

Toward Understanding the Genetic and Molecular Bases of the Eating and Cooking Qualities of Rice¹

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Rice (*Oryza sativa* L.) is a crucial cereal crop that feeds about half the world's population. New varieties with high yield potential, good quality, and high resistance to biotic and abiotic stresses are being bred and released continuously to meet the demand for more food arising from the rapid human population growth and a concurrent decrease in arable land. Improvement of rice quality has become one of the foremost considerations for rice buyers and breeding programs, especially its eating and cooking qualities because most rice is cooked and consumed directly.

Rice eating and cooking qualities are highly related to some easily measurable physicochemical properties: apparent amylose content (AAC), gel consistency, gelatinization temperature (GT), and pasting viscosity. All of these parameters reflect the starch functionality of the rice grain, but AAC is widely recognized as one of the most important determinants for various rice products (49). For cooked rice, grains with high AAC are dry, fluffy, separate, and hard, whereas those with low AAC are glossy, soft, and sticky. Rice eating qualities differ among varieties with similar AAC, however, which can be explained by differences in amylopectin structure (66,67,69). Thus, gel consistency, GT, and pasting viscosity can differentiate rice varieties with similar AAC, especially for waxy and high-amylose rice varieties (48).

Starch is composed of amylose and amylopectin and is synthesized under the



coordinated action of a wide array of enzymes (Fig. 1A). Amylose synthesis requires granule-bound starch synthase (GBSS), a product of the *Wx* gene, whereas starch branching enzymes, starch synthases, and starch debranching enzymes play major roles in the synthesis of amylopectin (45,64,76). Starch-synthesizing genes may contribute to variation in starch physicochemical properties because they affect the amount and structure of amylose and amylopectin in the rice grain. Genetic studies have shown that many starch physicochemical properties are controlled by the *Wx* and *SSIIa* genes (Fig. 1B). This article summarizes the current understanding of the genetic and molecular bases of the eating and cooking qualities of rice.

Apparent Amylose Content

AAC is one of the most important predictors of eating quality in rice. The AAC of milled rice may be classified as waxy (1–2%), very low (5–12%), low (12–20%), intermediate (20–25%), or high (>25%). Waxy rice is used in foods such as desserts and snacks. High-amylose cultivars are common among *indica* rice cultivars and are dry and fluffy when cooked, often becoming hard upon cooling. Low-amylose cultivars are soft and sticky and include nearly all temperate *japonica* rice cultivars. Intermediate-amylose rice is soft but not sticky and is preferred by most consumers.

Linkage mapping of the quantitative trait locus (QTL) for AAC in rice (1,5,10,30,35,51,52,71,74,87,89,97) has shown that it is largely controlled by the *Wx* locus on chromosome 6 (Fig. 1B). Two functional alleles of the *Wx* gene correspond to the AAC of *indica* and *japonica* rice varieties (70). *Wx^a* is widely distributed in *indica* rice, the subspecies with higher AAC, whereas *Wx^b* is mainly observed in *japonica* rice, the subspecies with low or intermediate AAC.

QTL analysis has shown that *Wx* is also responsible for super-long (or extra-long) unit chains in amylopectin (2). When the *Wx* gene was introduced into null-mutant waxy rice, AAC increased from undetectable amounts for nontransgenic waxy cultivars to 21.6–22.2% for transgenic lines. This increase was due, in part, to the presence of a significant amount of extra-long unit chains of amylopectin (7.5–8.4% of amylopectin weight) that was absent in nontransgenic *wx* cultivars. The study confirmed that *Wx* is responsible for biosynthesis of extra-long unit chains of amylopectin in rice (34).

Five functional markers in the *Wx* gene, a (CT)_n microsatellite (or simple sequence repeat), a 23 bp insertion/deletion sequence, and three single nucleotide polymorphism (SNP) markers, have been well characterized, with different alleles resulting in differing AAC (3,22,42,55) (Fig. 2A). Bligh et al. (22) first reported a (CT)_n microsatellite in the *Wx* gene located 55 bp upstream of the putative 5'-leader intron splice site and found four (CT)_n alleles (n = 10, 13, 18, and 20). Ayres et al. (3) identified eight alleles (n = 8, 11, 14, 16, 17, 18, 19, and 20) in 91 U.S. rice accessions; seven of the alleles explain 82.9% of the variation in AAC among 89 nonglutinous rice cultivars tested. Bergman et al. (21) reported six alleles (n = 10, 11, 14, 17, 18, and 20) in nonglutinous rice samples. Bao et al. (14) reported 10 alleles (n = 8, 10, 11, 12, 17, 18, 19, 20, 21, and 22) in 491 nonwaxy rice accessions that together explain 90.3% of the variation in AAC (Fig. 2B). Chen et al. (27)

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detected nine alleles (n = 8, 10, 11, 14, 16, 17, 18, 19, and 20) in 171 rice accessions that explain 75.6% of the variation in AAC among 152 nonglutinous accessions. In glutinous rice accessions, only four

classes of (CT)_n repeats, i.e., (CT)₁₆, (CT)₁₇, (CT)₁₈, and (CT)₁₉, have been detected (8,33,68).

