Quantitative Trait Loci for Antixenosis Resistance to Corn Earworm in Soybean

B. G. Rector,* J. N. All, W. A Parrott, and H. R. Boerma

ABSTRACT

Insect resistance in soybean, *Glycine max* (L.) Merr., is inherited quantitatively and has been difficult to incorporate into high-yielding cultivars. The objectives of this study were to: (i) use restriction fragment length polymorphism (RFLP) markers to identify quantitative trait loci (QTLs) for antixenosis (nonpreference) resistance to corn earworm, *Helicoverpa zea* Boddie, in soybean plant introductions PI171451 and PI227687, (ii) determine the location of the QTLs on the public soybean genetic linkage map, (iii) determine their relative magnitude and gene action, and (iv) compare them to insect resistance QTLs previously detected in PI229358. RFLP maps were constructed in two insect-susceptible × insect resistant soybean populations: Cobb × PI171451 and Cobb × PI227687. Insect resistance was measured either in the field or greenhouse as percentage defoliation by corn earworm on vegetative soybean plants. Marker-QTL associations were detected by interval mapping and confirmed by single-factor analysis of variance. Comparisons were made between QTLs identified in this study and those identified previously using PI229358 as the source of insect-resistant germplasm. Among the three resistant genotypes (PI171451, PI227687, and PI229358) a QTL on linkage group (LG) 'H' was shared among all three genotypes (accounting for an R^2 of 19, 9, and 17%, respectively), and a major QTL on LG 'M' was shared between PI171451 and PI229358 (R^2 = 37% for each). A minor QTL on LG 'C2' was identified which was unique to PI227687 (R^2 = 11%) and a minor QTL on LG 'D1' was identified which was unique to PI229358 (R^2 = 12%). In addition, a QTL was found on LG 'F' in the susceptible genotype, Cobb (R^2 = 20%). This QTL is in a region of the soybean genome which has been previously associated with a cluster of soybean pathogen-resistance loci.

INSECTICIDE APPLICATION is often required in the southeastern USA to reduce damage to soybean plants from defoliating insects (Hanthorne et al., 1982). Defoliating insects cost Georgia soybean growers an average of $6 million per year over a recent 7-yr period (1988-94) (Adams et al., 1989, 1991; Hudson et al., 1992a, b, 1993; McPherson et al., 1994, 1997). Plant resistance to insects (PRI) can be an effective component of an integrated pest management program. Utilization of soybean cultivars with high levels of PRI would reduce the need for chemical insecticide applications resulting in positive environmental and economic benefits.

The main sources of soybean germplasm with resistance to defoliating insects are plant introductions (PIs) with low agronomic value. Insect resistance in these PIs (PI171451, PI227687, and PI229358) was reported in the early 1970s (Van Duyn et al., 1971) and has been demonstrated against a number of defoliating insect species (Luedders and Dickerson, 1977; Lambert and Kilen, 1984a; All et al., 1989). Insect resistance in these PIs is inherited as a quantitative trait (Sisson et al., 1976; Luedders and Dickerson, 1977; Rufener et al., 1989; Kenty et al., 1996) and has been difficult to transfer into productive cultivars by conventional soybean breeding strategies. Only three soybean cultivars have been released with PRI derived from these PIs (Bowers, 1990; Hartwig et al., 1990, 1994). None of these cultivars has achieved popularity among soybean growers because of the difficulty of combining PRI with competitive yields. Screening for PRI in soybean is labor intensive and time consuming. Marker-assisted selection using DNA markers tightly linked to QTLs conditioning PRI would greatly enhance the efficiency of soybean breeders to introgress PRI into elite cultivars by allowing them to reduce the necessary number of insect bioassays and by significantly reducing linkage drag (Young and Tanksley, 1989).

Molecular markers have been used to study the quantitative inheritance of PRI in several crop species including maize (*Zea mays* L.) (Byrne et al., 1997), tomato (*Lycopersicon esculentum* Mill.) (Maliepaard et al., 1995), and potato (*Solanum tuberosum* L.) (Yencho et al., 1996). In addition, molecular markers have been used to mark QTLs for many agronomic traits of soybean, including seed weight (Mian et al., 1996b), seed protein and oil content (Lee et al., 1996c; Brummer et al., 1997), pod dehiscence (Bailey et al., 1997), water-use efficiency and leaf ash content (Mian et al., 1996a), hard seededness (Keim et al., 1990), plant height, lodging, and maturity (Lee et al., 1996a,b), sensitivity to the herbicide Chlorimuron (Mian et al., 1996c), and plant resistance to Phytophthora rot (*Phytophthora sojae* Auf. and Gender.) (Lohnes and Schmitthenner, 1997), sudden death syndrome [*Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burk.) Snyd. and Hans.] (Hnetkovsky et al., 1996), soybean mosaic-virus (Yu et al., 1994), root-knot nematodes [*Meloidogyne incognita* (Kofoid and White) Chitwood, and *M. javanica* (Treub) Chitwood] (Tamulonis et al., 1997a,b), and soybean cyst nematode (*Heterodera glycines* Ichinohe) (Mudge et al., 1997).

Antibiosis, antixenosis, and tolerance are the three principal modes of PRI (Painter, 1951; Kogan and Ortman, 1978). The PRI in each of these three PIs has both antibiotic and antixenotic properties (Lambert and Kilen, 1984a; All et al., 1989). Antibiosis describes resistance in which the insect's normal relationship with a host plant causes physiological or developmental detriment to the insect, whereas antixenosis, or nonpreference, describes resistance in which the insect is either

B.G. Rector, Dep. of Agronomy, Univ. Nebraska, Lincoln, NE 68583-1905; J.N. All, Dept. of Entomology, Univ. Georgia, Athens, GA 30602-2603; and W.A Parrott and H.R. Boerma, Dep. of Crop and Soil Sciences, Univ. Georgia, Athens, GA 30602-7272. Received 16 April 1998. *Corresponding author (brector@unlvm.unl.edu).

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Abbreviations: AFLP, amplified fragment length polymorphism; CEW, corn earworm; cM, centimorgan; LG, linkage group; PI, plant introduction; PRI, plant resistance to insects; QTL, quantitative trait locus; RFLP, restriction fragment length polymorphism; SSR, simple sequence repeat; USDA/ISU, United States Department of Agriculture/Iowa State University.

repelled from or not attracted to its normal host plant. Bioassays can be designed to infer individual modes of PRI. This study was designed to identify QTLs associated with antixenosis PRI in PI171451 and PI227687.

