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ORIGINAL PAPER

Mapping QTL main and interaction influences on milling quality in elite US rice germplasm

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Abstract Rice (*Oryza sativa* L.) head-rice yield (HR) is a key export and domestic quality trait whose genetic control is poorly understood. With the goal of identifying genomic regions influencing HR, quantitative-trait-locus (QTL) mapping was carried out for quality-related traits in recombinant inbred lines (RILs) derived from crosses of common parent Cypress, a high-HR US *japonica* cultivar, with RT0034, a low-HR *indica* line (129 RILs) and LaGrue, a low-HR *japonica* cultivar (298 RILs), grown in two US locations in 2005–2007. Early heading increased HR in the Louisiana (LA) but not the Arkansas (AR) location. Fitting QTL-mapping models to separate QTL main and QTL × environment interaction (QEI) effects and identify epistatic interactions revealed six main-effect HR QTLs in the two crosses, at four of which Cypress contributed the increasing allele. Multi-QTL models accounted for 0.36 of genetic and 0.21 of genetic × environment interaction of HR in MY1, and corresponding proportions of 0.25 and 0.37 in MY2. The greater HR advantage of Cypress in LA than in AR corresponded to a genomewide pattern of opposition of HR-increasing QTL effects by AR-specific effects, suggesting a selection strategy for improving this cultivar for AR. Treating year–location combinations as independent environments resulted in underestimation of QEI effects, evidently owing to lower variation among years within location than between location. Identification of robust HR QTLs in elite long-grain germplasm is suggested to require more detailed attention to the interaction of plant and grain development parameters with environmental conditions than has been given to date.

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Abbreviations

QTL	Quantitative-trait locus
GEI	Genetic × environment interaction
QEI	QTL × environment interaction
QQI	QTL × QTL interaction
QQEI	QQI × environment interaction
HR	Head-rice yield
RIL	Recombinant inbred line
MET	Multi-environment trial
MIM	Multiple-interval mapping

Introduction

High rice milling quality is required for exporting to the competitive world market and for sustaining domestic milling industries. In the US, which grows mostly long-grain *japonica* rice and exports about half of it, high production efficiency has produced record yields in recent years (Childs 2006), due in part to the development of cultivars with improved yield potential. But over the past 10 years the yield of whole-grain or head rice (HR)—the proportion of whole finished rice obtained from paddy rice—has remained static at around 60%. With milling quality in other rice-growing countries having improved, US rice no longer commands its former premium for grain quality. Because the market value of head rice is twice that of broken, HR is a high-priority objective for breeding programs.

Grain characteristics and nongenetic factors influence HR. Chalkiness, a white, opaque portion of the grain associated with loose packing of starch and protein particles, is influenced by temperature, light, wind, and humidity, and increases breakage during milling (Moldenhauer et al. 2004; Webb 1985); Slender or very long grains tend to break more easily, so that uniformity of grain size and shape is desired (Siebenmorgen and Meullenet 2004; van Ruiten 1985; Webb 1985), and width and thickness (transverse dimensions of the kernel) tend to correlate positively with HR (Zheng et al. 2007). Asynchrony of heading increases the number of immature and overripe grains in the sample, increasing breakage (Juliano and Bechtel 1985; van Ruiten 1985). Lower percentages of hull weight correspond to a larger rice kernel and are associated with higher milling yields (van Ruiten 1985). Weather conditions before harvest, moisture at harvest and during storage, and drying practices also influence milling (Kunze and Calderwood 1985; Siebenmorgen and Meullenet 2004). Ambient moisture changes can produce transverse fissures that increase kernel breakage in the mill. Endosperm is a triploid tissue and its traits are also subject to cytoplasmic effects (Zheng et al. 2007), so that the diploid genetic control model that is applied in standard QTL analyses may not adequately fit the more complex reality.

Many QTL mapping studies of rice quality-associated traits have been reported, almost all in Asian germplasm. The grass-genome database Gramene (http://www.gramene.org) (Ware et al. 2002) holds data for about 800 QTLs for about 60 such traits. For head rice, 17 QTLs were identified in four studies: in F_4 and RIL progenies of crosses between Chinese rice cultivars (Mei et al. 2002; Tan et al. 2001), a BC_2F_2 progeny of a cross between the elite *indica* IR64 and a Malaysian *O. rufipogon* (Septiningsih et al. 2003), and doubled-haploid lines from the cross of Brazilian *indica* cultivar Caiapo with a wild African *O. glaberrima* accession (Aluko et al. 2004). In the latter two crosses, all milling-yield-increasing effects came from the cultivated parent. For fissuring, 14 QTLs were identified in two studies: in doubled-haploid (DH) progeny derived from the cross of Reiho and Yamada-nishiki (Yoshida et al. 2002) and in the (Septiningsih et al. 2003) study. For chalkiness, 82 QTLs were reported for three crosses among Asian cultivars (Li et al. 2003a; Tan et al. 2000; Wan et al. 2005) though the Tan et al. study identified a major QTL on chromosome 5 and a recent study by (Zhou et al. 2009) has localized one to a small region on chromosome 7. For the highly heritable trait amylose content, 12 of 51 reported QTLs lie on chromosome 6, though not all at the same position. The *Wx* gene, encoding a starch-synthase enzyme that is a major determinant of amylose content, is located on this chromosome.

Milling-quality QTL results reported to date have had little or no influence on the improvement of public US rice cultivars. First, the germplasm used in these studies does not share close pedigree relationships with the US-adapted rice gene pool and is adapted to very different environments. Second, most QTL-mapping populations analyzed for these traits (Dong et al. 2004; Jiang et al. 2005; Lou et al. 2009; Mei et al. 2002; Tan et al. 2001) have been derived from different market classes, meaning that kernel shape varied sharply among mapping lines. Since milling yield is highest for short, uniformly shaped kernels and mill settings must be optimized for different kernel shapes, heterogeneity in grain dimensions will confound the assessment of genetic effects. Finally, the complexity and environmental sensitivity of milling-yield traits call for larger and better-designed experiments than have been conducted to date. Reports are common of unreplicated studies in one year and one location, using RIL sets as small as 71 lines. However, even in 308 testcross families from BC_3 progeny of an *O. sativa* × *O. glaberrima* cross, no QTLs for head rice were found by Li et al. (2004), with the authors advancing as possible reasons both unbalanced segregation and kernel shape heterogeneity. A recent report (Hao et al. 2009) of HR QTLs in 154 *indica* chromosome-segment substitution lines in the *japonica* cultivar Koshikihari pointed to

QTLs on chromosomes 1, 6, and 8 but gave modest statistical evidence.

QTL studies of milling quality in US-adapted germplasm have been few and inconclusive. Using a single year's unreplicated field data (Zheng et al. 2007) examined quality and grain shape in 231 introgression lines incorporating chromosome segments of Lemont, a long-grained US *japonica* cultivar, into Teqing, a medium-grained *indica* from China. Two QTLs for brown rice or milled rice recovery were accepted, of which the first coincided with a strong QTL for kernel length. But Lemont, although the higher-milling-yield parent, was the source of the HR-reducing alleles for both QTLs. A similar anomaly appeared in 137 RILs from a cross between Cypress and Panda, both long-grain *japonica* cultivars, grown in a single year in Beaumont, Texas. Here the only HR QTL reported was at the *Waxy* locus on chromosome 6 (Kepiro et al. 2007). But with the Cypress homozygote at this putative QTL showing a HR disadvantage of >2.5, this report fails to explain the 14% HR advantage of the Cypress over the Panda parent.

Improvement of HR requires the identification of QTL alleles and allele combinations conferring predictable advantages in cultivars with other desirable traits (such as long grains and cooking quality) grown in target environments. In analysis of multi-environment trials (METs), classical quantitative genetic methods partition trait variation into genetic (G), environmental (E), and G × E interaction (GEI) effects. Recent trends in QTL studies have been toward further decomposing the G component into main QTL (Q) and QTL × QTL (QQI) epistatic effects (Carlborg et al. 2003; Shimomura et al. 2001; Stylianou et al. 2006; Wei et al. 2010), and the GEI component into QTL × environment (QEI), and even QQ × E (QQEI) epistasis × environment interactions (Li et al. 2003b; Yang et al. 2007). It is also desirable to know what specific environmental characteristics underlie QEI. In rice, for example, there is evidence that high nighttime temperatures during late grain-maturing reduce HR (Cooper et al. 2008; Counce et al. 2005). The QTL profiles from most QTL-mapping software confound QTL main and QEI effects, and the most commonly used packages do not readily accommodate experimental factors or covariates other than genetic markers or are not adapted to MET designs.

Several recent MET-QTL-mapping studies have addressed the QEI challenge. Mixed-model approaches (Piepho 2000) allow specifying genetic correlations between environments (location–year combinations) and a Bayesian method (Bauer et al. 2009) accommodates multilocus tests. Environments are usually treated as either classification variables or, where an attempt is made (Boer et al. 2007; Malosetti et al. 2004) to identify specific environmental factors involved in QEI, as quantitative variables taking the same value for every entry grown in the environment and representing properties averaged over a specified time period. In any case, the still-common practice of conducting a separate QTL-mapping analysis in each of several environments prevents a rigorous partitioning of Q and QEI effects and favors unnecessarily arbitrary conclusions about their relative influence on traits.

In the present study, two populations of 129 and 298 RILs developed from the crosses of two US breeding lines with Cypress, a cultivar known for high and stable milling yield, were genotyped for sets of SSR loci and scored in replicated field plots in the US rice-growing states of Louisiana and Arkansas in 2005–2007 for agronomic properties, kernel dimensions, and milling-quality traits. The goals were to identify genetic factors predictive of HR in US long-grain rice cultivars and elucidate their interaction with environmental variation.

