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Fine Mapping of a Major Insect Resistance QTL in Soybean and its Interaction with Minor Resistance QTLs

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ABSTRACT

Utilization of native insect resistance genes can be an important component for managing insects in soybean [*Glycine max* (L.) Merr.]. A major quantitative trait locus (QTL-M) for insect resistance from PI 229358, controlling antibiosis and antixenosis, was previously identified on linkage group (LG) M and was found to increase the effectiveness of a *Bacillus thuringiensis* (Bt) transgene in soybean. The objectives of this study were to fine-map QTL-M using recombinant substitution lines (RSLs) identified from a 'Benning' backcross population, and to evaluate the main effects and the epistatic interactions between QTL-M and other resistance QTLs on LGs G and H using near-isogenic lines (NILs) in a Benning genetic background. The effect of QTL-M was still detectable in the Benning NILs when they were evaluated for resistance to corn earworm [CEW, *Helicoverpa zea* (Boddie)]. The two minor resistance QTLs only provided insect resistance when QTL-M was also present in the Benning NILs. The QTL-M was fine-mapped to an approximately 0.52-cM region after the first round of phenotyping the RSLs for resistance to CEW and soybean looper [SBL, *Pseudoplusia includens* (Walker)]. These results should increase the feasibility of cloning QTL-M and help guide the development of insect resistant soybean cultivars.

Classical breeding for insect resistance in soybean has faced several obstacles. First, since soybean resistance to insect defoliation is a quantitatively inherited trait (Sisson et al., 1976; Rufener et al., 1989), simple backcrossing has not been successful in transferring the full complement of resistance genes from unadapted germplasm accessions. In several insect resistance breeding programs based on phenotypic selection, only a major QTL on LG M was consistently selected, while minor insect resistance QTLs were often lost during breeding (Narvel et al., 2001), resulting in a resistance level lower than that of the original donor. Second, the main sources of insect resistance in soybean, the three Japanese plant introductions PI 229358, PI 227687, and PI 171451, are of low agronomic value, increasing problems with linkage drag (Van Duyn et al., 1971; Lambert and Tyler, 1999). Furthermore, the insect resistance in these PIs exhibits two distinct mechanisms: antibiosis (adverse effects on the insect life history), and antixenosis (discouragement of insect colonization and/or feeding) (Painter, 1951; Kogan and Ortman, 1978; Lambert and Kilen, 1984). The genetic independence of antibiosis and antixenosis has been suggested, although their effects may overlap (Painter, 1951; Rector et al., 1999, 2000). Depending on the phenotypic screen used, breeders often select for one of the two resistance modes, potentially resulting in the loss of resistance alleles for the other.

The difficulty of obtaining a high level of insect resistance in many crops via conventional breeding prompted genetic engineering with crystal protein genes from Bt (Stewart et al., 1996). However, the widespread use of Bt genes has raised issues about the evolution of Bt-resistant insect populations (Gould, 1998; Walker et al., 2002b). Pyramiding Bt genes and native resistance genes in the same plant increases the efficacy of both (Walker et al., 2002b, 2004). Therefore, the identification, characterization, and utilization of native soybean resistance genes could help the management of insect resistance to insecticidal proteins and broaden the resistance of plants with Bt genes.

Association of restriction fragment length polymorphism marker data with CEW defoliation and weight gain data from F_2–derived lines of a Cobb × PI 229358 population allowed Rector et al. (1998, 2000) to identify antixenosis QTLs on LGs M (QTL-M), D1b (QTL-D1b), and H (QTL-H). The QTLs for antibiosis were identified on LGs M and G (QTL-G), at which the resistance alleles were all contributed by PI 229358. In SoyBase (http://soybase.org; unverified future URL), QTL-M, QTL-H, and QTL-G were first listed as CEW 1- 1, CEW 1-2, and CEW 6-1, respectively. Of these, QTL-M, the major insect resistance QTL, was initially identified in an approximately 30-cM interval, with its peak at marker A584_4 on LG M. QTL-M contributed approximately 35 and 20% of phenotypic variations for antixenosis and antibiosis to CEW, respectively. It also enhances the effectiveness of a Bt *cry1Ac* transgene in soybean (Walker et al., 2004). Narvel et al. (2001) later presented evidence that QTL-M was in the approximately 8-cM region flanked by simple sequence repeat (SSR) markers Satt220 and Satt175. These authors also mapped QTL-H in the 0.5-cM Sat_122–Satt541 interval on LG H, QTL-D1b in the 3.7-cM Satt141–Satt290 interval on LG D1b, and QTL-G in the 4.4-cM Satt472–Satt191 interval on LG G.

