Rice Genomics and Genetics 2025, Vol.16, No.4, 219-236
http://cropscipublisher.com/index.php/rgg

Feature Review Open Access

Regulation of Starch Biosynthesis Pathway for Improved Grain Quality in Rice

Chengxi Wang, Xinguang Cai, Qiangsheng Qian
Modern Agricultural Research Center, Cuixi Academy of Biotechnology, Zhuji, 311800, Zhejiang, China
Corresponding email: qiangsheng.qian@cuixi.org

Rice Genomics and Genetics, 2025, Vol.16, No.4 doi: 10.5376/rgg.2025.16.0019
Received: 16 Jun., 2025
Accepted: 29 Jul., 2025
Published: 16 Aug., 2025

Preferred citation for this article:
Wang C.X., Cai X.G., and Qian Q.S., 2025, Regulation of starch biosynthesis pathway for improved grain quality in rice, Rice Genomics and Genetics, 16(4): 219-236 (doi: 10.5376/rgg.2025.16.0019)

Abstract The quality of rice mainly depends on the composition and structure of starch within its grains. The ratio of amylose to amylopectin content not only affects the steamed and cooked taste quality and nutritional value of rice, but also relates to the digestibility and glycemic index of rice and other health attributes. To meet the growing demand for high-quality rice, it is of great significance to conduct in-depth research on the molecular basis of starch biosynthesis in rice grains. This study reviews the biochemical mechanisms, genetic regulatory networks, and molecular breeding strategies of the rice starch synthesis pathway, introduces the functions and expression patterns of the main enzymes and related genes in starch synthesis during grain development, and summarizes the regulatory effects of transcription factors, non-coding Rnas, and epigenetic modifications on starch synthesis. This paper analyzes the key genetic loci and alleles that affect the ratio of amylose to amylopectin and the structure of starch grains. Combined with the research cases of typical gene mutants, it explores the strategies for improving the quality of rice grains through molecular breeding methods, including the application of new technologies such as molecular marker-assisted selection and gene editing. This study summarizes the latest progress in the regulation of starch synthesis, looks forward to its application prospects in the cultivation of high-quality rice in the future, and provides theoretical support and feasible molecular improvement strategies for the breeding of high-quality rice.

Keywords Rice; Starch synthesis; Amylose; Genetic regulation; Grain quality

1 Introduction

The main component of rice is starch, which accounts for approximately 80% of the dry weight of the grains. There are two types of starch: amylose and amylopectin. Their proportion will directly affect the taste and nutrition of the rice. Generally speaking, rice with a high content of amylose tends to have harder grains when cooked and dries out easily when cooled. Rice with less amylose and more amylopectin is more sticky and soft when cooked and tastes more glutinous. For instance, glutinous rice is almost entirely amylopectin and becomes very sticky when cooked. *Indica* rice with more than 25% amylose will be dry and hard when cooked. The composition of starch can also affect the digestion speed of rice. When there is a high amount of amylose, more resistant starch will be produced, which can lower the glycemic index of rice and be more friendly to diabetic patients. Apart from starch, the protein content and types in the grains also affect the texture and flavor of the rice. The composition of starch is a key factor determining the quality and nutrition of rice. Optimizing it not only makes the taste better but also meets people's demand for health.

Nowadays, people's living standards have improved, and their demands for the quality of rice are also getting higher and higher. In the past, breeding mainly focused on yield and paid insufficient attention to the improvement of rice quality, so there were not many high-quality rice varieties. The current challenge lies in how to ensure the output while making the rice taste better and more nutritious. The synthesis process of starch directly determines the amylose content, gel consistency and gelatinization characteristics of rice, all of which are important indicators affecting the quality of rice. Studying the biochemical processes and genetic regulation of starch synthesis can identify the key genes and their modes of action that affect rice quality, providing theoretical support for molecular breeding. For instance, the *Wx* gene controls the synthesis of amylose. Its different alleles can cause significant differences in the amylose content of rice, which is also one of the important reasons for the taste differences between *indica* rice and *japonica* rice (Huang et al., 2020). For instance, if the enzymes that regulate

the structure of amylopectin (such as starch synthase and branched enzymes) mutate, it will affect the digestion rate of starch and the formation of resistant starch (Zhou et al., 2022; Miura et al., 2024). Therefore, in-depth research on the gene functions and regulatory mechanisms in the starch synthesis pathway is conducive to precisely improving these quality traits in molecular breeding. In recent years, the development of new technologies such as genome editing has enabled us to specifically modify these genes and create high-quality functional alleles. This method can break through the limitations of long breeding cycles and strong randomness in traditional breeding, and cultivate new rice varieties that are both high-yielding and of high quality. It is evident that systematic research on the starch synthesis pathway in rice not only holds significant scientific importance but also has high application value.

This research will introduce the starch synthesis pathway at different levels, covering everything from biochemical mechanisms, genetic regulation to breeding applications. This research will first describe the biochemical reaction process and main metabolic links of starch synthesis in rice grains, then explain the key enzymes involved in starch synthesis and their functions, and also introduce the expression of these related genes during grain development. It will summarize the genetic mechanisms regulating starch synthesis and discuss how to adjust the ratio of amylose to amylopectin through genetic means. And the ultrastructure of starch granules, analyze the key loci and allelic variations that affect the physicochemical properties of starch. By combining some typical cases of genes and mutants, such as functional variations of the *Wx* gene and other starch synthase genes, to illustrate their impact on rice quality and the utilization of these superior alleles in high-quality rice breeding. The objective of this study is to review the significant progress made in rice starch synthesis and quality control in recent years, identify the current technical difficulties and scientific issues, and make prospects for future research directions and application prospects. It is hoped that this will provide theoretical basis and technical reference for accelerating the cultivation of high-quality new rice varieties that are both high-yielding and meet consumer demands.

2 Overview of the Starch Biosynthesis Pathway in Rice

2.1 Biochemical reactions and major metabolic steps in starch biosynthesis

The biosynthesis of rice grain starch is accomplished in the amyloid bodies (powdery bodies) of endosperm cells, involving a series of continuous enzymatic reactions. Firstly, sucrose, a product of photosynthesis, is converted into the substrate glucose-1-phosphate (G1P). Subsequently, under the action of ADP-glucose pyrophosphorylase (AGPase), a key initiating enzyme in starch synthesis, G1P reacts with ATP to form ADP-glucose (ADPG), which is the first rate-limiting step in starch synthesis. AGPase is composed of a heterotetramer consisting of two large subunits and two small subunits, and its activity regulation has a significant impact on the rate of starch synthesis (Dawar et al., 2013). The generated ADP-glucose serves as a glycoside donor and is utilized by subsequent enzymes to extend the glucan chain: Granulose-binding starch synthase (GBSS, also known as Wx protein) mainly catalyzes the synthesis of amylose, that is, adding the glucose group to the non-reducing end of the chain through an α-1, 4-glycosidic bond to form a long chain that is basically non-branched. Soluble starch synthases (SS, including ISO-SSIV equivalent enzymes) assist in prolonging the linear portion of amylopectin. When the glucan chain is extended to a certain length, starch branching enzymes (SBE, including SBEI, SBEIIb, etc.) will cut part of the α-1,4 bonds and connect the chain segments to another glucan chain through α-1,6-glycosidic bonds, forming side chains, thereby constructing the branched structure of amylopectin (Han et al., 2019). Meanwhile, starch debranching enzymes (DBE, including isoamylase ISA and starch debranching enzyme PUL) selectively hydrolyze inappropriate α-1,6 bonds, modifying the structure of amylopectin and making the branching chain arrangement more ordered and dense. These synthetic and modifying enzymes work in synergy to construct the semi-crystalline starch granule structure in rice grains. In addition, auxiliary enzymes such as starch phosphorylase and dismutase are involved in the conversion and balance of starch synthesis precursors, but their roles are relatively secondary. The rice starch synthesis pathway is a highly coordinated biochemical network, from substrate synthesis, chain extension to branching and modification, each step is interlinked and jointly determines the final starch yield and structural properties.

2.2 Key enzymes and their functions

The synthesis of rice starch involves multiple enzymes, each of which plays a different role in the pathway. A large number of studies on the genes encoding these enzymes have clarified their influence on the formation of rice quality. Adp-glucose pyrophosphorylase (AGPase) is the rate-limiting enzyme at the pathway initiation, consisting of a small subunit (such as the AgpS gene encoding) and a large subunit (AgpL encoding). Enhancing AGPase activity usually increases starch production, but excessively high activity may disrupt carbon allocation. Particle-binding starch synthase (GBSS), encoded by the Waxy (*Wx*) gene, is the sole enzyme for amylose synthesis. GBSS is located inside starch granules and is dedicated to catalyzing the extension of amylose. Therefore, the loss of *Wx* gene function will result in rice grains containing almost no amylose (i.e., sticky, commonly known as "glutinous rice"). The natural variation of the *Wx* gene directly determines the level of amylose content in rice and is an important genetic factor affecting the taste. The soluble starch synthase (SS) family includes various isoenzymes such as *SSI*, *SSII*, *SSIII*, and *SSIV*, and their functions are divided: *SSI* mainly extends short chains, *SSIIa* prefers to synthesize medium-length chains, and *SSIIIa* is responsible for the synthesis of longer chains, etc. Among them, *SSIIa*, also known as the *ALK* gene in rice, controls the distribution of amylose and amylopectin side chain lengths and has a significant impact on properties such as gel consistency and gelatinization temperature. Allelic variations in *SSIIa* among different rice varieties (such as the *ALK* difference between *indica* rice and *japonica* rice) lead to differences in cooking properties, which are utilized in breeding.

