Dominance Is the Major Genetic Basis of Heterosis in Rice as Revealed by QTL Analysis Using Molecular Markers

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ABSTRACT

A set of 194 F_7 lines derived from a subspecific rice cross showing strong F_1 heterosis was backcrossed to the two parents. The materials (388 BC_1F_7 lines, 194 F_8 lines, two parents, F_1) were phenotyped for 12 quantitative traits. A total of 37 significant QTLs (LOD $\geq$ 2.0) was detected through 141 RFLP markers in the BC_1F_7 populations. Twenty-seven (73%) quantitative trait loci (QTLs) were detected in only one of the BC_1F_7 populations. In 82% of these cases, the heterozygotes were superior to the respective homozygotes. The remaining 10 (27%) QTLs were detected in both BC_1F_7 populations, and the heterozygote had a phenotype falling between those of the two homozygotes and in no instances were the heterozygotes found to be superior to both homozygotes. These results suggest that dominance complementation is the major genetic basis of heterosis in rice. This conclusion was strengthened by the finding that there was no correlation between most traits and overall genome heterozygosity and that there were some recombinant inbred lines in the F_8 population having phenotypic values superior to the F_1 for all of the traits evaluated—a result not expected if overdominance was a major contributor to heterosis. Digenic epistasis was not evident.

HETEROSIS, or hybrid vigor, is a term used to describe the phenomenon in which the performance of an F_1, generated by crossing of two genetically different individuals, is superior to that of the better parent. Heterosis is a widely documented phenomenon in diploid organisms that undergo sexual reproduction. It was first observed in animals more than 1400 years ago (JI 1979) and later in plants from the experiments of hybridists in the 19th century (DARWIN 1876; ALLARD 1960).

The genetic basis of heterosis has been debated for more than 80 years and is still not resolved. Two major hypotheses have been promulgated to explain this phenomenon: the dominance hypothesis and the overdominance hypothesis. The dominance hypothesis, proposed by DAVENPORT in 1908, BRUCE (1910), and KEEBLE and PELLEW (1910), and later elaborated by JONES in 1917, supposes that heterosis is due to canceling of deleterious recessives contributed by one parent, by dominant alleles contributed by the other parent in the heterozygous F_1. The overdominance hypothesis, proposed by SHULL (1908) and EAST (1908), assumes that the heterozygous combination of the alleles at a single locus is superior to either of the homozygous combinations of the alleles at that locus. In the past, geneticists have found it difficult to resolve experimentally the dominance *vs.* overdominance controversy.

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The recent advent of molecular linkage maps has made it possible to detect and individually analyze the loci underlying heterosis. Using molecular markers, STUBER *et al.* (1992) were able to detect quantitative trait loci (QTLs) contributing to hybrid vigor in maize. Their results showed that the heterozygotes of most QTLs detected for grain yield had higher phenotypic values than those of either respective homozygotes, suggesting that overdominance is the principal factor controlling heterosis in this open-pollinated crop species.

Heterosis is the foundation of the great success of hybrid rice in China. From 1976, during which hybrid seeds were first released to rice farmers, to 1991 during which the planted acreage of hybrid rice accounted for 55% of total planted area of paddy rice in China, the cumulative increased grain yield from planting hybrid rice amounted to more than 200 million tons (YUAN 1992). It has been demonstrated empirically that hybrid rice varieties have 15–20% yield advantage over the best conventional inbred varieties using similar cultivation conditions (YUAN 1992). Encouraged by China's success in hybrid rice, the International Rice Research Institute (IRRI) resumed its research on hybrid rice in 1979, and scientists in India, Indonesia, the Philippines, South Korea, Japan, Malaysia, Thailand, USA, Brazil, Mexico and Vietnam have launched hybrid rice breeding programs, and India released its first commercial hybrids last year.

Currently, the highest yielding hybrids in rice involve crosses between the two cultivated subspecies of rice (indica and japonica). The goal of the study reported

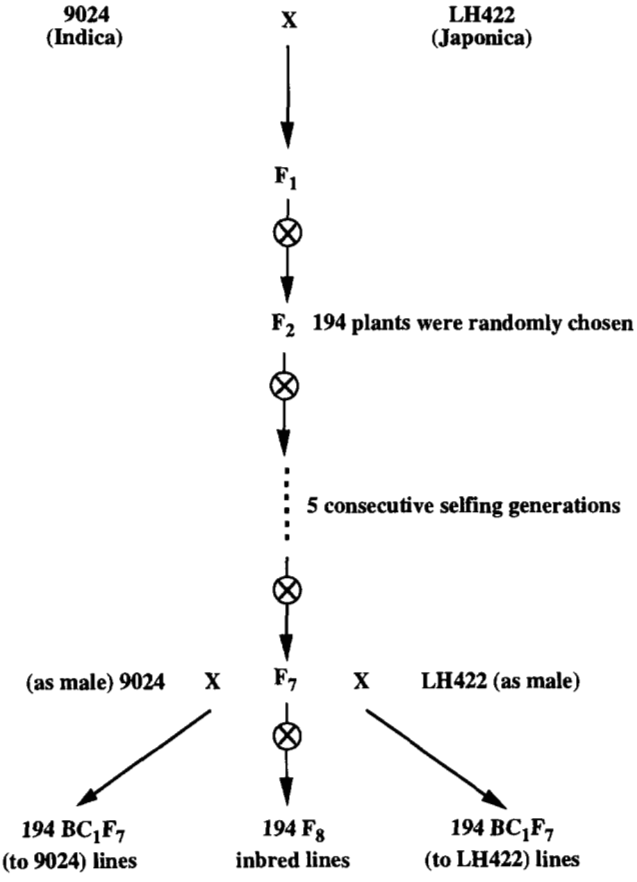

FIGURE 1.—Diagram showing procedures for developing experimental populations.

here was to use the molecular map of rice to determine the genetic basis of heterosis in one of the highest yielding indica × japonica hybrids.

