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Identification of QTLs affecting traits of agronomic importance in a recombinant inbred population derived from a subspecific rice cross

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Abstract To detect QTLs controlling traits of agronomic importance in rice, two elite homozygous lines 9024 and LH422, which represent the *indica* and *japonica* subspecies of rice (*Oryza sativa*), were crossed. Subsequently a modified single-seed-descent procedure was employed to produce 194 recombinant inbred lines (F_8). The 194 lines were genotyped at 141 RFLP marker loci and evaluated in a field trial for 13 quantitative traits including grain yield. Transgressive segregants were observed for all traits examined. The number of significant QTLs (LOD $\geqslant$ 2.0) detected affecting each trait ranged from one to six. The percentage of phenotypic variance explained by each QTL ranged from 5.1% to 73.7%. For those traits for which two or more QTLs were detected, increases in the traits were conditioned by *indica* alleles at some QTLs and *japonica* alleles at others. No significant evidence was found for epistasis between markers associated with QTLs and all the other markers. Pleitropic effects of single QTLs on different traits are suggested by the observation of clustering of QTLs. No QTL for traits was found to map to the vicinity of major gene loci governing the same traits qualitatively. Evidence for putative orthologous QTLs across rice, maize, oat, and barley is discussed.

Key words Rice · Subspecies · Recombinant inbred population · Molecular markers · QTLs

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Introduction

Tracking polygenes with genetic markers can be traced back to the early 1920s when Sax (1923) reported the association of quantitatively-inherited seed size with monogenes controlling seed coat pigmentation and pattern in bean. Subsequent reports of linkages between single gene markers and quantitative trait loci (QTLs) were made by Rasmusson (1935), Everson and Schaller (1955) and Thoday (1961). In these classical studies, morphological mutations were used as genetic markers; however, the nature of such genetic markers poses major limitations for the study of quantitative variation. Because only a few such markers are available in any given cross, and the effect of the marker genes on the quantitative traits is often larger than that of the linked QTLs, it is difficult to effectively and extensively study quantitatively inherited traits (Tanksley et al. 1989).

The recent advent of molecular markers in quantitative genetics greatly facilitates the study of complex, quantitatively inherited traits and has made it possible to dissect the polygenes for such traits into individual Mendelian factors. Using molecular linkage genetic maps and quantitative trait loci (QTLs) mapping technology, it is possible to estimate the number of loci controlling genetic variation in a segregating population and to characterize these loci with regard to their map positions in the genome, gene action, phenotypic effects, pleitropic effects, and epistatic interactions with other QTLs.

Since the introduction of molecular markers, RFLPs in particular, QTL mapping in numerous species (e.g., tomato, maize, wheat, bean, human, rat, pig, mice) and for various traits (e.g., plant status, yield and its components, quality traits, resistance, environmental stresses, hypertension, intelligence) has been well documented (for a review see Tanksley 1993b). In rice, QTLs for cooked kernel elongation (Ahn et al. 1993b), partial resistance to blast (Wang et al. 1994), heterosis (Xiao et al. 1995), plant height and heading date (Li et al. 1995; Xu

et al. 1993), yield components (Xu et al. 1995), and root morphological characters related to drought avoidance (Champoux et al. 1995) have been identified. These molecular markers can be employed to manipulate the selection of desired genotypes based on the marker alleles at these QTLs instead of expensive evaluations over locations and years.

In rice, hybridization between *indica* (tropical rice) and *japonica* (temperate rice), the two major subspecies of cultivated rice (*O. sativa*), has been important for the transfer of desired traits (e.g., resistance to diseases and pests, tolerance to environmental stresses, grain quality, yield potential) between these two cultivar types. Identification of QTLs underlying traits of agronomic importance in rice is a prerequisite for using molecular markers for rice genetic improvement.

In this study we have analyzed an RI population of 194 recombinant inbred lines derived from a cross between two elite inbred lines – one from *indica*, the other from *japonica*. Our objectives were to map and characterize the phenotypic effects of QTLs underlying 13 traits of agronomic importance that differentiate *indica* and *japonica* rice.

Materials and methods

Development of recombinant inbred population

Two elite homozygous lines 9024 [*indica* parent (*I*)] and LH422 [*japonica* parent (*J*)], representing the two major subspecies of *O. sativa*, were crossed in 1988 at the Hunan Hybrid Rice Research Center, China. Approximately 30 F_1 hybrid plants were obtained from this cross. F_1 selfed seeds were collected from those F_1 plants and grown in a field as the F_2 population; 194 F_2 plants were randomly chosen 2 weeks after transplanting. Approximately 100 seeds from each F_2 plant were individually harvested at maturity. Fifty plants from each of 194 F_3 seedlings were transplanted to the field. One plant from each plot was randomly chosen and approximately 100 seeds were harvested at maturity. This same procedure as used for the F_3 was continued to the F_7 except that approximately 500 seeds, instead of 100 seeds, were collected from each randomly chosen F_7 plant.

Field trial

The 194 F_8 lines, along with the two parental lines, were evaluated in the field in a randomized complete block design, with two replications (plots), in the summer of 1992 at the Hunan Hybrid Rice Research Center, China. Three-row plots were planted with nine plants per row. The middle five plants in the central row in each plot were used for data collection. The traits measured were: plant height (cm), days to heading, days to maturity, panicles per plant, panicle length (cm), spikelets per panicle, spikelet setting density (spikelets per cm), grains per plant, 1000-grain weight (g), spikelet fertility (as measured in percent seed set), spikelets per plant, grains per plant. Means over replications, for each trait, were used in data analyses.

Genotype determination

From each of the 194 F_8 lines, 30–40 seeds were sown in the greenhouse and seedlings were bulked for DNA extraction. RFLP genotypes were determined as previously described in McCouch et al. (1988). The 141 probes used were a subset of those previously mapped in two different rice mapping populations (Saito et al. 1991; Causse et al. 1994). Each of the probes used showed polymorphism between *I* and *J* in genomic DNA digests with at least one of the five restriction enzymes used.

Data analysis

The segregation ratio at each marker locus was statistically analyzed for deviation from the expected Mendelian segregation ratio (1:1) by χ^2 tests using Map Manager Version 2.5 (Manly 1993). The RI RFLP map was established using Mapmaker Version 3.0 (Lander et al. 1987; Lincoln et al. 1992a).

Genome composition was estimated using Hypergene (Young and Tanksley 1989). When consecutive markers bordering a region along the chromosome of an individual RI line had the same genotype (originating from the same parent), the estimates assumed that the region was comprised entirely of the parental genome. When consecutive markers bordering a region along the chromosome of an individual RI line showed a different genotype, the region was treated as being comprised of half of each parental genome.

The analyses of QTLs associated with markers for each trait were performed using two procedures: one-way analysis of variance (ANOVA) from Data Desk 4.0 (Data Description Inc. 1992) and interval mapping in MAPMAKER/QTL 1.1 program (Lincoln et al. 1992b; Paterson et al. 1988). A LOD score of 2.0 was used as the threshold for detecting QTL locations in the MAPMAKER/QTL 1.1 program. The proportion of the total phenotypic variation explained by each QTL was calculated as an iR^2 value (iR^2 = ratio of the sum of squares explained by the QTL to the total sum of squares). The total phenotypic variance explained was estimated by fitting a model including all putative QTLs for the respective trait simultaneously. A multiple QTL model was constructed for the same trait for which more than one significant LOD peak had been found on a chromosome to determine whether the chromosome possessed single or multiple QTLs. In interval analysis, data transformation for each trait was tried to improve the normality of the distributions of the traits and transformed data was also subjected to QTL detection. The transformed and untransformed data gave similar results in identifying QTLs; therefore, only the results from untransformed data are presented in this report.