Amyloidase (SBE) in rice includes *SBEI* and *SBEIIb*, etc. *SBEIIb* is the main branching enzyme in endosperm. The deficiency of *SBEIIb* will significantly increase the content of amylose and change the structure of amylopectin, resulting in a high amylose/resistant starch phenotype. However, it may also lead to seed shrinkage and poor quality. Starch debranching enzymes (DBE) include two types of enzymes: ISA and PUL. ISA (isoamylase) mainly prunes the branch points of amylopectin to form the double helix chains required for crystallization. Mutations with ISA defects are characterized by loose starch granule structure and powdery endosperm. PUL (pullulanase, also known as rate-limiting starch debranching enzyme) can also remove side chains, but its effect is more limited than that of ISA. Starch phospholysis enzymes may play a role in the initiation or degradation of starch synthesis, and dismutase (DPE) is involved in the transfer equilibrium of short chains such as maltotriose (Sun et al., 2017). The synergistic action of these enzymes ensures normal starch accumulation. Once the function of a key enzyme is lost, it often leads to abnormal composition and structure of starch, which in turn affects the appearance and quality of grains. For instance, mutations in the *Wx* gene cause the grains to appear opaque and pinkish white, and the absence of ISA also results in a milk-like endosperm. Therefore, these starch synthase coding genes are also major genes affecting rice quality, and their functional analysis and allelic variation utilization are the basis for molecular improvement of rice quality.

2.3 Expression patterns of starch biosynthesis-related genes during grain development

Rice starch is mainly synthesized during the grain filling process, and the spatiotemporal expression patterns of related genes determine the changes in enzyme activity at different developmental stages. Overall, most starch synthase genes start to be expressed early after pollination and reach their peak in the middle and late stages to ensure that a large amount of starch accumulates before the grains mature. For instance, the mRNA of the *Wx* gene begins to accumulate in large quantities 57 days after pollination and maintains high expression until the later stage of grouting. The GBSS protein encoded by it can be detected throughout the grouting period. This is consistent with the process in which amylose is mainly synthesized continuously in the middle and later stages (Ying et al., 2022; Ma, 2024). Among *SS* genes, *SSI* and *SSIIIa* genes are usually strongly expressed in the early and middle stages of grouting, providing enzymatic activity for the rapid synthesis of amylopectin. The expression of the *SSIIa* gene often persists in the middle and later stages, thereby affecting the formation of longer side chains and the binding of amylose. The expression peak of the branched enzyme *SBEIIb* gene also occurred in the middle of grouting, and its protein activity was relatively high at 715 days after grouting, and then gradually decreased. This indicates that during the critical period when starch accumulates in large quantities, the activity of branching enzymes is sufficient to ensure the reasonable branching of amylopectin. The expression of the de-branching enzyme ISA gene is relatively early, reaching its peak in the early stage of grouting. The presence of ISA protein

and its activity in the early stage helps to timely modify the newly formed branched structure and prevent the accumulation of excessive "redundant" branches. Similarly, the large and small subunit genes of AGPase initiate expression 3 to 5 days after pollination and maintain high activity for approximately 10 days, providing sufficient precursors for subsequent starch synthesis. Overall, the expression of starch synthesis-related genes in endosperm shows obvious stage-specific and coordinated development: first, substrate supply enzymes and branching/debranching enzymes are initiated, followed by a large amount of synthase expression, and linear and amylopectin accumulate simultaneously. In terms of spatial distribution, these genes are mainly highly expressed in the endosperm of grains, while their expression levels are very low in tissues such as seed coats and embryos. Some genes also show expression differences in different regions within the endosperm. For instance, there are differences in the activity of enzymes related to starch synthesis between the outer endosperm and the inner endosperm near the aleurone layer, which are believed to be associated with the formation of abdominal white granules (hollow and opaque granules). It should be noted that environmental factors can also affect the expression patterns of these genes. For instance, when grouting is carried out under high-temperature conditions, the expression levels of *Wx* and *SS* genes decline in the middle and later stages, which hinders the synthesis of amylose and long branched chains, and thus makes it easy for endosperm opacity and rice quality to deteriorate (Yamakawa and Hakata, 2010).

3 Genetic Regulation of the Starch Biosynthesis Pathway

3.1 Transcriptional regulation of gene expression

The expression of the starch synthase gene in rice endosperm is precisely regulated by multiple transcription factors. Previous studies have identified some transcription factors that are specifically expressed in endosperm and directly regulate starch synthesis genes. For instance, OsbZIP58 (also known as RISBZ1 or OsSPA) is a fundamental leucine zipper transcription factor that can bind to the conserved cis-elements of six starch synthase gene promoters, including Wx, SSI, and SBEI, significantly activating their transcription. The contents of amylose and amylopectin in the endosperm of the mutant with OsbZIP58 function deficiency both decreased, presenting a powdery and opaque "flour endosperm" phenotype, indicating that OsbZIP58 is a key positive regulatory factor for starch synthesis. Another member of the bZIP family, OsbZIP20 (RISBZ2), is also highly expressed in endosperm and can bind to the ACGT sequence of the promoter to promote the expression of some synthase genes (Wang et al., 2013). In addition to bZIP types, DOF transcription factors such as RPBF (Rice Prolamin Box Binding Factor) and GATA factors such as OsGATAI have also been found to be involved in regulating starch synthesis. RPBF and bZIP act synergically on the GCN4/P-box elements of gene promoters such as Wx to activate transcription. OsGATAI can jointly promote the expression of starch synthase genes with the NF-Y complex, thereby increasing starch accumulation. In terms of negative regulation, research has reported that the AP2/ereBP-like transcription factor OsRSR1 is a starch synthesis inhibitor: overexpression of OsRSR1 in endosperm can down-regulate the transcription of multiple enzyme genes such as *Wx* and *SSIIa*, resulting in a decrease in amylose and total starch content. The role of OsRSR1 is believed to respond to changes in endosperm sucrose levels, inhibiting starch synthesis genes when carbon sources are insufficient to prevent excessive accumulation. Some MADS-box transcription factors (such as OsMADS29) and NAC transcription factors (such as OsNAC20/26) are also involved in endosperm development and the accumulation of storage substances, and their mutants often affect the deposition of starch and storage proteins. It is worth mentioning that in recent years, through the analysis of abnormal mutant starch content, some new regulatory proteins have been discovered. For instance, the Flo2 gene encodes a TPR repeat protein. Although it is not an enzyme, its mutation leads to abnormal starch structure and opaque grains in the endosperm, indicating that FLO2 may affect starch accumulation by regulating the expression of genes related to starch synthesis or the assembly of enzyme complexes. For instance, the localization cloning of "dull endosperm" mutants such as Du1-Du16 has revealed multiple regulatory factors: Among them, Du12 and Du13 respectively encode a Feronia-like receptor kinase and a C2H2 zinc finger protein, both of which can positively regulate the expression of the *Wx* gene. After mutation, the content of amylose is significantly reduced. These findings have continuously improved the regulatory network for starch synthesis. The currently known transcriptional regulatory mechanisms show that the key genes of the starch synthesis pathway

are often synergistically controlled by multiple transcription factors, and there are also interactions and cascading relationships among different transcription factors. For instance, miR159-OsNF-YB1-OsbZIP58 constitutes a regulatory pathway that affects endosperm development and starch accumulation.

3.2 Regulatory roles of non-coding RNAs such as miRNAs and lncRNAs

Non-coding Rnas play a significant role in gene regulation during plant development. The synthesis of starch in rice endosperm is also influenced by some mirnas and long non-coding Rnas (lncrnas). In recent years, small RNA sequencing has revealed the expression of various mirnas during the grain filling stage and predicted that some mirnas may target starch synthase coding genes (Peng et al., 2013). For instance, miR159 and miR167 are important mirnas involved in the development of rice grains. Among them, miR159 indirectly affects the regulatory network of OsbZIP58 by down-regulating the transcription factor OsNF-YB1, thereby influencing the expression of starch synthesis genes. miR167 has been proven to directly target the OsARF12 transcription factor gene, forming the "MIR167-OSARF12" module, which participates in regulating the grain filling rate and grain weight of rice. Overexpression of OsARF12 can increase starch accumulation in grains, and its phenotype is similar to the effect of inhibiting miR167, indicating that miR167 negatively regulates the starch synthesis process by inhibiting OsARF12. Mir156/157, miR164, miR172, etc. have also been reported to be related to the formation of rice quality: miR156 targets *SPL* genes to affect grain size and indirectly change starch reservoir capacity, and miR164 may act on the starch synthesis pathway by regulating NAC transcription factors. Overall, the mechanism of action of miRNA is usually to cleave or inhibit target mRNA, thereby precisely controlling the production of corresponding enzymes or regulatory factors and adjusting the rate of starch synthesis in a timely manner at different developmental stages or under different environmental conditions. In contrast, research on the role of lncRNA in starch metabolism is still in its infancy. Some reports indicate that there are hundreds of specifically expressed lncrnas during the development of rice grains, some of which are co-expressed with starch synthase genes. For instance, some studies have identified an endosperm specific lncRNA LncYE1, and it is speculated that it regulates amylose content by influencing the mRNA splicing of key enzymes in starch synthesis. It has also been reported that long non-coding Rnas can act as "sponges" for mirnas. For instance, certain lncrnas competitively bind to miR156, thereby relieving the inhibition of miR156 on target transcription factors and indirectly promoting the synthesis of starch and other storage substances. Although there are not many specific examples, on the whole, non-coding Rnas provide additional dimensions for the multi-level regulation of starch synthesis. They can respond to rapid changes in external signals and integrate into the existing transcriptional regulatory network to achieve precise regulation of starch synthesis. In the future, through high-throughput sequencing and functional analysis, it is expected to discover more regulatory effects of miRNA/lncRNA on starch synthesis, thereby providing new strategies for improving rice quality.

