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Identification of QTLs for Cooking and Eating Quality of Rice Grain

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Abstract: The BIL (backcross inbred line) population derived from the cross between Koshihikari (good eating and cooking quality, japonica) and Kasalath (poor quality, indica) was used to analyze the QTLs for amylose content (AC), gelatinization temperature (GT), gel consistency (GC) and protein content (PC). Eight main-effect QTLs including 2 for AC, 3 for GT, 2 for GC and 1 for PC were identified. Moreover, 27 epistatic QTL pairs including 7 for AC, 5 for GT, 4 for GC and 11 for PC were also detected while for AC and GT, one main-effect QTL with a major gene was detected, respectively. Therefore, the main-effect QTL might be more responsible for the current variation than the epistatic QTL. The result indicated that the main-effect QTL is the primary genetic basis for those traits. However, for PC, the epistatic QTL explained a much greater portion of the total variation than main-effect QTL, suggesting that epistatic loci are the primary genetic basis for such trait. In the experiment, chromosome segment substitution lines (CSSLs) were used to confirm reliabilities of the main effect QTLs detected in BIL population. Of the 8 main-effect QTLs for 4 traits in BIL analysis, 6 were confirmed and 2 remained unconfirmed by CSSLs analysis.

Key words: rice quality; quantitative trait locus; molecular marker; chromosome segment substitution lines

Rice is one of the major crops served as the staple food of more than 50% of the world's population. Recently, more attention has been paid to rice quality, especially the cooking and eating quality than achieving the higher yield. The amylose content (AC), gelatinization temperature (GT) and gel consistency (GC) are the three major rice characteristics directly related to cooking and eating quality, responsible for the physical and chemical characteristics of the starch in the endosperm [1-4]. The GT is a physical characteristic responsible for cooking time and the capacity of absorbing water as cooking. The GC is responsible for softness in rice. The AC, the most important factor to rice quality, is negatively related to eating and cooking quality, including viscosity, transparency and milling quality. The protein content in rice is not only a good source of protein in food, but also an important factor influences the cooking and eating quality. Thus, the improvement of rice quality is a major goal in rice breeding programs. However, complex inheritance traits for rice quality led to the failure of breeding efforts. Therefore elucidation of their genetic basis would be of great help to accelerate the improvement of rice quality.

The genetic characters of the cooking and eating quality have been widely studied [5-9], but these gene loci have not been located on the chromosomes. The development of DNA markers and linkage maps of rice have provided new opportunities for the genetic analysis of rice [10]. Several experiments have been designed to detect the QTLs for rice traits, such as yield [11-12], heading date [13-14], salt tolerance [15-16]. The knowledge on QTLs analysis accumulated in these studies can be useful in rice breeding. The rice grain quality is also controlled by quantitative trait loci (QTLs) showing continuous phenotypic variation in rice progeny [17]. Recently, several studies reported the QTLs detected for rice grain quality by using different populations [18-25]. These results indicated that a major gene for AC tightly linked to *Wx* locus on the chromosome 6 or located at the same locus of *Wx* gene, while some minor QTLs also influenced AC. For GT, a major gene was found in the *alk* region, and some minor QTLs were detected. However, GC was only controlled by some minor QTLs.

Koshihikari is a famous japonica variety with

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excellent quality due to low AC, moderate GT and high GC. Although QTL analyses of these three traits have been carried out, the genetic analysis of AC, GT and GC in this variety has not been done yet. Therefore, we used the BIL (backcross inbred lines) population derived from a cross of Koshihikari and Kasalath, an indica variety possessing poor eating and cooking quality to detect the QTLs for AC, GT, GC and PC (protein content). Furthermore, the QTLs for cooking and eating quality detected in BIL population were confirmed by analysis of the CSSL (chromosome segments substitution lines) population. In addition, we found digenic epistatic loci associated with rice quality trait.

