ONLINE JOURNAL OF BIOLOGICAL SCIENCES

LITERATURE REVIEW

Molecular Genetic Basis of Starch Biosynthesis and Waxy Allelic Variation in Rice Grain Quality

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Abstract: The culinary, nutritional, and industrial properties of rice (*Oryza sativa* L.) grains are fundamentally governed by the structural arrangement of endosperm starch, a complex physiological phenotype shaped by genetic architecture and environmental conditions. This review provides a comprehensive synthesis of the molecular pathways driving starch biosynthesis in rice, centering on allelic diversity within the *Waxy* (*Wx*) locus, which codes for Granule-Bound Starch Synthase I (GBSSI). Sequence variations in *Wx* critically modulate amylose synthesis by altering transcription levels and post-transcriptional events, primarily pre-mRNA splicing accuracy. The resulting amylose-to-amylopectin ratio directly dictates grain firmness, retrogradation rates, digestibility, and postprandial metabolic responses. Although key biosynthetic enzymes have been characterized, the overarching regulatory network and its vulnerability to ambient temperature stress during grain maturation demand further elucidation. Harnessing modern molecular markers and genomic selection targeting these genetic networks offers promising avenues for precision breeding of rice cultivars tailored for specific end-use functionalities.

Keywords: *Oryza sativa*, Endosperm Starch, Waxy Locus, GBSSI Regulation, Amylose Dynamics, Precision Breeding

Received: 14-04-2026 | **Revised:** 29-06-2026 | **Accepted:** 21-07-2026 | **DOI:** 10.3844/ojbsci.2026.26.03.075

Introduction

Rice (*Oryza sativa* L.) serves as a vital nutritional foundation, sustaining a major fraction of the global population with daily dietary energy [1]. Increasing global demand for rice is accompanied by growing consumer expectations regarding grain quality [2,3]. Consequently, enhancing rice grain quality has become a central objective of modern rice breeding programs. Understanding the genetic and molecular mechanisms that determine the culinary and flavor properties of rice is essential for developing varieties with improved quality characteristics [4].

Comprising roughly 80-90% of the dry endosperm mass, starch acts as the core constituent defining processing, culinary performance, and nutritional traits. The amylose-to-amylopectin ratio strongly influences textural properties such as stickiness, firmness, and viscosity, as well as nutritional factors including digestibility and glycemic response [5-8].

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Grain quality traits are governed by complex genetic networks, among which the *Wx* locus, encoding GBSSI, functions as a primary genetic determinant of amylose accumulation [9-12]. Recent breakthroughs in molecular genetics and functional genomics have accelerated the discovery of key regulatory pathways, laying the groundwork for marker-assisted selection [13, 14]. Particular attention is given to biochemical properties of rice, such as amylose content, which determine consumer preference and the commercial value of the grain [15-17]. For instance, while some markets favor firm, non-sticky grains, regions such as China and several European nations evaluate quality primarily by the soft, sticky sensory characteristics of the cooked product [20].

The integration of molecular-genetic techniques with advanced biochemical analyses has elucidated the precise mechanisms of starch formulation and the structural organization of the endosperm. This multidisciplinary approach is pivotal not only for uncovering the fundamental determinants of grain quality, but also for engineering novel rice cultivars tailored to contemporary food industry standards [21, 22].

This review offers a comprehensive analysis of rice endosperm starch architecture and the enzymatic networks underlying its biosynthesis, emphasizing functional allelic variation within the *Waxy* (*Wx*) locus. By synthesizing recent breakthroughs, this work establishes a theoretical framework for deploying effective molecular breeding strategies to optimize starch-related rice quality traits.

Origin and Evolution of the Rice Genome in Relation to Grain Quality

Cultivated rice (*Oryza sativa* L.) is a monocotyledonous cereal belonging to the genus *Oryza* within the family *Poaceae* (Gramineae). This genus encompasses two domesticated species *O. sativa*, widely cultivated globally, and *O. glaberrima*, native to West Africa alongside a rich repertoire of wild relatives that collectively constitute an irreplaceable reservoir of genetic variation [23]. Morphological, biochemical, and genomic stratifications traditionally categorize *O. sativa* into distinct sub-species or varietal groups, primarily *indica*, *japonica*, and *javanica* (tropical *japonica*), each exhibiting divergent grain architectures, starch physicochemical behaviors, and agronomic performances.