Methods

Genetic materials

Cypress (PI 561734) is a long-grain, early-maturing semidwarf *japonica* variety with outstanding HR (characterized by insensitivity of HR to harvest date, leading to a wide "harvest window") derived from a cross of L-202 and Lemont, a cultivar well known in the southern US rice-growing states for good yield potential and high market quality. RT0034 is a semidwarf long-grain breeding line from RiceTec, Inc. (Alvin, TX, USA) derived from an *indica/japonica* cross, and has high yield potential but poor milling quality at about 50% HR. A cross between the two was advanced to the F_7 generation and 129 recombinant inbred lines (RILs) were extracted by single-seed descent until the F_{12}. LaGrue (PI 568891; pedigree Bonnet 73/Nova 76//Bonnet 73/3/Newrex) is a long-grain, early-maturing, high-yielding, and poor-milling *japonica* cultivar that lacks the *sd-1* semidwarfing allele. From the cross with Cypress, 298 F_6 RILs were selected for mapping. These populations will be abbreviated MY1 (Cypress × RT0034) and MY2 (Cypress × LaGrue).

Field planting and phenotypic evaluation

RILs of MY1 were planted in spring 2005, and of MY2 in 2006 and 2007, in Crowley, Louisiana and Stuttgart, Arkansas. MY1 was also planted in Beaumont, Texas and some agronomic but no milling data were collected because the planting was destroyed by disease. Plots of 1.2 m^2 were drill-seeded at a planting rate of 166 kg ha^{-1}. Standard cultural management practices were used to optimize yield while controlling weeds and pests. The experimental design was a randomized complete block

Table 1 Trait means and heritabilities for RIL populations MY1 and MY2

	2005						2007							
	AR			LA			AR			LA			h^2	
Trait	CY	RT	R/C	CY	RT	RT/CY	CY	LG	CY/LG	CY	LG	CY/LG	RT/CY	CY/LG
DTH (d)	80	77	75	91	88	92	82	83	81	83	85	84	0.68	0.48
DHV (d)	133	136	132	–	–	–	140	144	131	119	115	112	0.46	0.39
HT (cm)	–	–	–	92	98	100	105	126	117	103	111	113	0.43	0.72
KL (mm)	7.15	7.27	7.24	7.12	7.36	7.27	7.16	7.08	7.18	7.09	7.22	7.22	0.57	0.69
KW (mm)	2.13	1.98	2.07	2.16	1.98	2.08	2.13	2.12	2.14	2.20	2.22	2.23	0.77	0.66
AMY (%)	22.3	23.8	23.1	21.5	24.8	23.7	–	–	–	–	–	–	0.78	–
KTHK (mm)	1.65	1.60	1.64	1.70	1.59	1.63	–	–	1.70	–	–	1.80	0.58	0.67
HR (%)	56.8	46.9	44.5	64.6	51.8	47.4	60.5	53.5	56.9	68.9	48.6	61.1	0.07	0.17
TKW (g)	18.4	16.9	17.9	18.9	16.8	18.0	21.3	22.4	22.8	24.3	24.0	24.6	0.79	0.58
MDEG (*)	94	79.5	90.4	111.0	82.6	97.2	–	–	–	–	–	–	0.70	–
FBRK (%)	3.2	4.2	8.1	1.9	4.7	12.7	4.0	8.5	5.4	1.5	3.0	3.0	−0.01	0.10
IBRK (%)	11.1	13.4	17.1	6.6	17.1	22.4	7.8	7.5	9.1	7.0	8.0	10.5	0.16	0.21
FCRK (%)	1.9	1.6	3.4	0.9	4.3	13.5	2.8	2.5	3.0	1.0	2.0	3.5	0.24	0.21
ICRK (%)	11.1	9.0	16.6	12.4	29.0	35.2	20.3	17.5	22.8	36.0	56.5	35.0	0.16	0.17
FFIS (%)	5.1	5.8	11.5	2.8	9.0	26.2	6.5	11.4	8.9	2.7	6.4	7.6	0.10	0.16
IFIS (%)	22.2	22.4	33.7	19.0	46.1	57.6	31.6	26.2	35.2	48.8	78.7	54.9	0.24	0.21
KCHK (%)	2.8	3.7	5.1	3.6	5.1	7.1	3.4	3.6	3.7	3.1	8.8	4.1	0.30	0.18
KGRN (%)	1.4	1.6	1.7	1.9	1.8	2.2	1.5	1.4	1.6	1.2	1.7	1.7	0.10	0.11

CY Cypress, *RT* RT0034, *LG* LaGrue, *RT/CY and CY/LG* RILs from MY1 and MY2, respectively. *AR* Arkansas, *LA* Louisiana growing locations. For MY2, only 2007 means are shown, as a representative set. For cells with "–", data were not collected. *DTH* days to heading, *DHV* days to harvest, *HT* height, *KL* kernel length, *KW* kernel width, *AMY* amylose content, *KTHK* kernel thickness, *HR* head-rice yield, *TKW* thousand-kernel weight, *MDEG* milling degree, (*) units of measurement are on an arbitrary scale, *FBRK, IBRK* field- or induced-broken proportion, *FCRK, ICRK* field- or induced-cracked proportion, *FFIS, IFIS* field- or induced-fissured proportion, *KCHK* percent chalkiness, *KGRN* percent greenness, h^2 heritability, calculated as described in text

with two replicates and at least four checks, usually including the parental lines. Key traits recorded for this study are described in Table 1. Days to 50% heading (DTH) and days to harvest (DHV) were calculated from emergence, and plant height (HT) was determined approximately 2 weeks before harvest. Plots were harvested as they approached 20% grain moisture. Rough (unmilled) rice samples were dried to 12% moisture using an ambient-forced-air drier and were then stored in sealed plastic bags until phenotypic analysis.

Grain dimension measurements

For each plot, 100–150 whole brown rice kernels were produced with a Satake sheller model TH035A (Satake Engineering Co. Ltd., Tokyo, Japan). They were then scanned with a WinSeedle Pro 2005a^{TM} image analysis system (Regent Instruments Inc.; Sainte-Foy, Quebec, Canada) for determination of mean kernel length (KL), kernel width (KW), curved length, curved width, kernel surface area, and kernel volume. Kernel thickness of brown rice (KTHK) was measured with a digital micrometer on a subset of 20 kernels. Kernel weight (TKW) was measured for 100 brown-rice kernels.

Grain appearance

The brown-rice sample images used for grain dimension measurements were evaluated for chalk area (KCHK in MY1), green area (KGRN), and pale green area using the pixel-color classification function of the WinSeedle Pro 2005a^{TM} image-analysis system. Classification files were created with selected groups of pixels that exemplified the target color (white or green) of the trait, normal mature kernel color, and background. Because the method does not differentiate chalk according to location in the kernel, KCHK represents the percentage of kernel area showing any chalk (core, white back, or belly chalk). After milling, whole grain samples were scanned for percent chalk (KCHK in MY2), KL, and KW. Kernel translucency, whiteness, and milling degree (MDEG) were determined using a Satake Milling Meter, Model MM-1B (Satake Engineering Co., LTD., Tokyo, Japan) with approximately 25 g of milled rice. Whiteness and milling degree describe the completeness of bran removal from brown rice.

Induced grain-fissuring test

Rough rice subsamples (50 g) from the same lots sampled for milling were stored in sealed plastic bags (Minigrip) for fissuring evaluation. Aliquots of 2.5 g were weighed for field fissuring (untreated) and induced fissuring (treated) sets. To assess field fissuring, the samples were hulled by hand and then evaluated using a Petri dish placed over a light source (Fiber-Lite MI-150) directed at an approximately 45° angle. The proportions of immature or damaged (IMDAM), broken (FBRK), or cracked (FCRK) grains were determined. The sum of the latter two traits was used to determine the proportion of field fissured grain (FFIS). Samples were also scored for curvature of the grain using a scale of 0–4. For determining induced fissuring, plastic storage tubs (6.75 l, Rubbermaid) were filled with water to a depth of 2.5 cm and placed in an incubator (Precision Incubator 6LM) where the water was allowed to equilibrate to 50°C. Samples of rough rice were placed in wire-mesh seed holders and set on a 31.5 × 18 cm wire stacking shelf (Nexgen) that was inserted into the top of the storage tub, above the water. Lids were placed on tubs and sealed with tape. Tubs were placed in the middle two shelves of the incubator for 16 h. Samples were then removed from tubs, left to equilibrate to room temperature for 30 min, and then sealed in plastic bags (6.25 × 7.5 cm). Samples were hulled by hand and analyzed as described above for immature or damaged kernels and induced broken (IBRK), induced cracked (ICRK), and induced fissured (IFIS) grain. With this protocol 30 samples could be treated, hulled, and analyzed in a 24-hour period.

Grain chemistry

Apparent amylose content (AMY) was determined only in the MY1 cross (where the parents differed for the trait), using the modified iodine spectrophotometric method of (Pérez and Juliano 1978) with a continuous-flow analyzer (AutoAnalyzer 3 Seal Analytical, Mequon, WI, USA).

Milling measurements

The 125-g samples of rough rice were milled for 54 s using a McGill No. 2 mill (Rapsilver Supply Co Inc., Brookshire, TX, USA) with a 636-g weight located at the center position (12 cm) of the mill's saddle arm. The milled rice (whole plus broken kernels) was separated with a shaker/separator (#12 screen = 4.76 mm; Seedburo Equipment Co., Chicago, IL, USA); kernels with length greater than or equal to ¾ full length of a kernel were considered whole-grain head rice. The weight of grain recovered after milling and separating was used to calculate the percentage of HR, based on 125 g of rough rice. The percentage of "pre-broken" kernels in the brown rice after machine hulling was calculated using a WinSeedle Pro 2005a^{TM} image analysis based on the proportion of pixel area associated with broken brown rice kernels (lengths 1.3–5.3 mm) with respect to that of the total brown rice.