For each trait, two-way interactions were tested between significant markers associated with QTLs and all other marker loci in the genome using the PROC GLM in SAS (SAS Institute 1988). For example, let p_1, p_2, p_3, p_4 denote the phenotypic effects of RI lines with genotypes *II*;*II*, *II*;*JJ*, *JJ*;*II* and *JJ*;*JJ*, respectively. The null hypothesis (no epistasis) for such test is: $(p_1 + p_4) - (p_2 + p_3) = 0$, with one degree of freedom.

Results

Segregation of markers

Deviations from the expected 1:1 segregation ratio were significant for 52 loci (36.9%) at $P \leqslant 0.05$ and 42 loci (29.8%) at $P \leqslant 0.01$. The skewed loci corresponded to 13 linked chromosomal regions on ten different chromosomes (Fig. 1). Chromosomes 4 and 10 were the only chromosomes for which all loci showed normal segregation. All of the loci on chromosome 9 were skewed in favor of *I* alleles. All of the loci on chromosome 7 were skewed in favor of *J* alleles.

The average frequency of the heterozygous genotype per marker was 3.2% (range: 0–18.1%), higher than would be expected theoretically (1.6%). Fifty-nine (41.8%) of 141 markers (Fig. 1) exhibited an unexpectedly high frequency of marker heterozygosity ($P \leqslant 0.05$).

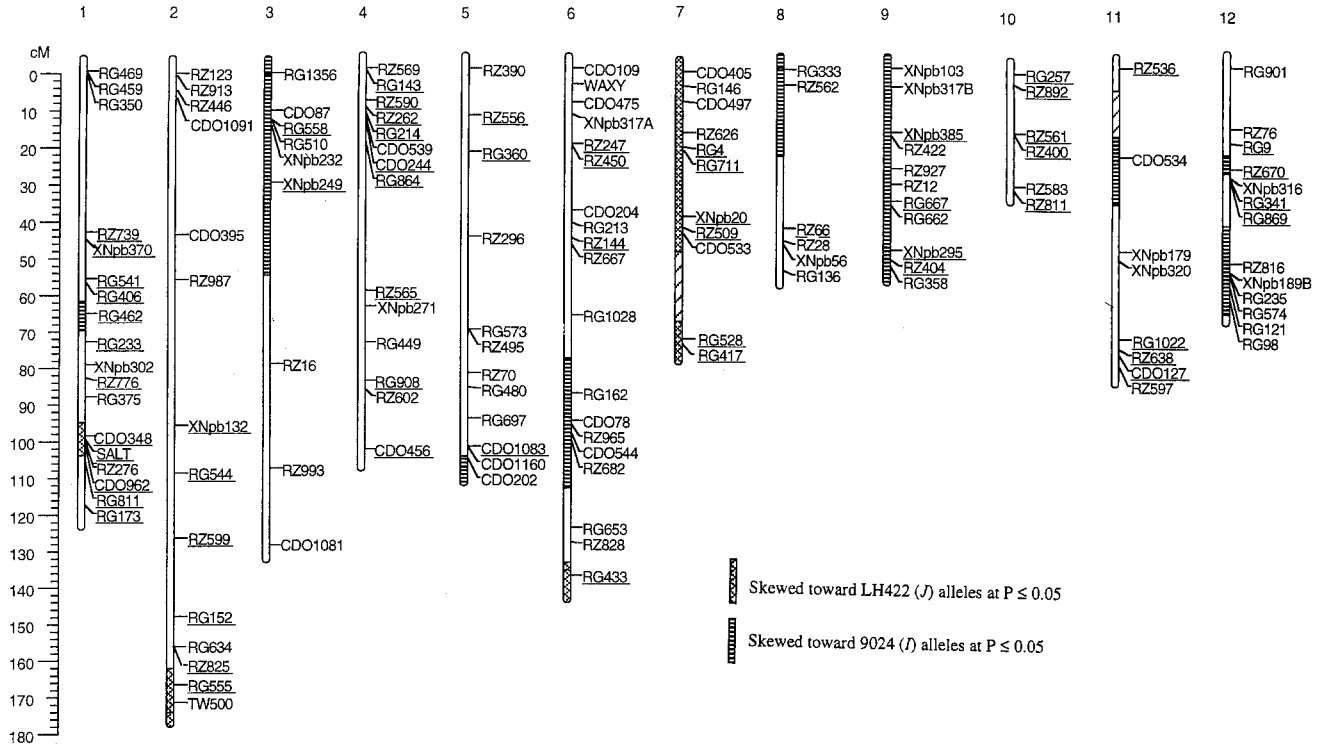


Fig. 1 Rice molecular genetic map showing segregation-distorted chromosomal regions, and marker loci (*underlined*) with significantly ($P \leq 0.05$) higher heterozygote frequency than expected. Map distances are in Kosambi (1944) centiMorgans. *Striped bars* on chromosomes 7 and 11 indicate markers are linked with LOD scores of less than 2.0

Genome composition of recombinant inbred lines

The percent of each parental genome in the recombinant inbred lines was estimated by Hypergene (Young and Tanksley 1989). The frequency distribution of these values for the *I* genome is shown in Fig. 2. The parental genome originating from the *I* parent ranged from 21.8% to 72.9%, with a mean of 52.7% which was not significantly different from the expected 50% at $P \leq 0.05$. The average percentage of overall genome heterozygosity per RI line was 3.6%, with a range from 0 to 35.7%.

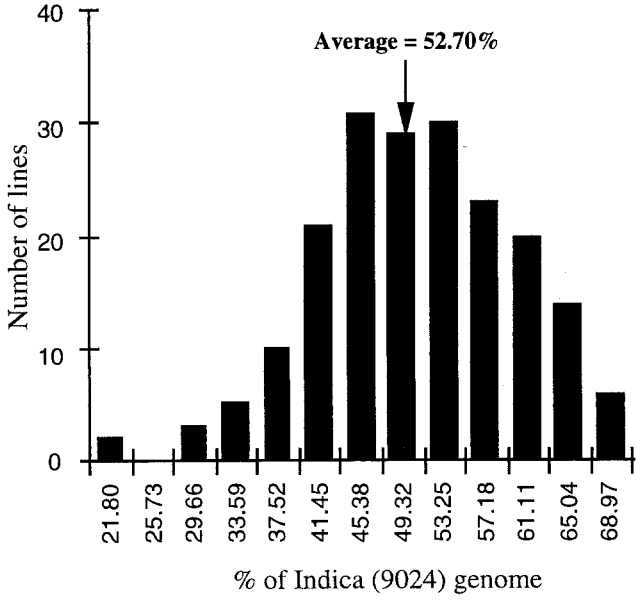


Fig. 2 Frequency distribution of the percentage *indica* (9024) genome in the 194 recombinant inbred lines

Transgressive segregation of traits

The frequency distribution of phenotypes for each trait in the F_8 lines is shown in Fig. 3. All traits approximately fit normal distributions. *I* and *J* had almost identical values for percent seed set and spikelets per plant; however, F_8 lines having phenotypic values greater than the higher parent and less than the lower parent (e.g., transgressive segregants) were observed for all traits.

