ONLINE JOURNAL OF BIOLOGICAL SCIENCES

LITERATURE REVIEW

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

Innabat Sartbayeva[1], Aigul Kuzembayevna Amirova*[2], Dana Mynbaeva[1], Aliya Meldebekova[2], Taira Kurbangaliyeva[1], Saule Atabayeva[2], Balnur Nurlybay[1], Nurlan Usenbekov[1], Bakdaulet Usenbekov*[1,2]

[1]Institute of Plant Biology and Biotechnology, 45 Timiryazev str., Almaty 050040, Kazakhstan
[2]Department of Molecular Biology and Genetics, Al-Farabi Kazakh National University, 71 Al-Farabi ave., Almaty 050040, Kazakhstan

*Corresponding Author: aigul_amir@mail.ru; bakdaulet7@yandex.kz

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].

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].

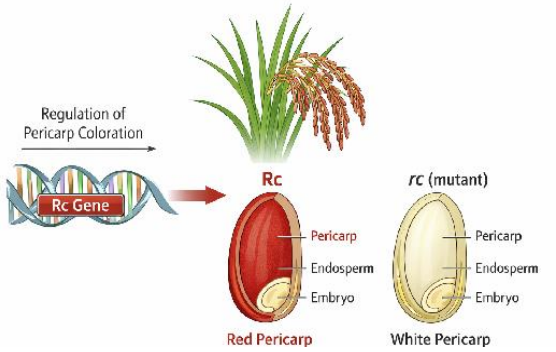

Fig. 1: Rc gene and rice pigmentation [35]

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].

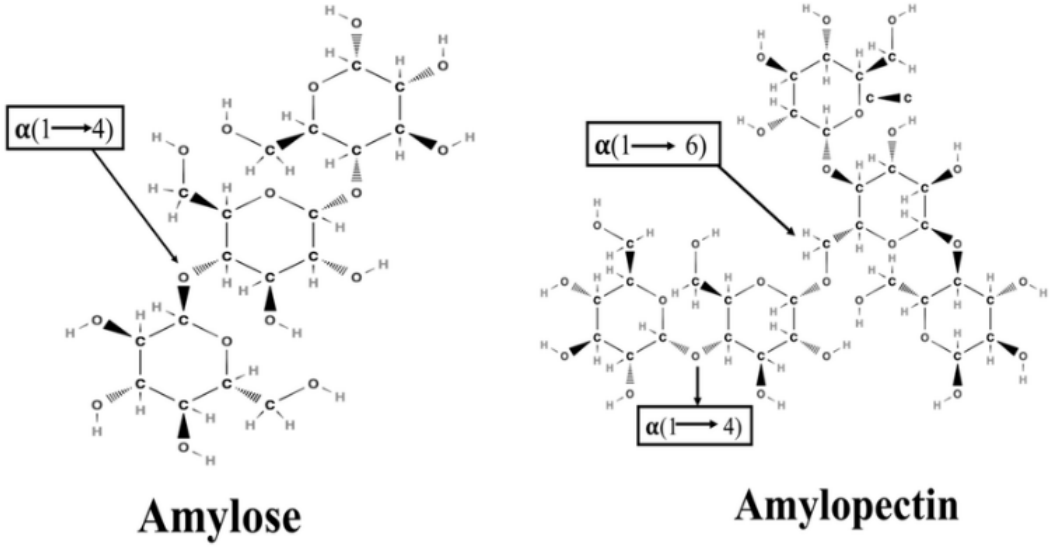

Fig. 2: Structural differences between amylose and amylopectin polymers [42]

DOI: 10.3844/ojbsci.2026.26.03.075

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].

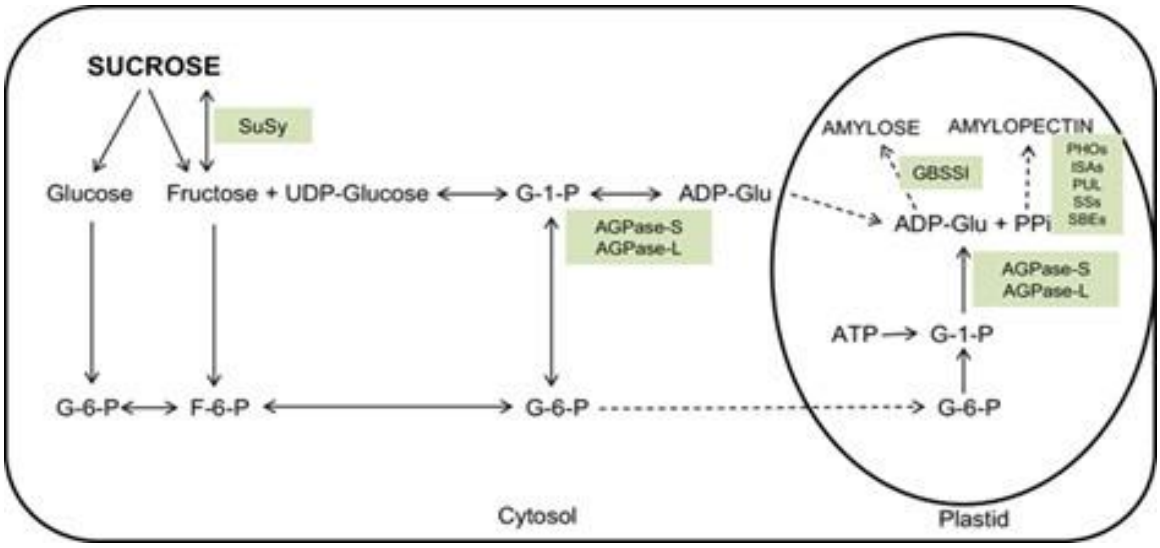

Fig. 3: Metabolic scheme of ADP-glucose formation and starch synthesis [57]

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.

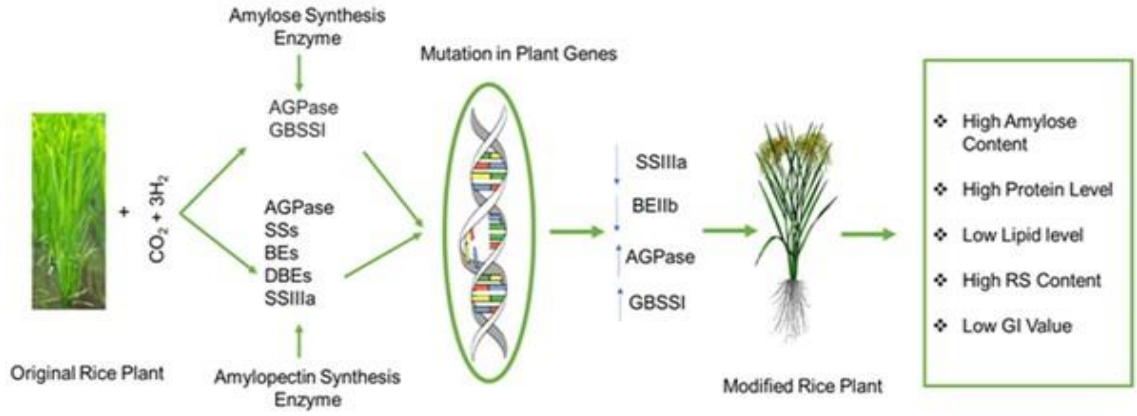

Fig. 4: Effects of reduced SSIIIa and BEIIb activities on starch structure and GI in rice [70]

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.

