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QTL mapping for the paste viscosity characteristics in rice (*Oryza sativa* L.)

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Abstract In order to understand the genetic basis of the paste viscosity characteristics (RVA profile, which is tested on the Rapid Visco Analyser) of the rice grain, we mapped QTLs for RVA profile parameters using a DH population derived from a cross between an *indica* variety, Zai-Ye-Qing 8 (ZYQ8), and a *japonica* variety, Jing-Xi 17 (JX17). Evidence of genotype-by-environment interaction was found by comparing the mapped QTLs between two locations, Hainan (HN) and Hangzhou (HZ). A total of 20 QTLs for six parameters of the RVA profiles were identified at least one location. Only the *waxy* locus (*wx*) located on chromosome 6 was detected significantly at both environments for five traits, i.e. hot paste viscosity (HPV), cool paste viscosity (CPV), breakdown viscosity (BDV), consistency viscosity (CSV) and setback viscosity (SBV). This locus explained 19.5%–63.7% of the total variations at both environments, suggesting that the RVA profiles were mainly controlled by the *wx* gene. HPV, CPV, BDV, CSV and SBV were also controlled by other QTLs whose effects on the respective parameter were detected only in one environment, while for the peak viscosity (PKV), only 2 QTLs, 1 at HN,the other at HZ, were identified. These results indicate that RVA profiles are obviously affected by environment.

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Introduction

The rice starch viscosity profile, also termed the RVA profile (because it is tested on the Rapid Visco Analyser, RVA), is a pasting curve generated from rice flour when the latter is subjected to a standard temperature-programmed heat-hold-cool-hold protocol. The RVA profile has proved useful in the estimation of rice eating and cooking quality in rice breeding programs (Juliano 1996; Shu et al. 1998; Bao 1999; Bao and Xia 1999). By analyzing the relationship between RVA profiles and other eating quality parameters, Shu et al. (1998) found that both setback viscosity (SBV) and consistency viscosity (CSV) had a significantly positive correlation with the apparent amylose content but a negative correlation with the adhesiveness of cooked rice. Because the amount of flour from a single seed is not enough for a viscosity test on the RVA, only a few investigations have been conducted on the genetic basis of the RVA profiles (Bao and Xia 1999). Gravois and Webb (1997) studied the inheritance of RVA profiles and suggested that peak viscosity (PKV), hot paste viscosity (HPV) and cool paste viscosity (CPV) are controlled by a single locus with additive effects. This suggestion was supported by both $F_{2:3}$ segregation analysis and diallel analysis (Gravois and Webb 1997). Recently, Bao and Xia (1999) proposed that the viscosity profile characteristics of *indica* rice are controlled by the effects of the seed itself, cytoplasm and maternal plant. However, these studies could not locate the possible genes controlling the RVA profiles on the rice chromosomes. Since starch viscosity tested on the RVA is a physiochemical property of rice starch, the genes involved in the pathways of starch biosynthesis in rice should have some effects on the inheritance of RVA profiles.

In the study presented here, a double haploid (DH) population derived from an *indica-japonica* combination

and its genetic linkage map were employed to map the locus underlying six parameters of RVA profiles at two locations. The results further revealed that the RVA profiles were controlled by quantitative trait loci (QTLs), of which a major QTL is the *waxy* gene (*wx*) on rice chromosome 6, which encodes the granule-bound starch synthase.

Materials and methods

Plant materials

A DH population consisting of 132 DH lines established in our laboratory was used in this study. This population was developed through anther culture of the F_1 hybrid between *indica* rice var. Zhai-Ye-Qing 8 (ZYQ8) and j*aponica* var. Jing-Xi 17 (JX17) (Zhu et al. 1993). Two parents and the DH lines were planted in three rows in Hainan (HN,18°N) in the late November of 1997 and in Hangzhou (HZ, 32°N) in early May of 1998. The rice plants of each line were harvested in late March (HN) and late September (HZ), 1998. Due to low seed setting in some lines, only102 lines in HN and 100 lines in HZ were finally used to test the RVA profiles.