There is a G/T SNP at the putative leader intron 5' splice site, and a G to T muta-

tion at this site reduces the efficiency of *Wx* pre-mRNA processing and, thus, results in lower levels of spliced mature mRNA, *Wx* protein, and AAC (23,25,39, 43,90). Waxy, low-amylose, and some in-

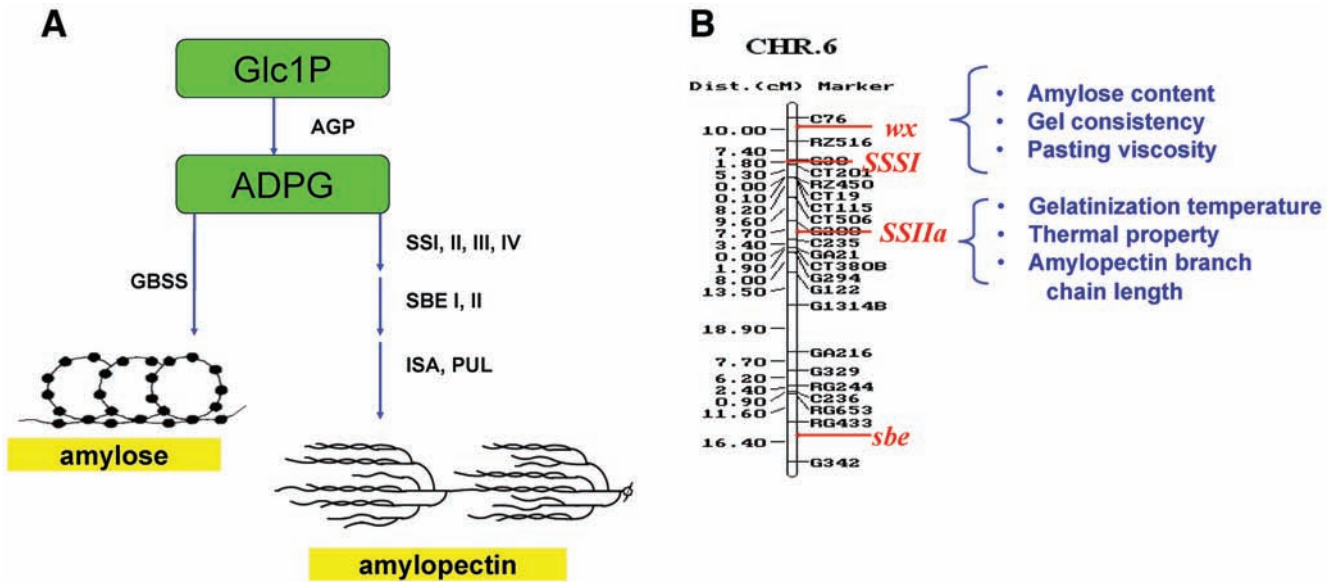


Fig. 1. Genetic basis of the eating and cooking qualities of rice. **A**, A simplified starch synthesis pathway of rice, and the enzymes and genes involved. AGP = ADP-glucose pyrophosphorylase; GBSS = granule-bound starch synthase, encoded by the *Wx* gene, which plays a unique role in the synthesis of amylose; SS = soluble starch synthase, which catalyzes the chain-elongation reaction; SBE = starch branching enzyme, which plays roles in the introduction of new branches; and ISA = isoamylase and PUL = pullulanase (ISA and PUL belong to starch debranching enzymes [DBE]), which play roles in removing improper branches of amylopectin. **B**, Location of some related genes (*Wx*, *SSI*, *SSIIa*, and *SBE1*) in chromosome 6, which show the importance of *Wx* and *SSIIa* to the genetic control of starch physicochemical properties.