MATERIALS AND METHODS

Development of experimental populations: Two elite homozygous lines, 9024 [*indica* parent (I)] and LH422 [*japonica* parent (J)], were crossed, using I as the female, to produce a F_1 hybrid. This hybrid yields on average 40% more than either parent (LO and YUAN 1987). From this F_1, 194 F_7 lines were developed through six consecutive selfing generations with each F_7 line tracing to a different F_2 plant. No conscious selection was made in any generation. A single plant from each of 194 F_7 lines was randomly chosen and was: backcrossed to each of the two parental lines to generate two BC_1F_7 lines and selfed to produce F_8 progeny. The procedures are outlined in Figure 1.

Phenotypic evaluation: The 388 BC_1F_7 backcross lines, 194 F_8 inbred lines, along with two parental lines and their F_1, were laid out in a field in a randomized complete block design with two replications (plots) for phenotypic evaluation in the summer season of 1992 at the Hunan Hybrid Rice Research Center, China. Twenty-seven plants (three lines × nine plants per line) were planted at a density of 300,000 plants per hectare in each of 1170 plots. The middle five plants in the central line of each plot were used for data collection. The 12 quantitative traits investigated were: plant height (centimeters) and panicle length (centimeters), which represent plant status; days to heading and days to maturity reflecting growth duration; panicles per plant, spikelets per panicle, grains per panicle, 1000-grain weight (g), spikelets per plant, grains per plant, which are directly related to grain yield; percent seed set indicating spikelet fertility; and grain yield (tons/hectare). Means over replications, for each trait, for each of two backcross populations, were used for QTL and other analyses.

RFLP linkage map construction: Seedlings of 30–40 selfed seeds (F_8) from a single plant of each F_7 line were used for bulk DNA extraction. A subset of 141 polymorphic RFLP markers was selected from the rice high-density molecular map (CAUSSE *et al.* 1994) to construct the linkage map of the recombinant inbred (RI) population. Because few heterozygotes (theoretically 1.5625%, in reality 3.23% averaged over 141 markers) for each marker were possible, the genotype heterozygous at a particular marker was treated as missing data. Recombination fractions between pairs of linked markers were calculated using both Map Manager Version 2.5 (MANLY 1993) and Mapmaker (LANDER *et al.* 1987; LINCOLN *et al.* 1992a). Both estimates were in good agreement. The RI-derived RFLP map reported here was constructed using MAPMAKER/EXP. Version 3.0 (LANDER *et al.* 1987; LINCOLN *et al.* 1992a) on a Sun II workstation. All RFLP markers were allocated to linkage groups by pairwise analyses with a threshold of LOD score 4.0. The framework of the map was established by analyses of highly informative and well spaced makers. The order of RFLP markers on each linkage group (frame) had an at least 1000-fold higher likelihood (LOD score 3.0) compared with any alternative order. The remaining markers were assigned to their corresponding linkage groups with a LOD score $\geq$ 2.0. The final order of markers on each linkage group was reconfirmed using "ripple" command with a LOD score $\geq$ 2.0.

QTL analyses: For simplicity and other purposes such as phenotypic comparison between heterozygous and homozygous genotypes, the allele at the *n*th locus from I is designated as *In*, *Jn* for the allele from J. The analyses of QTLs linked to markers for each trait in each of the two BC_1F_7 populations were performed using both single point analysis (TANKSLEY *et al.* 1982) and interval mapping (LANDER and BOTSTEIN 1989). Single point analysis for detecting the association of a marker with a QTL lying at or close to the marker in this study was tested using one-way analysis of variance (ANOVA) from Data Desk 4.0 (Data Description Inc. 1992) with each marker considered as a treatment with two levels and the phenotype of each trait as the dependent variable. This analysis involved comparing, for each trait and each RFLP marker, the phenotypic means of heterozygous and homozygous classes (*InJn vs. InIn* or *JnJn*) of BC_1F_7 lines, for the two BC_1F_7 populations. The difference between the phenotypic means of heterozygous and homozygous marker classes was used as an estimate of the phenotypic effect of different marker genotypes. The proportion of the total phenotypic variation explained by each marker associated with a QTL was calculated as an sR^2 value (sR^2 = ratio of the sum of squares explained by the marker locus to the total sum of squares). Interval mapping developed by LANDER and BOTSTEIN (1989), able to define the most likely position of a QTL and precisely estimate the phenotypic effect of the QTL if it does not lie exactly at the marker locus, was also employed for QTLs analysis for each trait. A LOD score threshold of 2.4 would be needed to test at the $P = 0.001$ level of significance per test, or $P = 0.05$ for the entire rice genome (LANDER and BOTSTEIN 1989). To reduce type II errors, a LOD score of 2.0 was chosen as the threshold for the analysis presented in this paper for declaring a QTL present or not in MAPMAKER/QTL 1.1 program (PATERSON *et al.* 1988; LINCOLN *et al.* 1992b). LOD peaks for each significant QTL were used to position the QTL on the RI/RFLP map. The proportion of the total phenotypic variation

TABLE 1

Means of quantitative traits over replications for F_1 and its parents and F_1 heterosis

Trait	9024 (I)	F_1	LH422 (J)	Heterosis (%) BP	Heterosis (%) MP
Plant height	94.20	114.30	104.00	9.90**	15.34**
Days to heading	83.00	86.00	86.00	0.00	1.78**
Days to maturity	118.00	129.00	125.00	3.20**	6.17**
Panicle length	21.98	25.09	23.88	5.07**	9.42**
Panicles per plant	11.40	10.80	8.60	−5.26	8.00*
Spikelets per panicle	118.07	126.52	151.16	−16.30**	−6.01
Grains per panicle	84.21	93.07	105.88	−12.10*	−2.08
Percent seed set	71.41	73.59	70.03	3.05	4.05
1000-grain weight	24.60	27.09	22.18	10.12**	15.83**
Spikelets per plant	1346.00	1366.42	1299.93	1.52	3.28
Grains per plant	959.99	1005.16	910.57	4.71	7.47*
Grain yield	6.53	7.88	6.02	20.67**	25.58**

BP, better parent; MP, middle parent. Significance for heterosis, $*P \leq 0.05$ and $**P \leq 0.01$.

