

Association Mapping of Starch Physicochemical Properties with Starch Biosynthesizing Genes in Waxy Rice (*Oryza sativa* L.)

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S Supporting Information

ABSTRACT: Waxy (glutinous) rice is widely used in traditional foods, and understanding the genetic bases of its diverse physicochemical properties will contribute to breeding of new waxy rice with unique qualities. The objective of this study was to investigate the genetic relationship between the starch biosynthesis related genes and the physicochemical properties of waxy rice using association mapping. A total of 36 molecular markers representing 18 genes were used to genotype 50 waxy rice accessions for which starch properties were previously available. Most of the starch properties differed between high and low gelatinization temperature (GT) groups, whereas most traits were similar between the low-GT indica rice and low-GT japonica rice, suggesting GT was the main determinant of the starch quality of waxy rice. Association mapping indicated that the starch properties of waxy rice were mainly controlled by *starch synthase IIa* (*SSIIa* or *SSII-3*, a major gene responsible for the gelatinization temperature) and *SSI*. It was found that gene–gene interactions were also important for the genetic control of starch properties of waxy rice. This study suggests that application of the functional SNPs of *SSIIa* in molecular breeding may facilitate quality improvement of waxy rice.

KEYWORDS: waxy rice, starch, physicochemical property, pasting viscosity, thermal property

■ INTRODUCTION

Although normal amylose rice is a staple food for half the world's population, waxy (glutinous) rice has important uses, mainly in processing of traditional foods (for example, Tangyuan and Zong-zi) and wine (for example, Shao-Xing yellow wine), and as an ingredient in some other products. Only in some mountainous regions of a few countries of the Indochina peninsula do people mainly utilize waxy rice as their daily dietary staple.^{1,2} Waxy rice starch contains essentially all amylopectin, with <2% amylose. Waxy rice is preferred to nonwaxy rice in specific food applications due to the physicochemical properties of its starch. If the biochemical and genetic mechanisms underlying the diverse physicochemical properties of waxy rice can be further clarified, this will allow us to understand the technological basis for its use and also facilitate breeding to create waxy rice cultivars with an extended range of starch properties.

In normal rice, amylose is considered to be the major contributor to its eating, cooking, and processing qualities, whereas in waxy rice, starch gelatinization, retrogradation, and rheological properties vary due to the differences in amylopectin structure. Waxy rices can be divided into two groups according to gelatinization temperature (GT), a high-GT group and a low-GT group.^{3–5} The low-GT waxy rice starches contain two major chain fractions upon debranching with isoamylase, whereas the high-GT starches contain three fractions.⁴ The waxy rice starches differ in amylopectin structure in terms of degree of polymerization (DP), average chain length, exterior chain lengths, and distribution of chains.⁶ The proportion of amylopectin chains with DP < 10 was negatively correlated with the onset temperature of starch gelatinization.⁷ The higher exterior chains (short and relatively longer short chains with DP ~ 19) confer

high-GT characteristics on waxy starches and promote crystallite formation.⁴ The amount of long chains is correlated positively with retrogradation enthalpy and gel firmness, and the ratio of short to long chains affected almost all of the rheological and retrogradation parameters.⁸ These results suggest that the amylopectin structure of waxy rice affects the gelatinization, rheological, and retrogradation properties of its starch.^{4,6,8}

Starch biosynthesis in the rice grain requires the functions of a series of enzymes, including ADP-glucose pyrophosphorylase (AGPase), starch synthase (SS), starch branching enzymes (SBE), and starch debranching enzymes (DBE).⁹ In the rice genome, there are 6 ADP-glucose pyrophosphorylase (AGPase) genes, 10 SS genes, 3 SBE genes, and 2 types of DBE, isoamylase (ISA) and pullulanase (PUL, also known as limit dextrinase or R-enzyme).^{9,10} In normal rice, the granule-bound starch synthase 1 (GBSS1) encoded by the *Waxy* (*Wx*) gene participates in amylose synthesis, whereas other isoforms of SS, BE, and DBE participate in amylopectin biosynthesis.¹⁰ Due to the absence of GBSS1, waxy rices are ideal materials to investigate which genes determine their starch functionalities.¹¹ Earlier work indicated that the starch physicochemical properties of waxy rice are associated with the microsatellite alleles of *wx*, *SSI*, and *SBE1*, all of which are located on chromosome 6.¹¹ Han et al. also indicated that alleles of *SBE1* and *SBE3* loci accounted for >40% of the observed variance of pasting viscosity parameters.¹² More recently, Yan et al. designed sequence-tagged sites (STS), cleaved amplified polymorphic sequence (CAPS) markers for 17 starch