Trait correlations

The correlation between traits was evaluated by regressing phenotypic values of one trait on those of another trait. The correlation coefficients among traits are presented in Table 1. For most of the correlations, the

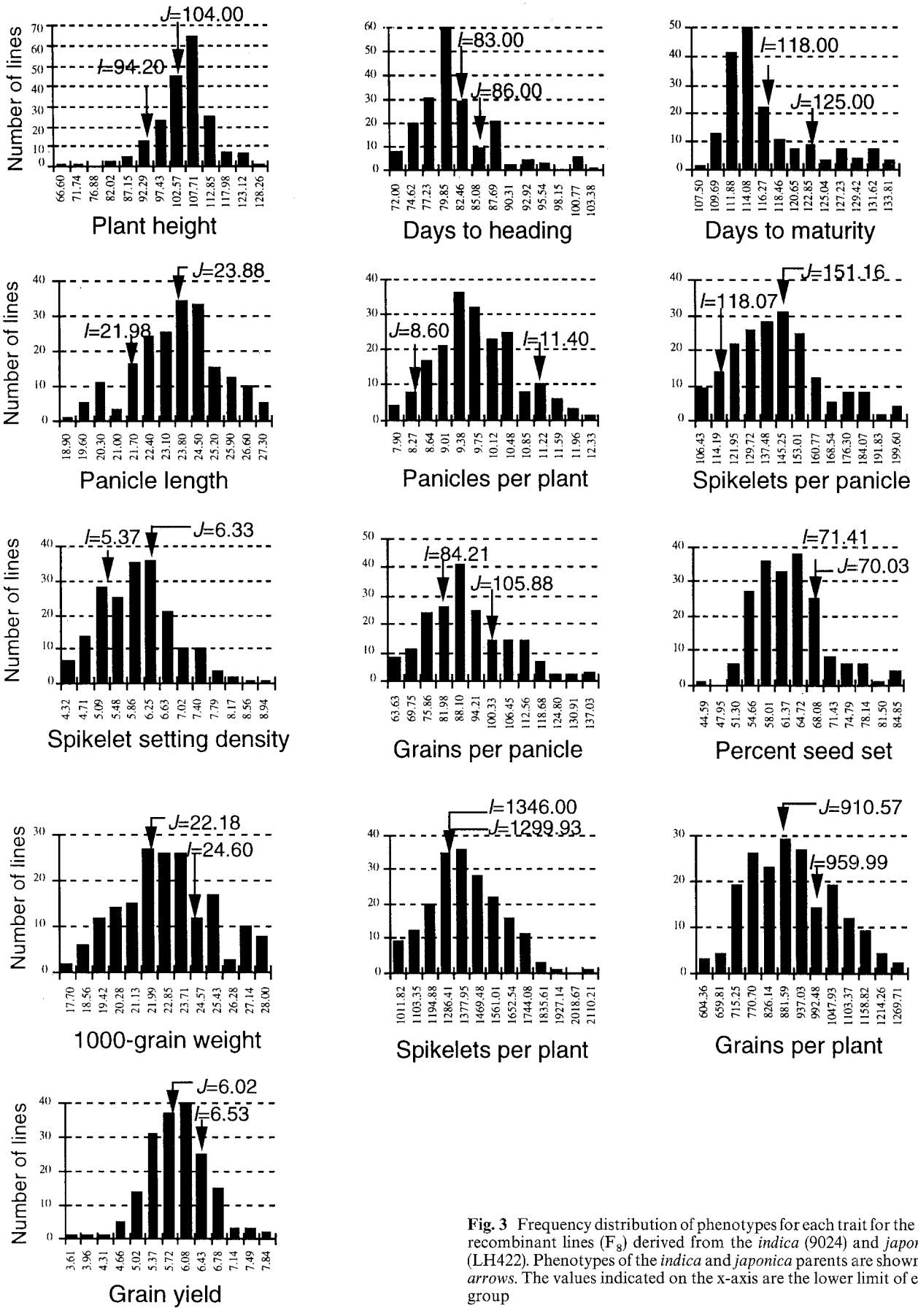


Fig. 3 Frequency distribution of phenotypes for each trait for the 194 recombinant lines (F_8) derived from the *indica* (9024) and *japonica* (LH422). Phenotypes of the *indica* and *japonica* parents are shown by *arrows*. The values indicated on the x-axis are the lower limit of each group

Table 1 Correlation coefficients (r) among traits in the 194 F_8 lines derived from the cross of *indica* (9024) × *japonica* (LH422)

Trait	1	2	3	4	5	6	7	8	9	10	11	12
Plant height (1)												
Days to heading (2)	0.295											
Days to maturity (3)	0.241	0.951										
Panicle length (4)	0.146	−0.084	0.047									
Panicles per plant (5)	−0.064	−0.112	−0.027	−0.004								
Spikelets per panicle (6)	0.172	0.059	0.089	0.304	−0.358							
Spikelet setting density (7)	0.079	0.093	0.044	−0.232	−0.357	0.854						
Grains per panicle (8)	0.069	0.011	0.018	0.183	−0.371	0.765	0.681					
Percent seed set (9)	−0.115	−0.063	−0.078	−0.127	−0.081	−0.169	−0.101	0.501				
1000-grain weight (10)	0.007	0.115	0.098	−0.196	−0.096	−0.507	−0.422	−0.608	−0.273			
Spikelets per plant (11)	0.141	−0.014	0.072	0.303	0.256	0.807	0.662	0.552	−0.232	−0.583		
Grain per plant (12)	0.045	−0.044	0.011	0.183	0.171	0.604	0.523	0.847	0.488	−0.703	0.731	
Grain yield (13)	0.116	0.109	0.106	0.121	0.204	0.356	0.302	0.545	0.368	−0.082	0.489	0.696

direction (+ or −) and degree of correlation was consistent with previous observations (Xiao and Yuan 1988). However, 1000-grain weight, one of the grain-yield components, was not significantly correlated with grain yield in this study. This may be due to the severe infertility observed in the F_8 population.

QTLs for traits

For each trait, the linkage of QTLs to molecular markers mapped in the RI population was assessed by single-point analysis and interval mapping. Because both analyses gave almost the same result in identifying QTLs for each trait, only the results from the interval analysis are presented here. The QTL plots are shown in Fig. 4.

Plant height

Six QTLs significantly affecting plant height were detected on chromosomes 1, 2, 5, 6, 7, and 8. *I* contributed alleles increasing plant height at *ph5* and *ph6* (chromosomes 5 and 6). At the other four QTLs (*ph1*, *ph2*, *ph7* and *ph8*), the alleles increasing plant height were contributed by *J*. The phenotypic effect of each QTL ranged from 4.8 to 6.5 centimeters. The percentage of phenotypic variance explained by each QTL ranged from 7.8 to 12.3%. Fitting the six QTLs simultaneously, 42.8% of the phenotypic variation could be accounted for. The sum of their absolute effects accounted for a difference of 24.2 cm in plant height.

Days to heading

Three QTLs were identified which significantly influenced days to heading. The locus *dth8* (chromosome 8) accounted for 51% of the total phenotypic variance such that the *I* allele in the homozygous state decreased days to heading by more than 9 days. The *J* alleles decreased days to heading at *dth3* and *dth11* (chromosomes 3 and 11). In total, the three QTLs explained 60.7% of the phenotypic variation and the sum of absolute effects amounted to 14.1 days.