References

1. Khush, G. S. (2023). Strategies for increasing the yield potential of cereals: Rice as a case study. *Nature Reviews Genetics*, *14*, 535–548.
2. Muthayya, S., Sugimoto, J. D., Montgomery, S., & Maberly, G. F. (2014). An overview of global rice production, supply, trade, and consumption. *Annals of the New York Academy of Sciences*, *1324*(1), 7–14. https://doi.org/10.1111/nyas.12540
3. Li, Q., Deng, F., Zeng, Y., Li, B., He, C., Zhu, Y., Zhou, X., Zhang, Z., Wang, L., Tao, Y., Zhang, Y., Zhou, W., Cheng, H., Chen, Y., Lei, X., & Ren, W. (2022). Low Light Stress Increases Chalkiness by Disturbing Starch Synthesis and Grain Filling of Rice. *International Journal of Molecular Sciences*, *23*(16), 9153. https://doi.org/10.3390/ijms23169153
4. Nawade, B., Shim, S.-H., Chu, S.-H., Zhao, W., Lee, S.-K., Somsri, A., Maung, T. Z., Kang, K. K., Kim, J. Y., Lee, C.-Y., Kim, M.-S., Baik, M.-Y., Jeon, J.-S., & Park, Y.-J. (2025). Integrative transcriptogenomic analyses reveal the regulatory network underlying rice eating and cooking quality and identify a role for alpha-globulin in modulating starch and sucrose metabolism. *Plant Communications*, *6*(5), 101287. https://doi.org/10.1016/j.xplc.2025.101287

5. Bao, J. S. (2012). Toward Understanding the Genetic and Molecular Bases of the Eating and Cooking Qualities of Rice. *Cereal Foods World*, *57*(4), 148–156. https://doi.org/10.1094/cfw-57-4-0148
6. Juliano, B. O. (2003). RICE. *Encyclopedia of Food Sciences and Nutrition*, 4995–5001. https://doi.org/10.1016/b0-12-227055-x/01025-7
7. Wang, L., Gong, Y., Li, Y., & Tian, Y. (2020). Structure and properties of soft rice starch. *International Journal of Biological Macromolecules*, *157*, 10–16. https://doi.org/10.1016/j.ijbiomac.2020.04.138
8. Bao, J., Kong, X., Xie, J., & Xu, L. (2004). Analysis of Genotypic and Environmental Effects on Rice Starch. 1. Apparent Amylose Content, Pasting Viscosity, and Gel Texture. In *Journal of Agricultural and Food Chemistry* (Vol. 52, Issue 19, pp. 6010–6016). https://doi.org/10.1021/jf049234i
9. Shewry, P. R., & Halford, N. G. (2002). Cereal seed storage proteins: structures, properties and role in grain utilization. In *Journal of Experimental Botany* (Vol. 53, Issue 370, pp. 947–958). https://doi.org/10.1093/jexbot/53.370.947
10. Nakamura, Y., Francisco, P. B., Hosaka, Y., Sato, A., Sawada, T., Kubo, A., & Fujita, N. (2005). Essential amino acids of starch synthase IIa differentiate amylopectin structure and starch quality between japonica and indica rice varieties. In *Plant Molecular Biology* (Vol. 58, Issue 2, pp. 213–227). https://doi.org/10.1007/s11103-005-6507-2
11. Zhong, H., Liu, S., Zhao, G., Zhang, C., Peng, Z., Wang, Z., Yang, J., & Li, Y. (2021). Genetic Diversity Relationship Between Grain Quality and Appearance in Rice. In *Frontiers in Plant Science* (Vol. 12, p. 708996). https://doi.org/10.3389/fpls.2021.708996
12. Yeilaghi, H., Fatima, I., Qazi, M., Qin, B., Bian, Z., Wong, M., Yu, L., Zhang, C., Arzani, A., Zhang, L., & Liu, Q. (2025). Amylose variation reveals a novel null wx allele in Malaysian rice. *Seed Biology*, *4*(1), e025. https://doi.org/10.48130/seedbio-0025-0023
13. Phing, W. C. L., Latif, M. A., Rafii, M. Y., Ismail, M. R., & Puteh, A. (2016). Advances to improve the eating and cooking qualities of rice by marker-assisted breeding. *Critical Reviews in Biotechnology*, *36*(1), 87–98. https://doi.org/10.3109/07388551.2014.923987
14. Yang, Q., Dong, F., Zhu, W., & Zhao, D. (2025). A distal regulatory element regulates Wx gene expression and the amylose content in rice. *Frontiers in Plant Science*, *16*, 1694305. https://doi.org/10.3389/fpls.2025.1694305
15. Fitzgerald, M. A., McCouch, S. R., & Hall, R. D. (2009). Not just a grain of rice: the quest for quality. *Trends in Plant Science*, *14*(3), 133–139. https://doi.org/10.1016/j.tplants.2008.12.004
16. Bao, J., & Bergman, C. J. (2018). Rice Flour and Starch Functionality. *Starch in Food: Structure, Function and Applications*, 373–419. https://doi.org/10.1016/b978-0-08-100868-3.00010-x
