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The main effects, epistatic effects and environmental interactions of QTLs on the cooking and eating quality of rice in a doubled-haploid line population

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Abstract Amylose content (AC), gel consistency (GC) and gelatinazation temperature (GT) are three important traits that influence the cooking and eating quality of rice. The objective of this study was to characterize the genetic components, including main-effect quantitative trait loci (QTLs), epistatic QTLs and QTL-by-environment interactions (QEs), that are involved in the control of these three traits. A population of doubled haploid (DH) lines derived from a cross between two *indica* varieties *Zhenshan 97* and *H94* was used, and data were collected from a field experiment conducted in two different environments. A genetic linkage map consisting of 218 simple sequence repeat (SSR) loci was constructed, and QTL analysis performed using QTLMAPPER 1.6 resolved the genetic components into main-effect QTLs, epistatic QTLs and QEs. The analysis detected a total of 12 main-effect QTLs for the three traits, with a QTL corresponding to the *Wx* locus showing a major effect on AC and GC, and a QTL corresponding to the *Alk* locus having a major effect on GT. Ten digenic interactions involving 19 loci were detected for the three traits, and six main-effect QTLs and two pairs of epistatic QTLs were involved in QEs. While the main-effect QTLs, especially the ones corresponding to known major loci, apparently played predominant roles in the genetic basis of the traits, under certain conditions epistatic effects and QEs also played important roles in controlling the traits. The implications of the findings for rice quality improvement are discussed.

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Introduction

One of the major problems of rice production in many of the rice-producing areas of the world is the improvement of grain quality. The cooking and eating quality of rice, which are among the most important components of grain quality, are largely determined by three primary physical and chemical characteristics of the starch in the endosperm: amylose content (AC) (Juliano 1985), gel consistency (GC) (Cagampang et al. 1973) and gelatinazation temperature (GT) (Little et al. 1958). Traditional genetic studies revealed that AC and GC are each determined by single gene inheritance (Bollich and Webb 1973; McKenzie and Rutger 1983; Kumar and Khush 1988; Chang and Li 1991; Tang et al. 1991), while the inheritance of GT involves one, two or more genes (McKenzie and Rutger 1983; Chang and Li 1991). Chang and Li (1991) suggested that additional minor genes or modifiers are also involved in the inheritance of these traits.

Molecular marker technology has facilitated our understanding of the genetic basis of complex quantitative traits. Recent results from molecular marker-based quantitative trait locus (QTL) analyses of AC, GC and GT have revealed that AC is mainly controlled by the waxy gene locus (*Wx*) on chromosome 6 (He et al. 1999; Tan et al. 1999; Bao et al. 2000; Lanceras et al. 2000; Septiningsih et al. 2003; Aluko et al. 2004), which encodes granule-bound starch synthase (Wang et al. 1990). However, the results of the QTL analyses of GC and GT were less clear. Tan et al. (1999) reported that a single locus in the *Wx* region controls both GC and GT, and Bao et al. (2000) and Lanceras et al. (2000) also detected the effect of the *Wx* region on GC. However, He et al. (1999) and Bao et al. (2002) showed that GC is controlled by two QTLs with minor effects. Moreover, the results of these two groups of investigators suggested that GT is specified by the genomic region corresponding to the alkali degeneration locus (*Alk*) encoding a soluble starch synthase II (SSSII) isoform, which was

recently cloned by Gao et al. (2003). To our knowledge, no study has been carried out on the effects of interactions between genes (epistasis) involved in the genetic basis of the cooking and eating quality traits, which have been found to be important in the expression of many other traits (Yu et al. 1997).

It is well documented that environmental conditions, especially temperature during grain filling, significantly affect the cooking and eating quality of rice (Suzuki et al. 1959; Chikubu et al. 1965; Ebata 1968; Resurreccion et al. 1977; Asaoka et al. 1984, 1985a, b; Umemoto et al. 1995). However, in all of the studies reported, data were collected in single plantings. No effort was made to control the effects of environmental conditions that were introduced by the segregation of phenological traits, such as flowering time that can differ by up to a month or more among lines in a single population (e.g. Tan et al. 1999). Moreover, to date, there is no reported study on the magnitudes of genotype-by-environment interactions in the development of the cooking and eating quality traits. Knowledge is also lacking as to the extent to which QTL-by-environment interactions (QEs) may affect the expression of these traits.

