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ORIGINAL PAPER

Genetic basis of 17 traits and viscosity parameters characterizing the eating and cooking quality of rice grain

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Abstract A recombinant inbred line population derived from a cross between Zhenshan 97 and Delong 208 was used to analyze the genetic basis of the cooking and eating quality of rice as reflected by 17 traits (or parameters). These traits include amylose content (AC), gel consistency (GC), alkali spreading value (ASV), cooked rice elongation (CRE), and 13 parameters from the viscosity profile. All the traits, except peak paste viscosity (PKV), time needed from gelatinization to peak (BAtime), and CRE, can be divided into two classes according to their interrelationship. The first class consists of AC, GC, and most of the paste viscosity parameters that form a major determinant of eating quality. The second class includes ASV, pasting temperature (Atemp) and pasting time (Atime), which characterize cooking process. We identified 26 QTL (quantitative trait locus or loci) in 2 years; nine QTL clusters emerged. The two major clusters, which correspond to the *Wx* and *Alk* loci, control the traits in the first and second classes, respectively. Some QTL are co-located for the traits belonging to the same class and also for the traits to a different class. The *Wx* locus also affects on ASV while the *Alk* locus also makes minor contributions to GC and some paste viscosity parameters. The QTL clusters on other chromosomes are similar to the *Wx* locus or *Alk* locus, although the variations they explained are relatively minor. QTL for CRE and PKV are dispersed and independent of the *Wx* locus. Low paste viscosity corresponds to low AC and soft gel, which represents good eating quality for most Chinese consumers; high ASV and low Atemp, together with reduced time to gelatinization and PKV, indicate preferred cooking quality. The genetic basis of Atemp, Atime, BAtime, peak temperature, peak time, paste viscosity at 95°C, and final paste viscosity is newly examined to reveal a complete and dynamic viscosity profile.

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Introduction

Rice is the staple food for about half of the world's population. Its grain quality has received increasing attention in recent years in many rice-producing countries including China. Cooking and eating quality is one of the most important components of grain quality (Juliano 1985; Unnevehr et al. 1992; Ge et al. 2005).

The eating and cooking quality of rice has usually been evaluated by three major physical and chemical characteristics of the starch as indirect indexes: amylose content (AC) (Juliano 1985), gel consistency (GC) (Cagampang et al. 1973), and alkali spreading value (ASV) (Little et al. 1958). ASV is generally considered to be inversely related to gelatinization temperature (GT). Most molecular marker-based QTL (quantitative trait locus or loci) analyses have focused on these indirect traits (He et al. 1999;

Tan et al. 1999; Bao et al. 2000a; Lanceras et al. 2000; Septiningsih et al. 2003; Aluko et al. 2004; Tian et al. 2005; Fan et al. 2005). Tan et al. (1999) proposed that the waxy gene (*Wx*) region (locus) controls all these three traits. He et al. (1999) also reported a major QTL with significant effect on AC at the *Wx* locus, but detected no QTL for GC on chromosome 6. However, they proposed that the *Alk* locus is the major QTL involved in the control of ASV. Umemoto et al. (2002) confirmed this and demonstrated that the *Alk* locus encodes the enzyme Soluble Starch Synthase IIa. Recently, Tian et al. (2005) found that the *Alk* locus predominantly controls the ASV, and the *Wx* locus is the major QTL specifying AC and a minor one for GC, but no effect on ASV. Fan et al. (2005) reported similar results. However, they also found that the *Wx* locus affects ASV, and proposed that the locus near *Alk* locus affects GC. As various investigations so far have shown different results about the genetic basis of AC, GC, and ASV, further studies using more populations are required to reveal a complete quality meaning of the *Wx* and *Alk* loci.

Other indirect traits, such as paste viscosity, a pasting curve generated from rice flour subjected to a standard temperature-programmed "heat-hold–cool-hold" protocol, have also been used to select rice varieties with desirable eating, cooking and processing properties (Juliano 1996; Shu et al. 1998). This method has become increasingly popular with the availability of a very effective instrument, Rapid Visco Analyser (RVA, Newport Scientific Pty Ltd., Warriewood, Australia), which has the virtues of being easy to operate, giving relatively rapid results, and requiring a small sample (Panozzo and McCormick 1993; Wrigley et al. 1996; Bao and Xia 1999). Compared with traditional indirect indexes, little work has been done to characterize the genetic control of the viscosity parameters, especially those in the gelatinization process during the heating period. In a preliminary study, Gravois and Webb (1997) found that the inheritance of paste viscosity profiles appears to be controlled by a single locus with additive gene effects. Bao et al. (2000b) investigated the genetic basis of the six paste viscosity parameters and found that the *Wx* locus at chromosome 6 was responsible for all parameters except peak viscosity (PKV). Bao et al. (2002) also simultaneously investigated the genetic basis of AC, GC, ASV, and the six paste viscosity parameters. Using the same population as Bao et al. (2000a, b), they also conducted a QTL analysis for a number of starch characters (Bao et al. 2003). However, due to the small population and lack of replications over environments, further studies are needed.

Among the direct traits for eating and cooking quality in rice, water absorption, cooked rice elongation (CRE), and volume expansion are the most commonly measured. However, only a few studies have discussed the genetic basis of these traits. Ahn et al. (1993) used restriction fragment length polymorphism markers to map the gene for cooked-kernel elongation in Basmati 370 rice to chromosome 8. Dong and Zheng (2002) identified three QTL each for steamed rice length and width. Recently, two separate studies identified a number of QTL for these characters (Ge et al. 2005; Tian et al. 2005). The authors proposed that the *Wx* region plays a major role in determining water absorption, CRE, and volume expansion.

In this study, QTL for the traits associated with AC, GC, ASV, viscosity parameters, and CRE were investigated simultaneously in a recombinant inbred line (RIL) population using data from 2 years. The objective of this study was to comprehensively characterize the genetic basis of the eating and cooking quality of rice in order to facilitate our future breeding of high-quality rice varieties.

Materials and methods

Mapping population and field experiment

The mapping population consisted of 188 rice RILs derived from a cross between Zhenshan 97 and Delong 208. The former is the female parent of a number of widely cultivated hybrids in China, which has good combining ability but poor quality. The latter is a local variety from Yunnan Province of Southwest China that has a number of characteristics desirable for cooked rice quality, such as low AC, high GC (soft gel), and high ASV (low GT), so the cooked rice is soft and moderately sticky. In addition, the cooked rice stays tender and glossy when cooled. These characteristics are highly preferred in many areas of Yunnan Province and in specialty restaurants.

The field experiments were conducted in the rice-growing seasons of 2004 and 2005 on the experimental farm of Huazhong Agricultural University, Wuhan, China. The sowing dates were May 25 in both years. Ten plants per line were transplanted in a single row with 16.5 cm between plants and 26.4 cm between rows. Field management essentially followed normal agricultural practice. The lines were harvested individually at maturity to prevent over-ripening.

Trait measurement

The head rice was prepared according to the methods described by Tan et al. (1999). The milled rice was ground into powder with an Udy Cyclone Sample Mill (Udy Corporation, Fort Collins, CO, USA), and was then sieved through a 100-mesh sieve. Samples were refrigerated until analysis.

