as means ± SD ($n = 20$). Asterisks indicate significant differences between ZHJ and large1-1. **, $P < 0.01$ compared with the wild type (ZHJ) by Student's t test. Bar in (A) and (B) = 2 mm; bar in (C) to (E) = 10 cm (Adopted from Lyu et al., 2020)

Lyu et al. (2020) found that the large1-1 variant of the ZHJ rice strain exhibits significant differences in grain size and plant morphology compared to the wild type. The study measured several parameters and revealed that large1-1 grains are notably longer and wider, and the 1000-grain weight is higher, indicating larger individual grain mass. Additionally, plants with the large1-1 mutation are taller, with increased panicle length and a higher number of both primary and secondary branches on the panicles. Furthermore, large1-1 plants produce more grains per panicle, demonstrating enhanced reproductive capability. These morphological and reproductive enhancements were statistically significant with a p-value of less than 0.01, suggesting that the large1-1 mutation positively influences both grain yield and overall plant structure (Haliru et al., 2020).

### 4.2 Success stories: field applications and yield improvements

Field applications of *GS2* gene manipulation have shown promising results in improving rice yield. For example, the study by Usman et al. (2021) utilized CRISPR/Cas9 to edit the *GS3* gene, which is closely related to *GS2*, resulting in increased grain length and weight (Figure 2). The mutants exhibited a 31.39% increase in grain length and a 27.15% increase in 1 000-grain weight compared to wild-type plants. This demonstrates the potential of gene editing technologies in enhancing rice yield through targeted manipulation of genes like *GS2*. Additionally, the study by Zhang et al. (2020) developed functional markers for 14 genes related to grain size, including *GS2*. These markers were used to genotype a global collection of rice cultivars, revealing significant trait contributions from *GS2*. The successful application of these markers in breeding programs has facilitated the selection of lines with superior grain yield and quality, underscoring the practical benefits of *GS2* gene manipulation in field conditions.

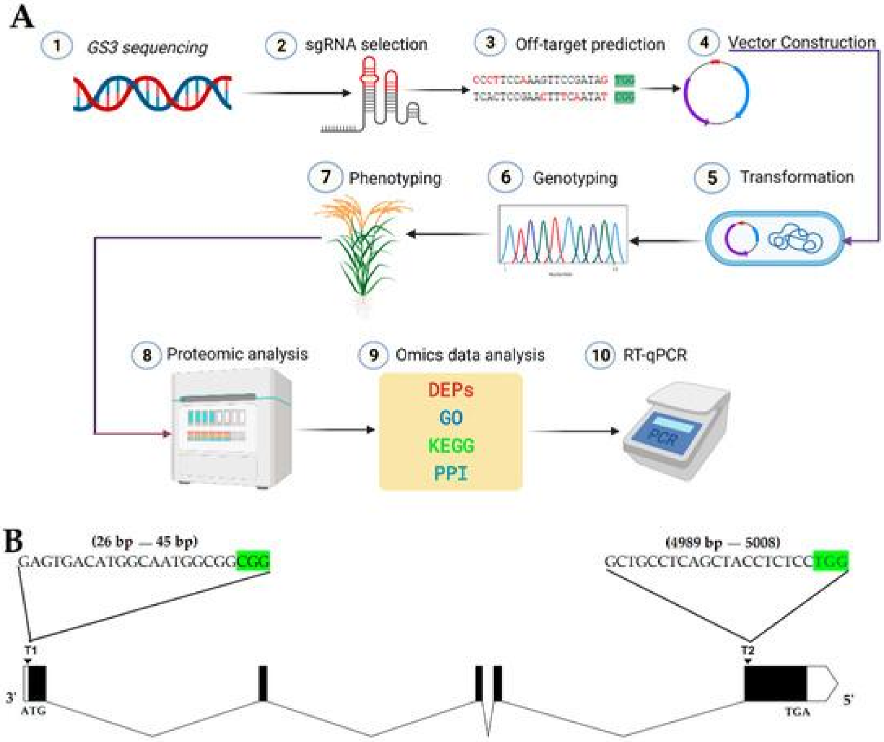

Figure 2 Schematic diagram of the procedure for CRISPR/Cas9-based generation of mutant plants and analysis of mutations and Diagram of *GS3* gene and positions of both target sites. ATG is the start codon (Adopted from Usman et al., 2021)