MATERIALS AND METHODS

Plant materials

The BIL population and CSSLs were selected by Yano's group at National Institute of Agrobiological Science, Japan [26-28]. Koshihikari, a good eating and cooking quality japonica rice, was crossed with Kasalath, a poor quality indica rice, and F_1 plant was backcrossed with Koshihikari to produce BC_1F_1 seeds, then the single-seed descent method (SSD) was applied to develop 182 BC_1F_9 lines (backcross inbred lines: BIL population) from BC_1F_1 plants [26-27]. The BIL population was used to develop the linkage map. The CSSLs (chromosome segments substitution lines) covering whole rice chromosomes developed from BC_4F_2 were selected by molecular marker-assisted selection. A target chromosome segment from donor parent, Kasalath was substituted in the recurrent parental (Koshihikari) background to form CSSLs [28]. The seeds of BILs, CSSLs and two parents were harvested to measure the quality traits.

Evaluation of rice grain quality

The four physicochemical parameters, AC, GT, GC and PC were measured. All measurements were conducted with two replications for each sample of the BIL population, CSSL population and the parents. The AC was measured following the procedure of Perez and Juliano [29], while the GT was analyzed by the method of Little et al [1]. The GC was measured according to the procedure of Cagampang et al [2], and the PC was measured by the Kjeldahl's method (Kjeltec System 1002, Tecator, Sweden).

Construction of linkage map and QTLs detection

A total of 162 RFLP markers and 2 CAPS markers distributed on all 12 rice chromosomes were selected in order to construct a linkage map using MAPMAKER/EXP 3.0 [30]. Mapping of QTL including main-effect QTL and epistatic QTL was carried out according to QTLMAPPER 1.0 [31]. The threshold was $P \leqslant 0.005$ for main-effect QTL and $P \leqslant 0.001$ for epistatic QTL [12]. The genetic parameters (effect and test statistics) with significant main-effect and epistatic QTL were estimated at the positions of respective LOD peaks in individual putative QTL regions by using QTLMAPPER1.0.

RESULTS

Performance of rice grain quality

The distributions of four traits in BIL population were showed in Fig. 1. The AC of two parents was significantly different, with 12.1% in Koshihikari and 23.8% in Kasalath. The segregation of AC in BIL population displayed a bimodal distribution with slightly transgressive segregation, suggesting that the AC is controlled by a major gene as well as by some minor modifying genes.

The mean value of GT for Koshihikari was 5.0, and 5.2 for Kasalath, with small difference between these parents. However, the GT in BIL population varied greatly, ranging from 3 to 7 (Fig. 1). The distribution was abnormal with a major peak at the high-GT region and relatively flat, extending from the low-GT to the intermediate-GT region. This showed that genes with large and small effect were involved in the segregation of GT.

For GC and PC, BIL population showed proximately normal distributions with transgressive segregations, indicating the polygenic inheritance of these traits.