RVA profile

The RVA paste viscosity was determined on a Rapid Visco Analyser according to the American Association of Cereal Chemists Standard Method AACC 61–02 (1995) as described by Bao and Xia (1999). The method specifies 3 g of rice flour in 25 ml of water. The analyser was a model RVA-3D (Newport Scientific Pty, Warriewood, Australia), which runs with Thermocline Windows control and analysis software, Version 1.2. Rice paste viscosity characteristics were described by three important measurements of the pasting curve: peak viscosity (PKV), hot paste viscosity (HPV) and cool paste viscosities (CPV). In addition, breakdown (BDV), setback (SBV) and consistency viscosity (CSV) could be derived from HKV minus HPV, CPV minus PKV and CPV minus HPV, respectively. All the viscosity parameters were measured in Rapid Visco Units (RVU) according to the American Association of Cereal Chemists Standard Method AACC 61–02 (1995).

Restriction fragment length polymorphsim (RFLP) map construction and QTL detection

The molecular linkage map referred to was that of Lu et al. (1996) and Xu et al. (1998). A total of 243 RFLP and microsatellite markers (Xu et al. 1998) distributed on all 12 rice chromosomes were selected to construct a rice linkage map using MAPMAKER/EXP version 3.0 (Lander et al. 1987; Lincoln et al. 1993a). Of the microsatellite markers, some that could only be mapped in the present population were denoted by GAXX, CTXX, ATTXX and ATCXX (Xu et al. 1998), whereas others that could be mapped in different populations were denoted as RMXX, which followed the Cornell nomenclature (Chen et al. 1997). Interval QTL mapping was carried out using the software MAPMAKER/QTL ver 1.1 (Lander and Botstein1989; Lincoln et al. 1993b). For a small DH population, a less stringent LOD threshold of 2.0 was used for declaring the presence of a putative QTL. In addition, the additive effect and the percentage of variation explanation of individual QTLs were also estimated.

Results and analysis

The performances of the RVA profiles of the parents and DH lines in two environments

The two parents showed significant differences in RVA profiles in both HN and HZ (Fig 1). For *indica* rice ZYQ8, all parameters in HN were much larger than those in HZ (Table 1), while for *japonica* rice JX17, some parameters were larger in HN than those in HZ but the others were not. All parameters of ZYQ8 were much larger than those of JX17 in both places except for the BDV. In HN, ZYQ8 and JX17 had similar values of BDV, but in HZ ZYQ8 showed a smaller value than JX17 (Table 1). The RVA profiles were continually distributed among the DH lines. Although some DH lines showed transgressive segregation for all the parameters (data not shown), the mean value of each parameter of the DH lines in both environments was near the mid-parent value (Table 1).

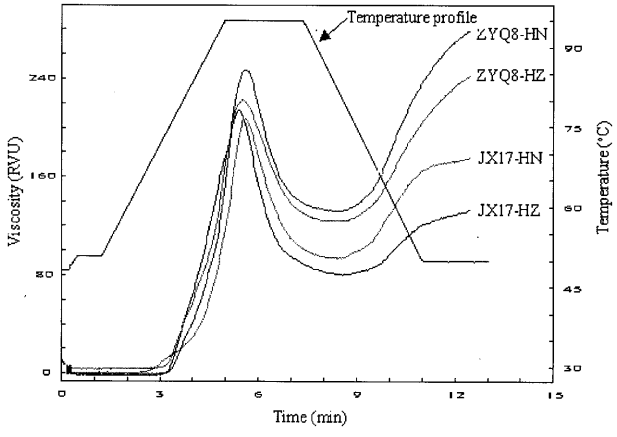

Fig. 1 The paste vicosity curves of the two parents, ZYQ8 and JX17, in two environments, Hainan (*HN*) and Hangzhou (*HZ*)

Table 1 RVA profile characteristics of the parents, ZYQ8 and JX17, and DH lines in two environments, HN and HZ (RVU)

Traits[a]	Hainan (HN)			Hangzhou (HZ)		
	ZYQ8	JX17	DH lines	ZYQ8	JX17	DH lines
PKV	247.0	206.9	209.8 ± 28.7	222.7	213.9	215.3 ± 30.5
HPV	131.9	93.8	108.5 ± 27.4	123.9	80.3	104.8 ± 21.2
CPV	279.4	174.6	216.8 ± 75.7	242.4	132.8	191.7 ± 55.5
BDV	115.1	113.2	101.3 ± 26.6	98.8	133.7	110.6 ± 28.8
CSV	147.5	80.8	108.3 ± 50.3	118.5	52.6	86.6 ± 37.5
SBV	32.4	–32.4	7.0 ± 70.6	19.7	–81.7	–23.6 ± 61.8

