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Analysis of Genotypic and Environmental Effects on Rice Starch. 1. Apparent Amylose Content, Pasting Viscosity, and Gel Texture

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Eight rice varieties with wide diversity in apparent amylose content (AC) were selected and planted in the early season and late season of Hangzhou and in the winter season of Hainan for two consecutive years to study the genotype × environment effects on the starch properties of the grain. Analyses of variance showed that AC, cool paste viscosity, breakdown viscosity, setback viscosity, peak time, gel hardness, adhesiveness, and cohesiveness were mainly affected by genotypic variation, whereas peak viscosity and hot paste viscosity were mainly affected by environmental variation. The year × season, year × variety, season× variety, and year × season × variety effects were significant for most traits, indicating significant genotype × environment interactions. AC was significantly correlated with all other parameters except PV. Because the *Wx* gene controls the synthesis of amylose in rice, the mechanism of how the environment affects starch properties is discussed in relation to *Wx* expression and regulation. The implications of the results for rice breeders and starch-based food manufacturers are discussed.

KEYWORDS: **Rice; starch; physicochemical property; pasting viscosity; gel texture; genotype; environment**

INTRODUCTION

Rice (*Oryza sativa* L.) is the principal staple food for half the world's population. Starch is the major chemical component of cereal grains, comprising ~90% of the dry weight of rice grain, so that starch properties determine various aspects of rice quality, especially eating and cooking quality. Previously established physicochemical criteria, such as apparent amylose content (AC), gelatinization temperature (GT), and gel consistency (GC) to evaluate the eating and cooking quality of rice actually reflect the starch properties (*1*). Other important starch property parameters, such as pasting viscosity characteristics (*2*−*5*), gel texture (*6*−*8*), thermal and retrogradation properties (*7*−*10*), and amylopectin structure (*10*, *11*), have been established to evaluate more precisely the end-use quality of cooked rice and starch-based food.

Starch properties are affected by heredity as well as environment. The genetic basis of the AC, GC, GT, pasting viscosity, gel texture, and thermal and retrogradation properties has been studied not only by using traditional quantitative genetic methods but also by recent molecular quantitative genetic approaches (*2*, *3*, *7*, *8*, *12*−*15*). Through quantitative trait locus (QTL) analysis, AC, GC, paste viscosity parameters, and gel texture are mainly controlled by the *Wx* locus as well as other minor QTLs (*3*, *8*, *14*). The *Wx* is known to encode granule-bound starch synthase (GBSS), which is responsible for the synthesis of amylose (*16*). On the other hand, GT and thermal properties are mainly controlled by the *alk* gene closely linked with *Wx* (*12*, *14*). These results indicate that rice genotypes harbor different alleles of the major genes or QTLs, contributing to the difference in the starch properties.

In addition to the genotypic differences, it is also well-known that starch properties vary in different climates and locations and in different seasons and years (*17*−*19*). Oh (*20*) studied the effect of seasonal variation on the physicochemical properties of milled rice by transplanting the rice at different times, finding that setback viscosity showed negligible seasonal variation, whereas AC, peak viscosity (PV), hot paste viscosity (HPV), and breakdown viscosity (BD) showed considerably larger seasonal variation. Studies on grain quality components of rice produced in the middle and southern plain areas (*21*) and in hilly and high altitude areas of Korea (*22*) also revealed significant varietal and locational variation and variety ×

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Table 1. Environmental Conditions for Early Season and Late Season in Hangzhou and Winter Season in Hainan in the Years 1998 and 1999[a]

		early season in Hangzhou				late season in Hangzhou				Hainan			
year		April	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	March
1998	mean temperature (°C)	19.2	22.3	25.1	30.5	30.4	24.5	21.0	15.8	22.0	21.1	21.9	25.3
	sunshine duration (h)	156.3	131.3	118.6	221.7	245.7	173.2	144.4	153.4	160.1	152.7	208.8	184.9
	rainfall (mm)	138.0	155.8	176.2	192.8	44.5	185.0	30.7	25.0	10.3	22.2	6.4	4.9
1999	mean temperature (°C)	16.3	22.0	23.5	26.8	27.7	26.6	20.3	12.9	19.4	20.9	21.2	24.1
	sunshine duration (h)	140.1	167.3	86.8	112.6	145.6	183.5	89.2	117.9	130.7	182.5	167.5	202.3
	rainfall (mm)	136.7	157.4	611.0	169.3	346.4	26.6	60.3	29.9	38.1	12.2	4.3	3.0