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). In cases where more than one peak was found on a chromosome for the same trait, multiple-QTL models were employed to determine whether the chromosome possessed single or multiple QTLs.

Epistasis analysis: Two-way interactions were performed between significant markers linked to QTLs and all other marker loci by the PROC GLM in SAS (SAS Institute 1988). For example, p_1, p_2, p_3, and p_4 were designated for the phenotypic effects of BC_1F_7 lines with genotypes *II;II, II;IJ, IJ;II* and *IJ;IJ,* respectively, in the population backcrossed to I (hereafter referred as to BC/I), and *JJ;JJ, JJ;IJ, IJ;JJ* and *IJ;IJ,* respectively, in the population backcrossed to J (hereafter referred as to BC/J). The null hypothesis (no epistasis) for this test is: $(p_1 + p_4) - (p_2 + p_3) = 0$, with a degree of freedom of 1 [$(2 - 1) \times (2 - 1) = 1$].

Relationship between genome heterozygosity and expression of traits: Hypergene (YOUNG and TANKSLEY 1989) was used for calculating genome ratios (percentage of total genome originated from one parental genome) for each line in the RI population. The rules for genome calculation are as followings: if two consecutive markers delimiting the chromosome region in the line were from the same parent, the interval between them was considered to have the parental genome. If an interval was bounded by consecutive markers with alleles from the two parents respectively, then one half of the interval was considered to be from one parent one half from the other parent. The genome heterozygosity of a BC_1F_7 line in the BC/I is equal to the percentage of J genome in the F_7 line which was used to be backcrossed to generate the BC_1F_7 line. The genome heterozygosity of a BC_1F_7 line in the BC/J is equal to the percentage of I genome in the F_7 line that was backcrossed to generate the BC_1F_7 line. The relationships between genome heterozygosity and expression of traits were tested by regressing trait values on the genome heterozygosity in the 194 BC_1F_7 lines for each of the two BC_1F_7 populations.

RESULTS

F_1 heterosis: The F_1 and parental means for each trait as well as the percent heterosis are given in Table 1. For heterobeltiosis (heterosis over the better parent), grain yield showed the strongest significant heterosis (20.6%), followed by 1000-grain weight (10.1%), plant height (9.9%), panicle length (5.0%), days to maturity (3.2%); number of grains per plant, percent seed set, and spikelets per plant also exhibited positive heterosis, but not significant; no heterosis was observed for days to heading; number of spikelets per panicle (−16.3%) and number of grains per panicle (−12.1%) showed significant negative heterosis. Panicles per plant, days to heading, and grains per plant, for which no heterobeltiosis was observed, showed significantly positive heterosis over the midparent. The grain yield is the function of three yield components: number of plants per unit area, number of grains per plant and grain weight. In the field trial of this study, the number of plants per unit area was held constant, *i.e.,* 300,000 plants per hectare. For the other two components, the increased grain weight, measured in 1000-grain weight, accounted for 73% of the increased grain yield in this heterotic F_1 hybrid (1.61 tons/Ha over the midparent or 1.35 tons/Ha over better parent), which benefited from heterosis, the other 27% was due to the increased number of grains per plant.

Genetic map: The genetic map shown in Figure 2 was based on 141 RFLP markers segregating in the RI population with 194 lines that served as the base population for generating the two backcross populations employed in this study. Those 141 markers are estimated to cover ~95% of the rice genome in comparison with the high density molecular map of rice (CAUSSE *et al.* 1994). All RFLP markers had been previously placed on either the SL map based on a interspecific BC population generated at Cornell University (CAUSSE *et al.* 1994) or the map derived from a inter subspecific (*indica/japonica*) F_2 population developed at National Institute of Agrobiological Resources, Japan (SAITO *et al.* 1991). The marker order on this RI map was in good agreement with that of the two maps mentioned above. A few exceptions were observed on this RI map as fol-

