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THE CONTRIBUTION OF TRANSGENIC RICE TO ENHANCE GRAIN YIELD

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Abstract *Breeders' main goal is to increase the proportion of high-quality rice produced overall. To create effective rice breeding strategies, possible yield-related loci have been mined. many researchers are using transgenic strategies as cutting-edge methods to increase rice productivity. Quantitative trait loci (QTLs) play a pivotal role in governing grain yield in Oryza sativa L., commonly known as rice. The genes contributing to QTLs that determine grain size, length, and weight have been successfully identified. Numerous genes are upregulated to enhance the overall yield of rice. Recent advancements have led to the discovery of genes and QTLs specifically associated with rice yield. Through an in-depth analysis of various yield characteristics, including grain weight, thousand grain weight, grain length, grain width, grain yield per plant, grain number per panicle, and panicles per plant, we conducted a comprehensive review using extensive literature research and public domain databases. Additionally, we explored the progress made in transgenic technology and advanced genomic techniques. The compiled information on genes and QTLs related to yield enhancement aims to provide a valuable resource. The integrated analysis of existing data on genes and/or QTLs provide evidence on potential combinations for creating superior genotypes that combine high yield across multiple traits. Integration of molecular markers, transgenic techniques and conventional breeding as discussed in this extensive review opens up the prospect of developing high yielding rice varieties.*

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Introduction

Rice (*Oryza sativa* L.), being one of the three major staple food crops, is essential for feeding more than half of the world's people. It is often called the "staple of life" giving evidence to the immeasurable role it plays in sustaining life in almost half of the world's population (Shrestha et al., 2020). This seemingly ordinary grain, brought forth from the rich fertile lands of Asia, has far surpassed its geographical origin to be known worldwide as a staple food (Haider et al., 2023). Its trajectory from humble beginnings to the present-day status of a daily dietary must-have reflects the delicate balance between nature and human innovativeness that has been sustaining civilizations for ages (Verma & Srivastav, 2020). Rice cultivating which has a history of over 10,000 years can be traced back to the Yangtze River basin in China. Over ages, its course has taken various continents, conforming to diverse climates and finally being a staple for cultures from the terraced fields of Southeast Asia to the vast plains of India and the flooded paddies of Japan (Sami et al., 2023). It is remarkable resilience and adaptability, which makes it grow in various conditions, that enabled it to fit into the social fabric of societies worldwide. It is not just a food item but a cultural and economic anchor (Birla et al., 2017). Through the course of generations, Oryza sativa and *Oryza glaberrima*, the two main cultivated rice species, have been subjected to selective breeding, which resulted in the development of the wide array of varieties with different traits (Sami et al., 2024). If the Basmati rice of the Indian subcontinent is aromatic, the sushi rice from Japan is short-grained, and sticky rice is essential in Southeast Asian cuisines, then rice is a canvas used by culinary traditions to paint their flavor profiles (Ito & Lacerda, 2019). Besides its nutritional and cultural significance, rice farming also influences land and ecosystems. The micro art of rice farming is composed of a sophisticated water management system where paddies are not just places of cultivation but are complex ecological systems (Mushtaq et al., 2023). The landscapes shaped by rice farming, from the terraced hillsides of China to the rice fields of the Mekong Delta, are the evidence of a coexistence where humans and nature are in harmony.

The cultural role of rice is far more than just a staple; it is a symbol that is deeply integrated into ceremonies, traditions, and folklore (Kim et al., 2020). Rice is linked with prosperity, fertility, and life in many Asian cultures. The planting and harvesting seasons are heavily ceremonial, serving to highlight the mutually beneficial relationship between the farmer and the land. In Asian wedding, where rice is a symbol of abundance and prosperity, the significance of rice as a cultural object is even more evident (Kaur et al., 2016). The rice impact, from the economic perspective, is also tremendous. The livelihoods of millions of agricultural workers depend on rice cultivation, which is at the heart of many economies. The global rice market is an ever-evolving entity, where nations such as India, China and Thailand are becoming the main drivers of the trade. However, the economic growth has its own problems, among which are sustainability, environmental impact, and the need for smart farming to cope with the ever-growing demand for this important cereal (Lakshmanan et al., 2015).