Many early QTL mapping studies in plants reported QTLs from populations of limited size and from statistical tests that produced high rates of Type I errors (Fasoula et al., 2004). Putative QTLs detected at a less stringent comparison-wise significance level ($P > 0.0001$) need to be further analyzed before use in marker-assisted selection (MAS) programs (Bernardo, 2004). The effects

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Abbreviations: Bt, *Bacillus thuringiensis*; CEW, corn earworm; LG, linkage group; MAS, marker-assisted selection; NIL, near-isogenic line; QTL, quantitative trait locus; RSL, recombinant substitution line; SBL, soybean looper; SSR, simple sequence repeat.

of true QTLs may not be detected when they are introduced into a genetic background different from the one in which they were mapped. The QTL-H was detected for antixenosis in mapping populations from the crosses Cobb × PI 229358, Cobb × PI 171451, and Cobb × PI 227687 (Rector et al., 1998, 1999). However, Walker et al. (2004) could not detect the main effect of QTL-H when the QTL was transferred into a genetic background of 'Jack' (Nickell et al., 1990). Therefore, the effect of a QTL in a population should be confirmed before pursuing fine mapping of the QTL in the population and before attempting to transfer the QTL using MAS.

The objectives of this study were (i) to fine-map QTL-M using RSLs identified from a Benning backcross population, and (ii) to evaluate the main effects of QTL-M, QTL-H, and QTL-G, and the epistatic interactions among these QTLs by analysis of NILs in a Benning genetic background.

MATERIALS AND METHODS

Plant Materials

Twenty-seven RSLs were initially identified from $BC_6F_{2:3}$ lines derived from Benning (7) × PI 229358. Benning is a Maturity Group VII soybean cultivar that is susceptible to defoliating insects (Boerma et al., 1997; Day et al., 1999). For each generation of the backcrosses, F_1 plants were screened and selected for the presence of PI 229358 marker alleles in the QTL-M region. These selected plants provided pollen for the next generation of backcrosses to Benning. The 27 RSLs were descended from plants which had undergone crossovers in the QTL-M region, and were also homozygous in the region. These RSLs were classified into four types based on their genotypes at Satt220, Satt536, and Satt175 (Fig. 1). Two Type I lines (RSL 26 and RSL 27), one Type II line (RSL 25), two Type III lines (RSL 1 and RSL 2), and 22 Type IV lines (RSL 3–24) were identified and used in a preliminary bioassay. Meanwhile, a B line (all Benning genome in the Satt220–Satt175 interval) and a P line (all PI 229358 genome in the Satt220–Satt175 interval) were also selected from the $BC_6F_{2:3}$ lines for use as controls. Four more Type II RSLs (RSL 30, RSL 31, RSL 32, and RSL 33) were later identified and were included in subsequent bioassays. DNA from an F_2 population derived from Cobb × PI 229358 (Rector et al., 1998) was used to initially map newly-identified polymorphic SSR markers in the QTL region (Song et al., 2004). Marker order and map distance in the Satt220–Satt536 interval were accurately determined using 92 recombinants (two plants derived from both recombinant gametes, and the rest from one recombinant gamete and one parental gamete) identified from 1911 $BC_7F_{1:2}$ plants.

Eight BC_5F_2–derived NILs were used to verify the main effects of QTL-M, QTL-H, and QTL-G, and to evaluate the epistatic interactions among the QTLs in a Benning genetic background (Table 1). The BC_5F_2 population was derived from Benning (6) × PI 229358, using MAS for the three QTLs in each backcross generation. The SSR markers used for each backcross generation and the final selection of each NIL were Satt220 and Satt175 flanking QTL-M, Sat_334 and Sat_118 flanking QTL-H, and Sct_199 and Satt191 flanking QTL-G, as well as Satt536, Sat_122, and Satt472 within the intervals of QTL-M, QTL-H, and QTL-G, respectively. This set of BC_5F_2–derived NILs represented all the possible combinations of PI 229358 homozygous alleles at the resistance QTLs on LGs M, H, and G (Table 1).