[a] Data were obtained from the Bureau of Meteorology of Hangzhou (Zhejiang) and Hainan, respectively.

location interaction variation. Imabayashi et al. (*23*) studied annual and locational variation in the physicochemical properties of rice, finding that amylograph characteristics and AC were affected more by genetic background than by environmental conditions. Variation among years in amylograph characteristics and AC was mainly affected by temperature during the ripening period (*23*). These studies suggest that the effect of environment on the starch properties is significant. However, no full data combining different years, seasons, or locations and genotype have been reported to date.

In China, more emphasis has recently been directed toward the improvement of the eating and cooking quality of rice. Understanding the magnitude of the effects of environmental conditions on starch properties will provide impetus to rice breeding in determining when and how to select starch properties during breeding progress. In the Zhejiang province of eastern China, *indica* rice is generally planted as an early crop, whereas *japonica* rice is generally planted as a late or intermediate crop. However, in a rice-breeding program, *indica* rice is also planted as a late crop right after the harvest of the early crop, which accelerates one generation. After the late crop, rice materials are sent to Hainan province, the southernmost province in China, to advance another generation. Because the environmental conditions differ dramatically between the early and late seasons of Zhejiang province and the winter season of Hainan province, how the environment affects rice starch quality is of great important to rice breeders. In addition, this information is also important to food processors to provide stable starch properties.

In the present paper, a total of eight rice varieties (or breeding lines) with wide variations in amylose content were planted during the early season of Zhejiang province, the late season of Zhejiang province, and the winter season of Hainan province for two consecutive years, and the genotypic, year, and environmental (season) effects on starch properties were studied.

MATERIALS AND METHODS

Plant Materials. Eight rice (*Oryza sativa* L.) genotypes were selected for this study: Zhefu 802 (P1), Jiayu 293 (P2), Zhefu 504 (P3), Jiayu 280 (P4), Zaojing T3 (P5), Zaoxiannuo (P6), Zaojingnuo (P7), and Zaojing T1 (P8). Among them, P5 and P8 are *japonica*, whereas the others are *indica*. All of the genotypes were grown in 1998−1999 in three seasons: the early season of Hangzhou (30° N, HZE) and the late season of Hangzhou (HZL) in Zhejiang province and the winter season in Hainan province (18° N, HN), China. For the early season in Hangzhou, the rice was sown in early April, transplanted on May 1, and harvested in mid-July. For the late-season crop, rice was sown around July 15, transplanted on August 5, and harvested in late October. For the winter-season crop in Hainan, rice was sown in late November, transplanted in late December, and harvested in late March or early April of the next year. The year 1998 denotes the three crops harvested in July and October 1998 and in late March 1999. The year 1999 denotes the three crops harvested in July and October 1999 and early April 2000. **Table 1** summarizes the major environmental conditions, that is, the mean temperature, total sunshine duration, and total rainfall for each month during rice growth.

After being air-dried and stored at room temperature for 3 months, the rice samples were stored in the cooling rooms at 3 °C until all six season rice samples were obtained. The samples were milled to white rice using a Satake rice machine (Satake Corp.) and then ground to flour in a Cyclone sample mill (UDY Corp., Fort Collins, CO).

Apparent Amylose Content (AC). AC was measured according to the method of Perez and Juliano (*24*).

Pasting Viscosity. Rice pasting properties were determined using a Rapid Visco Analyser (RVA, model 3-D, Newport Scientific, Warriewood, Australia) using American Association of Cereal Chemistry Standard Method 61-02 (*25*). Flour (3 g, 12% mb) was mixed with 25 g of 0.5 mM $AgNO_3$ solution in the RVA sample can. The RVA was run using Thermocline for Windows software (version 1.2). A programmed heating and cooling cycle was used; the samples were held at 50 °C for 1 min, heated to 95 °C in 3.8 min, held at 95 °C for 2.5 min before cooling to 50 °C in 3.8 min, and held at 50 °C in 1.4 min. The peak (PV), hot paste (holding) (HPV), and cool paste (final) (CPV) viscosities and their derivative parameters breakdown (BD, $= PV - HPV$) and setback (SB, $= CPV - PV$), and peak time (Ptime) were recorded. The viscosity was measured in Rapid Visco units (RVU).