3.3 Effects of epigenetic modifications (DNA methylation, histone modifications) on starch biosynthesis

In addition to transcriptional and post-transcriptional regulation, epigenetic modifications also affect the expression of starch synthesis genes, thereby indirectly influencing the quality of rice. Research has found that the DNA methylation level of the starch synthase gene promoter in the endosperm of rice is much lower than that in other tissues, which is conducive to the high-level expression of these genes in the endosperm. Especially, the promoter of the *Wx* gene has two very close CpG islands, and the methylation level in the endosperm is very low, which is related to the high expression and content of amylose. On the contrary, in some varieties with low amylose content (such as the Wx^b allele of *japonica* rice), the methylation level of the Wx promoter is high, the transcriptional amount decreases, and the synthesis of amylose is also restricted. Studies have found that altering the methylation levels of key gene promoters can, to a certain extent, adjust the composition of starch. For instance, treating pollinated rice ears with demethylating agents can increase the expression of the *Wx* gene and the content of amylose, which proves that DNA methylation silences the *Wx* gene (Tang et al., 2022). Recent studies on corn have also shown that the expression levels of starch synthesis-related genes (SSRGs) in endosperm are low when they are highly methylated, and genes with high expression often have low promoter demethylation or low genome methylation. This indicates that there might be a similar mechanism in rice. Environmental factors can also affect starch synthesis through epigenetic pathways. For instance, high

temperatures can reduce the histone acetylation levels of some starch synthase gene promoters, thereby inhibiting their expression and leading to a decrease in starch accumulation and a decline in grain transparency. Exogenous application of histone deacetylase inhibitors can restore the expression of these genes to a certain extent and alleviate the damage to rice quality caused by high temperature. Epigenetic mechanisms such as DNA methylation and histone modification are reversible and can also be inherited. Without altering the DNA sequence, they can adjust gene expression in response to environmental changes. In the future, regulating the epigenetic state of key genes through variety selection or biotechnological means is expected to improve the quality of rice. For instance, gene editing tools can be used to modify cis-elements or enzymes that affect methylation or acetylation, and to cultivate rice lines with low promoter methylation and high amylose content.

4 Molecular Mechanisms Regulating Starch Composition and Structure

4.1 Genetic basis of the amylose-to-amylopectin ratio

The ratio of amylose to amylopectin in rice is controlled by major genes and is also affected by the quantitative effect of multiple genes. The *Wx* gene is the major gene that determines the content of amylose. The Wx^a allele carried by common *indica* rice has a high post-transcriptional mRNA splicing efficiency due to the fact that the sequence of the filamentous resection site in intron 1 is GT, resulting in more GBSS enzymes and a relatively high content of amylose (generally >20%). However, the Wx^b allele carried by most *japonica* rice, due to the fact that this site is GC, leads to the retention of intron 1 in some transcripts, a decrease in the production of functional mRNA and enzymes, and an amylose content of only 10% to 18%. Therefore, the Wx^a/Wx^b variation is one of the main reasons for the quality differences of *indica* and *japonica* rice. Based on this genetic foundation, breeders have been able to rapidly distinguish *Wx* alleles through molecular markers and specifically select strains with the target amylose content. In addition to *Wx*, some secondary QTLS regulating amylose were also detected in different backgrounds. For instance, mutations such as Wx^{op} (opaque) were found in low-chain glutinous rice varieties from Japan and Wx^{mq} (medium quantity) in southern rice from the United States. These alleles each reduced the content of amylose and interacted with Wx^b and others to influence the final phenotype (Shao et al., 2020). In addition, the waxy mutant (wx) completely lacks GBSS enzyme activity, and its amylose content approaches zero, which is the reason for the formation of sticky rice and waxy corn, etc. The glutinous trait is controlled by the functional deletion allele of the (such as wx^g in hybrid glutinous rice in Yunnan, China), and wx can be introduced into varieties through conventional hybridization to achieve glutinous rice breeding. In terms of amylopectin, the *Alk* gene (*SSIIa*), *Wx* genewhich controls the ratio of long to short amylopectin, is another important locus. Among varieties with high linear chain content, carrying excellent Alk variations (such as alk alleles in *japonica* rice) can make the side chains of amylopectin shorter, resulting in a soft and glutinous gel texture. Even with a relatively high linear chain content, it can still maintain a relatively soft rice feel. Therefore, some varieties of medium and low straight-chain - soft rice types have achieved the ideal texture of being soft but not sticky by combining Wx^b (low straight-chain) and Alk superior grades. In recent years, new genes influencing the ratio of amylose to amylopectin have also been identified through genome-wide association analysis.

For instance, Wx^{lv} is an allele of the *Wx* ancestor from wild rice, which has the effect of reducing amylose content and improving taste. This allele has now been introduced into cultivated rice through hybridization and has been proven to reduce the content of amylose while increasing the gel consistency and improving the texture of cold rice. For instance, Mao et al. (2022) reported a new gene, *Wx410*, whose natural variation can up-regulate *Wx* expression and increase amylose content. In the future, it can also be used to enhance the amylose content of specific varieties. The genetic basis of the ratio of amylose to amylopectin in rice is mainly determined by major loci such as *Wx* and *Alk*, but there are also many minor genes and allelic variations that can fine-tune this ratio. By using molecular marker-assisted selection to polymerize favorable alleles or creating new allelic variations through gene editing (such as promoter editing for quantitatively regulating *Wx* expression levels), it is expected to achieve customized improvement of the starch composition ratio in rice. This molecular breeding strategy has successful examples both at home and abroad, providing the possibility to meet different consumption preferences.

4.2 Determinants of starch granule morphology and molecular structure

Starch in the endosperm of rice exists in the form of granules, and its morphology and internal molecular ultrastructure are influenced by both genetic and environmental factors. Rice starch particles are usually polygonal or irregularly spherical, with diameters generally ranging from 3 to 10 micrometers. The particle size varies slightly among different varieties. In terms of genetic factors, functional defects in genes of the starch synthesis pathway often lead to abnormal morphology of starch granules. For instance, due to certain regulatory factors or enzyme gene mutations in flo (floury) mutants, the starch granules are loosely arranged and the surface of the granules is rough, resulting in the endosperm being opaque and powdery. A typical example is the flo2 mutant, in which the starch granules in the endosperm are poorly developed and of uneven size, and the starch content is reduced by more than 30%. The TPR protein encoded by FLO2 is believed to be involved in the formation of the starch synthase complex, and the particle morphology is disordered after its absence. For instance, the mutant with ISA deficiency (referred to as isa or flo7 in Suzhou), due to the failure to modify the amylopectin dendritic points, forms many small and loose starch granules that accumulate in the cells, resulting in a distinct powdery texture in the endosperm. Therefore, the sound function of key enzymes is crucial for the formation of normal and dense starch granules. In addition to gene mutations, different alleles can also affect the characteristics of starch granules. For instance, varieties like Wx^a and Wx^b have different particle hardness and density. Particles with high amylose content are usually more compact and crystalline, while those with low amylose content have a slightly looser structure. In terms of environmental factors, temperature has a significant impact on the morphology of starch granules. High-temperature filling often leads to the reduction of starch particles and their irregular arrangement, which is one of the main reasons for the chalkiness of rice. At high temperatures, the activity of starch synthase decreases, especially the activities of SS and SBE are inhibited, resulting in the newly generated starch being short and unable to accumulate closely. Many tiny voids and changes in refractive index appear in the endosperm, which is known as chalkiness. Conversely, in a cool environment, endosperm filling is slow, and the starch granules tend to be larger and more regular with less chalkiness (Figure 1) (Huang, 2024; Zhao et al., 2024).