Days to maturity

Two putative QTLs were found for days to maturity. The QTL *dtm8* (chromosome 8) explained 73.7% of the total phenotypic variance with *I* alleles decreasing days to maturity by 12.5 days. In contrast, *J* alleles caused a reduction in days to maturity at *dtm11*. Together, the two QTLs explained 74.2% of the total phenotypic variation and accounted for 13.7 days.

Panicle length

Two QTLs were mapped for panicle length. At *pl9*, *I* alleles increased panicle length. However, at *pl7*, *J* alleles increased panicle length. The simultaneous fit of the two QTLs explained 13.8% of the total phenotypic variance and made a difference of 1.81 cm on panicle length.

Panicles per plant

Only one significant QTL (*ppp4*) was detected for panicles per plant. The *I* alleles increased the number of panicles per plant by 0.48 on average, and accounted for 7.4% of the total phenotypic variation.

Spikelets per panicle

Four QTLs were identified that significantly affected spikelets per panicle. *I* alleles at *spp4-1* (chromosome 4)

caused an increase in spikelets, whereas *J* alleles caused an increase at the other three QTLs (chromosomes 3, 4, 8). The simultaneous fit of all four QTLs explained 37.1% of the total phenotypic variation and the sum of absolute effects was 47.9 spikelets per panicle.

Spikelet setting density

Three QTLs were detected for spikelet setting density. *I* alleles increased spikelet setting density at *ssd10* (chromosome 10). *J* alleles increased spikelet setting density for the other two QTLs (*ssd3* and *ssd4*). The three QTLs together accounted for 33.6% of the total phenotypic variation and made a difference of 1.64 spikelets per cm of panicle.

Grains per panicle

Three QTLs significantly affected grains per panicle. An increase in grains per panicle was caused by *I* alleles at *gpp5* and by *J* alleles at *gpp3* and *gpp4*. The three QTLs together explained 45.8% of the total phenotypic variation. Their total absolute effects amounted to 35.1 grains on a per panicle basis.

Percent seed set

Only one significant QTL (*pss5*) was associated with spikelet fertility and *J* alleles decreased seed set by 5% at *pss5*. This QTL explained 12.3% of the total phenotypic variation.

1000-grain weight

Three QTLs affected grain weight. The *I* alleles at *gw3* and *gw4* (chromosomes 3 and 4) increased grain weight. In contrast, the *J* alleles were associated with increased grain weight at *gw5*. When fitted simultaneously, the three QTLs explained 35.2% of the total phenotypic variation. The sum of their absolute effects amounted to 4.6 g per 1000 grains.

Spikelets per plant

Four QTLs were associated with spikelets per plant. *I* alleles increased this parameter at *sppl9* (chromosome 9). The *J* alleles increased spikelets per plant at the other three QTLs (*sppl3*, *sppl4*, and *sppl11*). The four QTLs accounted for 26% of the total phenotypic variation and the total of their absolute effects was 378.4 spikelets per plant, when all four QTLs were fitted simultaneously.

Fig. 4 QTL plots based on MAPMAKER/QTL for each trait in the RI population. The *horizontal open bar* in the center of each plot represents the chromosome with both markers and map distances indicated. *Darkened bars* show LOD scores $\geqslant 2.0$ with the extensions representing LOD scores > 1.0 and < 2.0. The *triangles* indicate the map positions of the peak LOD scores. The names of the QTLs are given above the respective extensions and are based on the origins of chromosomes; for example, the QTL for plant height, on chromosome 1, is named *ph1*. The values on the right of each plot are phenotypic effects, variance explained (%), and peak LOD scores indicated from left to right. The signs, + (omitted) and −, preceding the phenotypic effects, indicate that the *J* alleles in a homozygous state had higher phenotypic effects than the respective *I* alleles in a homozygous state, and that the *J* alleles in a homozygous state had lower phenotypic effects than the respective *I* alleles in a homozygous state, respectively

Grains per plant

Three QTLs were identified for grains per plant. *I* alleles increased grains per plant at *gppl5* (chromosome 5). *J* alleles increased this trait at the other two QTLs (*gppl3*, *gppl4*). The three QTLs together explained 40.1% of the total phenotypic variation and the sum of absolute effects amounted to 305.5 grains per plant.

Grain yield

Two significant QTLs showed association with grain yield. *I* contributed alleles increasing grain yield at *gy12* (chromosome 12). In contrast, *J* alleles increased grain yield at *gy8*. The two QTLs cumulatively explained 13.6% of the total phenotypic variation. The sum of their absolute effects was 0.73 ton per hectare.

Epistasis

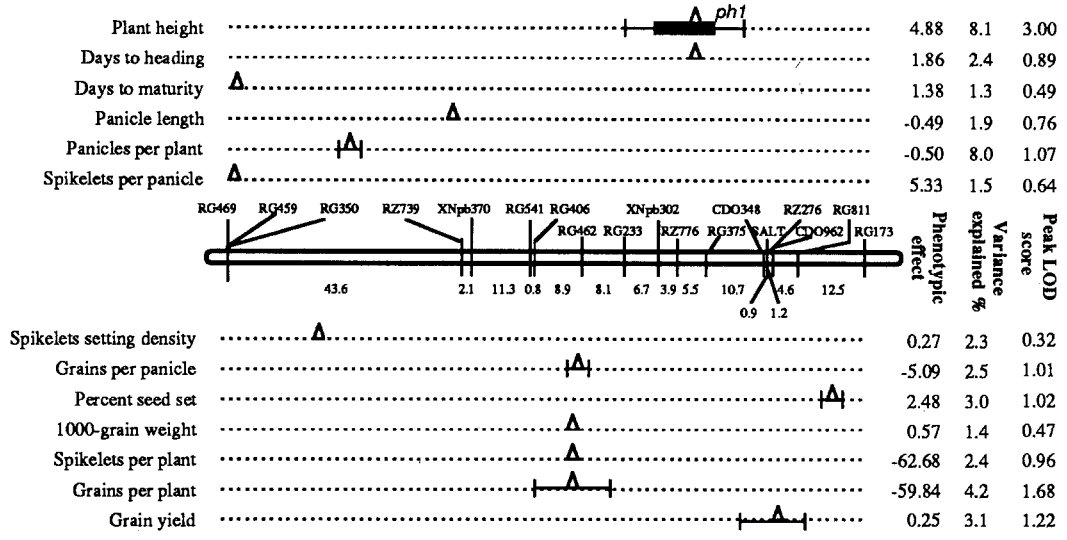
Markers linked to each of the 37 significant QTLs were tested for possible digenic interactions with all other markers. Only about 1% of the pairwise tests were significant at $P \leqslant 0.01$ significance level, indicating that digenic epistasis was not making a significant contribution to the traits examined.