Segment of LG M: Satt220 — 2.5 cM — Satt536 — 5.0 cM — Satt175				Expected phenotype(s) if QTL is in the interval:		
RSL Type				Satt220-Satt536	At Satt536	Satt536-Satt175
I.	-	+	+	R and S	R	R
II.	+	-	-	R and S	S	S
III.	-	-	+	S	S	R and S
IV.	+	+	-	R	R	R and S

+: PI 229358 allele present, -: PI 229358 allele absent; R=Resistance, S=Susceptible

Fig. 1. Genotypes of four types of Benning soybean recombinant substitution lines (RSLs) at three simple sequence repeat loci and expected phenotype(s) for each type if the quantitative trait loci were located at one of three possible locations. Map distances are estimates from Narvel et al. (2001).

Simple Sequence Repeat Genotyping

For the development of RSLs and NILs, DNA was extracted either from a quarter-seed chip with a modified protocol of Edwards et al. (1991) or from an unexpanded trifoliolate leaf with a modified CTAB procedure of Keim et al. (1988). For seed DNA extraction, the quarter-seed chips were placed in 1.1-mL 96-deepwell plates and soaked in 100 μL of distilled H_2O for >1 h. The softened seed chips were crushed, and 500 μL of extraction buffer [0.5% (w/v) sodium dodecyl sulfate; 250 m*M* NaCl; 200 m*M* Tris-HCl ph 7.5; and 25 m*M* ethylenediaminetetraacetic acid (EDTA)] was added. The samples were incubated at room temperature for 1 h and then centrifuged at 3600 rpm for 5 min. The supernatant was transferred to new 1.1-mL 96-deepwell plates, and two-thirds volume of isopropyl alcohol was added to precipitate DNA. The DNA pellets were washed with 70% (v/v) ethanol + 10 m*M* ammonium acetate and dissolved in tris-EDTA. For leaf DNA isolation, lyophilized leaves were ground in 1.1-mL, 96-deepwell plates and suspended in 700 μL of CTAB buffer [2% (w/v) hexadecyltrimethylammonium bromide; 1.4 *M* NaCl; 100 m*M* Tris-HCl pH 8.0; 20 m*M* ethylenediaminetetraacetic acid; and 1% (v/v) 2-mercaptoethanol].

The PCR reactions were prepared using the protocol described by Li et al. (2002). The sequences of all SSR primers

Table 1. Corn earworm defoliation and larval weight when fed on Benning soybean near-isogenic lines (NILs) with and without PI 229358 alleles at quantitative trait locus (QTL)-M, QTL-H, and QTL-G.

NIL	QTL-M	QTL-H	QTL-G	Defoliation	Larval weight
				%	mg
1	−†	−	−	30.6ab‡	140.7a
2	+	−	−	25.3bc	80.3b
3	−	+	−	32.1a	122.0a
4	−	−	+	32.1a	131.0a
5	+	+	−	21.0cd	79.8b
6	+	−	+	21.5cd	42.4c
7	−	+	+	28.1ab	125.9a
8	+	+	+	17.5d	56.5bc

† + and − indicate presence or absence of the resistance allele from PI 229358.

‡ Numbers followed by the same letters are not significantly different at the 0.05 probability level.

were obtained from the SoyBase website. The PCR amplicons were separated on an ABI PRISM 377 DNA sequencer (PE ABI, Foster City, CA), and marker data were analyzed with GeneScan v. 2.1 and Genotyper v. 2.5 software (PE ABI, Foster City, CA) using the procedures described by Li et al. (2002).