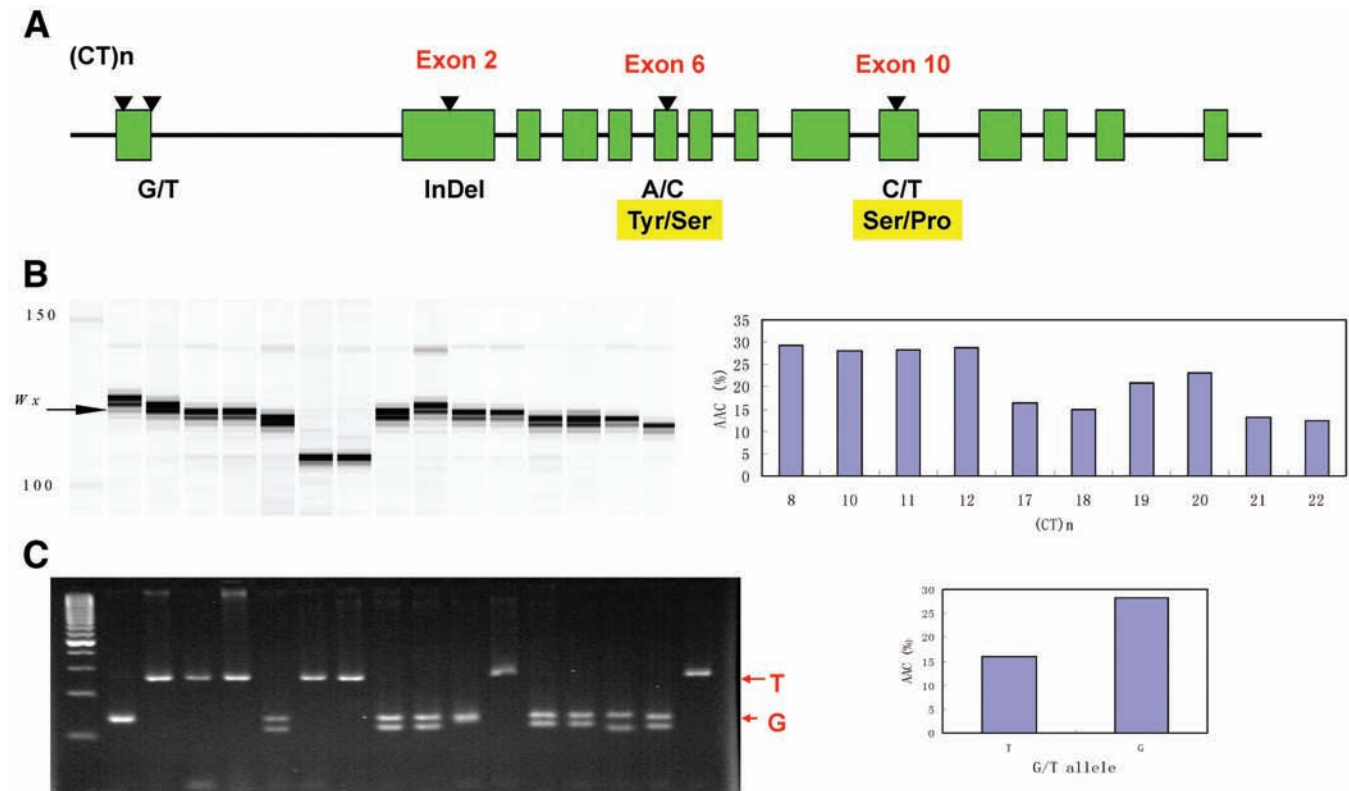


Fig. 2. Relationship between *Wx* alleles and apparent amylose content (AAC) in rice. **A**, *Wx* gene functional markers, a (CT)_n microsatellite, the G/T single nucleotide polymorphism (SNP) in intron 1, the 23 bp duplication in exon 2, an A/C SNP in exon 6, and a C/T SNP in exon 10; the change in amino acid is indicated under each SNP. **B**, Genotyping the (CT)_n microsatellite (left) and comparison of the AAC of each microsatellite class (right) (14). **C**, Genotyping the G/T SNP (left) and comparison of the AAC of each allele class (right) (14).

intermediate-amylose rice varieties have the T SNP allele, while some intermediate- and high-amylose rice varieties have the G allele (3,23,25,43). The G/T SNP explains 80–90% (3,14) of the total observed variation in AAC in nonwaxy rice accessions (Fig. 2C).

In waxy rice, the *wx* gene contains a 23 bp duplication in exon 2, resulting in a premature translation termination codon and loss of the function of GBSS. This 23 bp duplication is believed to be the cause of the glutinous trait (42,75,88). Larkin and Park (55) reported two additional functional SNPs in the *Wx* gene. One is an A/C SNP in exon 6, which causes an amino acid tyrosine (A)/serine (C) substitution; intermediate-AAC rice has the C SNP allele, while both A and C were identified in high- and low-AAC rice (55). The other is a C/T SNP in exon 10, which causes an amino acid serine (C)/proline (T) substitution; high-amylose, strong viscosity curve rice cultivars have a T allele, whereas the others have a C allele (55). Thus, both the exon 6 and exon 10 SNPs show a significant association with AAC (55). Chen et al. (27) reported that the SNPs at the leader intron splice site and in exon 6 together (the haplotypes) explain 86.7% of the variation in AAC and discriminate the three market classes of low-, intermediate-, and high-AAC rice from each other. By combining the SNPs at the leader intron splice, exon 6, exon 10, and the 23 bp duplication in exon 2, Teng et al. (75) identified five *Wx* haplotypes consistent with the five amylose classes: *wx* ($\approx 2\%$), *Wx*¹ (12.3–15.8%), *Wxg*¹ (21.9–23.6%), *Wxg*² (24.5–25.0%), and *Wxg*³ (25.8–26.2%).

It should be noted that other mutation sites in the *Wx* gene may lead to different starch functionalities. A SNP in exon 4, resulting in an amino acid substitution from aspartate to glycine, is associated with the opaque phenotype and defines the *Wx*^{op} allele (62). Very low AAC in some Yunnan rice landraces did not result from the above mutations, but from an A to G change at nucleotide position +497 from the start codon, resulting in an amino acid Asp/Gly substitution (59). This substitution has no detectable effect on GBSS activity in vitro but does affect the ability of GBSS to bind to starch granules, resulting in a reduction in AAC in rice. The presence of a tyrosine residue at GBSS position 224 correlates with the formation of large amounts of super-long chain amylopectin in rice cultivars containing *Wxa* (34).