Our specific objectives in this study were to: (i) identify QTLs in soybean associated with antixenosis resistance to defoliating insects in PI171451 and PI227687, (ii) determine the genomic location of these QTLs, (iii) quantify their relative magnitude and gene action, and (iv) compare these QTLs to those previously reported from PI229358 (Rector et al., 1998).

MATERIALS AND METHODS

Two soybean populations, derived from the crosses Cobb × PI171451 (Cross 1) and Cobb × PI227687 (Cross 2), were used to construct genetic linkage maps and to evaluate insect resistance. Cobb is susceptible to defoliation damage by the corn earworm (CEW), *Helicoverpa zea* Boddie, whereas PI171451 and PI227687 resist CEW damage (All et al., 1989). A total of 110 and 95 $F_{2:3}$ lines were developed from Crosses 1 and 2, respectively. Each line originated from a different F_2 plant grown at the University of Georgia Plant Sciences Farm near Athens, GA, in 1993. F_2 plants were derived from selfs of seven and eight F_1 plants from Crosses 1 and 2, respectively.

Leaves were harvested from F_2 plants for DNA isolation. DNA isolation, Southern blotting, and hybridization procedures have been described previously (Lee et al., 1996b). Approximately 400 previously mapped RFLP probes from various sources, including cDNA and/or genomic clones from soybean and other cultivated legumes were used to screen for RFLP between Cobb, PI171451, and PI227687. Five restriction enzymes (*Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, and *Taq*I) were used to identify RFLP. Polymorphic probes were used for genetic mapping. Linkage maps were constructed with marker data by the MAPMAKER/EXP computer program (Lander et al., 1987). For grouping linked markers, thresholds of a minimum LOD score of 3.0 and a maximum distance of 50 cM were used.

The insect bioassay applied to Cross 1 has been previously described (Rector et al., 1998). Briefly, the parents and $F_{2:3}$ lines were grown in hill plots in a randomized complete block design with four replications in 1995 at the University of Georgia Plant Sciences Farm. Each replication contained a hill of six plants from each $F_{2:3}$ line, plus six hills of six plants each from Cobb and PI171451. Hills were planted every 45 cm along rows spaced 76 cm apart. There was a single border row of an insect-susceptible cultivar, DPL726, which surrounded the experiment and was distinguishable by its narrow leaflet phenotype. A plastic, fine-meshed (1.5 mm^2) cage was constructed over the experiment to create conditions for an artificial insect infestation (Rowan et al., 1991). The cage prevented the invasion of predatory and parasitic insects. Corn earworm eggs were infested directly onto the plants at the V_4 stage of development (Fehr and Caviness, 1977). The eggs were applied in a corncob grits medium with a "bazooka" applicator (Wiseman et al., 1980) at a rate of approximately 150 eggs per plant per week for four consecutive weeks. The larvae were free to feed on the plants where they hatched, or to migrate to adjacent plants. Moths developing from these larvae were free to oviposit anywhere in the cage.

The Cross 2 insect bioassay was conducted in the greenhouse because of major field complications: The combination of a persistent fire ant infestation and residual levels of a systemic soil insecticide killed the CEW larvae in the first field planting. A subsequent planting and cage construction was partially destroyed by Hurricane Opal, and the moisture left by Hurricane Opal fostered an epizootic of *Nomurea rileyi* (Farlow) Samson, a fungal insect pathogen, which killed any remaining CEW in the repaired cage. In the greenhouse, an established bioassay (All et al., 1989) was employed. Briefly, nine replications consisting of four plants of each $F_{2:3}$ line and six cups of four plants each of Cobb and PI227687 were grown in 1.0-L polystyrene foam cups. At the V_2 stage of development (Fehr and Caviness, 1977; approximately 14 days after planting) each plant was infested with four neonate CEW larvae.

Visual percent defoliation ratings for both crosses were taken when the most susceptible genotypes had been significantly defoliated. The percent-defoliation scores were estimated by comparing leaf damage to defoliation standards (photographs of leaves with known percent leaf area removed by insect damage). Defoliation percentages for the standards were determined using a computerized leaf-area video digitization system (Hargrove and Crossley, 1988). The defoliation data were subjected to ANOVA (Proc GLM; SAS, 1988) assuming replications and lines as random effects. Variance component heritability estimates (h^2) were calculated for each population based on $F_{2:3}$ family means.

The presence of a QTL near an RFLP marker was tested by two different procedures. QTLs were detected by interval mapping (Lander and Botstein, 1989) with the MAPMAKER/QTL computer program (Lincoln et al., 1992). A LOD score of 2.0 was chosen as the minimum to declare the presence of a QTL in a given genomic region. This indicates that the chance of a QTL being present is 100 times more likely than a QTL not being present. The LOD score peak was used to estimate the most likely QTL position on the RFLP linkage map. The percentage of variance explained by each QTL (R^2) and the additive (a) and dominance (d) effects were estimated at each maximum likelihood QTL peak by the 'TRY' function

Table 1. RFLP markers associated with plant resistance to defoliation by *Helicoverpa zea* in three soybean populations based on MAPMAKER/QTL analysis.

		Cobb × PI171451 (CROSS 1)				Cobb × PI227687 (Cross 2)				Cobb × PI229358 (Rector et al., 1998)			
Map interval	LG	LOD	R^2	a†	d/a	LOD	R^2	a†	d/a	LOD	R^2	a†	d/a
			%				%				%		
A132T..A670T	C2	n.p.‡	–	–	–	2.2§	11	−2.6	−0.76	n.s.	–	–	–
Bng047D..A808V	D1	n.s.‡	–	–	–	n.s.	–	–	–	2.0§	10	−2.4	−0.13
B212V-1..A757V-2	F	4.8§	20	5.0#	−0.04	n.p.	–	–	–	n.p.	–	–	–
A1311..R249T	H	3.7§	19	−5.0	0.27	1.8§	9	−2.4	0.77	4.0§	16	−2.6	0.19
A584V..A226H	M	9.7§	37	−6.9	0.08	n.s.	–	–	–	10.1§	37	−4.1	−0.01

† Average change in percent defoliation for each PI allele.
‡ n.p. = no polymorphism was detected at this interval in this cross; n.s. = interval was polymorphic but not significantly associated with defoliation.
§ Significance confirmed with ANOVA ($P < 0.01$).
# Resistance allele for Cobb.

of the 'MAP' command in MAPMAKER/QTL. The average degree of dominance for each QTL was calculated as the ratio d/a (Table 1) and this value was used to determine the gene action (e.g., additive, dominant, recessive) of a QTL. In the second procedure, marker-QTL relationships were confirmed by a general linear model (PROC GLM, $P < 0.01$; SAS, 1988) in which the genotypic class (AA, Aa, aa) of each RFLP marker was the predictor variable, while the percent defoliation data was the response variable. Markers which did not display normal segregation (1:2:1) were not accepted ($\chi^2 < 0.05$) as significant marker-QTL associations. Alignment of linkage groups in comparative mapping was contingent on linkage groups sharing markers which were mapped by the same restriction enzyme and restriction fragment.