17. Wang, K., Wambugu, P. W., Zhang, B., Wu, A. C., Henry, R. J., & Gilbert, R. G. (2015). The biosynthesis, structure and gelatinization properties of starches from wild and cultivated African rice species (Oryza barthii and Oryza glaberrima). *Carbohydrate Polymers*, *129*, 92–100.
18. Lau, W. C. P., Rafii, M. Y., Ismail, M. R., Puteh, A., Latif, M. A., & Ramli, A. (2015). Review of functional markers for improving cooking, eating, and the nutritional qualities of rice. *Frontiers in Plant Science*, *6*, 00832. https://doi.org/10.3389/fpls.2015.00832
19. Rabiei, B., Valizadeh, M., Ghareyazie, B., & Moghaddam, M. (2004). Evaluation of selection indices for improving rice grain shape. *Field Crops Research*, *89*(2–3), 359–367. https://doi.org/10.1016/j.fcr.2004.02.016
20. Ferrero, N. N. (2004). Constraints and opportunities for the sustainable development of rice-based production systems in Europe. *FAO Rice Conference*, 12–13.
21. Sadhu, S., & Kole, P. C. (2024). Revolutionizing Rice Grain Quality: A Holistic Review Integrating Conventional and Molecular Approaches. *International Journal of Bio-Resource and Stress Management*, *15*(8), 01–12. https://doi.org/10.23910/1.2024.5489
22. Fujita, N., Miura, S., & Crofts, N. (2022). Effects of Various Allelic Combinations of Starch Biosynthetic Genes on the Properties of Endosperm Starch in Rice. *Rice*, *15*(1), 24. https://doi.org/10.1186/s12284-022-00570-8
23. Tripathy, K., Singh, B., Singh, N., Rai, V., Misra, G., & Singh, N. K. (2018). A database of wild rice germplasm of Oryza rufipogon species complex from different agro-climatic zones of India. *Database*, *2018*, bay058. https://doi.org/10.1093/database/bay058
24. Khush, G. S. (1997). Origin, dispersal, cultivation and variation of rice. *Plant Molecular Biology*, *35*(1–2), 25–34. https://doi.org/10.1023/a:1005810616885
25. Linares, O. F. (2002). African Rice (Oryza glaberrima): History and Future Potential. *Proceedings of the National Academy of Sciences*, *99*(25), 16360–16365. https://doi.org/10.1073/pnas.252604599
26. Ashfaq, M., Khan, A. S., Khan, S. H. U., & Ahmad, R. (2012). Association of Various Morphological Traits with Yield and Genetic Divergence in Rice (Oryza sativa. *International Journal of Agriculture & Biology*, *14*(1), 55–62.
27. Chang, T. T. (2003). Origin, Domestication, and Diversification, Rice: Origin, History, Technology, and Production. *Rice: Origin, History, Technology, and Production*, 3–25.
28. Huang, L., Tan, H., Zhang, C., Li, Q., & Liu, Q. (2021). Starch biosynthesis in cereal endosperms: An updated review over the last decade. *Plant Communications*, *2*(5), 100237. https://doi.org/10.1016/j.xplc.2021.100237
29. Hasan, S., Furtado, A., & Henry, R. (2022). Reticulate Evolution in AA-Genome Wild Rice in Australia. *Frontiers in Plant Science*, *13*, 767635. https://doi.org/10.3389/fpls.2022.767635
30. Vaughan, D. A., Lu, B.-R., & Tomooka, N. (2008). The evolving story of rice evolution. *Plant Science*, *174*(4), 394–408. https://doi.org/10.1016/j.plantsci.2008.01.016
31. Fornasiero, A., Wing, R. A., & Ronald, P. (2022). Rice domestication. *Current Biology*, *32*(1), R20–R24. https://doi.org/10.1016/j.cub.2021.11.025
32. Li, C., Zhou, A., & Sang, T. (2006). Rice Domestication by Reducing Shattering. *Science*, *311*(5769), 1936–1939. https://doi.org/10.1126/science.1123604
33. Tan, L., Li, X., Liu, F., Sun, X., Li, C., Zhu, Z., Fu, Y., Cai, H., Wang, X., Xie, D., & Sun, C. (2008). Control of a key transition from prostrate to erect growth in rice domestication. *Nature Genetics*, *40*(11), 1360–1364. https://doi.org/10.1038/ng.197
34. Hua, L., Wang, D. R., Tan, L., Fu, Y., Liu, F., Xiao, L., Zhu, Z., Fu, Q., Sun, X., Gu, P., Cai, H., McCouch, S. R., & Sun, C. (2015). LABA1, a Domestication Gene Associated with Long, Barbed Awns in Wild Rice. *The Plant Cell*, *27*(7), 1875–1888. https://doi.org/10.1105/tpc.15.00260