Amylose content, gel consistency, and alkali spreading value

AC was measured as described by Tan et al. (1999) and Tian et al. (2005), where the Automatic Recording Titrator (ART-3, Hirama Laboratories, Kanagawa, Japan) was used to analyze AC by the iodine titration method. Briefly, 20 mg rice flour was gelatinized overnight in 2 ml 1.0 N NaOH in a water bath at 56°C. After 8 ml of distilled water was added, the solution was boiled for 10 min and then cooled to room temperature. The cooled solution was defatted with 5 ml butanol:petroleum ether (1:3), after which 1.5 ml 0.4 N KI and 2–3 ml 1 N HCl were added. The AC was determined in duplicate with an ART-3 according to the manufacturer's instructions in which 1.57 mM KIO_3 was titrated to the solution. The titration terminal was automatically detected and the used volume of KIO_3 was transformed into AC.

GC analysis followed the methods described by Cagampang et al. (1973). Exactly 100 mg of milled rice flour was wetted with 0.2 ml of 95% ethanol containing 0.025% (w/v) bromthymol blue to prevent clumping in a 10 × 110-mm culture tube, to which 2.0 ml 0.2 N KOH was added. The tube was covered with a glass marble and boiled vigorously in a water bath for 8 min, making sure that the tube contents reach two-thirds the height of the tube. The tube was removed from the water bath and left at room temperature for 5 min, cooled in an ice-water bath for 20 min, and then laid down horizontally. The gel length was measured 1 h later as the distance from the bottom of the tube to the front of the gel migration. The gel length thus obtained provides a measurement of the GC: the longer the distance, the softer the gel.

ASV was assayed according to the method of Little et al. (1958). The method involves incubating six intact grains of milled rice in 10 ml of 1.7% KOH at 30°C for 23 h and measuring the degree of spreading on a seven-point scale: (1) grain not affected; (2) grain swollen; (3) grain swollen, collar incomplete and narrow; (4) grain swollen, collar complete and wide; (5) grain split or segmented, collar complete and wide; (6) grain dispersed, merging with collar; and (7), grain completely dispersed and intermingled. ASV is generally considered to be inversely related to GT such that a high ASV correlates with a low GT, or vice versa. The assay for each trait was conducted with two to four replicates for each line.

Viscosity profile (Micro viscoamylograph)

Viscosity profile was determined with a Micro Viscoamylograph (Ident No. 803201, Brabender, Germany), mainly according to the instruction manual, with reference to the American Association of Cereal Chemists (2000) Standard Method AACC 61-01 and 61-02. Fifteen grams of rice flour at 14% of moisture from each line was dispersed in 100 ml distilled water and subjected to gelatinization analysis. The temperature program was set as follows: heating from 30 to 95°C at 7.5°C/min, holding at 95°C for 5 min, then cooling from 95 to 40°C at 7.5°C/min and holding for 3 min.

Nine primary parameters can be obtained from the pasting curve or temperature profile of the diagram (Table 1; Fig. 1): Pasting temperature (temperature of the initial viscosity increase (gelatinization) at A point, designated as Atemp); Pasting time (time of the initial viscosity increase at A point, designated as Atime); Peak viscosity (first peak paste viscosity after gelatinization, point B, abbreviated as PKV); Peak temperature (temperature at PKV and designated as Btemp); Peak time (time to PKV and designated as Btime); Viscosity at 95°C (viscosity at the start of the holding period at 95°C, V95, point C); Hot paste viscosity (paste viscosity at the end of holding period at 95°C, HPV, point D); Cool paste viscosity (paste viscosity at the end of the cooling period, CPV, point E); and Final viscosity at 40°C (FV, paste viscosity at the end of the holding period at 40°C, point F). These parameters reflect the characteristics of cooked rice during the cooking and cooling processes. The parameters of paste viscosity were all in Brabender Units. From these primary parameters, three secondary parameters of paste viscosity, breakdown, setback, and consistency may be calculated (Table 1; Fig. 1): breakdown viscosity (BD) is the decrease in viscosity during cooking at 95°C (BD = PKV – HPV); setback viscosity (SB) is the viscosity when cooled to 40°C minus peak viscosity (SB = CPV – PKV); and consistency viscosity (CS) is the viscosity when cooled to 40°C minus final cooking viscosity at 95°C (CS = CPV – HPV). In addition, one secondary parameter of time—the time needed from initial viscosity increase to PKV (designated as BAtime)—can be calculated as peak time minus pasting time (BAtime = Btime – Atime). Atime, BAtime, V95 and FV were the new parameters firstly adopted by this study and the genetic basis of them, together with Atemp, Btemp, Btime, have not been studied in previous studies.

Cooked rice elongation

The analysis of CRE was modified slightly from the method described by Tian et al. (2005). Ten intact milled grains were selected randomly from each line and measured for length (before cooking length, BCL). After soaking in distilled water for 30 min, the grains were sandwiched between two pieces of wet filter paper and placed in a petri dish with an appropriate amount of water. Then the dish was placed in a covered container and steamed over boiling water for 10 min and simmered for 10 min (power shuts off). The cooked rice grain was transferred to another petri dish with a piece of dry filter paper on the bottom, then placed in a desiccator with a constant temperature (19°C)

Table 1 The parameters of viscosity profile investigated in this study

Traits abbr	Test points or formulation	Description (reference of terminology)
Atemp	A	Pasting temperature (61-02), temperature of initial viscosity increase (61-01)
Atime	A	Pasting time, time of initial viscosity increase
Btemp	B	Peak temperature, temperature at peak hot-paste viscosity (61-01)
Btime	B	Peak time (61-02), time required to reach peak hot-paste viscosity
PKV	B	Peak viscosity (61-02; Bao and Xia 1999), viscosity at peak hot past (61-01)
V95	C	Viscosity 95°C, viscosity at start of holding period at 95°C
HPV	D	Hot paste viscosity (Bao and Xia 1999), viscosity at end of holding period at 95°C (61-01), trough (61-02)
CPV	E	Cool paste viscosity (Bao and Xia 1999), viscosity at end of cooling to 40°C (61-01), final viscosity (61-02)
FV	F	Final paste viscosity at the end of final holding period at 40°C
BAtime	BAtime = Btime – Atime	Time needed from initial viscosity increase to peak viscosity
BD	BD = PKV – HPV	Breakdown (Bao and Xia 1999; 61-02), decrease in viscosity during cooking at 95°C
CS	CS = CPV – HPV	Consistency (Bao and Xia 1999), setback from trough (61-02), increase in viscosity during cooling period
SB	SB = CPV – PKV	Setback (Bao and Xia 1999; 61-01), setback from peak (61-02), CPV minus PKV

Note In the bracket are the references of the terminology of the parameters; 61-01 and 61-02 are the AACC methods and see in Materials and methods section for detail