QTLs analysis

Putative main-effect QTLs of BIL population

A linkage map with 162 RFLP markers and two

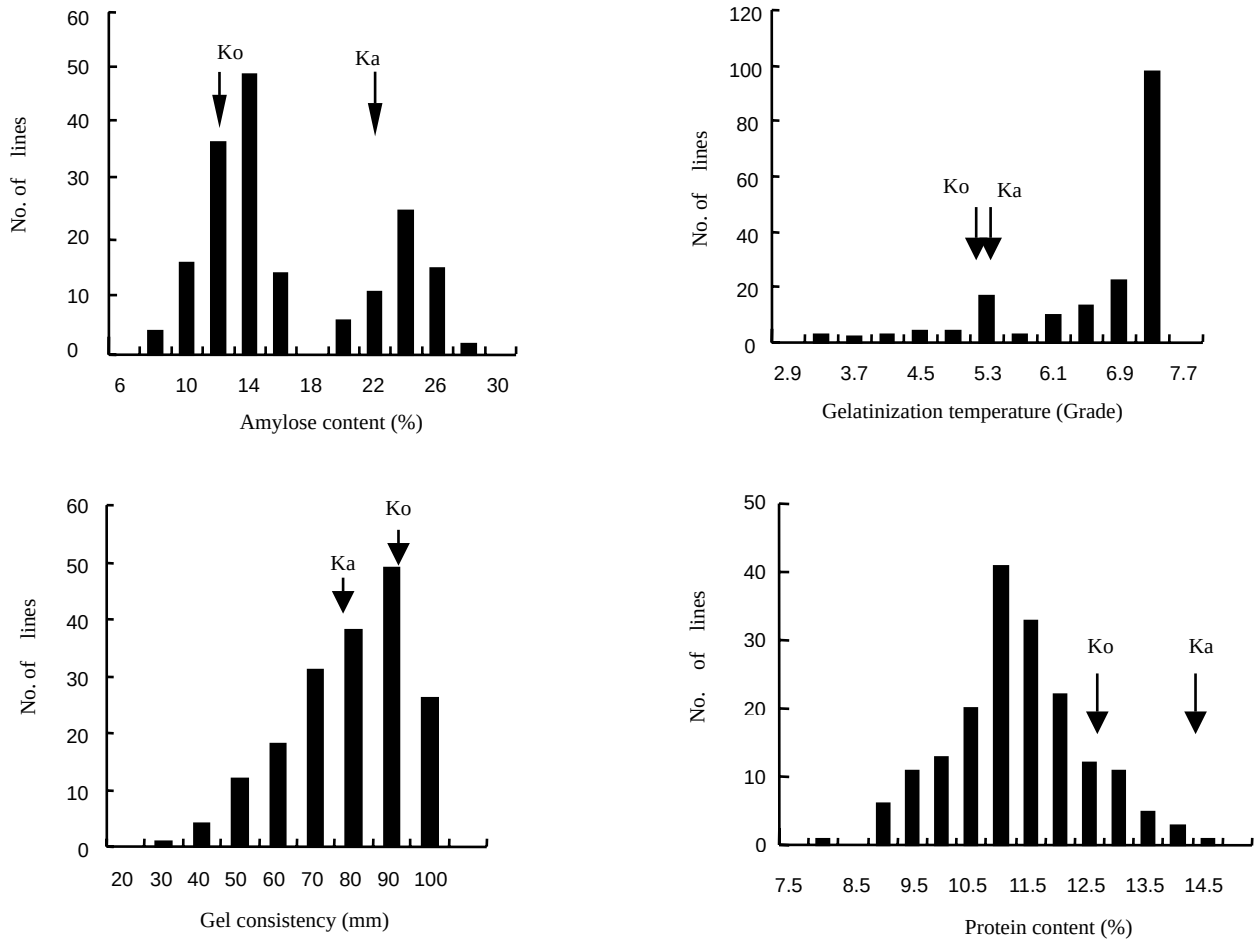

Fig. 1. Frequency distributions of 4 rice grain quality traits, amylose content (AC), gelatinization temperature (GT), gel consistency (GC) and protein content (PC) in BIL population.

Arrows indicate mean of traits for two parents. Ko and Ka indicate Koshihikari and Kasalath, respectively.

CAPS markers covering 12 chromosomes has been established to detect QTLs of rice quality traits (Fig. 2), with a total distance of 1269 cM and the average distance of 8 cM between the markers. The main-effect QTLs was identified by QTLMAPPER software [31] (Table 1, Fig. 2).

Two main-effect QTLs for AC were identified in BIL population and mapped on chromosome 2 and 6, respectively. The major QTL, *qAC-6* with very large effect explained 74.67% of the total variation, which was mapped between S1084 and R1952 on chromosome 6. The Kasalath allele increased the

Table 1. The putative main-effect QTLs in BIL population.

Trait	QTL	Chromosome	Interval	LOD	A [a]	R^2(%) [b]
Amylose content	*qAC-2*	2	R1843–G132	4.20	0.8623	5.83
	qAC-6	6	S1084–R1952	20.34	-3.0852	74.67
Gelatinization temperature	*qGT-3-1*	3	R663–S14055	3.85	-0.1907	3.14
	qGT-3-2	3	R2856–R3226	5.18	-0.3105	8.31
	qGT-6	6	G200–R2171	29.83	0.8644	64.42
Gel consistency	*qGC-2*	2	R712–R1843	6.38	6.0397	18.99
	qGC-3	3	R2856–R3226	4.73	4.9472	12.74
Protein content	*qPC-10*	10	R2194–R1629	5.04	-0.2611	14.31

[a] The QTL effect is associated with the Koshihikari allele (the effect due to substitution of the Kasalath allele by Koshihikari).
[b] Percent of total variance explained.