[a] For definition, see Materials and methods, RVA profile

Table 2 QTL analysis of the RVA profiles

Traits	Locus[a]	Chromosomes	Marker interval	Place[b]	LOD score	Additive effect	Percentage variation
PKV	qPKV-2	2	GA120-G357	HZ	2.18	22.8	14.0
	qPKV-12	12	RG413-G2140	HN	2.39	–18.6	10.6
HPV	qHPV-6-1	6	*Wx*	HN	8.13	–31.7	33.6
				HZ	4.14	–18.8	19.5
	qHPV-6-2	6	CT506-C235	HZ	2.3	–14.6	11.6
CPV	qCPV-1	1	C949-C385	HZ	2.08	–35.8	10.5
	qCPV-6-1	6	*Wx*	HN	13.86	–115.0	57.9
				HZ	9.39	–70.1	39.6
	qCPV-6-2	6	CT506-C235	HZ	2.56	–40.2	12.8
BDV	qBDV-1	1	RG612-C131	HN	2.66	20.7	14.5
	qBDV-5	5	RG573-C624	HN	2.29	18.7	10.4
	qBDV-6	6	*Wx*	HN	6.24	28.0	27.8
				HZ	7.27	31.1	29.0
	qBDV-7	7	RG769-RG528	HZ	2.14	21.3	10.4
	qBDV-12	12	ATT42A-G196	HN	2.66	18.7	12.3
CSV	qCSV-1	1	C949-C385	HZ	2.06	–23.7	10.0
	qCSV-6-1	6	*Wx*	HN	16.31	–80.1	63.7
				HZ	10.95	–50.5	45.0
	qCSV-6-2	6	CT506-C235	HZ	2.05	–24.8	10.7
	qCSV-7	7	TCT122-RG769	HZ	2.18	–27.7	10.4
SBV	qSBV-1	1	RG612-C131	HN	2.09	–48.9	11.5
	qSBV-5-1	5	C624-G81	HN	2.29	–49.0	10.1
	qSBV-5-2	5	RG470-GA41	HZ	2.10	–51.0	13.3
	qSBV-6	6	*Wx*	HN	14.65	–106.0	56.8
				HZ	8.16	–70.3	32.5

[a] QTL nomenclature follows that of McCouch et al. (1997)
[b] HN and HZ, Hainan and Hangzhou, respectively