In addition to genetic effects, AAC is also affected by environment (11), especially the growing temperature during grain filling. AAC increases when rice is grown at cooler temperatures. *Wx* gene expression is activated reversibly in response to cool temperatures (18°C), and it is the *Wx* promoter that is responsive to cool temperatures (40). The G/T SNP at the alternate splicing site is also temperature dependent. At 25°C and particularly at 32°C, the amount of mature GBSS mRNA in developing seeds is dramatically reduced (53). This is one of the reasons that low-amylose varieties are particularly temperature sensitive. The steady state level of GBSS mRNA is essentially identical over this temperature range for rice containing the G SNP allele.

Gelatinization Temperature

GT is another important quality predictor for the cooking quality of rice. Low-GT rice needs less energy input during cooking than high-GT rice and takes less time to cook. GT can be determined directly using a polarizing microscope with a heating stage or by differential scanning calorimetry, which measures the gelatinization transition temperature; onset, peak, and conclusion temperatures; and enthalpy of gelatinization (17). For rice, GT is measured indirectly by the degree of disintegration of milled rice in 1.7% KOH after 23 hr at 30°C using a 1–7 scale proportionate to the degree of spreading, where scales 2–3, 4–5, and 6–7 represent high (>74°C), intermediate, and low (<70°C) GT, respectively (57). GT can also be indirectly predicted by the pasting temperature (PT) measured using a rapid viscosity analyzer (RVA) (18). The proportion of chains with degree of polymerization (DP) ≤ 10 to those with DP ≤ 24 in amylopectin molecules may be a good means of classifying rice starch into long, short, and intermediate amylopectin structures (63). The ratio is negatively correlated with onset temperature of gelatinization, whereas there is no correlation between the amylose content and thermal properties of starch (63).

To date, linkage mapping studies have identified only one major QTL, i.e., the alkali degeneration (*alk*) locus on chromosome 6, as being primarily responsible for different gelatinization temperatures in diverse rice germplasm (1,10,13,30,35,52, 79,89) (Fig. 3A). Map-based cloning of the *alk* locus provided evidence that the gene encoding SSIIa is the major gene responsible for GT (31). QTL mapping revealed

that amylopectin chain length distribution is also controlled by the *SSIIa* locus (84). The function of *SSIIa* is to elongate the short A and B1 chains with DP < 10 to form long B1 chains of amylopectin (65). Genetic engineering through introduction of the *indica* rice active *SSIIa* gene into *japonica* rice increased GT and produced longer amylopectin side chain length (32,65).

Several research groups worked on identification of the functional SNPs that cause amino acid substitution within the *SSIIa* gene (15,26,65,85,86,91). There are four SNPs in the *SSIIa* gene (Fig. 3A). The first one is at 264 bp in exon 1 of AY423717, where a change from G to C results in the change of glutamate to aspartate. The second site is at 3799 bp, where glycine encoded by GGC is replaced by serine encoded by AGC. The third site is at 4198 bp, where valine encoded by GTG is replaced by methionine encoded by ATG. The fourth site is at 4330 bp, where glycine-leucine encoded by GGGCTC is replaced by glycine-phenylalanine encoded by GGGTTTC. *SSIIa* gene fragment-shuffling experiments showed that only the third and fourth SNPs are functional, and the third SNP (G/A) is crucial for SSIIa activity. The enzyme is inactive when it is an A SNP (coding for methionine) regardless of which GC/TT allele in the fourth SNP is present (65).

Genotyping of 65 rice accessions for the first three SNPs revealed four haplotypes; however, GT and chain length distribution were not haplotype specific (85). Further genotyping of the fourth SNP (i.e., GC/TT) among the same rice accessions resulted in five haplotypes in combination with the previously identified haplotypes (86). The last two SNPs were strongly associated with the chain length distribution of amylopectin and GT of rice flour. Later studies support the evidence that the GC/TT SNP is strongly associated with GT (15,91) (Fig. 3B). This GC/TT polymorphism alone can differentiate rice with high or intermediate GT (possessing the GC allele) from those with low GT (possessing the TT allele), explaining 62.4% of the total variation in PT (15) (Fig. 3C). Few rice varieties containing the GC allele have a low-GT phenotype, which can be explained by the A SNP allele they carry in the third SNP (85,91). However, it should be mentioned that the A allele of the third SNP (G/A) is quite rare in natural populations. The frequency of allele A is 1 in 30 rice varieties (15), 9 in 180 rice varieties (26), 127 in 1,543 rice varieties (29), 5 in

65 rice varieties (85), and 13 in 73 rice varieties (91). It should also be noted that the genetic control of intermediate-GT rice starch remains unknown. Intermediate-GT rice is characterized by more chains of DP24-35, which may be synthesized by other enzymes (29).

A mechanism by which *SSIIa* determines high or low GT of rice starch has been proposed (20). The *SSIIa* activity determines the chain length of amylopectin, and the latter determines whether the GT is high or low: low-GT starch has amylopectin with more short chains, while high-GT starch has amylopectin with more long chains. Genotypic differences in the *SSIIa* sequence play an important role in determining the functionality of *SSIIa*, while the modifier genes, transcription factors, and enzyme-enzyme interactions may regulate the expression of *SSIIa* and *SSIIa* activity. Environmental factors also affect GT, resulting in a difference of up to 10 degrees Celsius between some rice starches (92). Environmental factors, especially temperature during grain filling, can modify the expression of the *SSIIa* gene (93) and, hence, modify amylopectin chain length (41,73,83). These previous studies show that the amount of short chains in the amylopectin increases sig-

nificantly, whereas the amount of long chains decreases in rice grown at low temperatures compared with rice grown at higher temperatures (41,73,83).