Polymorphism screening and marker genotyping

DNA was extracted from 20 mg of dried leaf tissue as described in (Kepiro et al. 2007). SSR markers were amplified and scored following (Fjellstrom et al. 2004). SSR primer sequence information was obtained from the Gramene database (http://www.gramene.org), except for two markers having the following primer sequences: CON673, forward CGTACTTGCCACCGTAAG, reverse TTGATAGGCAATGTTTCTCC; and AL120885, forward TTTTCGGACTTTTGTGTGTC, reverse AGAATAAGTT TGGCAGCATTG. SSR primers were synthesized by Integrated DNA Technologies (Coralville, IA, USA).

The MY1 parents were surveyed with a set of 640 SSRs. Of these 490 or 0.77 were polymorphic and 152 were selected to be evenly spaced, where possible, at 5 to 10-cM intervals along the rice reference map (McCouch et al. 2002). On the MY2 parents 787 SSRs were screened, with only 309 or 0.39 polymorphic, yielding 106 cleanly amplifying and scorable markers.

Statistical properties of traits and markers

Trait heritabilities were calculated from an ANOVA fitting effects of RIL, environment, and RIL × environment, as $\sigma_g^2/(\sigma_g^2 + \sigma_{ge}^2 + \sigma_\varepsilon^2)$ where the g and e subscripts refer to genetic (RIL) and environment (year/location combination) and variance components are computed from equating observed to expected mean squares. Marker segregation was evaluated by chi-square test against expected ratios.

Correlation analysis

Pearson's correlations were calculated for all trait means within locations. Pairs with $r > 0.8$ in both locations showed very similar genome-wide QTL interval-mapping profiles and were judged to measure the same phenotype. Examples were milling degree and kernel whiteness or kernel dimensions measured on brown or milled rice or on a straight or curved basis. Only one trait of such pairs was retained.

Linkage mapping

Marker linkage maps were constructed with CarthaGène (de Givry et al. 2005). Marker order was generally as

expected from the rice genetic and physical maps (McCouch et al. 2002; Ware et al. 2002).

Statistical analysis

Adjustment of milling scores

Milling was conducted over a 2-week period. With MY2, as a control for HR variation introduced by instrument or ambient variation, a Cypress check sample was milled after approximately every 24 samples, and for each day all samples estimated to have been milled on that day were standardized to the check mean for that day.

QTL mapping

All traits were analyzed by multiple-interval mapping (MIM) (Kao et al. 1999) applied to one environment at a time with QGene 4.5 (Joehanes and Nelson 2008). MIM fits a multiple-QTL model that includes the QTL position being tested while accounting for trait variation due to QTLs located elsewhere. It generally affords more accurate and precise QTL location and effect estimation than the commonly used composite interval mapping (CIM) method, which employs only marker and not QTL cofactors. LOD thresholds were established from 1,000 permutations of the trait data for sample traits and typically ranged from 2.5 ($p < 0.05$) to 3.5 for single-trait MIM.

For the HR trait of primary interest, QTL models incorporating environmental along with genetic terms to explain phenotypic variation were fitted with SAS 9.1.3 (SAS Institute 2004) PROC MIXED or PROC GLM. The general form of the models was

$$Y_{ijk} = \mu + Q_{il} + L_j + Y_k + L_jY_k + Q_{il}L_j + Q_{il}Y_k + Q_{il}L_jY_k + \sum_c (B_{ic} + B_{ic}L_j) + \varepsilon_{ijkl} \quad (1)$$

where effects are defined as follows: μ the general mean, L_j the jth location, Y_k the kth year (included only for MY2, since MY1 was evaluated in a single year); Q_{il} the lth QTL genotype of the ith RIL, represented by the genotypic expectation at the tested QTL position, calculated with QGene by the method of Jiang and Zeng (1997) at 5-cM intervals on the genetic map; B_{ic} the effect of the cth QTL-cofactor genotype for the ith RIL (fitted as a random effect) and ε_{ijkl} the residual variation. Also fitted were two variant models: 1(a), in which year–location combinations and their QTL interactions were represented by effects E_{jk} and Q_{il}*$E_{jk\cdot}$, and 1(b), in which year and all of its interactions were nested in location, as done by von Korff et al. (2008). Cofactors numbered five or six QTL positions or markers identified by MIM, and if lying within 15 cM of the QTL position being fitted were excluded from the model. Models were fitted at all QTL and marker positions in turn: 414 in MY1 and 340 in MY2. F statistics and additive-effect estimates for main and interaction effects were collected from SAS output with Perl scripts and plotted (after conversion of F to LOD) against the corresponding QTL positions on a genetic map with gnuplot (http://www.gnuplot.info). With two environments, only one QEI effect was estimable and is that of location AR relative to LA. Permutation testing to establish an acceptance threshold for Type I error control was carried out as follows: the original data set for the target trait (HR) was shuffled 500 times, exchanging only the vectors of phenotypes for each RIL across all environments to preserve the correlations across environments. For each shuffled data set, 100 QTL positions were randomly selected for model fitting, and the maximum F value for a chosen main or interaction effect was recorded, followed by ordering and selection of the appropriate percentile values as cutoffs.

For MY2 only, model (1) was expanded to test QTL × QTL interaction (QQI) and epistasis × environment interactions (QQEI) by inclusion of all pairwise combinations of different QTLs.

$$Y_{ijk} = \mu + Q_{1il} + Q_{2il} + L_j + Y_k + L_jY_k + Q_{1il}L_j + Q_{2il}L_j + Q_{1il}Q_{2il} + Q_{il}Q_{2il}L_j + Q_{il}Q_{2il}LY_{kj} + \varepsilon_{ijkl}$$

Here interactions involving year were omitted, since they were found in all models to be negligible. The same model was also fitted after substitution of an E_{jk} term as described for model (1). Permutation analysis was as for oneway mapping except that only 50 QTL positions were chosen at each shuffle, in view of the quadratically higher computational burden.

For determining the proportions of genetic variance explained by the QTLs, model (1) was refitted once with the Q terms replaced by G (i.e. RIL) and once with all of the declared QTLs and interactions included. Genetic variance explained was computed for the lth QTL QTLs as SS(Q_l)/SS(G), where SS denotes Type III sum of squares and (Q_l) and (G) denote, respectively, the main QTL or RIL effects.

For MY2 only, the dataset was prepared for QTLNetwork 2.1 (Yang et al. 2007) with the purpose of identifying loci involved in QEI, QQI epistasis, and QQEI (epistasis by environment) effects. This software fits a mixed model with environment as a random effect and includes background markers to absorb polygenic QTL variance, but does not support separation of year and location effects. It was for approximate comparison with this approach that we also fitted models 1(a) and 1(b).

Results

Marker coverage and segregation distortion

The total lengths of the MY1 and MY2 genetic maps were 1,370 and 1,210 cM, representing 90% that of the high-density rice map of (Harushima et al. 1998) but with respective average interval lengths of 9 and 10 cM. Gaps represented absence not of coverage but of polymorphism. For example, on chromosome 11 in MY2 showing only four mapped SSRs, only 13 of the 61 SSRs screened were polymorphic between the parents, with most of these clustered in a few segments or showing unsatisfactory amplification or scoring characteristics. In a separate study (Zhao et al. 2010) of single-nucleotide polymorphisms (SNPs) typed in assorted rice cultivars, only 12 of 127 SNPs on this chromosome distinguished the MY2 parents, and the spatial clustering of SNP polymorphism was similar to that of the SSRs (unpublished data of W. Solomon et al.). There were no SSR gaps larger than 5 cM and only five larger than 3 cM (based on the rice reference map). The patterns on other chromosomes were similar.

Both populations showed segregation distortion (Fig. 1). In MY1, only markers on parts of chromosomes 1, 2, and 6 segregated at near the expected 1:1 proportion for homozygous genotypes. The overall marker segregation ratio, based on summation over all genotypes of each of the three classes, was 63:34:3 for the RT0034 (RR) and Cypress (CC) homozygotes and the heterozygote (RC) in that order. The most extreme segregation in favor of RT0034 was 112:9, at RM26821 on chromosome 11. Only five markers showed segregation favoring the Cypress allele. In MY2, overall marker segregation was 43:42:15 (CC: LaGrue LL: heterozygote LC), giving an excess of heterozygosity corresponding more closely to an F_4 than to the F_6 generation. Distortion was not seen on all chromosomes and did not favor one parent consistently as in MY1.

Trait statistics

We focus here only on the traits for which QTLs were accepted. Except for the kernel-damage traits chalkiness, green color, and broken, cracked, and fissured kernels, which were right skewed in both populations, the traits showed symmetrical, unimodal distributions (not shown). All transgressed the parent distributions to some degree in some environment, although the highest HR score in the RILs barely exceeded that of Cypress. Means and heritabilities are shown in Table 1. Heritability was high for kernel-dimension traits, DTH, height, milling degree, TKW, and amylose content, but low for HR and other milling-quality traits. The RIL means generally exceeded either parental mean for the kernel-damage traits.

In the trait correlations, the most striking finding was the reversal across locations, in both populations, of correlation between HR and DTH. In LA, HR was favored by earlier and in AR by later heading (Table 2). As might be expected from this, HR scores were uncorrelated across locations (although in MY2 they showed $r = 0.46$ and 0.48 between years within the two locations). Kernel width and thickness were more intercorrelated than either with length. HR was negatively correlated with length and TKW, consistent with observations that the longer and larger kernels in a segregating population sustain more damage under the same mill settings than the shorter and smaller ones. HR was also negatively correlated with kernel-damage traits, slightly more with the field-observed than with the induced traits (represented by ICRK and IFIS).