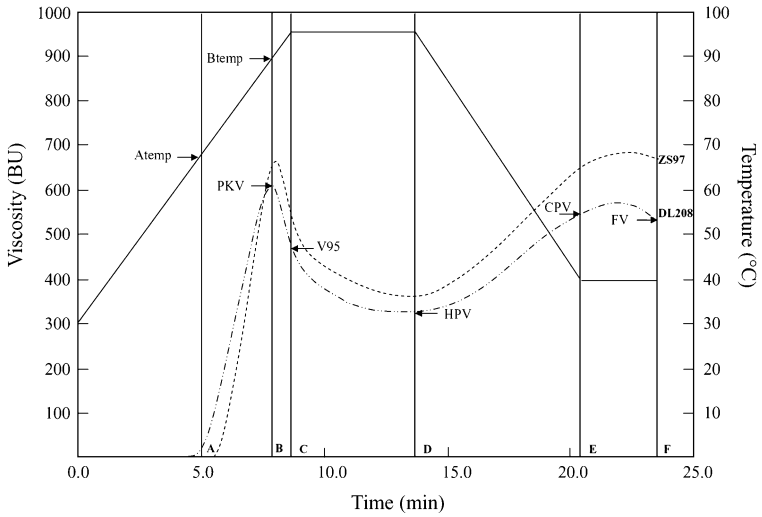

Fig. 1 Parameters of the viscosity profile (Take Delong 208 in 2004 as an example; see Table 1 for abbreviation and descriptions of the traits and parameters). The diagram with two *y*-axes (*left y*-axis: torque = viscosity; *right y*-axis: nominal temperature) and an x-axis (test time in [min]). *Note* The viscosity value of the variety in *pasting curve* (*solid line*) at a certain time point appears on the Viscosity axis, and the corresponding temperature is in the *temperature profile* (*dotted line*) and appears on the temperature axis

for about 30 min to remove the adhered water on the grain surface. The desiccated cooked rice grains from each line were put on a glass plate and scanned with a scanner. The scanned picture was measured for length in Photoshop (Adobe Systems incorporated USA), referred to as after cooking length (ACL). The CRE was calculated as: CRE = (ACL – BCL)/BCL × 100%.

DNA markers and assays

One hundred eighty polymorphic simple sequence repeat (SSR) markers covering all 12 chromosomes were used to genotype the population. The primers of the RM-series were designed according to Temnykh et al. (2000, 2001), and those of the MRG-series were according to the rice genome sequences of the Monsanto Company (McCouch et al. 2002). The SSR assay was performed essentially as described by Jiang et al. (2004).

Data analysis

Mapmaker 3.0 was used to construct a genetic linkage map (Lincoln et al. 1992). The average of the measurements for each line was used for QTL analysis. The means of the traits were used to identify QTL with composite interval mapping with QTLCartographer 2.0 (Zeng 1994). The

thresholds (2.28–2.60 for these traits in 2004 and 2.27–2.55 for these traits in 2005) of the QTL were obtained at $P = 0.01$ by 1,000 random permutations of the trait values. QTL with LODs that were slightly below the thresholds in 1 year but exceeded the thresholds in the other year were also included. The peak points of the LR in the linkage map were taken as the putative positions of the effects, and additive effects were taken from the points showing the largest effects. The relative contribution of a QTL was calculated as the proportion of phenotypic variance explained by the QTL. The statistical package Statistica (StatSoft 1991) was used to conduct the statistical analyses, including *t* test, correlation analysis and principal component analysis.

Results

Phenotypic variation in parents and the population

In both years, significant phenotypic differences were detected between the parents using a *t* test for AC, GC and ASV ($P < 0.01$), in which Zhenshan 97 had higher AC but lower ASV and GC (Table 2). The two parents showed significant differences in most parameters from viscosity profiles in both years (Table 2; Fig. 1). All parameters of Zhenshan 97 except for Btemp, Btime, BAtime, PKV, and BD were much larger than those of Delong 208 in both years. Zhenshan 97 had a lower value for BAtime in both years. The values of Btemp and Btime in Zhenshan 97 were larger than Delong

Table 2 Descriptive statistics of the rice cooking and eating quality traits (parameters) in parents and RIL population observed in 2004 (upper) and 2005 (lower) (SD standard deviation)

Traits[a]	Parent (Mean ± SD)		RIL population		
	Zhenshan 97	Delong 208	Mean ± SD	Range	h^{2b}
AC (%)	27.25 ± 1.05	13.43 ± 0.69	22.64 ± 7.08	9.18–31.53	96.8
	27.58 ± 1.08	12.43 ± 0.66	20.86 ± 8.39	6.59–31.78	
GC (mm)	28.00 ± 0.50	46.00 ± 0.92	33.38 ± 8.78	24.00–56.00	87.3
	26.00 ± 0.48	47.24 ± 0.98	35.18 ± 9.96	22.67–57.50	
ASV	3.10 ± 0.37	6.00 ± 0.00	4.50 ± 1.78	1.69–7.00	95.3
	3.04 ± 0.32	6.00 ± 0.00	4.18 ± 2.10	1.33–7.00	
Atemp (°C)	74.5 ± 1.1	69.5 ± 1.1	72.8 ± 3.7	64.4–79.1	88.3
	75.0 ± 0.6	67.9 ± 1.1	72.4 ± 3.8	65.5–77.6	
Atime (min)	5.78 ± 0.05	5.10 ± 0.08	5.53 ± 0.51	4.46–6.58	87.9
	5.86 ± 0.13	4.88 ± 0.06	5.49 ± 0.51	4.71–6.42	
Btemp (°C)	87.8 ± 0.4	87.8 ± 1.6	88.0 ± 2.0	81.9–92.2	57.6
	84.9 ± 1.0	82.5 ± 0.6	83.5 ± 3.1	72.8–89.5	
Btime (min)	7.86 ± 0.05	7.89 ± 0.23	7.83 ± 0.32	6.70–8.60	71.2
	7.42 ± 0.11	6.90 ± 0.14	7.24 ± 0.46	5.79–7.96	
BAtime (min)	2.08 ± 0.09	2.79 ± 0.18	2.28 ± 0.54	1.17–3.63	79.2
	1.56 ± 0.13	1.87 ± 0.29	1.73 ± 0.66	0.67–3.17	
PKV (BU)	645.3 ± 13.2	604.4 ± 27.0	623.9 ± 51.9	478.0–757.0	61.2
	634.1 ± 19.4	683.6 ± 31.7	694.3 ± 35.8	591.0–769.5	
V95 (BU)	505.7 ± 10.5	476.1 ± 32.4	498.3 ± 69.4	328.5–679.5	83.5
	463.9 ± 17.1	378.1 ± 32.4	479.8 ± 81.2	295.0–649.0	
HPV (BU)	356.3 ± 22.9	332.6 ± 22.2	342.4 ± 49.3	233.0–457.0	84.9
	294.3 ± 13.2	242.4 ± 13.6	311.0 ± 50.8	184.5–436.0	
CPV (BU)	649.3 ± 22.9	558.5 ± 18.7	613.3 ± 101.3	424.0–857.5	92.0
	616.8 ± 16.1	450.3 ± 23.9	615.0 ± 114.2	389.0–836.7	
FV (BU)	660.0 ± 14.0	536.9 ± 19.9	595.6 ± 104.0	407.0–835.5	92.6
	594.4 ± 9.0	444.5 ± 25.4	586.4 ± 115.0	384.0–836.5	
BD (BU)	288.3 ± 26.6	271.1 ± 21.6	280.2 ± 69.5	144.0–446.7	62.3
	340.3 ± 13.8	440.9 ± 27.6	387.1 ± 56.0	263.0–521.0	
CS (BU)	289.3 ± 2.5	224.2 ± 19.5	268.8 ± 59.1	164.0–401.0	88.9
	322.5 ± 15.5	206.8 ± 14.7	300.4 ± 65.0	193.0–424.0	
SB (BU)	4.0 ± 26.1	–45.8 ± 15.9	–8.5 ± 110.1	–233.5 to 189.0	87.3
	–17.2 ± 20.0	–233.4 ± 25.9	–85.3 ± 114.4	–292.0 to 121.3	
CRE (%)	104.3 ± 2.0	115.0 ± 2.1	102.1 ± 14.6	76.1–134.7	84.5
	100.2 ± 1.9	117.0 ± 2.1	103.3 ± 14.4	69.5–138.2	