Discussion

QTLs for traits

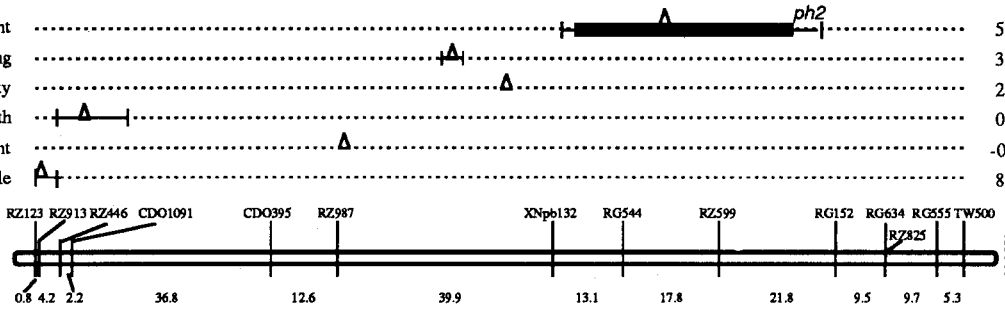
The number of significant QTL(s) per trait ranged from one to six. The percentage of phenotypic variance explained by each QTL ranged from 5.1% to 73.7% with an average of 13.8%. When two or more QTLs were found to affect a trait, increases in the trait were conditioned by *I* alleles at some loci and *J* alleles at others. In no instance were the allelic effects in the same direction for all QTLs for a trait. The proportion of the total

Chromosome 1



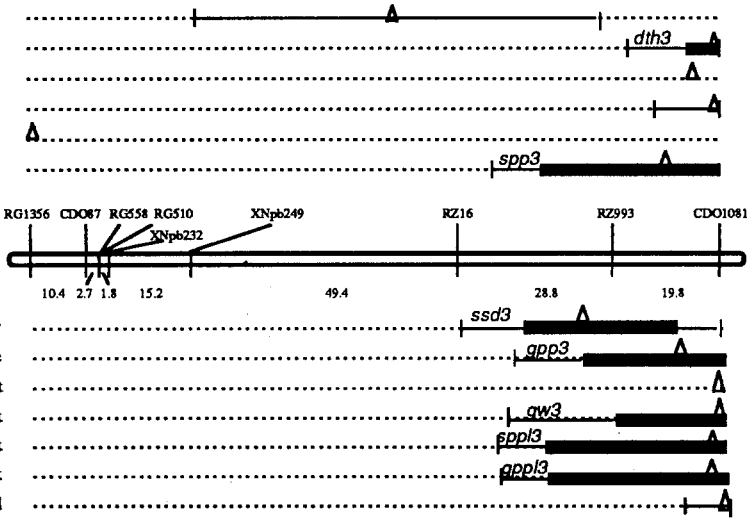
	Phenotypic effect	Variance explained %	Peak LOD score
Plant height	4.88	8.1	3.00
Days to heading	1.86	2.4	0.89
Days to maturity	1.38	1.3	0.49
Panicle length	-0.49	1.9	0.76
Panicles per plant	-0.50	8.0	1.07
Spikelets per panicle	5.33	1.5	0.64
Spikelets setting density	0.27	2.3	0.32
Grains per panicle	-5.09	2.5	1.01
Percent seed set	2.48	3.0	1.02
1000-grain weight	0.57	1.4	0.47
Spikelets per plant	-62.68	2.4	0.96
Grains per plant	-59.84	4.2	1.68
Grain yield	0.25	3.1	1.22

Chromosome 2

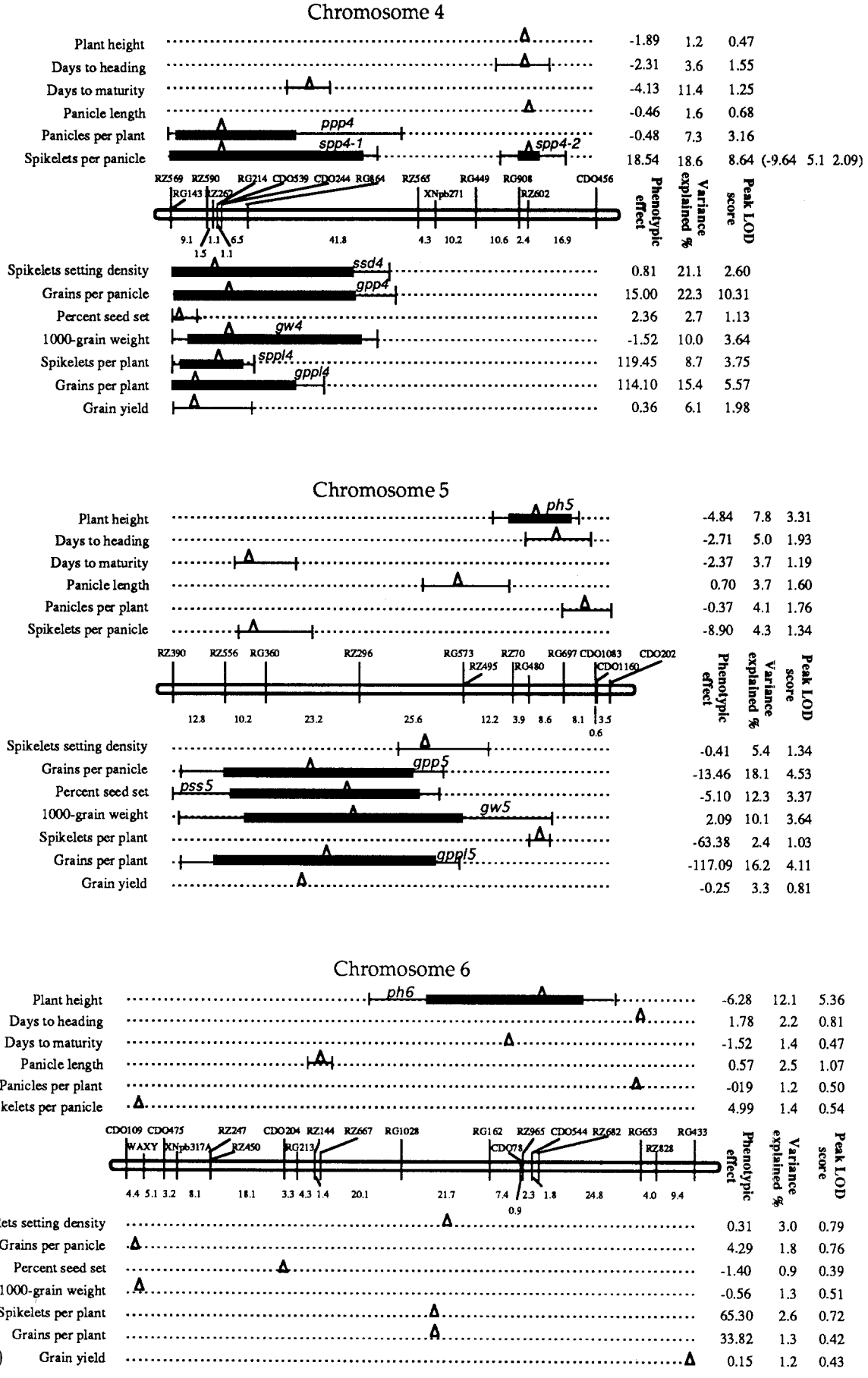


	Phenotypic effect	Variance explained %	Peak LOD score
Plant height	5.85	11.5	3.62
Days to heading	3.19	7.0	1.00
Days to maturity	2.29	3.5	0.50
Panicle length	0.69	3.6	1.54
Panicles per plant	-0.19	1.2	0.49
Spikelets per panicle	8.62	4.1	1.76
Spikelets setting density	0.38	4.6	1.60
Grains per panicle	5.83	3.4	1.42
Percent seed set	-1.24	0.8	0.31
1000-grain weight	-0.99	4.2	1.64
Spikelets per plant	80.55	4.0	1.70
Grains per plant	53.39	3.4	1.42
Grain yield	0.19	1.9	0.58