Gel Texture. The resulting flour gels from RVA analysis were kept in the RVA canister, sealed with Parafilm, and held at room temperature (20−25 °C) for 24 h (*6*). Texture profile analysis was carried out on a TA-XT2*i* texture analyzer (Texture Technologies Corp., Scarsdale, NY) equipped with the Texture Expert software program (version 5.16). A standard two-cycle program was used to compress the gels for a distance of 15 mm at a 4 mm/sec speed using a 7-mm cylindrical probe with a flat end. Texture parameters of hardness (HD, *g*), adhesiveness (ADH, *g.s*), and cohesiveness (COH) were derived from the instrument software.

Statistical Analysis. All of the starch property parameters were measured in duplicate. All of the data analyses were performed with SAS program version 8 (SAS Institute Inc., Cary, NC). Means and ranges were determined using Proc means for the eight genotypes, two years, and three seasons. Analysis of variance (ANOVA) was carried out to determine genotypic and environmental variation among starch properties using the general linear model procedure (Proc glm). Mean squares were used to calculate F statistics for tests of significance. The total variation was the sums of all mean squares of the main and interaction effects. The percentage of the total variation for a specific effect was calculated by dividing its mean square by the total variation. Proc corr was used to examine correlations between these traits.

RESULTS

Amylose Content. ANOVA indicated that AC differed dramatically among genotypes, because the genotypic variation accounted for 95% of the total variation (**Table 2**). Variations for season, year × season, season × genotype, and year × season × genotype were also significant ($P < 0.001$), although in total they accounted for only 5% of the total variation. The year and year × genotype interaction effects were not significant (**Table 2**).

The rice materials selected for this study represented a wide variation in AC: two waxy rices (1.5% AC), two low-amylose

Table 2. Mean Square Values from Analysis of Variance for Apparent Amylose Content, Pasting Viscosity, and Gel Texture Parameters[a]

source	df	AC	PV	HPV	CPV	BD	SB	Ptime	HD	ADH	COH
year	1	0.1	3987.4***	1062.7***	1173.2***	930.6***	837.8***	0.01	90.3***	6.5	0.001
season	2	47.9 ***	14910.2***	7764.5***	9939.2***	1990.6***	742.8***	0.04***	88.5***	117.6	0.001
genotype	7	1042.3***	2300.7***	2807.8***	33555.9***	4872.7***	33016.8***	5.15***	2012.3***	3922.3***	0.083***
year × season	2	3.1***	2633.8***	600.1***	2136.2***	733.5***	3456.4***	0.06***	5.6**	47.1	0.005**
year × variety	7	0.2	124.1***	74.9***	153.7***	144.2***	224.0***	0.01**	15.2***	33.9	0.002**
season × genotype	14	3.3***	630.3***	230.7***	602.1***	305.3***	374.8***	0.07***	36.3***	302.8***	0.001
year × season × genotype	14	0.9***	186.2***	135.5***	137.0***	73.7***	86.6***	0.02***	10.9***	96.4*	0.002***

[a] Abbreviations: df, degree of freedom; AC, apparent amylose content; PV, peak viscosity; HPV, hot paste viscosity; CPV, cool paste viscosity; BD, breakdown; SB, setback; Ptime, peak time; HD, hardness; ADH, adhesiveness; COH, cohesiveness. *, **, and *** indicate significance at 0.05, 0.01, and 0.001 levels, respectively.