[a] See Table 1 for the abbreviations and descriptions of the traits

[b] Heritability (%) calculated as $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2/n)$, where σ_g^2 is the genotypic variance, σ_e^2 is the error variance, n is the number of environments

208 in 2005, but the same as Delong 208 in 2004 (Table 2). For PKV and BD, Zhenshan 97 had higher values in 2004, but lower values in 2005. Furthermore, unlike the more or less bimodal segregation for most viscosity parameters, Btemp, Btime, and PKV exhibited approximately normal distributions in the RIL population (data not shown). For CRE, Delong 208 had a higher value in both years. The RIL population showed transgressive segregations in both directions for all the traits. The estimated heritabilities (h^2) of the traits and parameters were relatively high ranging from 57.6% for Btemp to 96.8% for AC, indicating that genetic components accounted for most of the phenotypic variations (Table 2).

Correlations of traits and parameters

Pairwise correlation coefficients between the traits and parameters were generally consistent both years (Table 3). All the traits, except PKV, BAtime, and CRE, could be divided into two classes according to the correlation coefficients and distributions of the trait scores resolved by principal component analysis (Tables 3, 4; Fig. 2). Class I consisted of AC, GC, and most of parameters from the viscosity profile; class II included ASV, Atemp, and Atime. Most traits in each class correlated significantly with each other, but certain levels of correlations were also detected between the traits in different classes. Thus, AC and GC together with the paste viscosity parameters in class I describe the viscosity characteristics, while ASV, Atemp, and Atime in class II describe the gelatinization characteristics. PKV and CRE were not closely related to the traits in the two classes, suggesting that they are relatively independent of either group.

Linkage map

A linkage map was constructed, consisting of 180 SSR markers spanning a total of 1817.2 cM, with an average interval of 11.8 cM between adjacent markers (Fig. 3). The marker orders in the map agreed well with those of Temnykh et al. (2000, 2001).

Quantitative trait locus analysis

Amylose content, gel consistency, and alkali spreading value

Three QTL were detected for AC in 2004 and one in 2005, accounting for 90.3 and 86.2% of the phenotypic variation respectively (Table 5; Fig. 3). The largest one found in both years corresponded to the *Wx* locus (the locus tightly linked with the gene *Wx*, marked by MX21) on chromosome 6. The other two were detected in 2004 only, mapping on chromosomes 2 and 9, where no QTL for AC were previously reported.

Two QTL for GC were detected in 2004 and three in 2005, respectively (Table 5; Fig. 3). Of the two QTL detected in both years, one corresponded to the *Wx* locus showing the largest effect; the allele from Delong 208 increased the gel length by 7.64 and 8.15 mm in 2004 and 2005, respectively. The other QTL corresponded to the *Alk* locus (the locus tightly linked with the gene *Alk*, RM276–RM549), which has often been reported as controlling ASV (Kudo 1968; Harushima et al. 1998; Umemoto et al. 2002). An additional QTL located on chromosome 1 with minor effect was detected in 2005.

Two QTL were identified for ASV in both 2004 and 2005, collectively explaining 95.5 and 92.9% of the phenotypic variation, respectively (Table 5; Fig. 3). The QTL with a major effect corresponded to the *Alk* locus; the allele from Delong 208 increased ASV by 1.78 in 2004 and 2.01 in 2005. The one with a minor effect corresponded to the *Wx* locus; the allele from Delong 208 decreased ASV. The effect of the *Wx* locus for ASV was also detected in two previous studies (Tan et al. 1999; Fan et al. 2005); in both cases Zhenshan 97 was used as a parent, and the allele from Zhenshan 97 (higher AC) contributed to the increase of ASV.

The parameters of the viscosity profile

Pasting temperature and pasting time

Two each QTL for Atemp were detected in 2004 and in 2005, collectively explaining 69.6 and 89.3% of the phenotypic variation, respectively (Table 5; Fig. 3). One QTL corresponding to the *Alk* locus on chromosome 6 was detected in both years. The other one in 2004 was located in MRG4499–RM445 and the other one in 2005 was located in RM2224–MRG7006. Although the QTL were closely linked with each other on chromosome 7, the opposite effects of the two QTL indicated that they marked different genes. For Atime, two QTL were identified in 2004 and one in 2005, explaining 62.2 and 84.0% of the phenotypic variation, respectively (Table 5; Fig. 3). Again, the QTL corresponding to the *Alk* locus had a major effect on the trait. The QTL flanked by MRG4499–RM445 detected in 2004 had a simultaneous effect on both Atemp and Atime.

Peak temperature, peak time, and time needed from initial viscosity increase to PKV

Two QTL for Btemp were detected in both 2004 and 2005, collectively explaining 27.7 and 45.8% of the total phenotypic variation respectively (Table 5; Fig. 3). One of the

Table 3 Coefficients of pairwise correlations of eating and cooking traits of rice grain in an RIL population observed in 2004 (upper) and 2005 (lower)

	AC	GC	ASV	Atemp	Atime	Btemp	Btime	BAtime	PKV	V95	HPV	CPV	FV	BD	CS	SB
GC	–0.74**															
	–0.72**															
ASV	0.02	–0.32**														
	0.09	–0.40**														
Atemp	0.15	0.18	–0.81**													
	0.07	0.29**	–0.86**													
Atime	0.14	0.20*	–0.81**	0.97**												
	0.02	0.32**	–0.88**	0.97**												
Btemp	0.56**	–0.49**	0.01	0.13	0.08											
	0.66**	–0.60**	0.09	–0.01	0.01											
Btime	0.74**	–0.54**	–0.05	0.22**	0.21*	0.89**										
	0.67**	–0.61**	0.05	0.05	0.07	0.96**										
BAtime	0.34**	–0.53**	0.72**	–0.77**	–0.80**	0.43**	0.38**									
	0.49**	–0.65**	0.69**	–0.69**	–0.69**	0.69**	0.64**									
PKV	–0.14	0.08	0.32**	–0.39**	–0.37**	–0.38**	–0.30**	0.16								
	0.07	0.04	0.08	–0.12	–0.12	–0.01	0.03	0.11								
V95	0.79**	–0.63**	0.29**	–0.10	–0.10	0.38**	0.62**	0.48**	0.30**							
	0.77**	–0.60**	0.31**	–0.26**	–0.26**	0.66**	0.70**	0.68**	0.34**							
HPV	0.82**	–0.60**	0.13	0.12	0.10	0.51**	0.70**	0.32**	0.04	0.88**						
	0.79**	–0.64**	0.28**	–0.20*	–0.20*	0.71**	0.75**	0.67**	0.27**	0.94**						
CPV	0.89**	–0.73**	0.25**	–0.01	–0.02	0.54**	0.74**	0.46**	0.01	0.89**	0.89**					
	0.86**	–0.71**	0.30**	–0.20*	–0.20*	0.74**	0.78**	0.70**	0.08	0.90**	0.95**					
FV	0.86**	–0.72**	0.30**	–0.08	–0.08	0.47**	0.68**	0.48**	0.08	0.90**	0.85**	0.99**				
	0.82**	–0.71**	0.36**	–0.27**	–0.28**	0.69**	0.73**	0.73**	0.11	0.90**	0.94**	0.98**				
BD	–0.69**	0.58**	0.15	–0.40**	–0.37**	–0.62**	–0.70**	–0.07	0.71**	–0.34**	–0.62**	–0.58**	–0.52**			
	–0.79**	0.72**	–0.23**	0.16	0.15	–0.75**	–0.76**	–0.66**	0.37**	–0.65**	–0.75**	–0.85**	–0.82**			
CS	0.81**	–0.73**	0.30**	–0.11	–0.13	0.46**	0.63**	0.48**	0.00	0.75**	0.65**	0.92**	0.93**	–0.45**		
	0.85**	–0.72**	0.30**	–0.19*	–0.20*	0.70**	0.73**	0.67**	–0.05	0.81**	0.85**	0.97**	0.95**	–0.85**		
SB	0.87**	–0.70**	0.06	0.18*	0.16	0.63**	0.78**	0.30**	–0.43**	0.63**	0.75**	0.87**	0.83**	–0.87**	0.82**	
	0.85**	–0.74**	0.23**	–0.13	–0.13	0.77**	0.78**	0.67**	–0.15	0.77**	0.84**	0.95**	0.92**	–0.95**	0.97**	
CRE	0.27*	–0.30*	–0.22	0.11	0.07	0.46**	0.37**	0.20	–0.14	0.15	0.21	0.27*	0.23	–0.29*	0.26*	0.29*
	0.23	–0.23	–0.25*	0.09	0.09	0.42**	0.36**	0.17	–0.10	0.16	0.18	0.25*	0.21	–0.23	0.23	0.25*