RESULTS AND DISCUSSION

The mean *H. zea* defoliation ratings for Cobb and PI171451 in Cross 1 were 63 and 17%, respectively. The $F_{2:3}$ lines showed a continuous range of defoliation from 16 to 48%. However, there was one line which had a mean defoliation rating of 70% (Fig. 1) but this was not significantly different from the susceptible parent Cobb.

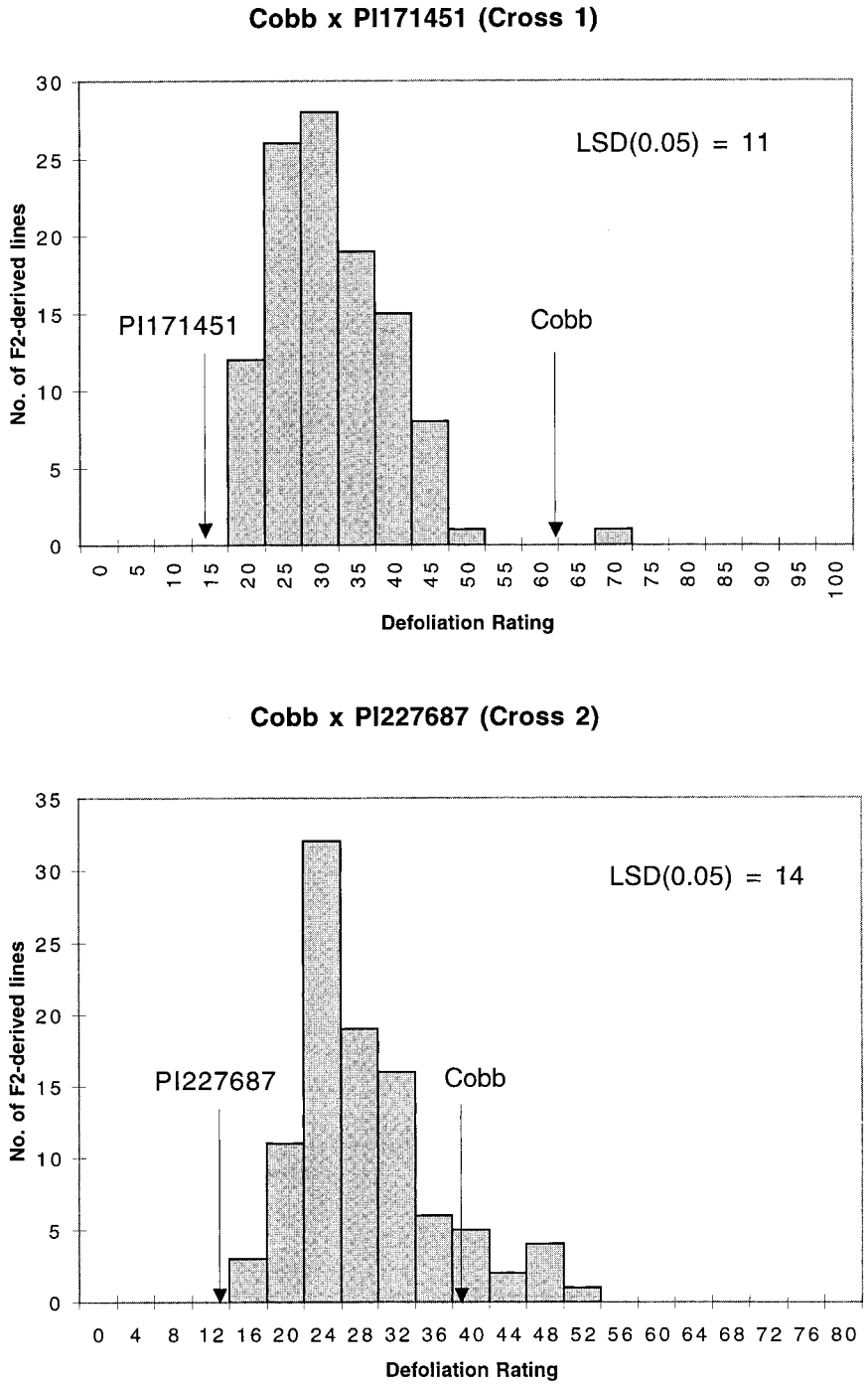

Fig. 1. Distribution of F_2-derived lines of the crosses Cobb × PI171451 and Cobb × PI227687 on the basis of percent defoliation by CEW.

There was no significant transgressive segregation detected for greater defoliation than Cobb or less defoliation than PI171451. In Cross 2, the $F_{2:3}$ lines also showed a continuous range of defoliation by *H. zea*, from 16 to 52% (Fig. 1). The mean defoliation ratings for Cobb and PI227687 were 38 and 14%, respectively. There was no transgressive segregation in this population. The heritability estimates for antixenosis in Cross 1 and Cross 2 were 80% (based on a mean of four replications) and 65% (based on a mean of nine replications), respectively.

Linkage maps were constructed with RFLP markers in Crosses 1 and 2. In Cross 1, the map consisted of 85 markers on 21 linkage groups with 15 unlinked markers. This map covered 1113 cM. In Cross 2, the map consisted of 120 markers on 25 linkage groups with 13 markers unlinked. It covered 1470 cM. In both maps, linkage groups were associated with named linkage groups on the USDA/Iowa State University (USDA/ISU) public soybean genetic linkage map (Shoemaker and Specht, 1995) through comparative mapping.

Three QTLs for antixenosis were detected in Cross 1 (Table 1). They were detected on linkage groups (LGs) 'F', 'H', and 'M' of the USDA/ISU map. These three QTLs were detected in the intervals between markers B212V-1 and A757V-2 on LG 'F', markers A131I and R249T on LG 'H', and markers A584V and A226H on LG 'M', and accounted for 20, 19, and 37% of total phenotypic variation for resistance, respectively. Two QTLs for antixenosis were detected in Cross 2 (Table 1). These were in the intervals between markers A132T-1 and A670T on LG 'C2' and markers A131I and R249T on LG 'H'. These two QTLs accounted for 11 and 9% of total genetic variance for PRI, respectively. The LOD peak for the QTL on LG 'H' in Cross 2 was just below threshold (LOD = 1.8), but this QTL was considered significant because of its significant *F*-test value ($P = 0.01$) and because of the presence of resistance alleles at this locus in both PI171451 and PI229358 (Table 1; Rector et al., 1998).