Chromosome 3



	Phenotypic effect	Variance explained %	Peak LOD score
Plant height	5.93	11.7	1.97
Days to heading	-3.17	6.9	2.83
Days to maturity	-1.49	1.5	0.39
Panicle length	0.72	3.9	1.60
Panicles per plant	0.16	0.7	0.29
Spikelets per panicle	15.03	12.3	3.53
Spikelets setting density	0.55	9.7	2.60
Grains per panicle	12.01	14.3	4.45
Percent seed set	2.32	2.6	0.98
1000-grain weight	-1.91	15.3	6.00
Spikelets per plant	136.69	11.4	3.60
Grains per plant	113.29	15.1	5.50
Grain yield	0.26	3.8	1.37



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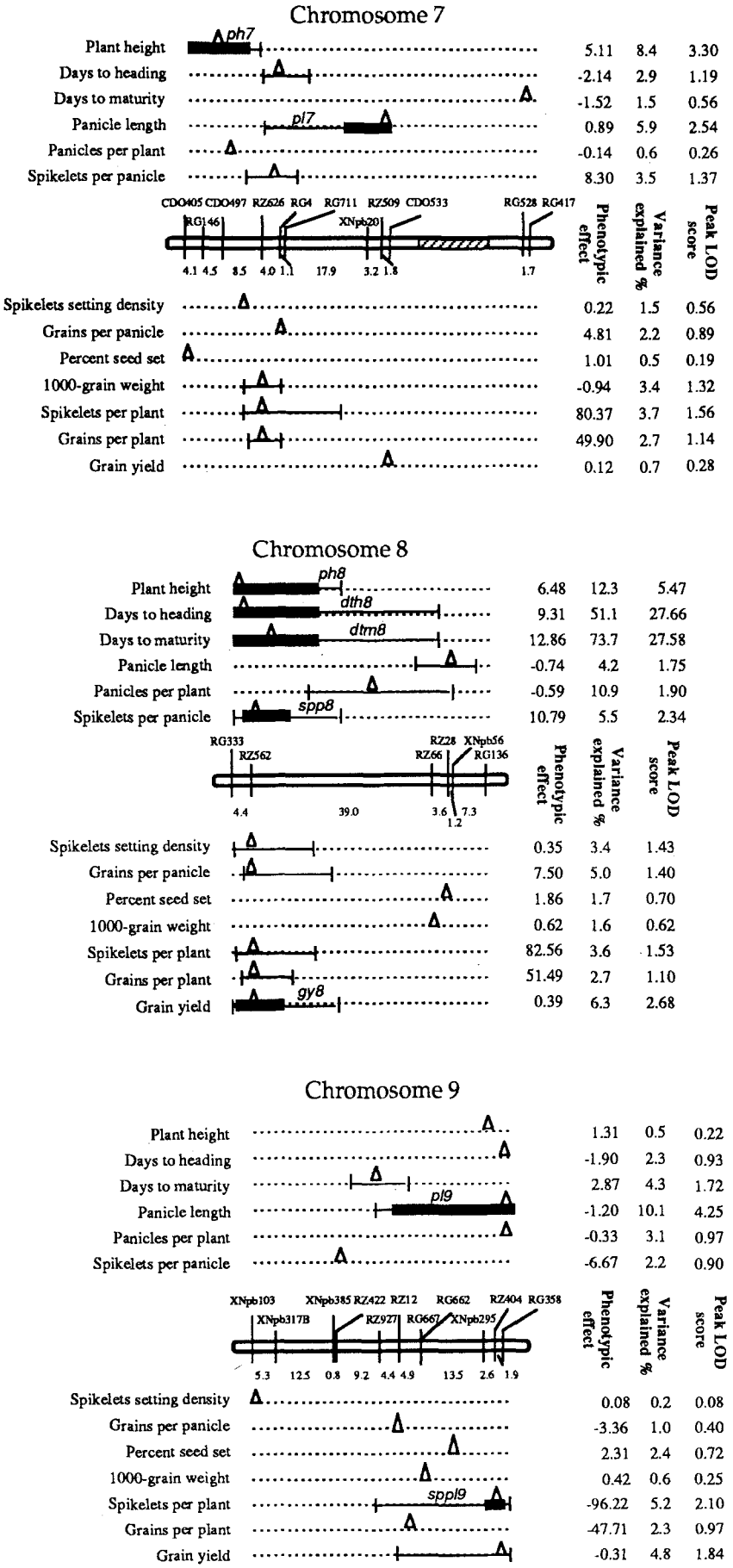

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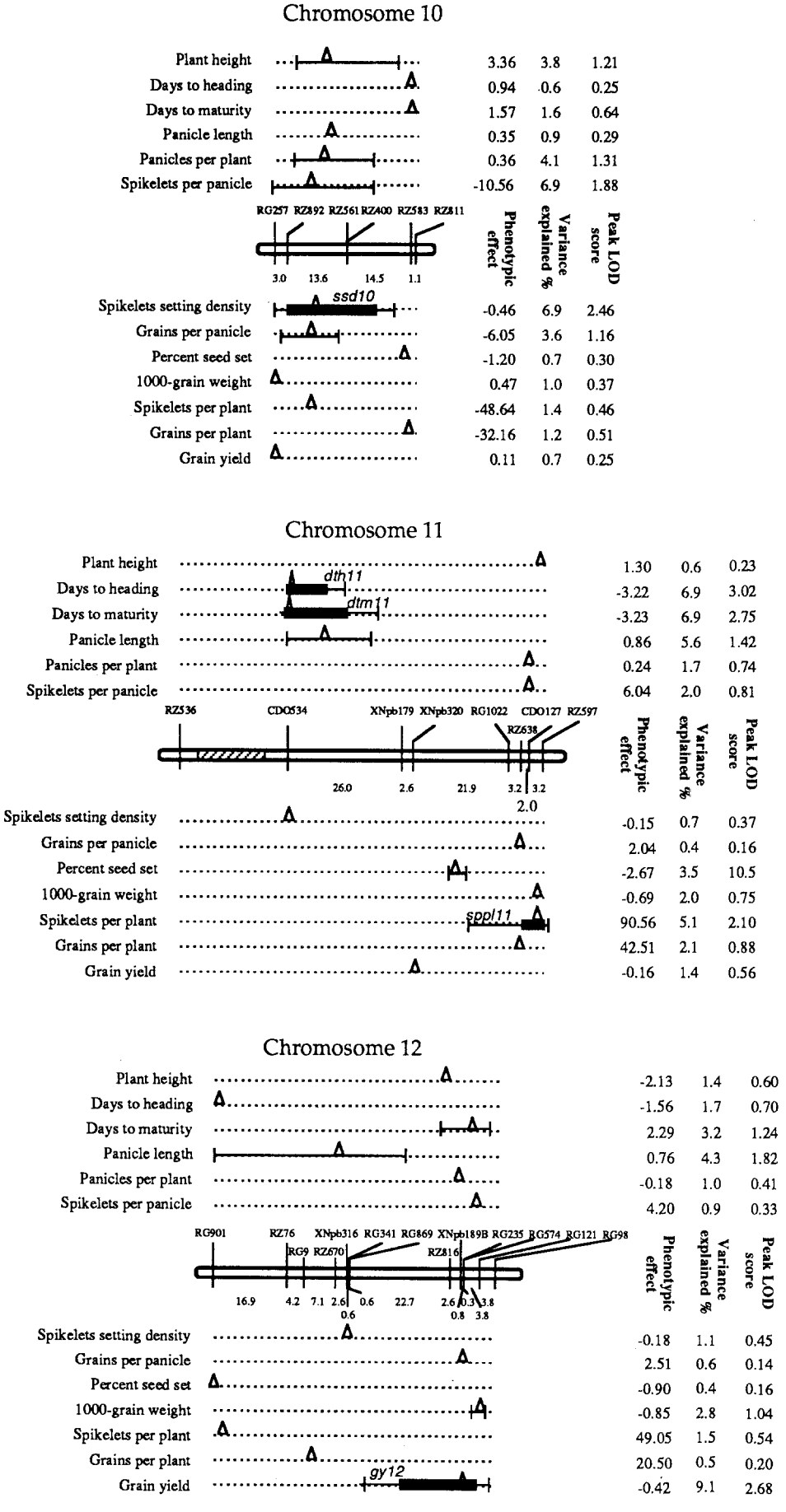