35. Sweeney, M., Thomson, M. J., Cho, Y.-G., Park, Y.-J., Williamson, S., Bustamante, C. D., & McCouch, S. R. (2005). Global dissemination of a single mutation conferring white pericarp in rice. *PLoS Genetics*, *preprint*(2007), e133. https://doi.org/10.1371/journal.pgen.0030133.eor
36. Fujino, K., Wu, J., Sekiguchi, H., Ito, T., Izawa, T., & Matsumoto, T. (2010). Multiple introgression events surrounding the Hd1 flowering-time gene in cultivated rice, Oryza sativa L. *Molecular Genetics and Genomics*, *284*(2), 137–146. https://doi.org/10.1007/s00438-010-0555-2
37. Oikawa, T., Maeda, H., Oguchi, T., Yamaguchi, T., Tanabe, N., Ebana, K., Yano, M., Ebitani, T., & Izawa, T. (2015). The Birth of a Black Rice Gene and Its Local Spread by Introgression. *The Plant Cell*, *27*(9), 2401–2414. https://doi.org/10.1105/tpc.15.00310
38. Si, L., Chen, J., Huang, X., Gong, H., Luo, J., Hou, Q., Zhou, T., Lu, T., Zhu, J., Shangguan, Y., Chen, E., Gong, C., Zhao, Q., Jing, Y., Zhao, Y., Li, Y., Cui, L., Fan, D., Lu, Y., … Han, B. (2016). OsSPL13 controls grain size in cultivated rice. *Nature Genetics*, *48*(4), 447–456. https://doi.org/10.1038/ng.3518
39. Xu, H., Xu, S., Xu, Y., Jiang, Y., Li, T., Zhang, X., Yang, J., & Wang, L. (2024). Relationship between the physicochemical properties and amylose content of rice starch in rice varieties with the same genetic background. *Journal of Cereal Science*, *118*, 103932. https://doi.org/10.1016/j.jcs.2024.103932
40. Yu, H., & Wang, T. (2016). Proteomic Dissection of Endosperm Starch Granule Associated Proteins Reveals a Network Coordinating Starch Biosynthesis and Amino Acid Metabolism and Glycolysis in Rice Endosperms. *Frontiers in Plant Science*, *7*, 00707. https://doi.org/10.3389/fpls.2016.00707
41. Ferreira, S. J., Senning, M., Fischer-Stettler, M., Streb, S., Ast, M., Neuhaus, H. E., Zeeman, S. C., Sonnewald, S., & Sonnewald, U. (2017). Simultaneous silencing of isoamylases ISA1, ISA2 and ISA3 by multi-target RNAi in potato tubers leads to decreased starch content and an early sprouting phenotype. *PLOS ONE*, *12*(7), e0181444. https://doi.org/10.1371/journal.pone.0181444
42. Maji, B. (2019). Introduction to natural polysaccharides. *Functional Polysaccharides for Biomedical Applications*, 1–31. https://doi.org/10.1016/b978-0-08-102555-0.00001-7
43. Zhou, H., Xia, D., Zhao, D., Li, Y., Li, P., Wu, B., Gao, G., Zhang, Q., Wang, G., Xiao, J., Li, X., Yu, S., Lian, X., & He, Y. (2021). The origin of Wxla provides new insights into the improvement of grain quality in rice. *Journal of Integrative Plant Biology*, *63*(5), 878–888. https://doi.org/10.1111/jipb.13011
44. Khatami, M. H., Barber, W., & Haan, H. W. de. (2021). Using geometric criteria to study helix-like structures produced in molecular dynamics simulations of single amylose chains in water. *RSC Advances*, *11*(20), 11992–12002. https://doi.org/10.1039/d1ra00071c
45. Varghese, S., Awana, M., Mondal, D., Rubiya, M. H., Melethil, K., Singh, A., Krishnan, V., & Thomas, B. (2023). Amylose–Amylopectin Ratio. *Handbook of Biopolymers*, 1305–1334. https://doi.org/10.1007/978-981-19-0710-4_48
46. Tian, Y., Liu, X., Kirkensgaard, J. J. K., Khakimov, B., Enemark-Rasmussen, K., Hebelstrup, K. H., Blennow, A., & Zhong, Y. (2024). Characterization of different high amylose starch granules. Part I: Multi-scale structures and relationships to thermal properties. *Food Hydrocolloids*, *146*, 109286. https://doi.org/10.1016/j.foodhyd.2023.109286
47. Fernandes, J.-M., Madalena, D. A., Pinheiro, A. C., & Vicente, A. A. (2020). Rice in vitro digestion: application of INFOGEST harmonized protocol for glycemic index determination and starch morphological study. *Journal of Food Science and Technology*, *57*(4), 1393–1404. https://doi.org/10.1007/s13197-019-04174-x
48. Trinh, K. S. (2015). Recrystallization of starches by hydrothermal treatment: digestibility, structural, and physicochemical properties. *Journal of Food Science and Technology*, *52*(12), 7640–7654. https://doi.org/10.1007/s13197-015-1945-6
49. Huang, M., Hu, L., Chen, J., & Cao, F. (2022). In vitro testing indicates an accelerated rate of digestion of starch into glucose of cooked rice with the development of low amylose rice in China. *Food Chemistry: X*, *14*, 100278. https://doi.org/10.1016/j.fochx.2022.100278
50. Gu, X., Wang, P., Huang, J., Chen, S., Li, D., Pu, S., Li, J., & Wen, J. (2024). Structural and physicochemical properties of rice starch from a variety with high resistant starch and low amylose content. *Frontiers in Nutrition*, *11*, 1413923. https://doi.org/10.3389/fnut.2024.1413923
51. Shao, Y., Peng, Y., Mao, B., Lv, Q., Yuan, D., Liu, X., & 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
52. James, M. G., Denyer, K., & Myers, A. M. (2003). Starch synthesis in the cereal endosperm. *Current Opinion in Plant Biology*, *6*(3), 215–222. https://doi.org/10.1016/s1369-5266(03)00042-6
53. Cai, T., Sun, H., Qiao, J., Zhu, L., Zhang, F., Zhang, J., Tang, Z., Wei, X., Yang, J., Yuan, Q., Wang, W., Yang, X., Chu, H., Wang, Q., You, C., Ma, H., Sun, Y., Li, Y., Li, C., … Ma, Y. (2021). Cell-free chemoenzymatic starch synthesis from carbon dioxide. *Science*, *373*(6562), 1523–1527. https://doi.org/10.1126/science.abh4049
54. Li, C., Luo, J.-X., Zhang, C.-Q., & Yu, W.-W. (2020). Causal relations among starch chain-length distributions, short-term retrogradation and cooked rice texture. *Food Hydrocolloids*, *108*, 106064. https://doi.org/10.1016/j.foodhyd.2020.106064
55. Tuncel, A., Kawaguchi, J., Ihara, Y., Matsusaka, H., Nishi, A., Nakamura, T., Kuhara, S., Hirakawa, H., Nakamura, Y., Cakir, B., Nagamine, A., Okita, T. W., Hwang, S.-K., & Satoh, H. (2014). The Rice Endosperm ADP-Glucose Pyrophosphorylase Large Subunit is Essential for Optimal Catalysis and Allosteric Regulation of the Heterotetrameric Enzyme. *Plant and Cell Physiology*, *55*(6), 1169–1183. https://doi.org/10.1093/pcp/pcu057
56. Ohdan, T., Francisco, P. B., Sawada, T., Hirose, T., Terao, T., Satoh, H., & Nakamura, Y. (2005). Expression profiling of genes involved in starch synthesis in sink and source organs of rice. *Journal of Experimental Botany*, *56*(422), 3229–3244. https://doi.org/10.1093/jxb/eri292
57. Thitisaksakul, M., Jiménez, R. C., Arias, M. C., & Beckles, D. M. (2012). Effects of environmental factors on cereal starch biosynthesis and composition. *Journal of Cereal Science*, *56*(1), 67–80. https://doi.org/10.1016/j.jcs.2012.04.002
58. Tetlow, I. J., & Bertoft, E. (2020). A Review of Starch Biosynthesis in Relation to the Building Block-Backbone Model. *International Journal of Molecular Sciences*, *21*(19), 7011. https://doi.org/10.3390/ijms21197011