Transgenic Rice

People are working hard to make sure there's enough food for everyone in the world. The way we talk about farming is changing because of new technologies. Among them, the transgenic or the genetically modified (GM) crops have become a critical factor, which are changing the conventional agricultural systems. In this research, we plunge into the world of transgenic rice and its revolutionary influence on agricultural systems, highlighting the advances in improving yield to tackle the ever-increasing needs of a burgeoning population (Todaka et al., 2015). Rice, which is a dietary staple for billions, has always been in the focus of research aimed at increasing productivity. Transgenic rice, modified to express features such as pest resistance, tolerance to environmental stress, and improved nutritional content, offers a solution to the problems that farmers face all over the world. While dealing with transgenic rice, we discover a narrative of ingenuity, dispute, and the possibility of transforming the whole world's food system (Yamori et al., 2016). The genetic modification of the rice involves the insertion of specific genes that provide the desired characteristics, usually taken from other organisms. The objective of this scientific effort is to fortify rice plants with increased pest and disease resistance, tolerance to harsh climate conditions, and better nutrition profiles, which will in turn improve yields and make the crop more resilient to the changing environment (James et al., 2018).

One of the major reasons for the development of transgenic rice is the desperate need to increase global food production. With a population expected to reach over 9 billion by 2050, traditional agricultural practices are faced with the challenge of providing sufficient food. Transgenic rice presents an opportunity for the future by providing a means of crop improvement that exceeds the limitations of traditional breeding, enabling the desired changes to be made more accurately and precisely (Dhungana et al., 2015). The development of transgenic rice is not without controversies, since discussions on safety, environmental concerns and ethical issues are still ongoing. Critics express worries about unforeseen consequences and the possible long-term implications of putting genetically modified organisms into the environment. Nevertheless, supporters claim that highly tested and regulated transgenic rice varieties are safe, highlighting the advantages for both farmers and consumers (Liu et al., 2016). However, the frequent appearance of the erratic and extreme weather conditions is a big threat to the rice production (Muthayya et al., 2014). In order to meet these challenges, breeders are encouraged to develop innovative breeding technologies that enhance yield as well as environmental sustainability. The key feature of this molecular breeding is the accurate identification of QTLs linked to grain quality and nutrition (Zuo et al., 2021). These genetic markers provide the important hints to the molecular basis of the favorable rice traits. The objective is to analyze and manipulate the genetic components that determine these characteristics, thus, the development of superior rice varieties with high-quality nutrition. A previous study has highlighted the importance of a more sophisticated way of utilizing the minor QTLs by considering the complicated pattern of interaction of multiple minor genes that control rice quality. Through uncovering the molecular mechanism and exploiting the advantages of these genetic factors, breeders will not only enhance rice quality but also set the stage for a new era of environmentally friendly and sustainable farming practices (Bai et al., 2012).

The distinctive genes responsible for regulating quantitative trait loci (QTLs) associated with grain size and weight have been successfully pinpointed. Among these, the gene pair GL7/GW7 (S. Wang et al., 2015; Y. Wang et al., 2015), as well as GS9 (Abaza et al.), demonstrated precise control over grain length (GL) and grain width (GW) without influencing the total grain weight (TGW). Conversely, the gene GSA1 exhibited effects on both GL and GW, displaying consistent allelic directions, and remarkably influenced the overall TGW (Dong et al., 2020). Genes named GS5 (Li et al., 2011), GW2 (Song et al., 2007), TGW2 (Ruan et al., 2020), GSE5 (Duan et al., 2017), GW6 (Gao et al., 2021; Munir et al., 2020) and GW8 (Wang et al., 2012)probably regulated GW and TGW. other genes, namely OsGRF4 (Gao et al., 2021), qTGW1.2b (Chan et al., 2021), GS2/GL2 (Che et al., 2015; Hu et al., 2015), OsLG3 (Yu et al., 2017) qLGY3/OsLG3b (Liu et al., 2018; Yu et al., 2018), GS3(Zeng et al., 2020), SG3 (Li et al., 2020), qGL3/GL3.1 (Qi et al., 2012; Zhang et al., 2012), GW6a (Song et al., 2015), TGW6 (Ishimaru et al., 2013), qTGW3 (Hu et al., 2018), GL6 (Wang et al., 2019), qGL5 (Qiao et al., 2021) and GLW7 (Si et al., 2016) primarily governed by TGW and GL. Except for chromosomes 4, 10, 11, and 12, all of the rice chromosomes include these genes. A new gene resource for molecular breeding that will increase grain weight and size is made available via the cloning of OsMADS56 (Zuo et al., 2021). Through the use of Ho-LAMap or genome-wide association analyses, GSE5, GLW7, and OsLG3 were cloned (Duan et al., 2017; Si et al., 2016; Yu et al., 2017). Increased panicle length and larger grains were seen in osmyb30 mutants (Zeng et al., 2020; Zuo et al., 2021). In comparison to the wild type, the ospin5b knockdown mutant had longer panicles and more tillers (Zeng et al., 2020).