Lower defoliation ratings were associated with the PI parent allele of all marker-QTL combinations in Crosses 1 and 2 except for the QTL on LG 'F' in Cross 1, which draws PRI from the Cobb allele (Table 1; Fig. 2). Although the small population sizes in this study may bias the estimates of both additive and dominance genetic effects (Beavis, 1998), degree of dominance data at QTL peaks in Cross 1 indicate that the resistant allele at the QTL on LG 'M' acts in an additive manner (d/a ≈ 0) (Table 1; Fig. 3), the resistant allele at the LG

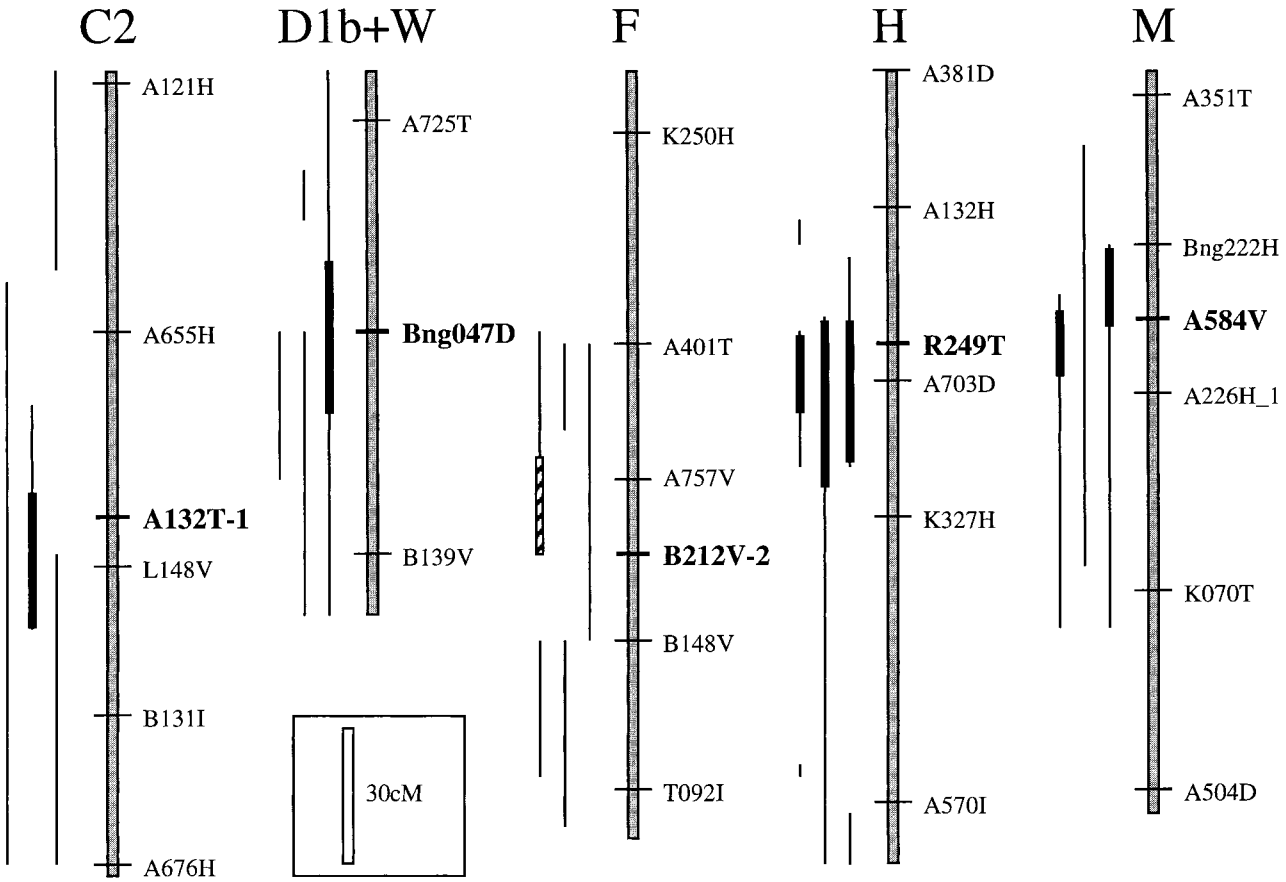

Fig. 2. Confidence intervals for CEW defoliation resistance QTLs in three soybean crosses. Shaded bars represent soybean linkage groups (LGs) with RFLP markers from the USDA/ISU public soybean genetic linkage map (Shoemaker and Specht, 1995) given for reference (plain text). Listed in boldface are the markers which are closest to the QTL LOD peak(s) and are specific to the maps constructed for this study. LG designations are adapted from the public soybean SSR map (P. Cregan, pers. comm.). Vertical lines to the left of each LG approximate the portion(s) of each LG mapped in the soybean crosses Cobb × PI171451, Cobb × PI227687 (this study), and Cobb × PI229358 (Rector et al, 1998) (left to right, respectively). Bars superimposed on these lines represent confidence intervals for CEW defoliation resistance QTLs. A solid bar indicates that the resistance allele is inherited from the PI parent. A hatched bar indicates that the resistance allele is inherited from Cobb. Refer to text for the relative magnitude of the QTLs.

'H' QTL exhibits partial dominance ($1 > d/a > 0$) (Table 1; Fig. 3), and the resistant allele at the QTL on LG 'F' is partially recessive ($-1 < d/a < 0$). In Cross 2, the resistant allele at the QTL on LG 'H' exhibits partial dominance while the QTL on LG 'C2' exhibits partial recessive gene action (Table 1).

The resistance allele from PI227687 at the QTL on LG 'H' has a higher degree of dominance than the homologous alleles from PI171451 and PI229358 (Table 1). A comparison of the mean defoliation ratings for the genotypic classes of marker R249T in each of the three crosses (Fig. 3) suggests that the resistant allele is dominant in the PI227687 genetic background, but not in PI171451 or PI229358. Assuming the insect resistance QTL in these three PIs was derived from a common source, this difference in gene action could be an indication of the extent of divergence that has occurred between these genotypes since acquisition of this locus. This is consistent with the relative amounts of polymorphism which exist between the three PIs (data not shown) and with the presumed difference in collection sites of these genotypes. PI227687 was collected on or near Okinawa, whereas PI171451 was collected near Tokyo, Japan, and the collection site for PI229358 is listed as 'Japan' (USDA Germplasm Resources Information Network, 1997).