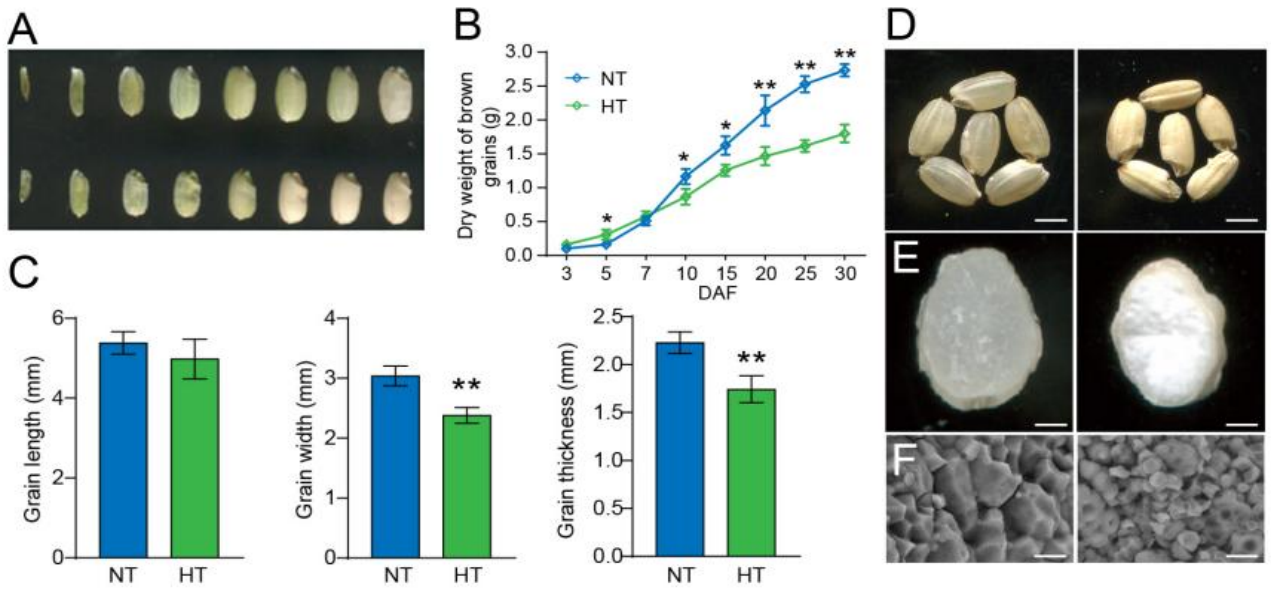

Figure 1 Characterization of cv. NJ1 under normal-temperature (NT) and high-temperature (HT) treatment (Adopted from Zhao et al., 2024)

Image caption: (A) Dry brown grains of NJ1 under NT (above) and HT (below) at various stages of development. (B) Weight of dry brown grains of NJ1 under NT and HT at various stages of grain filling. DAF, days after fertilization. (C) Grain length, width, and thickness of NJ1 under NT and HT. (D) Comparison of phenotype of cross-sections of NJ1 mature brown grains between NT and HT; scale bar, 5 mm. (E) Phenotypic comparison of the NJ1 brown grains between NT and HT; scale bar, 5 mm. (F) Scanning electron microscopy (SEM) images of transverse sections of NJ1 endosperm under NT and HT; scale bar, 10 μM. Data in (B,C) are presented as mean from three replicates. Asterisks indicate statistical significance between NT and HT determined by Student's t-tests (* $p < 0.05$; ** $p < 0.01$) (Adopted from Zhao et al., 2024)

The moisture and nutrient status can also indirectly change the particle morphology by influencing the grouting rate and enzyme activity. In terms of molecular structure, the branching density of amylopectin and the length of amylose also affect the crystallinity and morphology of the particles. When the proportion of branched-chain and long-chain grains is high (such as high straight-chain rice), there are more and denser grain crystal areas, but they are also more brittle and prone to cracking. During rice milling, it is easy to produce white grains with a white belly. On the contrary, when the side chains are dominant (such as in glutinous rice), there are no double helical crystal regions inside the grains, and they are amorphous. The glutinous rice grains are semi-transparent but unstable for storage and prone to aging and regeneration. The size, shape and internal structure of starch granules are jointly determined by internal genes and the external environment. By cultivating varieties with low chalkiness and dense structure, the rice milling yield and appearance quality can be improved. By regulating the crystallinity of the particles, the texture and digestive characteristics of the rice can be changed. Therefore, in the breeding of rice quality, the morphology and structure of starch particles should be comprehensively considered, and genetic improvement should be combined with the optimization of the cultivation environment to produce high-quality rice with smooth grain surface, high transparency and suitable taste.

4.3 Key loci involved in altering the physicochemical properties of starch

The physicochemical properties of rice starch (such as gelatinization temperature, gel consistency, resistant starch content, etc.) are largely controlled by some key gene loci. Allelic variations at these loci can significantly alter the properties of starch, thereby influencing the cooking and nutritional quality of rice. In addition to the aforementioned *Wx* and *Alk* genes, another typical example is the *SSIIIa* gene. *SSIIIa* plays a role in the synthesis of amylopectin long chains. The loss of its function will lead to changes in the distribution of amylopectin length and a decrease in gel consistency, but on the other hand, it can increase the content of resistant starch. Studies have shown that *SSIIIa* mutants (such as ae variants) have slightly increased amylose content and form more long-chain amylopectin. The texture of the rice is slightly harder but its ability to resist enzymatic digestion is enhanced, which can reduce the blood sugar response. Therefore, *SSIIIa* is regarded as a site that can be used to increase the content of resistant starch (Zhou et al., 2022).

Another key locus is the Pullulanase gene (PUL), which encodes amyloid-limiting debranching enzyme. The high-resistant starch rice cultivated in Japan (such as Gao Kang No.1) has significantly increased the resistant starch content in the endosperm by knocking out the PUL gene. The PUL mutation slightly increases the branching of amylopectin but its arrangement is disordered, thus making more of it difficult to be digested in the intestine and achieving the goal of reducing GI. However, the absence of PUL can also cause the rice to become brittle, which needs to be comprehensively weighed against other characteristics. For instance, the ISA1 gene variation (i.e., the flo7 mutation) not only causes a powdery appearance but also significantly increases the proportion of amylose in grains and resistant starch, which may have application value in specific functional health foods. Apart from enzyme-coding genes, some regulatory gene loci also affect the properties of starch. For instance, in the aforementioned Flo2 mutant, although the total starch content decreased, the proportion of amylose increased and the consistency of the gel decreased. This is because Flo2 affects the efficiency of the entire enzyme complex. Similarly, the natural variation of the high-temperature inducible factor OsGDT1 can alleviate the adverse effects of high temperature on starch quality and is regarded as a site with excellent heat resistance.

In addition, in terms of the nutritional quality of rice, a notable site is SuS2 (sucrose synthase 2) : studies have shown that its allelic variation affects the conversion efficiency of sucrose to starch during grain filling, thereby indirectly influencing the starch accumulation rate and grain weight, and having an impact on both yield and quality. By analyzing key sites and creating allelic variations, breeders can more flexibly improve the specific physicochemical properties of starch. For instance, by polymerizing the combination of Wx^b (low linear chain) + high-quality *Alk* allele + weakly functional *SSIIIa* allele, it is expected to cultivate an ideal quality variety with medium linear chain, thick and soft viscosity, and moderate resistant starch.

5 Case Studies of Representative Genes and Mutants

5.1 *Wx* gene and regulation of amylose content

The Waxy (*Wx*) gene is a core gene that controls the synthesis of amylose in rice and plays a decisive role in the formation of grain quality. The *Wx* gene encodes particle-binding starch synthase (GBSS), and its function is directly related to the level of amylose content. There are various *Wx* allelic variations in rice. Among them, the loss-in-function (wx) allele reduces the amylose content within the grains to less than 2%, endowing the grains with a sticky texture (such as in glutinous rice and glutinous corn). Another important variation is the aforementioned Wx^a and Wx^b: *Indica* rice carrying Wx^a has 25% amylose, and the rice is drier and harder.

Japonica rice with 15% straight chains carrying Wx^b has soft and sticky rice. Therefore, in hybrid breeding, the selection of the *Wx* allele can effectively alter the rice quality of the offspring. For instance, introducing Wx^a into *japonica* rice can enhance amylose content and improve the overly soft and sticky texture. Conversely, introducing Wx^b into *indica* rice can reduce amylose and make the rice softer. In addition to natural alleles, modern technology has also achieved targeted modification of the *Wx* gene. CRISPR/Cas9 gene editing was used to knock out the *Wx* gene, resulting in a series of artificial glutinous rice materials.

For instance, research teams from Japan and China reported almost simultaneously that CRISPR was used to cause the deletion of exon 1 of *Wx* or the generation of an early stop codon, obtaining phenotypically stable glutinous rice strains. These edited glutinous rice varieties contain nearly zero amylose, have a soft and sticky texture, and are no different from natural glutinous rice. Moreover, they do not require years of hybridization and homozygism, which greatly accelerates the targeted breeding of glutinous rice varieties. In terms of increasing amylose, researchers have also attempted to enhance *Wx* expression or alter regulatory elements through gene editing. For instance, a team edited a microsatellite polymorphism ($(CT)_n$ repeat) on the *Wx* promoter, obtaining a series of repeat variations of different lengths, which led to gradient changes in *Wx* expression and amylose content, achieving fine regulation of the softness and hardness of rice (Figure 2) (Huang et al., 2020).