QTL mapping results

QTL results are shown in Table 3 and Figs. 2, 3, and 4. QTLs were accepted on 5 of the 12 chromosomes in MY1 and 8 in MY2. Graphical results comparing the several mapping approaches are presented only for the trait HR.

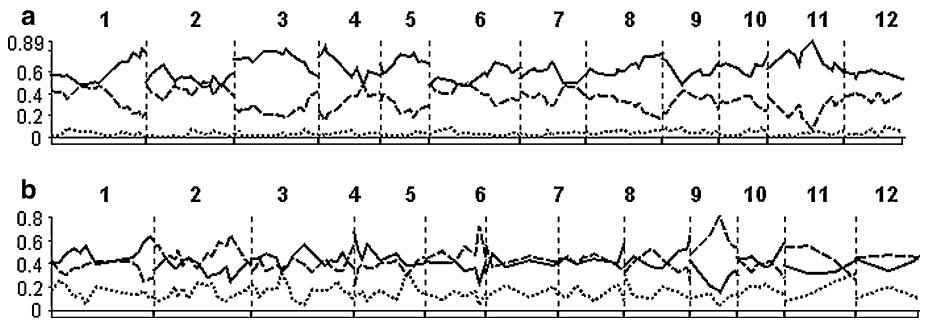

Fig. 1 Marker segregation in Cypress × RT0034 (MY1) and Cypress × LaGrue (MY2) rice RILs. *Numbered* vertical divisions represent chromosomes on the genetic map. *Y*-axis labels are proportions of the three non-missing genotypes, calculated at each marker. *Broken line*: Cypress; *dotted line*: heterozygote; *solid lines*: **a** RT0034 (MY1), **b** LaGrue (MY2)

Table 2 Correlations between traits in RIL populations MY1 and MY2

LA/AR	DTH	HT	KL	KTHK	KW	TKW	AMY	HR	FCRK	ICRK	FFIS	IFIS	IMDAM	KCHK	MDEG
a. MY1															
DTH	***0.71***	*0.40*	0.02	0.17	*0.38*	0.29	0.16	*0.52*	−0.26	−0.23	*−0.44*	*−0.33*	−0.18	−0.24	−0.11
HT	*0.41*	***0.58***	−0.05	0.21	*0.39*	0.28	−0.04	−0.26	−0.03	−0.16	−0.01	−0.19	0.04	0.03	0.13
KL	−0.19	−0.11	***0.85***	0.12	−0.24	*0.49*	0.11	−0.10	0.01	0.10	0.07	0.26	−0.12	−0.21	−0.17
KTHK	0.04	−0.03	0.14	***0.70***	0.27	*0.63*	0.04	−0.03	0.15	0.12	0.24	0.18	0.07	0.18	0.18
KW	0.29	0.10	−0.22	*0.42*	***0.86***	*0.55*	−0.05	*−0.30*	0.07	−0.04	0.19	−0.05	*0.37*	*0.31*	*0.45*
TKW	0.07	0.06	*0.48*	*0.73*	*0.62*	***0.81***	0.07	−0.17	0.12	0.09	0.25	0.20	0.16	0.11	*0.31*
AMY	*0.37*	0.05	0.22	−0.02	0.05	0.16	***0.81***	0.19	0.03	0.18	−0.06	0.13	−0.11	−0.29	0.02
HR	*−0.56*	0.21	−0.26	0.29	*0.32*	0.20	−0.07	**−0.04**	*−0.37*	−0.20	*−0.57*	*−0.51*	*−0.33*	−0.13	−0.09
FCRK	*0.30*	−0.19	0.15	0.03	−0.19	0.04	−0.17	*−0.32*	***0.41***	0.20	*0.78*	0.21	0.22	−0.05	0.03
ICRK	−0.02	−0.08	0.15	0.01	−0.15	−0.02	0.11	−0.04	0.30	**0.26**	0.26	*0.81*	−0.14	−0.09	0.04
FFIS	0.39	−0.26	0.22	−0.01	−0.17	0.05	−0.08	*−0.54*	*0.88*	0.26	**0.22**	*0.47*	0.15	0.16	0.25
IFIS	0.05	−0.10	0.29	−0.04	−0.14	0.01	0.23	−0.27	*0.34*	*0.85*	*0.42*	***0.33***	−0.09	0.01	0.17
IMDAM	*0.55*	*0.34*	−0.06	0.07	−0.07	0.04	−0.08	*−0.72*	0.22	−0.18	*0.34*	−0.03	**0.21**	*0.15*	*0.00*
KCHK	0.23	0.19	*−0.35*	0.21	0.29	0.03	−0.07	*−0.47*	−0.09	−0.28	0.01	−0.15	*0.58*	***0.58***	*0.70*
MDEG	0.18	0.12	0.02	0.25	*0.40*	0.23	0.12	*−0.52*	−0.15	−0.22	0.04	−0.04	*0.54*	0.77	**0.77**

LA/AR	DTH	DHV	HT	KL	KW	KTHK	FTKW	ITKW	HR_06	HR_07	FCRK	FFIS	IFIS	KCHK
b. MY2 (representative year 2007)														
DTH	***0.69***	*0.80*	−0.16	0.26	0.15	0.15	0.10	0.12	0.23	0.26	−0.01	*−0.32*	−0.12	*−0.61*
DHV	*0.59*	***0.55***	*−0.39*	0.06	0.16	0.16	0.15	−0.04	0.28	*0.33*	−0.06	−0.29	−0.16	*−0.58*
HT	*−0.37*	*−0.42*	***0.80***	*0.30*	−0.21	−0.21	0.06	0.08	−0.24	*−0.35*	0.08	0.16	0.12	0.22
KL	0.09	−0.02	0.25	***0.85***	0.22	*0.34*	*0.55*	*0.49*	*−0.32*	−0.25	0.08	0.12	0.14	−0.07
KW	0.16	0.09	−0.14	0.20	***0.81***	*0.61*	*0.51*	*0.45*	0.11	0.01	0.25	0.04	0.18	−0.04
KTHK	0.04	0.00	−0.13	*0.33*	*0.57*	***0.80***	*0.60*	*0.54*	0.14	0.12	0.22	0.07	0.16	−0.17
FTKW	*0.35*	*0.30*	−0.12	*0.44*	*0.39*	*0.48*	***0.67***	*0.81*	−0.13	−0.12	0.22	0.28	*0.32*	0.09
ITKW	*0.31*	0.27	−0.07	*0.64*	*0.69*	*0.62*	*0.55*	***0.52***	−0.10	−0.11	0.18	0.25	*0.32*	0.08
HR_06	*−0.35*	−0.15	−0.06	*−0.32*	−0.12	−0.12	*−0.32*	−0.28	**0.14**	*0.46*	−0.05	*−0.36*	−0.22	*−0.32*
HR_07	*−0.40*	*−0.36*	0.05	−0.22	−0.13	−0.11	−0.23	−0.20	*0.49*	**0.04**	−0.28	−0.28	−0.22	*−0.36*
FCRK	0.29	0.28	0.07	0.18	0.25	0.25	0.29	*0.36*	*−0.41*	*−0.57*	***0.33***	0.24	0.26	0.04
FFIS	0.10	0.27	0.01	0.21	0.26	0.19	*0.43*	*0.41*	−0.28	−0.20	*0.41*	**0.14**	*0.61*	*0.53*
IFIS	−0.12	−0.13	0.01	0.07	0.21	0.18	*0.47*	*0.43*	0.11	*−0.31*	0.11	*0.32*	***0.42***	*0.35*
KCHK	0.19	*0.37*	−0.02	0.18	0.27	0.06	0.28	0.22	−0.29	*−0.39*	0.28	*0.50*	0.22	**0.08**

Lower left triangular matrix: within location LA; upper right: within location AR; main diagonal: between LA and AR. Shading: correlations of absolute value at least 0.3 ($p \ll 0.005$; this threshold was arbitrarily selected to show most prominent patterns). Trait abbreviations are as in Table 1; HR_06 and HR_07 refer to years 2006 and 2007

Head-rice yield

In the MY1 cross, conventional QTL analysis by MIM suggested location-specific QTLs linked in repulsion on chromosome 8. However, the LA-expressed QTL lay within a few cM of a major days-to-heading QTL where RT0034 contributes the earliness allele. In contrast, in a region on chromosome 9 that showed submarginal effects from Cypress in both locations, the multi-environment model supported a QTL main effect at this position as well as a RT0034 QTL on chromosome 6 (Fig. 3a). In most chromosomal regions influencing HR, the QEI effect in AR opposed the QTL main effect (Fig. 3b). Two-way (QQ) interaction analysis was performed, but is not reliable in populations of this size (Yang et al. 2007), especially in the presence of severe segregation distortion, and is not reported. A model fitting location and main effects from these two and three less significant QTLs, as well as main and QEI effects for a DTH QTL, accounted for 0.36 of genetic and 0.21 of genetic × environment interaction (GEI) in MY1 (Table 3a).