*, ** Significance at $P < 0.05$ and $P < 0.01$, respectively

Table 4 Standardised loadings of the traits on the first three principal components (The absolute values of marked loadings are > 0.65)

Traits	Principal components in 2004			Principal components in 2005		
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3
AC	0.87*	–0.12	0.14	0.84*	–0.36	0.05
GC	–0.73*	–0.16	0.07	–0.80*	–0.05	0.19
ASV	0.20	0.79*	–0.15	0.40	0.83*	–0.10
Atemp	0.01	–0.94*	0.24	–0.30	–0.92*	0.07
Atime	–0.01	–0.94*	0.28	–0.31	–0.92*	0.07
Btemp	0.68*	–0.20	–0.46	0.81*	–0.28	–0.08
Btime	0.83*	–0.25	–0.20	0.82*	–0.32	–0.02
BAtime	0.51	0.73*	–0.37	0.82*	0.46	–0.07
PKV	–0.15	0.61	0.67*	0.09	0.16	0.96*
V95	0.84*	0.22	0.41	0.89*	0.01	0.36
HPV	0.87*	–0.05	0.28	0.92*	–0.06	0.29
CPV	0.96*	0.07	0.19	0.97*	–0.09	0.09
FV	0.94*	0.15	0.24	0.96*	0.00	0.11
BD	–0.71*	0.51	0.32	–0.88*	0.16	0.38
CS	0.86*	0.16	0.12	0.94*	–0.09	–0.05
SB	0.90*	–0.23	–0.10	0.95*	–0.19	–0.17
CRE	0.22	–0.12	–0.25	–0.02	–0.49	–0.03
Variation explained	8.13	3.87	1.54	9.78	3.25	1.39
Proportion of total	0.48	0.23	0.09	0.58	0.19	0.08

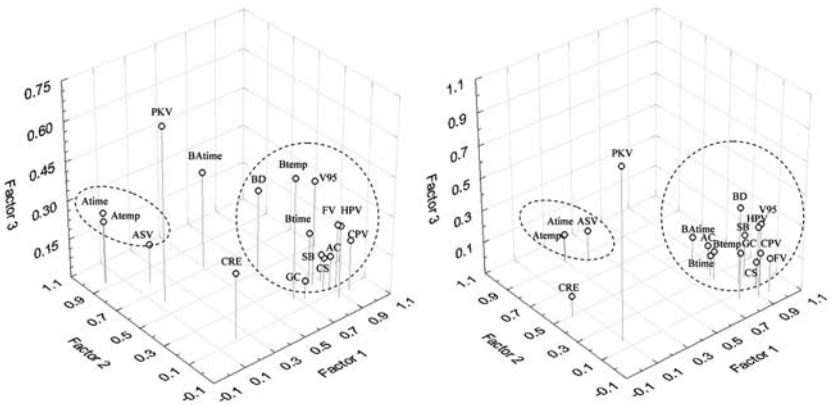

Fig. 2 3D scatterplots of the eating and cooking quality traits and viscosity parameters of the rice grain resolved by principal component analysis (set factor 1, 2, 3 as *x*, *y*, *z* axes; *left* 2004; *right* 2005)

QTL for Btemp corresponding to the *Wx* locus was detected in both years. Two QTL for Btime were detected in 2004 and three in 2005, collectively explaining 34.7% and 48.5% of the phenotypic variation. Again the QTL around the *Wx* locus had a major effect on the trait and was consistently detected in both years.

Four QTL were detected for BAtime in 2004 and two in 2005, collectively explaining 70.2 and 78.8% of the phenotypic variation, respectively (Table 5; Fig. 3). Two of the QTL were common across 2 years, one of which corresponded to the *Alk* locus where the allele from Zhenshan 97 decreased the trait value while the other corresponded to the *Wx* locus where the allele from Zhenshan 97 increased the trait value. Two additional QTL on chromosomes 5 and 7 were only significant in 2004.

Peak viscosity, viscosity at 95°C, and hot paste viscosity

Three QTL were detected for PKV in 2004 and four in 2005, collectively explaining 29.5 and 22.3% of the phenotypic variation, respectively (Table 5; Fig. 3). The QTL on chromosome 7 was significant at $P = 0.01$ in 2004 but at only $P = 0.05$ in 2005. The effects on chromosome 9 detected in RM108–RM553 in 2004 ($P = 0.01$) and in RM257–RM410 in 2005 ($P = 0.05$) were likely due to the same QTL.

Four QTL were detected for V95 in 2004 and six in 2005, collectively explaining 74.2 and 71.9% of the phenotypic variation, respectively (Table 5; Fig. 3). Three QTL on chromosomes 5 and 6 were consistently detected in both years, although the effects of two QTL in 1 year were only significant at $P = 0.05$. Two QTL were detected for HPV in