Rice yield and quality improved as a result of the expression of GWD1 and OSL2 (Wang et al., 2021). Overexpression of OsCPK18 boosts rice yield (Li et al., 2022). Grain yield was increased by the (NtPT1) gene, which accumulates phosphate (Clement et al., 2017; Park et al., 2010). The ongoing search for new genes controlling these properties has led to the development of transgenic rice plants that carry genes like the microRNA OsmiR397, which increases yield by up to 25% in field trials by boosting grain size and panicle branching (Clement et al., 2017; Zhang et al., 2013). The TIFY11B gene increased grain size and yield by demonstrating higher carbohydrate buildup in the vegetative organ (Hakata et al., 2012). The transgenic lines with both GNP1 and NAL1 genes showed higher GNP and grain yield compared to the transgenic lines with GNP1 or NAL1 alone. The use of the two genes in the introgression lines together enhanced the GNP. These findings should be useful in pyramiding GNP1 and NAL1 in rice breeding for high yield (Wang et al., 2020).

In the rice plant, the promoter D18 led to a significant expression of osa-MIR156f in the stem. Transgenic lines exhibited more tillers with an average height. Besides, there was a drastic rise in grain yield (Liu et al., 2019). There are six OsSUS genes that are singly overexpressed in transgenic rice lines. This results in a drastic increase in grain weight. The transgenic hulls of OsSUS3 show larger cell clusters during hull growth and development thanks to the acceleration of cell division and the corresponding cellulose synthesis (Fan et al., 2019). OsFWL4 governs tillering and plant yield in rice and the Agrobacterium-mediated CRISPR/Cas9 system can produce rice plants with specified mutations in the T1 generation (Gao et al., 2020) gene OsMPH1 is overexpressed. On the other hand, down-regulation of OsMPH1 results in more plant height and grain yield in rice plants. The rice height-regulating gene that produces a member of the MYB family of transcription factor. OsMPH1 overexpression in plants led to the increased plant height by expanding internodes cells (Zhang et al., 2017). The overexpression of the DEP1 allele is accompanied by an increase in the grain size, which results in large grains, whereas a decrease in the grain size is caused by the under-expression of this allele, which leads to the development of small grains. The interaction between DEP1 and RGB1 is critical in triggering the grain growth process, whereas the regulation of grain size is achieved by GS3, which exerts its effect by inhibiting the activity of DEP1. Enlargement of grain size is a consequence of DEP1 allele expressed at a higher level, which in turn leads to the emergence of larger grains, while the DEP1 allele, on the other hand, results in the formation of smaller grains. It is the complex mechanism of grain development, which takes place through the cooperative engagement of DEP1 and RGB1, that acts as a booster for vigorous grain growth. In addition to this, the control of the grain size is governed by GS3, that influences DEP1 (Zhao et al., 2019). Among the number of variables improved in transgenic rice overexpressing gene OsSGL are the grain number per panicle, length and width, and the yield, which increased significantly (Wang et al., 2016).

Rice yield-related attributes are associated with a slew of genes in genome-wide association

OsMADS56 has been identified just as a causative gene driving a rice QTL for grain size and weight. The result was originally verified using NIL populations that were just OsMADS56-segregated, and it was later validated using gene target mutagenesis. MADS-box protein, encoded by OsMADS56, has four domains: the M, I, C-terminal, and K domains (Zuo et al., 2021). Irrespective of whether the mutation occurred at target A (observed in mutants S1, S2, and S3), target C (notably in D2-1), or concurrently at both targets B and C, a consistent reduction in both grain weight and size was observed across all knock-out mutants of OsMADS56TQ (comprising D1-1, D1-2, and D2-2). This uniformity in the phenotypic outcome underscores the significant role of OsMADS56TQ in regulating these crucial agronomic traits, regardless of the specific mutation site. The findings demonstrated that grain size and weight can be affected by change of sequence in any domain of the coding area and thus the OsMADS56 gene, which is similar to some genes duplicated for grain weight and size, such as GS5 (Li et al., 2011), GW8 (Wang et al., 2012), GS2 (Hu et al., 2015), GL2 (Che et al., 2015), GL3.1 (Qi et al., 2012), and GLW7 (Si et al., 2016), was connected with an increase in the expression of grain size and weight (Zuo et al., 2021). Under long-day settings, overexpression of OsMADS56 caused a delay in heading via the OsLFL1-Ehd1 pathway (RYU et al., 2009). To investigate the association between heading and expression of OsMADS56 and heading date genes in rice, plants were grown under natural day conditions. The expression of OsMADS56 was significantly higher in NIL-TQ and ZY179 than in NIL-IR and D1-2. Leaf samples that were gathered while the plant was transitioning from the vegetative to the reproductive phase were analyzed using RNA-seq. It was also found that NIL-TQ and ZY179 induced more heading than NIL-IR and D1-2 did. Although our findings also indicate that OsMADS56 controls the heading of rice, the relationship between the upregulated OsMADS56 expression and the early heading is different. Moreover, no obvious difference between EHD1 and HD1 expression was found, indicating that OsMADS56 may be involved in the additional, unrecognized pathways (Zuo et al., 2021).