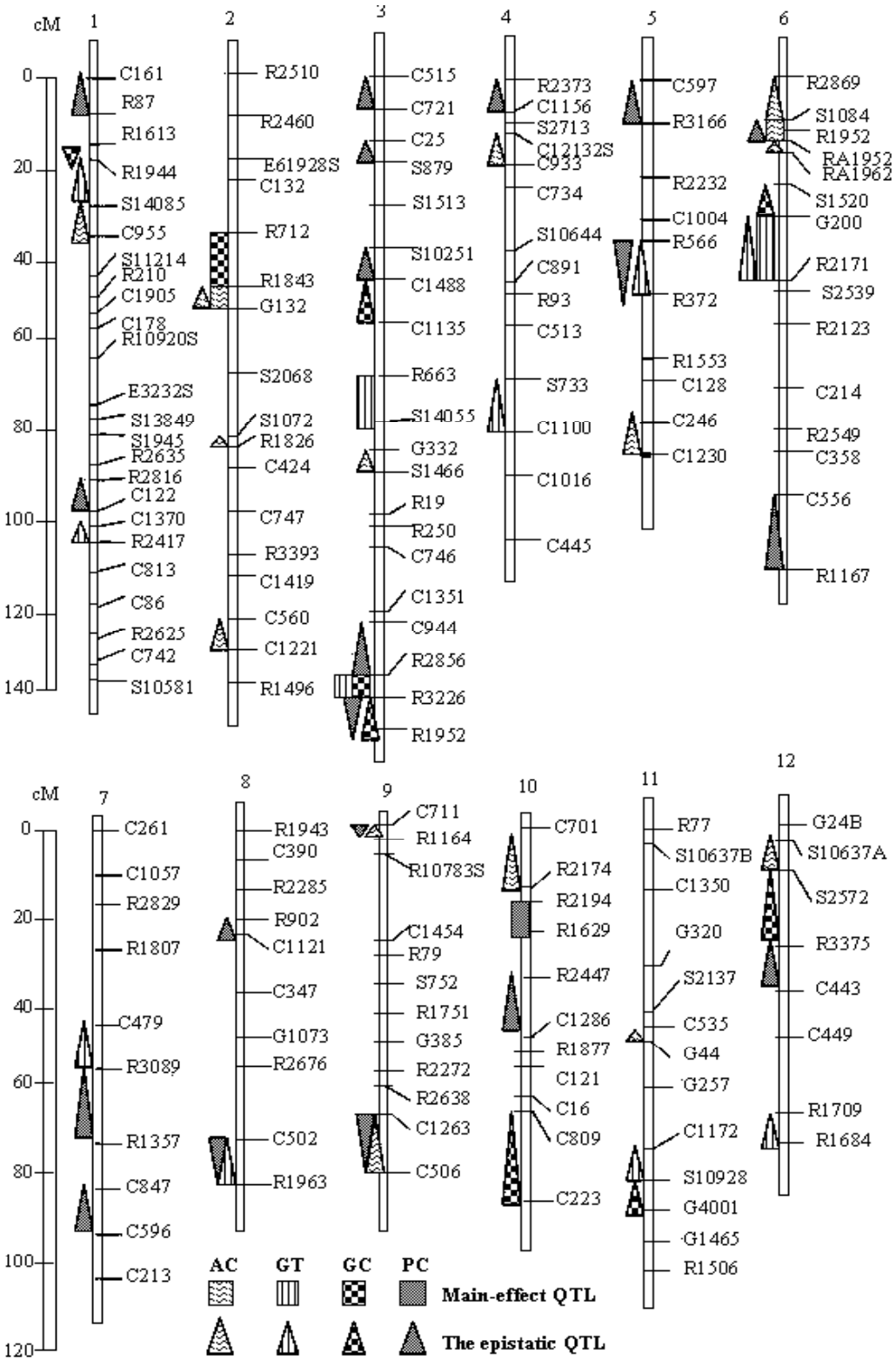

Fig. 2. The genomic locations of the main-effect QTL and the epistatic QTL affecting AC, GT, GC and PC in BIL population.

amylose content at this locus. However, the other minor one only explained 5.83% of the total variation with negative effect at locus by Kasalath allele.

Three main-effect QTLs were detected for GT on chromosomes 3, 3, 6, respectively. A major QTL, *qGT-6* on chromosome 6 in the vicinity of *alk* explained 64.42% of the total variation. The other two minor QTLs, *qGT-3-1* and *qGT-3-2* explained 3.14%

and 8.31% of the total variation, respectively. The Kasalath allele at *qGT-6* lowered GT but the alleles at *qGT-3-1* and *qGT-3-2* raised GT.