In the study reported here, we attempted to characterize the genetic basis of AC, GC and GT by analyzing the QTLs of main effects and epistatic effects, and their environmental interactions, using a population of doubled haploid (DH) lines derived from a cross between two *indica* varieties. The field experiment was carried out under two very different environmental conditions for the expression of endosperm traits. However, within each of these environments, we staggered the plantings in order to synchronize the grain filling period of the lines so that the development of the endosperm would occur under relatively uniform environmental conditions. The results of these experiments should increase our understanding of the genetic components of grain quality and the mode of their environmental interactions in determining AC, GC and GT, which in turn will help rice breeders formulate strategies for improving the cooking and eating qualities of rice.

Materials and methods

The mapping population and the field experiment

The mapping population consisted of 92 rice (*Oryza sativa* L.) DH lines obtained by anther culture of the F_1 from a cross between two *indica* varieties, *Zhenshan 97*, the female parent for a number of widely cultivated hybrids in China, and *H94*, a variety with good grain quality. To synchronize the grain filling, we grouped the parents and DH lines into three bulks on the basis of their flowering time (determined from data collected prior to the experiment, both on Hainan Island in the spring, and in Wuhan in the summer, 2002), such that 18, 60 and 14 lines were allocated to bulk 1, bulk 2 and bulk 3, respectively. The field experiment was conducted in the rice-growing season of 2003 on the experimental farm of Huazhong Agriculture University, Wuhan, China. Two batches of plantings provided two environment treatments for the population using the flowering time as the indicator. The first batch targeted the flowering time of the population in a relatively short period in July, which will be referred to as the July heading group, and the second batch targeted the flowering time of the population in September, which will be referred to as the September heading group. For the first batch, the sowing dates were May 25 for bulk 1, May 16 for bulk 2 and May 10 for bulk 3. Additionally, the lines in bulk 3 received an 8-h daylength treatment from June 15 to July 5 (plants in the field were covered with a black cloth from 16:00 to 8:00 the following day). For the second batch, the three bulks were planted on June 21, June 11 and May 10, respectively. Since the temperature and daylength in July (high temperatures and long days) are very different from those of September (relative low temperatures and short days), this staggered planting system placed the flowering and graining filling periods of the population in two very different environments.

Each bulk was planted with two replications in each sowing, with each replicate containing ten plants per line transplanted in a single row with 16.5 cm between plants and 26.4 cm between rows. Field management essentially followed normal agricultural practice, with fertilizer applied (per hectare) as follows: 48.75 kg N, 58.5 kg P and 93.75 kg K as the basal fertilizer; 86.25 kg N at the tillering stage; 27.6 kg N at the booting stage.

Trait measurement

Approximately 40 days after heading, the ten plants in each row were harvested and threshed in bulk and the rough rice air-dried and stored at room temperature for 3 months before milling. Milled rice was ground to flour and used for measuring the AC and GC following the methods described by Tan et al. (1999) and Cagampang et al. (1973), respectively. Milled rice was also used for assaying ASV (alkali spread value) according to the method of Little et al. (1958). ASV is inversely related to GT such that a high ASV indicates a low GT, and vice versa. The assay for each trait was conducted with two replicates for each filled replicate, resulting in four data points per line per planting.

DNA markers and assays

In total, 218 polymorphic SSR (simple sequence repeat) markers covering all 12 chromosomes were used for genotyping the population. The markers of the RM-series were designed according to Temnykh et al. (2000, 2001), and those of the MRG-series according to the rice genome sequences of the Monsanto Company

(McCouch et al. 2002). The SSR assay was carried out essentially as described by Jiang et al. (2004). Apicule color controlled by the *C* gene was used as a morphological marker in the map construction.