Table 3. Mean and Range (in Parentheses) of Apparent Amylose Content, Pasting Viscosity, and Gel Texture Parameters of Different Genotypes at Different Years and Seasons[a,b]

genotype[c]	AC (%)	PV (RVU)	HPV (RVU)	CPV (RVU)	BD (RVU)	SB (RVU)	Ptime (min)	HD (*g*)	ADH (*g.s*)	COH
P1	25.4 a (24.3–27.7)	183.7 b (160.4–231.9)	142.7 ab (121.8–164.2)	285.4 a (266.1–338.1)	41.0 e (28.5–67.7)	101.7 a (88.8–108.8)	6.08 a (5.9–6.35)	35.6 a (29.6–37.9)	–47.1 d (–69.9 to –19.0)	0.546 c (0.52–0.58)
P2	25.7 a (24.2–27.4)	208.7 a (182.3–246.7)	152.0 a (136.2–166.3)	299.9 a (285.7–333.1)	56.7 d (46.1–80.5)	91.2 a (79.6–103.4)	6.08 a (5.9–6.15)	36.5 a (27.3–43.5)	–52.2 d (–69.1 to –29.7)	0.555 c (0.53–0.59)
P3	13.9 c (11.1–15.6)	214.9 a (182.2–253.3)	115.9 c (97.2–128.9)	205.6 c (186.7–232.3)	99.0 a (91.6–118.0)	–9.4 c (–34.9 to 16.8)	5.76 b (5.65–5.85)	9.0 c (7.9–9.9)	–14.0 ab (–21.2 to –9.3)	0.661 b (0.63–0.69)
P4	14.4 c (11.8–16.9)	213.4 a (176.3–249.2)	114.4 c (92.4–135.0)	202.3 c (178.3–229.4)	99.0 a (82.0–120.8)	–11.1 cd (–39.5 to 13.8)	5.78 b (5.70–5.90)	8.4 cd (7.0–9.7)	–12.7 ab (–14.8 to –10.4)	0.674 b (0.62–0.70)
P5	17.6 b (15.5–18.7)	216.9 a (184.5–252.8)	147.5 a (116.8–168.8)	253.1 b (215.1–276.3)	69.4 bc (59.8–90.5)	36.2 b (4.8–48.1)	6.19 a (6.05–6.40)	13.3 b (8.6–18.4)	–22.2 bc (–24.6 to –19.5)	0.663 b (0.63–0.72)
P6	1.6 d (1.5–1.8)	200.5 ab (181.8–217.1)	125.2 bc (108.6–141.0)	165.2 d (144.8–190.6)	75.3 b (66.4–85.8)	–35.4 e (–49.7 to –15.6)	4.66 c (4.50–4.9)	4.7 d (3.2–5.6)	–4.8 a (–6.2 to –3.6)	0.772 a (0.74–0.83)
P7	1.5 d (1.2–1.9)	180.9 b (158.8–202.9)	117.8 c (98.6–136.4)	156.5 d (133.2–185.4)	63.1 cd (49.9–71.1)	–24.4 de (–36.5 to –0.6)	4.63 c (4.40–4.90)	4.6 d (3.5–5.4)	–4.9 a (–9.9 to –1.8)	0.769 a (0.72–0.85)
P8	19.0 b (16.9–20.6)	202.0 ab (170.2–247.4)	140.2 ab (112.9–169.1)	244.4 b (211.3–267.3)	61.8 cd (51.6–78.4)	42.4 b (19.9–56.3)	6.21 a (6.0–6.4)	16.6 b (11.1–19.5)	–25.0 c (–28.4 to –20.0)	0.645 c (0.62–0.68)
year										
1998	14.9 a	209.1 a	135.3 a	230.0 a	73.8 a	26.9 a	5.68 a	17.0 a	–22.6 a	0.664 a
1999	14.8 a	196.2 b	128.6 a	223.1 a	67.6 b	20.9 a	5.67 a	15.1 b	–23.1 a	0.657 a
season[d]										
HZE	13.5 b	209.2 a	141.3 a	234.6 a	67.9 b	25.6 a	5.67 a	14.7 b	–23.6 a	0.666 a
HZL	15.3 a	178.5 b	114.0 b	206.3 b	64.6 b	27.8 a	5.64 a	15.6 ab	–20.7 a	0.653 a
HN	15.9 a	220.2 a	140.6 a	238.7 a	79.6 a	18.5 a	5.71 a	17.9 a	–24.3 a	0.663 a

[a] Different letters in the same column indicate significant difference at the 0.05 level. [b] See **Table 2** for definitions of parameters. [c] P1, Zhefu 802; P2, Jiayu 293; P3, Zhefu 504; P4, Jiayu 280; P5, Zaojing T3; P6, Zaoxiannuo; P7, Zaojingnuo; P8, Zaojing T1. [d] HZE, early season in Hangzhou; HZL, late season in Hangzhou; HN, winter season in Hainan.

rices (14%), two intermediate-amylose rices (18%), and two high-amylose varieties (25%) (**Table 3**), so it was not surprising that AC was mainly affected by the genotypic variation.