Gel Consistency

Gel consistency is a commonly used indirect indicator of eating and cooking qualities tested in breeding programs for rice improvement. The gel consistency test measures the displacement, after 1 hr, of a gel made from rice flour and KOH. For rice varieties with a hard texture, the gel is compact, flowing only a small distance, but for those with a soft texture, the gel is viscous and displaces up to 10 cm within 1 hr (24). Gel consistency is categorized as hard (26–40 mm), medium (41–60 mm), and soft (61–100 mm).

Linkage mapping has shown that the gel consistency is mainly controlled by the *Wx* gene (5,7,10,30,36,51,56,74,79,89,97). Association mapping for gel consistency led to the same conclusion (80). Map-based cloning of the *qGC-6* locus indicated that *Wx* is the major gene responsible for gel consistency (72). Transgenic complementation experiments confirmed that *Wx* may contribute equally to grain amylose content and gel consistency (72). The exon 10 SNP of *Wx* provides the

genetic basis for gel consistency and is also associated with the proportion of amylose bound to amylopectin, the proportion of amylose that is able to leach, and gel hardness (81). Rice varieties with SNP allele C at exon 10 produce soft, viscous gels and have a soft texture when cooked, but with high retrogradation. Rice varieties with SNP allele T produce a short, firm gel and have a firm texture when freshly cooked, with little change in texture over storage (81).

Pasting Viscosity

Pasting viscosity is another parameter used to differentiate rice varieties with similar AAC and is commonly measured with an RVA (Rapid Visco Analyser, Newport Scientific Pty. Ltd.). An RVA records viscosity continuously as the temperature is increased, held constant for a time, and then decreased (Fig. 4A). During the initial step, viscosity increases rapidly with the increase of temperature as the granule swells. Peak viscosity (PV) is reached when granule swelling is balanced with the granules broken by stirring. With continued stirring, more granules rupture and fragment, causing a further decrease in viscosity. With cooling, some starch molecules partially

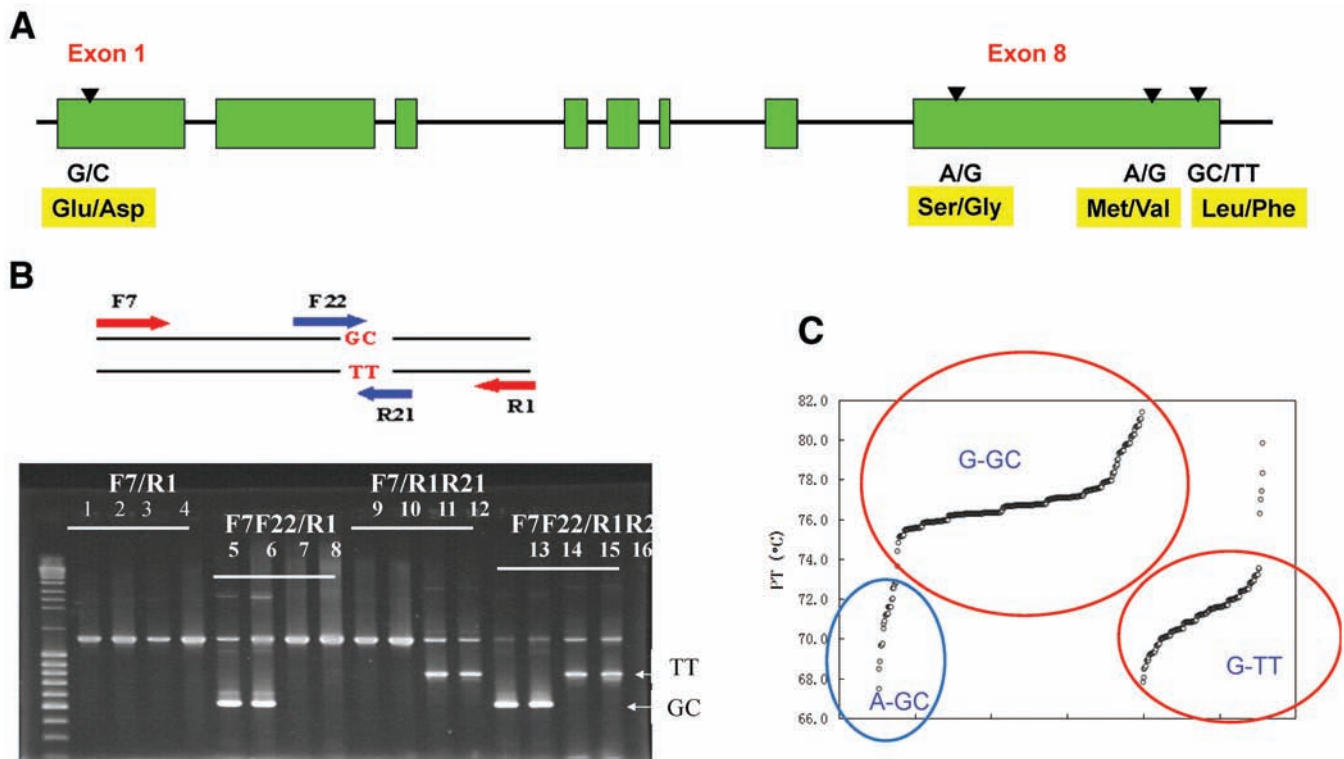


Fig. 3. Relationship between *starch synthase IIa* (*SSIIa*) alleles and gelatinization temperature. **A**, Four functional single nucleotide polymorphisms (SNPs) of *SSIIa*; the change in amino acid is indicated under each SNP. **B**, Strategy for genotyping the GC/TT SNPs (top) and gel showing different PCR products amplified by different combinations of primers (bottom) (15). **C**, Relationship between pasting temperature (PT) and the haplotypes of the third (G/A) and fourth (GC/TT) SNPs (15).