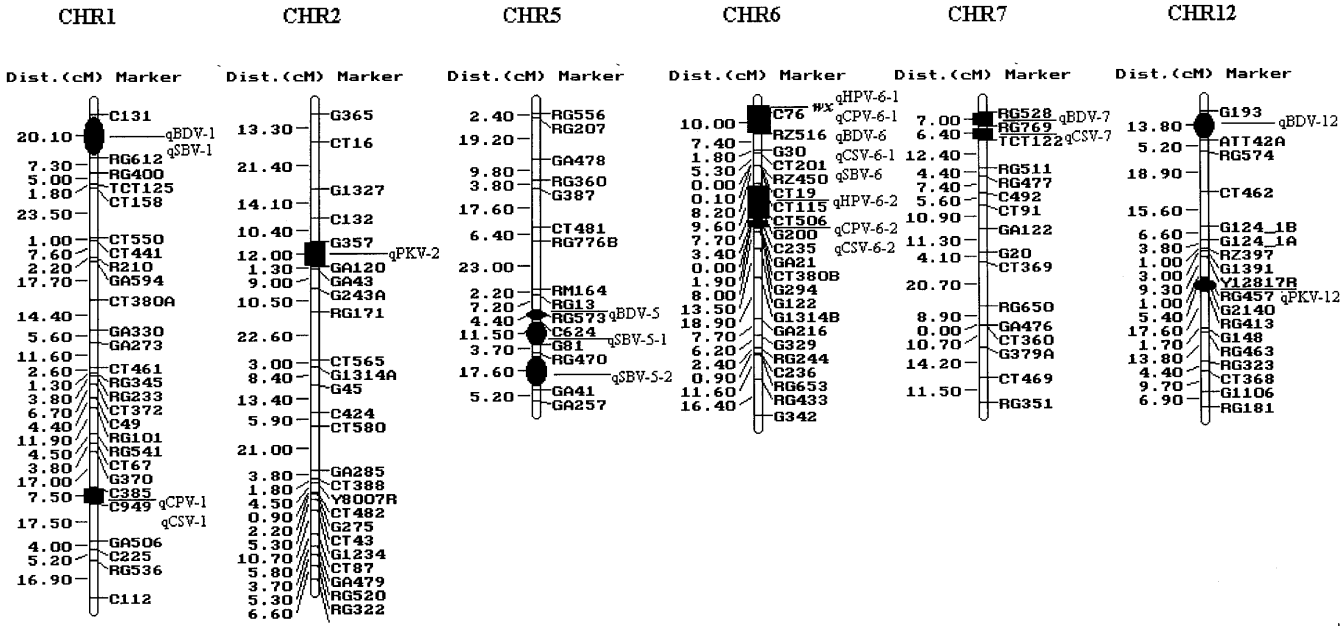

Fig. 2 Some of the rice chromosomes associated with rice paste viscosity characteristics in the ZYQ8/JX17 DH population. Distances in Kosambi centiMorgans (*cM*) are shown on the *left* of each chromosome. The major genes and QTL positions for rice paste viscosity parameters are shown on the *right* of each chromosome

QTL analysis for RVA profile parameters

A rice linkage map was constructed using this DH population, and 243 markers evenly distributed on all 12 chromosomes were selected for QTL identification for the rice RVA profile with MAPMAKER/QTL software. The QTL positions and their biometrical parameters are shown in Table 2 and Fig. 2.

Peak viscosity

For PKV, 2 QTLs were identified, each being significant in only one environment. The QTL, qPKV-2, on chromosome 2 was detected in HZ; this locus from JX17 could increase the PKV by 22.8 RVU and explain 14.0% of the total variation. However, this locus could not be detected in HN, where another QTL, qPKV-12, was identified.

The positive effect of qPKV-12 from ZYQ8 could increase PKV by 18.6 RVU.

Hot paste viscosity

For HPV, a QTL, qHPV-6–1, was detected in the interval on chromosome 6 where the *wx* gene was also located in both environments. It explained 33.5% (HN) and 19.5% (HZ) of the total variation. It should be noted that He et al. (1999) mapped an allele of *wx* as a major QTL that could account for 91.1% of the phenotypic variation of amylose content in the same population of ZYQ8/JX17. Therefore, qHPV-6–1 should also be an allele for *wx*. The allele from ZYQ8 could increase the HPV by 31.7 RVU in HN and 18.8 RVU in HZ. In addition, there was another QTL, qHPV-6–2, on chromosome 6, which was detectable only in HZ. This QTL was located between CT506 and C235, where the gene *alk* (alkali degeneration) is situated. Interestingly, He et al. (1999) identified *alk* as a major QTL for one of the rice grain quality parameters, alkali spreading score. Therefore, qHPV-6–2 should be the same locus as the *alk* gene.

Cool paste viscosity

For CPV, 3 QTLs were identified in two environments. In HN, only the interval of the *wx* gene was significant; this locus explained as much as 57.9% of the total variation. Therefore, this QTL, qCPV-6—1, should also be the *wx* locus. The other 2 QTLs, qCPV-1 and qCPV-6–2, on the chromosome 1 and 6, respectively, were detected in only HZ, and again the qCPV-6–2 was located in the interval of the *alk* gene.

Breakdown viscosity

Five QTLs were found for BDV in both environments. A major QTL, qBDV-6, located in the interval of *wx*, explained 27.8% of the total variation in HN and 29.0% in HZ. The other 4 QTLs, qBDV-1, qBDV-5, qBDV-7 and qBDV-12 were located on the chromosomes 1, 5, 7 and 12, respectively, and they could interpret 14.5%, 10.4%, 10.4% and 12.3% of the total variation, respectively. qBDV-5 shared the same interval with the minor QTL for amylose content that was also detected in the abovementioned study by He et al. (1999).