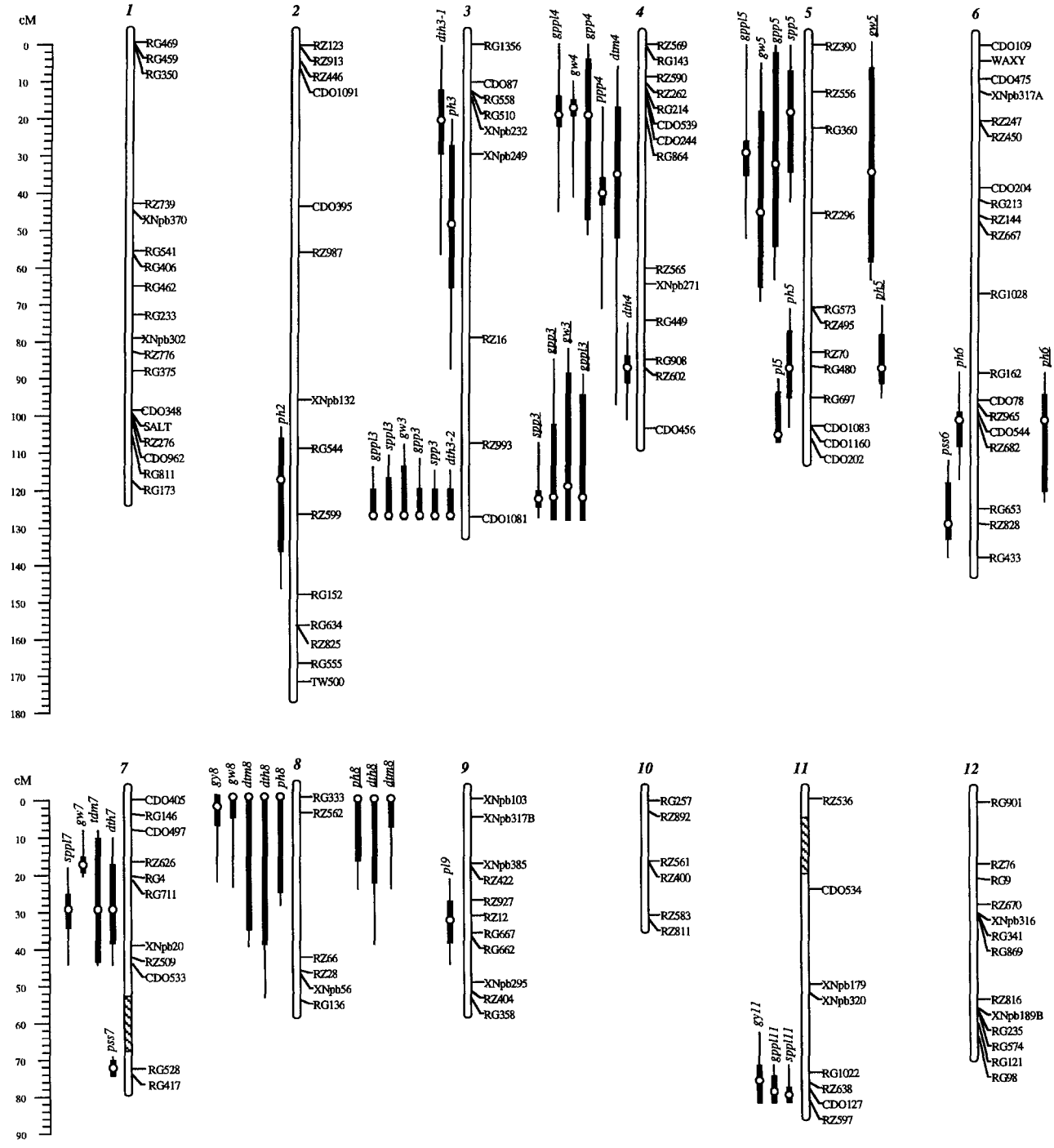

FIGURE 2.—Genetic linkage map of rice based on 194 recombinant inbred lines derived from an Indica (9024) × Japonica (LH422) cross. Distances were given in Kosambi centiMorgans. RG, rice (IR36) genomic; RZ, rice cDNA; CDO, oat cDNA; XNpb, rice (Nipponbare) genomic; TW500, rice cDNA; WAXY and SALT are known genes. Stripped bars on chromosomes *7* and *11* indicated markers are linked with LOD scores <2.0. Darkened bars show LOD scores ≥ 2.0 with the extensions representing LOD scores >1.0 and <2.0. 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, bordered by RG544 and RZ599 on chromosome *2*, is named *ph2*. Map positions (○) are shown of the peak LOD scores that are the most likely positions for the putative QTLs. QTLs underlined were detected in both populations.

lows: (1) the single copy clone, RZ262, which was mapped to a position close to the lower end of chromosome *4* on the SL map was assigned to the upper end of chromosome *4* on the RI map, (2) linked markers RG213 and RZ667 on chromosome *6* and RZ562 and RG333 on chromosome *8* on SL map were reversed in order based on our mapping analysis, and (3) RZ825, CDO204, CDO109, RG634, and RG98, which were assigned on chromosomes *1, 2, 3, 7* and *11*, respectively, on SL map, were placed on chromosomes *2, 6, 6, 2* and

12 respectively on the RI map. We checked the mapping films and found those five clones had two or more copies on the both mapping populations. Presumably, different copies of the same clone were mapped on the two mapping populations.

Mapping QTLs underlying traits: Each trait was subjected to QTL detection based on single point analysis (one-way ANOVA) and interval mapping for each of the two BC_1F_7 populations. The results for each trait, for each of the two BC_1F_7 populations are presented in Table 2. Single point analysis and interval analysis gave basically the same result in detecting QTLs for each trait, but single point analysis usually underestimated the phenotypic effect of a QTL that did not lie exactly at the marker locus. This can be seen for the QTL for plant height on chromosome *3* and the QTLs for days to maturity and panicles per plant on chromosome *4* in the BC/I. Because the interval mapping more precisely estimates the phenotypic effects of the QTLs, all further analyses were based on the results output from the interval mapping.

Plant height: Five QTLs were detected in the BC/I. For three of these QTLs (chromosomes *2, 3,* and *8*), the heterozygotes increased plant height compared with the respective homozygotes. The QTL bordered by markers XNpb249 and RZ16 on chromosome *3* accounted for 26% of the total phenotypic variation. For the other two QTLs (chromosomes *5* and *6*), the heterozygotes caused a decrease in plant height.

Three QTLs in approximately the same map position (chromosomes *5, 6* and *8*) were also detected in the BC/J. For the QTLs on chromosomes *5* and *6,* the heterozygotes were superior to the homozygotes—a result compatible with additive gene action. The remaining QTL on chromosome *8* resulted in the heterozygote with reduced height, also suggesting additive gene action.

Days to heading: Three QTLs were revealed in the BC/I. For the two QTLs on chromosomes *3* and *4,* the heterozygotes reduced days to heading. The heterozygote for the QTL on chromosome *8,* which contributed to 36.6% of the total phenotypic variation, increased days to heading. In the BC/J, three QTLs were detected on chromosomes *3, 7* and *8.* The two QTLs on chromosomes *3* and *7* increased days to heading in the heterozygotes; the QTL on chromosome *8* was found at the same map position as the QTL in the BC/I. However, in this case, the heterozygote decreased days to heading suggestive of additive gene action.