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Table 1. Comparisons of Physicochemical Properties of Waxy Rice Genotypes between Different GT Groups and Subspecies^a

	high-GT (<i>n</i> = 15)		low-GT (<i>n</i> = 41)		indica (<i>n</i> = 34)		japonica (<i>n</i> = 22)		low-GT indica (<i>n</i> = 19) ^b	
	mean	range	mean	range	mean	range	mean	range	mean	range
<i>T₀</i> (°C)	72.5 ^{*c}	69.0–74.5	60.9	57.6–63.5	65.8 [*]	57.6–74.5	61.2	59.3–63.0	60.5	57.6–63.5
<i>T_p</i> (°C)	77.9 [*]	76.2–79.0	68.8	66.9–70.5	72.6 [*]	66.9–79.0	69.1	67.3–70.5	68.5	66.9–70.2
ΔH_g (J/g)	9.3 [*]	7.4–10.2	8.1	5.9–9.4	8.5	5.9–10.2	8.3	7.4–9.4	7.9	5.9–9.1
$\Delta T_{1/2}$ (°C)	6.3	5.4–7.8	8.6 [*]	7.3–10.5	7.5	5.4–10.5	8.7 [*]	7.9–9.7	8.5	7.3–10.5
ΔH_r (J/g)	6.1 [*]	5.1–7.1	1.5	1.0–2.0	3.5 [*]	1.1–7.1	1.5	1.0–2.0	1.5	1.1–2.0
R%	66.6 [*]	52.4–77.1	18.6	10.3–25.1	40.1 [*]	14.1–77.1	18.1	10.3–23.4	19.2	14.1–25.1
PV (RVU)	164	144–186	169	115–241	171	137–241	163	115–198	176	137–241
HPV (RVU)	102 [*]	91–116	88	57–126	95 [*]	65–126	86	57–102	90	65–126
CPV (RVU)	128.8 [*]	114.3–145.2	112	73–163	121 [*]	83–163	109	73–126	115	83–163
BD (RVU)	61.9	50.3–77.1	82 ^c	48–115	76	49–115	77	48–96	87 [*]	49–115
SB (RVU)	–34.4 [*]	–41.8 to –26.5	–58	–84 to –24	–50	–84 to –25	–54	–72 to –29	–62	–84 to –24
FSV (mL/g)	18.9 [*]	16.2–21.3	17.2	14.1–20.9	18.3 [*]	14.3–21.3	16.7	14.1–18.8	17.7 [*]	14.3–20.9

^aData were adapted from Bao et al.⁵ ^bThis group also compared with the *japonica* group. ^cAn asterick indicates significance at *P* < 0.05 between pairwise groups (high-GT vs low-GT, and indica vs japonica).

synthesis-related genes (SSRG), and analyzed their associations with pasting viscosity parameters in waxy rice.¹³ Their results showed that 10 SSRGs are involved in controlling the rapid visco analyzer (RVA) profile parameters, and the PUL gene plays an important role in the control of peak viscosity, hot paste viscosity, cool paste viscosity, breakdown viscosity, peak time, and paste temperature in glutinous rice.¹³ However, the genetic control of other starch properties, such as thermal and retrogradation properties in waxy rice, has not been reported.

In this study, markers designed by Yan et al. and by ourselves for SSRGs were employed to genotype 50 waxy rice accessions exhibiting a diversity of starch physicochemical properties, as previously reported.^{5,11,13,14} The objective of this study was to carry out association mapping of starch pasting, gelatinization, retrogradation properties, and swelling volume with SSRGs to understand the genetic control of these starch quality parameters in waxy rice background. The results will provide information for marker-assisted breeding for the improvement of waxy rice grain quality.

MATERIALS AND METHODS

Rice Materials and Physicochemical Properties. All of the waxy rice materials (varieties, landraces, and breeding lines) were obtained from various rice research centers and rice breeding programs. The physicochemical property data of 56 waxy rices reported in a previous paper⁵ were used in this study (Supporting Information Supplementary Table 1). Due to the lack of seed viability, only 50 waxy accessions (i.e., excluding the accessions of w17, w19, w22, w25, w49, and w56; Supporting Information Supplementary Table 1) were available for DNA extraction, genotyping with new markers, and association mapping in this study.

DNA Extraction. Five seedlings of each accession were used for genomic DNA extraction using the CTAB method.¹⁵

Genotyping of the SSRGs. The markers tagged for SSRG developed by Yan et al., Bao et al., and Lu et al. were used to genotype the 50 rice accessions.^{11,13,14} The primer sequences are listed in Supporting Information Supplementary Table 2. Each 20 μ L PCR reaction consisted of 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X 100, 2 mM MgCl₂, 0.1 mM dNTPs, 200 nM primers, 1 unit of *Taq* polymerase, and 50 ng of genomic DNA. All amplifications were performed on an MG96G thermal cycler (Hangzhou LongGene Scientific Instruments Co. Ltd., Hangzhou, China) under the following conditions: (1) predenature at 95 °C for 5 min; (2) 35 cycles of run, each followed by denature at 95 °C for 1 min, anneal at 55–60 °C for 45 s, and extension at 72 °C for 1 min; (3) final extension at 72 °C for 10 min. The PCR products were separated by electrophoresis either in

1.5% agarose in 0.5 \times tris-borate EDTA (TBE) buffer or in 8% denaturing polyacrylamide gel (PAGE) with 3.4% cross-linker (ratio of bis(acrylamide) to acrylamide) in 1.0 \times TBE buffer.