Antixenosis Tests

Antixenosis of both the RSLs and NILs was evaluated under greenhouse conditions with CEW and/or SBL using the bioassay described by All et al. (1989). Both CEW and SBL eggs were provided by the Crop Protection and Management Unit (USDA-ARS, Tifton, GA). Briefly, one seed was planted per 450-mL polystyrene foam cup with three holes punched in the bottom and filled with Fafard 2 mix (Conrad Fafard, Agawam, MA). Only cups with healthy seedlings were used in the assays, and these cups were arranged in a randomized complete block design with 15 replications. The cups were placed in a stainless steel pan measuring 4.9 m long by 1.2 m wide by 8 cm deep, and the pan was filled with 2 cm of water. For the QTL evaluation in the NILs, Benning and PI 229358 were used as controls. For the QTL-M fine mapping tests, four genotypes (Benning, PI 229358, the B line, and the P line) were included as controls. The original assay design of All et al. (1989) was modified by surrounding each block with border plants of alternating resistant and susceptible genotypes. Four neonate (<5 h old) larvae were placed on an unexpanded trifoliolate leaf of each plant using a 000-size camel's hair brush when plants were at the V2 stage (Fehr et al., 1971). The percentage leaf area consumed (defoliation) was visually estimated 10 d after infestation. Defoliation estimates were made by at least three different researchers, and the mean of the estimates for each experimental unit was used in the ANOVA.

Antibiosis Tests

Antibiosis of both the RSLs and NILs to CEW was evaluated in a growth chamber, using a procedure from Walker et al. (2002a). The growth chamber was maintained at 27°C and 85% ambient humidity, and a 14-h photoperiod was maintained with fluorescent and incandescent lights providing approximately 40 μmol photons m^{-2} s^{-1}. Newly expanded trifoliolate leaves were collected from either growth chamber- or greenhouse-grown plants. One whole trifoliolate leaf was placed in a Petri dish (100 × 25 mm), and infested with three neonate larvae. A fresh leaf was added to each dish after 4 d, and the feeding was stopped (about 6 d after infestation) by moving all the dishes to 4°C once the leaf tissue in any one of the dishes was completely consumed. An hour later, larvae were transferred to empty Petri dishes, frozen at −20°C, and weighed. For fine mapping of QTL-M, the procedure was modified by moving the three larvae into three separate dishes, with one leaflet from a trifoliolate leaf per dish, to prevent cannibalism. Controls included were the same as those used in the antixenosis tests. The bioassays were set up as randomized complete block designs with 10 replications. The data were recorded as the average weight of larvae surviving from the three reared in a single dish for evaluation of QTL effects, or in three dishes for fine mapping QTL-M.

Data Analyses

The procedure described by Zhu and Kaeppler (2003) was used to map newly identified polymorphic SSR markers in the QTL regions using MAPMAKER/EXP 3.0b (Lander et al., 1987). The percentage defoliation and the average larval weights were first tested for normality by using the Univariate Procedure (Proc Univariate) of SAS (SAS Institute, 1988). Analysis of variance for the traits was conducted with the General Linear Model Procedure (Proc GLM) of SAS (SAS Institute, 1990).

RESULTS AND DISCUSSION

Fine Mapping of QTL-M

The strategy to fine-map QTL-M began by determining whether the QTL is in the Satt220–Satt536 interval or the Satt536–Satt175 interval (Fig. 1). In a preliminary experiment, 27 RSLs were evaluated for antixenosis to CEW under greenhouse conditions (data not shown). In the RSL Type I class, RSL 26 was clearly susceptible to CEW, whereas RSL 27 was resistant when compared with the controls. Recombinant substitution line 25 in the Type II class was susceptible, as were the two Type III lines. Among the 22 lines in the Type IV class, two (RSL 13 and RSL 14) were moderately susceptible, while the rest were relatively resistant. Therefore, additional tests for antibiosis and antixenosis to CEW and antixenosis to SBL were performed on two Type I lines (RSL 26 and RSL 27) and two Type IV lines (RSL 13 and RSL 14) to confirm their phenotypes. Meanwhile, all the RSLs were genotyped with newly reported SSR markers Satt323, Satt626, Sat_258, and Satt702 in the vicinity of QTL-M to better characterize the positions of the crossovers in the region (Song et al., 2004).

The results from these additional tests confirmed the phenotypic differences between RSL 26 (susceptible) and RSL 27 (resistant) found in the initial study; however, RSL 13 and RSL 14 were resistant on the basis of these assays (data not shown). Genotyping results indicate that RSL 26 contained a recombination event between SSR markers Sat_258 and Satt702, while RSL 27 was homozygous for PI 229358 DNA in the Satt323–Satt702 interval and had a recombination event between Satt220 and Satt323 (Fig. 2). This finding explains the phenotypic differences between RSL 26 and RSL 27 found in the initial study and in these additional tests. These additional results suggested that QTL-M is located in the Satt220–Satt536 interval.