59. Chang, H., Bai, J., Zhang, H., Huang, R., Chu, H., Wang, Q., Liu, H., Cheng, J., & Jiang, H. (2023). Origin and evolution of the main starch biosynthetic enzymes. *Synthetic and Systems Biotechnology*, *8*(3), 462–468. https://doi.org/10.1016/j.synbio.2023.05.006
60. Edwards, A., Marshall, J., Denyer, K., Sidebottom, C., Visser, R. G. F., Martin, C., & Smith, A. M. (1996). Evidence That a 77-Kilodalton Protein from the Starch of Pea Embryos Is an Isoform of Starch Synthase That Is Both Soluble and Granule Bound. *Plant Physiology*, *112*(1), 89–97. https://doi.org/10.1104/pp.112.1.89
61. Hylton, ChristopherM., Denyer, K., Keeling, PeterL., Chang, M.-T., & Smith, AlisonM. (1996). The effect of waxy mutations on the granule-bound starch synthases of barley and maize endosperms. *Planta*, *198*(2), 230–237. https://doi.org/10.1007/bf00206248
62. Wang, K., Hasjim, J., Wu, A. C., Li, E., Henry, R. J., & Gilbert, R. G. (2015). Roles of GBSSI and SSIIa in determining amylose fine structure. In *Carbohydrate Polymers* (Vol. 127, pp. 264–274). https://doi.org/10.1016/j.carbpol.2015.03.081
63. Maung, T. Z., Chu, S.-H., & Park, Y.-J. (2021). Functional Haplotypes and Evolutionary Insight into the Granule-Bound Starch Synthase II (GBSSII) Gene in Korean Rice Accessions (KRICE_CORE). In *Foods* (Vol. 10, Issue 10, p. 2359). https://doi.org/10.3390/foods10102359
64. Huang, J., Liu, F., Zhang, J., Tang, B., Deng, J., Shi, T., Zhu, L., Li, H., & Chen, Q. (2025). Identification of the Granule-Bound Starch Synthase (GBSS) Genes Involved in Amylose Biosynthesis in Tartary Buckwheat (Fagopyrum tataricum (L.) Gaertn.). In *Plants* (Vol. 14, Issue 2, p. 203). https://doi.org/10.3390/plants14020203
65. Hirose, T., & Terao, T. (2004). A comprehensive expression analysis of the starch synthase gene family in rice (Oryza sativa L.). In *Planta* (Vol. 220, Issue 1, pp. 9–16). https://doi.org/10.1007/s00425-004-1314-6
66. Pandey, M. K., Rani, N. S., Madhav, M. S., Sundaram, R. M., Varaprasad, G. S., Sivaranjani, A. K. P., Bohra, A., Kumar, G. R., & Kumar, A. (2012). Different isoforms of starch-synthesizing enzymes controlling amylose and amylopectin content in rice (Oryza sativa L.). In *Biotechnology Advances* (Vol. 30, Issue 6, pp. 1697–1706). https://doi.org/10.1016/j.biotechadv.2012.08.011
67. Zhang, H., Jang, S.-G., Lar, S. M., Lee, A.-R., Cao, F.-Y., Seo, J., & Kwon, S.-W. (2021). Genome-Wide Identification and Genetic Variations of the Starch Synthase Gene Family in Rice. *Plants*, *10*(6), 1154. https://doi.org/10.3390/plants10061154
68. Irshad, A., Guo, H., Rehman, S. U., Wang, X., Wang, C., Raza, A., Zhou, C., Li, Y., & Liu, L. (2021). Soluble Starch Synthase Enzymes in Cereals: An Updated Review. In *Agronomy* (Vol. 11, Issue 10, p. 1983). https://doi.org/10.3390/agronomy11101983
69. Talamphai, S., Arshad, K., Tan, X., Surasilp, T., Tasanasuwan, P., & Sinumporn, S. (2021). Genetic diversity analysis of starch synthesis related genes (SSRGs) in rice varieties from Thailand, Laos and Yunnan province of China. *Journal of Genetics And*, *14*(1), 18–24. https://doi.org/10.14456/gag.2021.3
70. Farooq, M. A., & Yu, J. (2025). *Starches in Rice: Effects of Rice Variety and Processing/Cooking Methods on Their Glycemic Index*. Foods. https://doi.org/10.3390/foods14122022
71. Zhou, H., Wang, L., Liu, G., Meng, X., Jing, Y., Shu, X., Kong, X., Sun, J., Yu, H., Smith, S. M., Wu, D., & Li, J. (2016). Critical roles of soluble starch synthase SSIIIa and granule-bound starch synthase Waxy in synthesizing resistant starch in rice. In *Proceedings of the National Academy of Sciences* (Vol. 113, Issue 45, pp. 12844–12849). https://doi.org/10.1073/pnas.1615104113
72. Tsuiki, K., Fujisawa, H., Itoh, A., Sato, M., & Fujita, N. (2016). Alterations of starch structure lead to increased resistant starch of steamed rice: Identification of high resistant starch rice lines. In *Journal of Cereal Science* (Vol. 68, pp. 88–92). https://doi.org/10.1016/j.jcs.2016.01.002
73. Zhao, X., Mei, T., & Cui, B. (2024). Lipids-modified starch: Advances in structural characteristic, physicochemical property, and application. In *Food Research International* (Vol. 197, p. 115146). https://doi.org/10.1016/j.foodres.2024.115146
74. Kaur, B., Ranawana, V., Teh, A., & Henry, C. J. (2015). The Glycemic Potential of White and Red Rice Affected by Oil Type and Time of Addition. In *Journal of Food Science* (Vol. 80, Issue 10, pp. H2316–H2321). https://doi.org/10.1111/1750-3841.13070
75. Farooq, A. M., Dhital, S., Li, C., Zhang, B., & Huang, Q. (2018). Effects of palm oil on structural and in vitro digestion properties of cooked rice starches. *International Journal of Biological Macromolecules*, *107*, 1080–1085. https://doi.org/10.1016/j.ijbiomac.2017.09.089
76. Badoni, S., Pasion-Uy, E. A., Kor, S., Kim, S.-R., Tiozon, R. N., Misra, G., Buenafe, R. J. Q., Labarga, L. M., Ramos-Castrosanto, A. R., Pratap, V., Slamet-Loedin, I., Steimker, J. von, Alseekh, S., Fernie, A. R., Kohli, A., Khush, G. S., & Sreenivasulu, N. (2024). Multiomics of a rice population identifies genes and genomic regions that bestow low glycemic index and high protein content. *Proceedings of the National Academy of Sciences*, *121*(36), e2410598121. https://doi.org/10.1073/pnas.2410598121
77. Crofts, N., Itoh, A., Abe, M., Miura, S., Oitome, N. F., Bao, J., & Fujita, N. (2019). Three Major Nucleotide Polymorphisms in the Waxy Gene Correlated with the Amounts of Extra-long Chains of Amylopectin in Rice Cultivars with S or L-type Amylopectin. *Journal of Applied Glycoscience*, *66*(1), 37–46. https://doi.org/10.5458/jag.jag.jag-2018_005
78. Chen, Z., Lu, Y., Feng, L., Hao, W., Li, C., Yang, Y., Fan, X., Li, Q., Zhang, C., & Liu, Q. (2020). Genetic Dissection and Functional Differentiation of ALKa and ALKb, Two Natural Alleles of the ALK/SSIIa Gene, Responding to Low Gelatinization Temperature in Rice. *Rice*, *13*(1), 39. https://doi.org/10.1186/s12284-020-00393-5
79. Crofts, N., Hareyama, K., Miura, S., Hosaka, Y., Oitome, N. F., & Fujita, N. (2022). Effect of Heading Date on the Starch Structure and Grain Yield of Rice Lines with Low Gelatinization Temperature. *International Journal of Molecular Sciences*, *23*(18), 10783. https://doi.org/10.3390/ijms231810783
80. Crofts, N., Abe, N., Oitome, N. F., Matsushima, R., Hayashi, M., Tetlow, I. J., Emes, M. J., Nakamura, Y., & Fujita, N. (2015). Amylopectin biosynthetic enzymes from developing rice seed form enzymatically active protein complexes. In *Journal of Experimental Botany* (Vol. 66, Issue 15, pp. 4469–4482). https://doi.org/10.1093/jxb/erv212
81. Umemoto, T., Horibata, T., Aoki, N., Hiratsuka, M., Yano, M., & Inouchi, N. (2008). Effects of Variations in Starch Synthase on Starch Properties and Eating Quality of Rice. In *Plant Production Science* (Vol. 11, Issue 4, pp. 472–480). https://doi.org/10.1626/pps.11.472
82. Okamoto, K., Kobayashi, K., Hirasawa, H., & Umemoto, T. (2002). Structural Differences in Amylopectin Affect Waxy Rice Processing. In *Plant Production Science* (Vol. 5, Issue 1, pp. 45–50). https://doi.org/10.1626/pps.5.45