Computer simulations of QTL studies using small populations (100 F_2 progeny) to search for QTLs with small effects (>10% R^2 per QTL) suggest that errors in estimating variance explained and degree of dominance can be great (Beavis, 1998). Thus, the small QTLs detected in Cross 2 could be false positives. However, since the LG 'H' QTL was also detected in Cross 1 and Cobb × PI229358 (Rector et al, 1998), its legitimacy is likely. The large LG 'M' QTL found in Cross 1 is also confirmed in Cobb × PI229358.

A three-way multiple regression analysis including the three significant markers from Cross 1 produced an R^2 value of 84%. This is similar to the sum of the R^2 values of the individual QTL markers ($\Sigma = 76\%$) and to the observed heritability estimate ($h^2 = 80\%$). This suggests that there is little genetic variance for PRI remaining to be explained in this cross although the small population sizes used in this study may have inflated the R^2 estimates made for these QTLs (Beavis, 1998).

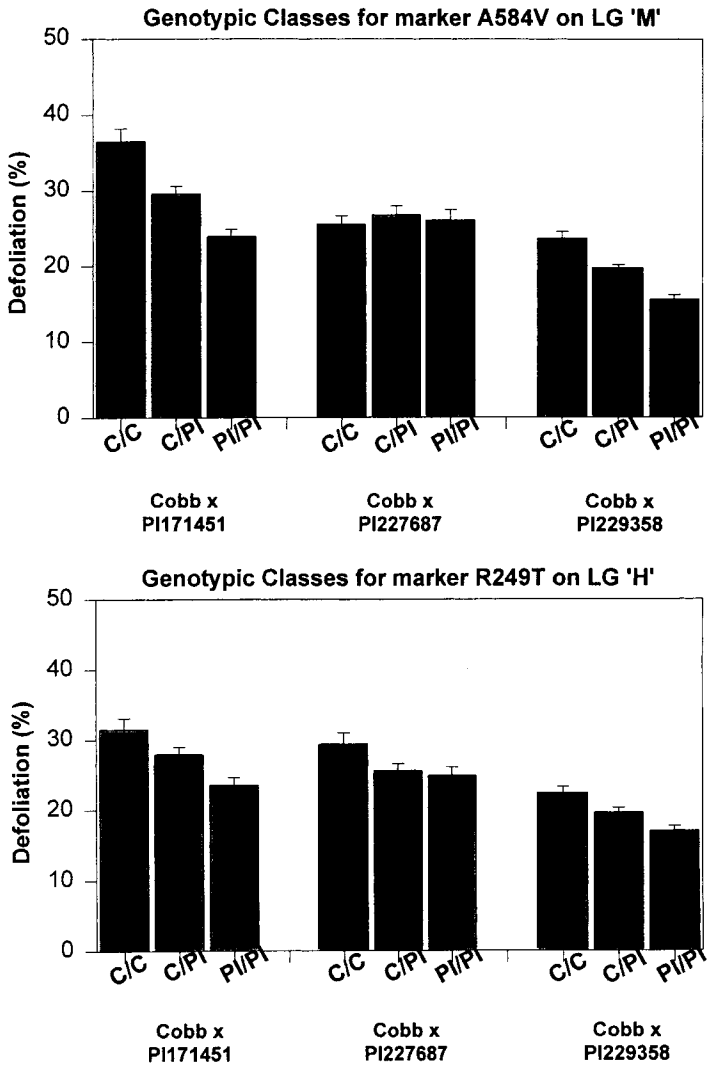

Fig. 3. Effect of allele substitution, across genotypes, at the soybean antixenosis QTLs linked to markers A584V on LG 'M' and R249 on LG 'H'. Standard error bars are included.

Two minor QTLs associated with antixenosis were detected in Cross 2. The combined R^2 value for these two QTLs ($\Sigma = 20\%$) is much lower than the heritability estimate for PRI in this cross ($h^2 = 65\%$). RFLP markers linked to QTLs identified in Cross 1 and in Cobb × PI229358 (Rector et al., 1998) have been mapped in Cross 2, but of these, PRI has been associated only with marker R249T on LG 'H' (Table 1; Fig. 3). The absence of resistance alleles at the other loci in Cross 2 may be due to inadequate sampling of the gamete pool in the mapping population due to its small size (Beavis, 1998). It is also possible that additional QTLs for PRI could be discovered in Cross 2 with the addition of more markers to its genetic map. A more saturated map should be possible with the use of newer marker systems such as simple-sequence repeat (SSR or 'microsatellite') markers and amplified fragment length polymorphism (AFLP). A recently published AFLP map in soybean (Keim et al., 1997) contains 650 AFLP markers and spans over 3400 cM, whereas the RFLP map constructed in Cross 2 has only 118 markers and less than 1500 cM. Thus, approximately half of the soybean genome, or less, has been mapped in this cross. Microsatellite primers from a 600-marker, 2800 cM USDA soybean genetic map (Cregan et al., 1997) will be publicly available soon. With the availability of these new marker systems, it should be possible to search for PRI QTLs in regions of the genome for which RFLPs were not available in Cross 2.

Genetic mapping with molecular markers enables the comparison of soybean PRI QTLs with other soybean pest resistance QTLs. Insect resistance QTLs can also be compared across legume species through comparative mapping. If borne out, these comparisons can give insight into possible relationships between pest resistance QTLs. The resistance allele of one of the QTLs in Cross 1, on LG 'F', came from the susceptible parent, Cobb (Fig. 2). It is located in a region of LG 'F' which has been associated with a number of other soybean pest-resistance QTLs. These include QTLs for plant resistance to viruses, fungi, nematodes, and a bacterium (Tamulonis et al., 1997b).

Attempts were made, through comparative mapping, to associate the QTLs detected in this study with each other through investigation of previously reported duplicated regions in the soybean genome (Shoemaker et al., 1996). No such associations were found. Nor could any associations be made between the QTLs found in this study and previously mapped insect-resistance genes in mungbean [*Vigna radiata* (L.) Wilczek] (Young et al., 1992; Boutin et al., 1995) and cowpea (*V. unguiculata* L. Walpers) (Menancio-Hautea et al., 1993; Myers et al., 1996).

Candidate regions for disease resistance genes have been marked on the soybean genetic map on the basis of homology to cloned disease resistance genes and resistance gene subunits from other plants (Kanazin et al., 1996; Yu et al., 1996). Resistance gene analogs (RGAs) have been detected on LGs 'M', 'H', and 'C2' (Kanazin et al., 1996) raising the possibility of an association of these RGAs with resistance to defoliating insects, although specific map location data for the RGAs was not available for further comparison.