The average AC levels were similar in the two years, 14.9% for 1998 and 14.8% for 1999 (**Table 3**). However, AC differed significantly across seasons: 13.5% for season HZE, 15.3% for season HZL, and 15.9% for HN (**Table 3**). For all of the nonwaxy rices, AC in HZE was the lowest in the two years, whereas the highest AC was either in HZL or in HN (**Figure 1**). The range of variation in AC was different between waxy and nonwaxy rices. For waxy rice, there was only as much as 0.7% difference across seasons, whereas for nonwaxy rice there was as much as 5% difference due to environmental effects (**Figure 1**).

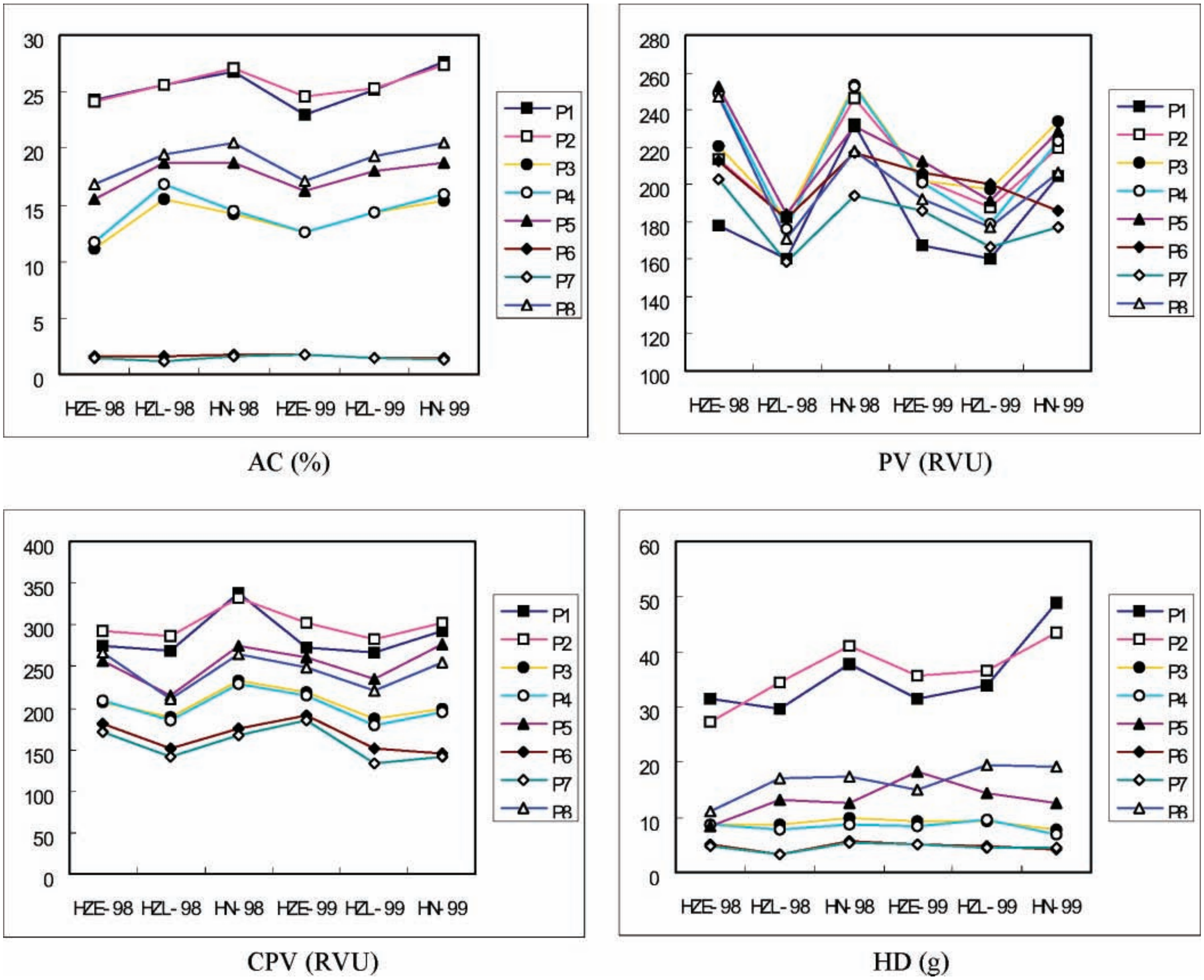
Pasting Properties. ANOVA indicated that all of the variation components for pasting properties were significant ($P < 0.001$) (**Table 2**). However, the variation due to season for PV and HPV accounted for 60.2 and 61.3% of the total variation, respectively, suggesting that PV and HPV were mainly affected by seasonal effects, although year, genotype, and year × season effects also accounted for a large portion of the total variation. On the other hand, genotypic effects on CPV, BD, SB, and Ptime were significant, accounting for 70.4, 53.8, 85.2, and 96.1% of the total variation, respectively (**Table 2**).

