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Quantitative Trait Loci Mapping for Appearance Quality in Short-Grain Rice

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ABSTRACT

Rice (*Oryza sativa* L.) appearance quality, as defined by both grain shape and lack of chalkiness, are key breeding goals throughout the world's rice-producing areas. To understand the genetic effects on rice appearance quality, we grew a double-haploid (DH) population derived from the cross between two short-grain rice cultivars, *japonica* Chunjiang 06 (CJ06) and *indica* Taichung Native 1 (TN1), in both subtropical Hangzhou and tropical Hainan, China. Transgressive segregation was noted for all measured traits: grain length (GL), grain width (GW), length/width ratio (LW), percentage of kernels with chalk (CK), average chalk size (CS) per kernel, and chalkiness degree (CD, calculated as $CK \times CS$). High positive correlations observed between the chalkiness measures (CK, CS, and CD) with GL and GW indicate that grain shape may have pleiotropic effects on grain chalkiness. Nineteen main-effect QTL and nine epistatic interaction pairs were detected for grain chalkiness and shape. Quantitative trait loci (QTL) were on all 12 chromosomes and explained 8.0 to 23.6% of the phenotypic variance observed under the two environments. One chalkiness QTL and one shape QTL had main effects in both locations. However, when epistasis was considered, seven of 10 identified main-effect regions also had epistatic effect on either grain chalkiness or shape. This demonstrated that epistasis plays an important role in determining grain chalkiness, shape, and the association between chalkiness and shape. The identified QTL and epistatic interactions provide further understanding of the genetic basis of grain appearance quality and assistance in improving grain appearance quality of rice through breeding.

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Abbreviations: CD, chalkiness degree; CK, percentage of kernels with chalk; CS, average chalk size per kernel; CSSL, chromosome segment substitution line; DH, double-haploid; GL, grain length; GW, grain width; HN, Hainan, China; HZ, Hangzhou, China; LW, length/width ratio; SSR, simple-sequence repeat; STS, sequence tag site.

RICE is the staple food for half of the world's population (Hao and Lin, 2010; Guo et al., 2013), and high yield and good quality are the two main goals of rice breeding to increase production. In general, rice grain quality, which is governed by multiple genes termed as QTL (Liu et al., 2013), is mainly determined by four indexes, namely, appearance, cooking and eating quality, nutritional value, and milling quality (Li et al., 2003). Grain chalkiness and shape, including CK, CD, CS, GL, GW, and LW, are crucial aspects of grain appearance quality considered by consumers. Low CK, CD, and CS values are favored by most consumers, whereas grain shape preference greatly varies depending on the regions and customs (Fitzgerald et al., 2009). For example, people in southern China's Guangdong prefer rice with slender shape, while the round grain is the favorite in eastern China.

Eighty-two QTL for grain chalkiness have been identified using diverse populations (<http://archive.gramene.org>) including recombinant inbred line populations derived from intra- (Mei et al., 2013) and inter-subspecific crosses (Lou et al., 2009), $F_{2,3}$ populations (Tan et al., 2000), backcross inbred line population (Li et al., 2003), chromosome segment substitution line (CSSL) population (Wan et al., 2005), DH populations (He et al., 1999; Jiang et

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al., 2005), etc. However, there is rare research on the interaction effect between grain shape and grain chalkiness.

Grain shape is also an important component of the appearance quality in rice, affecting the market demand and consumption. Its genetic basis has been extensively reported in the last decade (Song et al., 2007; Ikeda et al., 2013). Because of its high heritability, substantial progress has been achieved in breeding for three major rice shapes based on LW and short, medium, and long grain and in identification of QTL affecting grain shape (Zhang et al., 2013). To date, >500 QTL for grain shape have been reported across all 12 chromosomes of rice (<http://www.gramene.org>). At least seven of these QTL have been cloned, and these include *GW2*, *GS3*, *GL3.1*, *GS5*, *qSW5/GW5*, *TGW6*, and *GW8*. *GW2* encodes an unknown RING-type protein with E3 ubiquitin ligase activity. Loss of *GW2* function increases cell numbers and results in a wider husk; the grain milk filling rate is also accelerated, thereby enhancing the GW, grain weight, and grain yield (Song et al., 2007). The *gl3.1/qgl3* allele with weaker dephosphorylation activity accelerates cell division, consequently resulting in a longer grain and higher yield (Qi et al., 2012; Zhang et al., 2012). *TGW6* encodes an indole-3-acetic acid-glucose hydrolase protein, and loss of *TGW6* function increases GL and GW as well as enhances grain yield (Ishimaru et al., 2013).