Genotypic Data Analysis. The polymorphic information content (PIC) of each marker was calculated with PowerMarker 3.25.¹⁶ Dendrograms were generated using the unweighted pair-group method with arithmetic means (UPGMA) based on Nei's genetic distance in PowerMarker 3.25.^{16,17} The cluster image was viewed in MEGA4.0.¹⁸

Population Structure and Association Mapping. The analysis of population structure (*Q*) was performed using the STRUCTURE program,¹⁹ based on 455 polymorphic amplified fragment length polymorphism (AFLP) and intersimple sequence repeat (ISSR) bands.²⁰ The most probable number of subpopulation was chosen according to ΔK , which is an ad hoc quantity related to the second-order change of the log probability of data with respect to the number of clusters inferred by Structure.²¹ The kinship coefficients (*K*) between accessions were estimated using the SPAGeDi program (v. 1.2 g) with the same set of AFLP and ISSR bands.²² Association between marker alleles and different starch physicochemical property data was performed with TASSEL version 2.1 software, taking the gross level population structure (*Q*) and kinship (*K*) into account.^{23,24} The *P* value (marker) determining whether a marker (QTL) is associated with the trait and the *R*² (marker) indicating the fraction of the total variation explained by the marker were reported.

Statistical Analysis. A *t* test and analysis of variance (ANOVA) using the general linear model were performed with the SAS System for Windows version 8 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Phenotypes of Starch Properties among Waxy Rice.

The starch physicochemical properties of each waxy were reported before.⁵ All of the waxy rice could be divided into high-GT and low-GT classes. The high-GT waxy had a peak temperature (*T_p*) ranging from 76.2 to 79 °C, whereas the low-GT rice ranged from 66.9 to 70.5 °C. Many starch properties differed between the two classes (Table 1). All accessions could be divided into indica and japonica subspecies, and dramatic differences in starch physicochemical properties were found between indica and japonica, particularly as all of the high-GT rices were indica rice. However, when low-GT indica and low-GT japonica were compared, most traits did not differ (Table 1). These results indicated the overall great importance of gelatinization temperature to the starch quality of waxy rice.

Genotypes of Starch Biosynthesizing Genes, Population Structure, and Kinship. All 36 gene-specific marker loci are polymorphic, with polymorphic information content (PIC)

ranging from 0.0348 (marker SSI3-1) to 0.5409 (marker SSI-3; Supporting Information Supplementary Figure 2 and Supplementary Table 2). Cluster analysis based on these markers

revealed two groups representing indica and japonica rice (Figure 1a), indicating that the starch genes in the rice genome have already diverged into indica and japonica subspecies.