In addition, after introducing the wild rice *Wx* ancestor allele Wx^{lv} into cultivated rice, it was found that the amylose content could be reduced by approximately 5 percentage points, while the viscoelasticity of the rice was also enhanced. This indicates that some excellent *Wx* alleles have potential in improving the quality of rice. In breeding practice, molecular markers have been utilized to efficiently screen *Wx* alleles. For instance, in hybrid offspring, through functional markers of the *Wx* gene, individual plants carrying waxy alleles, low linear alleles and high linear alleles can be accurately distinguished, accelerating the aggregation of quality traits (Yao et al., 2010). In conclusion, whether it is the utilization of traditional variations or the creation of modern gene editing, the mechanism by which *Wx* genes regulate amylose content has been deeply mastered and successfully applied to rice quality breeding. From the cultivation of pure *japonica* glutinous rice to the development of soft rice with moderate straight-chain in hybrid *indica* rice, the *Wx* gene is a key regulatory target. It can be expected that with the enrichment of understanding of the *Wx* allelic variation effect and the application of new technologies, people will be able to cultivate more rice varieties that meet different consumer demands.

5.2 Functional characterization of SBE and SS gene families

In addition to *Wx*, functional variations of other enzyme-coding genes in the starch synthesis pathway also have a significant impact on rice quality. The research on the starch branched enzyme (SBE) and soluble starch synthase (SS) families has provided us with multiple gene targets for improving the structure and properties of starch. The classic "high amylose corn" is achieved by mutating the SBEIIb gene (amylomaize, ae mutation) in corn, which increases the amylose content to over 50% (Han et al., 2022). Similarly, knocking out the homologous SBEIIb gene in rice can significantly increase the content of amylose and resistant starch in grains. A research team from NIAS in Japan silenced rice SBEIIb using RNAi technology, obtaining high-amylose rice with an amylose content of over 30%. At the same time, the starch digestion rate was significantly reduced, which is conducive to the development of low-glycemic index varieties.

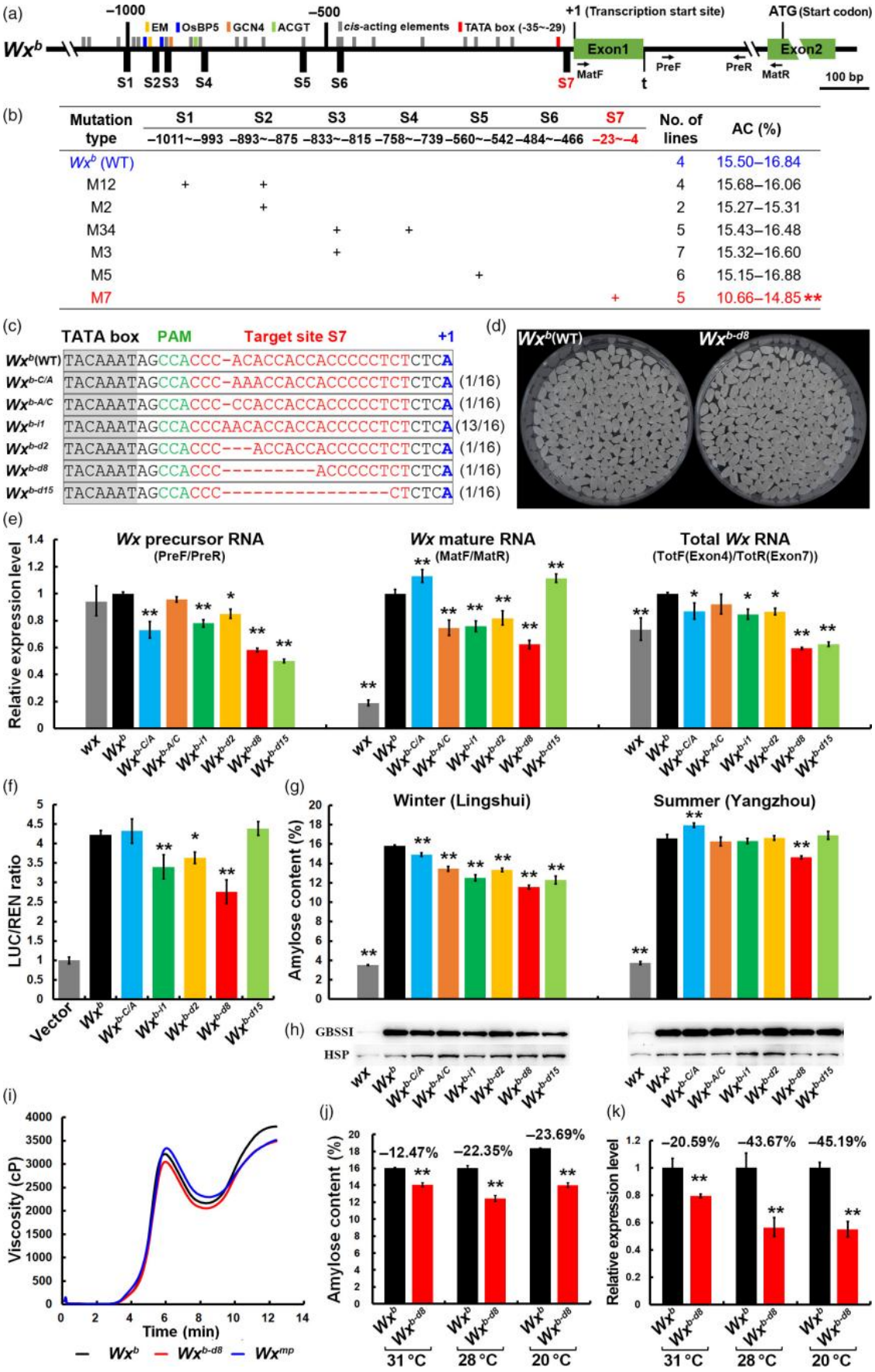

Figure 2 CRISPR/Cas9 system editing of the *Wx* promoter to create novel *Wx* alleles to fine-tune expression and amylose content (Adopted from Huang et al., 2020)

However, the absence of SBEIIb is also accompanied by negative effects such as slight shrinkage of the endosperm of rice grains and decreased transparency. Scientists have further utilized gene editing to precisely regulate the expression of SBEIIb, hoping to increase resistant starch while reducing the adverse effects on appearance. Some progress has been made in this regard. For instance, through gene editing, some functions of SBEIIb have been partially lost rather than completely absent, resulting in improved materials that balance both appearance and nutrition. Among *SS* genes, *SSIIa* and *SSIIIa* have a significant impact on the structure of amylopectin and are the focus of research. The aforementioned *Alk* (*SSIIa*) allelic variation determines the lower gelatinization temperature and soft texture of *japonica* rice. In breeding, superior alleles of *japonica* rice (such as alk/alk) are often selected in combination with low straight-chain to improve the quality of *indica* rice. The alteration of SSIIIa function can be used to increase the content of resistant starch. Fujita et al. (2007) created SSIIIA-deficient rice and wheat mutants. The results showed that the proportion of long branched chains in the endosperm increased, resistant starch significantly improved, and the GI value decreased simultaneously. However, the absence of SSIIIa makes the rice slightly harder in texture.

During breeding, it needs to be combined and adjusted with other variations such as *Wx* and *SSIIa* to achieve a balanced taste and nutrition. In recent years, Chinese scholars have also reported the role of SSIVb in starch synthesis: although SSIV is mainly responsible for regulating the number of starch granules, its mutation can reduce the number of starch granules in endosperm, but the difference in particle size and total starch content is not significant, and its impact on quality is limited. It is worth mentioning that bifunctional enzymes are also under research. For instance, the phosphoric acid accumulated on the surface of starch granules can promote the swelling and gelatinization of starch, and altering the enzyme activity can affect this property. A deletion mutation of a phosphatase called PHS2 (the homolog of Starch Excess4 in *Arabidopsis thaliana*) leads to an increase in the degree of starch phosphorylation and a slight decrease in the gelatinization temperature of rice.

All these indicate that through functional identification, we are constantly exploring new gene targets that can be used to improve the properties of starch. In the future, through the integration of multiple gene variations, such as *Wx* low straight-chain + high-quality Alk+ weak functional SBEIIb and other combined breeding methods, it is expected to cultivate ideal rice varieties with soft texture, low cold rice hardness and high resistant starch content. It can be foreseen that with the in-depth identification of functional genes, we will establish a more abundant "gene toolbox" to provide support for the targeted improvement of different rice quality traits.

5.3 Utilization of superior alleles in high-quality rice breeding

Utilizing the superior alleles of the aforementioned key genes to cultivate high-quality rice is one of the important strategies in modern molecular breeding. Traditionally, the superior quality genes of different rice varieties can be introduced into the main cultivated varieties through hybridization. For instance, the Japanese "Koshihikari" *japonica* rice is of excellent quality, among which the low-linear Wx^b and soft alk alleles play a decisive role in its soft and glutinous texture. In recent years, breeders have introduced these alleles into high-yield background varieties through backcrossing and transfer, thereby fostering new varieties that are both high-yielding and have good palatability.

In China, the Wx^b (low straight-chain) allele has been introduced into many restorer lines of hybrid *indica* rice, significantly improving the quality of hybrid rice. For instance, combinations like Taiyou 390 ensure yield while reducing amylose content to around 16%, meeting the standards of high-quality rice. For instance, the fragrant rice variety has developed fragrant glutinous rice by taking advantage of the functional deletion allele of the *OsBADH2* gene (which enables rice to synthesize the aromatic substance 2-AP) and combining it with the glutinous wx allele, thus meeting the market's dual demands for flavor and texture.