Two main-effect QTLs for GC accounting for 18.99% and 12.74% of the total variation were found on chromosomes 2 and 3, respectively. The Kasalath alleles in both loci lowered the GC value.

Only one putative QTL associated with PC resulted in 14.31% of total variation, which was detected and mapped on chromosome 10. The allele of Kasalath at the locus increased the protein content.

Confirming the main-effect QTLs of BIL population by using chromosome segments substitution lines (*CSSLs*)

The AC, GT, GC and PC of CSSL were investigated to confirm the existence of the main-effect QTLs detected in BIL population. The CSSL contained a chromosomal segment with a target QTL of donor parent, Kasalath, with maximun chromosomal segments of recurrent parent, Koshihikari. The main-effect QTL detected in BIL population was confirmed by the comparison of the value of grain quality trait between the CSSL and recurrent parent, Koshihikari.

A major QTL, *qAC-6* for AC was detected within S1084-R1952 on chromosome 6 (Table 1). The data showed that the Kasalath allele of *qAC-6* markedly increased AC, and the three CSSLs spanning the entire Kasalath chromosome 6 with overlapping segments were obtained (Table 2). The AC value of CSSL6-1 harboring of the Kasalath segment with *qAC-6* was 24.2%, which was significantly higher than that of the control, Koshihikari (13.1%). Therefore, the main-effect QTL was confirmed by CSSL analysis. Another main-effect QTL for AC, *qAC-2*, Kasalath allele weakly reduced AC and the AC value of CSSL2-1 was similar to that of the control. Therefore, the effect of *qAC-2* was not confirmed by CSSL.

The Kasalath alleles at both two main-effect QTLs for GT, *qGT-3-1* and *qGT-3-2* had shown a positive effect for GT (Table 1). The *qGT-3-1* resided in overlapping segment of CSSL3-1 and CSSL3-2, while the *qGT-3-2* resided in segment of CSSL3-2. Both the CSSLs had increased GT relatively to Koshihikari. Therefore, the two QTLs were confirmed by CSSLs. However, a major QTL, *qGT-6* located on chromosome 6 had negative effect for GT. Of 3 CSSLs for chromosome 6, only CSSL6-2 carried the *qGT-6* with lower GT value than the control. Therefore, this putative QTL was confirmed by CSSL.

The Kasalath alleles at both main-effect QTLs for GC, *qGC-2* and *qGC-3* was mapped in R712-R1843 region on chromosome 2 and in R2856-R3226 region on chromosome 3 with negative effects, respectively. The values of GC for the CSSL2-1 and CSSL3-2 containing the Kasalath

Table 2. The mean of traits in the CSSL (chromosome segment substitution lines) carried the Kasalath (donor parent) chromosome segment of the target region.

Line No.	Chr	Position (cM) [a]	Substituted region	Trait	Line No.	Chr	Position (cM) [a]	Substituted region	Trait
Amylose content (AC)					Gelatinization temperature (GT)				
CSSL2-1	2	2.5–60.3	R2510–G132	14.1%	CSSL3-1	3	1.9–73.5	C515–R663	6.8 Grade
CSSL2-2	2	60.3–107.7	G132–C747	15.2%	CSSL3-2	3	30.0–159.5	S1513–R1925	7.0 Grade
CSSL2-3	2	79.0–154.7	C499–C1470	13.6%	CSSL6-1	6	3.0–33.5	R2869–G200	5.0 Grade
CSSL6-1	6	3.0–33.5	R2869–G200	24.2%	CSSL6-2	6	33.3–90.3	G200–R2549	4.0 Grade
CSSL6-2	6	33.3–90.3	G200–R2549	12.2%	CSSL6-3	6	50.4–123.1	R2171–R1167	6.1 Grade
CSSL6-3	6	50.4–123.1	R2171–R1167	12.7%	Koshihikari				5.0 Grade
Koshihikari				13.1%	Kasalath				5.2 Grade
Kasalath				23.8%	Gel consistency (GC)				
Protein content (PC)					CSSL2-1	2	2.5–60.3	R2510–G132	85.5 mm
CSSL10-1	10	2.0–41.8	C701–C1268	11.1%	CSSL2-2	2	60.3–107.7	G132–C747	88.0 mm
CSSL10-2	10	29.8–68.4	R2447–R716	8.5%	CSSL2-3	2	79.0–154.7	C499–C1470	74.5 mm
CSSL10-3	10	41.8–82.9	C1286–C223	9.4%	CSSL3-1	3	1.9–73.5	C515–R663	74.5 mm
Koshihikari				12.9%	CSSL3-2	3	30.0–159.5	S1513–R1925	75.5 mm
Kasalath				14.1%	Koshihikari				90.5 mm
					Kasalath				76.0 mm