In MY2 the correspondence between HR and DTH was even stronger than in the smaller MY1 population, even though the range in DTH was much lower. In the LA location, most HR QTLs evident in a single-environment

Table 3 QTLs showing main or QEI effects in RIL populations MY1 and MY2

Name	Chr	Pos	LH	RH	LOD	Add	PVE	Add*	GVE*	QEI	GEIVE
a. MY1 QTLs											
qamy_6.1	6	8	RM19226	RM190	31.8	−1.00	0.73				
qamy_6.2	6	36.9	RM2615	CON673a	6.1	−0.31	0.16				
qdth_6.1	6	8	RM19226	RM190	5.7	−2.77	0.05				
qdth_8.1	8	35.7	RM8266	RM22559	11.8	3.45	0.16	−3.45	0.03	4.38	0.1
qfis_6.1	6	106	RM5371	RM3430	4.6	4.69	0.10				
qgrn_8.1	8	32.3	RM5647	RM8266	5.2	0.40	0.14				
qhr_6.1	6	38.4	RM2615	CON673a	2.6	−1.75	0.12	−1.12	0.07	−1.4	0.01
qhr_9.1	9	61.4	RM566	RM1189	2.9	2.65	0.16	2.84	0.12	−0.81	0
qimd_8.1	8	35.7	RM8266	RM22559	7.0	3.17	0.20				
qkl_3.1	3	15.6	RM569	RM3117	5.5	−0.12	0.16				
qkl_3.2	3	109	RM5626	RM1292	6.3	−0.15	0.19				
qkthk_9.1	9	49.9	RM24201	RM566	5.0	0.02	0.15				
qkw_2.1	2	45.8	RM12885	RM1190	5.7	0.03	0.17				
qkw_8.1	8	39.8	RM8266	RM22559	9.2	0.05	0.34				
qkwt_8.1	8	45.4	RM22559	RM44	5.1	−0.04	0.10				
qmdeg_2.1	2	59.5	RM1190	RM13366	7.4	6.11	0.24				
qmdeg_3.1	3	84.4	RM1334	RM8208	4.2	3.47	0.08				
qmdeg_6.1	6	36.9	RM2615	CON673a	5.5	5.56	0.17				
qpht_3.1	3	164	RM3684	RM3585	8.8	4.81	0.08				
qpht_8.1	8	33.6	RM8266	RM22559	6.6	4.14	0.06				
b. MY2 QTLs											
qbrk_2.1	2	95	RM3688	RM3355	5.9	−0.61	0.07				
qbrk_3.1	3	162	RM3684	RM3585	4.8	−0.56	0.03				
qcrk_10.1	10	76	RM1108	RM5494	5.7	−1.38	0.10				
qcrk_3.1	3	44	RM232		4.1	1.40	0.09				
qdth_2.1	2	95	RM3688	RM3355	7.3	−0.78	0.10	1.4	0.0	−2.16	0.04
qdth_3.1	3	6	RM22		5.7	0.99	0.07	−1.2	0.03	1.23	0.04
qdth_7.1	7	59	RM1186	RM1204	4.0	−0.70	0.05				
qdth_8.1	8	104	RM149	RM5493	9.8	1.00	0.16	−1.1	0.0	1.55	0.06
qdth_10.1	10	76	RM1108	RM5494	10.2	−1.04	0.17				
qdtm_1.1	1	147	RM1339		9.3	1.38	0.15				
qfis_2.2	2	95	RM3688	RM3355	4.1	−3.34	0.06				
qfis_10.1	10	76	RM1108	RM5494	5.6	−1.69	0.09				
qhr_1.1	1	161	RM3482	RM5794	4.7	0.81	0.08	0.28	0.01	0.52	0
qhr_5.1	5	96	RM1090	RM5575	2.59	−0.49	0.08	−2.8	0.03	1.4	0.01
qhr_9.1	9	5.2	RM8206	RM3912	4.1	0.62	0.08	0.61	0.03	0	0
qhr_10.1	10	76	RM1108	RM5494	8.3	1.73	0.12	2.4	0.07	−2.4	0.09
qkl_1.1	1	163	RM3482	RM5794	15.3	−0.11	0.19				
qkl_3.1	3	100	RM5626	RM6736	6.1	0.08	0.07				
qkl_4.1	4	101	RM3217	RM8217	8.0	−0.08	0.09				
qkl_10.1	10	44	RM25470	RM1873	4.6	−0.07	0.03				
qkthk_3.1	3	98	RM15281	RM5626	10.7	0.02	0.12				
qkthk_3.2	3	158	RM3684	RM3585	9.3	−0.02	0.11				
qkthk_4.1	4	101	RM3217	RM8217	5.3	−0.01	0.05				
qkthk_10.1	10	9.6	RM7545	RM3311	7.5	0.01	0.08				
qkw_1.1	1	45	RM5735	RM580	5.8	0.02	0.07				
qkw_2.1	2	43	RM6911	RM12987	9.2	0.02	0.10				

Table 3 continued

Name	Chr	Pos	LH	RH	LOD	Add	PVE	Add*	GVE*	QEI	GEIVE
qkw_2.2	2	95	RM3688	RM3355	11.0	−0.03	0.13				
qkw_3.1	3	118	RM5626	RM6736	4.9	0.02	0.08				
qkw_7.1	7	2.5	RM3394		4.3	0.02	0.07				
qkw_10.1	10	66	RM1108	RM5494	9.5	−0.03	0.11				
qkwt_2.1	2	43	RM6911	RM12987	3.6	0.36	0.06				
qkwt_2.2	2	95	RM3688	RM3355	4.9	−0.41	0.07				
qkwt_3.1	3	120	RM5626	RM6736	9.8	0.59	0.15				
qkwt_3.2	3	162	RM3684	RM3585	8.1	−0.43	0.08				
qkwt_4.1	4	101	RM3217	RM8217	4.4	−0.29	0.06				
qpht_1.1	1	147	RM1339		56.8	−10.6	0.64				
qpht_2.1	2	95	RM3688	RM3355	5.8	2.44	0.02				
qpht_8.1	8	100	RM149	RM5493	7.8	−2.99	0.01				

a MY1; **b** MY2. *Chr* chromosome, *Pos* position in cM on the rice reference map, *LH, RH* left-and right-hand markers bounding the QTL interval, *LOD, Add* the maximum LOD score and the corresponding additive effect in the interval in any single-environment MIM analysis. Positive values correspond to increasing effects from the Cypress allele at the QTL; negative values to increasing effects from the other parent. *PVE* phenotypic variance explained, estimated as r^2 statistic from CIM, *GVE* genetic variance explained. Add*, GVE, QEI, and GEIVE refer to a multi-QTL model for trait HR only, and denote additive (main QTL) effect, GVE in the full model, QTL × location effect difference (AR minus LA), and proportion of genetic × location interaction variance explained, calculated as described in the text

MIM profile (not shown) coincided with DTH QTLs on chromosomes 2, 3, 7, 8, and 10 (Fig. 4c) at which the earliness and HR-increasing allele is contributed by the same parent. Because in AR none of these putative HR QTLs from LA was expressed in either year, these regions are marked by strong QEI peaks in the multi-environment model (Fig. 4a). As in MY1, the QEI effect in AR opposed the QTL main effect in most regions influencing HR. In AR, none of these putative HR QTLs from LA is expressed in either year, but there is evidence for HR QTLs on chromosomes 1 and 9 and elsewhere. At all these putative HR or DTH QTLs, the homozygote difference was <2% HR, except near the largest DTH QTL on chromosome 10, showing a difference of 3.4% (Table 3b). Addition of DTH as a covariate to the model did not change the profile much (not shown), indicating that the influence of heading time was swamped by that of location.

Together the QTL-mapping approaches indicated that chromosomes 1, 5, 9, and 10 carry QTLs with main genetic effects on HR in MY2, with only the chromosome-5 QTL carrying an HR-increasing LaGrue allele, and that the crossover effects of QTLs lying on chromosomes 2, 7, 8, and 10 (terminal region) are due largely to variation in DTH. As was evident from the trait correlations, QTL × location interaction dominated the QEI effect, with LOD profiles for QTL × year and QTL × location × year close to the baseline and omitted from the figures. When year–location combinations were modeled as individual environments, QEI effect estimates were markedly lower, whereas the model nesting year in location agreed well with model (1) with respect to main QTL effects (Fig. 4e).

The two-way QQI scan (Fig. 5) revealed three QQI and four QQEI effects accepted at the 0.05α threshold. Symmetry around the diagonal suggests that like the main effects, the QQ interactions were subject to environmental variation. A chromosome-4 region with a minor main effect displays an interaction with regions on chromosomes 1 and 8 near DTH QTLs, but the other interaction effects show no clear correspondence with main-effect QTLs.

Main-effect QTL estimates from the QTLNetwork 2.1 mixed-model (VI) analysis agreed in general with those from the other analyses. QTLNetwork does not generate a separate F or LOD profile for QEI. Five epistatic interactions were reported for the QTLNetwork model including all locations and years. However, they showed *P* values up to 0.35, and when separate models were fitted for the 2 years, only three of the four reported for 2007 and neither of the two reported for 2006 were in this set. But the set did include three of the two-way interactions identified by our approach.

The general genetic model accounted for 66% of total variation of HR in MY2. The joint model constructed for prediction of HR in MY2 fitted 32 effects including 19 main QTL (Q), six QEI, three QQI, and four QQEI effects. Most of the main effects were included only because the corresponding interactions were in the model. The *Q* accounted for 19.5% and QQI effects for 5.1% of genetic variation, while QEI and QQEI effects accounted for 24.4% and 12.5% of GEI variation.