In addition to the conventional hybridization to polymerize superior alleles, new molecular approaches are also accelerating this process. Molecular marker-assisted selection (MAS) has been widely used to track important sites such as *Wx*, *Alk*, and *BADH2*. Through marker detection, individuals carrying the target allele can be precisely selected in the early generation, significantly enhancing breeding efficiency. For instance, in the project

to improve the quality of Indian *indica* rice, by using the linkage markers of *Wx* and *Alk*, the excellent alleles of two seats were successfully introduced into the high-yield background together, thereby significantly reducing the amylose content and increasing the gel consistency, achieving a notable improvement in rice quality (Figure 3) (Gao et al., 2024). Gene editing technology offers a new approach to the utilization of superior alleles: it can directly create the desired allelic variations in an existing variety without introducing an exogenous genetic background, thereby avoiding long-term backcrossing. For instance, for a common high-straight-chain rice, by knocking out the *Wx* gene with CRISPR/Cas9, isogenic homozygotes of glutinous rice alleles can be obtained within one generation. For instance, by fine-tuning the *Wx* promoter through gene editing, a series of *Wx* allelic variants with different expression intensities were obtained, achieving continuous adjustment of amylose content. Within the same variety, a series of materials with high, medium and low amylose content were created.

This is equivalent to obtaining the allelic variation that requires multiple varieties in traditional breeding with just one click, which is very convenient for breeding. In addition, a foreign research team used CRISPR to simultaneously edit the *Wx* and *OsBADH2* genes, cultivating a new material called "fragrant glutinous rice" with both glutinous texture and fragrance. This requires complex hybridization and multiple generations of selection in traditional breeding, while gene editing can achieve the superposition of two superior traits with a single mutation, demonstrating great potential. It can be foreseen that with the mature application of gene editing technology, we will be able to combine high-quality alleles more freely. For instance, the polymerization of multiple traits such as "low linear chain + high resistant starch + aromatic" will be more convenient, thus enabling the cultivation of functional high-quality rice varieties that meet the needs of special groups. Fully exploring and utilizing the excellent alleles that affect the quality of rice, along with advanced molecular polymerization methods, will greatly promote the breeding process of high-quality rice and meet people's expectations for rice to be "delicious and healthy".

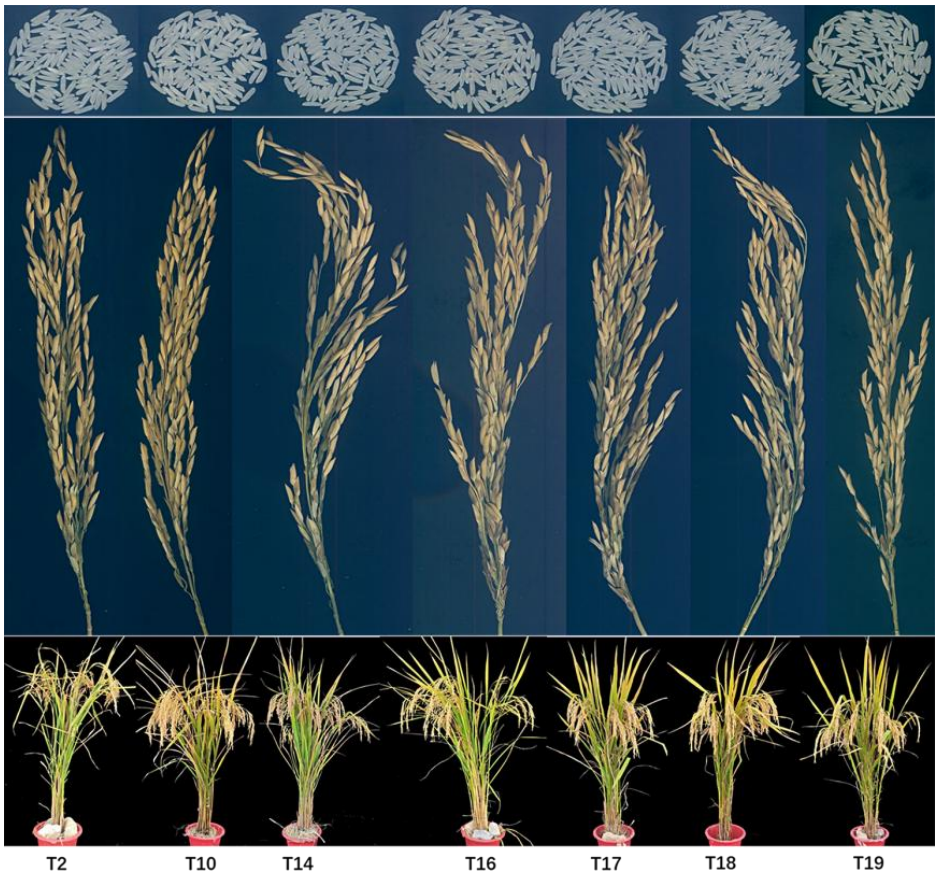

Figure 3 The combinations of hybrid rice with high quality. (a) The appearance of rice; (b) The characteristics of rice panicles; (c) The plant type characteristics of rice (Adopted from Gao et al., 2024)

6 Strategies for Grain Quality Improvement Based on Starch Biosynthesis Regulation

6.1 Application of marker-assisted selection (MAS) and gene editing technologies

The major genes related to the starch synthesis pathway, such as *Wx*, *Alk*, *BADH2*, etc., provide clear selective targets in the improvement of rice quality. Molecular marker-assisted selection (MAS) has been widely used to track the superior alleles of these genes. In breeding practice, by developing molecular markers closely linked to target genes, breeders can identify the genotype of plants at the seedling stage, thereby significantly improving the selection efficiency. For instance, in response to different alleles of the *Wx* gene (such as distinguishing Wx^a from Wx^b, *wx*, etc.), researchers have established functional InDel and SSR markers to achieve precise selection of amylose content traits. In a high-yield *indica* rice background, by using MAS to select the low straight-chain alleles of Wx^b and eliminate Wx^a, an improved variety with reduced amylose content and softer rice was successfully cultivated while maintaining the yield of the original variety (Yang et al., 2019). For instance, for the *Alk* gene in *japonica* rice that determines the gel consistency, the designed CAPS marker can effectively distinguish between high gel consistency (soft rice) and low gel consistency (hard rice) alleles, thereby guiding the introduction of soft rice alleles into the target variety (Gao et al., 2011). In conclusion, MAS makes the selection of complex quality traits efficient and reliable, especially in the utilization of heterosis and multi-gene aggregation breeding. Molecular markers can simultaneously track multiple quality genes, increasing the probability of aggregation. In the past decade, the rise of gene editing technologies (such as CRISPR/Cas9) has opened up new paths for quality improvement. Compared with traditional mutagenesis, gene editing can precisely alter the gene loci of interest, thereby directly obtaining the desired allelic variations. For the major genes in rice quality, gene editing can instantaneously "knock out" or modify the target genes in one variety without introducing additional exogenous DNA. For instance, as mentioned earlier, by knocking out the *Wx* gene with CRISPR, a common rice can be quickly transformed into glutinous rice. Knocking out the *BADH2* gene can endow rice with a fragrant aroma. In terms of enhancing the taste of steamed and cooked rice, Japanese scholars have utilized gene editing to destroy two negative effect genes in large-grain *indica* rice with poor taste, significantly improving the texture without losing yield. Gene editing can also be used to precisely regulate the expression level or activity of quality genes without complete knockout. For instance, by editing the repetitive sequences of the *Wx* promoter, different degrees of expression inhibition were achieved, and a series of materials with gradually changing amylose content were obtained. This makes it possible to develop rice of various textures. It can be foreseen that in the future quality breeding of rice, the organic combination of conventional MAS and emerging gene editing will significantly enhance the efficiency and accuracy of breeding. For clear major effect loci, superior alleles can be screened from germplasm resources through MAS. For ideal alleles that do not exist in germplasm, they can be directly created through gene editing and introduced into breeding materials. The combined application of these two methods is expected to cultivate new rice materials that meet the target quality indicators in a targeted manner within one or two generations, thereby accelerating the breeding process of high-quality rice varieties.