[a] Indicate position in linkage map from left marker to right marker at substituted region.

segments with *qGC-2* and *qGC-3*, respectively, were lower than that of the control. These results could verify the existences of the two main-effect QTLs.

Only one main-effect QTL for PC was detected on chromosome 10 and the Kasalath allele at this QTL increased PC. However, the value of PC in CSSL10-1 carrying the Kasalath allele of this QTL was similar to that of the control. So, this QTL could not be confirmed by the CSSL analysis.

Epistatic QTLs associated with the four quality traits in the BIL population

The 27 digenic epistatic QTL pairs associated with rice grain quality are shown in Table 3 and Fig. 2. For AC, 7 pairs of the epistatic QTL were detected, which together explained 17.2% of the total phenotypic variation in BIL, while the two of the epistatic QTL effects were negative and the remaining five were positive. On chromosomes 1, 2, 5, 12, 6 and 6, six of the epistatic QTL had shown significant main effects on AC. The alleles at two QTL loci reducing AC were from the Kasalath, while the other four alleles were from the Koshihikari.

During the experiment, five pairs of epistatic QTL associated with GT of the BIL were identified, which together explained 15.7% of the total phenotypic variation. One of the epistatic QTL effects was negative and the remaining four were positive. Only one of the epistatic QTL on chromosome 6 had significant main effect on GT, at which the Kasalath allele resulted in the reduction of GT.

For GC, four pairs of epistatic QTL were detected, which explained 26.6% of the total phenotypic variation in BIL. One of the epistatic QTL effect was positive and the remaining three were negative. Only one of the epistatic QTL on chromosome 3 had significant main effect on GC, at which the Kasalath allele reduced GC.

Table 3. Digenic epistatic QTL in BIL population.

Trait	Chr	QTL_i marker interval	Chr	QTL_j marker interval	LOD	A_i	A_j	AA_{ij}	R^2 (%)
AC	1	S14085–C955	2	R1843–G132	9.96	1.0637***	1.4818***	-1.0823***	4.00
AC	2	S1072–R1826	11	C535–G44	4.88	0.1382	0.0745	0.5418**	1.00
AC	2	C560–C1221	3	G332–S1466	5.55	0.2965	-0.1820	0.6151***	1.29
AC	4	C12132–C933	9	C711–R1164	3.40	-0.0533	-0.2856	0.5670**	1.10
AC	5	C246–C1230	12	S10637–S2572	4.25	-0.3774*	-0.4928*	0.7236***	1.79
AC	6	R2869–S1084	6	RA1952–RA1962	65.36	-1.2032***	-3.0113***	-1.4377***	7.05
AC	9	C1263–C506	10	C701–R2174	3.73	0.0967	-0.0321	0.5319**	0.97
GT	1	R1944–S14085	5	R566–R372	3.87	-0.0269	-0.0516	0.2348**	3.71
GT	1	C1370–R2417	11	C1172–S10928	6.02	-0.0845	0.0004	-0.1937***	2.52
GT	4	S733–C1100	8	C502–R1963	2.84	-0.045	-0.0288	0.1820*	2.23
GT	6	G200–R2171	11	C1172–S10928	32.36	0.8766***	-0.1139	0.2579***	4.74
GT	7	C479–R3089	12	R1709–R1684	4.34	-0.0140	0.0010	0.1933*	2.51
GC	1	R1613–R1944	6	S1520–G200	3.94	0.0890	0.7091	-4.8328*	7.24
GC	3	C1488–C1135	6	S1520–G200	9.51	5.4999***	0.9382	-6.2531***	12.11
GC	3	R3226–R1925	11	S10928–G4001	3.10	-1.6422	0.2002	-3.0895*	2.96
GC	10	C809–C223	12	S2572–R3375	2.96	-0.7567	0.1554	3.7073*	4.26
PC	1	C161–R87	4	R2373–C1156	2.90	0.0347	0.0537	-0.2191*	3.44
PC	1	R2816–C122	7	R3089–R1357	3.69	0.0486	0.1449	-0.2864**	5.88
PC	3	C515–C721	7	R3089–R1357	5.28	-0.2416**	0.0578	0.2174**	3.39
PC	3	C25–S879	9	C1263–C506	9.02	0.3029***	0.0740	0.2015**	2.91
PC	3	S10251–C1488	8	R902–C1121	3.60	-0.0675	-0.0801	0.2478**	4.40
PC	3	C944–R2856	5	R556–R372	3.36	-0.0686	0.0620	0.2185*	3.42
PC	3	R3226–R1925	12	R3375–C443	3.08	-0.0556	0.0533	0.1951*	2.73
PC	5	C597–R3166	6	C556–R1167	3.99	0.0029	-0.1246	0.2658**	5.06
PC	6	S1084–R1952	10	R2447–C1286	7.17	-0.0056	-0.0083	-0.3049***	6.66
PC	6	C556–R1167	8	C502–R1963	2.95	-0.1763*	-0.1035	0.2716**	5.29
PC	7	C847–C596	9	C711–R1164	5.24	0.1516*	0.0665	-0.3130***	7.02