reassociate to form a precipitate or gel (this step is called retrogradation) in which amylose molecules aggregate into a network, embedding remnants of starch granules.

Many studies have found that pasting profile parameters are significantly correlated with AAC (4,10,16,28,82,89). Genetic analysis using the QTL mapping approach revealed that the RVA parameters are mainly controlled by the *waxy* gene, which is responsible for amylose synthesis (4,6,10,54,82,89). Association mapping confirmed that *Wx* is the major gene responsible for variation in pasting viscosity parameters among diverse rice varieties (14,19,28). In a cross between two varieties with similar high AAC, but with different paste viscosity properties, Traore et al. (82) found that the exon 10 SNP marker was associated with most RVA pasting measurements, as well as the proportion of soluble to insoluble apparent amylose. In a population derived from two parents with similar intermediate AAC, QTLs other than the *Wx* locus were associated with RVA pasting viscosities, and two might be located close to the *starch branching enzyme 1* (*SBE1*) and *SBE3* loci (9).

The pasting viscosity profile of waxy rice is very different from nonwaxy rice (12) (Fig. 4B). Since GBSS is not active in waxy rice, genes other than *Wx* are expected to control the pasting viscosity of waxy rice. Comparing the starch physicochemical properties among different microsatellite groups in *SBE1* and *soluble starch synthase 1* (*SSS*), waxy rices with the *SBE-A* allele have higher PV, hot paste

viscosity (HPV), and cold paste viscosity (CPV) than those with other alleles, and those with the *SSS-B* allele have higher HPV and CPV than other alleles (8). Han et al. (33) indicated that nucleotide polymorphisms in both *SBE1* and *SBE3* loci account for 70% of the observed variation in HPV and CPV and for 40% of the observed variation in PV. Yan et al. (94) conducted association analysis for pasting viscosity parameters of waxy rice using starch synthesis-related gene markers. The analysis showed that 10 gene markers were involved in controlling pasting viscosity parameters. Among these, the *pullulanase* gene plays an important role in control of PV, HPV, CPV, breakdown viscosity, peak time, and PT in glutinous rice. In contrast, Zhang (96), also genotyped the gene markers and *SSIIa* SNPs found in the waxy rice reported in Bao et al. (12), and association mapping revealed that *SSIIa* is a major gene for pasting viscosity and thermal (gelatinization) properties in the waxy rice studied.

Genetic Interactions

Genetic linkage mapping studies have shown that *Wx* not only controls AAC, gel consistency, and pasting viscosity, but also affects GT (52,89); the QTL cluster at the *SSIIa* locus also contains individual QTLs for gel consistency and some paste viscosity parameters (89). Association mapping has shown that *Wx* not only affects AAC and gel consistency as a major gene, but that it also regulates GT as a minor one; *SSIIa* plays an essential role not only in controlling GT, but AAC and gel consistency as well (80). Association mapping

also revealed that the *Wx* gene is strongly associated with PT, and *SSIIa* is strongly associated with AAC (47). Gao et al. (32) introduced the *SSIIa* gene into low-GT rice through transgenic engineering and found that it also affects AAC, gel consistency, and pasting properties, suggesting that *SSIIa* is a modifier gene for AAC, gel consistency, and pasting properties in rice.

Despite the progress made in understanding the mechanism that underlies the complex network of genetic control of the eating and cooking qualities of rice, the exact roles played by *Wx* and *SSIIa* remain to be untangled. One possibility is that the complicated effects of *Wx* and *SSIIa* are derived from their close physical position in chromosome 6 (Fig. 1B), as suggested by the strong linkage disequilibrium for all starch-related traits. Another is that there is true interaction between *Wx* and *SSIIa* in starch biosynthesis. Genetic transformations conducted by Tian et al. (80) and Gao et al. (32) provide supporting evidence for the latter hypothesis.