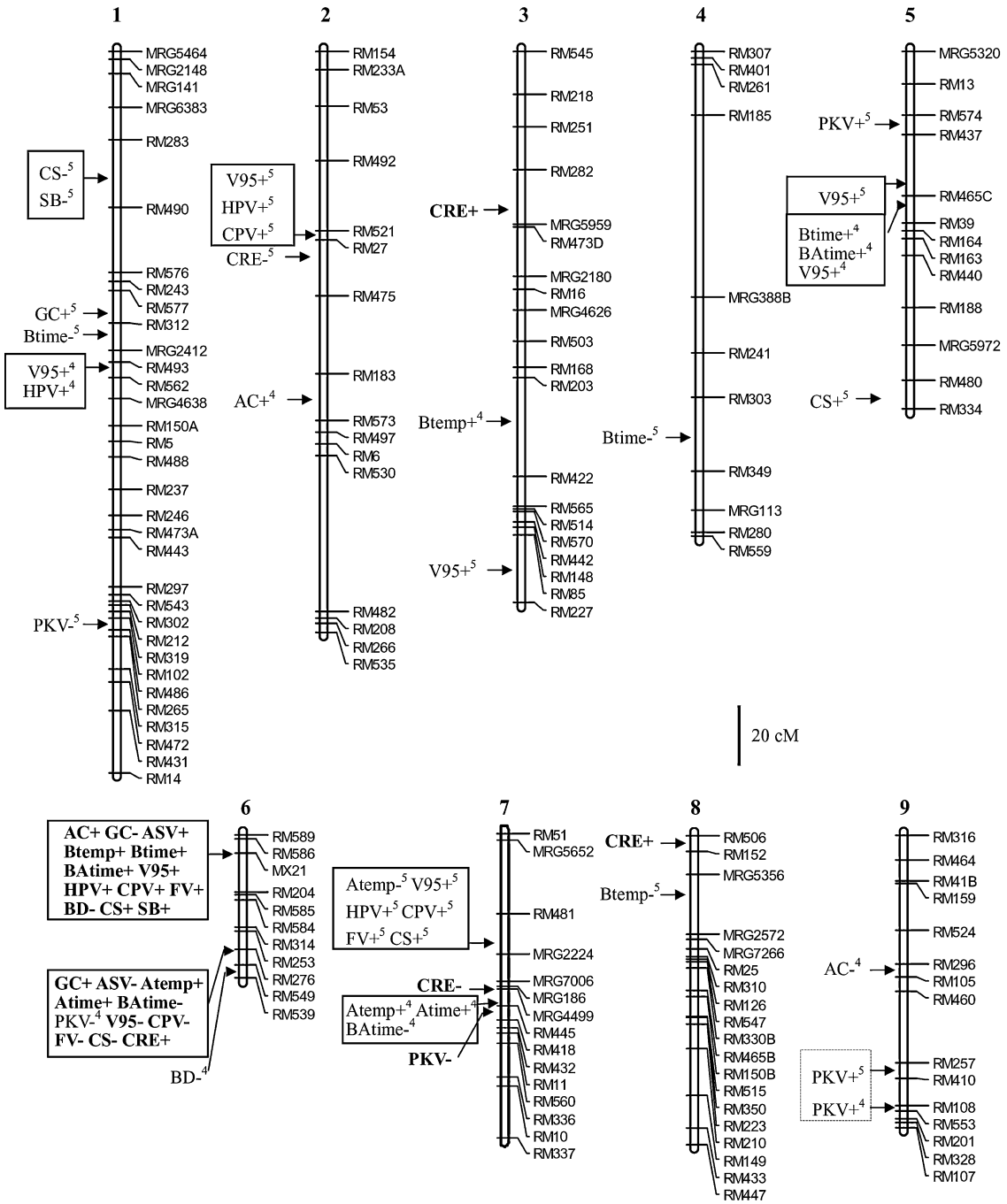

Fig. 3 Distribution of the QTL for eating and cooking quality traits in the linkage map. The marker name is shown on the right of the chromosome. "+" and "–" following the abbreviation of traits indicate the directions of additive effects of the alleles from Zhenshan 97. The *superscript 4 or 5* indicates the year (2004 or 2005) in which the QTL is detected; the letters in *bold* indicate that the QTL are detected in both years. See Table 1 for abbreviations and descriptions of the traits and parameters

2004 and three in 2005, collectively explaining 60.6 and 68.0% of the phenotypic variation, respectively. Again, the largest effect on both traits in both years was from the QTL corresponding to the *Wx* locus, with the positive allele from Zhenshan 97. In addition, the QTL on chromosome 1 affected both traits in 2004, while the two QTL on chromosomes 2 and 7 affected both traits in 2005.

Cool pasteviscosity and final viscosity at 40°C

Two QTL for CPV were detected in 2004 and four in 2005, collectively explaining 88.1 and 86.8% of the phenotypic variation, respectively (Table 5; Fig. 3). Two QTL for FV were detected in 2004 and three in 2005, collectively explaining 83.2 and 84.2% of the phenotypic variation,

Table 5 QTL identified for the rice eating and cooking quality traits in RIL population using QTLCartographer 2.0

Traits	2004					2005				
	Chr	Interval	LOD	Add	Var %	Chr	Interval	LOD	Add	Var %
AC	2	RM183–RM573	2.9	0.74	1.1					
	6	RM586–MX21	85.0	6.91	88.0	6	MX21–RM204	85.0	8.15	86.2
	9	RM296–RM105	2.9	–0.76	1.2					
GC						1	RM577–RM312	2.6	2.03	3.8
	6	RM586–MX21	29.7	–7.64	69.8	6	RM586–MX21	22.9	–8.15	53.5
	6	RM276–RM549	4.5	2.44	7.2	6	RM276–RM549	4.2	2.88	7.1
ASV	6	MX21–RM204	10.8	0.53	7.7	6	MX21–RM204	6.9	0.61	7.1
	6	RM276–RM549	73.0	–1.78	87.8	6	RM276–RM549	52.5	–2.01	85.8
Atemp	6	RM276–RM549	36.4	3.03	64.0	6	RM276–RM549	51.7	3.63	85.2
	7	MRG4499–RM445	4.9	0.90	5.6	7	MRG2224–MRG7006	2.8	–0.79	4.1
Atime	6	RM276–RM549	31.9	0.43	56.2	6	RM276–RM549	49.6	0.49	84.0
	7	MRG4499–RM445	5.1	0.13	6.0					
Btemp	3	RM203–RM422	2.7	0.80	9.3					
	6	MX21–RM204	6.3	0.94	18.4	6	MX21–RM204	17.0	2.11	39.6
						8	MRG5356–MRG2572	2.6	–0.82	6.2
Btime	5	RM465C–RM39	2.7	0.07	4.5	1	RM312–MRG2412	2.7	–0.12	5.9
						4	RM303–RM349	2.5	–0.11	5.0
	6	MX21–RM204	14.6	0.18	30.2	6	MX21–RM204	15.6	0.31	37.6
BAtime	5	RM465C–RM39	2.3	0.11	3.6					
	6	MX21–RM204	11.7	0.26	19.6	6	MX21–RM204	16.9	0.44	37.9
	6	RM276–RM549	22.5	–0.36	40.7	6	RM276–RM549	17.2	–0.45	40.9
	7	MRG4499–RM445	5.0	–0.14	6.3					
PKV	6	RM276–RM549	3.2	–15.04	8.0	1	RM102–RM486	3.9	–14.13	6.8
						5	RM574–RM437	3.6	13.87	6.6
	7	RM445–RM418	4.3	–20.00	12.3	7	RM445–RM418	2.1	–12.79	6.1
	9	RM108–RM553	4.2	16.04	9.2	9	RM257–RM410	2.1	9.29	2.8
V95	1	RM493–RM562	2.8	13.71	3.6	2	RM521–RM27	3.1	18.3	4.4
						3	RM85–RM227	2.7	21.92	6.3
	5	RM465C–RM39	2.1	10.81	2.3	5	RM437–RM465C	2.7	21.12	6.0
	6	MX21–RM204	33.2	58.23	63.1	6	MX21–RM204	22.2	63.81	46.6
	6	RM276–RM549	4.6	–17.04	5.2	6	RM276–RM549	2.4	–17.32	3.8
						7	RM481–MRG2224	3.0	18.75	4.8
HPV	1	RM493–RM562	2.5	9.29	3.3	2	RM521–RM27	3.2	10.52	3.8
	6	MX21–RM204	30.3	38.26	57.3	6	MX21–RM204	28.5	42.98	58.5
						7	RM481–MRG2224	3.5	12.81	5.7
CPV						2	RM521–RM27	2.2	17.43	2.2
	6	MX21–RM204	55.2	97.69	84.3	6	MX21–RM204	40.6	110.09	77.1
	6	RM276–RM549	4.9	–20.99	3.8	6	RM276–RM549	2.0	–17.05	1.9
						7	RM481–MRG2224	5.0	28.16	5.6
FV	6	MX21–RM204	49.1	96.83	76.9	6	MX21–RM204	37.8	108.54	73.9
	6	RM276–RM549	7.7	–27.80	6.3	6	RM276–RM549	5.4	–30.36	6.2
						7	RM481–MRG2224	3.2	23.66	4.1
BD	6	RM586–MX21	16.2	–44.26	36.1	6	MX21–RM204	32.6	–51.07	72.5
	6	RM549–RM539	2.3	–14.45	4.0					