A_i and A_j are the main effects of the loci *i* and *j*, and AA_{ij} is the epistatic effect between loci *i* and *j*. R^2(%) is the proportion of the total phenotypic variation explained by the epistatic QTL. * Significant at $P<0.05$; ** Significant at $P<0.001$, *** Significant at $P<0.0001$.

The 11 pairs of epistatic QTL were identified for PC, which explained 50.2% of the total phenotypic variation in BIL. Four of the epistatic QTL effects were negative and the remaining seven were positive, while four of the epistatic QTL on chromosome 3, 3, 6 and 7 had significant main effects on PC. The Kasalath alleles at two of the QTL reduced PC, while the Kasalath alleles at the other two of the QTL increased PC.

DISCUSSION

The AC, GT and GC are the three most important traits in determination of cooking and eating quality of rice, and the PC is one of the important traits that determine rice nutritional quality. In this study, a permanent mapping population, the BIL population (Koshihikari/Kasalath), was used to analyze the main-effect and epistatic QTLs, which control the rice grain quality. In addition, the CSSLs were used to confirm the main-effect QTLs identified in the BIL population.

During the experiment, we found 8 different main-effect QTLs including 2 for AC, 3 for GT, 2 for GC and 1 for PC at 6 chromosomal regions controlling complex traits related to rice grain quality (Table 1, Fig. 2). There have been several reports on QTL analysis of rice grain quality [18-25]. Several groups reported that a major QTL for AC was mapped on *Wx* locus region of chromosome 6 [18-20, 22-25]. We also found that a major QTL for AC, *qAC-6* was located on chromosome 6. Based on the comparison of chromosomal position of *qAC-6* and the QTL reported, *qAC-6* is likely to be located on the same locus of the QTL. We also detected one minor QTL on chromosome 2, with similar locus to one minor QTL detected by Tan et al [19]. Moreover, one major QTL with very large effect, *qGT-6* for GT detected in this study was on the same locus as *alk*, which was found in previous studies [10, 18, 20, 23-25]. But we didn't find report on the genes located in the same or similar region of the other two minor QTLs for GT identified on the chromosome 3 in this study, so the 2 minor QTLs were newly identified. One QTL for GC, *qGC-2* detected on chromosome 2 might be the same loci detected by He et al [18], Li et al [23] and Tian et al [25]. Another QTL, *qGC-3* was newly detected in this study. Tan et al [21] detected 2 minor QTLs for the protein content, which were located on chromosomes 6 (in the *Wx* gene region) and 7, respectively [21]. However, we did not detect these 2 QTLs, while another QTL for PC was detected on chromosome 10. In conclusion, based on the comparison of chromosomal location, we found that some QTLs are likely in the same locus as in previous study. However, it is difficult to determine whether both QTLs are in the same locus or are tightly linked. Therefore, further analysis, including fine mapping of both QTLs using common markers, cloning and sequence comparison of these QTLs, will be required to answer these questions.