Image caption: (A) Schematic diagram of the procedure for CRISPR/Cas9-based generation of mutant plants and analysis of mutations. Two sgRNAs were selected using the CRISPR-GE online web-based tool, and vector was constructed. Agrobacterium-mediated transformation was performed, and T0 plants were regenerated. Later generations were produced by self-pollination, and genotyping was performed using target-specific primers. The phenotypic data of mutant and wild-type (WT) plants were recorded and further analyzed. The proteomic analysis was also performed, and RT-qPCR was performed to assess the GS3 expression level and validate the proteomic data. (B) Diagram of GS3 gene and positions of both target sites. ATG is the start codon; TGA is the stop codon; green highlighted CGG and TGG are the PAM sequences; the white boxes at extreme left and right represent the untranslated (UTR) regions, the black boxes represents the exons; black lines in between the exon regions represent the intron regions, T1 and T2 represent target 1 and target 2, respectively (Adopted from Usman et al., 2021)

Usman et al. (2021) found that the use of CRISPR/Cas9 technology effectively generates mutant plants by targeting specific genes. In their study, they selected two sgRNAs to create mutations in the GS3 gene, which influences grain size. The vector construction and Agrobacterium-mediated transformation led to the successful regeneration of T0 plants. Subsequent self-pollination produced later generations, which were genotyped to confirm the presence of mutations. Phenotypic analysis compared mutant plants to wild-type (WT) plants, and the results showed significant differences in growth and development. Proteomic analysis and RT-qPCR were used to assess GS3 expression levels, validating the impact of the mutations on plant phenotype. This approach demonstrates the precision and effectiveness of CRISPR/Cas9 in plant genetic engineering, providing a robust method for studying gene function and improving crop traits.

### 4.3 Comparative analysis with non-*GS2* modified rice varieties

Comparative studies between *GS2*-modified and non-modified rice varieties have provided insights into the effectiveness of *GS2* manipulation. The study by Gull et al. (2019) investigated the contribution of multiple genes, including *GS2*, to grain size and weight in 204 diverse rice germplasms. The results showed that *GS2* was significantly associated with grain length, width, and thickness, indicating its pivotal role in determining grain size. The non-*GS2* modified varieties displayed a wide range of variability in these traits, highlighting the potential for improvement through *GS2* manipulation.Furthermore, the study by Ngangkham et al. (2018) examined the influence of seven known grain size-regulating genes, including *GS2*, in 89 rice germplasms. The findings revealed that *GS2*, along with other genes, showed strong associations with grain size traits. The non-modified varieties exhibited less favorable grain size characteristics compared to those with targeted gene modifications, demonstrating the advantages of *GS2* manipulation in achieving desired agronomic traits. In summary, the manipulation of the *GS2* gene has shown significant potential in improving rice yield through various genetic and molecular approaches. Field applications and comparative analyses further support the effectiveness of *GS2* gene modification in enhancing grain size and weight, making it a valuable target for rice breeding programs.

## 5 Technological Advances in Studying the *GS2* Gene

### 5.1 Recent developments in genetic engineering and CRISPR

Recent advancements in genetic engineering, particularly the CRISPR/Cas9 system, have significantly enhanced our ability to manipulate genes associated with grain size in rice. CRISPR/Cas9 has been effectively used to edit multiple genes, including *GS3* and *GL3.1*, which are closely related to grain size and weight. For instance, simultaneous editing of *GS3* and *GL3.1* in rice resulted in mutants with larger grains, although it also led to a reduction in grain number and overall yield, highlighting the complexity of genetic interactions in grain size regulation (Chen et al., 2020). Additionally, CRISPR/Cas9-mediated mutagenesis of the *GS3* gene has been shown to increase grain length and weight by regulating proteins involved in cellular processes such as cysteine proteinase inhibitors and ubiquitin-related proteins (Usman et al. 2021). These studies underscore the potential of CRISPR/Cas9 in creating rice varieties with improved grain characteristics.