Improving grain quality in conjunction with increasing grain yield is difficult because increased grain size may reduce grain quality (Song et al., 2007; Sakamoto and Matsuoka, 2008; Li et al., 2014). Several cloned QTL are associated with grain shape, and their physiochemical properties may have pivotal roles in the genetic control of grain chalkiness in rice (Mei et al., 2013). For instance, *GW2*, a major QTL for GW and weight, significantly increases the percentage of chalky grain (Song et al., 2007). Moreover, the major QTL for grain chalkiness, *Chalk5*, is closely associated with two major QTL for GW and weight, namely, *qSW5/GW5* and *GS5*. A plant with *Chalk5/GS5/gw5* produces chalky and wide grains and a plant with *chalk5/g5/GW5* produces nonchalky and narrow grains (Li et al., 2014).

Studies showed that rice grain appearance traits are simultaneously controlled by triploid endosperm genes, cytoplasmic genes, maternal genes (Shi and Zhu 1996), and their genotype \times environment (GE) interaction effects (Shi et al., 1999). Shi et al. (2000) found grain shape to be mostly controlled by genetic main effects but also by genotype \times environment interaction effects. Zhang et al. (2006) showed the predominant role of additive gene action for development of rice grain shape and the important roles of both maternal and cytoplasm genetic heritabilities. Thus, the stability of QTL under different environments is critical in determining the usefulness of a QTL for marker-assisted breeding (Peyman et al., 2009).

Based on a DH population derived from a cross between the *japonica* cultivar CJ06 and *indica* cultivar TN1, we previously identified QTL for eating and cooking quality (Leng et al., 2014). It was shown that epistatic QTL and environmental interactions also affected the two rice quality traits in that study, that is, apparent amylose content and gelatinization temperature. In the current study, the same mapping population was used to identify QTL for specific aspects of rice grain appearance quality including the CK, CS, CD, GL, GW, and LW of brown rice were further performed. Chromosomal regions containing loci with digenic epistatic effects, as well as those having main effects, were determined.

MATERIALS AND METHODS

Planting Materials and Field Trials

The DH population consisting of 116 lines was previously developed via anther culture of an F_1 hybrid between the *japonica* cultivar CJ06 and *indica* cultivar TN1 (Sogawa et al., 2004), which segregates for eating and cooking quality (Leng et al., 2014) and for chlorophyll content (Huang et al., 2015). Grain for appearance quality evaluation was produced in two diverse field locations in China with three replications each. For each study, uniform seeds of all DH lines and parents were soaked in distilled water in the dark at 30°C for 2 d and were germinated in distilled water at 35°C for 12 h. The germinated seeds were then sown in a seedbed located in the paddy field. The 25-d-old seedlings were transplanted into a 20 by 20 cm grid pattern in the flooded paddy. Plots consisted of four rows containing six plants each. Taking into account the impact on the intake and outfall from irrigation, three replications of each line were arranged in a randomized block design, blocking in the direction of water depth differences from the water intake to the outflow ends of the paddy. The field plots were supplemented with 150 kg N ha⁻¹ (as urea) applied in three splits in a ratio of 5:3:2 at basal, tillering, and earing stage, respectively. Potassium at 130 kg K₂O ha⁻¹ was applied in two splits: 50% as basal dressing and 50% as topdressing at earing in the form of KCl. Phosphorus at 60 kg P₂O₅ ha⁻¹ in the form of calcium superphosphate was applied as basal. The field management was performed as described by Wang et al. (2014) with slight modification. Field trials were conducted in Hangzhou (HZ) (119°54' E, 30°04' N) from May to October 2012. Because of frequent multistage typhoons during the rainy season (from June to October) in Hainan (HN) (110°00' E, 18°31' N), the experiment was conducted from December 2012 to April 2013 in HN.

Evaluation of Rice Grain Chalkiness and Shape

Approximately 40 d after flowering, four plants in the middle of each block were harvested together, bulk-threshed, air-dried, and stored at room temperature for 3 mo before milling. About 30 g of grain was dehulled using a huller (SDL-A; CNRRI) and milled using a JMJ-100 rice miller (CNRRI). The evaluation of GL, GW, LW, CK, CD, and CS were executed according to National Standard of People Republic of China (1999) and Zeng et al. (2002) with slight modification. Ten whole (unbroken)

milled grains were lined up and measured with a vernier caliper to determine their GL and GW. The total length and width of 10 kernels for three replications were measured and then used to calculate average per-kernel values. The LW was calculated as GL divided by GW. To determine CK, 100 random kernels per grain sample were determined to be chalky or vitreous using a chalkiness visualizer constructed at the China National Rice Research Institute (National Standard of People Republic of China, 1999). In addition, 20 chalky grains were then selected at random, and the ratio of the area of chalkiness to the area of the whole endosperm for each grain was estimated by human visual assessment as viewed on the chalkiness visualizer. This determination of the size of the chalky area per grain is hereafter called chalky size (CS). The value of CS was multiplied by CK to obtain CD. All six traits were measured on three replications per line for each field location.