Members of the genus *Oryza* are broadly distributed across tropical and subtropical environments, occupying a wide array of ecological niches. Benefiting from a remarkably compact diploid genome (~430 Mb) alongside well-characterized genetic resources, rice serves as a premier model organism for investigating monocot genomics and cereal biology. From an agricultural footprint perspective, rice cultivation accounts for nearly 11% of global arable land [1, 24], emphasizing its strategic role in food security.

The diversity of species within the genus *Oryza* reflects a broad evolutionary continuum that includes both domesticated rice and closely related wild taxa, encompassing domesticated lines as well as ancestral wild species including *O. rufipogon*, *O. nivara*, *O. barthii*, and *O. longistaminata*. These wild genetic resources provide valuable reservoirs of alleles that can be exploited in molecular breeding to broaden the genetic base of cultivated rice cultivars and enhance tolerance against adverse environmental stresses and biological threats (Table 1).

While *O. sativa* is globally distributed and extensively cultivated across Asia, the Americas, Europe, and Africa, the cultivation of *O. glaberrima* remains largely restricted to its native West African domains. However, traditional *O. glaberrima* landraces are progressively being superseded throughout the African continent by higher-yielding *O. sativa* cultivars and elite interspecific hybrids [25].

Grounded in extensive genetic plasticity, the genus *Oryza* incorporates both diploid and polyploid cytotypes. The genomic framework of cultivated *Oryza sativa* is strictly diploid, comprising a chromosome complement of 2n = 24 [26]. Diploid representatives including *O. sativa*, *O. rufipogon*, and *O. nivara* share an AA-genome architecture (2n = 24), whereas tetraploid lineages (2n = 48) evolved through historical interspecific hybridization events coupled with genome duplication mechanisms. Such genomic events contributed to the accumulation of allelic diversity, which formed the basis for the development of variability in grain quality traits such as endosperm structure, starch content, and finished product texture [27, 28].

Evolutionary trajectories within the genus *Oryza* have been continually shaped by reticulate processes, including adaptive divergence, interspecific hybridization, and introgressive gene flow, giving rise to intricate genomic architectures. Among the defined genomic groups, the AA-genome lineage holds paramount evolutionary and breeding significance, as it encompasses domesticated rice alongside its immediate wild progenitors, *O. rufipogon* and *O. nivara* [24, 29]. Crucially, this specific genome

clade served as the evolutionary cradle for foundational alleles that were subsequently targeted by human selection during domestication to govern key grain quality phenotypes.

Table 1: Taxonomic grouping, chromosomal numbers, and geographical distribution of representative species within the genus Oryza [23]

Group	Species	Chromosome number (2n)	Geographic distribution
I. Sativa group	O. sativa L.	24	Worldwide; originally South and Southeast Asia
	O. nivara	24	South and Southeast Asia
	O. rufipogon	24	South and Southeast Asia, Southern China
	O. meridionalis	24	Tropical Australia
	O. glumaepetula	24	Tropical America
	O. glaberrima	24	Tropical West Africa
	O. barthii	24	West Africa
	O. longistaminata	24	Tropical Africa
	O. punctata	24	East Africa
	O. rhizomatis	24	Sri Lanka
II. Officinalis group	O. minuta	48	Philippines, New Guinea
	O. malamphuzaensis	48	Kerala and Tamil Nadu (India)
	O. officinalis	24	South and Southeast Asia
	O. alta	48	South and Southeast Asia
	O. grandiglumis	48	South America
III. Meyeriana group	O. granulata	24	South and Southeast Asia
	O. meyeriana	24	Southeast Asia
IV. Ridleyi complex	O. longiglumis	48	Indonesia, New Guinea
	O. ridleyi	48	Southeast Asia
V. Unclassified	O. brachyantha	24	West and Central Africa
	O. schlechteri	48	Indonesia, New Guinea

The domestication history of rice represents a classic example of parallel evolutionary trajectories originating across two distinct geographical hubs. Asian rice (*O. sativa*) diverged from its ancestral wild progenitors (*O. rufipogon* and *O. nivara*) across South and Southeast Asian regions, whereas African rice (*O. glaberrima*) was independently domesticated in West Africa from its wild ancestor *O. barthii* [30, 31]. This process involved directional selection for traits directly and indirectly associated with grain quality, including reduced grain shattering, modifications in endosperm structure, and improved processing and sensory properties. These changes were accompanied by the fixation of specific alleles, resulting in modern varieties with distinct quality properties.