Data analysis

A genetic linkage map was constructed using MAPMAKER 3.0 (Lincoln et al. 1992). The average of the four measurements for each line in each heading group was used for QTL analysis. QTLMAPPER VER. 1.6 (Wang et al. 1999) based on a mixed linear model approach (Zhu and Weir 1998), which estimates main-effect and digenic epistatic QTLs as well as predicting QE interaction effects simultaneously, was employed to assess QTLs conditioning the grain quality traits with heading times as the environments. In this analysis, likelihood ratio (LR) and *t*-statistics were combined for testing hypotheses about QTL effects (including additive effects and digenic interactions) and QE interactions. Estimates of QTL effects (additive and epistasis) were obtained by the maximum-likelihood estimation method, while QE effects (additive × environment interaction and epistasis × environment interaction) were predicted using an adjusted unbiased predictor. The LR value corresponding to $P = 0.005$ (equivalent to LOD = 4.03 for $df = 6$) was used as the threshold for claiming the presence of putative main or epistatic QTLs. The significance of the QTL effects, including additive effect, additive-by-additive epistatic effect, additive-by-environment interaction effect and epistasis-by-environment interaction effect, was further tested by running the sub-menu of the Bayesian test ($P < 0.005$). 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. When a QTL was involved in more than one epistasis, its position and additive effect were taken from the point showing the largest effect. The relative contribution of a genetic component was calculated as the proportion of phenotypic variance explained by that component in the selected model.

Results

Measurements and segregations of the traits in the two heading groups

The staggered plantings of the DH lines resulted in heading from July 12 to August 3 (average daily temperature: 32°C; daylength: 13.8 h) for the first batch of the three bulks of the population, and from September 2 to September 20 (average daily temperature: 27°C; daylength: 12.6 h) for the second batch of the three bulks.

Significant differences between the parents were detected using a least significant difference (LSD) test at the 0.01 probability level for all three traits in both heading groups, in which *Zhenshan 97* had higher AC, shorter (harder) gel and lower ASV (higher GT) than *H94*. The DH population showed transgressive segregations in both directions for all three traits in both heading groups (Table 1). In addition, for *H94*, the LSD test detected significant differences ($P < 0.01$) between the early and late plantings for all three traits, while only ASV differed significantly ($P < 0.05$) between the two plantings of *Zhenshan 97*.

A two-way ANOVA showed that for all of the traits, as well as genotype differences, the differences were highly significantly between the two heading groups (environments) and that the genotype-by-environment interactions were also highly significant (Table 2).

The DH population showed bimodal segregations for all three traits (Fig. 1). The two categories in each of the three traits fit well to a 1:1 ratio (Table 3), indicating the involvement of a major gene and some minor genes for each trait.

Linkage map

A linkage map consisting of 219 markers spanning a total of 1,646.2 cM was constructed (Fig. 2), with chromosomes 9 and 11 each separated into two linkage groups. The marker order in the map was in good agreement with that of Temnykh et al. (2000, 2001).

Main-effect QTLs and epistatic effects

Amylose content

Four main-effect QTLs for AC were resolved (Table 4, Fig. 2) that collectively explained 58.7% of the total phenotypic variation. The major QTL, *ac6a*, flanked by RM190 and RM587 on chromosome 6, corresponds with the waxy gene (*Wx*) region and accounted for 54.87% of the total phenotypic variation. The allele from *Zhenshan 97* increased AC by 5.83% (additive effect), which is in good agreement with previous findings (e.g. Tan et al. 1999). The other three main-effect QTLs for AC were mapped on chromosomes 6, 11 and 12, respectively, where no QTLs for AC have yet been reported. The effects of these three QTLs were relatively small, with their contributions ranging from 0.85% to 1.85% of the phenotypic variation. At *ac6b*, the allele from *Zhenshan 97* contributed to the increase of AC, whereas the alleles of *ac11* and *ac12* from *H94* increased AC.