83. Sartbayeva, I., Usenbekov, B., Rysbekova, A., Kazkeyev, D., Zhanbyrbayev, Y., Batayeva, D., Berkimbay, K., Zhambakin, K., Matsuba, S., & Umemoto, T. (2017). Haplotype Analysis of Wx and Alk Genes and Amylopectin Chain-Length Distribution among Kazakhstan Glutinous Rice Lines. In *Japan Agricultural Research Quarterly: JARQ* (Vol. 51, Issue 2, pp. 147–153). https://doi.org/10.6090/jarq.51.147
84. Nishi, A., Nakamura, Y., Tanaka, N., & Satoh, H. (2001). Biochemical and Genetic Analysis of the Effects ofAmylose-Extender Mutation in Rice Endosperm Get access Arrow. In *Plant Physiology* (Vol. 127, Issue 2, pp. 459–472). https://doi.org/10.1104/pp.010127
85. Fujita, N., Yoshida, M., Kondo, T., Saito, K., Utsumi, Y., Tokunaga, T., Nishi, A., Satoh, H., Park, J.-H., Jane, J.-L., Miyao, A., Hirochika, H., & Nakamura, Y. (2007). Characterization of SSIIIa-Deficient Mutants of Rice: The Function of SSIIIa and Pleiotropic Effects by SSIIIa Deficiency in the Rice Endosperm. In *Plant Physiology* (Vol. 144, Issue 4, pp. 2009–2023). https://doi.org/10.1104/pp.107.102533
86. Satoh, H., Shibahara, K., Tokunaga, T., Nishi, A., Tasaki, M., Hwang, S.-K., Okita, T. W., Kaneko, N., Fujita, N., Yoshida, M., Hosaka, Y., Sato, A., Utsumi, Y., Ohdan, T., & Nakamura, Y. (2008). Mutation of the Plastidial α-Glucan Phosphorylase Gene in Rice Affects the Synthesis and Structure of Starch in the Endosperm. *The Plant Cell*, *20*(7), 1833–1849. https://doi.org/10.1105/tpc.107.054007
87. Umemoto, T., Terashima, K., Nakamura, Y., & Satoh, H. (1999). Differences in Amylopectin Structure Between Two Rice Varieties in Relation to the Effects of Temperature During Grain-Filling. *Starch - Stärke*, *51*(2–3), 58–62. https://doi.org/10.1002/(sici)1521-379x(199903)51:2<58::aid-star58>3.0.co;2-j
88. Inouchi, N., Ando, H., Asaoka, M., Okuno, K., & Fuwa, H. (2000). The Effect of Environmental Temperature on Distribution of Unit Chains of Rice Amylopectin. *Starch - Stärke*, *52*(1), 8–12. https://doi.org/10.1002/(sici)1521-379x(200001)52:1<8::aid-star8>3.0.co;2-q
89. Gao, Z., Zeng, D., Cui, X., Zhou, Y., Yan, M., Huang, D., Li, J., & Qian, Q. (2003). Map-based cloning of the ALK gene, which controls the gelatinization temperature of rice. *Science in China Series C*, *46*(6), 661–668.
90. Gao, Z., Zeng, D., Cheng, F., Tian, Z., Guo, L., Su, Y., Yan, M., Jiang, H., Dong, G., Huang, Y., Han, B., Li, J., & Qian, Q. (2011). ALK, The Key Gene for Gelatinization Temperature, is a Modifier Gene for Gel Consistency in Rice (Oryza sativa L.). *Journal of Integrative Plant Biology*, *53*(9), no-no. https://doi.org/10.1111/j.1744-7909.2011.01065.x
91. Gong, D., Zhang, X., He, F., Chen, Y., Li, R., Yao, J., Zhang, M., Zheng, W., & Yu, G. (2023). Genetic Improvements in Rice Grain Quality: A Review of Elite Genes and Their Applications in Molecular Breeding. *Agronomy*, *13*(5), 1375. https://doi.org/10.3390/agronomy13051375
92. Nakamura, Y., Takeichi, T., Kawaguchi, K., & Yamanouchi, H. (1992). *Purification of two forms of starch branching enzyme (Q-enzyme) from developing rice endosperm*. Physiologia Plantarum. https://doi.org/10.1034/j.1399-3054.1992.840302.x
93. Mizuno, K., Kawasaki, T., Shimada, H., Satoh, H., Kobayashi, E., Okumura, S., Arai, Y., & Baba, T. (1993). *Alteration of the structural properties of starch components by the lack of an isoform of starch branching enzyme in rice seeds*. Journal of Biological Chemistry. https://doi.org/10.1016/s0021-9258(17)46738-x
94. Fujita, N., Toyosawa, Y., Utsumi, Y., Higuchi, T., Hanashiro, I., Ikegami, A., Akuzawa, S., Yoshida, M., Mori, A., Inomata, K., Itoh, R., Miyao, A., Hirochika, H., Satoh, H., & Nakamura, Y. (2009). Characterization of pullulanase (PUL)-deficient mutants of rice (Oryza sativa L.) and the function of PUL on starch biosynthesis in the developing rice endosperm. In *Journal of Experimental Botany* (Vol. 60, Issue 3, pp. 1009–1023). https://doi.org/10.1093/jxb/ern349
95. Utsumi, Y., Utsumi, C., Sawada, T., Fujita, N., & Nakamura, Y. (2011). Functional Diversity of Isoamylase Oligomers: The ISA1 Homo-Oligomer Is Essential for Amylopectin Biosynthesis in Rice Endosperm . In *Plant Physiology* (Vol. 156, Issue 1, pp. 61–77). https://doi.org/10.1104/pp.111.173435
96. Yamanaka, S., Nakamura, I., Watanabe, K. N., & Sato, Y.-I. (2004). Identification of SNPs in the waxy gene among glutinous rice cultivars and their evolutionary significance during the domestication process of rice. In *Theoretical and Applied Genetics* (Vol. 108, Issue 7, pp. 1200–1204). https://doi.org/10.1007/s00122-003-1564-x
97. Qu, J., Xu, S., Zhang, Z., Chen, G., Zhong, Y., Liu, L., Zhang, R., Xue, J., & Guo, D. (2018). Evolutionary, structural and expression analysis of core genes involved in starch synthesis. In *Scientific Reports* (Vol. 8, Issue 1, p. 12736). https://doi.org/10.1038/s41598-018-30411-y
98. Jeon, J.-S., Ryoo, N., Hahn, T.-R., Walia, H., & Nakamura, Y. (2010). Starch biosynthesis in cereal endosperm. In *Plant Physiology and Biochemistry* (Vol. 48, Issue 6, pp. 383–392). https://doi.org/10.1016/j.plaphy.2010.03.006
99. Xu, L., You, H., Zhang, O., & Xiang, X. (2020). Genetic Effects of Soluble Starch Synthase IV-2 and It with ADPglucose Pyrophorylase Large Unit and Pullulanase on Rice Qualities. In *Rice* (Vol. 13, Issue 1, p. 46). https://doi.org/10.1186/s12284-020-00409-0
100. Zhou, H., Wang, B., Liu, Y., Ouyang, L., Zhou, D., He, H., Liu, J., Hu, J., & He, X. (2022). Effects of Four Non-Synonymous SNPs of SSIIa Gene on Amylopectin Structure and Gelatinization Characteristics in Rice. In *Starch - Stärke* (Vol. 74, Issues 7–8, p. 2100198). https://doi.org/10.1002/star.202100198
101. Crofts, N., Satoh, Y., Miura, S., Hosaka, Y., Abe, M., & Fujita, N. (2022). Active-type starch synthase (SS) IIa from indica rice partially complements the sugary-1 phenotype in japonica rice endosperm. In *Plant Molecular Biology* (Vol. 108, Issues 4–5, pp. 325–342).https://doi.org/10.1007/s11103-021-01161-9
102. Crofts, N., Domon, A., Miura, S., Hosaka, Y., Oitome, N. F., Itoh, A., Noge, K., & Fujita, N. (2022). Starch synthases SSIIa and GBSSI control starch structure but do not determine starch granule morphology in the absence of SSIIIa and SSIVb. In *Plant Molecular Biology* (Vol. 108, Issues 4–5, pp. 379–398). https://doi.org/10.1007/s11103-021-01197-x
103. Hirano, H.-Y., & Sano, Y. (1991). *Molecular Characterization of the waxy Locus of Rice (Oryza sativa)*. Plant and Cell Physiology. https://doi.org/10.1093/oxfordjournals.pcp.a078186
104. Wang, Z., Wu, Z., Xing, Y., Zheng, F., Guo, X., Zhang, W., & Hong, M. (1990). *Nucleotide sequence of rice waxy gene*. Nucleic Acids Research. https://doi.org/10.1093/nar/18.19.5898
105. Wang, Z., Zheng, F., Shen, G., Gao, J., Snustad, D. P., Li, M., Zhang, J., & Hong, M. (1995). The amylose content in rice endosperm is related to the post-transcriptional regulation of the waxy gene. *The Plant Journal*, *7*(4), 613–622. https://doi.org/10.1046/j.1365-313x.1995.7040613.x