So-called “domestication genes” play a key role in understanding domestication by determining phenotypic differences between cultivated forms and their wild ancestors (*O. rufipogon*). These traits include loss of grain shattering, changes in pericarp color, plant architecture, and inflorescence structure, as well as increased yield and improved agronomic performance. Despite the polygenic nature of many of these traits, several major genes have been identified, including *Sh4, Rc, PROG1*, and *LABA1* [32-34]. Although these genes do not directly regulate the biochemical composition of the grain, they indirectly influence grain quality through their effects on plant architecture, inflorescence structure, and grain characteristics. For example, the *Rc* gene affects pericarp pigmentation, which in turn influences the nutritional value, antioxidant properties, and consumer appeal of rice (Figure 1) [35].

Several studies suggest that certain alleles, such as mutations in *Rc*, may have spread between genetic groups via introgression, highlighting the role of hybridization in the evolution of cultivated rice. Thus, the current genetic structure of *O. sativa* likely reflects a combination of independent domestication events, subsequent hybridization, and directional selection. Importantly, many alleles associated with domestication and grain quality were already present in wild populations of *O.*

rufipogon. This indicates that natural genetic variation preceded domestication and was later exploited by humans during breeding. Through selection and introgression, these alleles became fixed and widely distributed in cultivated rice [35-38].

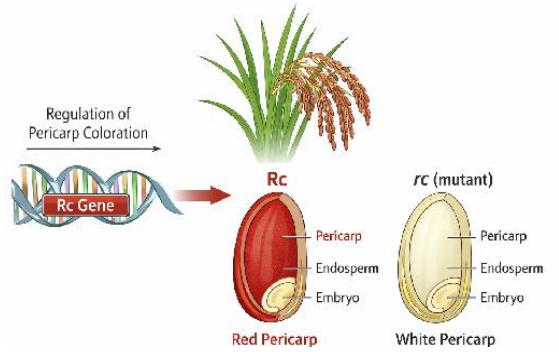

Fig. 1: Rc gene and rice pigmentation [35]

Thus, evolutionary processes including divergence, hybridization, introgression, and directional selection during domestication shaped the genetic foundation of the genus *Oryza* and promoted the accumulation of allelic diversity associated with grain quality traits. Concurrently with the selection of agronomic traits controlled by primary domestication genes, alleles regulating endosperm development and its biochemical composition underwent progressive fixation. This dual selective pressure ultimately yielded modern rice cultivars with highly diverse physicochemical grain properties [39].

Because starch represents the overwhelmingly dominant constituent of the rice endosperm, its supramolecular structure and biochemical ratios inherently govern the processing behaviors, culinary parameters, and nutritional profiles of the grain. Consequently, a comprehensive dissection of the pathways mediating starch biosynthesis is paramount to deciphering the genetic and molecular network that shapes overall grain quality attributes.

Starch Structure and Biosynthesis as the Basis of Rice Grain Quality

As the principal constituent of the rice endosperm, starch fundamentally dictates the processing functionality, cooking performance, and nutritional attributes of the grain. The structural and molecular properties of starch underlie variations in grain quality, including texture, stickiness, glycemic index, and consumer characteristics.

Structurally, the mature caryopsis of rice comprises the embryo, surrounding aleurone and bran tissues, and the starchy endosperm, with the latter acting as the primary sink tissue for reserve carbohydrate deposition [40]. Starch serves as the primary reserve substance, meeting the energy needs of the embryo during germination [41].