Starch biosynthesis in the cereal endosperm is orchestrated by complex interacting combinations of multiple isoforms of starch synthase and branching and debranching enzymes, resulting in a fine amylopectin structure (45,76). Biochemical and yeast two-hybrid experiments have identified multienzyme complexes (protein-protein interactions) in wheat and maize endosperms during grain filling (37,38,77,78). The multienzyme complex components in the normal maize endosperm include SSI, SSIIa, and SBEIIb (58). Based on the fact that *Wx* and *SSIIa* exert joint control over both AAC and GT and

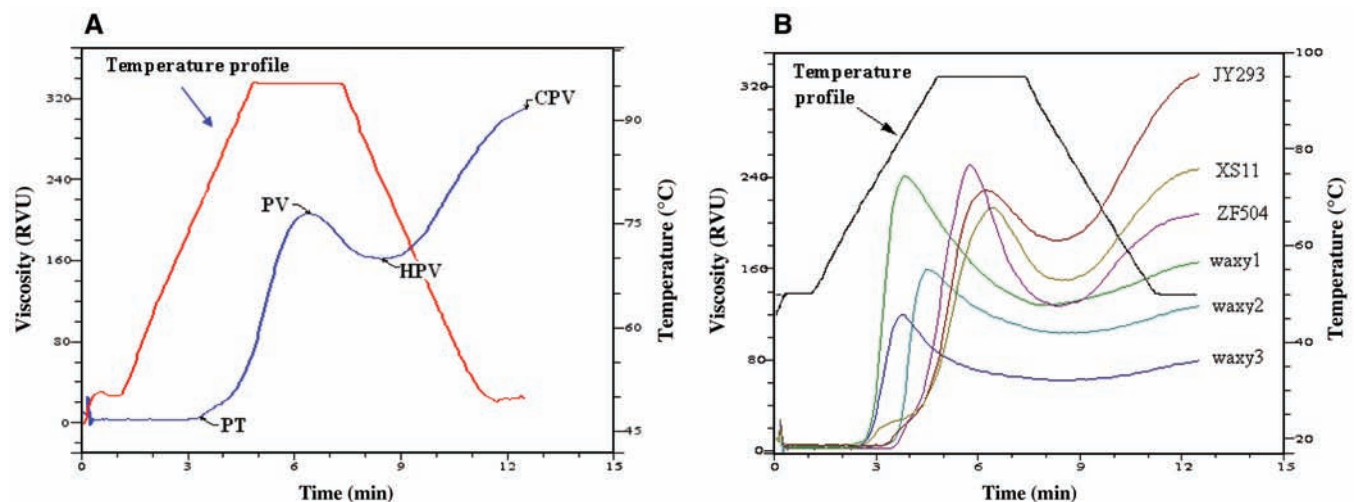


Fig. 4. Rapid Visco Analyser (RVA) pasting viscosity profile for rice starch. **A**, Pasting viscosity parameters: PV = peak viscosity; HPV = hot paste viscosity; CPV = cold paste viscosity; and PT = pasting temperature. Breakdown, setback, and consistency viscosities are the differences between PV and HPV, PV and CPV, and CPV and HPV, respectively. **B**, Typical RVA profiles for waxy and nonwaxy starches; waxy rice RVA traces are very different from those for nonwaxy rice (12).

that complex AAC and GT combinations exist in rice germplasm, I propose a multi-enzyme complex model to explain the interaction between GBSS (*Wx* protein) and *SSIa* protein and how they contribute to diverse combinations of AAC and GT in rice germplasm (Fig. 5).

High-AAC rice usually has intermediate or low GT, whereas low-AAC or waxy rice usually has high or low GT among rice germplasm. In contrast, it is difficult to find the combinations of high-AAC and high-GT or low-AAC and intermediate-GT rice (48,49). Suppose *SBEIIb*, *SSI*, and *SSIa* are the major multi-enzyme complex components in intermediate-GT (*SSIa*-active) rice; the high activity of GBSS (*Wx* protein) in the stroma negatively regulates the function of the multi-enzyme complex, resulting in synthesis of high-AAC, intermediate-GT starch (Fig. 5A). However, it remains to be determined whether GBSS is attached to amylopectin to synthesize super-long chains of amylopectin or to synthesize amylose. In low-AAC, high-GT rice, due to lower GBSS activity, the higher activity of the multi-enzyme complex allows the synthesis of more chains with DP > 12, thus producing high-GT starch (Fig. 5B). It is plausible that this step needs to recruit other enzymes, such as *SSIIa*.

Low-GT rice can have high, intermediate, low, or zero (waxy rice) AAC. Due to the dysfunction of *SSIa*, the compo-

nents that comprise the multi-enzyme complex are unknown and, thus, need to be studied further. This complex is not affected by GBSS and produces amylopectin with more short chains (DP6–12), resulting in low-GT rice (Fig. 5C). In summary, the model highlights the complex combinations of AAC and GT in different rice germplasm and the genetic effects of *Wx* and *SSIa* on the eating and cooking qualities of rice.

Molecular Breeding

All of the information discussed above has direct applications, i.e., to help breeders design breeding lines using marker-assisted selection (MAS) techniques. There have been some successful cases of improvement of the eating and cooking qualities of rice using molecular markers, especially for breeding high-quality hybrid rice. The *Wx* marker has been used to improve the eating and cooking qualities of hybrid rice such as Shanyou 63, a hybrid derived from Zhenshan 97A (a male-sterile line) and Minghui 63 (a restorer line) (98). Liu et al. (60) successfully transferred a SNP (*Wx*-G/T) to the maintainer line Longtefu B and Zhenshan 97B using MAS, which led to a good quality rice with intermediate AAC. Jin et al. (46) used the functional markers of *Wx*, *SSIa*, and *fragrance* genes to improve another maintainer line, I132B, for hybrid rice breeding, producing

a low-AAC, low-GT, fragrant rice. Yi et al. (95) successfully improved the fragrance and intermediate AAC of a Myanmar rice cultivar, and Jain et al. (44) successfully improved the resistance to brown planthopper and eating quality of a Thailand aromatic rice using MAS.