Data and Quantitative Trait Loci Analysis

Population distribution and correlation analyses were performed using SAS version 8.0 (SAS Institute, 1999). The linkage map constructed using Mapmaker/EXP version 3.0 (Xu et al., 1998). The marker dataset and linkage map comprising 188 simple-sequence repeats (SSRs) plus 19 sequence tag site (STS) loci were previously developed by Leng et al. (2014) with some modification. The QTL and epistatic effects were determined using QTLNetwork version 2.0 in this DH population (Yang and Zhu, 2005) based on a mixed linear model (Wang et al., 1999). A threshold of $P < 0.005$ was used to determine the putative QTL. The digenic epistatic effects were scanned for all 12 chromosomes, and interactions between pairs of loci were declared significant when they exceeded a threshold of $P < 0.005$. The genetic parameters, additive effects, and percentages of variation accounted for by each QTL were also estimated using QTLNetwork version 2.0. The identified QTL were named according to the standard nomenclature (McCouch, 2008).

RESULTS

Phenotypic Variation in Parents and Double-Haploid Lines

Figure 1 and Table 1 summarize the grain appearance quality of the parents and DH population. All measured traits exhibited significant differences between parents under each of the two different field locations. The chalkiness of the grains of the *japonica* parent CJ06 (values of CK, CS, and CD) were lower when grown in the subtropical HZ location than when grown in the more tropical HN location (Table 1). In contrast, the grain chalkiness of the *indica* parent, TN1, was only slightly different between HZ and HN locations (Fig. 1; Table 1). The grain shape of both TN1 and CJ06 were similar between the two growing locations, with TN1 grains being more slender than CJ06 grains under both HZ and HN conditions (Table 1). This indicates that grain shape is less sensitive to environment than chalkiness.

For each trait, the distribution in the DH population between the 2 yr was similar (Fig. 1A–1F). A deviated bimodal distribution of the phenotypic values for CK, CS,

and CD was observed (Fig. 1A–1C), indicating that those measures of chalkiness were controlled by a major gene plus additional minor modifying genes. The continuous normal distribution for GL, GW, and LW in the DH population indicated that those traits were also controlled by multiple genes (Fig. 1D–1F). Transgressive variation was observed for all measured traits in the DH population except for CK.

Correlation Analysis of Traits

The correlations between grain appearance traits under the two growing conditions are presented in Table 2. The chalkiness traits, including CK, CS, and CD, were positively correlated with each other in both HZ and HN but were more strongly correlated in HZ than HN (Table 2). For example, the correlation coefficient for CK and CD was 0.827 in HZ but 0.487 in HN (Table 2). On the contrary, the correlation between CK and GL in HN was higher than in HZ, and while the correlation between CK and LW was a highly significant, -0.384 in HN, these traits were not correlated in HZ (Table 2).

Main-Effect Quantitative Trait Loci for Grain Appearance Quality

A total of 19 main-effect QTL, including 10 for chalkiness and nine for shape, were detected on chromosomes 2, 5, 6, 7, 8, 10, 11, and 12 under two different environments (Fig. 2; Table 3). These main-effect QTL spanned the F -values from 9.5 to 20.4 with additive effect ranging from 8.6 to 25.5% (Table 3). For all QTL associated with grain chalkiness, regardless of which environment they were detected in, the CJ06 allele was associated with reduced chalkiness. The QTL $qCK10$ was detected under both environments. This QTL explains 14.5 and 15.3% of the total variance for this trait in HZ and HN, respectively (Table 3). Another CK QTL, $qCK7n$, explained more phenotypic variance than $qCK10$ in the HN environment, but its lack of effectiveness in HZ suggests it is not a robust CK QTL. While each location identified a different single QTL for CS, each CS QTL was collocated with a CK locus effective in that same environment.

Four and five QTL for grain shape were identified in HZ and HN, respectively, but only one shape QTL ($qGW6$) had main effect on GW under both environments. $qGW6$ increased GW ~ 0.10 and 0.14 mm and explained 10.2 and 9.1% of the total variance in GW (Fig. 2; Table 3). The region between RM492 and RM145 on chromosome 2 showed main effects on GL, GW, and LW in HN but did not appear to have any effects in the HZ data.