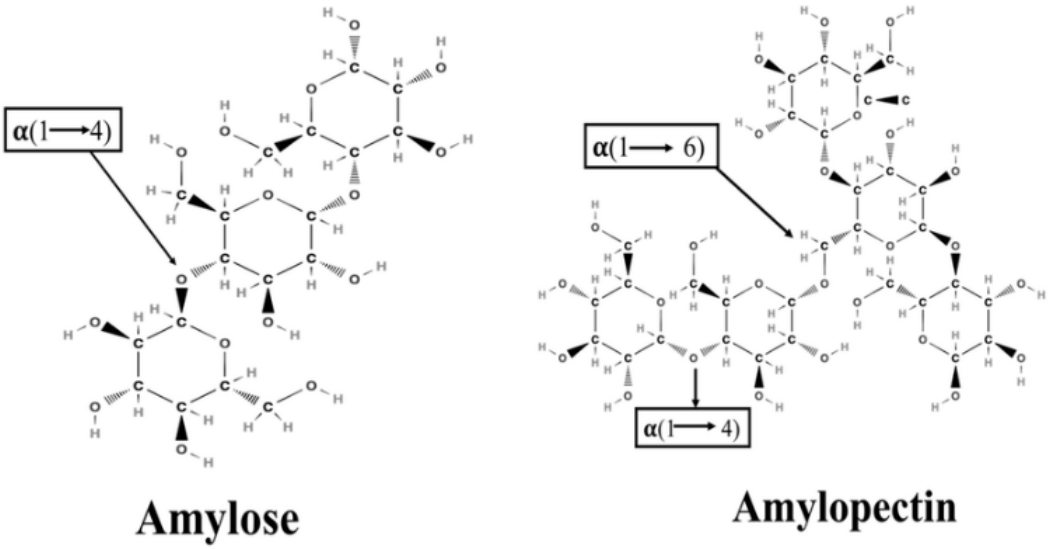

Fig. 2: Structural differences between amylose and amylopectin polymers [42]

The amylose-to-amylopectin ratio serves as a pivotal parameter governing rice quality. A high amylose concentration is associated with the formation of a crisp texture in cooked rice and a lower glycemic index, while a predominance of amylopectin results in stickiness and softness [45, 46]. Furthermore, amylose is capable of retrograding to form resistant starch, thereby slowing down digestive kinetics and having a positive effect on metabolic health [47-50].

Starch Biosynthesis and Its Impact on Rice Grain Properties

Starch synthesis in the rice endosperm is governed by the concerted action of several enzyme classes, including ADP-glucose pyrophosphorylase (AGPase), Starch Synthases (SS), Granule-Bound Starch Synthase 1 (GBSSI), starch branching enzymes (SBEs), and debranching enzymes (DBEs) [51-53].

The structural architecture of starch specifically the amylose amylopectin ratio, glucan chain-length distribution, and branching frequency is collectively shaped by these enzymes, directly dictating the cooking, processing, and nutritional attributes of the rice grain [54].

The process begins with photosynthetic products formed during CO_2 fixation in the Calvin cycle, where triose phosphates serve as precursors for glucose-1-phosphate formation. ADP-glucose, the fundamental glucosyl donor for starch polymerization, is generated by AGPase through the reaction of glucose-1-phosphate with ATP. The small subunit of the enzyme (AGPS, including AGPS2a) performs the catalytic function, while the large subunits (AGPL1, AGPL3, and AGPL4) provide allosteric regulation of the enzyme complex's activity (Figure 3) [55, 56].

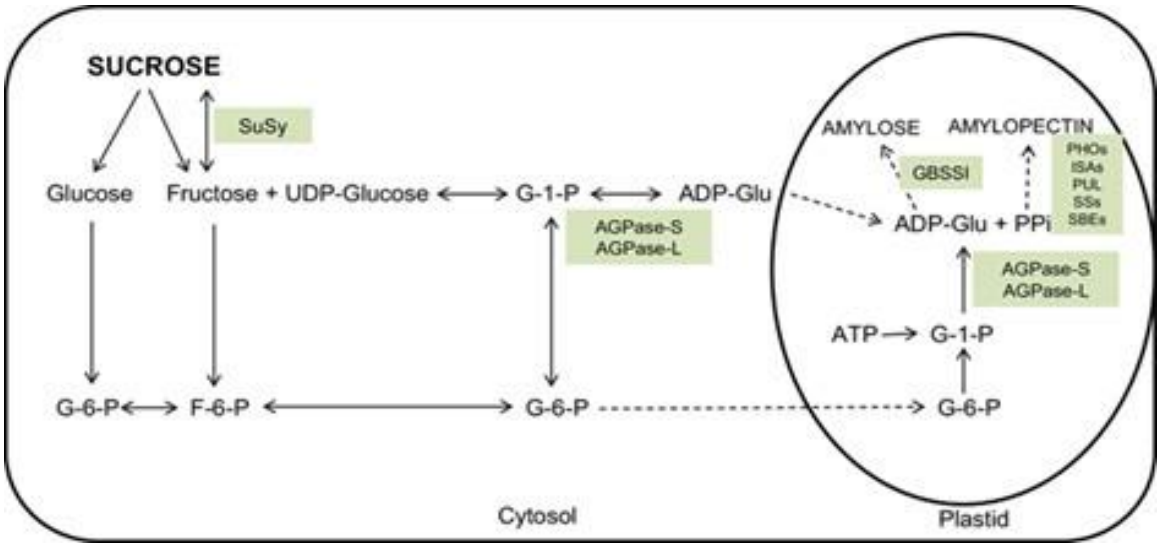

Fig. 3: Metabolic scheme of ADP-glucose formation and starch synthesis [57]

Abbreviations: ADP, adenosine diphosphate; ADP-Glu, ADP-glucose; AGPase, ADP-glucose pyrophosphorylase; ATP, adenosine triphosphate; F-6-P, fructose-6-phosphate; G-1-P, glucose-1-phosphate; G-6-P, glucose-6-phosphate; GBSSI, granule-bound starch synthase 1; ISAs, isoamylase-type starch debranching enzymes; PHO, alpha-glucan phosphorylase; PPi, inorganic diphosphate; PUL, pullulanase; SBEs, starch branching enzymes; SS, starch synthases; SuSy, sucrose synthase; UDP, uridine diphosphate.