Epistatic effects were detected for five pairs of loci (Table 4), but only one pair of loci involved a main-effect QTL. Parental two-locus genotypes appeared to increase AC for three of the five pairs, while recombinant two-locus combinations increased AC for the remaining two pairs. In total, epistatic interactions accounted for 3.1% of the phenotype variation.

Table 1 Descriptive statistics of the grain-quality traits for the parents and the DH population observed in the two environments (July and September heading groups, 2003) (SD standard deviation)

Traits[a]	Heading group	Parent (mean ± SD)		DH population			
		Zhenshan 97	*H94*	Mean ± SD	Range	Skewness	Kurtosis
AC (%)	July	26.17 ± 1.01	13.23 ± 0.98	20.94 ± 7.10	8.90–30.50	−0.17	−1.62
	Sept	27.86 ± 1.31	17.11 ± 0.90	23.63 ± 5.57	12.73–31.23	−0.25	−1.37
GC	July	27.00 ± 0.50	47.50 ± 1.32	37.88 ± 12.33	25.50–72.00	0.62	−0.96
	Sept	27.80 ± 0.76	55.70 ± 1.26	39.16 ± 13.29	26.50–77.50	0.68	−0.8
ASV	July	3.06 ± 0.38	5.31 ± 0.26	4.53 ± 1.52	2.42–7.00	0.23	−1.56
	Sept	3.64 ± 0.38	5.90 ± 0.20	4.95 ± 1.58	2.42–7.00	−0.12	−1.56

[a]Abbreviations are described in the Materials and methods. GC is measured as gel length (in millimeters)

Table 2 Summary of effects resolved by two-way ANOVA of the three traits of the DH population measured in two environments

Traits	Variation[a]	*df*	MS	*F*	*P*
AC	G	83	315.70	128.66	0.0000
	E	1	1445.01	588.91	0.0000
	G × E	83	9.34	3.18	0.0000
	Error	504	2.45		
GC	G	83	1261.61	269.23	0.0000
	E	1	109.29	23.32	0.0000
	G × E	83	75.43	16.10	0.0000
	Error	504	4.69		
ASV	G	84	19.00	156.08	0.0000
	E	1	18.06	148.41	0.0000
	G × E	84	0.41	3.36	0.0000
	Error	510	0.12		

[a] G, Genotype; E, environment; G × E, genotype-by-environment interaction

Gel consistency

The three main-effect QTLs for GC were all mapped on chromosome 6 (Table 5, Fig. 2) and collectively explained 44.4% of the phenotypic variation. All of the alleles increased the gel length and, consequently, the consistency, and all were contributed by *H94*. The position of the QTL with the largest effect, *gc6b*, accounted for 32.52% of the trait variation and corresponded well to the *Wx* locus. This is likely the same locus as reported previously (Tan et al. 1999). The *H94* allele at this locus increased the gel length by 11.87 mm. The QTL *gc6a* also had a sizable effect and accounted for 10.88% of the variation, with the allele from *H94* increasing gel length by 6.87 mm. While *gc6b* corresponded to a QTL (*qGC-6a*) reported previously by Li et al. (2003), *gc6c* has not been detected in previous studies.

An epistatic effect was detected involving a main-effect QTL and a locus that did not show main effect (Table 5). The epistatic interaction accounted for 1.45% of the phenotype variation.

Gelatinazation temperature (GT)

Five QTLs were resolved for GT as measured by ASV, collectively explaining 78.5% of the phenotypic variation (Table 6, Fig. 2). The QTL with the largest effect *asv6c*,