Consistency viscosity

In HN, only 1 QTL, qCSV-6–1, in the interval of *wx* was detected to be significant for the consistency viscosity. It could explain as much as 63.7% of the total variation. The allele from *indica* parent ZYQ8 could increase the CSV by 80.1 RVU. In HZ, the effect of qCSV-6–1 was also detected, explaining 45% of the total variation and increasing the CSV by 50.5 RVU. Therefore, qCSV-6–1 should be considered to be an allele of wx. Additionally, the other 3 QTLs, qCSV-1, qCSV-6–2 and qCSV-7, on chromosomes 1, 6 and 7, respectively, were also identified in HZ, and each could explain about 10% of the total variation. qCSV-6–2 was in the interval of *alk*, and qCSV-7 was closely linked to qBDV-7.

Setback viscosity

A total of 4 QTLs were detected for SBV. One QTL, qSBV-1, showed its effect only in HN. The other 2 QTLs, qSBV-5–1 detected in HN and qSBV-5–2 in HZ, were closely linked on chromosome 5. It is noted that qSBV-1 and qSBV-5–1 were mapped to the same regions as qBDV-1 and qBDV-5, respectively. In addition, a QTL, qSBV-6, located in the interval of *wx*, could account for more than 30% of the total variation in both places.

Discussion

Because the amount of rice flour obtained from a single seed is not enough for a test of paste viscosity, very little information on the genetic basis of paste viscosity has been available so far. Elucidation of the genetic basis of the RVA profiles will assist the rice breeding process. The genetic homology of the plants of each DH line allowed us to test the starch viscosity on the RVA provided approximately 3 g of rice sample could be collected from each line. Recently, Gravois and Webb (1997) revealed that paste viscosity appeared to be controlled by one major gene with an additive effect. On the basis of our mapping QTLs for each of six RVA profile parameters in two locations, we conclude that the gene suggested by Gravois and Webb (1997) must be the *wx* gene on chromosome 6, which encodes the granule-bound starch synthase. The effects of the *wx* gene were significantly detected for five parameters, HPV, CPV, BDV, CSV and SBV, though they were also affected by other minor QTLs (Table 2). Since none of these minor QTLs could be detected in both HN and HZ, we suggest that the RVA profiles were affected by genotype × environment interaction effects. This suggestion is in agreement with Gravois and Webb (1997) who found that both the year × entry and year × GCA (general combining ability) for PKV, HPV and CPV were significant at the 0.01 probability level.

Rice grain quality is an endosperm trait, with its inheritance being rather complicated because the genetic expression for an endosperm trait in cereal seeds is conditioned not only by the triploid endosperm genotype but also by the diploid maternal genotype and additional possible cytoplasm differences (Zhu and Weir 1994; Bao 1999; Bao and Xia 1999). Zhu and Weir (1994) proposed an endosperm model for analysis of the cytoplasm and maternal plant effects. Mo (1995) put forward a mating

design and a corresponding statistical method, with which the genetic effects of the endosperm and maternal genotype as well as cytoplasm differences can be independently tested. However, neither model can be used to identify the related genes on rice chromosomes. It seems that the RVA profiles are also controlled by both seed direct effects and maternal plant effects (Bao and Xia 1999). However, in the present study, we were unable to dissect the additive effects from the seed and maternal plant.

Shu et al. (1998) found that the BDV of rice grains with accepted good eating quality is higher than 100 RVU, while the SBV is below 25 RVU. Our present results suggest that *indica-japonica* hybridization is a good way to breed good quality rice. When the *wx* gene from *japonica* rice is introduced into the *indica* background, the SBV decreases and BDV increases (Table 2). These changes in the RVA pasting profiles indicate that the eating quality has been improved as compared to the quality of the *indica* rice. Ayres et al. (1997) reported a polymorphic microsatellite marker closely linked to the *wx* gene; this marker can be applied to rice eating quality improvement with marker-assisted selection. Such selection will achieve the desired RVA profile characteristics as well as the desired apparent amylose content in the improved rice according to our present study and He et al. (1999). The marker-assisted selection toward these targets is at present underway in our laboratory.

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