The QTL detection was usually interfered by background loci in primary populations (such as F_2, BC_1, RIL, or BIL population). In this experiment we used chromosome segment substitution lines (CSSLs) to confirm the reliabilities of the main effect QTLs for rice grain quality trait detected in BIL population. The CSSLs with a uniform genetic background can minimize the genetic effects background. Among 8 main-effect QTLs for 4 grain quality traits, 6 were confirmed and only the remaining 2 were not confirmed by CSSLs analysis, indicating that most of the main-effect QTLs detected in the BIL population were credible. Such credible QTLs can be used for map-based cloning. Previously, QTLs for rice heading date, yield and salt tolerance have been successfully cloned by map-based cloning [32-34]. Another advantage of the CSSLs is to identify QTL that was not detected in primary populations. For example, no main-effect QTL for GC on the Kasalath chromosomal region (C499–C1470) in the BIL analysis was observed, however, the GC value of CSSL2-3 (74.5 mm) was lower than that of the control (90.5 mm) (Table 2), indicating an additional main-effect QTL for GC might exist in C499–C1470 region, which is in agreement with the result of Li et al [23]. Eshed and Zamir also reported 2 QTLs, which had not been previously detected in primary population, were identified by ILs (introgression lines, IL is similar to CSSL) analysis [35]. These results indicated that the CSSLs were useful not only in confirmation of main-effect QTLs, but also for detection of main-effect QTL, which was not detected

in primary populations (such as F_2, BC_1, BIL or RI).

Most of the previous studies were focused on detection the main-effect QTL for quality traits of rice grain. However, detection of epistatic QTL was rarely carried out [24]. Some groups performed identification of main-effect QTL and epistatic QTL for rice yield components, and found few main-effect QTL with several epistatic QTL pairs [11-12]. These results indicated that the epistatic QTL explained a much greater portion of the total variation than main-effect QTL for quality traits. A similar case was also found in this study. For example, only one minor main-effect QTL for PC was detected and together explained only 14.3% of the total phenotypic variation (Table 1), while 11 epistatic QTL pairs for PC were detected and together explained 50.2% of the total phenotypic variation (Table 3). The epistatic loci are the primary genetic basis for some agronomic traits, e.g. yield components and grain quality traits, which are controlled by multi-loci with complex heredity. Therefore, the epistasis should be carefully considered in breeding process. However, for AC and GT, the main-effect QTL explained a greater portion of the total variation than the epistatic QTL, suggesting that the main-effect QTL is the primary genetic basis for these traits. Therefore, these traits have shown higher heritability and are relatively readily improved in rice breeding program.

Among the 27 epistatic QTL pairs, no occurrence between main-effect QTLs, 3 occurred between a main-effect QTL and a "background" locus, and the remaining 24 occurred between complementary loci. These results revealed that most epistasis occurred between complementary loci, which is in agreement to the findings by Li et al and Luo et al [11-12]. Moreover, among 8 main-effect QTLs, 3 were also epistatic QTL. One was *qAC-2* located on R1843–G132 region of chromosome 2, which interacted with other locus affecting AC, one was *qGT-6* located on G200–R2171 interval of chromosome 6, which interacted with other locus affecting GT (Table 3, Fig. 2). However, *qAC-6* for AC located on *Wx* gene region (S1084–R1952) of chromosome 6 was an epistatic QTL with large effect on PC. Tan et al [21] detected a main-effect QTL in the *Wx* gene region, responsible for the protein content, which had a large effect. Our results is inconsistent with the previous reports that starch synthetase, one of the milled rice proteins is correlated with amylose content, is embedded in the starch granule [36-38]. We have noted that the total effects of the epistatic QTLs were much smaller than those of the main-effect QTLs for AC and GT. However, the significant epistatic interactions were dominant for GC and PC. Based on these results, we can conclude that epistatic effects between the loci are very important trait for which effects of the main-effect QTLs are small. Furthermore, the epistasis can result in the heterosis [11-12], which is important in breeding. Therefore, the epistatic interactions must be in consideration to improve the grain quality of rice.

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