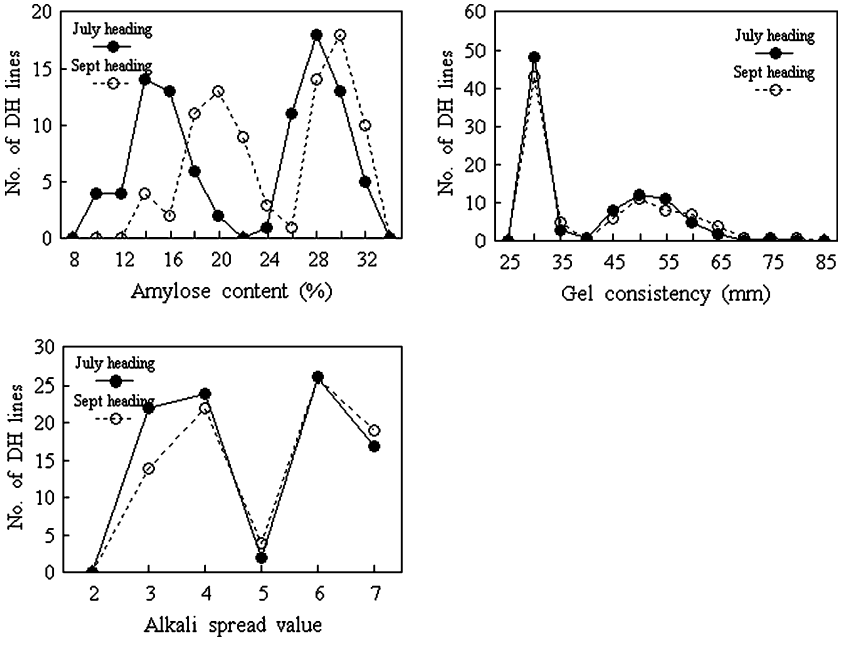

Fig. 1 Distribution of the AC, GC and GT measurements of the DH population in the two heading groups

Table 3 Chi-square test for the expected 1:1 ratio of single-locus segregation for the three traits in the DH population planted in the two environments

Traits	Heading group	Number of lines	Number of lines	χ^2	*P*
AC	July	43 (≤ 20%)	48 (> 20%)	0.18	0.675
	September[a]	42 (≤ 24%)	43 (> 24%)	0.01	0.914
GC	July	51 (≤ 35 mm)	40 (> 35 mm)	1.10	0.295
	Septembert[a]	48 (≤ 40 mm)	38 (> 40 mm)	0.94	0.332
ASV	July	46 (≤ 4)	45 (> 4)	0.00	1.000
	September[a]	40 (≤ 5)	45 (> 5)	0.19	0.664

[a] One line in the July heading group and eight lines in the September heading group did not produce enough seeds for taking measurements

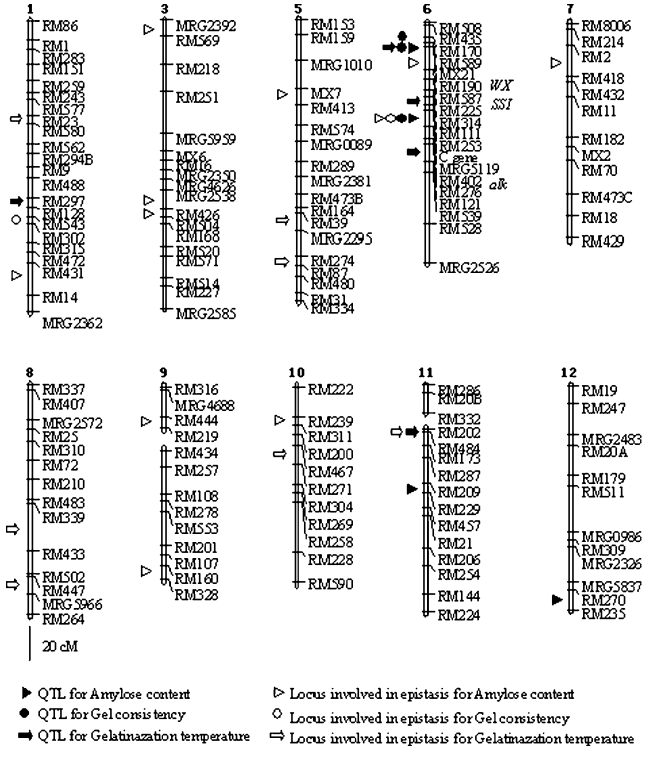

Fig. 2 Distribution of main-effect QTLs and epistatic QTLs on the linkage map as detected by QTLMAPPER