6.2 Strategies for multi-gene coordinated improvement of starch quality

The cooked taste and nutritional quality of rice are often the result of the combined effect of multiple starch-related traits. Therefore, multi-gene collaborative improvement is needed to achieve an overall enhancement. For instance, merely reducing the content of amylose might make the rice soft but overly sticky, which is not accepted by some consumers. If the proportion of long-chain amylopectin is appropriately increased while reducing the linear chain (increasing the viscosity of the gel), a balance can be struck between soft stickiness and elasticity. This requires the simultaneous regulation of multiple genes such as *Wx* and *SSIIa*. The strategy of aggregating multiple genes through conventional hybridization has been achieved to a certain extent, such as the combination of high-yield genes and high-quality genes in the cultivation of "super rice". However, more efficient multi-gene improvement can be achieved by using gene-edited components or stacks. In 2019, researchers from IRRI used CRISPR/Cas9 to simultaneously edit the quality genes of two restorer lines - *Wx* from an sterile line material and *BADH2* from a restorer line. Eventually, they combined stickiness and aroma to cultivate the world's first hybrid rice with both fragrant and glutinous lines. This achievement proves that through

one transformation and one generation, multiple genes can be specifically modified from different parents, and then hybridized and combined to obtain hybrids that simultaneously possess two superior traits. Similarly, a team from Huazhong Agricultural University in China has also explored the quality improvement of a multi-gene editing stack: they simultaneously knocked out the *Wx*, *SSIIb*, and *SSIIIa* genes in a backbone restorative line, endowing it with the characteristics of low linear and high branched long chains. Then, based on this, they introduced aromatic mutations to obtain a new rice variety with a low GI and prominent aroma. It can be imagined that in the future, by flexibly choosing different gene combinations, various characteristic functional rice varieties can be customized and cultivated. For instance, to meet the needs of the diabetic population, a gene package combining weak *Wx* alleles with highly resistant starch mutations (such as SBEIIb knockout) can be used to lower the GI. To meet the dietary habits of different regions, a set of high straight chain, soft and thick, and fragrant gene can be combined to balance the texture and flavor of dry rice. When improving multi-gene collaboration, it is also necessary to pay attention to the mutual balance of the effects of different genes (Tang et al., 2022). For instance, while enhancing resistant starch, it is also necessary to take into account both yield and taste. When designing the combination, it is essential to retain the necessary enzyme activity to avoid extreme traits. Through reasonable gene "superposition" and genetic engineering methods, we are expected to create "tailor-made" ideal rice varieties, shifting from a single pursuit of yield to a balance between yield and quality, and providing consumers with more diverse and healthier staple food choices.

6.3 Optimizing pathways by integrating metabolic engineering and traditional breeding

Improving the quality of rice is a systematic project. Relying solely on molecular-level improvements often needs to be combined with conventional breeding and cultivation measures to achieve the best results. Therefore, when applying the research results of starch synthesis regulation to breeding practice, it is necessary to comprehensively consider the combination of metabolic engineering and traditional breeding. Firstly, after molecular breeding creates high-quality genotypes, traditional breeding methods such as hybridization and backcrossing remain important means to transfer these genotypes to varieties with excellent agronomic traits. For instance, low-linear glutinous rice mutants were obtained through gene editing, but the background might be experimental materials that were susceptible to diseases or had low yields. In such cases, backcrossing was needed to introduce the glutinous rice alleles into the background of the main cultivated variety, and then the agronomic traits were restored through field selection. In this process, traditional breeding experience and field phenotypic selection are extremely crucial, as quality is only a part of the overall traits of a variety. Secondly, quality traits are largely influenced by the environment and cultivation conditions, and the so-called "variety × environment interaction" is significant. Therefore, when applying high-quality genotypes, it is necessary to combine them with appropriate cultivation management. For instance, high-amylose and high-resistant starch varieties can achieve better quality performance in a cooler environment. However, in a high-temperature environment, cultivation improvements (such as watering to cool down and timely harvesting) are needed to prevent chalkiness and texture deterioration. In addition, when promoting nutritionally fortified functional rice (such as varieties rich in resistant starch), it is also necessary to gradually guide consumers' dietary habits. The quality can be gradually transitioned through hybridization of traditional varieties to make them accepted by the general public. Therefore, new varieties produced by metabolic engineering usually need to go through the refinement of traditional breeding steps, including the improvement of agronomic traits, multi-environment test identification, and the matching of cultivation techniques, before they can become truly valuable varieties. Fortunately, with the development of modern breeding theory, the concept of "collaborative improvement of multiple target traits" has been deeply rooted in people's minds. In the overall goal of genetic improvement of rice, yield, resistance and quality are taken into account in a coordinated manner, and various breeding innovation teams are also building comprehensive genetic engineering breeding plans that are "high-yielding, stress-resistant and high-quality". For instance, among the major molecular design breeding projects implemented in China in recent years, there is a plan to cultivate new varieties of ideal plant types that balance both yield and rice quality. By using gene aggregation methods, multiple yield QTLS are crossed and polymerized with quality sites such as *Wx* and *ALK*, and then precisely selected and bred with the help of molecular markers. This reflects the deep integration of traditional breeding

practices and modern molecular methods. Looking ahead, we should guide the cultivation of high-quality rice varieties with a systems perspective: we should not only leverage the power of metabolic engineering improvement in the starch synthesis pathway, but also attach importance to field trials and the control of comprehensive traits through traditional methods. Only by taking a three-in-one approach of "molecular design + conventional improvement + optimized cultivation" can the high-quality genes in the laboratory be truly transformed into practical achievements such as increased field yields, enhanced aroma in rice buckets, and enhanced flavors on dining tables (Zhong et al., 2023).

7 Concluding Remarks

In recent years, functional genomics and gene editing technologies have developed rapidly. People's understanding of the starch synthesis pathway in rice is also deeper than before. New regulatory factors and metabolic mechanisms are constantly being discovered, which makes the original regulatory network more complete. In the field of genetic research, scientists have identified many new genes and alleles that affect starch synthesis. For instance, Du and flo genes that regulate amylose, as well as long-chain sucrose transporters that affect the structure of amylopectin, etc. All of these were determined through mutant cloning methods. These achievements have made us more clearly aware that the regulation of starch synthesis is extremely complex. In terms of trait inheritance, people have gained a clearer understanding of the quantitative genetic basis of starch quality. By using genome-wide association analysis (GWAS) and genome-wide selection, researchers have elucidated the genetic structures of important traits such as amylose content, gel consistency, and chalkiness rate, and have also identified many new quantitative regulatory loci. For instance, Indian scientists analyzed 3,000 core germplasms and identified 11 key loci related to resistant starch and predicted glycemic index, providing a reference target for improving low-GI rice. In terms of regulatory mechanisms, the roles of epigenetics and non-coding Rnas have attracted increasing attention. New sequencing techniques have mapped out the specific methylation map of endosperm and long non-coding RNA lineages. Although their specific functions still need further research, these results have already suggested that there may be more levels in the regulation of starch synthesis. In the field of synthetic biology, there have also been notable advancements. A Chinese research team has for the first time achieved the artificial synthesis of starch in vitro, converting CO_2 into starch molecules in one step. Although this method cannot be implemented in plants at present, it theoretically offers new possibilities for the future modification or reconstruction of crop starch synthesis pathways through metabolic engineering.

Although there have been many advancements in the research on the regulation of rice starch synthesis, there are still some unsolved problems. First, the basic mechanism is still not clear enough. In the starch synthesis pathway, how different key enzymes assemble into complexes on the particle surface and work together is currently only speculated by models, and there is no direct evidence yet. To understand this issue, more advanced in-situ analysis techniques are needed. Second, there is insufficient understanding of the signal regulation of endosperm development. We know that the hormones and nutritional signals of plants can affect starch accumulation, but the specific process is still unclear. For instance, it is not yet fully understood how they function through the transcription factor network or by regulating enzyme activity. For instance, it is still unclear which signaling pathways will inhibit the expression of the amylase gene under high-temperature stress. Thirdly, it is difficult to balance output and quality. In breeding, many mutations that can improve the quality of rice can lead to a decrease in yield. Varieties with high amylose or high resistant starch are often accompanied by a decrease in 1000-grain weight and insufficient grain filling. How to break this negative correlation and achieve both high quality and high yield still requires new ideas. Perhaps the traits of different mechanisms can be combined, such as enhancing the grain filling strength while improving starch, but there are still few successful cases in this regard. Fourth, the quality standards are not uniform. Consumers' demands vary greatly and are subjective. It cannot be measured by a single indicator. The improvement of main parameters such as amylose has been relatively mature, but the sensory properties of rice, such as aroma, texture and graininess, are more complex, and the involved components and related genes are still not clear enough. Fifth, there are difficulties in promoting the technology. The potential of gene editing is great, but it is currently difficult and costly to stably edit multiple gene loci at one time in crops. For instance, the efficiency of editing more than three sites at once is not high, and the homozygous screening of

multiple mutants is also very troublesome. At the same time, the regulatory policies and public acceptance of gene-edited crops will also affect the speed of their promotion. To comprehensively enhance the quality of rice, we must not only tackle these fundamental issues in scientific research but also continuously innovate and optimize in technology.

Looking ahead, there will be several promising research directions and application prospects regarding the regulation of rice starch synthesis and the improvement of grain quality. Firstly, in basic research, with the development of single-cell omics and real-time imaging technology, we are expected to map the spatiotemporal regulation of endosperm starch accumulation. By analyzing the transcriptional dynamics of different cellular levels of endosperm during the grouting period through single-cell RNA sequencing, the differential regulation of starch synthesis in different sites can be understood, guiding further pathways to reduce chalkiness. Observation of the formation of enzyme complexes in living endosperm using high-resolution microscopic imaging will reveal the direct interaction relationship among starch synthases. This will fill the gap in the coordination mechanism of starch synthase. Secondly, in terms of breeding applications, molecular design breeding will enter a new stage of intelligent design and refined improvement. By leveraging big data and computational models, the effects of different quality genes can be integrated to optimize the design of variety traits based on the preferences of specific ecological regions or consumer groups. For instance, by using models to predict the most suitable combination of straight-chain content and gel consistency under the climate of a certain region, and then working backward to infer the required genotype combination, it can guide breeding practice. Secondly, gene editing technology is expected to break through current limitations and achieve simultaneous targeted editing of multiple genes and targeted recombination of the genome.