### 5.2 Advances in genomic sequencing techniques for *GS2*

The advent of high-throughput genomic sequencing techniques has revolutionized the study of genes like *GS2*. Techniques such as genome-wide association studies (GWAS) and single nucleotide polymorphism (SNP) arrays have been instrumental in identifying quantitative trait loci (QTLs) associated with grain size. For example, a GWAS identified the novel grain size gene *OsSNB*, which negatively regulates grain size, and demonstrated that knockout mutants of this gene exhibit increased grain length, width, and weight (Figure 3) (Ma et al., 2019). Similarly, chromosome segment substitution lines (CSSLs) have been used to map QTLs for grain size, leading to the identification of genes such as *OsGH3.13*, which influences grain length and weight (Tan et al., 2021). These genomic tools provide a comprehensive understanding of the genetic basis of grain size and facilitate the identification of candidate genes for targeted breeding.

Ma et al. (2019) found that the *OsSNB* gene plays a critical role in rice plant development. Through the application of CRISPR/Cas9 technology, they successfully generated knockout mutant lines (KO1 and KO2) and overexpression lines (OE1 and OE2) to study the gene's function. The relative expression levels of *OsSNB* were significantly altered in these transgenic lines compared to the wild type (WT). Specifically, overexpression lines exhibited elevated *OsSNB* expression, while knockout lines showed reduced expression. The phenotypic analysis revealed notable differences in plant morphology and growth between the transgenic lines and WT plants, highlighting the impact of *OsSNB* manipulation. This study underscores the importance of *OsSNB* in regulating key developmental processes in rice and demonstrates the potential of gene editing technologies in agricultural biotechnology.