Downstream of ADP-glucose synthesis, three primary enzyme classes drive the intra-plastid assembly of amylose alongside amylopectin [58]. The enzymatic steps of this pathway are primarily mediated by SS, SBEs, and DBEs. Each of these groups includes multiple tissue-specific and developmentally regulated isoforms that provide fine regulation of starch structure [59].

*Amylose biosynthesis. A*mylose is synthesized primarily by GBSS, which catalyzes the formation of the largely unbranched glucan chains constituting the amylose fraction of starch. In rice, GBSSI is encoded by the *Wx* gene and is predominantly active in the developing endosperm [60-62]. A second isoform, GBSSII, is mainly associated with non-storage tissues and photosynthetic organs [51, 63, 64]. The rice GBSSII transcript shows approximately 70% sequence homology with GBSSI [52, 65].

Amylopectin biosynthesis. Starch structure assembly within rice caryopsis is determined through the concerted interplay among a multienzyme complex including SS, SBEs, and DBEs. Soluble SS isoforms residing in the plastid stroma catalyze amylopectin α-1,4-glucan chain extension. Rice harbors 11 distinct SS genes, including GBSSI, GBSSII, SSI through SSIVb, and SSV [66-68].

A central role during amylopectin assembly is played by soluble starch synthases SSI-SSIII, which function in conjunction with branching and debranching enzymes [69]. During synthesis, they sequentially lengthen α-glucan chains, forming fractions with different Degrees of Polymerization (DP). In this case, SSI predominantly synthesizes short glucan chains (DP 8-12), SSII synthesizes medium-length branches (DP 12-30), while SSIII synthesizes extended chains (DP ≥ 30) [69]. Among these enzymes, SSIIIa and BEIIb are of particular interest because alterations in their activities markedly affect starch structure, resistant starch accumulation, and Glycemic Index (GI), as illustrated in Fig. 4.

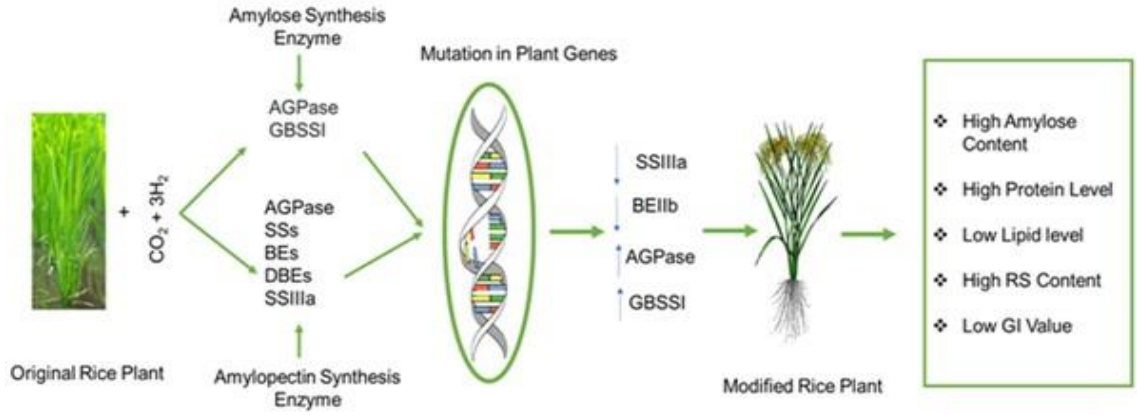

Fig. 4: Effects of reduced SSIIIa and BEIIb activities on starch structure and GI in rice [70]

As illustrated in Fig. 4, reduced SSIIIa and BEIIb activities result in structural changes in starch that ultimately contribute to the low-GI phenotype of rice. Reduced BEIIb activity decreases the degree of amylopectin branching, resulting in an increased frequency of long glucan branches, whereas reduced SSIIIa activity alters the elongation and distribution of long amylopectin polymers, thereby further modifying starch architecture. The effect of SSIIIa on resistant starch accumulation is particularly pronounced in genotypes carrying the Wx^a allele [71], while mutations in BEIIb, especially in combination with reduced SSI activity, further enhance resistant starch accumulation by modifying amylopectin chain-length distribution [72]. Together, these changes promote the formation of starch with a more ordered crystalline structure and increased amylose-lipid complex formation, making starch less susceptible to enzymatic hydrolysis. Consequently, resistant starch accumulates, glucose is released more gradually during digestion, and the postprandial glycemic response is reduced, resulting in the low-GI phenotype depicted in Fig. 4 [73-76].