Several variants of the models described above were fitted; for example cofactor QTLs were treated as fixed instead of random or were omitted; only replicate was

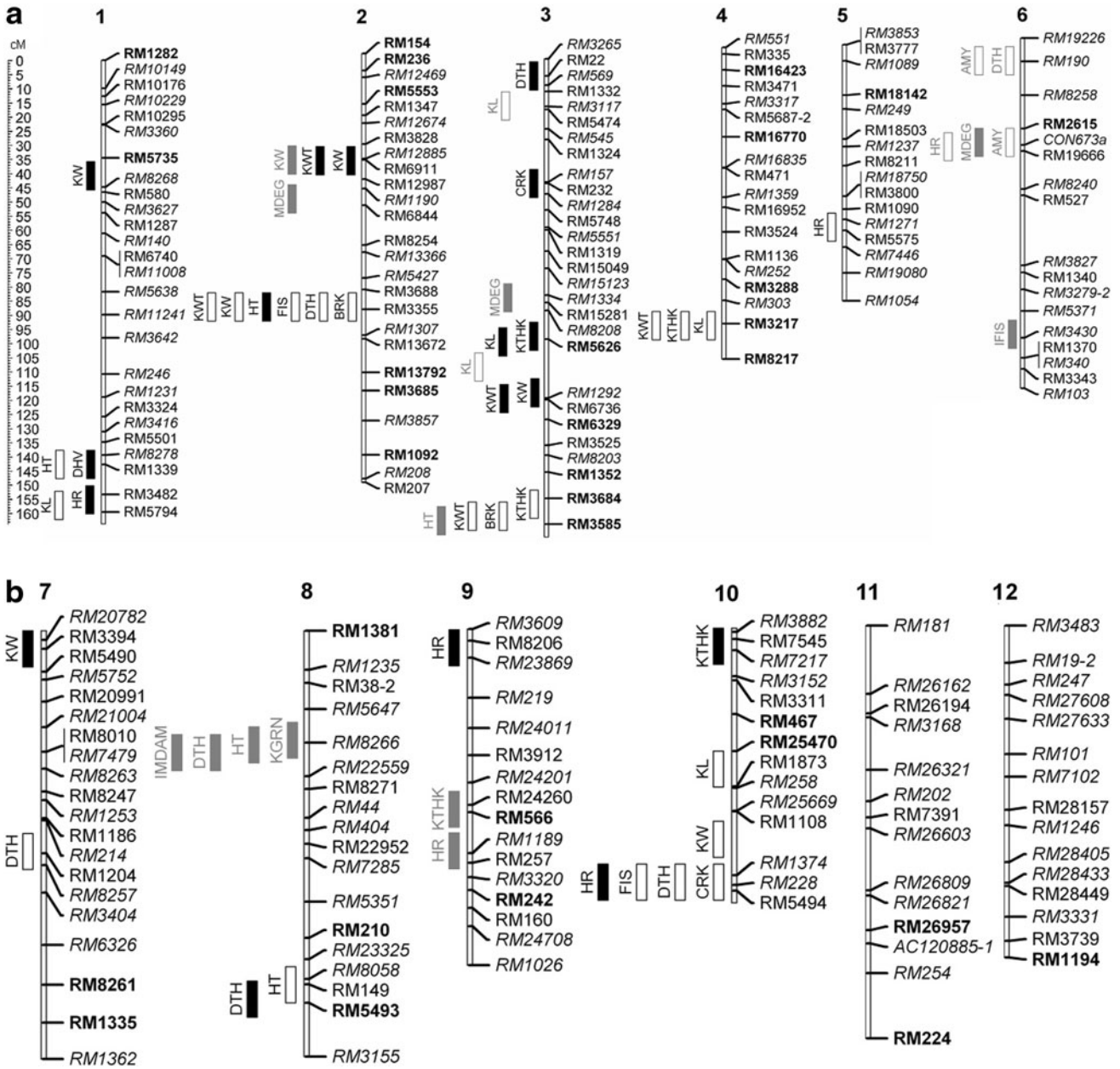

Fig. 2 Combined genetic maps of Cypress × RT0034 (MY1) and Cypress × LaGrue (MY2) rice RILs, showing main-effect QTLs. Reference-map positions are used for all loci (see text). *Boldface* are common to both maps; *italic*, present only in MY1 map; *plain*, present only in MY2 map. *Vertical bars*, all of about 10 cM length, represent approximate QTL locations. *Gray* coloring distinguishes MY1 QTLs; *black*, MY2. *Solid* and *open bars* denote trait-increasing effect from, respectively, Cypress or the other parent. Trait abbreviations are as described in text. Figure was adapted from output of MapChart (Voorrips 2002)

treated as random; all effects were treated as fixed. The QTL results were insensitive to these choices and are not reported.

DTH and DHV

In MY1 one chromosome-8 QTL expressed in all three planting environments gave a Cypress homozygote heading-date difference of up to 6.9 days (Table 3a). In MY2 this QTL did not appear and four different consistent QTLs were declared on chromosomes 2, 3, 8, and 10 with Cypress contributing the lateness allele only on chromosome 8 and no allele effect greater than about 1 day. All DTH were associated with DHV effects. Because delayed heading should lead to delayed harvest date in the absence of genes for accelerated grain filling, these loci were accordingly not counted as independent DHV QTLs, except at the chromosome-1 locus mentioned

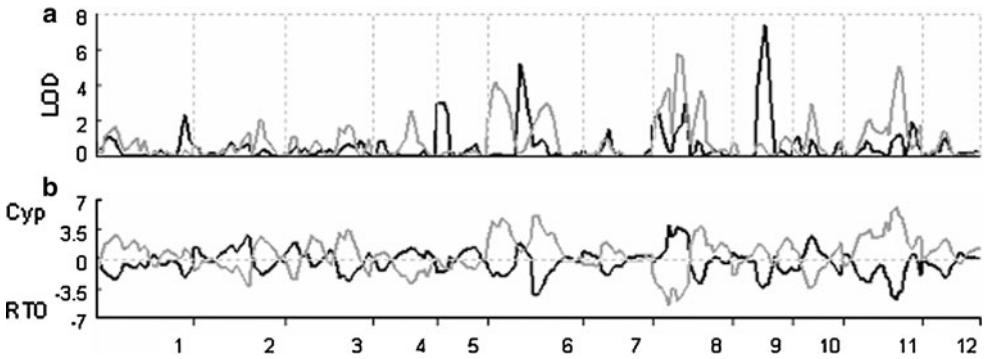

Fig. 3 QTL and QEI profiles of head-rice yield (HR) in Cypress × RT0034 (MY1) rice RILs. **a** LOD profile from simultaneous fitting of QTL main (*black contour*) and QTL × location (*gray contour*) effects, with QTL and marker cofactors and location interactions. **b** Additive-effect profile corresponding to **a**, where QTL location effect is expressed as AR effect–LA effect. *Vertical dotted lines* demarcate chromosomes

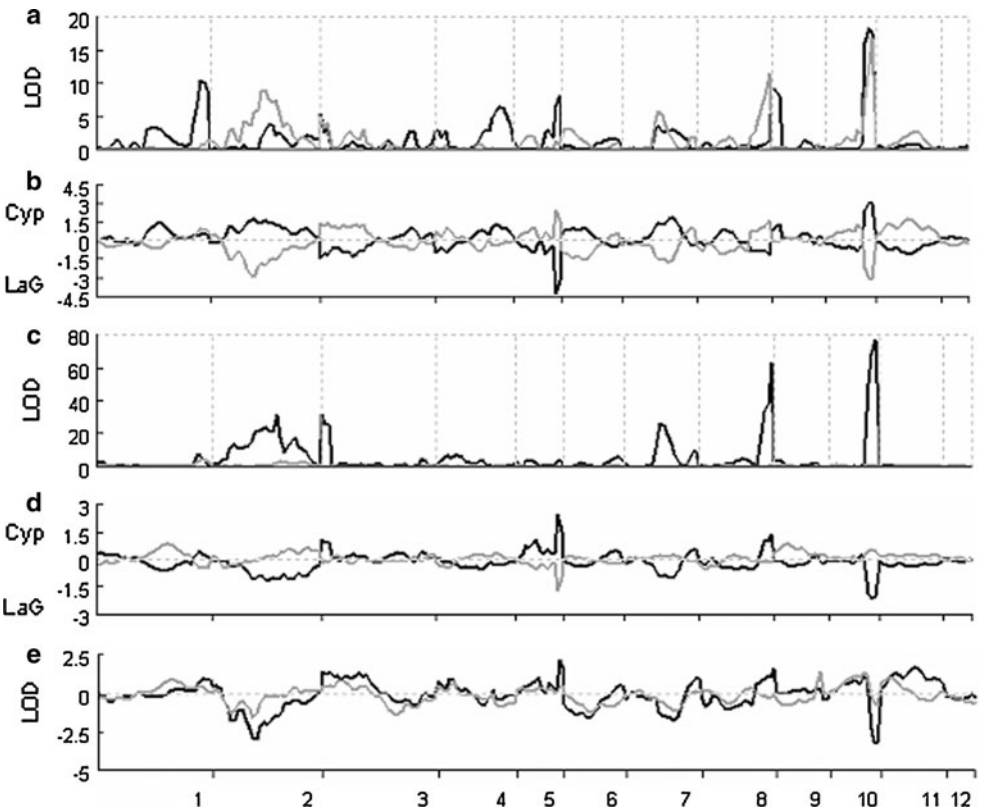

Fig. 4 Multienvironment QTL main-effect and QEI profiles of head-rice yield (HR) and DTH in Cypress × LaGrue (MY2) rice RILs in years 2006 and 2007. *Vertical dotted lines* demarcate chromosomes. Permutation $\alpha = 0.05$ thresholds are shown as *horizontal dotted lines*. **a** LOD profiles for QTL main (*black contour*) and QTL × location (*gray contour*) effects on HR; **b** additive effects corresponding to **a**; **c**, **d** as **a** and **b** except for trait DTH; **e** as **b** but comparing QTL × location effect when year and its QTL interactions are nested in location (*black contour*) with that when each year–location combination is treated as a distinct environment (*gray contour*)

above. Here a DTH effect was seen only in LA in 1 year while DHV was consistently extended by up to 3 days per Cypress allele.