Comparison of the insect resistance QTLs identified in this study and those reported previously in the soybean cross Cobb × PI229358 (Rector et al., 1998) reveal that one major QTL on LG 'M' was detected in both the Cobb × PI171451 and Cobb × PI229358 populations and a minor QTL on LG 'H' was detected in crosses of Cobb to all three resistant PIs (Figs. 2, 3). All other QTLs were found in only one genotype each (Fig. 2). However, in the case of the QTL on LG 'F' in Cross 1, the allele for resistance was derived from the susceptible parent Cobb. Both PI227687 and PI229358 shared the same RFLP bands as Cobb for marker B212V-1, therefore it could not be mapped in crosses between these genotypes. The map of LG 'F' in Cobb × PI229358 has polymorphic markers in the region containing the QTL on LG 'F' detected in Cross 1 (Fig. 2). The presence of an insect resistance allele from Cobb at this locus in Cross 1, and the lack of a QTL detected in the same region in Cobb × PI229358 (Rector et al., 1998) suggests that PI229358 and Cobb share this PRI allele. The map of LG 'F' in Cross 2 does not cover the region containing the QTL detected in Cross 1 (Fig. 2).

There were areas of the Cross 1 genetic map that contained polymorphic markers whose segregation was unusual. Certain $F_{1:2}$ families did not segregate at all for these markers when they were expected to show a 1:2:1 segregation. Other $F_{1:2}$ families appeared to segregate 1:1 when either no segregation or 1:2:1 segregation was expected (data not shown). The causes of these unusual segregation phenomena were not known. There were no significant differences in defoliation among F_1-derived families. The regions of unusual segregation were localized to linkage groups that were not associated with PRI in Cross 1 and there is no indication that there is any connection between the unusual segregation in Cross 1 and PRI in Cobb or PI171451.

Lambert and Kilen (1984b) suggested that PRI could be increased by the incorporation of resistance genes from all three PIs into a single cultivar. Until now, this has not been considered feasible because of the lack of information on the number of genes for resistance from each PI and the amount of linkage drag that would be encountered in a breeding program using all three insect-resistant PIs as donor parents. Through QTL analysis we have identified molecular genetic markers linked to insect resistance QTLs in crosses of Cobb to each of the three PIs, including QTLs unique to each cross. Marker-assisted selection for PRI in soybean using the most robust marker-QTLs reported here and previously (Rector et al., 1998) may make pyramiding resistance genes from all three sources possible.

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REFERENCES

Adams, D.B., R.M. McPherson, D.C. Jones, and D.L. Horton. 1989. Soybean Insects. p. 36–37. *In* G.K. Douce and R.M. McPherson (ed.) Summary of losses from insect damage and costs of control in Georgia, 1988. Georgia Agric. Exp. Stn. Spec. Publ. 64, Athens, GA.

Adams, D.B., R.M. McPherson, D.C. Jones, and D.L. Horton. 1991. Soybean Insects. p. 30–31. *In* G.K. Douce and R.M. McPherson (ed.) Summary of losses from insect damage and costs of control in Georgia, 1989. Georgia Agric. Exp. Stn. Spec. Publ. 70, Athens, GA.

All, J.N., H.R. Boerma, and J.W. Todd. 1989. Screening soybean genotypes in the greenhouse for resistance to insects. Crop Sci. 29:1156–1159.

Bailey, M.A., M.A.R. Mian, T.E. Carter, Jr., D.A. Ashley, and H.R. Boerma. 1997. Pod dehiscence of soybean: Identification of quantitative trait loci. J. Heredity 88:152–154

Beavis, W.D. 1998. QTL analyses: Power, precision, and accuracy. *In* A.H. Paterson (ed.) Molecular dissection of complex traits. CRC Press. p. 145–162.

Boutin, S.R., N.D. Young, T.C. Olson, Z.H. Yu, R.C. Shoemaker, and C.E. Vallejos. 1995. Genome conservation among three legume genera detected with DNA markers. Genome 38:928–937.

Bowers, Jr., G.R. 1990. Registration of 'Crockett' soybean. Crop Sci. 30:427.

Brummer, E.C., G.L. Graef, J. Orf, J.R. Wilcox, and R.C. Shoemaker. 1997. Mapping QTL for seed protein and oil content in eight soybean populations. Crop Sci. 37:370–378.

Byrne, P.F., M.D. McMullen, B.R. Wiseman, M.E. Snook, T.A. Musket, J.M. Theuri, N.W. Widstrom, E.H. Coe. 1997. Identification of maize chromosome regions associated with antibiosis to corn earworm (Lepidoptera: Noctuidae) larvae. J. Econ. Entomol. 90: 1039–1045.

Cregan, P.B., K.G. Lark, J.E. Specht, R.C. Shoemaker, A.L. Bush, T. Jarvik, and A.L. Kahler. 1997. An integrated SSR/RFLP/RAPD molecular genetic linkage map of soybean. p. 153. *In* Agronomy Abstracts. ASA, Madison, WI.

Fehr, W.R. and C.E. Caviness. 1977. Stages of soybean development. Iowa Coop. Ext. Serv. Spec. Rep. 80.

Hanthorne, M., C. Osteen, R. McDowell, and L. Robertson. 1982. 1980 Pesticide use on soybeans in the major producing states. AGES 820106. USDA-ARS, Washington, DC.

Hargrove, W.H., and D.A. Crossley. 1988. Video digitizer for the rapid measurement of leaf area lost to herbivorous insects. Ann. Entomol. Soc. Am. 81:593–598.

Hartwig, E.E., L. Lambert, and T.C. Kilen. 1990. Registration of 'Lamar' soybean. Crop Sci. 30:231.

Hartwig, E.E., T.C. Kilen, and L.D. Young. 1994. Registration of 'Lyon' soybean. Crop Sci. 34:1412.

Hnetkovsky, N., S.J.C. Chang, T.W. Doubler, P.T. Gibson, and D.A. Lightfoot. 1996. Genetic mapping of loci underlying field-resistance to soybean sudden death syndrome (SDS). Crop Sci. 36:393–400.

Hudson, R.D., D.C. Jones, and R.M. McPherson. 1992a. Soybean Insects. p. 30–31. *In* G.K. Douce and R.M. McPherson (ed.) Summary of losses from insect damage and costs of control in Georgia, 1990. Georgia Agric. Exp. Stn. Spec. Publ. 77, Athens, GA.

Hudson, R.D., D.C. Jones, and R.M. McPherson. 1992b. Soybean Insects. p. 44–45. *In* R.M. McPherson and G.K. Douce (ed.) Summary of Losses from Insect Damage and Costs of Control in Georgia, 1991. Georgia Agricultural Experiment Stations Special Publication no. 81, Athens, GA.