PV of different genotypes ranged from 180 RVU in P7, a waxy rice, to 214.9 RVU in P3, a low-amylose rice, a range of 34 RVU (**Table 3**). The average PV across different years and genotypes was lowest in HZL season (178 RVU) and highest in HN (220 RVU) (**Table 3**; **Figure 1**). Although the PV was significantly different in the two years, the difference was relatively small, 209 RVU in 1998 versus 196 RVU in 1999 (**Table 3**).

The range of HPV for different genotypes was 38 RVU, ranging from 114 RVU for P4 to 152 RVU for P2 (**Table 3**). Similar to PV, season had great effects on the variation in HPV, being lowest in HZL and highest in HZL or HN. The mean HPV was similar in the two years (**Table 3**).

Unlike PV and HPV, CPV was mainly affected by genotypic variation. There was a wide difference among different genotypes (**Table 3**; **Figure 1**), the highest being 300 RVU for P2, a high-amylose rice, and the lowest being 157 RVU for P7, a waxy rice. The season also significantly affected CPV, accounting for 20.8% of the total variation (**Table 2**). HZE and HN had higher mean CPV than HZL, but no difference in CPV was found between the two years (**Table 3**; **Figure 1**).

BD and SB were derived from PV minus HPV and CPV minus PV, respectively. However, ANOVA indicated that they were mainly affected by genotypic variation, more like CPV



AC (%)

PV (RVU)

CPV (RVU)

HD (g)

Figure 1. Genetic and environmental variation in some properties of starches from rice genotypes: P1, Zhefu 802; P2, Jiayu 293; P3, Zhefu 504; P4, Jiayu 280; P5, Zaojing T3; P6, Zaoxiannuo; P7, Zaojingnuo; P8, Zaojing T1; HZE, early season in Hangzhou; HZL, late season in Hangzhou; HN, Hainan; AC, amylose content; PV, peak viscosity; HPV, hot paste viscosity; HD, hardness.

than like PV or HPV. The lowest BD was 41 RVU for P1, a high-amylose variety, and the largest was from P3 and P4, the low-amylose varieties. Conversely, P1 had the highest SB, whereas the two waxy rices had the lowest SB. For BD, the seasonal variation accounted for 22% of the total variation (**Table 2**), and HN had higher mean BD than HZE and HZL (**Table 3**). Although variation due to year was significant ($P <$ 0.001, **Table 2**) and BD was significantly different in the two years (**Table 3**), the difference was quite small, only 6 RVU. The year × season interaction effects for SB were quite large, accounting for 8.5% of the total variation (**Table 2**). However, the average SB was not significantly different in different years or seasons (**Table 3**).

Peak time (Ptime) is the time at which the viscosity reaches its peak. Due to lack of amylose, waxy rice had a characteristic RVA profile and reached the peak very quickly (4.6 min) (**Table 3**). ANOVA showed that Ptime was mainly affected by genotypic variation, whereas other sources accounted for only a very small part (**Table 2**). The average Ptime was almost the same in different years or seasons (**Table 3**).