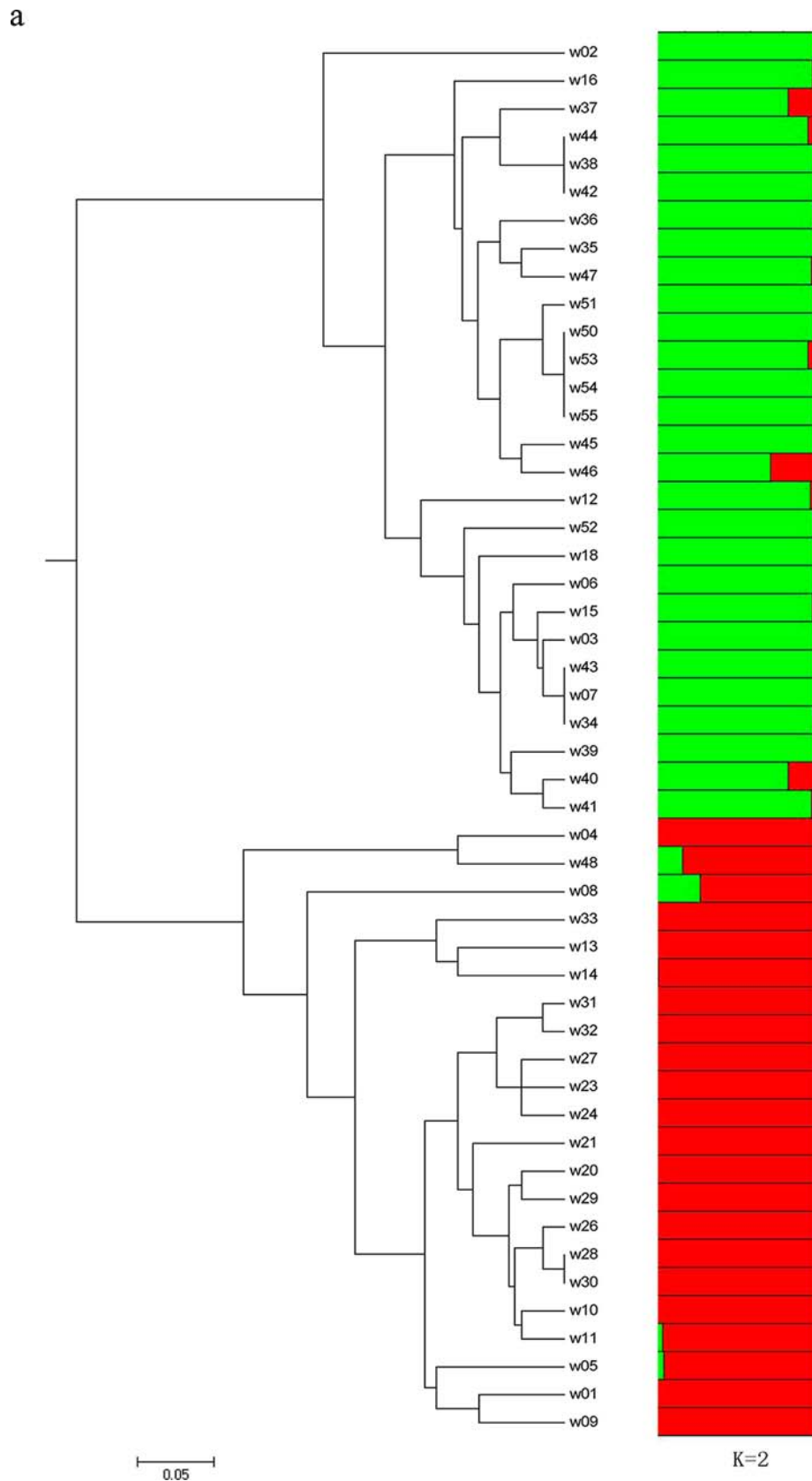


Figure 1. continued

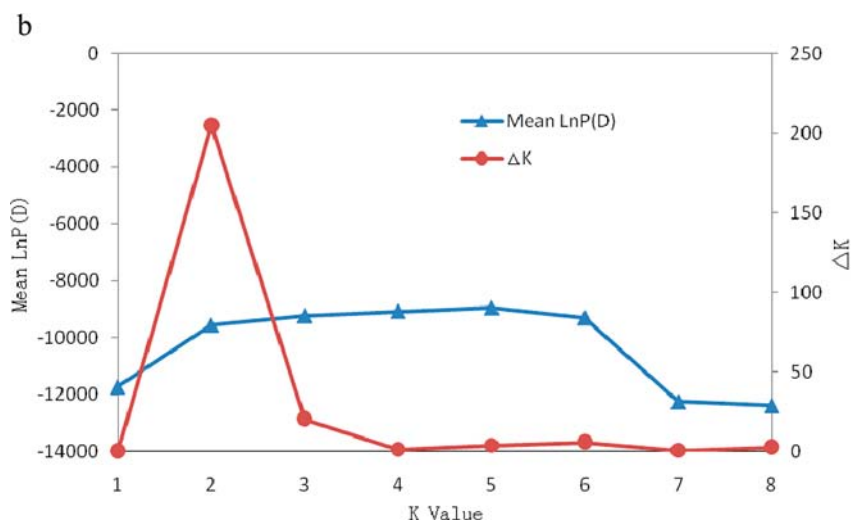


Figure 1. Genetic structure of waxy rice: (a) UPGMA tree of 50 accessions based on starch gene markers (left) and the genetic structure revealed by 455 AFLP and ISSR polymorphic markers (right); (b) estimate of the population structure of 50 waxy rice with K from 1 to 8, showing that $K = 2$ (two subgroups) is the optimal population structure.

Genetic structure analysis with AFLP and ISSR polymorphic bands showed that the likelihood was maximized and α minimized when the number of populations was set at 2. Calculation of ΔK also showed that it peaked at $k = 2$, indicating two subpopulations (Figure 1b). When k was 2, all of the waxy rice could be divided into indica and japonica rice (Figure 1a), which matched well with the groups revealed on the basis of starch gene alleles (Figure 1a). Thus, the population structure (Q) data at $k = 2$ were used in association mapping.

Relative kinship estimates based on the AFLP and ISSR data showed that 51% of the pairwise kinship estimates were equal to 0, suggesting that about half of the total pairs of accessions had no relationship with each other. Thirty-one percent of the estimates were <0.3 , indicating a weak relationship in this rice panel (Figure 2).

Association of Phenotype–Genotype in Waxy Rice.

Association analysis showed that there are eight genes significantly associated ($P < 0.05$) with starch quality traits in glutinous rice, that is, *AGP_{sma}*, *GBSSII*, *SSI*, *SSII-1*, *SSII-3* (*SSIIa*), *SBE1*, *SBE3*, and *ISA* (Table 2). Among them, *SSII-3* and *SSI* have a greater impact on glutinous rice starch quality than the others. Gelatinization temperature and retrogradation were mainly controlled by *SSII-3* and *SSI*, whereas the RVA viscosity parameters were controlled by many genes (Figure 3).