Hudson, R.D., D.C. Jones, and R.M. McPherson. 1993. Soybean Insects. p. 40–41. *In* R.M. McPherson and G.K. Douce (ed.) Summary of losses from insect damage and costs of control in Georgia, 1992. Georgia Agric. Exp. Stn. Spec. Publ. 83, Athens, GA.

Kanazin, V., L.F. Marek, and R.C. Shoemaker. 1996. Resistance gene analogs are conserved and clustered in soybean. Proc. Natl. Acad. Sci. (USA) 93:11746–11750.

Keim, P., B.W. Diers, and R.C. Shoemaker. 1990. Genetic analysis of soybean hard seededness with molecular markers. Theor. Appl. Genet. 79:465–469.

Keim, P., J.M. Schupp, S.E. Travis, K. Clayton, T. Zhu, L. Shi, A. Ferreira, and D.M. Webb. 1997. A high-density soybean genetic map based on AFLP markers. Crop Sci. 37:537–543.

Kenty, M.M., K. Hinson, K.H. Quesenberry, and D.S. Wofford. 1996. Inheritance of resistance to the soybean looper in soybean. Crop Sci. 36:1532–1537.

Kogan, M. and E.E. Ortman. 1978. Antixenosis — a new term proposed to replace Painter's 'Nonpreference' modality of resistance. Bull Entomol. Soc. Am. 24:175–176.

Lambert, L., and T.C. Kilen. 1984a. Multiple insect resistance in several soybean genotypes. Crop Sci. 24:887–890.

Lambert, L., and T.C. Kilen. 1984b. Influence of three soybean plant genotypes and their F1 intercrosses on the development of five insect species. J. Econ. Entomol. 77:622–625.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln, and L. Newburg. 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181.

Lander, E.S., and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199.

Lee, S.H., M.A. Bailey, M.A.R. Mian, T.E. Carter, D.A. Ashley, R.S. Hussey, W.A. Parrott, and H.R. Boerma. 1996a. Molecular markers associated with soybean plant height, lodging, and maturity across locations. Crop Sci. 36:728–735.

Lee, S.H., M.A. Bailey, M.A.R. Mian, E.R. Shipe, D.A. Ashley, W.A. Parrott, R.S. Hussey, and H.R. Boerma. 1996b. Identification of quantitative trait loci for plant height, lodging, and maturity in a soybean population segregating for growth habit. Theor. Appl. Genet. 92:516–523.

Lee, S.H., M.A. Bailey, M.A.R. Mian, T.E. Carter, E.R. Shipe, D.A. Ashley, W.A. Parrott, R.S. Hussey, and H.R. Boerma. 1996c. RFLP loci associated with soybean seed protein and oil content across populations and locations. Theor. Appl. Genet. 93:649–657.

Lincoln, S., M. Daly, and E. Lander. 1992. Mapping genes controlling quantitative traits with MAPMAKER/QTL. Whitehead Institute Tech Rept 2nd ed. Whitehead Institute, Cambridge, MA.

Lohnes, D.G. and A.F. Schmitthenner. 1997. Position of the Phytophthora resistance gene Rps7 on the soybean molecular map. Crop Sci. 37:555–556.

Luedders, V.D., and W.A. Dickerson. 1977. Resistance of selected soybean genotypes and segregating populations to cabbage looper feeding. Crop Sci. 17:395–396.

Maliepaard, C., N. Bas, S. van Heusden, J. Kos, G. Pet, R. Verkerk, R. Vrielink, P. Zabel, and P. Lindhout. 1995. Mapping of QTL for glandular trichome densities and *Trialeurodes vaporarium* (greenhouse whitefly) resistance in an F_2 from *Lycopersicon esculentum* × *Lycopersicon hirsutum* f. *glabratum*. Heredity 75:425–433.

McPherson, R.M., R.D. Hudson, and D.C. Jones. 1994. Soybean Insects. p. 38–39. *In* G.K. Douce and R.M. McPherson (ed.) Summary of losses from insect damage and costs of control in Georgia, 1993. Georgia Agric. Expe. Stn Spec. Publ. 87, Athens, GA.

McPherson, R.M., R.D. Hudson, and D.C. Jones. 1997. Soybean Insects. p. 37–38. *In* G.K. Douce and R.M. McPherson (ed.) Summary of losses from insect damage and costs of control in Georgia, 1994. Georgia Agric. Expe. Stn Spec. Publ. 89, Athens, GA.

Menancio-Hautea, D., C.A. Fatokun, L. Kumar, D. Danesh, and N.D. Young. 1993. Comparative genome analysis of mungbean (*Vigna radiata* L. Wilczek) and cowpea (*V. unguiculata* L. Walpers) using RFLP mapping data. Theor. Appl. Genet. 86:797–810.

Mian, M.A.R., M.A. Bailey, D.A. Ashley, R. Wells, T.E. Carter, W.A. Parrott, and H.R. Boerma. 1996a. Molecular markers associated with water-use efficiency and leaf ash in soybean. Crop Sci. 36: 1252–1257.

Mian, M.A.R., M.A. Bailey, J.P. Tamulonis, E.R. Shipe, T.E. Carter, W.A. Parrott, D.A. Ashely, R.S. Hussey, and H.R. Boerma. 1996b. Molecular markers associated with seed weight in 2 soybean populations. Theor. Appl. Genet. 93:1011–1016.

Mian, M.A.R., E.R. Shipe, J. Alvaernaz, J.D. Mueller, D.A. Ashely, and H.R. Boerma. 1996c. RFLP analysis of chlorimuron ethyl sensitivity in soybean. J. Heredity 88:38:41.

Mudge, J., P.B. Cregan, J.P. Kenworthy, W.J. Kenworthy, J.H. Orf, and N.D. Young. 1997. Two microsattelite markers that flank the major soybean cyst nematode resistance locus. Crop Sci. 37: 1611–1615.