At that time, we will be able to simultaneously modify more than ten quality-related loci within one breeding cycle, just like "building with blocks", to construct the ideal genotype. In the longer term, synthetic biology may bring about revolutionary changes. For instance, if the synthetic starch pathway can be grafted onto plants, enabling crops to produce starch with special structures through metabolism (such as highly branched and hard-to-digest resistant starch), it will open up a brand-new path for quality improvement. Of course, such a prospect still requires long-term efforts. In terms of actual production, the promotion of high-quality rice also faces some challenges, which require the joint efforts of scientific research and industry to address. On the one hand, it is necessary to enhance the planting benefits of high-quality rice varieties. By improving their stress resistance and the upper limit of yield, farmers should be encouraged to grow high-quality varieties to meet market demands. On the other hand, it is necessary to enhance brand building and consumer education to make the public aware of the value of high-quality rice (such as the health benefits of low-GI rice, etc.), and form a positive market-driven mechanism. It can be foreseen that as people's pursuit of the quality and health of staple foods continues to rise, high-quality rice will have a broad market prospect. Through in-depth scientific research and advancements in breeding techniques, we are confident in cultivating more new rice varieties that are both delicious and nutritious, making contributions to ensuring food security and the health of all people. In the near future, functional rice that is "delicious and does not cause weight gain" and local specialty rice that is "fragrant, glutinous and tasty" are all expected to appear on the dining tables of thousands of households, bringing people a better dining experience and nutritional benefits.

Acknowledgments

We would like to express our gratitude to the reviewers for their valuable feedback, which helped improve the manuscript.

Conflict of Interest Disclosure

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

References

Dawar C., Jain S., and Kumar S., 2013, Insight into the 3D structure of ADP- glucose pyrophosphorylase from rice (*Oryza sativa* L.), Journal of Molecular Modeling, 19: 3351-3367.
https://doi.org/10.1007/s00894-013-1851-7

Fujita N., Yoshida M., Kondo T., Saito K., Utsumi Y., Tokunaga T., Nishi A., Satoh H., Park J.H., and Jane J.L., 2007, Characterization of SSIIIa‐ deficient mutants of rice: the function of SSIIIa and pleiotropic effects by SSIIIa deficiency in the rice endosperm, Plant Physiology, 144(4): 2009-2023.
https://doi.org/10.1104/pp.107.102533

Gao J., Gao L., Chen W., Huang J., Qing D., Pan Y., Ma C., Wu H., Zhou W., Li J., Yang X., Dai G., and Deng G., 2024, Genetic effects of grain quality enhancement in *indica* hybrid rice: insights for molecular design breeding, Rice, 17: 39.
https://doi.org/10.1186/s12284-024-00719-7

Gao Z.Y., Zeng D.L., Cheng F.M., Tian Z.X., Guo L.B., Su Y., Yan M.X., Jiang H., Dong G.J., Han B., Li J.Y., and Qian Q., 2011, *ALK*, the key gene for gelatinization temperature, is a modifier gene for gel consistency in rice, Journal of Integrative Plant Biology, 53(9): 756-765.
https://doi.org/10.1111/j.1744-7909.2011.01065.x

Han H., Yang C., Zhu J., Zhang L., Bai Y., Li E., and Gilbert R.G., 2019, Competition between granule-bound starch synthase and starch branching enzyme in starch biosynthesis, Rice, 12: 96.
https://doi.org/10.1186/s12284-019-0353-3

Han J., Guo Z., Wang M., Liu S., Hao Z., Zhang D., Yong H., Weng J., Zhou Z., Li M., and Li X., 2022, Using the dominant mutation gene Ae1-5180 (amylose extender) to develop high-amylose maize, Molecular Breeding, 42: 57.
https://doi.org/10.1007/s11032-022-01323-7

Huang Y.M., 2024, Genomic insights into grain size and weight: the *GS2* gene's role in rice yield improvement, Plant Gene and Trait, 15(3): 141-151.
https://doi.org/10.5376/pgt.2024.15.0015

Huang L., Li Q., Zhang C., Chu R., Gu Z., Tan H., Zhao D., Fan X., and Liu Q., 2020, Creating novel *Wx* alleles with fine‐ tuned amylose levels and improved grain quality in rice by promoter editing using CRISPR/Cas9 system, Plant Biotechnology Journal, 18(11): 2164-2166.
https://doi.org/10.1111/pbi.13391

Ma H.L., 2024, Advanced genetic tools for rice breeding: CRISPR/Cas9 and its role in yield trait improvement, Molecular Plant Breeding, 15(4): 178-186.
https://doi.org/10.5376/mpb.2024.15.0018

Mao H., Peng Y., Mao B.G., Shao Y., Zheng W.J., Hu L.M., Zhou K., and Zhao B.R., 2022, Function and effect analysis of a new gene *Wx410* regulating amylose synthesis in rice, Chinese Journal of Rice Science, 36(6): 579-585.
https://doi.org/10.16819/j.1001-7216.2022.220103

Miura S., Ohnishi S., Kurata N., and Yamazaki K., 2024, Mutations in *Starch-Branching Enzyme 2a* increase resistant starch content in barley endosperm, Theoretical and Applied Genetics, 137: 1021-1030.
https://doi.org/10.1007/s00122-024-04725-7

Peng T., Sun H., Du Y., Zhang J., Li J., Liu Y., Zhao Y., and Zhao Q., 2013, Characterization and expression patterns of microRNAs involved in rice grain filling, PLOS ONE, 8(1): e54148.
https://doi.org/10.1371/journal.pone.0054148

Shao Y., Peng Y., Mao B., Lv Q., Yuan D., Liu X., and Zhao B., 2020, Allelic variations of the *Wx* locus in cultivated rice and their use in the development of hybrid rice in China, PLOS ONE, 15(5): e0232279.
https://doi.org/10.1371/journal.pone.0232279

Sun Y., Jiao G., Liu Z., Zhang X., Li J., Guo X., Du W., Du J., Francis F., Zhao Y., and Xia L., 2017, Generation of high‐ amylose rice through CRISPR/Cas9‐ mediated targeted mutagenesis of starch branching enzymes, Frontiers in Plant Science, 8: 298.
https://doi.org/10.3389/fpls.2017.00298

Tang S., Yang C., Wang D., Deng X., Cao X., and Song X., 2022, Targeted DNA demethylation produces heritable epialleles in rice, Science China Life Sciences, 65: 753-756.
https://doi.org/10.1007/s11427-021-1974-7

Wang J.C., Xu H., Zhu Y., Liu Q.Q., and Cai X.L., 2013, OsbZIP58, a basic leucine zipper transcription factor, regulates starch biosynthesis in rice endosperm, Journal of Experimental Botany, 64(11): 3453-3466.
https://doi.org/10.1093/jxb/ert187

Yamakawa H., and Hakata M., 2010, Atlas of rice grain-filling-related gene expression: a platform to identify grain quality determinants, Plant Molecular Biology, 72: 513-528.
https://doi.org/10.1007/s11103-009-9603-5

Yang G.L., Chen S.P., Chen L.K., Gao W.W., Huang Y.T., Huang C.H., Zhou D.H., Wang J.F., Liu Y.Z., Huang M., Xiao W.M., Wang H., Guo T., and Chen Z.Q., 2019, Development and utilization of functional KASP markers to improve rice eating and cooking quality through MAS breeding, Euphytica, 215: 66.
https://doi.org/10.1007/s10681-019-2392-7

Yao S., Chen T., Zhang Y.D., Zhu Z., Zhao L., Zhao Q.Y., Zhou L.H., and Wang C.L., 2010, Pyramiding of translucent endosperm mutant gene Wx‐ mq and rice stripe disease resistance gene Stv‐ b^i by marker‐ assisted selection in rice (*Oryza sativa*), Chinese Journal of Rice Sciences, 24(4): 341-347.
https://doi.org/10.3969/j.issn.1000-7240.2010.04.005

Ying Y., Xu F., Zhang Z., Tappiban P., and Bao J., 2022, Dynamic change in starch biosynthetic enzymes complexes during grain-filling stages in BEIIb active and deficient rice, International Journal of Molecular Sciences, 23(18): 10714.
https://doi.org/10.3390/ijms231810714

Zhao S.L., Cao R.J., Sun L.H., Zhuang D.Y., Zhong M., Zhao F.L., Jiao G.A., Chen P.F., Li X.W., Duan Y.Q., Li X.X., Tang S.Q., Ni S., Hu P.S., and Wei X.J., 2024, An integrative analysis of the transcriptome and proteome of rice grain under high temperature stress during grain filling, Plants, 13(23): 3309.
https://doi.org/10.3390/plants13233309

Zhong Q., Jia Q., Yin W., Wang Y., Rao Y., and Mao Y., 2023, Advances in cloning functional genes for rice yield traits and molecular design breeding in China, Frontiers in Plant Science, 14: 1206165.
https://doi.org/10.3389/fpls.2023.1206165

Zhou H., Ding W., Ding Y., and Jenkins T., 2022, Resistant starch formation in rice: genetic regulation and beyond, Trends in Food Science & Technology, 124: 32-44.
https://doi.org/10.1016/j.tifs.2022.05.005