OsPIN5b and GS3 gene impact on rice yield

Genetic modifications were performed on two key genes—OsPIN5b, which is responsible for the panicle length, and GS3, which regulates the grain size—to improve the yield of rice. The sites targeted for editing were OsPIN5b-site1, OsPIN5b-site2, GS3-site1, and GS3-site2 (Zeng et al., 2020). The CRISPR/Cas9 system was used in the T0 generation to get significant editing efficiency. Importantly, OsPIN5b-site1 showed editing efficiency of 53%, OsPIN5b-site2 at 42%, GS3-site1 at 66%, and GS3-site2 at 63%. These findings further support the effectiveness of the CRISPR/Cas9 system in the precise editing of target genes and indicate a great potential for the creation of rice mutants with enhanced yield (Ma & Liu, 2016). The results also showed that the two mutants T2 generations had higher yields than the wild type (Zeng et al., 2020).

Table 1: The significant QTNs for grain length

Trait	QTN	Chromosome	position	Reported genes
Grain Length	qGL-3-3	3	16,699,322	GS3
	qGL-3-4	3	16,717,839	GS3
	qGL-3-5	3	16,911,337	GS3
	qGL-3-6	3	35,509,618	qTGW3
	qGL-5-1	5	5,371,587	GSE5/GW5

HDR3 controls the size and weight of rice grains.

The rice grain size and yield are controlled by the histone acetyltransferase GRAIN WEIGHT 6a (GW6a). Despite the lack of identification of the specific gene regulatory network that modulates grain size by GW6a, it has been confirmed that GW6a interacts with HDR3 (HOMOLOG OF DA1 ON RICE CHROMOSOME 3). HDR3 serves as a ubiquitin receptor, encompassing the ubiquitin-interacting motif, thus establishing its role in the mechanism associated with GW6a's regulation of grain size. In contrast, to control lines, overexpressed HDR3 plants produced wide grains, while HDR3 knockout lines produce reduced grains. According to cytological evidence, HDR3 affects grain size similarly to GW6a via changing the proliferation of cells in spikelet hulls. In transgenic rice plants, the HDR3 gene was overexpressed, endowing them with the capacity to modulate grain size. Through phenotypic evaluation and rigorous statistical analysis, it was established that the grains in the HDR3 overexpressing (HDR3-OE) plants exhibited a remarkable 20% increase in size compared to their non-transgenic counterparts. This augmentation in size was attributed to a 10% increase in length, a 5% expansion in width, while the grain thickness remained unaltered. In contrast, two different transgenic plants (hdr3-1 and hdr3-2) with HDR3 gene editing using the CRISPR/Cas9 technique produced seeds that were obviously smaller than the standard. Accordingly, phenotypic tests revealed that the hdr3 grains were 15% lighter and 10% shorter than the control seeds, with no change in grain thickness. These findings together suggested that HDR3 regulates rice grain size and weight positively (Gao et al., 2021).

GWD1 and Osl2 expression evaluation in rice

First, Analysis using qRTPCR was conducted to measure GWD1 expression levels. Both newborn seedlings and fully grown plants' leaf blades showed the highest levels of GWD1 expression (Hirose et al., 2013). Examined Osl2's expression pattern next. It is aimed to use Osl2's promoter shows the expression of GWD1 in rice, boosting the expression of GWD1 in leaves at the late stage of filling of grains, because Osl2 is mostly expressed in leaves and its expression is substantially increased in rice leaf senescence. According to qRTPCR findings, Osl2 is mostly expressed in rice leaves and has low rates of expression in stems, blades, roots, and growing seeds. In particular, when the rice seeds matured, Osl2 expression in the rice leaves gradually increased. The Osl2 promoter was thus appropriate for driving GWD1 expression in rice and should aid in improving the transfer of photosynthetic result from source tissue to sink tissues during the time of filling of grains (Wang et al., 2021).