Digenic Epistasis Quantitative Trait Loci for Grain Appearance Quality

QTLNetwork, a methodology for systematically mapping QTL based on the mixed-model approaches, has been used for QTL analysis and digenic epistasis analysis (Wang et al.,

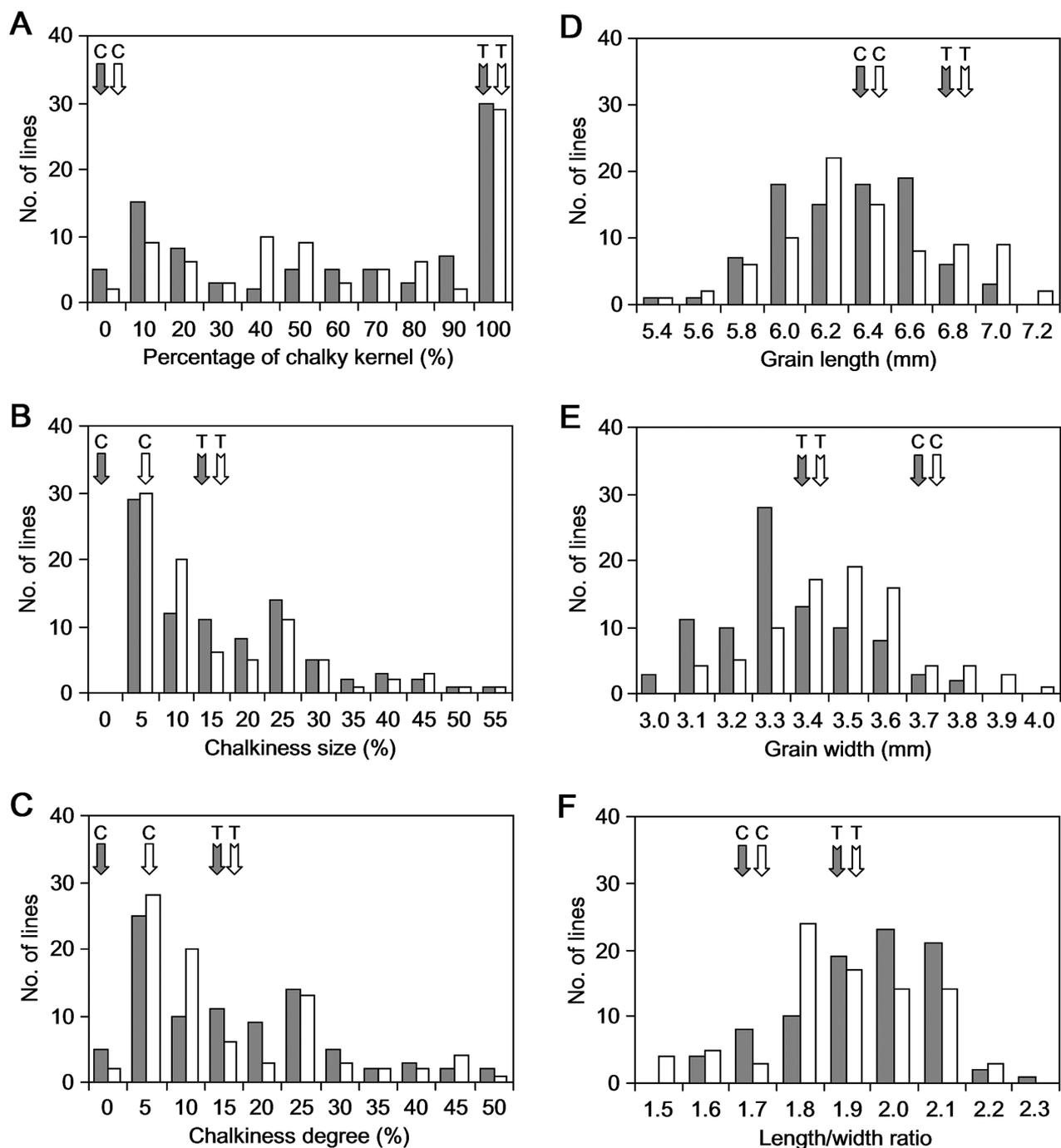


Fig. 1. Frequency distributions of the chalky kernel, chalkiness size, chalkiness degree, grain length, grain width, and length/width ratio in double-haploid population. Grey columns and arrows indicate the rice grown in Hangzhou in 2012. Empty columns and arrows indicate the rice grown in Hainan in 2013. Arrows with smooth end indicate CJ06; arrows with notch end indicate TN1. The capital C and T indicate CJ06 and TN1, respectively.