accounting for 38.1% of the trait variation, was mapped between RM276 and RM121 on chromosome 6, which corresponded to the *Alk* locus (Kudo 1968; Harushima et al. 1998; Umemoto et al. 2002). The H94 allele at this locus increased ASV by 1.15. Two other major QTLs for ASV were also detected on chromosome 6. The one located in the vicinity of the *Wx* locus, *asv6a*, accounted for 14.2% of the trait variation with the H94 allele decreasing ASV by 0.7. The other, *asv6b*, was located in between of *Alk* and *Wx* that seemed to correspond to the position of a locus encoding soluble starch synthase I (SSSI) (Tanaka et al. 1995). This QTL accounted for 19.6% of the trait variation, with the H94 allele increasing ASV by 0.8. Thus, the alleles with positive

Table 4 Main effects, epistatic effects and environmental interactions of QTLs detected by two-locus analysis using QTLMAPPER for AC at LOD = 4.03 (equal to a chi-square value for *df* = 6 at *P* = 0.005). General contributions: additive (A): h^2 a = 58.7%; epistasis: h^2 aa = 3.1%; QE interactions: h^2 ae = 6.1%; h^2 aae = 2.0%

Ch-Ini[a]	Flanking markers	QTL	Ch-Inj[a]	Flanking markers	QTL	LOD	$a_{i<}^b$	h^2 a_i^c	a_j^b	h^2 a_j^c	aa_{ij}^c	h^2 a_{ij}^c	ae_i^d	h^2 ae_i^e	ae_j^d	h^2 ae_j^e	aae_{ij}^d	h^2 aae_{ij}^e	h^2 total[f]
1–20	RM431-RM14		9–3	RM444-RM219		5.6					0.55	0.49					−1.08	0.94	1.43
2–12	RM327-RM475		11–8	RM209-RM229	*ac11*	10.8			−1.07	1.85					1.93	3.00			4.85
3–1	MRG2392-RM569		6–12	*C* gene-MRG5119	*ac6b*	6.2			0.83	1.10	0.45	0.33							1.43
3–10	MRG2538-RM426		10–2	RM239-RM311		6.8					−0.63	0.64							0.64
3–11	RM426-RM504		7–3	RM2-RM418		5.0					0.63	0.65					1.12	1.01	1.66
5–4	MX7-RM413		9–12	RM160-RM328		6.5					−0.79	1.00							1.00
6–6	RM190-RM587	*ac6a*	10–4	RM200-RM467		65.8	5.83	54.87					−1.62	2.11					56.98
8–1	RM337-RM407		12–11	RM270-RM235	*ac12*	7.9			−0.73	0.85					1.54	0.95			1.80

[a]Ch-Ini and Ch-Inj represent the chromosome number-interval of the points being tested in the analysis

[b]a_i and a_j are the additive effects of the test points *i* and *j*, respectively. Positive values of a_i and a_j imply that the *Zhenshan 97* genotype has a positive effect on that trait

[c]aa_{ij} is the effect of additive-by-additive interaction between points *i* and *j*; a positive value indicates that the parental two-locus genotypes have a positive effect and that the recombinants had a negative effect

[d]ae_i, $ae_j$and aae_{ij} are effects of the environmental interaction of locus *i*, *j* and epistasis, respectively; a negative value indicates that the effect in the early heading group is larger than in the late heading group

[e]h^2 a_i, h^2 a_j, h^2 aa_{ij}, h^2 ae_i, h^2 ae_j and h^2 aae_{ij} are the percentages of the phenotypic variations explained by a_i, a_j, aa_{ij}, ae_i,, ae_j and aae_{ij}, respectively

Table 5 Main effects, epistatic effects and environmental interactions of QTLs detected by two-locus analysis using QTLMAPPER for GC at LOD = 4.03 (equal to a chi-square value for $df = 6$ at $P = 0.005$). General contributions: Additive (A): h^2 a = 44.4%; Epistasis: h^2 aa = 1.5%; QE interactions: h^2 ae = 1.1%; h^2 aae = 0