OsCPK18 and OsCPK4 control defense and yield-related features

In earlier studies, it was revealed that CALCIUM DEPENDENT PROTEIN KINASE 18 (OsCPK18) and MITOGEN-ACTIVATED PROTEIN KINASE 5 (OsMPK5) in rice engage in reciprocal phosphorylation, leading to the positive regulation of OsMPK5 and subsequent suppression of rice immunity. Our recent findings extend this understanding by identifying a new facet of their roles. Specifically, we discovered that OsCPK18 and OsCPK4 play a positive modulatory role in traits associated with plant yield and height. This adds a nuanced layer to the functional dynamics of these proteins, emphasizing their multifaceted contributions to key agricultural attributes beyond their previously identified roles in immune regulation. Analysis shows that OsMPK5 and OsCPK18 differently control development-related genes but synergistically regulate genes linked to defense. OsMPK5 phosphorylates the C-terminal threonine (T505) and serine (S512) residues of OsCPK18 and OsCPK4, respectively, according to in vitro and in vivo kinase experiments. OsCPK18T505D, whose kinase activity mimicked T505 phosphorylation by replacing T505 with aspartic acid, was less sensitive to calcium than wild-type OsCPK18. The edited MAPK phosphorylation motif in OsCPK18 and its paralog OsCPK4 was shown to boost both rice yield and immunity. This editing technique inhibits OsMPK5-mediated phosphorylation but keeps the kinase activity of calcium intact. The changes of the last seven amino acids in OsCPK18 decrease its binding with OsMPK5. The results indicate that application of CRISPR/Cas9-mediated construction of phosphorylation pathways could simultaneously increase crop yield and resistance. This novel strategy creates a new regulatory loop that fine-tunes the balance between growth and defense by remodeling the activity of OsCPK18/4 (Li et al., 2022) (Clement et al., 2017).

By CRISPR/Cas9 gene editing, the phosphorylation motif of MAPK of OsCPK18 has been successfully mutated. Furthermore, the MAPK phosphorylation pattern of OsCPK4 was modified in that OsMPK5 phosphorylates OsCPK4 at S512, which is similar to the case of OsCPK18. It is worth mentioning that the CRISPR/Cas9 editing scope is limited by certain features, such as the editing area and the PAM requirements. These considerations emphasize the accuracy and limitations on the use of the CRISPR/Cas9 technology for the targeted genetic modifications and no suitable gRNAs that can guide the Cas9 nuclease or base editor to alter the codons encoding the MAPK phosphorylation residues of OsCPK18 or OsCPK4 can be found (Zhu et al., 2020). Alternatively, gRNAs could be used to accurately insert frameshift mutations before T505 and S512 in OsCPK18 and OsCPK4, respectively. This would stop OsMPK5 from being phosphorylated by breaking the S/T-P motif. The final 10 and 7 amino acids of OsCPK18 and OsCPK4 would be changed by these frameshift mutations. Phenotypic studies were conducted using homozygous edited lines that had frameshift mutations in some alleles. Notably, OsCPK18-GE3 (2 bp), OsCPK18-GE8 (1 bp), OsCPK4-GE1 (2 bp), and OsCPK4-GE3 (4 bp) were the focus of these genetic modifications. It's crucial to highlight that alterations were confined to the C-terminal variable peptide beyond T505 and S512. Intriguingly, all plants with edited phosphorylation motifs exhibited enhanced disease resistance and increased yield, pointing towards the potential benefits of these genetic modifications in bolstering both immunity and crop productivity (Li et al., 2022).

TIFY11B gene expression influence yield

The accumulation of phosphate resulted in heightened grain production within transgenic rice plants that were engineered to overexpress the tobacco high-affinity phosphate transporter (NtPT1) gene (Park et al., 2010). The complex property of rice yield is related to a different physiological characteristics, including grain size, grain weight, panicle, tiller, and spikelet quantity. In an ongoing effort to identify new genes that control these properties, transgenic rice plants have been created that contain genes like the microRNA OsmiR397, which increases yield by up to 25% in a field trial by boosting the size of the grain and the number of panicle branches (Zhang et al., 2013). The rice lines that carried the TIFY11B gene exhibited more carbohydrate accumulation in the vegetative organ than the wild type, suggesting that the improved carbohydrate assimilation was responsible for the increase in grain size and yield (Clement et al., 2017; Hakata et al., 2012).

GNP1 and NAL1 Combinations Determine the Rice Grain Number per Panicle

As a significant factor in both Xian and geng genetic backgrounds, the NAL1 locus revealed its importance in the context of Genetic Network Programming (GNP). Prior research focused on various rice lines that carried different alleles, closely isogenic strains or transgenic varieties with different genetic backgrounds. Notably, the NAL1 allele of the Xian and geng rice cultivars has the most conserved alterations in the coding region, characterized by a single-base substitution. The genetic variations sometimes exhibited different effects on the development of the traits like the panicle type, the leaf type, and the plant morphology. Furthermore, the expression of GNP1, mediated by alterations in the promoter sequence, was found to be increased, which in turn led to the increase in grain number, thus confirming the involvement of GNP1 in shaping grain-related characteristics (Fujita et al., 2013; Takai et al., 2013; Xu et al., 2015; Yano et al., 2016; Zhang et al., 2014). Among the indica (Xian) and japonica (geng) subpopulations, the NAL1 gene had the greatest effect on Grain Number per Panicle (GNP). Interestingly, the occurrence of GNP1 was extremely rare in the Xian background, where the superior GNP1 allele (GNP1-6) was only present in 4.0% of the 198 germplasms and also showed negligible impact in the geng subpopulation. In addition, the transgenic lines with both NAL1 and GNP1 showed significant increases in GNP (15.5%-25.4% and 11.6%-15.9% greater, respectively) and grain yield (5.7%-9.0% and 8.3%-12.3% greater, respectively) compared to the transgenic lines with either NAL1. The synergistic effects of both genes in the introgression lines within the japonica background notably enhanced GNP, offering valuable insights for rice breeding programs focused on achieving high yield (Fujita et al., 2013; Takai et al., 2013; Wang et al., 2020; Zhang et al., 2014).