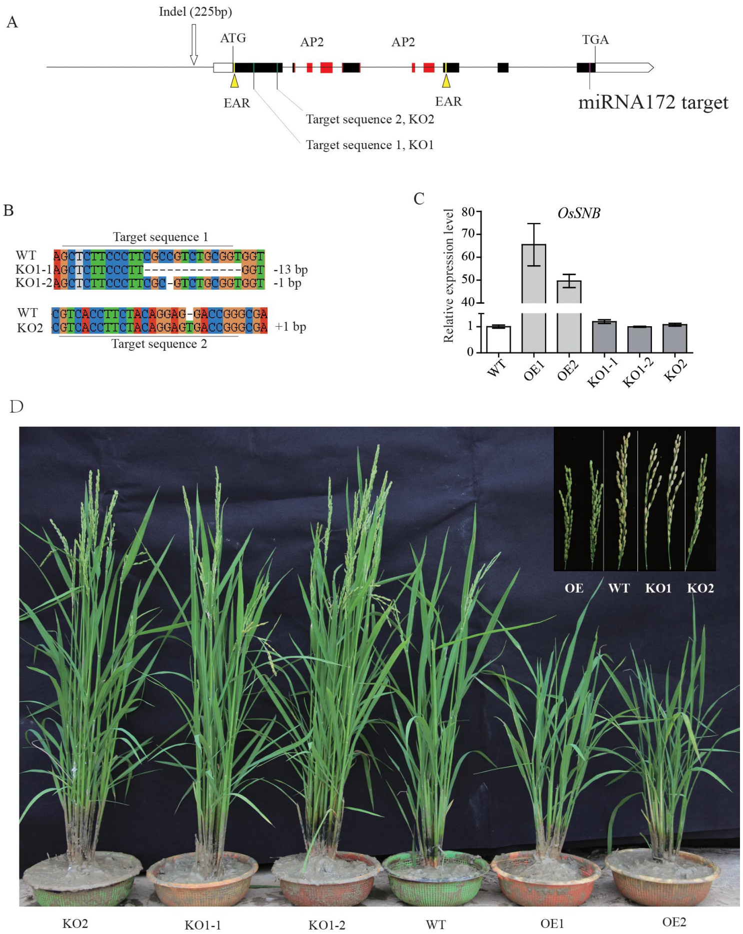

Figure 3 Molecular identification of *OsSNB* transgenic plants (Adopted from Ma et al., 2019)
Image caption: (A) The structure of *OsSNB*. (B) The knockout mutant plants were obtained by CRISPR/Cas 9 technology. (C) Relative expression levels of *OsSNB* in transgenic plants. WT: wildtype; OE1, OE2: overexpression lines; KO1-1, KO1-2, KO2: knockout mutant lines. (D) Phenotypes of transgenic rice plants and WT plants (Adopted from Ma et al., 2019)

### 5.3 Bioinformatics tools for analyzing *GS2*-related data

Bioinformatics tools play a crucial role in analyzing the vast amount of data generated from genomic studies of the *GS2* gene. Tools for proteomic analysis, such as isobaric tags for relative and absolute quantitation (iTRAQ), have been used to identify differentially expressed proteins in GS3 mutants, revealing key proteins involved in cellular processes and metabolic pathways (Usman et al., 2021). Additionally, bioinformatics approaches have been employed to analyze the genetic variations and allele contributions of multiple genes related to grain size. For instance, insertion/deletion (InDel) markers have been used to study the genetic diversity and marker-trait associations in rice germplasm, providing insights into the genetic regulation of grain size and weight (Yan et al., 2023). These bioinformatics tools are essential for integrating and interpreting complex datasets, thereby advancing our understanding of the genetic mechanisms underlying grain size regulation. In summary, the integration of genetic engineering, advanced genomic sequencing techniques, and bioinformatics tools has significantly advanced the study of the *GS2* gene and its role in rice yield improvement. These technological advances provide powerful means to dissect the genetic architecture of grain size and develop high-yielding rice varieties through precise genetic modifications (Wang et al., 2021).

## 6 Breeding Strategies Involving the *GS2* Gene

### 6.1 Incorporating *GS2* insights into conventional breeding programs

The *GS2* gene has been identified as a significant regulator of grain size and weight in rice, making it a valuable target for conventional breeding programs. Studies have shown that *GS2*, along with other genes such as *GS3*, *GW8*, and *GL7*, plays a crucial role in determining grain length, width, and thickness, which are key traits for improving rice yield and quality (Zhong et al., 2019). By selecting rice varieties that exhibit favorable alleles of the *GS2* gene, breeders can enhance grain size and weight, thereby increasing overall yield. The integration of *GS2* insights into conventional breeding can be achieved through phenotypic selection and cross-breeding of high-yielding varieties with desirable grain traits (Mishra et al., 2022).

### 6.2 Molecular marker-assisted selection for *GS2* traits

Molecular marker-assisted selection (MAS) is a powerful tool for incorporating *GS2* traits into rice breeding programs. InDel markers have been successfully used to identify and select for favorable alleles of the *GS2* gene, along with other grain size-related genes such as *GS3*, *GW8*, and *GL72*. This approach allows for the precise selection of genotypes with desirable traits, thereby accelerating the breeding process. The use of MAS can help in the development of rice varieties with improved grain size and weight, which are essential for meeting market demands and enhancing yield (Gull et al., 2019). Additionally, the application of CRISPR/Cas9 technology to edit genes like *GS3* and *GL3.1* has shown potential in rapidly improving grain size, although it may have some trade-offs in terms of grain quality and yield (Chen et al., 2020).