Textural Properties. The textural parameters HD, ADH, and COH of the starch gel were mainly affected by genotype (**Table 2**), which accounted for 89.1, 86.7, and 87.4% of the total variation, respectively. The HD was also influenced by the year and season, whereas ADH and COH were not. As expected, because the waxy starch pastes form very weak gels, the HD was the smallest (4.6 g) for them among all of the genotypes (**Table 3**). The high-amylose rice had the highest gel HD (**Table 3**). In contrast, the gel of waxy rice had a higher ADH and COH than did nonwaxy rice gels. HD was significantly different in the two years and three seasons (**Table 3**; **Figure 1**). The HN season had a higher HD than HZE and HZL. However, the gel ADH and COH levels were similar across different years and seasons (**Table 3**).

Correlation Analysis. The starch property parameters of eight genotypes either in a full data set from six seasons among two years ($n = 48$) or in an averaged data set from six seasons ($n = 8$) were used to correlation analysis (**Table 4**). AC was not correlated with PV, but was significantly correlated with nearly all of the other parameters (**Table 4**). PV was correlated with only HPV, CPV, and BD with the full data set ($n = 48$) but had no correlation with all parameters with the genotypic average data set ($n = 8$). HPV was correlated with CPV, SB, HD, ADH, and COH in the full data set. However, in the

Table 4. Correlation Analysis of Starch Properties of Rice Genotypes with the Full Data from All Seasons (above Diagonal, $n = 48$) and with Genotype Mean Data (below Diagonal, $n = 8$)[a,b]

parameter	AC	PV	HPV	CPV	BD	SB	Ptime	HD	ADH	COH
AC		0.103	0.461**	0.892***	–0.319*	0.880***	0.908***	0.849***	–0.817***	–0.909***
PV	0.226		0.591***	0.335*	0.633***	–0.17	0.255	–0.036	–0.051	–0.007
HPV	0.710*	0.048		0.774***	–0.25	0.501***	0.469***	0.525***	–0.581***	–0.368*
CPV	0.968***	0.161	0.853**		–0.340*	0.871***	0.805***	0.847***	–0.835***	–0.811***
BD	–0.384	0.651	–0.727*	–0.537		–0.686***	–0.144	–0.547***	0.497***	0.346*
SB	0.916**	–0.102	0.847**	0.965***	–0.714*		0.708***	0.905***	–0.846***	–0.845***
Ptime	0.923**	0.453	0.616	0.855**	–0.156	0.742*		0.607***	–0.645***	–0.769***
HD	0.870**	–0.144	0.769*	0.921**	–0.684	0.967***	0.623		–0.904***	–0.852***
ADH	–0.910**	0.046	–0.803*	–0.957***	0.642	–0.977***	–0.698	–0.993***		0.796***
COH	–0.980***	–0.107	–0.667	–0.953***	0.433	–0.932***	–0.836**	–0.927***	0.947***	

[a] *, **, and *** indicate significance at 0.05, 0.01, and 0.001 levels, respectively. [b] See **Table 2** for definitions of parameters.

genotypic average data set, HPV had no correlation with COH, but had negative correlation with BD ($r = -0.727$, $P < 0.05$). CPV was correlated with all other parameters in the full data set, but was not correlated with PV and BD in the genotypic average data set. Similarly, the correlation of BD with Ptime, HD, ADH, and COH was not convincing because the coefficients were significant in only one set of data. However, the significant correlations of SB with BD, Ptime, HD, ADH, and COH were consistent and so were the correlations of HD with ADH and COH (**Table 4**).