Natural allelic variation in the *SSIIa* (*ALK*) gene represents a primary determinant shaping amylopectin structure as well as starch physicochemical properties [22,7 7]. Typical indica cultivars carrying highly active SSIIa alleles accumulate a greater proportion of medium-length amylopectin branches (DP 13-24), resulting in an L-type structural pattern of amylopectin as well as higher gelatinization temperatures [78]. In contrast, *japonica* cultivars with less active *SSIIa* alleles contain more short chains, exhibit an S-type amylopectin structure, and show lower gelatinization temperatures [79, 80]. Complete or partial loss of SSIIa activity subsequently elevates the proportion of short-chain amylopectin fractions while significantly reducing starch thermal gelatinization properties [81-92]. Consequently, allelic variation in SSIIa, together with gene polymorphisms coding for starch synthases, SBEs, and PUL, contributes substantially to variation in starch thermal behavior, retrogradation, rheological characteristics, digestibility, and grain quality traits [93-95]. Although enzymes involved in amylopectin biosynthesis shape the fine structure of starch, amylose accumulation is predominantly controlled by GBSSI, encoded by the *Wx* locus. Consequently, allelic variation at the *Wx* locus represents an important genetic determinant of starch composition and rice grain quality.

Allelic Diversity and Molecular Regulation of the Rice *Wx* Locus

The *Wx* locus, encoding GBSS, exerts a pivotal function in controlling amylose synthesis. The expression level as well as allelic diversity at this locus determine amylose accumulation in the endosperm and, consequently, the culinary properties of

the grain. The Wx locus stands among the most extensively investigated quality-related loci across rice. Its nucleotide sequence was determined in the early 1990s, which allowed the identification of a number of functionally significant mutations affecting gene expression and enzyme activity [22, 96-103]. Early molecular studies of the *Wx* gene have led to the design of various DNA marker systems used for genotyping and Marker Assisted Selection (MAS) of rice varieties.

Amylose accumulation in rice endosperm is regulated by the *Wx* locus mapped to chromosome 6, which encompasses 14 exons and 13 introns. This gene displays strict spatial expression in the developing seed endosperm. The transcription start site is located in exon 1, while the translation initiation codon is situated in exon 2. The primary 3.3 kb transcript undergoes post-transcriptional processing to produce a mature 2.3 kb mRNA that is translated into the GBSS protein. In glutinous rice and high-amylose genotypes, the 3.3 kb as well as 2.3 kb transcript variants are preferentially formed, respectively, whereas in varieties with medium amylose levels, co-expression of both forms is observed [105,106]. Structural variations across the exon-intron organization of the *Wx* gene can profoundly impact both its transcript level and the catalytic properties of the encoded protein. Within rice populations, numerous allelic variants of the *Wx* locus have been uncovered, featured by specific Single Nucleotide Polymorphisms (SNPs) that correlate with variations in amylose content [107-112]. Identification of functional polymorphisms in the *Wx* gene has become the basis for designing genomic markers extensively utilized in breeding schemes for predicting grain quality at early stages of plant development.

Allelic variations of the *Wx* gene exert a central function in shaping the diversity of amylose content [113,114]. To date, functional alleles of the *Wx* gene have been documented, encompassing Wx^{lv}, Wx^{a}, Wx^{in}, Wx^{b}, $Wx^{op/hp}$, Wx^{mq}, Wx^{mp}, Wx^{mw}, as well as *wx*, which are associated with diverse amylose types found in different rice varieties [107-118]. Discrimination among these alleles is achieved via six functional polymorphic markers, specifically Int1-1, Ex2-112, Ex4-53, Ex4-77, Ex6-62, and Ex10-115 (Table 2).

Table 2: Functional alleles of the rice *Wx* locus: genomic polymorphisms and corresponding amylose phenotypes [117, 118].