Fig. 4 (Continued)

phenotypic variance explained, when all the QTLs for a single trait were fitted simultaneously, varied from 7.4% for panicles per plant up to 74.2% for days to maturity.

Only two significant QTLs were detected for grain yield. They cumulatively explained 13.6% of the total phenotypic variation. Due to the mapping population size, only the QTLs having large phenotypic effects were detected. Most QTLs affecting grain yield probably had smaller effects which were undetected at the threshold set for QTL declaration.

Although 1000-grain weight and grains per plant are the two components of grain yield, there were no similarities in the locations of the significant QTLs for 1000-grain weight and grain yield, or for grains per plant and grain yield. This could be due to the counterplay between the increase in one of the two components and decrease in another by QTL(s) in the same location. Three significant QTLs were found for each of the two yield components, 1000-grain weight and grains per plant. *J* alleles at *gppl3* and *gppl4* (chromosomes 3 and 4) increased grains per plant. However, *J* alleles at *gw3* and *gw4* (at the same positions on the chromosomes) decreased 1000-grain weight. *J* alleles at *gw5* (chromosome 5) increased 1000-grain weight, but *J* alleles at *gppl5* mapping to the same location decreased grains per plant. Grains per plant and 1000-grain weight were highly correlated ($r = -0.70$), consistent with the QTL mapping results. This could be accounted for by pleiotropic effects of single QTLs rather than linked QTLs as discussed in later section.

QTLs mapping to known major genes

Coincidental map locations of QTLs and major genes affecting the same trait have been reported in several instances in maize (Beavis et al. 1991; Edwards et al. 1992; Veldboom et al. 1994). These observations have been interpreted to support the hypothesis that QTLs are allelic with major genes affecting the same trait (Robertson 1985). Such associations were not found in the current study. None of the QTLs for plant height map to the location of the semi-dwarf gene *sd-1*, a major gene controlling plant height which is widely used by rice breeders for the development of high-yielding va-rieties (Cho et al. 1994), or to another dwarfing gene locus. Also, there is no correspondence between QTLs for days to heading and major photoperiod or photosensitive genes that have been previously placed on rice genetic maps (Causse et al. 1994). The QTL (*dth8*) on chromosome 8, which explains more than 50% of the total phenotypic variance, may represent a major gene which was not previously identified or characterized. Likewise, none of the QTLs for the other traits examined were associated with previously reported major genes exerting qualitative effects on the same traits.

Complementary action of genes from the two parents is the major genetic basis of transgressive segregants

Transgressive segregation is the term used to describe the phenomenon in which individuals in segregating populations out-perform the parents. It has been observed in the progeny of inter- and sub-specific crosses of rice, but the underlying genetic basis of this phenomenon has not been experimentally determined. Transgressive segregants were observed for all traits examined in the current study (Fig. 3). For those traits for which two or more significant QTLs were detected, both parents were found to possess QTL alleles which increased phenotypic values (Fig. 4). The occurrence of such transgression could be directly associated with the inheritance of complementary QTL alleles from the two parents. For example, six significant QTLs were detected for plant height. *I* alleles were associated with an increase in plant height at two of the six QTLs, and *J* alleles at the other four. When graphical genotypes of the transgressive segregants were constructed based on their marker genotypes, transgressive segregants taller than the *J* parent were found to possess "tall" alleles at four or more of the plant-height QTLs. In contrast, transgressive segregants shorter than the *I* parent possessed "short" alleles at four or more of the six QTLs.

Correlated traits often have QTLs sharing similar genomic locations

As demonstrated by Abler et al. (1991), Paterson et al. (1991) and Veldboom et al. (1994), correlated traits often have QTLs mapping to the same chromosomal locations. The same trend was observed in the current study. For example, days to heading and days to maturity were highly correlated ($r = 0.95$) and had two QTLs with large effects which were found at approximately the same map locations. Moreover, the allelic effects were the same (i.e., decreasing or increasing the phenotypic values) for the QTLs for each trait. Grains per panicle and 1000-grain weight showed a highly negative correlation ($r = -0.61$), and each had three QTLs in similar locations. In this case, the QTLs acting on both traits had opposite effects. QTLs sharing similar locations were observed for the other correlated traits, and the directions of the correlations were consistent with the effects of the QTLs on the traits (Table 1 and Fig. 4).

Trait correlations may result from either pleiotropic effects of single genes or from tight linkage of several genes controlling the traits. Pleiotropy was suggested at several chromosomal regions in the current study. The region near RZ562 on chromosome 8 exerted a large effect on days to heading, days to maturity, spikelets per panicle, and grain yield (Fig. 4). Moreover, *J* alleles in this region conditioned an increase in each of these

traits. Days to heading and days to maturity had very similar QTL likelihood plots which were different from the QTL likelihood plots of plant height, spikelets per panicle, and grain yield (Fig. 4). This suggests that one QTL simultaneously affecting days to heading and days to maturity is closely linked with the QTLs in that region affecting the other three traits. A similar trend for days to heading and days to maturity was observed in the region near CDO534 on chromosome 11 with *I* alleles conditioning increased values for these two traits. In maize, pleiotropic effects of QTLs controlling flowering traits has been suggested (Veldboom et al. 1994).

A highly negative correlation between 1000-grain weight and grains per plant has been noted by rice breeders and geneticists. Such a correlation was also observed in this study. This molecular-marker based QTL analysis sheds light on the genetic basis of this observation. The three QTLs (chromosomes 3, 4, and 5) with significant effects on 1000-grain weight, which together explained 35.2% of the total phenotypic variance, coincided with the three significant QTLs involved in grains per plant, which explained 40.1% of the total phenotypic variance. Moreover, these QTLs had opposite allelic effects on the two traits (Fig. 4), consistent with the direction of the trait correlation (Table 1), It is very unlikely that the three opposing QTLs for these two traits are independent and closely linked in cis. Instead, our results suggest that single QTLs with pleiotropic effects is the more likely explanation of the highly negative correlations between 1000-grain weight and grains per plant. Pleiotropy could also account for the other highly significant correlations among traits directly related to grain yield, such as spikelets per panicle, grains per panicle and spikelets per plant, observed in this study.