Table 5 continued

Traits	2004					2005				
	Chr	Interval	LOD	Add	Var %	Chr	Interval	LOD	Add	Var %
CS						1	RM283–RM490	2.5	–10.47	2.2
						5	RM480–RM334	3.0	12.75	3.6
	6	MX21–RM204	39.3	53.66	75.1	6	MX21–RM204	43.8	63.45	73.1
	6	RM276–RM549	4.5	–15.02	5.8	6	RM276–RM549	3.0	–12.31	3.2
						7	RM481–MRG2224	3.4	13.11	3.7
SB						1	RM283–RM490	2.7	–18.81	2.3
	6	MX21–RM204	36.0	92.72	68.1	6	MX21–RM204	44.7	113.08	78.0
CRE						2	RM27–RM475	2.7	–4.35	9.5
	3	RM282–MRG5959	5.0	6.86	21.4	3	RM282–MRG5959	6.7	6.15	18.5
	6	RM276–RM549	5.7	6.33	18.5	6	RM276–RM549	3.0	3.67	6.2
	7	RM186–MRG4499	3.1	–4.43	8.8	7	RM186–MRG4499	6.6	–5.53	15.3
	8	RM506–RM152	5.4	6.34	16.5	8	RM506–RM152	2.9	3.92	7.2

Additive: the additive effects of QTL. Positive values of additive effects indicate that the Zhenshan 97 genotype have a positive effect on that trait

% Variance: the percentage of the phenotypic variation explained by the QTL

* Significant at $P = 0.05$

respectively. The QTL profiles for these two interrelated traits were very similar. The two QTL on chromosome 6, one with a major effect and the other with a minor effect, simultaneously affected the two traits in both years, while the one on chromosome 7 was only detected in 2005 and affected both traits.

Breakdown, consistency and setback

Two QTL for BD were detected in 2004 and one in 2005, collectively accounting for 40.1 and 72.5% of the phenotypic variation, respectively (Table 5; Fig. 3). Two QTL were detected for CS in 2004 and five in 2005, collectively explaining 80.9 and 85.8% of the phenotypic variation, respectively. One QTL was detected for SB in 2004 and two in 2005, collectively explaining 68.1 and 80.3% of the phenotypic variation, respectively.

Again, the QTL corresponding to the *Wx* locus was the largest for all three traits in both years. The allele from Zhenshan 97 showed a negative effect on BD, but a positive effect on CS and SB. Also, the QTL corresponding to the *Alk* locus affected BD and CS in 2004, and that on chromosomes 1 affected CS and SB in 2005. In addition, the one on chromosome 7, which was significant at $P = 0.01$ for CS in 2005, also showed significant effects on the other two traits at $P = 0.05$ in the same year (data not shown).

Cooked rice elongation

Four QTL for CRE were identified in 2004 and five in 2005, collectively explaining 65.2 and 56.7% of the total phenotypic variation, respectively (Table 5; Fig. 3). Four QTL on chromosomes 3, 6, 7, and 8 were consistently detected in both years. The effects of the QTL were relatively minor, and the positive alleles were dispersed between the two parents. These QTL were independent of the *Wx* locus, which differs from the results reported by Tian et al. (2005) and Ge et al. (2005) who showed that the *Wx* locus affected CRE. The QTL on chromosomes 2, 3, and 9 co-mapped with at least one QTL for cooked rice traits, including length elongation, width expansion, shape, weight, and water absorption of cooked rice detected in previous studies (Dong and Zheng 2002; Ge et al. 2005; Tian et al. 2005). The QTL on the short arm of chromosome 8 was near the QTL for cooked-kernel elongation in Basmati 370 rice detected by Ahn et al. (1993).

QTL co-localization

Comparison of the identified QTL revealed 9 QTL clusters for these traits that are distributed on six chromosomes (Fig. 3). The QTL for the traits in the same class are often clustered into the same chromosomal regions. The QTL clusters corresponding to the *Wx* locus and *Alk* locus were the two predominant ones detected for most of the traits. The QTL clusters corresponding to *Wx* locus simultaneously controlled AC, GC, ASV and most viscosity parameters, but had no effect on PKV and CRE, and the QTL corresponding to *Alk* locus played a role in ASV, GC, Atemp, and most viscosity parameters. The positive alleles in the QTL cluster corresponding to the *Wx* locus came from Zhenshan 97 for almost all the traits except GC and

BD, and the positive alleles of the cluster corresponding to the *Alk* locus came from Delong 208 for almost all the traits except GC, ASV, and Atime. Co-localization was found at these two major loci and other loci as well such as those on chromosomes 1, 2, 5, and 7. When we lowered the threshold of LOD values to $P = 0.05$, we found a wider co-localization (data not shown). For example, the QTL for Btemp in RM203-RM442 on chromosome 3 was also found for Btime, BD, CS, and SB, the QTL for Btemp in MRG5356–MRG2572 on chromosome 8 was coincident with the QTL for GC, BAtime, Btime, PKV, BD, CS and SB (all near MRG5356), and the QTL for AC in RM296–RM105 on chromosome 9 might affected GC, Btemp, BAtime, and BD. Co-location of the QTL for the same class was also found in previous studies (Bao et al. 2000b; Bao et al. 2002). Bao et al. (2000b) found that *Wx* locus affected HPV, CPV, BD, CS, and SB, and *Alk* locus affected HPV, CPV, and CS. The QTL cluster for CS and SB in RM283–RM490 on chromosome 1 in the present study might be the same as the QTL cluster for BD and SB as reported by Bao et al. (2000b), and the QTL cluster in RM481–MRG2224 on chromosome 7 appears to be consistent with the QTL cluster for AC, GC, PKV, and HPV as reported by Bao et al. (2002). In addition, we found QTL co-location for the traits belonging to different categories. For example, the *Wx* locus affected ASV, and the QTL cluster at *Alk* locus also contains individual QTL for GC and some paste viscosity parameters, the traits belonging to Class I. The QTL clusters on other chromosomes have characteristics similar to the locus *Wx* or *Alk*, although the variations they explained, such as the QTL cluster on chromosome 7 (MRG4499–RM445), were relatively small.