Ch-Ini[a]	Flanking markers	QTL	Ch-Inj[a]	Flanking markers	QTL	LOD	a_i^b	h^2	a_i^e	a_j^b	h^2	a_j^e	aa_{ij}^c	h^2	a_{ij}^e	ae_i^d	h^2	ae_i^e	ae_j^d	h^2	ae_j^e	aae_{ij}^d	h^2	aae_{ij}^e	h^2 total[f]
1–16	RM543-RM302		6–12	*C* gene-MRG5119	*gc6c*	5.9				−2.08	1.00	2.50	1.45												2.45
3–11	RM426-RM504		6–6	RM190-RM587	*gc6b*	29.6				−11.87	32.52								2.96	0.73					33.25
5–11	RM164-RM39		6–3	RM170-RM589	*gc6a*	7.3				−6.87	10.88								−1.96	0.32					11.20

[a–e]See footnotes in Table 4

Table 6 Main effects, epistatic effects and environmental interactions of QTLs detected by two-locus analysis using QTLmapper for ASV (GT) with the LOD threshold 4.03 (equal to a chi-square value for $df = 6$ at $P = 0.005$). General contributions: Additive (A): h^2 a = 78.5%; epistasis: h^2 aa = 5.4%; QE interactions: h^2 ae = 0.3%; h^2 aae = 0

Ch-Ini[a]	Flanking markers	QTL	Ch-Inj[a]	Flanking markers	QTL	LOD	a_i^b	h^2	a_i^e	a_j^b	h^2	a_j^e	aa_{ij}^c	h^2	a_{ij}^e	ae_i^d	h^2	ae_i^e	ae_j^d	h^2	ae_j^e	aae_{ij}^d	h^2	aae_{ij}^e	h^2 total[f]
1–8	RM23-RM580		5–12	RM39-MRG2295		7.7							−0.28	2.21											2.21
1–14	RM297-RM128	*asv1*	12–8	RM309-MRG2326		11.8	0.35	3.52											0.13	0.11					3.63
3–14	RM520-RM571		6–15	RM276-RM121	*asv6c*	46.2				−1.15	38.12														38.12
5–14	RM274-RM87		8–9	RM339-RM433		4.6							0.26	1.92					0.14	0.15					2.07
6–6	RM190-RM587	*asv6a*	8–12	RM447-MRG5966		27.1	0.70	14.22					0.14	0.53											14.75
6–10	RM111-RM253	*asv6b*	10–1	RM222-RM239		31.3	−0.83	19.58																	19.58
10–4	RM200-RM467		11–4	RM202-RM484	*asv11*	7.9				−0.33	3.06		−0.16	0.76											3.82

[a–e]See footnotes in Table 4

effects at these three loci on chromosome 6 are dispersed in the two parental lines, with alleles from H94 increasing ASV at two QTLs, and decreasing ASV at one QTL.

The remaining two QTLs, *asv1* and *asv11*, with small effects, were mapped on chromosomes 1 and 11, respectively. The allele from *Zhenshan 97* was in the direction of increasing ASV at one QTL and decreasing ASV at the other. Both of these QTLs have not been identified in previous studies.

Epistatic effects were detected in four pairs of loci, two of which involved main-effect QTLs (Table 6). Parental two-locus genotypes for two of the pairs increased ASV, while recombinant two-locus combinations increased ASV for the other two pairs. In total, epistatic interactions accounted for 5.4% of the phenotype variation.

QTL-by-environment interactions

For AC, environmental interactions were detected for three main-effect QTLs and two pairs of the epistatic QTLs (Table 4). In total, the QEs explained 8.1% of the phenotype variation.

For GC (Table 5), two main-effect QTLs were detected that interacted with environments, while no interaction between epistatic QTLs and environment was detected. These QEs explained 1.1% of the phenotype variation.

For GT (Table 6), only one main-effect QTL interacted with the environments, and the QE accounted only for 0.3% of the variation.