Putative orthologous QTLs across rice, maize, oat, and barley

The plant family Gramineae contains more than 10, 000 species, including many of economical importance such as rice, maize, wheat, oats, barley, sorghum and sugar-

Table 2 Putative orthologous QTLs across rice and some other cereal crops

QTL detected in rice				Possible homologous QTL found in other cereal crops			
Chrom.	Marker linked	Trait	Reference	Crop	Chrom.	Trait	Reference
1	RZ776	Plant height	This study	Maize	3	Plant height	Stuber et al. 1992
				Maize	8	Plant height	Beavis et al. 1991, 1994;Koester et al. 1993; Stuber et al. 1992
				Barley	3	Plant height	Hayes et al. 1993; Olufomote 1994
2	RZ599	Plant height	This study	Barley	6	Plant height	Hayes et al. 1993
2	RZ260	Plant height	Li et al. 1995	Maize	5	Plant height	Beavis et al. 1991
				Barley	6	Plant height	Hayes et al. 1993
3	RZ16	Plant height	Xiao et al. 1995	Maize	1	Plant height	Beavis et al. 1991, 1994; Schön et al. 1994; Stuber et al. 1992
				Maize	5	Plant height	Beavis et al. 1994
3	RG348	Plant height	Li et al. 1995	Maize	1	Plant height	Schön et al. 1993
				Maize	9	Plant height	Koester et al. 1993; Stuber et al. 1992
5	RZ70	Plant height	This study	Maize	6	Plant height	Koester et al. 1993; Veldboom et al. 1994
				Maize	8	Plant height	Beavis et al. 1991, 1994; Koester et al. 1993
6	CDO78	Plant height	This study	Maize	9	Plant height	Beavis et al. 1991, 1994; Schön et al. 1994
				Barley	1	Plant height	Hayes et al. 1993; Olufomote 1994
7	CDO405	Plant height	This study	Maize	7	Plant height	Schön et al. 1994; Veldboom et al. 1994
8	RZ562	Plant height	This study	Maize	1	Plant height	Koester et al. 1993
3	CDO1081	Days to heading	This study	Maize	1	Days to tassel	Stuber et al. 1992
				Maize	9	Days to flowering	Koester et al. 1993
				Maize	9	GDD to silk emergence	Veldboom et al. 1994
				Barley	4	Heading date	Hayes et al. 1993
				Hexaploid oat	5	Heading date	Siripoonwiwat 1995
4	RZ602	Days to heading	Xiao et al. 1995	Barley	2	Heading date	Hayes et al. 1993
6	CDO109	Days to heading	Li et al. 1995	Barley	1	Heading date	Hayes et al. 1993
7	CDO533	Days to heading	Xiao et al. 1995	Maize	2	GDD to silk emergence	Veldboom et al. 1994
8	RZ562	Days to heading	This study	Maize	1	Days to flowering	Koester et al. 1993
3	CDO1081	Grain weight	This study	Maize	1	Kernel weight	Doebley et al. 1994
				Maize	9	Kernel weight	Schön et al. 1994
4	CDO539	Grain weight	This study	Maize	2	Kernel weight	Doebley et al. 1994
				Maize	10	Kernel weight	Schön et al. 1994
				Barley	2	Test weight	Olufowote 1994
5	RZ296	Grain weight	This study	Maize	6	Kernel weight	Schön et al. 1994; Veldboom and Lee 1994
7	RZ626	Grain weight	Xiao et al. 1995	Maize	7	Kernel weight	Schön et al. 1994
4	RZ590	Grain yield	This study	Barley	2	Grain yield	Hayes et al. 1993; Olufomote 1994

cane. Despite different chromosome numbers and ploidy levels of these species, homoeologous relationships of rice, maize, wheat, oats and barley chromosomes have been defined by using a common set of cDNA clones (Ahn and Tanksley 1993; Ahn et al. 1993 a; Van Deynze et al. 1995 a, b). Thus, it is possible to compare the locations of QTLs affecting similar traits in different grass species for which QTL mapping has been performed. Some of the QTLs detected in this study, as well as other work with rice, show similarities in the locations of QTLs for the same or corresponding traits in maize, oat and barley, and are presented in Table 2.

Plant height

The QTL near RZ776 on rice chromosome 1 appears to lie in a conserved region on the homoeologous chromosomes of maize, chromosomes 3 and 8, and barley chromosome 3. The QTL near RZ599 on rice chromosome 2 may correspond to a similar QTL on barley chromosome 6. The map position of the QTL near RZ260 on rice chromosome 2 was found to coincide with QTLs on maize chromosome 5 and barley chromosome 6. The QTL near RZ16 on rice chromosome 3 was found to be in a conserved region on maize chromosomes 1 and 5. The QTL near RG348 on rice chromosome 3 may match a similar QTL on maize chromosomes 1 and 9. The QTL near RZ70 on rice chromosome 5 was detected in the conserved regions of maize chromosomes 6 and 8. The QTL near CDO78 on rice chromosome 6 seems to match a similar QTL on the homoeologous chromosomes of maize 9, and barley 1. The QTL near CDO405 on rice chromosome 7 appears to lie in the conserved region of maize chromosome 7. The QTL near RZ562 on rice chromosome 8 corresponds to a similar QTL on maize chromosome 1.

Days to heading

Days to heading in rice corresponds to flowering traits (days to tassel or flowering, growing degree days to anthesis, growing degree days for silk emergence) in maize and to heading date in barley and oat. The QTL near CDO1081 on rice chromosome 3 is coincidental with a similar QTL on maize homoeologous chromosomes 1 and 9, barley 4, and hexaploid oat 5 which is homoeologous to diploid oat chromosome F. The map position of the QTL near RZ602 on rice chromosome 4 matches a similar QTL on barley chromosome 2. The QTL on rice chromosome 6 was in the conserved region on barley chromosome 1. The QTL on rice chromosome 7 coincided with a similar QTL on maize chromosome 2. The QTL near RZ562 on rice chromosome 8 was also detected on the conserved region of maize chromosome 1.

1000-grain weight

This trait in rice corresponds to kernel weight in maize and test weight in barley. The QTL near CDO1081 on rice chromosome 3 was in the conserved regions on homoeologous maize chromosomes 1 and 9. The map location of the QTL near CDO539 on rice chromosome 4 matches a similar QTL on homoeologous chromosomes of maize, 2 and 10, and barley 2. The QTL on rice chromosome 5 is coincidental with a similar QTL on maize chromosome 6. The map position of the QTL on rice chromosome 7 was found to correspond to that of the QTL on maize chromosome 7.

Grain yield

A less significant (LOD = 1.98) QTL (Fig. 4) on rice chromosome 4 matches a similar yield-QTL on barley chromosome 2.

As discussed above and shown in Table 2, four QTLs affecting plant height, one QTL for days to heading, and two QTLs for grain weight detected in rice were found to be duplicated in the maize genome, indicating that the expression of a quantitative trait is also affected by the number of copies of a QTL. This raises the question of how the performance of a quantitative trait is affected by two or more copies of a QTL.

The coincidence of QTL map positions among these distinct species suggests that these loci controlling quantitative variation may trace back to their common ancestor. These loci may also represent key rate-limiting genes in a wide variety of cereal crops.

Evidence for orthologous QTLs across grass species suggests that it should be possible to predict QTLs affecting similar traits of agronomic importance in other cereal crops by comparing map positions of QTLs in one such species with the others. Such evidence also implies that QTL cloning in one species may help in the isolation of similar QTLs from related species. In addition, QTLs isolated from one species might be transferred through genetic transformation to improve other cereal crops of economical importance in the family Gramineae.

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