Thermal Properties. Thermal properties T_o , T_p , and T_c were associated with *wx*, *SSI*, and *SSII-3*, and the three genes clustered in a line on chromosome 6. *wx* and *SSII-3* explained 16–17% of total phenotypic variance of the traits, whereas *SSI* explained nearly 10% of the total variance. The enthalpy (ΔH_g) was controlled by *wx* and *SSII-3*, which explained 10 and 5% of the total variance, respectively. $\Delta T_{1/2}$ was associated with *wx*, *SSI*, *AGP_{sma}*, and *SSII-3*. *AGP_{sma}* contributed 5% to the total variance.

Retrogradation Properties. Similar to the thermal properties, both the enthalpy of retrogradation (ΔH_r) and retrogradation percentage (R%) were associated with the *wx*–*SSI*–*SSII-3* cluster (Table 2).

Pasting Viscosity Parameters. All of the pasting viscosity traits were associated with *wx*, which explained 11–19% of the total variance of each trait. All of the traits except peak viscosity (PV) and breakdown viscosity (BD) were also associated with

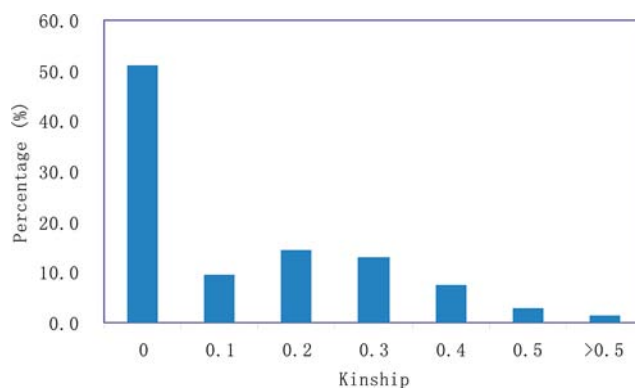


Figure 2. Distribution of the estimated pairwise relative kinship between 50 waxy rice accessions. Values are from SPAGeDi estimates based on a data set of 455 AFLP and ISSR bands.

SSI, which explained 6–11% of the total variance. In addition to these two common loci, PV was associated with *SSII-1*, *SBE1*, and *ISA*. Hot paste viscosity (HPV) and cool paste viscosity (CPV) were also associated with *SSII-1* and *SSII-3*. BD was associated with *SBE3*, *ISA*, and *SSII-3*. Setback (SB) was associated with *SBE3*, *ISA*, and *SSII-3*. Peak time (Ptime) and pasting temperature (PT) were associated with *SSII-3* (Table 2).

Flour Swelling Volume. Flour swelling volume (FSV) was associated with *SBE1*, *GBSSII*, *SSII-1*, and *SSII-3*, and in total, all of the loci explained 21% of the total variance (Table 2).

Gene Interaction Analysis. The starch property parameters may also be controlled by gene–gene interaction, that is, epistatic effects. Except for ΔH_g , BD, and SB, all of the other traits were controlled by two loci epistatic effects ($P < 0.001$) (Supporting Information Supplementary Table 3; Figure 4), suggesting that the genetic mechanism of starch quality in glutinous rice is quite complex.

Thermal Properties. Although *GBSSII*, *AGP_{lar}*, and *SSII-2* were not directly associated with the thermal properties (Table 2), their interactions did have effects on T_o , T_p , T_c , and $\Delta T_{1/2}$. It was evident that *SSI* was the most important player for these interactions; its interaction with *AGP_{sma}* was detected for T_o , T_p , and T_c and $\Delta T_{1/2}$, and its interaction with *GBSSII* was detected for T_o , T_p , and T_c (Figure 4). Each interaction accounted for

Table 2. Summary of the Result of Association Mapping of Starch Quality Parameters in Waxy Rice^a