Discussion

The most important outcome of the present investigation is the characterization of the relative importance of the main-effect QTLs, epistatic QTLs and their environmental interactions in controlling the expression of the three traits that are critical in determining the cooking and eating quality of rice grains. Our results clearly show that for each of the traits, the main-effect QTLs were the most important determinants, accounting from 44.4% of the phenotypic variation in GC to 78.5% of the variation in GT. Moreover, the effects of individual QTLs were also highly variable, explaining from as little as 0.85% of the variation (a gene with very minor effect) in AC to as much as 54.87% of the variation (a major gene) also in AC.

Similarly, the effects of epistasis also varied considerably among the traits, although the total effects of the epistatic QTLs were much smaller than those of the main-effect QTLs. The effects of epistasis were the most prominent in GT, accounting for 5.4% of the phenotypic variation, followed by AC, in which epistatic interactions accounted for 3.1% of the variation. Epistatic interactions accounted for the smallest portion of the variation in GC.

The three traits also differed considerably with respect to the effects of QEs. QE had the largest effect on AC, accounting for 8.1% of the phenotypic variation, followed by GC, in which QE accounted for 1.1% of the variation. Very little of the phenotypic variation observed for GT was due to QE.

Based on these results, we conclude that although the major genes (or major QTLs) are frequently the most important determinants of the quality traits, interactions between genes of minor effects or even interactions between ones that do not have effects detectable by single-locus analysis may have sizable effects on quality traits. Moreover, the effects of both major and minor genes are also sometimes subject to environmental modifications, which can cause dramatic differences in the phenotypic effects of the genes. In addition, the effects of epistatic QTLs and QEs explain the genetic basis of the continuity in the distribution curves of these traits in the segregation population, as observed in this study, and also the quantitative differences in these traits among different varieties.

The results of this investigation should be compared those of previous studies. Sano (1984) suggested that there were two functional alleles at the *Wx* locus—Wx^a and Wx^b. The Wx^a allele, specifying a high AC, occurs in most of *indica* rice varieties, while Wx^b, specifying low AC, occurs predominantly in *japonica* rice. Temperature has also been shown to affect the activity of the Wx^b allele such that cool temperatures lead to higher AC in mature seed (Umemoto et al. 1995; Suzuki et al. 2002). Recent investigations have revealed that the expression of the Wx^bgene can be reversibly activated in response to cool temperature conditions (Hirano and Sano 1998), while the Wx^a gene does not respond to cool temperature (Sano et al. 1991). In the present study, both of the parents are *indica* rice. *Zhenshan 97*, which shows high AC and presumably carries the Wx^a allele, showed no significant difference in AC when headed at different time periods, whereas *H94*, which shows low AC and presumably carries the Wx^b allele, showed a significantly higher AC at lower temperatures. This is consistent with the activation of the Wx^b allele by cool temperature conditions.

Tan et al. (1999) reported that the *Wx* region on chromosome 6 simultaneously controls the three traits AC, GC and GT. We also found the *Wx* region to have the largest effects on AC and GC, while the genomic region corresponding to the *Alk* locus, previously reported to have a major effect on GT (He et al. 1999), exhibited the largest effect on GT. As one of the parents of the experimental populations, *Zhenshan 97*, was common to both studies, we can conclude that the difference in the major locus for GT detected in both studies was mainly the result of the alleles at the *Alk* locus between the other two parents, *H94* and *Minghui 63*.

These results also have significant implications in rice quality improvement programs. It is apparent that in addition to the major genes, attention should also be directed to the effects of minor QTLs, epistatic QTLs and QEs. The information obtained in this study should be very useful for manipulating the QTLs for these traits by molecular marker-assisted selection. Moreover, marker-assisted selection will be particularly useful for breaking the unfavorable linkage of the genes, such as in the case of the three major QTLs for GT on chromosome 6 detected in this study. This would enable all of the favorable alleles to be combined in a single individual, which would be impossible to attain using conventional methods.

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