Table 2 The significant QTNs for grain thickness

Trait	QTN	Chromosome	position	Reported genes
Grain Thickness	qGT-5-4	5	23,605,308	OsSNAT1
	qGT-6-2	6	19,652,114	OsSPDS2

Osa-MIR156f Expression Controlled by D18 Promoter to Control Plant Architecture and Yield

On chromosome 8 of Nippon bare, two big repeat segments contain two neighboring copies of the osa-MIR156f gene. Pre-miR156f can form a conventional hairpin, and mature osa-miR156f is a 20 Nt fragment. Important roles for t osa-MIR156f in plant architecture and rice tillering (Liu et al., 2015). osa-MIR156f pro: GUS (-glucuronidase) adhesins from Nippon bare were introduced into transgenic lines, and the expression of osaMIR156f during development and rice growth was tracked. A histochemical investigation revealed that the GUS expression was limited in both space and time. Adolescent tissues with high GUS expression included the shoot apex, axillary bud, young spike, and young glamorous flower. Rice with constitutive overexpression of osa-MIR156 may experience significant problems in tillering and panicle development by sharply downregulating the level of SPL14 (L. Wang et al., 2015; Xie et al., 2006).

OsGA2ox1 was expressed ectopically through the D18 promoter in shoots, resulting in a semi-dwarf morphology with regular flower and grain development. The shoot's pinnacle was marked by the expression D18 (Sakamoto et al., 2003). To decrease the negative effects of excessively expressed osa-MIR156f on rice growth and development, the D18 promoter was employed to trigger osa-MIR156f activity in rice. synthetic lines Most of the D18pro: osa-MIR156f lines did not exhibit faulty phenotypes when compared to UBQpro: osa-MIR156f transformants and WT. A typical D18pro: the osa-MIR156f transgenic line was chosen because it produced tillers that were more efficient than those of the wild type. Gene expression study at the booting stage showed that D18-transgenic stems and basal nodes had higher transcriptional levels of pri-miR156f than the wild type, but that roots, leaves, and panicles did not differ from the wild type In D18 transgenic plants, pri-miR156f and osa-miR156 levels in the shoot were markedly upregulated. According to these findings, the D18 promoter can accurately control the tissue-specific expression of osa-MIR156f in stem cells. The outcomes suggested that osa-MIR156f expression modulation might be useful in rice genetic breeding. In this study, a unique method for controlling he potential for grain yield and rice plant morphology were developed (Liu et al., 2019).

Grain Weight Improvement in All OsSUSs Transgenic Rice Plants

The SUS (Sucrose Synthase) family in plants exhibited distinct categorization into four clades through phylogenetic analysis. Notably, Clade II comprised monocot members, housing rice representatives such as OsSUS1, OsSUS2, and OsSUS3. In contrast, Clades III and IV featured a mix of monocot and dicot members, including OsSUS4, OsSUS5, and OsSUS6. Clade I, on the other hand, was exclusively composed of dicot species. Examining the gene expressions of six OsSUSs through a microarray study revealed nuanced patterns. OsSUS1 demonstrated low expression levels in the leaf and sheath, spiking during seed germination, imbibition, and in young seedlings, panicle, and young branches. In contrast, OsSUS2 exhibited upregulation across almost all tissues, particularly during the initial stages of endosperm development. OsSUS4 displayed exclusive expression in the endosperm, while OsSUS3 primarily manifested expression in the spikelet and endosperm. Moreover, OsSUS5 and OsSUS6 exhibited relatively modest expression levels across a variety of tissues. The study proceeded to generate transgenic rice plants expressing each OsSUS constitutively to delve into the roles of OsSUS in rice development. The selection of overexpressed transgenic lines for OsSUS1-6 (OE, with two lines for each SUS) was based on significantly elevated OsSUS1-6 transcription levels compared to ZH11. The transgenic lines harboring OsSUSs displayed grain yields per plant that were 8–15% higher than those observed in the ZH11 and empty vector (EV) controls. Statistical analysis revealed no significant changes in tiller quantity per plant, seed number per panicle, or seed setting rate between the control and OsSUSs transgenic lines. However, a notable increase of 14–18% in the 1000-grain weight was observed in the transgenic plants compared to controls, with weights ranging from 26.24 g and 26.19 g in the ZH11 and EV to 29.87–31.03 g in the OsSUSs transgenic lines.