1999; Gao et al., 2004; Huang et al., 2015). To understand the genetic components of the aforementioned traits, we further estimated the digenic epistatic effects of CK, CS, CD, GL, GW, and LW. Four and five pairs of digenic epistasis were detected in HZ and HN, respectively (Fig. 2; Table 4). The epistatic interaction explained 8.0 to 23.6% of the phenotypic variance. Most of the epistasis occurred between a locus that had a main effect on one or more traits in this study at the single-locus level and a locus that

did not show a significant main effect (Fig. 2). Analysis of epistasis also revealed significant association between chalkiness and shape among the QTL than was seen in main effects. For example, the digenic interaction for CD found in HZ was between two QTL found to have main effects on shape but not on chalkiness. In fact, two of the four digenic effects on chalkiness involved loci having main effects on grain shape but not chalkiness, and one of the four digenic interactions identified for grain shape

Table 1. Statistical analysis of grain appearance quality by two parents and their double-haploid (DH) population in Hangzhou (2012) and Hainan (2013)

| Year | Trait† | Parents (mean ± SD) | | | DH population | | | |
|--------------------|---------|---------------------|-------------|---------|---------------|-----------|----------|----------|
| | | CJ06 | TN1 | P-value | Means ± SD | Range | Skewness | Kurtosis |
| Hangzhou (2012) | CK (%) | 0.0 | 99.0 ± 0.8 | <0.0001 | 55.8 ± 38.0 | 0.0~100.0 | -0.24 | -1.61 |
| | CS (%) | 0.0 | 15.5 ± 2.7 | <0.0001 | 14.9 ± 12.0 | 1.5~55.0 | 1.08 | 0.90 |
| | CD (%) | 0.0 | 15.5 ± 3.2 | <0.0001 | 14.4 ± 12.4 | 0.0~54.6 | 1.01 | 0.67 |
| | GL (mm) | 6.32 ± 0.10 | 6.62 ± 0.09 | 0.0153 | 6.21 ± 0.32 | 5.38~6.98 | -0.02 | -0.37 |
| | GW(mm) | 3.60 ± 0.07 | 3.39 ± 0.08 | 0.0028 | 3.29 ± 0.18 | 2.94~3.73 | 0.29 | -0.23 |
| | LW | 1.75 ± 0.06 | 1.96 ± 0.06 | 0.0012 | 1.89 ± 0.15 | 1.54~2.22 | -0.41 | -0.43 |
| Hainan (2013) | CK (%) | 3.0 ± 1.1 | 97.2 ± 1.5 | <0.0001 | 58.4 ± 34.8 | 0.0~100.0 | -0.24 | -1.42 |
| | CS (%) | 5.0 ± 1.8 | 18.5 ± 2.6 | <0.0001 | 13.0 ± 12.3 | 1.5~56.0 | 1.41 | 1.49 |
| | CD (%) | 5.1 ± 1.5 | 18.9 ± 3.1 | <0.0001 | 12.8 ± 12.4 | 0.0~55.9 | 1.39 | 1.42 |
| | GL (mm) | 6.37 ± 0.11 | 6.65 ± 0.10 | 0.0218 | 6.27 ± 0.40 | 5.39~7.19 | 0.22 | -0.41 |
| | GW(mm) | 3.62 ± 0.06 | 3.38 ± 0.05 | 0.0049 | 3.43 ± 0.19 | 3.02~3.92 | 0.18 | 0.19 |
| | LW | 1.76 ± 0.08 | 1.97 ± 0.08 | 0.0015 | 1.83 ± 0.17 | 1.45~2.17 | -0.30 | -0.27 |

† CK, chalky kernel; CS, chalkiness size; CD, chalkiness degree; GL, grain length; GW, grain width; LW, length/width ratio.

Table 2. Trait correlations for grain appearance quality related traits from double-haploid population derived from the cross of CJ06 × TN1 observed in Hangzhou (upper) and Hainan (lower).

| Trait† | CK | CS | CD | GL | GW |
|--------|--------------------|--------------------|-------------------|----------------------|----------------------|
| CS | 0.551** 0.366** | | | | |
| CD | 0.827** 0.487** | 0.367** 0.311* | | | |
| GL | 0.532** 0.613** | -0.164 -0.322* | 0.359** -0.008 | | |
| GW | 0.448** 0.499** | -0.217 -0.341** | 0.302* -0.015 | -0.702** -0.667** | |
| LW | -0.221 -0.384** | 0.462** 0.261 | -0.240 -0.001 | 0.937** 0.911** | -0.721** -0.664** |

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† CK, chalky kernel; CS, chalkiness size; CD, chalkiness degree; GL, grain length; GW, grain width; LW, length/width ratio.

involved loci having main effects on chalkiness but not for shape. The two pairs of epistatic interaction identified for GL were estimated to explain 21.4 and 23.6% of the total phenotypic variation for GL in HZ and HN, respectively. For GL, the epistasis detected was of both larger and more consistent effect within and between environments than the two main effects GL QTL were. It appears that epistasis plays an important role in GL in our population.