### 6.3 Challenges and opportunities in GS2-based breeding

While the incorporation of *GS2* into rice breeding programs offers significant opportunities for yield improvement, several challenges must be addressed. One major challenge is the complex interaction between multiple genes that regulate grain size and weight. For instance, the interaction between *GS2* and other genes such as *OsOFP19*, which plays an antagonistic role, complicates the breeding process (Huang et al., 2022). Additionally, the pleiotropic effects of gene editing, as observed with the simultaneous knockout of *GS3* and *GL3.1*, can lead to unintended consequences on other agronomic traits (Li et al., 2021). Despite these challenges, the opportunities for *GS2*-based breeding are substantial. The identification of favorable alleles and their incorporation into breeding programs can lead to the development of high-yielding rice varieties with improved grain size and weight . Furthermore, the use of advanced molecular techniques such as MAS and CRISPR/Cas9 can enhance the efficiency and precision of breeding efforts, ultimately contributing to global food security.

## 7 Impact of *GS2* Research on Rice Agriculture

### 7.1 Implications of *GS2* studies for rice yield enhancement

Research on the *GS2* gene has significantly advanced our understanding of grain size regulation in rice, which is a critical determinant of yield. Studies have shown that *GS2*, along with other genes, plays a pivotal role in controlling grain length, width, and weight. For instance, the study by Ngangkham et al. (2018) demonstrated that *GS2*, among other genes, is associated with grain length (GL), grain width (GW), and grain thickness (GT), which collectively influence the thousand grain weight (TGW). This highlights the potential of *GS2* in breeding programs aimed at improving rice yield by selecting for favorable grain size traits. Additionally, the research by supports the importance of *GS2* in grain size regulation, showing that it is one of the key genes influencing grain length and width, which are crucial for yield enhancement (Anant et al., 2021).

### 7.2 Role of *GS2* in meeting global food security challenges

The *GS2* gene's role in regulating grain size has broader implications for global food security. As the global population continues to grow, there is an increasing demand for higher crop yields. The ability to manipulate grain size through genetic means, such as targeting the *GS2* gene, offers a promising strategy to meet this demand. The study by Huang et al. (2022) highlights the potential of *GS2* in improving grain size through its interaction with brassinosteroids (BRs), which are known to enhance cell expansion in spikelet hulls. This suggests that *GS2* can be a target for genetic modifications to produce rice varieties with larger grains, thereby increasing overall yield. Furthermore, the research by Lyu et al. (2020) indicates that the *GS2* gene, along with other genes in the *GSK2* signaling pathway, can be manipulated to achieve desired grain size and weight, which is essential for enhancing food production and ensuring food security (Ramayya et al.,2021).

### 7.3 Strategies for scaling *GS2* research to benefit global rice production

To maximize the benefits of *GS2* research for global rice production, several strategies can be employed. First, the development of molecular markers for *GS2* can facilitate the selection of desirable traits in breeding programs. This approach allows for the efficient identification and incorporation of favorable *GS2* alleles into new rice varieties. Second, the use of advanced genome editing technologies, such as CRISPR/Cas9, can be leveraged to create targeted mutations in the *GS2* gene to enhance grain size and yield, as shown by Usman et al. (2021). These technologies enable precise modifications, leading to the rapid development of high-yielding rice varieties. Lastly, integrating *GS2* research with other agronomic practices and breeding strategies, such as marker-assisted selection and pyramiding of multiple yield-related genes, can further enhance the impact of *GS2* on rice production. The study by Zhang et al. (2020) emphasizes the importance of combining multiple gene targets to achieve a balance between grain yield and quality, which is crucial for the success of rice breeding programs. In conclusion, the *GS2* gene holds significant promise for improving rice yield and addressing global food security challenges. By employing molecular markers, genome editing technologies, and integrated breeding strategies, the benefits of *GS2* research can be scaled to enhance global rice production (Radchenko et al., 2022).