Table 3 The significant QTNs for 1000-grain weight

Trait	QTN	Chromosome	position	Reported genes
1000-Grain Weight	qTGW-1-1	1	4,853,002	GW5L
	qTGW-3-1	3	16,776,481	GS3
	qTGW-4-2	4	32,409,784	FLO2

Transgenic OsSUS3 lines were produced, and they considerably boosted SUS enzyme activities and OsSUS3 expression levels as measured by Q-PCR and Q-PCR, respectively. Transgenic rice lines considerably outperformed ZH11 and EV controls in terms of grain yield and 1000-grain weight (p 0.05 or 0.01) This study studied the grain length, width, and thickness in the transgenic rice plants because 1000-grain weights were dramatically altered. There was a noticeable increase in grain length (10–13%), a modest rise in grain width (3-5%), and no discernible differences in grain thickness. As a result, the findings suggested that OsSUS3 overexpression might greatly improve grain weight in transgenic rice lines by primarily increasing grain length and width (Cho et al., 2011; Fan et al., 2019).

Table 4 The significant QTNs for grain width

Trait	QTN	Chromosome	position	Reported genes
Grain Width	qGW-4-1	4	26,662,080	STRK1
	qGW-5-1	5	5,359,498	GSE5/GW5
	qGW-5-2	5	5,371,587	GSE5/GW5
	qGW-9-2	9	1,318,664	BC12
	qGW-10-1	10	22,500,927	OsCAO1

The OsFWL4 gene controls tillering and plant yield in rice

The Agrobacterium-mediated CRISPR/Cas9 system was employed to introduce alterations in the rice FWL family genes, facilitating the study of mutant phenotypes in two specific genes, OsFWL1 and OsFWL4. The findings of this study demonstrate that the OsFWL1 gene is an important regulator of tiller number and final plant yield in rice, thereby highlighting its positive effect on these agronomic parameters. On the other hand, OsFWL4 gene comes out as a negative regulator, suggesting its inhibitory function in controlling tiller number and plant yield in rice. The tiller number of the mutants was increased by 45.9% compared to the WT OsFWL4 gene. Mutants also had broader flag leaves. Microscopic observations of the leaf epidermal cells in mutants revealed that the increase in leaf width was due to an increase in cell number rather than cell size. Hence, OsFWL4 might be detrimental to cell proliferation during the growth of leaves and tillers. The grain yield per plant increased by up to 35.8% in the mutants, suggesting that the OsFWL4 gene is useful for breeding (Gao et al., 2020).

Rice grain yield is enhanced by OsMPH1 regulation of plant height.

An OsMPH1 gene, a newly identified rice gene, has been identified as a novel regulator of plant height that acts via the production of a MYB transcription factor. The overexpression of OsMPH1 led to an increase in both yield and plant height, mainly due to the increased cell length of internodes. The OsMPH1 seems to be involved in cell development as shown by its effect on the expression of different wall-associated kinase genes. The gene, found at the locus LOC_Os06g45890, has proved its function of longitudinally lengthening internode cells, thereby leading to the overall increase in plant height. Moreover, the study showed that plants with OsMPH1Es (edited) had a phenotype similar to that of plants with OsMPH1 overexpression, while plants with OsMPH1V (vector) and OsMPH1-RNAi (RNA interference) had the opposite phenotype. The investigation extended to other essential rice yield characteristics, such as tiller count and grain weight. Although the 1000-grain weight indicated no significant difference between OsMPH1V, OsMPH1E, and wild-type plants, statistical analysis indicated that OsMPH1Es had fewer tillers than OsMPH1Vs and the wild-type. Further assessment of actual grain yield demonstrated that OsMPH1E plants exhibited grain yields between 45% and 50% higher than those of wild-type plants. The increased grain production in OsMPH1Es appears to be attributed to their longer growing time and the presence of more primary and secondary branches (Huang et al., 2015; Zhang et al., 2017).

DEP1 regulates grain quality and Yield

In rice, a notable quantitative trait locus associated with Nitrogen Use Efficiency (NUE) has been identified and found to be identical to the DEP1 gene. The DEP1 protein is a part of the heterotrimeric G protein, an important element in nitrogen signaling and carbon metabolism. Rice plants with the dep1 allele, which is a gain-of-function mutation, display specific features, such as upright panicles, higher GS (glutamine synthetase) activity, and enhanced nitrogen uptake, even under conditions of limited nitrogen supply. These changes are responsible for enhanced NUE and consequently lead to higher grain yield in rice plants carrying dep1 allele (Ashikari et al., 1999; Sun et al., 2014).