DISCUSSION

A total of 19 main-effect QTL and nine pairs of digenic epistasis interactions for appearance quality were identified across all 12 chromosomes under two different conditions. However, only one chalkiness and one shape loci had main effects were detected in both locations. The loci lacking a main effect in one location were sometimes identified as having an epistatic effect for that trait instead. It showed that the expression of QTL for rice appearance quality was affected by environments as well as epistasis, which is in

agreement with previous observations by Li et al. (2003). The QTL for different traits showed different stability, which is in agreement with the results of QTL identification for rice agronomic traits (Lu et al., 1997; Li et al., 2003).

Wan et al. (2005) identified 22 QTL for the appearance quality distributed in 12 intervals using the CSSLs derived from the cross 'Asominori' × 'IR24': five were overlapped or adjacent to the loci in this study. The intervals of RM492 to RM145 on chromosome 2 and RM6836 to RM527 on chromosome 6 were similar to their previous report. Wan et al. (2005) also reported three QTL in the region of chromosome 8 flanked by RM1376 and RM4085. Zhou et al. (2009a) found QTL for percentage of chalky grains that were similarly mapped to the CD, CK, and CS we identified on chromosomes 6 and 7. Zhou et al. (2009b) further detected two QTL for percentage of chalky kernels and grain weight next to the region of RM6737 to RM1108. We also identified two novel loci for grain chalkiness (*qCD8h* and *qCK11n*) that have not yet been previously reported. The digenic epistasis may possess an important function as well as the main-effect QTL to a certain extent. The epistatic interactions for the six components of the appearance quality were detected either in HZ or in HN, and the epistatic effects for GL were higher than those of a single main QTL both in HZ and HN.

The pleiotropic effects or the tight linkage of genes could cause QTL to affect related traits mapped and anchored into similar regions. Among the main-effect QTL and digenic epistasis found, 34 were located in 12 intervals or adjacent regions. For instance, three QTL related to the grain shape and two epistatic loci mapped between RM492 and RM145 on chromosome 2. In addition, *qCK7n*, *qCS7n*, and *qCD7n* were anchored between RM3555 and RM1306 on chromosome 7. Zhou et al. (2009a) also identified a major QTL, *qPGWC7*, for the percentage of grain chalkiness and fine-mapped it within the 44-kb region. Additionally, four, three, five, and two