Because wide diversity exists in consumer preferences for rice eating quality, the starch quality of rice preferred by different consumers also varies. Diverse foods made from rice, whether traditional or new, exist in different markets. The starch quality of the rice used for food processing also differs. Therefore, rice breeders must have a broad knowledge of the existing starch properties of different rice cultivars and consumer preferences before applying marker-assisted breeding to achieve desired starch properties.

Future Trends

Great progress has been made in our understanding of the genetic and molecular bases of the eating and cooking qualities of rice. *Wx* is the major gene controlling AAC, gel consistency, and pasting viscosity; it also regulates GT as a minor gene. *SSIa* is the major gene controlling GT and amylopectin branch chain length distribution; it also affects AAC, gel consistency, and pasting viscosity as a minor gene. The molecular mechanisms controlling the effects of the environment on AAC and GT have been clarified. Molecular breeding using *Wx* and *SSIa* functional markers has resulted in new high-quality rice cultivars (breeding lines). However, there are four major problem areas that challenge researchers working on the molecular genetics of rice eating and cooking qualities.

- 1) To date the genetic basis of intermediate-GT rice is still unknown. The third and fourth SNPs of *SSIa* can only differentiate low GT from high (or intermediate) GT rice. The molecular difference between high- and intermediate-GT rice remains to be characterized. The proposed multi-enzyme complex model aims to explain the underlying mechanism, but further experimental evidence is needed.
- 2) Another combination of AAC and GT, e.g., high AAC and high GT (50), may exist. If it does, questions about the genetic basis of this combination would arise.
- 3) The genetic basis of pasting viscosity derived from similar AAC needs to be further characterized. Although the

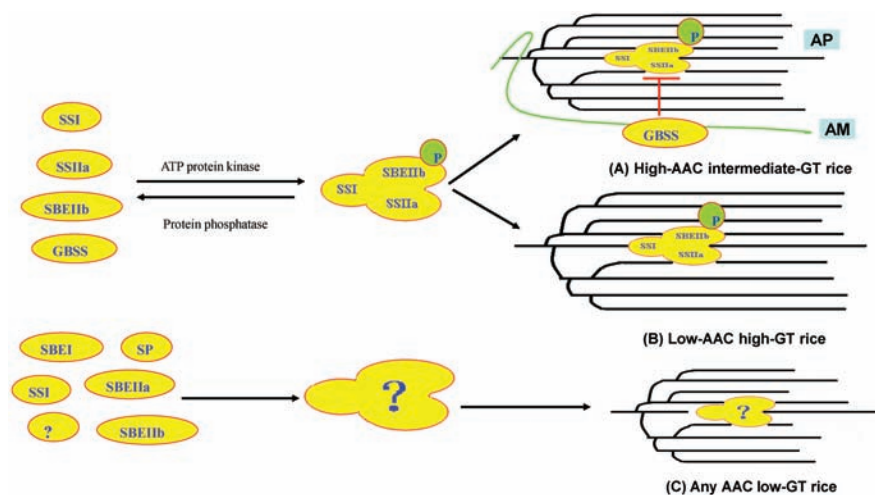


Fig. 5. Model of a multi-enzyme complex (protein–protein interactions) formed among amylopectin-synthesizing enzymes in rice endosperm that explains the AAC-GT combinations. **A**, In starch synthase IIa (*SSIa*) active endosperm, the major form of starch branching enzyme (*SBEIIb*) forms a phosphorylation-dependent protein complex with *SSI* and *SSIa*. However, the high activity of granule-bound starch synthase (*GBSS*) represses the function of the complex, leading to synthesis of high-apparent amylose content (AAC) intermediate-gelatinization temperature (GT) starch. **B**, In low-AAC or waxy rice, the repression of *GBSS* is removed, and the function of the complex elongates more chains with a degree of polymerization > 12, leading to the production of high-GT starch. **C**, In low-GT rice, due to the loss of *SSIa*, the components of the complex are unknown, but the complex is not affected by *GBSS* and produces amylopectin with more short chains (DP6–12), resulting in low-GT rice. AM = amylose; AP = amylopectin.

exon 10 SNP of *Wx* provides a good indication of the pasting profiles resulting from high AAC (82), the RVA parameters in populations derived from parents with intermediate or low AAC have been mapped to loci other than the *Wx* locus (9,61). Because RVA pasting viscosities are widely used by breeders and food processors, further studies of their genetic basis are needed.

- 4) Increasing use of molecular markers to design cultivars with desired eating and cooking qualities is expected in rice breeding programs worldwide. Targeting more traits with more markers, such as *Wx*, *SSIIa*, and *fragrance* (46), is becoming popular in rice breeding programs. Because yield and other agronomic or resistance traits are also very important, MAS for quality and yield should be considered together in the future. Strategies for more effective selection should be developed when many markers are used at the same time. More importantly, genomics-based MAS should gain in popularity as genome-wide resequencing technology becomes less expensive. New rice cultivars with both high quality and high yield potential are expected to be developed in the next decade.

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