Culture	Allele	Genetic variability in the *Wx* gene				Endosperm phenotype
		Intron1 (G/T)	Exon 6 (A/C)	Exon 4-53 (A/C)	Exon 10 (T/C)	
Rice	Wx^{a}	G	A	-	T	High amylose content
	Wx^{lv}	G	A	-	C	High amylose content
	Wx^{in}	G	A	-	C	Medium amylose type
	Wx^{b}	T	A	-	C	Low amylose content
	$Wx^{mw/la}$	-	C	-	-	Low amylose content
	$Wx^{op/hp}$	G	C	-	-	Very low amylose content
	Wx^{mp}	T	-	A	-	Very low amylose content
	wx	T	C	-	C	Glutinous

Note: A dash ("–") denotes undisclosed or uncharacterized nucleotide states at the respective loci in referenced literature

The Wx^{a} gene variant possesses an efficient splicing motif within its initial intron (AGG TATA), which ensures proper mRNA processing and results in intermediate to high amylose levels. High-amylose rice cultivars are characterized by the accumulation of significant amounts of fully spliced GBSS transcripts, including the functional 2.3-kilobase *Wx* transcript. In contrast, low-amylose cultivars accumulate both spliced GBSS mRNA alongside incompletely spliced transcripts retaining the first intron, as well as an unspliced 3.3-kb precursor RNA. The Wx^{b} gene variant carries a single nucleotide substitution (a G-to-T single-base change) within this splice site, resulting in a sequence change from AGGTATA to AGTTATA [105, 108, 115, 119, 120].

Mutations affecting the *Wx* locus can reduce the efficiency of pre-mRNA processing or alter GBSSI activity, thereby limiting amylose synthesis. In tropical glutinous rice, a direct 23-bp duplication has been identified in the coding region of the *waxy* allele [109]. This alteration severely impairs GBSSI function, leading to very low amylose accumulation and the characteristic opaque endosperm and sticky texture of cooked rice [109, 120].

Studies have identified ancestral alleles, such as Wx^{lv}, which modulate grain flavor by influencing the amylose chain length. The Wx^{lv} allele is characteristic of most wild rice varieties. Unmutated forms of the Wx^{lv} and Wx^{a} alleles provide starch amylose contents above 25%, but their effects on starch suspension viscosity may differ [113].

It was found that all studied cultivars exhibiting medium amylose levels harbored a genetic variant with cytosine in the SNPs of exons 6 and 10, while varieties with high amylose content were typically characterized by the presence of adenine in the SNP of exon 6 alongside either cytosine or thymine at the exon 10 SNP locus [116].

The C/T SNP (Pro415Ser) at the Ex10-115 locus in exon 10 significantly influences the synthesis of very long amylopectin chains in the endosperm of non-glutinous rice [77].

The Wx^{in} and $Wx^{op/hp}$ alleles are characterized by functional polymorphisms at Ex6-62 and Ex4-77. The A/C substitution (Tyr224Ser) at Ex6-62 is associated with intermediate amylose levels, whereas the A/G substitution (Asp166Gly) at Ex4-77 is associated with cloudy endosperm and very low amylose content [111, 116, 121]. The Wx^{mp} allele, a derivative of Wx^{b}, contains a G-to-A single-base substitution (Arg158His) located within Ex4-53, resulting in reduced amylose accumulation [109].

The rare Wx^{mw} allele, derived from the naturally occurring Wx^{in} and Wx^{b} alleles, has received considerable attention because of its association with favorable amylose levels, improved cooking properties, and greater grain transparency [111]. A new *Wx* allele, named Wx^{la}, which combines Wx^{b} and Wx^{in}, has been discovered [122]. The Wx^{b} and $Wx^{mw/la}$ alleles in rice are associated with low amylose content (14-15%), while the Wx^{1-1}, Wx^{mp}, Wx^{mq}, and $Wx^{op/hp}$ alleles cause very low amylose content (8-12%) [77].

With the emergence of novel genetic alleles, a relationship was demonstrated between variation in amylose content and polymorphism at the *Wx* locus [113,114]. An updated hierarchy was published in descending order: Wx^{lv} (>25%), Wx^{a} (20-25%), Wx^{in} (18-22%), Wx^{b} (15-18%), Wx^{mw} (10-14%), Wx^{mq} (8-12%), Wx^{op} (5-10%), and *wx*, with these alleles contributing to differences in rice texture, adhesiveness, and cooking quality [110-113, 123, 124]. Collectively, the known *Wx* alleles contribute substantially to variation in amylose concentration, underscoring their importance in rice breeding and genetic research [124].