trait	locus	F	P	R ²	trait	locus	F	P	R ²
<i>T_o</i>	<i>wx</i>	10.4477	2.45 × 10 ⁻⁵	0.1696		<i>SSII-1</i>	5.544	0.0228	0.0506
	<i>SSI</i>	7.3301	0.0017	0.0998		<i>SSII-3</i>	6.431	0.0146	0.0577
	<i>SSII-3</i>	29.6995	1.81 × 10 ⁻⁶	0.1599		CPV	<i>wx</i>	7.424	3.84 × 10 ⁻⁴
<i>T_p</i>	<i>wx</i>	9.5038	5.60 × 10 ⁻⁵	0.1606	<i>SSI</i>		9.671	0.0032	0.0814
	<i>SSI</i>	6.6396	0.0029	0.0928	<i>SSII-1</i>		5.3064	0.0257	0.0484
	<i>SSII-3</i>	29.7418	1.79 × 10 ⁻⁶	0.1605	<i>SSII-3</i>	4.512	0.0389	0.0418	
<i>T_c</i>	<i>wx</i>	7.9838	2.25 × 10 ⁻⁴	0.1478	BD	<i>wx</i>	4.3115	0.0093	0.1187
	<i>SSI</i>	4.9928	0.0109	0.0759		<i>SBE3</i>	6.0803	0.0174	0.0609
	<i>SSII-3</i>	31.1664	1.15 × 10 ⁻⁶	0.1696		<i>ISA</i>	9.0266	0.0043	0.0856
ΔH_g	<i>wx</i>	3.4891	0.0232	0.1007	SB	<i>wx</i>	7.2893	4.37 × 10 ⁻⁴	0.1628
	<i>SSII-3</i>	4.5156	0.0389	0.0468		<i>SSI</i>	6.2465	0.004	0.1063
$\Delta T_{1/2}$	<i>wx</i>	6.8666	6.61 × 10 ⁻⁴	0.1282		<i>SBE3</i>	4.798	0.0335	0.0461
	<i>SSI</i>	7.4093	0.0016	0.0994		<i>ISA</i>	8.3462	0.0058	0.075
	<i>AGP_{sma}</i>	6.8695	0.0118	0.052		<i>SSII-3</i>	10.3467	0.0023	0.0898
	<i>SSII-3</i>	10.6217	0.0021	0.0752		Ptime	<i>wx</i>	6.6415	8.26 × 10 ⁻⁴
ΔH_r	<i>wx</i>	9.0595	8.34 × 10 ⁻⁵	0.1475	<i>SSI</i>		9.92	2.62 × 10 ⁻⁴	0.1163
	<i>SSI</i>	4.6074	0.015	0.0654	<i>SSII-3</i>		27.2455	3.99 × 10 ⁻⁶	0.1416
	<i>SSII-3</i>	19.0918	6.83 × 10 ⁻⁵	0.1132	PT	<i>wx</i>	12.3499	5.06 × 10 ⁻⁶	0.1911
R%	<i>wx</i>	6.8213	6.91 × 10 ⁻⁰⁴	0.1227		<i>SSI</i>	8.7041	0.0049	0.0661
	<i>SSI</i>	4.2672	0.02	0.0614		<i>SSII-3</i>	23.6569	1.33 × 10 ⁻⁵	0.1417
	<i>SSII-3</i>	18.8413	7.50 × 10 ⁻⁵	0.1123	FSV	<i>SBE1</i>	4.1021	0.0229	0.0676
PV	<i>wx</i>	3.9748	0.0135	0.1143		<i>GBSSII</i>	5.3584	0.025	0.0457
	<i>SSII-1</i>	4.5481	0.0382	0.0481		<i>SSII-1</i>	4.4588	0.0401	0.0387
	<i>SBE1</i>	5.0594	0.0292	0.053		<i>SSII-3</i>	7.4042	0.0091	0.0608
	<i>ISA</i>	9.4577	0.0035	0.0914	HPV	<i>wx</i>	6.8577	6.67 × 10 ⁻⁴	0.1505
<i>SSI</i>	7.9521	0.007	0.0694	<i>SSI</i>		7.9521	0.007	0.0694	

^a*T_o*, onset temperature; *T_p*, peak temperature; *T_c*, conclusion temperature; ΔH_g , enthalpy of gelatinization; $\Delta T_{1/2}$, width at half-peak height; ΔH_r , enthalpy of retrograded starch; R%, percent of retrogradation; PV, peak viscosity; HPV, hot paste viscosity; CPV, cool paste viscosity; BD, breakdown; SB, setback; Ptime, peak time; PT, pasting temperature; FSV, flour swelling volume.

19–27% of the total variance (Supporting Information Supplementary Table 3).

Retrogradation Properties. The genetic interaction network for ΔH_r and R% involved *SSI*, *GBSSII*, *AGP_{sma}*, and *AGP_{lar}*, representing a total of four pairs of interactions (Figure 4; Supporting Information Supplementary Table 3). *SSI* was significantly associated with the two traits (Table 2). The epistatic effects contributed 19–25% of the total variance to the trait (Supporting Information Supplementary Table 3).

Pasting Viscosity Parameters. Only interactions *PUL* versus *AGP_{iso}* and *AGP_{sma}* versus *SSI* were found to control PV and CSV. HPV and CPV were controlled by three pairs of interactions, with *PUL* as the central player. Ptime and PT were controlled by two pairs of interactions involving *AGP_{sma}*, *SSI*, and *GBSSII*.

Flour Swelling Volume. FSV was controlled by the interaction effects of *GBSSII* and *AGP_{sma}* (Figure 4), which explained 19.7% of the total variance (Supporting Information Supplementary Table 3).