The transgenic lines have considerably higher expression levels of GS1;1, GS1;2, NADH-GOGAT1, NADH-GOGAT2, AS, and PEPC1 when compared to the wild type. The lines with overexpressed dep1 showed more primary and secondary panicle branches, shorter plant height, higher grain density, and panicle length, and erect panicle types. In comparison to the wild type, transgenic lines under LN conditions demonstrated enhanced grain yields of 27.07% and 34.53%, which were mostly related to the increased biomass, number of panicles per plant, and number of grains per panicle. Due to the increased grain counts per panicle and harvest index, Grain yields from transgenic lines increased by 14.82% and 13.08% than those of the wild type under HN (high nitrogen) circumstances (Němec & Zachariáš, 2018; Yi et al., 2011; Zhao et al., 2019).

OsSGL expression aided in grain development and cell growth

In transgenic plants carrying the OsSGL gene, we investigated how OsSGL impacts crop growth and stress tolerance. At the vegetative growth stage, the phenotypic analysis revealed that transgenic lines had a generally common phenotype. Surprisingly, throughout plant reproductive development, overexpressed plants showed changes in a variety parameter such as panicle design, plant height, grain length, and grain weight under field settings. More main and secondary branches were formed by OE panicles than WT, which resulted in a 22.2% increase in panicle length and an average increase of 25.7% in the number of grains per panicle. In general, the 93-11-OE grains were 16.3% heavier, 8.6% narrower, and 24.8% longer. In order to control grain weight, OsSGL might even contribute to the accumulation of dry matter during grain milk filling. Reverse genetics was used to find and clone OsSGL, a novel rice gene that responds to pleiotropic stress. Grain length and number per panicle expansions were two agronomic characteristics of rice that were considerably and favorably impacted by overexpression of OsSGL. Rice's two agronomic metrics were gains in grain length and grains per panicle is used to demonstrate how yield-related genes can be isolated (Wang et al., 2016; S. Wang et al., 2015; Wu et al., 2016).

Table 5 The significant QTNs for yield per plant

Trait	QTN	Chromosome	position	Reported genes
	qTGW-7-2	7	18,895,502	OsSPL13
	qTGW-7-3	7	19,391,625	OsSPL13
	qTGW-8-2	8	26,309,952	GW8/OsSPL16/qGW8
Yield Per Plant	qTGW-11–1	11	18,100,034	OsBDG1
	qYPP-4-2	4	25,868,074	LABA1
	qYPP-5-2	5	25,806,082	OsRab7
	qYPP-8-2	8	19,396,188	PAY1
	qYPP-11–1	11	994,705	ONAC122

Conclusions

The process of cloning has been instrumental in acquiring a novel gene resource, facilitating molecular design breeding strategies focused on enhancing various essential agronomic traits in rice. This includes but is not limited to increasing grain size, weight, thousand grain weight, grain width, grain length, grains per plant, grain number per panicle, and the number of panicles per plant. Utilizing genome editing methods, the insertion or creation of novel alleles has been implemented, leading to the generation of significant phenotypic variation. This approach not only offers an efficient means of employing genome editing techniques but also serves as a valuable tool for augmenting the genetic diversity of rice. The application of these advancements holds promise for the development of rice varieties with improved characteristics and increased agricultural productivity.

Future prospective

The conversion of C_3 crops into C_4 crops has long been a goal of botanists and breeders, and more recently, new chances have arisen due to the development of genetic engineering. Increased photosynthetic efficiency for higher grain yield Rice plants are members of the C_3 family, which has poorly functioning photosynthetic mechanisms. When the ratio of O_2 to CO_2 is greater in C3 plants, such as rice, the oxygenase activity of *RubisCO* takes over, triggering the energy-intensive photorespiration pathway that breaks down the poisonous phosphoglycerate that is generated. Due to their two-celled C_4 system, these plants are able to concentrate CO_2, which serves as a storage space for the Rubisco enzyme as compared to C_4 plants. Scientists have taken on the difficult task of engineering rice with overexpressed C4 enzymes in the hopes of enhancing photosynthetic capacity and grain yield because they are fascinated by the C_4 photosynthesis machinery's efficiency (Ermakova et al., 2020).

Abréviations

QTLs	Quantitative trait loc
GL	Grain Length
GW	Grain Width
TGW	Thousand grain weight
GY	Grain Yield per Plant
GNP	Grain number per Panicle
NPP	Number of panicles per plant
Nt	Nucleotides

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Author's contributions

MMJ, AS, MZH, AA, MHA, and SN wrote the initial draft of manuscript. MA, AA, and RB edit the manuscript for final submission. All authors have read and approved the final manuscript.

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Consent for Publication

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Competing interests

The authors declare that they have no competing interests.