Days to maturity: Two QTLs were found in the BC/I. The heterozygote for the QTL on chromosome *4* shortened growth duration; while for the QTL on chromosome *8,* to which 41.6% of the total phenotypic variation was attributable, the heterozygote lengthened growth duration. In the BC/J, two QTLs were identified. The heterozygote of the QTL on chromosome *7* increased growth duration; but the QTL, which was detected and mapped to the same chromosomal location on chromosome *8* in the BC/I, shortened growth duration.

Panicle length: Only two QTLs, on chromosomes *4* and *8,* were detected in the BC/I and the BC/J, respectively; and for both QTLs the heterozygotes demonstrated increased panicle length compared with homozygotes.

Panicles per plant: Only one QTL (chromosome *4*) was detected in the BC/I, and heterozygote had fewer panicles per plant. No QTL was found over the threshold set for declaration in the BC/J.

Spikelets per panicle: One QTL was found on chromosome *3* in the BC/I for which the heterozygote had increased spikelets per panicle. Two QTL were revealed in the BC/J. For the QTL on chromosome *3,* which was found in the same map position as in BC/I, the heterozygote decreased spikelets per panicle. For the QTL on chromosome *5,* the heterozygote had increased spikelets per panicle.

Grains per panicle: Two QTLs were detected on chromosomes *3* and *4* in the BC/I, and their heterozygotes had increased grains per panicle. Two QTLs were found in the BC/J. For the QTL at the same map position as in BC/I on chromosome *3,* the heterozygote of the QTLs decreased grains per panicle in comparison with the homozygote. The heterozygote of the QTL on chromosome *5* enhanced grains per panicle.

Percent seed set: Two QTLs were mapped to chromosome *6* in the BC/I and to chromosome *7* in the BC/J, respectively. For the QTLs, the heterozygote of the QTL raised seed set rate compared with homozygotes.

1000-grain weight: Three QTLs were detected in the BC/I. For the QTL on chromosome *3,* the heterozygote lowered grain weight, while the heterozygotes of QTLs on chromosomes *5* and *8* increased grain weight. Four QTLs were identified in the BC/J. For the QTLs on chromosomes *3, 4* and *7,* the heterozygotes enhanced grain weight compared with their corresponding homozygotes. The heterozygote of the QTL on chromosome *5* decreased grain weight. The QTLs on chromosomes *3* and *5* were detected in the two backcross populations and had the same map positions.

Spikelets per plant: Three QTLs were identified in the BC/I on chromosomes *3, 5* and *11.* In all of cases, the heterozygotes of the QTLs had an increased spikelets per plant. No significant QTL for this trait were found in the BC/J.

Grains per plant: Three QTLs on chromosomes *3, 4* and *11* were detected in the BC/I, and all the heterozygotes increased grains per plant. Two QTLs were found for the BC/J. For the QTL on chromosome *3* (found at the same map position as in the BC/I), the heterozygote reduced grains per plant, when compared to the homozygote. The heterozygote of the QTL on chromosome *5* enhanced grains per plant.

Grain yield: Two significant QTLs were found—one

TABLE 2

Characteristics of QTLs detected affecting traits in populations backcrossed to 9024 and LH422