106. Umeda, M., Ohtsubo, H., & Ohtsubo, E. (1991). Diversification of the rice Waxy gene by insertion of mobile DNA elements into introns. *The Japanese Journal of Genetics*, *66*(5), 569–586. https://doi.org/10.1266/jjg.66.569
107. Zhang, C., Yang, Y., Chen, S., Liu, X., Zhu, J., Zhou, L., Lu, Y., Li, Q., Fan, X., Tang, S., Gu, M., & Liu, Q. (2021). A rare Waxy allele coordinately improves rice eating and cooking quality and grain transparency. *Journal of Integrative Plant Biology*, *63*(5), 889–901. https://doi.org/10.1111/jipb.13010
108. Sato, H., Suzuki, Y., Sakai, M., & Imbe, T. (2002). Molecular Characterization of Wx-mq, a Novel Mutant Gene for Low-amylose Content in Endosperm of Rice (Oryza sativa L.). *Breeding Science*, *52*(2), 131–135. https://doi.org/10.1270/jsbbs.52.131
109. Wanchana, S., Toojinda, T., Tragoonrung, S., & Vanavichit, A. (2003). Duplicated coding sequence in the waxy allele of tropical glutinous rice (Oryza sativa L.). In *Plant Science* (Vol. 165, Issue 6, pp. 1193–1199). https://doi.org/10.1016/s0168-9452(03)00326-1
110. Mikami, I., Uwatoko, N., Ikeda, Y., Yamaguchi, J., Hirano, H. Y., Suzuki, Y., & Sano, Y. (2008). Allelic diversification at the wx locus in landraces of Asian rice. In *Theoretical and Applied Genetics* (Vol. 116, Issue 7, pp. 979–989). https://doi.org/10.1007/s00122-008-0729-z
111. Liu, L., Ma, X., Liu, S., Zhu, C., Jiang, L., Wang, Y., Shen, Y., Ren, Y., Dong, H., Chen, L., Liu, X., Zhao, Z., Zhai, H., & Wan, J. (2009). Identification and characterization of a novel Waxy allele from a Yunnan rice landrace. In *Plant Molecular Biology* (Vol. 71, Issue 6, pp. 609–626). https://doi.org/10.1007/s11103-009-9544-4
112. Yang, J., Wang, J., Fan, F., Zhu, J., Chen, T., Wang, C., Zheng, T., Zhang, J., Zhong, W., & Xu, J. (2013). Development of AS-PCR marker based on a key mutation confirmed by resequencing of Wx-mp in Milky Princess and its application in japonica soft rice (Oryza sativa L.) breeding. In *Plant Breeding* (Vol. 132, Issue 6, pp. 595–603). https://doi.org/10.1111/pbr.12088
113. Zhang, C., Zhu, J., Chen, S., Fan, X., Li, Q., Lu, Y., Wang, M., Yu, H., Yi, C., Tang, S., Gu, M., & Liu, Q. (2019). Wxlv, the Ancestral Allele of Rice Waxy Gene. In *Molecular Plant* (Vol. 12, Issue 8, pp. 1157–1166). https://doi.org/10.1016/j.molp.2019.05.011
114. Biselli, C., Cavalluzzo, D., Perrini, R., Gianinetti, A., Bagnaresi, P., Urso, S., Orasen, G., Desiderio, F., Lupotto, E., Cattivelli, L., & Valè, G. (2014). Improvement of marker-based predictability of Apparent Amylose Content in japonica rice through GBSSI allele mining. In *Rice* (Vol. 7, Issue 1, p. 1). https://doi.org/10.1186/1939-8433-7-1
115. Cai, X., Wang, Z., Xing, Y., Zhang, J., & Hong, M. (1998). Aberrant splicing of intron 1 leads to the heterogeneous 5′ UTR and decreased expression of waxy gene in rice cultivars of intermediate amylose content. In *The Plant Journal* (Vol. 14, Issue 4, pp. 459–465). https://doi.org/10.1046/j.1365-313x.1998.00126.x
116. Larkin, P. D., & Park, W. D. (2003). Association of waxy gene single nucleotide polymorphisms with starch characteristics in rice (Oryza sativa L.). In *Molecular Breeding* (Vol. 12, Issue 4, pp. 335–339). https://doi.org/10.1023/b:molb.0000006797.51786.92
117. Chen, F., Song, Y., Jiang, Y., Cao, P., Yu, Y., Dong, M., Xie, Y., Yuan, C., Zhu, Y., & Qiao, Z. (2025). Research Progress on the Regulatory Mechanism of the Waxy Gene in Rice Starch Synthesis. *Current Issues in Molecular Biology*, *47*(9), 678. https://doi.org/10.3390/cimb47090678
118. Yaqoob, K., Galani, S., Ali, K., Kraehenbuehl, R., & Steele, K. A. (2025). Association of KASP with starch-trait diversity in modern rice grown in Pakistan. *Cereal Research Communications*, *53*(4), 2141–2158. https://doi.org/10.1007/s42976-025-00675-y
119. Bligh, H. F. J., Larkin, P. D., Roach, P. S., Jones, C. A., Fu, H., & Park, W. D. (1998). Use of alternate splice sites in granule-bound starch synthase mRNA from low-amylose rice varieties. *Plant Molecular Biology*, *38*(3), 407–415. https://doi.org/10.1023/a:1006021807799
120. Isshiki, M., Morino, K., Nakajima, M., Okagaki, R. J., Wessler, S. R., Izawa, T., & Shimamoto, K. (1998). A naturally occurring functional allele of the rice waxy locus has a GT to TT mutation at the 5′ splice site of the first intron. *The Plant Journal*, *15*(1), 133–138. https://doi.org/10.1046/j.1365-313x.1998.00189.x
121. Mikami, I., Aikawa, M., Hirano, H.-Y., & Sano, Y. (1999). Altered tissue-specific expression at the Wx gene of the opaque mutants in rice. *Euphytica*, *105*(2), 91–97. https://doi.org/10.1023/a:1003457209225
122. Zhou, L., Chen, S., Yang, G., Zha, W., Cai, H., Li, S., Chen, Z., Liu, K., Xu, H., & You, A. (2018). A perfect functional marker for the gene of intermediate amylose content Wx-in in rice (Oryza sativa L.). *Crop Breeding and Applied Biotechnology*, *18*(1), 103–109. https://doi.org/10.1590/1984-70332018v18n1a14
123. Suto, M., Ando, I., Numaguchi, K., & Nobuyuki. (1996). Horisue, Breeding of low amylose content paddy rice variety" milky queen" with good eating quality. *Jpn J Breed*, *46*(1), 221.
124. Di Marco, A. E., Ixtaina, V. Y., & Tomás, M. C. (2022). Analytical and technological aspects of amylose inclusion complexes for potential applications in functional foods. In *Food Bioscience* (Vol. 47, p. 101625). https://doi.org/10.1016/j.fbio.2022.101625
125. Li, H., Prakash, S., Nicholson, T. M., Fitzgerald, M. A., & Gilbert, R. G. (2016). The importance of amylose and amylopectin fine structure for textural properties of cooked rice grains. In *Food Chemistry* (Vol. 196, pp. 702–711). https://doi.org/10.1016/j.foodchem.2015.09.112
126. Kaur, B., Ranawana, V., & Henry, J. (2016). The Glycemic Index of Rice and Rice Products: A Review, and Table of GI Values. In *Critical Reviews in Food Science and Nutrition* (Vol. 56, Issue 2, pp. 215–236). https://doi.org/10.1080/10408398.2012.717976
127. Kumar, A., Sahoo, U., Baisakha, B., Okpani, O. A., Ngangkham, U., Parameswaran, C., Basak, N., Kumar, G., & Sharma, S. G. (2018). Resistant starch could be decisive in determining the glycemic index of rice cultivars. In *Journal of Cereal Science* (Vol. 79, pp. 348–353). https://doi.org/10.1016/j.jcs.2017.11.013
128. Harris, K. F. (2019). An introductory review of resistant starch type 2 from high-amylose cereal grains and its effect on glucose and insulin homeostasis. In *Nutrition Reviews* (Vol. 77, Issue 11, pp. 748–764). https://doi.org/10.1093/nutrit/nuz040
129. Hu, P., Zhao, H., Duan, Z., Linlin, Z., & Wu, D. (2004). *Starch digestibility and the estimated glycemic score of different types of rice differing in amylose contents*. Journal of Cereal Science. https://doi.org/10.1016/j.jcs.2004.06.001
130. Adegoke, T. V., Wang, Y., Chen, L., Wang, H., Liu, W., Liu, X., Cheng, Y.-C., Tong, X., Ying, J., & Zhang, J. (2021). Posttranslational Modification of Waxy to Genetically Improve Starch Quality in Rice Grain. In *International Journal of Molecular Sciences* (Vol. 22, Issue 9, p. 4845). https://doi.org/10.3390/ijms22094845