Discussion

Many studies have been devoted to the genetic basis of the cooking and eating quality of rice. AC is mainly controlled by the *Wx* region on chromosome 6 (He et al. 1999; Tan et al. 1999; Bao et al. 2000a; Lanceras et al. 2000; Septiningsih et al. 2003; Aluko et al. 2004). However, the other characteristics of *Wx* locus and the results of the QTL analyses of GC and ASV (GT) were less clear. Tan et al. (1999) reported that *Wx* region also controls both GC and ASV, and other studies also detected *Wx* effect on GC (Bao et al. 2000a; Lanceras et al. 2000; Tian et al. 2005; Fan et al. 2005). However, only Fan et al. (2005) confirmed the "three in one" function of *Wx* locus as reported by Tan et al. (1999); that is, the *Wx* locus also affects ASV. Fan et al. (2005) also proposed that a new locus near the *Alk* locus affects GC. Our results clearly support Fan et al. (2005) as we detected the all related QTL at two loci at $P = 0.01$ and a QTL for GC at the *Alk* locus.

Our study goes further than the confirming of the "three in one" characteristic of the *Wx* locus and the "two in one" characteristic of the *Alk* locus. In the present study, the genetic basis of cooking and eating quality traits in rice, reflected by AC, GC, ASV, CRE, and the parameters of the viscosity profile, was dissected simultaneously with an RIL population developed from two parents with contrasting phenotypes. We identified 26 distinct QTL for 17 traits (or parameters) in the 2 years at $P = 0.01$. This allowed us to investigate the relationship between traits and compare their QTL. The 17 traits, except PKV, BAtime, and CRE, could be divided into two classes by principal component analysis. The first class consists of AC, GC, and most of the paste viscosity parameters that form a major determinant of eating quality. The second class includes ASV, Atemp, and Atime, which characterize the cooking process. Our results clearly showed that the QTL corresponding to the *Wx* locus simultaneously controls the traits in the first class and also has a minor effect on ASV. The QTL corresponding to the *Alk* locus plays a major role in determining the traits in the second class and a minor role in GC and most of the paste viscosity parameters. Interestingly, the QTL clusters on the other chromosomes have similar characteristics as the *Wx* or *Alk* locus, although the variations they explained are relatively minor.

The evidence from the principal component analysis and QTL co-localization indicated that the quality of cooked rice can be well evaluated by viscosity parameters. AC, GC, and ASV have long been used as indexes of rice cooking and eating quality (Juliano 1985; Webb 1991). Our study showed that the lower values of primary paste viscosity parameters, except PKV, correspond to lower AC and longer and softer gel that represent good eating quality for most Chinese consumers, while lower pasting temperature and time correspond to higher ASV that represents a suitable cooking quality. Among the secondary paste viscosity parameters, previous studies indicated that BD and SB are highly correlated with eating quality (Shu et al. 1998; Larkin and Park 2003), such that rice varieties with relatively good eating quality have higher BD but lower lower SB (Shu et al. 1998). Our results showed that BD and SB are two main members in the first class as indicated by principal component analysis; BD has a negative value (like GC) and SB has a positive value (like AC). Our results are generally consistent with previous studies (Shu et al. 1998; Larkin and Park 2003). However, CPV might be better than BD and SB, since the later two were related to PKV (BD = PKV – HPV, SB = CPV – PKV) and the trait (PKV) has relatively low heritability.

One feature of the present study is its investigation of the genetic basis of the seven parameters of the viscosity profile, namely, Atemp, Atime, Btemp, Btime, BAtime, V95, and FV (most were the parameters in the heating period of

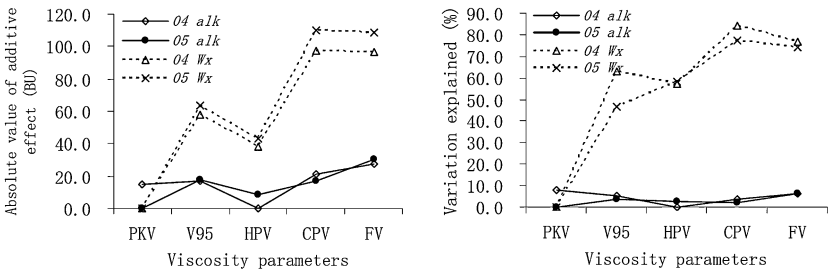

Fig. 4 QTL dynamics showing the additive effect (*left*) and variations explained (*right*) by putative QTL corresponding to the *Wx* and *Alk* loci on the paste parameters during different periods of viscosity analysis

viscosity analysis that had not been investigated previously), and this provided a more complete description of the process and enabled us to characterize of the dynamic nature of the QTL. The heating process can be divided into two successive periods: from the commencement of heating to the initial viscosity increase and from the initial viscosity increase to PKV. Interestingly, The alleles of the QTL corresponding to the *Alk* locus acted in opposite directions in the two successive periods, such that an allele shortening the pasting time would prolong the time needed from the initial viscosity increase to PKV, or vice versa. The QTL corresponding to the *Alk* locus for Atime and BAtime had similar magnitudes of additive effects but in opposite directions in both 2004 (0.43 and –0.36) and 2005 (0.49 and –0.45). Such effects would cancel each other, so no significant effects of *Alk* locus were detected for time and temperature at peak viscosity. The effect of *Wx* locus was not detected for Atime but was for BAtime, and might indicate that *Wx* locus takes effect after the initial viscosity increase. This could explain that the time and temperature at PKV stage were largely dependent on the QTL at the *Wx* locus. Like the paste viscosity parameters after the onset of peak viscosity, we think that these time- and temperature-related parameters in the heating process also suggested structural differences of grain starch in the various genotypes. We further investigated the QTL dynamics during the period from peak viscosity to the final cooling viscosity by quantifying the effects of the QTL corresponding to the *Wx* and *Alk* loci on paste viscosity parameters (Fig. 4). In general, the *Wx* locus made a major contribution to the paste viscosity parameters after the PKV stage while *Alk* locus played a minor role through the whole process (Fig. 4). In both years, the effect of the *Wx* locus was not detected for PKV, but it increased to a considerable amount at 95°C and maintained a high level at the holding stage, then its effects increased again at the cooling stage (Fig. 4).

Our results have clear implications for rice quality breeding programs. First, the availability of closely linked markers on both sides of the *Wx* locus or *Alk* locus will greatly facilitate the precise replacement of the alleles of the poor-quality parent using marker-assisted selection. The parent Delong 208 used in the present study is a specialty *indica* rice cultivar in China with low AC, soft gel, and low GT. Its cooked rice stays tender, moderately sticky and glossy even after cooling. Thus, Delong 208 provided a desirable parent for improving the quality of rice by introducing its *Wx* and *Alk* alleles into the breeding lines. Second, both *Wx* and *Alk* loci can be the well-defined targets for association analysis of sequence variation with the traits characterizing the eating and cooking quality of rice. Ayres et al. (1997) found that the CT repeat and a single nucleotide polymorphism (SNP) at the 5′ leader intron splice site of *GBSS* (*Wx*) accounted for more than 85% of the variation in AC in an extended pedigree of 89 US rice cultivars. Umemoto and Aoki (2005) reported the two SNPs of *SSIIa* (*Alk*) haplotypes alter the starch gelatinization and starch association of the enzyme. Further studies are needed to investigate the sequence variation at both *Wx* and *Alk* loci and its association with the traits characterizing the eating and cooking quality of rice grain and thus facilitate the breeding of these traits.

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