Trait	Population	QTL	Markers bordering the QTL	*P* value	Peak LOD	iR^2 (%)	Phenotypic effect	Phenotype comparison of different genotypes
Plant height	BC/I	*ph2*	RG544-RZ599	0.0001	3.69	11.40	4.35	*IJ* > *II*
		ph3	XNpb249-RZ16	0.0005	3.69	26.00	6.63	*IJ* > *II*
		ph5	RG480-RG697	<0.0001	5.20	12.20	−4.53	*IJ* < *II*
		ph6	RZ682-RG653	0.0011	2.28	5.30	−3.12	*IJ* < *II*
		ph8	RG333-RZ562	<0.0001	6.73	15.10	5.37	*IJ* > *II*
	BC/J	*ph5*	RG480-RZ70	0.0001	3.36	8.20	3.80	*IJ* > *JJ*
		ph6	RZ682-RG653	<0.0001	4.92	11.10	4.61	*IJ* > *JJ*
		ph8	RG333-RZ562	<0.0001	3.94	9.00	−4.26	*IJ* < *JJ*
Days to heading	BC/I	*dth3-1*	CDO1081-RZ993	0.0006	2.47	6.10	−1.45	*IJ* < *II*
		dth4	RZ602-CDO456	0.0011	2.32	5.40	−1.38	*IJ* < *II*
		dth8	RG333-RZ562	<0.0001	18.29	36.60	3.85	*IJ* > *II*
	BC/J	*dth3-2*	XNph232-XNpb249	0.0011	2.68	8.10	1.13	*IJ* > *JJ*
		dth7	RG711-XNpb20	0.0008	3.16	10.20	1.24	*IJ* > *JJ*
		dth8	RG33-RZ562	<0.0001	5.69	12.70	−1.47	*IJ* < *JJ*
Days to maturity	BC/I	*dtm4*	RG864-RZ565	0.0007	2.99	16.80	−3.11	*IJ* < *II*
		dtm8	RG333-RZ562	<0.0001	20.50	41.60	5.30	*IJ* > *II*
	BC/J	*dtm7*	CDO533-RZ509	0.0001	3.43	10.00	1.91	*IJ* > *JJ*
		dtm8	RG333-RZ562	0.0012	2.62	6.10	−1.58	*IJ* < *JJ*
Panicle length	BC/I	*pl5*	CDO1160-CDO202	0.0004	3.20	7.80	0.75	*IJ* > *II*
	BC/J	*pl9*	RZ12-RG667	0.0004	2.74	6.60	0.78	*IJ* > *JJ*
Panicles per plant	BC/I	*ppp4*	RG864-RZ565	0.0181	2.14	18.30	−0.64	*IJ* < *II*
Spikelets per panicle	BC/I	*spp3*	CDO1081-RZ993	0.0006	2.65	6.50	9.62	*IJ* > *II*
	BC/J	*spp3*	CDO1081-RZ993	0.0044	2.02	7.00	−10.84	*IJ* < *JJ*
		spp5	RG360-RZ556	0.0019	2.55	7.30	11.08	*IJ* > *JJ*
Grains per panicle	BC/I	*gpp3*	CDO1081-RZ993	0.0009	2.50	6.20	5.73	*IJ* > *II*
		gpp4	CDO244-RG864	0.0001	3.18	8.50	6.68	*IJ* > *II*
	BC/J	*gpp3*	CDO1081-RZ993	<0.0001	5.35	16.70	−10.26	*IJ* < *JJ*
		gpp5	RG360-RZ296	<0.0001	4.49	17.30	10.44	*IJ* > *JJ*
Percent seed set	BC/I	*pssr6*	RZ828-RG653	0.0011	2.52	6.20	2.44	*IJ* > *II*
	BC/J	*pssr7*	RG528-RG417	0.0007	2.22	5.30	2.98	*IJ* > *JJ*
1000-grain weight	BC/I	*gw3*	CDO1081-RZ993	<0.0001	3.87	9.60	−1.00	*IJ* < *II*
		gw5	RZ296-RG360	<0.0001	4.36	11.60	1.11	*IJ* > *II*
		gw8	RG333-RZ562	0.0004	2.55	6.10	0.86	*IJ* > *II*
	BC/J	*gw3*	CDO1081-RZ993	<0.0001	7.84	25.00	1.74	*IJ* > *JJ*
		gw4	RG864-CDO244	0.0043	2.00	5.20	0.80	*IJ* > *JJ*
		gw5	RZ296-RG360	0.0009	3.21	12.40	−1.22	*IJ* < *JJ*
		gw7	RZ626-RG4	0.0038	2.00	4.70	0.78	*IJ* > *JJ*
Spikelets per plant	BC/I	*sppl3*	CDO1081-RZ993	0.0006	2.60	6.50	86.96	*IJ* > *II*
		sppl5	RG711-XNpb20	0.0078	2.15	7.30	93.69	*IJ* > *II*
		sppl11	RZ597-CDO127	0.0016	2.18	5.10	76.94	*IJ* > *II*
Grains per plant	BC/I	*gppl3*	CDO1081-RZ993	0.0012	2.37	6.00	49.77	*IJ* > *II*
		gppl4	CDO244-RG864	0.0011	2.16	5.70	48.50	*IJ* > *II*
		gppl11	RZ597-CDO127	0.0006	2.55	6.00	49.72	*IJ* > *II*
	BC/J	*gppl3*	CDO1081-RZ993	<0.0001	5.53	17.60	−93.72	*IJ* < *JJ*
		gppl5	RG360-RZ296	0.0035	2.13	8.10	63.60	*IJ* > *JJ*
Grain yield	BC/I	*gy11*	RZ638-CDO127	0.0011	2.64	6.80	0.32	*IJ* > *II*
	BC/J	*gy8*	RZ562-RG333	0.0008	2.49	6.30	−0.33	*IJ* < *JJ*

P value refers to the probability that the marker listed on the left and having a higher sR^2 does not have effect on the trait. The signs, + (omitted) and − preceding phenotypic effects indicate that the heterozygote had a higher phenotypic effect than the respective homozygote, and the heterozygote had a higher phenotypic effect than the respective homozygote, respectively. *I* and *J* in the genotypes represent the alleles of the locus originating from 9024 and LH422, respectively.

TABLE 3

Correlation coefficients (r) between the genome heterozygosity and traits in the 194 BC_1F_7 families for the two BC_1F_7 populations

Trait	BC/I	BC/J
Plant Height	0.204**	0.081
Days to heading	−0.004	0.021
Days to maturity	−0.027	0.026
Panicle length	0.143*	−0.021
Panicles per plant	−0.082	−0.048
Spikelets per panicle	0.062	−0.013
Grains per panicle	0.069	−0.026
Percent seed set	0.028	−0.016
1000-grain weight	0.068	0.099
Spikelets per plant	0.026	−0.041
Grains per plant	0.037	−0.057
Grain yield	0.091	0.017

* $P \leq 0.05$ and ** $P \leq 0.01$.

on chromosome *11* in the BC/I and one on chromosome *8* in the BC/J, respectively. For the QTL on chromosome *8*, the heterozygote decreased grain yield with comparison with the homozygote. The heterozygote of the QTL on chromosome *11* increased grain yield.

The fact that only two significant QTLs were detected for grain yield may be because of severe spikelet infertility (as measured in percent seed set), which was observed in both backcross populations. Percent seed set ranged from 47 to 77% in BC/I and from 42 to 83% in BC/J. Greater than 95% of both BC/I and BC/J lines showed lower percent seed set than the F_1. There was no correlation ($r = 0.062$ in the BC/I and 0.090 in the BC/J) between grain yield and 1000-grain weight, which made >73% contribution to the F_1 heterosis of grain yield.

Epistasis: Markers associated with each of the 47 significant QTLs were tested for possible two-way interactions with all other markers in the genome. A total of 6580 two-way tests was performed. Only 5.40, 1.18 and 0.17% of pairwise tests were significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively, which were close to the frequencies that would be expected by chance. The markers with significant interactions were rarely found coinciding with other markers associated with other QTLs for the same trait.

Relationship between traits and genome heterozygosity: The effect of genome heterozygosity on the performance of each trait for the two BC populations was evaluated by regressing the trait value of each BC_1F_7 line on its percentage of genome heterozygosity. The r value (correlation coefficient) obtained from such regression analysis should reflect the importance of heterozygosity *per se* to the expression of a particular trait. Only for plant height and panicle length, were there significant positive relationships with genome heterozygosity in the BC/I (see Table 3). All other traits for the both populations and plant height and panicle length for the BC/J showed no relationship between the genome heterozygosity and trait performance, indicating that the overall genome heterozygosity alone had little effect on trait expression in both BC_1F_7 populations.