Kernel dimension

For the three dimension traits, five QTLs were found in MY1 and 14 in MY2. All but two width (KW) QTLs were expressed in all years and locations. In MY1, the increasing alleles for KL and KW matched those expected from the parents: RT0034 has a longer and narrower grain than Cypress. In contrast, in MY2 the parental effects varied at the QTLs for each trait, consistent with the more similar shapes of Cypress and LaGrue. Two dimension QTLs, a KW QTL on chromosome 2 and a KL QTL on chromosome 3, appeared common to the two Cypress crosses. Interestingly, at the second common QTL the increasing effect was from RT0034 in MY1 but from Cypress in MY2, indicating that the three parents all carry distinct alleles for this QTL.

No dimension QTLs were found near HR QTLs, in agreement with the generally low correlations between

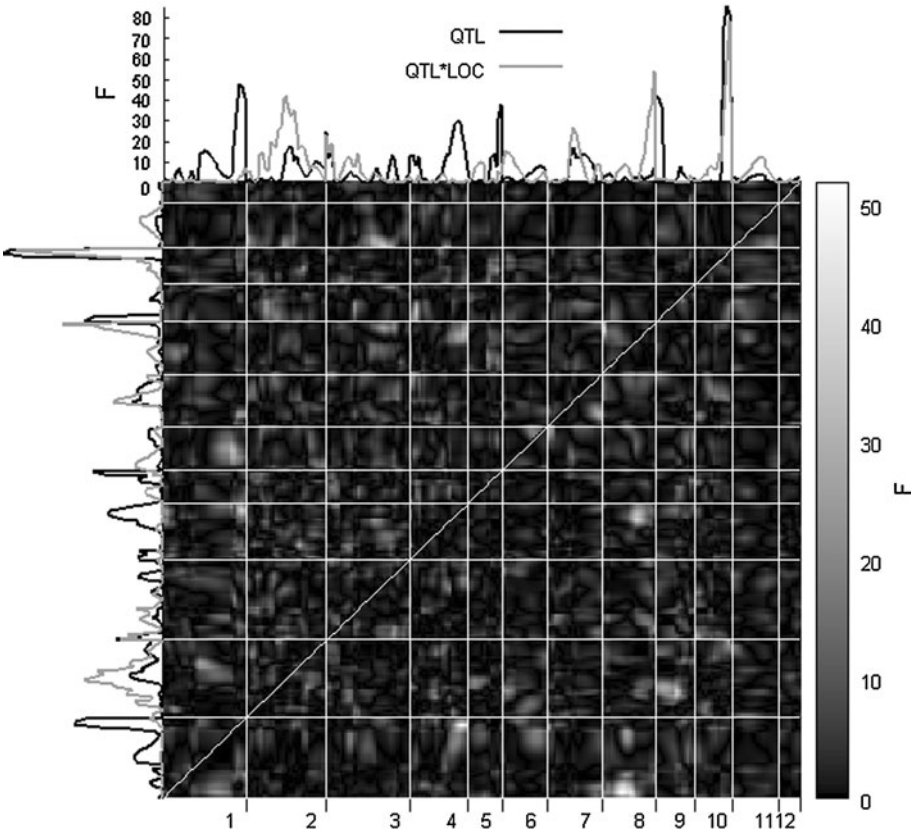

Fig. 5 Heat map from one- and two-way QTL scans for head-rice yield (HR) in Cypress × LaGrue (MY2) rice RILs grown in 2 years and locations. *Upper left* triangular portion of the square plot shows *F* statistics from all QTL × QTL interaction tests and *lower right*, those from QTL × QTL × location interaction tests, with all statistics based on the same degrees of freedom. Marginal plots (identical to those in Fig. 4a though showing *F* rather than LOD) show one-way QTL scans using the same model except for the QTL interactions; the *black contour* represents main and the *gray*, QTL × location effect

dimension traits and HR (Table 2) and the similar dimensions of the parents evident from Table 1.

Plant height

In the MY1 cross with RT0034, which like Cypress carries the major semidwarfing gene *sd-1* on chromosome 1, only two height QTLs were detected and only in Texas. In MY2, the main height determinant was *sd-1*, with the Cypress allele conferring a 10.6-cm height reduction. Two other QTLs on chromosomes 2 and 8 conferred opposite effects of 3 cm or less. These coincided with heading-date QTLs, with height increasing as heading time decreased, a more pronounced effect in location LA.

Amylose content

Only MY1 was assayed for amylose, since the MY2 parents did not differ for this trait. The *Wx* locus on chromosome 6 accounted for most of the variation, with the RT0034 allele increasing amylose by 1% and a lesser QTL on the same chromosome adding 0.3%.

Milling traits other than head rice

In MY1 six QTLs were declared for traits associated with kernel milling effects, but milling degree (MDEG) was the only trait showing consistency across locations. At all three MDEG QTLs, the high-milling parent Cypress contributed the increasing allele (conferring whiter grain due to more complete bran removal), but this was true also of the induced-fissuring QTL on chromosome 6. A MDEG QTL from Cypress lies at the same position as the chromosome-6 HR QTL from RT0034. In MY2, six QTLs were declared for field or induced kernel-damage traits, with the increasing effect from the lower-milling LaGrue parent at five of these. However, none was expressed in both growing locations. In contrast, 5 TKW QTLs were consistently expressed, but all coincided with kernel-dimension QTLs.

Discussion

Model-fitting choices that affect QEI analysis

In this study, following the common practice of treating location–year combinations as individual environments as is frequently done for MET analyses, e.g. Maccaferri et al. (2008), produced in our analysis markedly lower estimates of QEI for HR from models in which both year and location (and their interaction term) were included. This is not surprising in view of the much higher correlation, for this low-heritability trait, between years within location than

between locations. Because this same correlation structure is likely to be seen in larger MET-QTL studies using widely disparate environments, we suggest that analysts routinely make this simple comparison. The second variant model we assessed, with years nested in locations, is more attractive than the alternatives on purely statistical grounds, since the information contained in a year effect is confined to the location in which it was measured. The practical benefit of using this model seemed negligible in our study, but might be more pronounced in a larger one.

Contributions of main and interaction effects of HR QTLs

Conventional single-variable QTL-mapping methods, represented by MIM, identified HR QTLs only in individual year × location combinations in either of these two crosses of the high-milling Cypress with low-milling rice cultivars. A naïve conclusion would have been that under these experimental conditions, genetic variation in HR was due only to GEI. An additional confounding point was added by the reversal of correlation between milling yield and earlier heading in the two growing locations, such that earlier heading was advantageous in Louisiana but neutral in Arkansas. Rice breeders know already that escape from damaging high nighttime temperatures in July and August is favored by rapid heading in the early-planted Louisiana fields and by slower heading in the later-planted more northerly Arkansas fields. In a controlled phytotron study, HR of cultivars Cypress and Bengal remained stable with increasing night temperatures, while that of LaGrue and other cultivars declined (Cooper et al. 2008). But the proposed mechanisms (increased kernel chalkiness, change in amylopectin chain, change in kernel thickness distribution) by which these temperatures might affect HR were not supported in their study, nor in ours.

The separation of QTL main and location-interaction effects also accounts for the more extreme performance differences between Cypress and either of the other parents in LA than in AR. As noted in the Results, location interactions (except at the QTLs on chromosomes 6 and 9 in MY1, and 1 and 9 in MY2) invariably opposed the QTL main effect in AR (Figs. 3b, 4b). A practical application of this finding, if it is supported by further trials, might be to develop an improved cultivar for the Arkansas rice-growing region by marker-assisted selection for the corresponding alleles or further development of selected RILs in these populations. In MY2, for example, regions on chromosomes 2, 3, 4, 6, 7, and 11 that contain no loci accepted as HR QTLs, and in four of which the LaGrue allele is beneficial in AR, might be used. Since most of these regions are not implicated in early heading, this strategy would not amount to manipulating earliness, which we have already seen was beneficial for HR in LA but neutral in AR. Breeders focus early-generation breeding efforts on their local rice-growing regions, although they test advanced lines throughout the southern growing region for broader adaptation.

Few QTLs common to two Cypress-derived RIL populations

None of seven heading-time or six HR QTLs were shared between the crosses, although Cypress is a parent common to both. The chromosome-9 Cypress HR QTLs (Fig. 2) are convincingly separated. Only two apparently common QTLs were identified, both for kernel dimension. At the second QTL, the Cypress allele showed a positive effect in one cross and a negative in the other, suggesting an allelic series.

What can explain such QTL diversity between progenies with a common parent? For the original multiple-cross design of the RiceCAP project (http://www.uark.edu/ua/ricecap) of which this study was a part, the parents had in fact been selected for just such diversity, with the goal of finding multiple genetic contributors to HR. Indeed, RT0034 showed the same level of genetic divergence from LaGrue, 0.77 of 616 tested SSRs, as from Cypress. Moreover, at 276, or 0.45, of these SSRs all three parents carried a different allele, and at only 30 of the SSRs did RT0034 and LaGrue share an allele that was not shared by Cypress. We might thus expect only one in twenty biallelic QTLs to be common to the two crosses, while detection of a multiallelic QTL might be expected only in a highly heritable trait controlled by few genes—for example, kernel dimension.

Still, although the wider MY1 cross showed twice the polymorphism level of MY2, it revealed only half as many QTLs. This result is likely the consequence of small population size, compounded by segregation distortion.

Segregation distortion

Marker segregation distortion is common in *indica* × *japonica* crosses (Fukuta et al. 2000; Harushima et al. 1998; Wang et al. 1994) and has been attributed (Lin et al. 1992) to pollen sterility resulting from lethal genetic interactions. Either parental allele may be favored, depending on the map region, but skewing toward the *indica* allele, as seen in MY1 in our study, is most common. In QTL mapping studies using wide crosses with wild species, distortion is also common. It frequently but not always (Aluko et al. 2004; Septiningsih et al. 2003) favors the cultivated parent, possibly in part because of selection for adaptation to the experimental environment. Since *indica* germplasm is more vigorous and has higher tillering

ability than most US cultivars, we speculate that the predominance of *indica* alleles in MY1 is a result of higher fitness and inadvertent selection of predominantly *indica* recombinants during population development by single-seed descent. Whatever the genetic bases of distortion and irrespective of its direction, the imbalance of marker class sizes inflates sampling variation of the QTL test statistic (shown as higher permutation thresholds), reducing power of QTL detection. This bias toward *indica* alleles may account for the invisibility in MY1 of most Cypress QTLs detected in MY2.