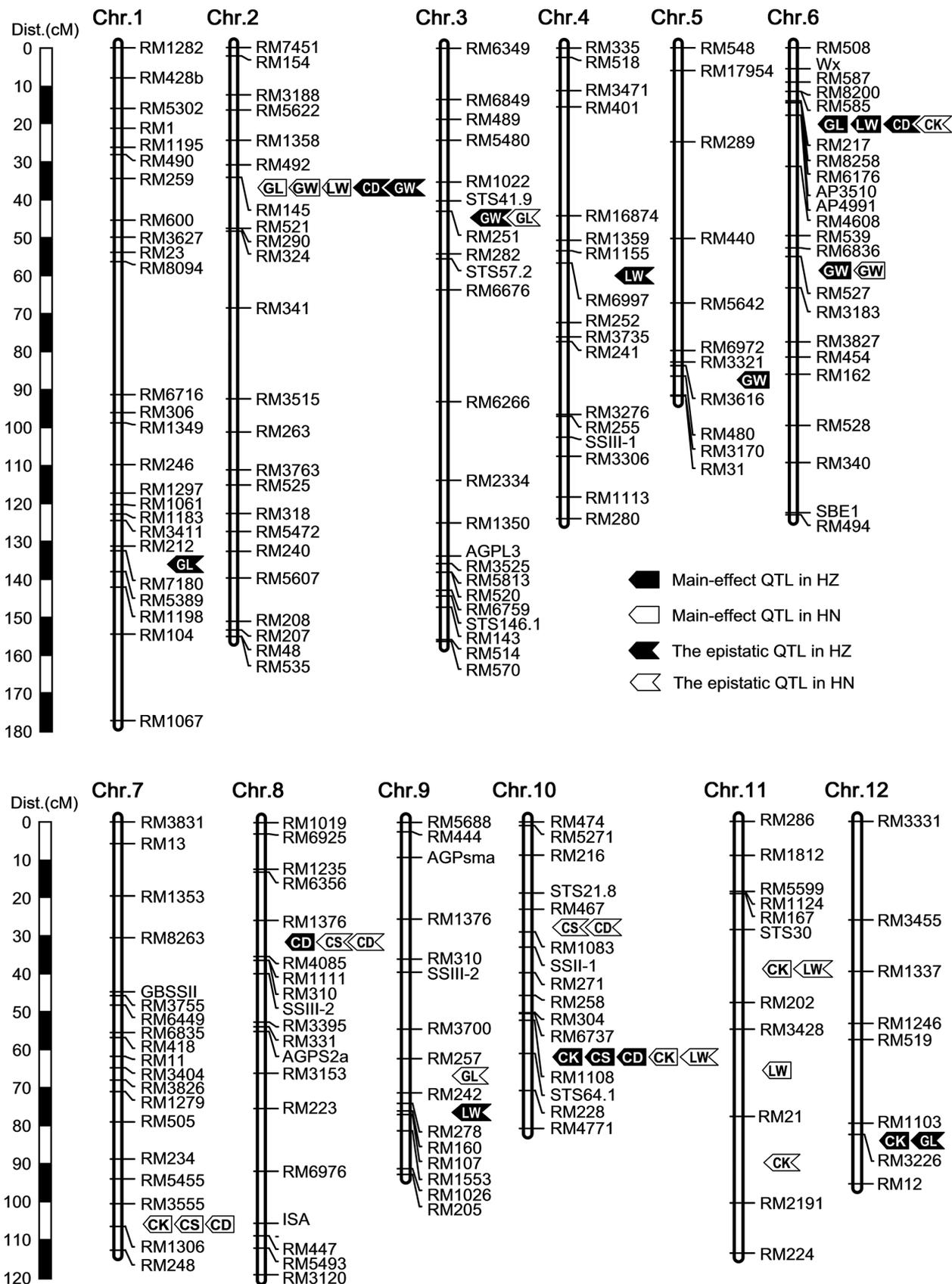


Fig. 2. Locations of main- and epistasis-effect quantitative trait loci (QTL) for chalky kernel, chalkiness size, chalkiness degree, grain length, grain width, and length/width ratio on the linkage map. Solid arrows indicate QTL identified in Hangzhou. Open arrows indicate QTL identified in Hainan. Arrows with same notch end indicate epistasis interaction QTL. The bar on the left indicates the genetics distance on the linkage map. The markers starting with RM are simple-sequence repeat markers and all others are sequence tag site markers.

Table 3. Quantitative trait loci (QTL) for grain appearance quality in double-haploid population of CJ06 × TN1.

| Trait | QTL | Chromosome | Marker interval | Additive effect | $H^2(A)†$ % | F-value | P-Value |
|--------------------|---------------|------------|-----------------|-----------------|----------------|---------|-----------------------|
| Hangzhou | | | | | | | |
| Chalky kernel | <i>qCK10</i> | 10 | RM6737–RM1108 | –14.1 | 14.5 | 14.1 | 6.3×10^{-4} |
| | <i>qCK12h</i> | 12 | RM1103–RM3226 | –13.4 | 13.0 | 16.9 | 1.2×10^{-3} |
| Chalkiness size | <i>qCS10h</i> | 10 | RM6737–RM1108 | –13.9 | 14.7 | 14.6 | 8.1×10^{-4} |
| Chalkiness degree | <i>qCD8h</i> | 8 | RM1376–RM4085 | –11.5 | 13.3 | 12.5 | 6.9×10^{-4} |
| | <i>qCD10h</i> | 10 | RM6737–RM1108 | –15.3 | 15.1 | 15.7 | 8.4×10^{-4} |
| Grain length | <i>qGL6h</i> | 6 | RM585–RM217 | –0.23 | 17.2 | 19.2 | 2.7×10^{-5} |
| Grain width | <i>qGW5h</i> | 5 | RM3321–RM3616 | 0.12 | 16.6 | 17.4 | 5.3×10^{-5} |
| | <i>qGW6</i> | 6 | RM6836–RM527 | 0.10 | 10.2 | 13.0 | 1.9×10^{-3} |
| Length/width ratio | <i>qLW6h</i> | 6 | RM585–RM217 | –0.05 | 12.2 | 16.1 | 3.8×10^{-4} |
| Hainan | | | | | | | |
| Chalky kernel | <i>qCK7n</i> | 7 | RM3555–RM1306 | –18.3 | 24.5 | 17.8 | $<1.0 \times 10^{-5}$ |
| | <i>qCK10</i> | 10 | RM6737–RM1108 | –14.5 | 15.3 | 18.9 | $<1.0 \times 10^{-5}$ |
| | <i>qCK11n</i> | 11 | STS30–RM202 | –12.2 | 10.9 | 14.7 | 1.2×10^{-5} |
| Chalkiness size | <i>qCS7n</i> | 7 | RM3555–RM1306 | –6.3 | 25.5 | 17.4 | $<1.0 \times 10^{-5}$ |
| Chalkiness degree | <i>qCD7n</i> | 7 | RM3555–RM1306 | –6.1 | 23.9 | 15.4 | 1.2×10^{-5} |
| Grain length | <i>qGL2n</i> | 2 | RM492–RM145 | –0.29 | 18.2 | 13.3 | $<1.0 \times 10^{-5}$ |
| Grain width | <i>qGW2n</i> | 2 | RM492–RM145 | 0.14 | 8.6 | 9.5 | 4.9×10^{-3} |
| | <i>qGW6</i> | 6 | RM6836–RM527 | 0.14 | 9.1 | 10.8 | 4.3×10^{-3} |
| Length/width ratio | <i>qLW2n</i> | 2 | RM492–RM145 | –0.07 | 16.2 | 18.9 | $<1.0 \times 10^{-5}$ |
| | <i>qLW11n</i> | 11 | RM3428–RM21 | 0.06 | 14.5 | 20.4 | 2.8×10^{-5} |