Trait expression in the recombinant inbred lines: Figure 3 shows the distribution of phenotypic means over replications for each trait in the recombinant inbred (F_8) population. For each of all traits evaluated, there were some recombinant lines having a higher phenotypic value than the F_1 hybrid (see Figure 3). For grain yield, which showed the strongest significant heterosis in the F_1, there were two RI lines having grain yields (8.17 tons/Ha and 8.20 tons/Ha), which are significantly ($P \leq 0.05$) higher than the F_1's (7.88 tons/Ha). This is despite the fact that 91.10% of the recombinant lines experienced spikelet fertility problems (which is a common phenomenon observed in progeny of *indica/japonica* cross, and caused by incompatibility between *indica* and *japonica*), *i.e.*, seed set rates were lower than the F_1's. The occurrence of some recombinant lines having a higher phenotypic value than the F_1 hybrid was because of genetic reasons rather than environmental noise, as our experiment was designed to control environmental noise and experimental errors, and data used for the analyses were means obtained from a replicated trial rather than from single plants in the BC_1 or F_2 populations. This was clearly demonstrated by the fact that the proportions of phenotypic variance explained by genetic to the total phenotypic variance ranged from 81.13% for panicles per plant to 99.88% for days to heading estimated by two-way ANOVA analyses from the PROC GLM in SAS (SAS institute 1988) and that there was no significant difference ($P \leq 0.05$) between two replications for all traits studied.

DISCUSSION

Genetic basis of heterosis: An RI population, which allowed more recombinational segregation of linked QTLs than F_2 and backcross populations, was employed to serve as the base population for producing the two backcross populations used in this study. Phenotypes were investigated in the backcross populations in a replicated yield trial in Hunan, China where hybrid rice was originally developed. Using QTL data from these combined populations, it was possible to estimate the difference in the phenotypic means of heterozygotes (*IJ*) and homozygotes (*II* or *JJ*) over all portions of the genome. The QTL mapping results revealed the following: (1) most of significant QTLs (27/37 = 73%) were detected in only one of the two backcross populations. In 82% of these cases the heterozygotes had higher phenotypic values than their respective homozygotes. (2) 10 QTLs were detected with significance in both backcross populations and each pair was mapped to the same chromosomal location, and in each of all

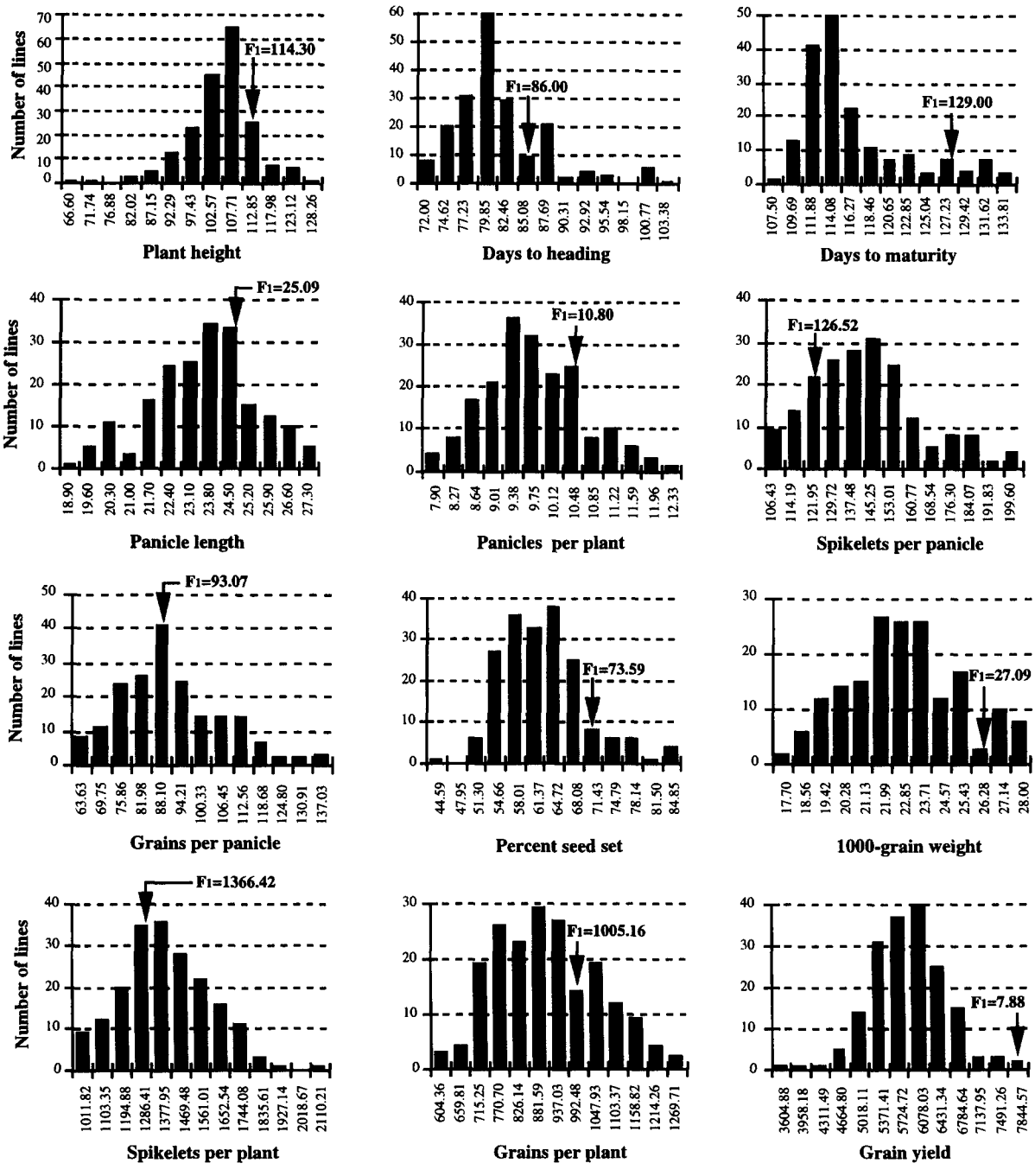

FIGURE 3.—Frequency distribution of phenotypes for each trait for the 194 recombinant lines (F_8) derived from the 9024 and LH422. Phenotypes for F_1 are shown by arrows. The values indicated in the *x*-axis are the lower limit of each group.