DISCUSSION

Many genetic studies have been carried out for starch quality parameters in nonwaxy rice, with the results providing the

genetic basis for molecular improvement of grain quality.²⁵ It is widely accepted that the *Wx* gene is responsible for the amylose content, gel consistency, gel texture, and pasting viscosity parameters, whereas the *SSIIa* gene is responsible for gelatinization temperature and thermal properties.²⁵ Retrogradation properties are controlled by both *Wx* and *SSIIa*.²⁶ However, this is not the case for waxy (glutinous) rice because it lacks the *GBSSI* enzyme owing to the defective *Wx* gene. Elucidation of the genetic mechanism underlying the starch quality of waxy rice has attracted much research attention recently.^{2–6,11–13}

Analysis of phenotypic diversity showed that all waxy rice could be divided into two groups based on the GT, a high-GT group (*T_p* from 76.2 to 79 °C) and a low-GT group (*T_p* from 66.9 to 70.5 °C) (Table 1). Most other traits differed significantly between these two GT groups, and also most of the other traits were similar between low-GT indica and low-GT japonica rice (Table 1). These results show that GT mainly determines the starch quality of waxy rice. The GT of rice starch is mainly determined by the alkaline disintegration gene (*alk*), which encodes soluble *SSIIa* (or *SSII-3*) enzyme. Different alleles of *SSIIa* are responsible for differences in

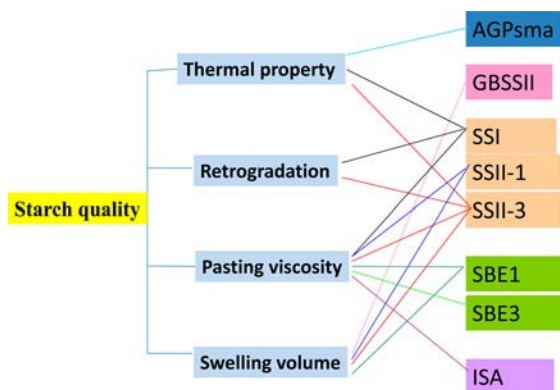


Figure 3. Summary of the starch property–gene association mapping results in waxy rice.

amylopectin structure in that functional SSIIa elongates the short chains within clusters (A+B1 chains) of amylopectin, thus

resulting in a higher GT.²⁷ Association mapping of the thermal, retrogradation, pasting viscosity, and flour swelling volume in this waxy rice panel showed that they were strongly associated with SSIIa, a major gene responsible for GT and thermal properties in rice (Table 2). GT is mainly determined by two (GC/TT and G/A) of four nonsynonymous SNPs in the SSIIa gene.^{28–30} These two functional SNPs, GC/TT and G/A, have been genotyped in this study (Supporting Information Supplementary Table 2). The current results also show that these two SNPs are suitable for predicting GT as shown in the previous studies^{28–30} and are also important markers for molecular breeding of starch quality in waxy rice.

Although the *Wx* gene is defective in waxy rice, the markers in *wx* are still found to be strongly associated with almost all starch quality parameters. Another interesting finding is that *SSI* is also significant for many traits (Table 2). Due to the fact that the *wx*, *SSI*, and *SSI-3* are located in line on chromosome 6 with physical distances of 1.5 and 3.5 Mb between, association