Inferences about genetic control from correlation and QTL pleiotropy

It is of interest to know whether any trait correlations can be explained in part by shared pleiotropic QTLs. Such correlation might arise from direct control by a QTL of both traits independently, indirect genetic control of one trait via the other, or more complex mixtures of causal relationships, including other QTLs, traits, and environment.

Some measured traits have clear arithmetical relationships with more elementary traits, some examples being DHV with DTH; or kernel volume, surface area, and length:width ratio with kernel length, width, or thickness. HR showed pleiotropy with all measurements of broken kernels, at the respective map location giving the highest QTL signal for either growing environment (results not shown). Since HR is defined as the proportion of milled kernels above 75% full length, the broken-kernel proportion is in effect its complement. Colocation of QTLs for such traits has been reported in other studies (Dong et al. 2004; Jiang et al. 2005). It is possible to eliminate purely arithmetical pleiotropy by substituting for a trait the residuals from its regression on the others in the correlation set and ignoring QTLs that can be "regressed away". Though even "derived" traits were subjected to QTL mapping, we chose not to record QTLs for such traits unless they differed from QTLs for the primary traits. Only one such case was found: on chromosome 1 in MY2, where a QTL was strongly expressed for DHV in all environments but for DTH in only one. The kernel-weight trait TKW was allowed its own QTLs even though all five observed TKW QTLs in MY2 and one in MY1 coincided with kernel-dimension traits and all disappeared in the regression test (results not shown). In principle kernel density might be expected to vary independently of volume, though in this material it did not.

The QTLs identified in MY2 as influencing phenology show different kinds of action. The major chromosome-10 DTH QTL has no effect on plant height, while at three other heading-time or days-to-maturity QTLs, RILs with the allele for taller stature also mature earlier. This negative association is reflected in the negative trait correlations in MY2. But the two traits are positively correlated in MY1, suggesting that an explanation based on "vigor" QTLs would not hold generally.

Negligible effects of milling-time variation

Although the repeated Cypress checks showed appreciable HR variation across the two-week milling period especially in 2007 (data not shown), the QTL LOD and additive-effect profiles for raw and check-adjusted HR in every environment showed negligible change around the QTLs described above for this trait (results not shown). In other areas of the map the profiles were reduced in height. Such adjustment thus may be expected to reduce potential false QTL assignments but not to increase sensitivity. Thus E or GEI variation for HR arising from the actual milling operation appears to be of little concern for these analyses.

Low potential of induced-fissuring assay

The rationale for the induced-fissuring assay is that often rice is not harvested at the optimum moisture content of 17% and while drying down in the field may undergo repeated re- and dehydration cycles resulting in severe breakage in susceptible genotypes such as LaGrue. Imposing this stress in a controlled way on grain harvested, over a period of several days, at a near-optimum moisture level for each RIL and thus having undergone minimal hydration stress in the field was intended to expose QTLs for HR stability over varying harvest dates. Such QTLs, which might not coincide with QTLs for other components of HR, could spare breeders the necessity of testing breeding lines for HR stability by successive harvests at declining moisture levels. However, while induced fissuring showed slightly higher heritability than field fissuring in both populations (Table 1), no stable QTLs for the trait were found. In general, in both populations field fissuring and cracking were more highly correlated with HR than the artificially induced traits. Thus the assay as performed here did not show promise as a selection method for HR stability.

QTLs reported previously

For several traits consistently expressed across locations in the RIL populations, nearby (within 10 cM) or coinciding QTLs have been previously reported in other *indica* or *japonica* rice accessions. Amylose QTLs on chromosome 6 were reported in DH progeny from two *indica* cultivars, Zhenshan 97 and H94 (Fan et al. 2006), and in Chinese elite rice hybrid Shanyou 63 (Tan et al. 2000). QTLs for

DTH near the MY1 QTLs on chromosome 6 and 8 have been reported in (Lin et al. 2000; Wang et al. 2002; Xing et al. 2001; Yamamoto et al. 2001; Yano et al. 1997). The kernel-width QTL on chromosome 8 in MY1 was 7 cM from QTLs identified in an elite Chinese hybrid cultivar (Xing et al. 2002). The thickness QTL on chromosome 5 is close to one reported in a DH progeny of Japanese cultivars Reiho and Yamada-nishiki (Yoshida et al. 2002). However, the kernel-length QTL on chromosome 3 is not near the recently cloned (Fan et al. 2006) *GS3* gene for this trait.

HR QTLs in the *O. sativa* × *O. glaberrima* BC_3F_1 progeny of Aluko et al. (2004) were located at about the same positions on chromosome 6 and 8 as MY1 QTLs for this trait. For the milling-degree QTL on chromosome 3, a nearby QTL for chalkiness was reported in the *indica* × *japonica* DH lines of Li et al. (2003a). In MY1, the milling-degree QTL on chromosome 6 that coincides with a HR QTL contributed by the lower-HR parent lies at the major amylose QTL and may be identical to the QTL reported by Kepiro et al. (2007).

Conclusions

1. In replicated two-location tests of 129 MY1 RIL progeny evaluated in 1 year and 298 MY2 RILs evaluated in 2 years for HR and related traits, HR showed low heritability and correlated oppositely with DTH in the two locations, underlining the sensitivity of this trait to environmental conditions. Since in both populations HR QTLs were expressed inconsistently in the two growing locations, conventional location-specific analysis was inadequate for distinguishing QTL main from QEI effects.
2. An array of joint analyses indicated for MY1 two main-effect QTLs on chromosomes 6 and 9 and for MY2 four different QTLs on chromosomes 1, 5, 9, and 10, with the superior-milling parent Cypress contributing the favorable allele at four of the six. Multiple-QTL models accounted for about a fourth of genetic and a third of GEI variation in HR. It might appear that higher marker density could have increased these proportions by sampling genotypic variation through more of the genome, but SSR polymorphism for the mapping parents was not found in large tracts of the genetic map.
3. The common parent, Cypress, did not appear to contribute common QTL alleles for most traits in the two crosses: an observation that may be explained by the diversity of the other two parents used and that suggests the importance of genetic background and complex gene interactions in the control of these traits.
4. Deliberate use of long-grain × long-grain crosses between genetically divergent parents of similar maturity characteristics appears to have minimized confounding effects of grain dimensions on milling quality, judging from the differing map locations of dimension and milling QTLs. However, in the *indica* × *japonica* MY1 cross, QTL detection for lowly heritable traits such as HR was disfavored by the approximate 2:1 skewing of marker alleles towards the *indica* parent, combined with the relatively small population size.
5. HR QTL detection was robust to nonexperimental variation introduced during milling.
6. No HR-predictive advantage was seen from laboratory induced-fissuring tests applied to field-grown rice in the genetic materials used in these experiments.
7. The genome-wide opposition of AR-location-specific to main QTL effects suggests a strategy for targeted improvement of Cypress-related cultivars for the AR region and identifies genomic regions for selection and further testing.

While ours is among the largest studies yet reported for elucidating the genetic basis of HR and partially accounts for the known superiority of the higher-milling cultivar Cypress, we suggest that like all previous studies with which we are familiar, it lacks the statistical power to support any model of candidate genes and interactions that might be constructed. Methods recently developed for identification of QTL interactions of predictive value (Dudley and Johnson 2009; Xu and Jia 2007) seem preferable for building QTL models based on larger datasets. In a recent perspective (Yamamoto et al. 2009) cite a dozen previous studies reporting epistatic effects in rice and urge that confirmation has become more important than estimation of minor QTL effects, QQI, and QEI. They propose for this the use of sets of chromosome substitution lines in parallel with conventional mapping populations. But this approach will still face the masking of allelic diversity in individual crosses and the incomplete accounting for genetic variation (not only for HR but for highly heritable traits) that we have seen even when DNA-level variation has been exhaustively sampled. We believe that further investigation of rice milling yield should hinge on the collection of data from large, densely genotyped populations with limited segregation distortion and tested in multiple environments over several years—unfortunately not a cheap proposition. For the loss of QTL detection sensitivity and specificity resulting from distorted allele frequencies, we see no available statistical remedy short of increasing the experiment size, though a recent review (Zhang and Gai 2009) hints at the application of viability-locus modeling to QTL mapping.

Plant response may be better characterized with respect to short-term than to longer-term average environmental influences, both because responses may differ depending on growth stage and because entries in a genetic study may occupy different growth stages on a given day. For example, heading and harvest dates rarely coincide for all entries, though a growth or yield response such as to drought may differ sharply pre- or post-anthesis. This suggests the partitioning of GEI variation among genotypes into two components: that due to differing developmental stages and that due to differing response at the same stages. An initial attempt at such an analysis was made in our study (though not reported here), but did not prove fruitful, perhaps owing to the small sample of environments. Because of the stage specificity of plant response, it seems attractive to incorporate into QTL-mapping models at least heading date as a covariate, and possibly even weather or soil conditions during comparable growth stages of different entries. Daily, hourly, or even more frequent weather data are commonly collected at agricultural experiment stations. The exploitation of such data in imaginative statistical models, combined with plant sampling during the growing season, might aid in identifying the key environmental causes of low HR and of other traits showing high GEI. For HR, one approach might be the measurement and QTL mapping of kernel anatomical and physiological traits connected with moisture diffusivity of bran and endosperm that might influence HR stability in varying temperature and humidity regimes.

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