cases the phenotype of the heterozygote fell between those of the two homozygotes. This result suggests that the complementation of dominant (including partial dominant) alleles at different loci in the F_1 hybrid is the major contributor to F_1 heterosis.

This conclusion is supported by the general lack of significant correlations between genome heterozygosity and the phenotypic traits. Finally, one of the predictions of the dominance hypothesis is that true inbreeding individuals, like F_1 in vigor, can be obtained from its segregating populations. The prediction was met in this experiment for each of all traits including grain yield, because at least two recombinant inbred lines were observed whose phenotypes exceeded that of the F_1. This result may be attributed to the segregation and recombination of genes at different loci in the seven meiosis experienced during the population development in this study. These lines of evidence reinforce the conclusion that the genetic basis of heterosis in the F_1 hybrid examined is largely due to dominance.

The degree of the correlation between the genome heterozygosity and a phenotypic traits reflects the im-

portance of overall genome heterozygosity to the trait expression. For most of traits, such correlation is very low and not significant (see Table 3). This is consistent with QTLs mapping results. As shown in Table 2, for most of traits, not all QTLs detected for the trait had higher phenotypes in heterozygotes than in respective homozygotes, *i.e.,* for some of the QTLs, heterozygotes showed higher phenotypes than the respective homozygotes; for the other of the QTLs, the heterozygotes exhibited lower phenotypes than the respective homozygotes. Therefore, the overall genome heterozygosity would show no correlation with the trait.

Digenic interactions between markers associated with significant QTLs and all other markers were not found significant in this study. This suggests that strong epistasis is not likely to be involved in this study. However, as discussed by TANKSLEY (1993), marker-based QTL studies are inherently inefficient at detecting epistasis and one cannot exclude the possibility that some level of epistasis is occurring.

Genotype by environment interaction is interesting to geneticists and breeders. Since molecular markers were introduced in quantitative genetics, a number of QTL studies have been carried out to detect possible QTL by environment interaction. While QTL by environment interaction has been detected in some instances, it is usually of the type where QTLs found in one of environments, differ in the magnitude of their effects in different environments. To our knowledge, there are no instances where the gene action of a QTL has changed, *e.g.,* change from dominance to recessiveness, partial dominance to overdominance, from one environment to another. Although the conclusion that heterosis in rice is largely because of dominance is drawn from QTL study in one environment, we believe that this conclusion is likely to extend to other environments.

Comparison with maize: The conclusion that heterosis in rice is largely due to dominance contrasts with QTL studies in maize which suggest that overdominance is implicated as the prominent factor conditioning heterosis (STUBER *et al.* 1992). One possible explanation for this difference is that maize actually possesses a large number of genes for which alleles interact in a truly overdominant manner whereas rice does not. Rice and maize are both members of the Gramineae, evolved from a common ancestor and share many orthologous genes (AHN and TANKSLEY 1993). For maize alone to harbor alleles that are truly overdominant would be remarkable. An alternative explanation is that maize does not contain a higher frequency of overdominant alleles and that the observed overdominant gene action detected in QTL studies is due to pseudo-overdominance or the occurrence of dominant and recessive alleles in coupling at closely linked loci (CROW 1952).

In this regard, it is important to note that the QTLs discovered from mapping studies are defined with only limited resolution. From a primary mapping study it is normally not possible to localize a QTL to a region <10 cM. This leaves open the possibility that an overdominant QTL may actually be a deleterious recessive allele at one locus in cis with a beneficial dominant allele at a closely linked locus. This would be detected in a mapping study as a QTL with overdominant gene action. This phenomenon was termed pseudo-overdominance and has been acknowledged as a possible explanation for some of the overdominant gene action observed in maize (STUBER *et al.* 1992). One might predict that pseudo-overdominance would be more likely to occur in plants in which deleterious recessive alleles are more abundant. Breeding and genetic studies would suggest that deleterious recessives are more frequent in maize and other out-crossing species than in self-pollinated species like rice (ALLARD 1960).

The difference in the reproductive biology of maize and rice could account for the greater accumulation of deleterious recessives in maize than in rice. In maize and other out-crossing species, recessive alleles are usually masked by their corresponding dominant counterparts. In rice and other self-pollinated species, populations and individuals are more highly inbred, a condition in which deleterious recessive mutations are more likely to be eliminated by natural and artificial selections.

To distinguish definitively overdominance from pseudo-overdominance will require fine mapping of QTLs displaying overdominant gene action. In the case of pseudo-overdominance, it should be possible to break the tight linkage of the dominant and recessive alleles which would result in loss of the observed overdominance behavior. In the case of true single gene overdominance, fine mapping will more precisely define the position of the locus, but the overdominant gene action will persist. In the past, fine mapping of overdominant loci was impractical. Now, with the availability of high-density molecular linkage maps, fine mapping is a feasible proposition and the hypothesis that overdominant QTLs in maize are a result of tight linkage of dominant and recessive alleles can be tested empirically (PATERSON *et al.* 1989; JANSEN and STAM 1994).

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