## 8 Future Directions in Rice Genetic Research

### 8.1 Potential for new discoveries in grain size and weight genes

The genetic basis of grain size and weight in rice is complex and involves multiple genes and pathways. Recent studies have identified several key genes and quantitative trait loci (QTLs) that regulate these traits. For instance, the TGW2 gene has been shown to influence grain width and weight through cell proliferation and expansion in glumes (Ruan et al., 2020). Similarly, the GSK2-OML4 pathway negatively regulates grain size and weight by restricting cell expansion in the spikelet hull (Lyu et al., 2020). The *GW6* gene, which is regulated by gibberellins, also plays a significant role in increasing grain width and weight by promoting cell expansion (Shi et al., 2020). These discoveries highlight the potential for identifying new genes and pathways that can be targeted to improve rice yield.

### 8.2 Collaborative efforts to enhance rice genetics research

Collaborative efforts among researchers, institutions, and countries are crucial for advancing rice genetics research. The study of multiple allelic combinations of genes, such as *DEP1*, *GS7*, *GS3*, *GW8*, *GL7*, *GS5*, and *GW2*, has shown that these genes interact in complex ways to regulate grain size and yield (Ngangkham et al., 2018). Additionally, the use of diverse rice germplasms and advanced genetic tools, such as InDel markers, has facilitated the identification of key genes and their contributions to grain size and weight. Collaborative projects, such as the multiparent advanced generation intercross (MAGIC) populations, have also been instrumental in identifying QTLs and SNPs associated with grain size traits (Zhou et al., 2021). These efforts underscore the importance of international collaboration in accelerating the discovery and application of genetic improvements in rice.

### 8.3 Emerging technologies and their potential impact

Emerging technologies, such as CRISPR/Cas9 genome editing and proteomic analysis, hold great promise for rice genetic research. The CRISPR/Cas9 system has been successfully used to edit the GS3 gene, resulting in increased grain length and weight (Yang et al., 2020). Proteomic analysis has further revealed the involvement of proteins related to cysteine proteinase inhibitors and ubiquitin-related proteins in regulating grain size (Yan et al., 2023). Additionally, the identification of functional markers for genes related to grain size, such as *GW2*, *GS2*, and *GL3.1*, can expedite the selection of superior rice lines in breeding programs (Usman et al. 2021). These technologies offer powerful tools for dissecting the genetic and molecular mechanisms underlying grain size and weight, and for developing high-yielding rice varieties.

## 9 Concluding Remarks

The *GS2* gene has been identified as a significant regulator of grain size and weight in rice. Studies have shown that *GS2* is associated with multiple grain traits, including grain length (GL), grain width (GW), and grain thickness (GT). Specifically, *GS2* influences these traits by modulating cell expansion and proliferation within the spikelet hulls, which directly impacts the overall grain size and weight. The gene's role in these processes underscores its importance in determining the final yield of rice crops.

This study has provided valuable insights into the genetic mechanisms underlying grain size and weight regulation in rice. By elucidating the role of *GS2* and its interaction with other genes and pathways, such as the *GSK2-OML4* pathway and the *GW2-WG1-OsbZIP47* regulatory module, we have expanded our understanding of how grain size can be manipulated to enhance rice yield. The identification of key alleles and their contributions to grain traits offers practical applications for rice breeding programs aimed at developing high-yielding varieties with desirable grain characteristics. Additionally, the use of InDel markers and other genetic tools has demonstrated the potential for precise selection and breeding strategies to improve grain size and weight.

Future research should focus on further dissecting the molecular pathways involving *GS2* and its interactions with other genes to fully understand the regulatory networks controlling grain size and weight. Investigating the environmental factors and their influence on *GS2* expression and function could provide additional insights into optimizing rice yield under varying conditions. Moreover, expanding the genetic diversity of studied germplasms and incorporating advanced genomic techniques, such as CRISPR/Cas9, could facilitate the development of rice varieties with enhanced grain size and yield. Finally, integrating these findings into practical breeding programs and field trials will be crucial for translating genetic insights into tangible yield improvements in rice cultivation.

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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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