DISCUSSION

The starch physicochemical traits of rice grain are affected by heredity as well as environment. The *Wx* locus, which is the major QTL associated with AC, can be identified in all environments, whereas other QTLs with minor effects are not (*12*). The present study showed that AC was mostly subject to genotypic variation, which is in agreement with previous results (*26*, *27*). These studies indicated that different alleles of the *Wx* gene correspond to different AC (*26*, *27*). Genetic analysis has also shown that the genotype × environmental effect for AC is significant (*28*). The nonwaxy rice had different AC levels when planted in different seasons (**Table 3**). It is reported that the same cultivar grown in different environments may vary by up to 6% in AC (*29*). The major environmental factor is temperature during seed development (*30*, *31*). The temperatures during plant growth, particularly during seed development, in the three seasons fluctuate greatly (**Table 1**), so that the effect of temperature on the synthesis of AC is distinct. The mechanism of how the temperature regulates the synthesis of amylose has been clarified (*30*, *31*). As in AC, the amount of GBSS increases at cool temperatures when compared with plants grown under warmer conditions (*30*). *Wx* gene expression was activated reversibly in response to cool temperatures, as noted by higher levels of *Wx* transcript at cooler temperatures. The longer rice plants were exposed to cool temperatures, the higher the levels of *Wx* protein and AC. In transgenic plants with the β-glucuronidase gene under the control of the *Wx* gene promoter, enhancement of β-glucuronidase activity was also detected at cooler temperatures, suggesting that the *Wx* promoter is temperature sensitive (*30*). The single nucleotide polymorphism of AG$\underline{\text{G}}$TATA and AG$\underline{\text{T}}$TATA at the leader intron 5′ splice site displays differential temperature sensitivity (*31*). Cultivars with the sequence AG$\underline{\text{T}}$TATA were reported to have a substantial increase in accumulation of mature *Wx* transcripts at 18 °C compared to at 25 or 32 °C (*31*). The Rice genotypes Zhefu 802 (P1) and Jiayu 293 (P2) have the AG$\underline{\text{G}}$TATA sequence, whereas the others have the AG$\underline{\text{T}}$TATA sequence (*32*) in the present study.

In a diallele analysis, it is found that the inheritance of paste viscosity profiles appeared to be controlled by a single locus with genes with additive effects (*2*, *13*). It is also found that these parameters are subject to environmental effects (*13*). Through QTL mapping, all of the RVA pasting viscosity parameters are mapped at the *Wx* locus except for PV (*3*). These parameters are significantly correlated with AC either in the all-data or in the averaged data systems (**Table 4**). However, PV was mapped at different loci in two environments (*3*) and had no correlation with AC in the present study (**Table 4**), suggesting that it is under other genetic control and is mainly affected by environment. In addition to the genetic effects, environment still exerts a great influence on the pasting viscosity parameters, particularly PV and HPV, as shown in the present study (**Table 2**). The variations of season and year for these two parameters are larger than those of genotypic variation (**Table 2**). The reason PV and HPV are susceptible to environmental effects is not clear. For other parameters that were mainly affected by the genotypic variation (**Table 2**), part of the mechanism of how environment regulates these viscosity parameters should be the same as for AC. However, many factors other than AC are reported to influence the RVA pasting viscosity parameters, such as protein and lipids (*33*). Proteins contribute to peak height, offset thixotropy, and contribute to the final viscosity, and removing lipids alters the structure of the paste significantly, which consequently alters viscosity curves (*33*). Shi et al. indicated that protein content was also affected by genotype × environment interaction effects (*34*). It might be deduced that differences in the total protein content arising from the environment may influence RVA pasting viscosity parameters. The drying condition (*11*) and storage condition (*35*) would modify the RVA viscosity to some extent, so the fine mechanism would be unexpectedly complex and requires further study.

Vandeputte et al. indicated that gel textural characteristics were related to absolute, free, and lipid-complexed AC (*36*). This is supported by the present study, in which the gel textural properties were significantly correlated with AC ($P < 0.01$). Therefore, it is logical that these parameters were mainly affected by the genotypic variation as for AC. Bao et al. also indicated that gel HD and ADH were mainly controlled by the *Wx* locus, whereas COH was controlled by a genomic locus between *Wx* and *alk* (*8*). So far, few studies have been made on the effects of environmental conditions on gel textural properties, but the mechanism by which the environment regulates HD should be similar in part to that for AC. The present study showed that HD was influenced by year and season, whereas ADH and COH were quite stable across different years and seasons.

The present study has implications for rice breeders and processors. Rice planted in different seasons will vary greatly in starch properties. Hence, rice breeders should carefully select the starch properties of breeding lines in suitable conditions. PV and HPV could not be efficiently selected when rice is planted in different seasons, whereas BD and SB are useful across different seasons. For food processors, the best way to keep the desired quality of the starch-based food is to use rice produced under the same environment (year, season, or location) so as to maintain minimum variation in starch pasting and textural properties of a genotype. It is apparent that even the same rice variety harvested during different seasons or from different locations cannot guarantee the same starch qualities.

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On August 30, 2004, values in columns SB and ADH of Table 3 were corrected from the original posting of August 20, 2004.

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