† A, heritability from additive effect.

Table 4. Epistasis effects for grain appearance quality in double-haploid population of Chunjiang06 × TN1.

| Trait† | Chromosome | Marker interval | Chromosome | Marker interval | P-value | Epistasis (AA)‡ | H^2 (AA) % |
|-----------------|------------|-----------------|------------|-----------------|----------------------|-----------------|-----------------|
| Hangzhou (2012) | | | | | | | |
| CD | 2 | RM492–RM145§ | 6 | RM585–RM217§ | 2.6×10^{-3} | 4.7 | 12.6 |
| GL | 1 | RM212–RM7180 | 12 | RM1103–RM3226¶ | $<1 \times 10^{-5}$ | –0.26 | 21.4 |
| GW | 2 | RM492–RM145§ | 3 | STS41.9–RM251 | 3.3×10^{-3} | 0.09 | 9.3 |
| LW | 4 | RM1155–RM6997 | 9 | RM242–RM278 | 1.1×10^{-3} | 0.05 | 10.5 |
| Hainan (2013) | | | | | | | |
| CK | 6 | RM585–RM217§ | 11 | RM21–RM2191§ | 2.0×10^{-4} | 10.5 | 8.0 |
| CS | 8 | RM1376–RM4085¶ | 10 | RM467–RM1083 | 1.5×10^{-3} | –3.9 | 9.7 |
| CD | 8 | RM1376–RM4085¶ | 10 | RM467–RM1083 | 1.1×10^{-3} | –4.0 | 10.5 |
| GL | 3 | STS41.9–RM251 | 9 | RM257–RM242 | $<1 \times 10^{-5}$ | 0.38 | 23.6 |
| LW | 10 | RM6737–RM1108¶ | 11 | STS30–RM202¶ | 3.5×10^{-4} | 0.06 | 13.6 |

† CK, chalky kernel; CS, chalkiness size; CD, chalkiness degree; GL, grain length; GW, grain width; LW, length/width ratio.

‡ AA, heritability from the interaction are all additive effects.

§ Locus found to have main effect on grain shape.

¶ Locus found to have main effect on grain chalkiness.

QTL or interaction loci were anchored in cluster on chromosomes 6, 8, 10, and 12, respectively.

Grain chalkiness is closely related to grain shape. The correlations ranged from 0.613 between CK and GL to 0.384 between CK and LW. Coincidence of QTL location and genetic effect is expected for positively related traits. The present results agree well with this expectation. Although there was little coincidence of main-effect QTL between shape and chalkiness, with inclusion of loci with digenic interaction, we then found more agreement between both traits and field locations. The regions with main effect on grain shape on chromosome 2 and

the upper of chromosome 6 also showed epistatic effect on grain chalkiness. The intervals on chromosome 10, 11, and 12 carrying main-effect chalkiness loci had digenic epistasis for grain shape. It indicates that increasing GW also generally increased the grain chalkiness. Conversely, decreasing GW also reduced the chalkiness. Thus, a compromise between GW and chalkiness for rice improvement is necessary. Particularly for the improvement of short-grain rice lines, it is difficult to decrease endosperm chalkiness while also increasing grain weight.

The results in this study have significant implications for those attempting to breed for improved rice

appearance quality. In addition to the major genes, attention should also be given to the effects of minor and epistatic QTL. The information obtained from this study can be used to manipulate QTL for the aforementioned traits through molecular marker-assisted selection. Reducing the GW almost certainly reduced the chalkiness, as indicated by the colocation of the QTL for these two traits. The results in this study could help breeders identify, and pyramid favorable alleles with marker-assisted selection, those QTL that impart desired shape without causing detrimental effects on chalkiness.

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