Myers, G.O., C.A. Fatokun, and N.D. Young. 1996. RFLP mapping of an aphid resistance gene in cowpea (Vigna unguiculata L. Walp). Euphytica 91:181–187.
Painter, R.H. 1951. Insect resistance in crop plants. Macmillan & Co., New York.
Rector, B.G., J.N. All, W.A. Parrott, and H.R. Boerma. 1998. Identification of molecular markers associated with quantitative trait loci for soybean resistance to corn earworm. Theor. Appl. Genet. 96:786–790.
Rowan, G.B., H.R. Boerma, J.N. All, and J. Todd. 1991. Soybean cultivar resistance to defoliating insects. Crop Sci. 31:678–682.
Rufener, G.K., S.K. St. Martin, R.L. Cooper, and R.B. Hammond. 1989. Genetics of antibiosis resistance to Mexican bean beetle in soybean. Crop Sci. 29:618–622.
SAS. 1988. SAS/STAT user's guide, version 6.03. SAS Institute, Cary, NC.
Shoemaker, R.C. and J.E. Specht. 1995. Integration of the soybean molecular and classical genetic linkage maps. Crop Sci. 35:436–446.
Shoemaker, R.C., K. Polzin, J. Labate, J. Specht, E.C. Brummer, T. Olson, N. Young, V. Concibido , J. Wilcox, J.P. Tamulonis, G. Kochert, and H.R. Boerma. 1996. Genome duplication in soybean (*Glycine* subgenus *Soja*). Genetics 144:329–338.
Sisson, V.A., P.A. Miller, W.V. Campbell, and J.W. Van Duyn. 1976. Evidence of inheritance of resistance to the mexican bean beetle in soybeans. Crop Sci. 16:835–837.
Tamulonis, J.P., B.M. Luzzi, R.S. Hussey, W.A. Parrott, and H.R. Boerma. 1997a. RFLP mapping of resistance to southern root-knot nematode in soybean. Crop Sci. 37:1903–1909.
Tamulonis, J.P., B.M. Luzzi, R.S. Hussey, W.A. Parrott, and H.R. Boerma. 1997b. DNA markers associated with resistance to Javanese root-knot nematode in soybean. Crop Sci. 37:783–788.
USDA Germplasm Resources Information Network. 1997. Database Management Unit. National Germplasm Resources Laboratory. ARS Beltsville, MD. http://www.ars-grin.gov/.
Van Duyn, J.W., S.G. Turnipseed, and J.D. Maxwell. 1971. Resistance in soybean to the Mexican bean beetle. Crop Sci. 11:572–573.
Wiseman, B.R., F.M. Davis, and J.E. Campbell. 1980. Mechanical infestation device used in fall armyworm plant resistance programs. Fla. Entomol. 63:425–438.
Yencho, G.C., M.W. Bonierbale, W.M. Tingey, R.L. Plaisted, and S.D. Tanksley. 1996. Molecular markers locate genes for resistance to the Colorado potato beetle, *Leptinotarsa decemlineata*, in hybrid *Solanum tuberosum* × *S. berthaultii* potato progenies. Entomol. Exp. Appl. 81:141–154.
Young, N.D., and S.D. Tanksley. 1989. RFLP maps and the concept of graphical genotypes. Theor. Appl. Genet. 77:95–101.
Young, N.D., L. Kumar, D. Menancio-Hautea, D. Danesh, N.S. Talekar, S. Shanmugasundarum, and D.H. Kim. 1992. RFLP mapping of a major bruchid resistance gene in mungbean. Theor. Appl. Genet. 84:839–844.
Yu, Y.G., M.A. Saghai Maroof, G.R. Buss, P.J. Maughan, and S.A. Tolin. 1994. RFLP and microsatellite mapping of a gene for soybean mosaic-virus resistance. Phytopathology 84:60–64.
Yu, Y.G., G.R. Buss, and M.A. Saghai Maroof. 1996. Isolation of a superfamily of candidate disease-resistance genes in soybean based on a conserved nucleotide-binding site. Proc. Natl. Acad. Sci. (USA) 93:11751–11756.

Molecular Marker-Assisted Dissection of Genotype × Environment Interaction for Plant Type Traits in Rice (*Oryza sativa* L.)

Juqiang Yan,* Jun Zhu, Cixin He, Mebrouk Benmoussa, and Ping Wu

ABSTRACT

A doubled haploid (DH) population of 123 lines from IR64/Azucena was used to analyze the genotype × environment (GE) interaction for eight plant type traits in rice (*Oryza sativa* L.). The total genetic effects were partitioned into genetic main effects and GE interaction effects. These two kinds of predicted effects were used in mapping quantitative trait loci (QTLs). Four to nine QTLs affecting different plant type traits were detected. Results indicated that all common QTLs detected in both environments were controlled by genetic main effects and some also by GE interaction effects. Some genomic regions identified significant QTL in only one environment; some also showed genetic main effects. Those QTLs with genetic main effects could be used in marker-assisted selection across environments. For some other map regions, QTLs were controlled by only GE interaction effects without genetic main effects. Those QTLs could be included in marker-assisted selection only for specific environments. In most cases, the pairs of traits with a high genetic correlation shared more common QTL regions than those pairs of traits with a lower genetic correlation.

PLANT TYPE is one of the most important traits to rice breeders. The component traits contributed to plant type are plant height (culm length), tiller number, culm angle (tiller angle), leaf dimensions and angles, and panicle characteristics (Chang and Li, 1991). Since the end of the 1950s, high-yielding rice varieties of reduced plant height with high lodging resistance, favorable plant type, and high harvest index have been released in almost all rice growing countries (Ming, 1987). These semidwarf, high-yielding varieties with improved plant type have played a vital role in the food sufficiency for countries where rice is a staple food. The yield potential of semidwarf indica rice is about 10 Mg/ha (IRRI, 1995). The further increase in yield potential is limited for semidwarf rice varieties because of (i) limited sink size, (ii) high percentage of unproductive tillers, (iii) short grain-filling duration, (iv) early senescence, and (v) susceptibility to lodging (IRRI, 1995). In recent years, tropical japonica varieties have been used for breeding new plant type lines (IRRI, 1995). Some elite breeding lines of new plant type with short stature, sturdy stems, larger panicles, reduced tillering, and with erect dark green and thick leaves, have been developed (IRRI, 1995). Most plant type traits in rice are quantitatively inherited and their performances are greatly affected by the envi-

Juqiang Yan, Dep. of Biological Science, Texas Tech. Univ., Lubbock, TX 79409-3131 and Agronomy Dep., Zhejiang Univ., Hangzhou, Zhejiang, China; Jun Zhu, Cixin He and Mebrouk Benmoussa, Agronomy Dep., and Ping Wu, Biological Science Dep., Zhejiang Agric. Univ., Hangzhou, 310029, China. Received 18 May 1998. *Corresponding author (jqyan_98@yahoo.com).

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Abbreviations: GE, genotype × environment interaction; QTLs, Quantitative trait loci; DH, double haploid; RFLP, restriction fragment length polymorphism; LOD, likelihood ratio; RAPD, random amplified polymorphic DNA; SD, standard deviation; cM, centimorgan; PH, plant height; PN, panicle number; MTN, maximum tiller number; PPT, percentage of productive tillers; TA, tiller angle; FLL, flag leaf length; FLW, flag leaf width; FLA, flag leaf angle.