Thus, variations in the Wx locus affect not only gene expression but also post-transcriptional processes, including pre-mRNA splicing and mRNA stability, ultimately determining the amount of GBSSI synthesized and the resulting amylose content.

These differences in amylose accumulation have important consequences for the nutritional and metabolic properties of rice grain. High-amylose genotypes, primarily associated with the unmutated Wx^{lv} and Wx^{a} alleles, contain a greater proportion of linear amylose chains that undergo retrogradation during cooling, promoting the formation of enzyme-resistant structures classified as Resistant Starch type 3 (RS3) [125,126]. These structures reduce enzymatic accessibility to starch and slow glucose release during gastrointestinal digestion, thereby contributing to a lower glycemic response [127,128].

Conversely, in low-amylose (Wx^{b}, Wx^{mw}, Wx^{mq}) and glutinous (*wx*) varieties, the restriction or complete loss of GBSSI activity leads to a predominantly amylopectin-rich, amorphous starch structure. Due to an abundance of exposed terminal branches, this starch matrix is highly susceptible to rapid amylase hydrolysis, leading to an elevated GI and minimal RS content [126,129], which is an important consideration in dietary management of diabetes [130]. Concurrently, this structural organization yields the characteristic soft and sticky texture after cooking [131].

Collectively, these findings have substantially advanced our comprehension of the molecular regulatory mechanisms governing rice grain traits and have established the foundation for modern precision breeding strategies. Consequently, contemporary rice breeding increasingly integrates GWAS approaches, CRISPR/Cas9-based genomic editing, and MAS to accelerate the generation of varieties with enhanced grain quality and enhanced nutritional value.

To achieve a stable low-GI and high-RS phenotype without compromising essential agronomic traits, modern rice breeding programs must transition from single-locus selection to an integrated precision framework. While conventional MAS and gene pyramiding are highly effective for tracking major loci like *Wx* and *ALK* (*SSIIa*) [132-135], expanding the genetic architecture of starch quality relies on GWAS and haplotype mining to identify novel, superior allele combinations across diverse germplasm [136]. Precision genome editing via CRISPR/Cas9 directly complements these discovery platforms by enabling targeted, transgene-free modifications of key branching enzymes. For instance, targeted knockout of SBEIIb, especially when stacked with specific starch synthase mutations, significantly shifts starch architecture toward a high-amylose and high-resistant-starch profile [137-141]. Furthermore, fine-tuning *Wx* expression through cis-regulatory promoter editing offers a powerful strategy to systematically modulate amylose content while mitigating the pleiotropic tradeoffs such as reduced grain weight and increased chalkiness commonly observed in traditional high-RS genotypes [142-144].

Conclusion

Rice grain quality is governed by the synchronized control of starch synthesis, in which polymorphism within the *Wx* gene and its interaction with other starch biosynthetic genes determine amylose accumulation, starch structure, and cooking quality. Advances in molecular genetics, functional genomics, and precision breeding have substantially improved our insights into these regulatory pathways, facilitating the creation of more efficient breeding strategies. The integration of functional markers, GWAS approaches, genomic prediction, and CRISPR/Cas9 genome editing provides unprecedented opportunities to optimize amylose content, resistant starch accumulation, and glycemic properties while maintaining agronomic performance. However, challenges remain in translating genomic discoveries into stable breeding outcomes across diverse environments and in balancing grain quality with yield stability. Prospective investigations must prioritize integrating multi-omics approaches, genomic prediction, and precision genome engineering to identify robust gene networks and develop rice cultivars that simultaneously satisfy consumer preferences, nutritional demands, and climate resilience.

Acknowledgment

Thank you to the publisher for their support in the publication of this research article. We are grateful for the resources and platform provided by the publisher, which have enabled us to share our findings with a wider audience. We appreciate the efforts of the editorial team in reviewing and editing our work, and we are thankful for the opportunity to contribute to the field of research through this publication.

Funding Information

This research was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan Grant AP 26104353 "Development of a rice line with increased amylose content using marker-associated selection (MAS)".

Author’s Contributions

Innabat Sartbayeva: Drafting the article.
Aigul Amirova: Conceptualization, formal analysis.
Aliya Meldebekova and Saule Atabayeva: Formal analysis, resources.
Taira Kurbangaliyeva: Formal analysis, resources, edited.
Balnur Nurlybay, Dana Mynbaeva and Nurlan Usenbekov: Resources.
Bakdaulet Usenbekov: Supervision, reviewed and approved the final manuscript, funding.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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