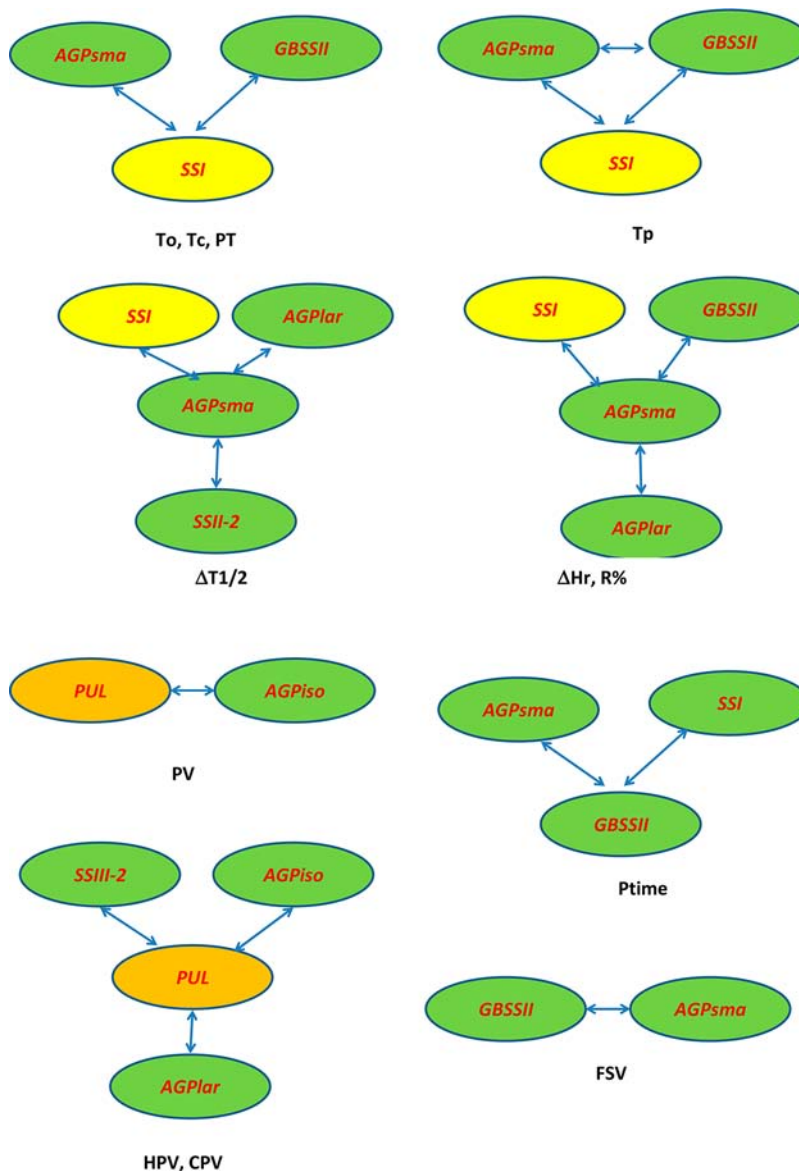


Figure 4. Diagram showing the genetic interactions controlling starch quality parameters. The arrows between any two genes indicate the existence of an interaction between them (also see Supporting Information Supplementary Table 3). The importance of *SSI* and *PUL* is highlighted.

of *wx* markers with starch qualities could be explained by its linkage disequilibrium with the functional genes *SSI* and *SSIIa*.

The genetic control of starch paste viscosity in glutinous rice was studied by Yan et al. using candidate gene association mapping.¹³ This indicated that 10 of 17 SSRGs were involved in controlling the pasting viscosity parameters, and the *PUL* gene was identified as playing an important role in the control of peak viscosity (PV), hot paste viscosity (HPV), cool paste viscosity (CPV), breakdown viscosity (BD), peak time, and paste temperature in glutinous rice. This result is contrary to ours, where the *SSIIa* (*SSII-3*) was the most important player. Two reasons may explain this discrepancy. One is that different waxy rice accessions were used in the association mapping. Yan et al. used more japonica rice (78) than indica rice (20) and also included some javanica rice (4), intermediate rice (8), and African cultivated rice *O. glaberima* (8).¹³ A rice panel with a few complex rice accessions may cause false discovery in association mapping derived from rare samples with distinct starch characters. The present study used 28 indica rices and 22 japonica rices. The relatively balanced sample size in association mapping may reduce the possibility of false discovery. The other reason is that the method used for measuring PV is different. During measurement, we used AgNO₃ to inactivate the α -amylase, which is abundant in waxy rice. Zhu et al. found that amylase activity was one of the main causes of the pasting property differences among waxy rice starches and flours.³¹ Yan et al. have not inactivated α -amylase, presuming that all of their samples had similar α -amylase activities.¹³ Other than this difference, both studies found that *SSII-3* was associated with pasting time, and *SBE3* and *ISA* were associated with SB.

Epistasis is the interaction of alleles at different loci.³² Epistatic effects have been considered as important for control of complex traits.^{13,33} In an attempt to better understand the genetic control of the physicochemical properties of waxy rice, the digenic epistatic relationships among the SSRGs were revealed in this study (Figure 4). Although thermal and retrogradation properties were mainly controlled by *SSIIa*, they were also governed by various interactions among *SSI*, *GBSSII*, *AGPlar*, and *AGPsm*. Yan et al. found the RVA pasting parameters of BD, PT, and Ptime were controlled by gene-gene interactions involving *AGPlar*, *SSI*, and *PUL*, etc.¹³ They found that *PUL* especially is involved in the interactions for all three traits. We also found that the epistatic effects displayed by *PUL* are involved in the control of PV, HPV, and CPV. The presence of epistatic effects for rice quality implies that manipulation of a few major-effect loci may not achieve the goal of improvement of rice grain quality. Future work should be performed to understand the mechanism underlying the genetic network responsible for starch quality.

Waxy rice is widely applied in diverse food processings, but the quality of foods made from waxy rice has been little studied. Future work may focus on the genetics-structure-functionality relationships to clarify what genetic factors determine the fine structure of amylopectin and how the latter determines the functionality of the food. The genetic loci identified in this study, especially *SSIIa*, are useful in molecular breeding of high-quality waxy rice, but the genetic interactions among different loci should be taken into account.

In conclusion, wide genetic differences in starch physicochemical properties exist in waxy rice accessions, with pasting temperature and thermal properties (gelatinization temperature) the most distinct. These starch properties of waxy rice are mainly controlled by the *SSIIa* (*SSII-3*), then by *SSI*. Gene

interactions are also important for genetic control of starch properties of waxy rice. Application of the functional SNPs of *SSIIa* in molecular breeding may facilitate quality improvement of waxy rice.

■ ASSOCIATED CONTENT

📄 Supporting Information

Supplementary Tables 1–3 and Supplementary Figure 1. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

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