131. Patindol, J., Gu, X., & Wang, Y. (2010). *Chemometric analysis of cooked rice texture in relation to starch fine structure and leaching characteristics*. Starch - Stärke. https://doi.org/10.1002/star.200900181
132. Sharma, N., & Khanna, R. (2020). Rice Grain Quality: Current Developments and Future Prospects. *Recent Advances in Grain Crops Research*, 89367. https://doi.org/10.5772/intechopen.89367
133. Xu, Y. (2010). Developing marker-assisted selection strategies for breeding hybrid rice. *Plant Breeding Reviews*, *33*, 73–174.
134. S.Cho, K., Kongsil, P., Wangsawang, T., & Sreewongchai, T. (2020). Marker-assisted pseudo-backcross breeding for improvement of amylose content and aroma in Myanmar rice cultivar Sinthukha. *ScienceAsia*, *46*(5), 539. https://doi.org/10.2306/scienceasia1513-1874.2020.070
135. Sreewongchaı, T., Wangsawang, T., Wangsawang, S., Matthayatthaworn, W., Kumdee, O., & Cho, K. S. (2024). Improvement of High Amylose Content in Ch1 Rice Variety by Marker Assisted Pseudo-Backcross Breeding. *Turkish Journal Of Field Crops*, *29*(1),
136. Selvaraj, R., Singh, A. K., Singh, V. K., Abbai, R., Habde, S. V., Singh, U. M., & Kumar, A. (2021). Superior haplotypes towards development of low glycemic index rice with preferred grain and cooking quality. *Scientific Reports*, *11*(1), 10082. https://doi.org/10.1038/s41598-021-87964-8
137. Sun, Y., Jiao, G., Liu, Z., Zhang, X., Li, J., Guo, X., Du, W., Du, J., Francis, F., Zhao, Y., & Xia, L. (2017). Generation of High-Amylose Rice through CRISPR/Cas9-Mediated Targeted Mutagenesis of Starch Branching Enzymes. *Frontiers in Plant Science*, *8*, 00298. https://doi.org/10.3389/fpls.2017.00298
138. Tanaka, Y., Takahashi, K., Kato, J., Sawazaki, A., Akasaka, T., Fujita, N., Kumamaru, T., Saito, Y., Shirouchi, B., & Sato, M. (2018). Starch synthase IIIa and starch branching enzyme IIb-deficient mutant rice line ameliorates pancreatic insulin secretion in rats: screening and evaluating mutant rice lines with antidiabetic functionalities. *British Journal of Nutrition*, *119*(9), 970–980. https://doi.org/10.1017/s0007114518000314
139. Yan, J., Yu, J., Shen, H., Zhou, L., Chen, Z., Fan, X., Li, Q., Zhang, C., Liu, Q., Huang, L., & Liu, Q. (2025). *Creating a Superior Wx Allele with Temperature-Responsive Amylose Regulation and a Novel Transcriptional Pattern in Rice via CRISPR/Cas9-Mediated Promoter Editing*. Foods. https://doi.org/10.3390/foods14081330
140. Zhao, P., Liu, Y., Deng, Z., Liu, L., Yu, T., Ge, G., Chen, B., & Wang, T. (2024). *Creation of novel Wx allelic variations significantly altering Wx expression and rice eating and cooking quality*. Journal of Plant Physiology.
141. He, L., Xiufeng, L., Yang, X., Hualong, L., Mingliang, H., Xiaojie, T., Zhenyu, W., Xiuju, W., Qingyun, B., & Jie, Y. (2020). *High-Efficiency Reduction of Rice Amylose Content via CRISPR/Cas9-Mediated Base Editing*. Rice Science. https://doi.org/10.1016/j.rsci.2020.09.001
142. Yang, J., Guo, X., Wang, X., Fang, Y., Liu, F., Qin, B., & Li, R. (2022). *Development of Soft Rice Lines by Regulating Amylose Content via Editing the 5'UTR of the Wx Gene*. International Journal of Molecular Sciences. https://doi.org/10.3390/ijms231810517
143. Zhang, Q., Zhang, S., Yu, X., Wei, X., Huang, X., & Zhou, X. (2022). Fine-tuning grain amylose contents by genome editing of Waxy cis-regulatory region in rice. *Molecular Breeding*, *42*(12), 72. https://doi.org/10.1007/s11032-022-01342-4
144. Zhu, J., Wang, Z., Zhao, R., Li, W., Gan, T., Wan, J., Sun, H., Liu, Y., Wei, M., Xu, H., Luo, T., Hua, Y., Li, S., Fu, Y., & Li, P. (2025). Creation of TGMS Lines of Waxy Rice with Elite Physicochemical Properties of Starch via Waxy Gene Editing. *Foods*, *14*(20), 3530. https://doi.org/10.3390/foods14203530