If QTL-M were located in the Satt220–Satt536 interval, RSLs in the Type II class would be expected to differ in response to insect feeding as well (Fig. 1). To confirm this, four more Type II RSLs (RSL 30, RSL 31, RSL 32, and RSL 33) were identified and included in further bioassays. Recombinant substitution line 33 is clearly resistant, while RSL 30, RSL 31, and RSL 32 are susceptible (Fig. 2). Collectively, individual Type I and Type II RSLs displayed both resistant and susceptible responses to insect feeding, Type III RSLs were all susceptible, and Type IV RSLs were all resistant. These new results corroborate that QTL-M is located in the Satt220–Satt536 interval.

The differential resistance reactions of the two Type I lines (RSL 26 and RSL 27) and the two Type II lines (RSL 30 and RSL 33) provide strong evidence that QTL-M is located in an approximately 0.52-cM region flanked by Sat_258 and Satt702 (Fig. 2). Since RSL 26 is susceptible, QTL-M cannot be in the Satt702–Satt536

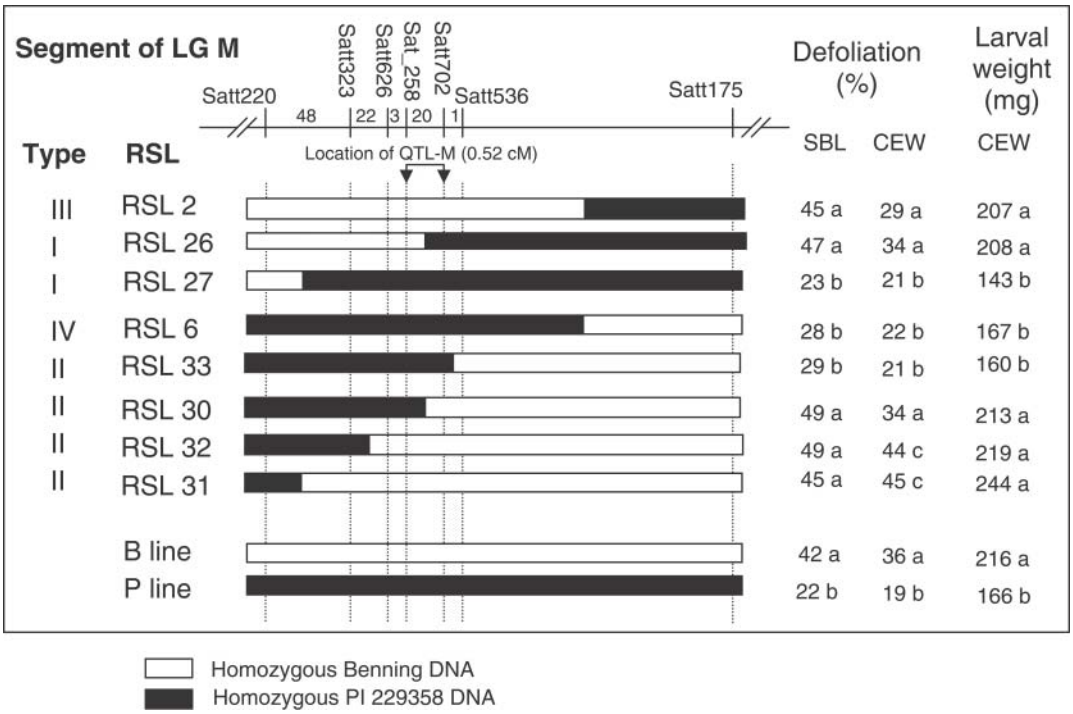

Fig. 2. Graphical genotypes of some representative Benning soybean RSLs in the quantitative trait locus (QTL)-M region on linkage group (LG) M, and most likely location of QTL-M in the Sat_258–Satt702 interval. Means followed by the same letters are not significantly different at the 0.05 probability level. The numbers of recombinant gametes in the Satt220–Satt536 interval in the 92 critical recombinant plants identified from 1911 $BC_7F_{1:2}$ plants are indicated in the marker intervals. The map distance (centimorgans) was calculated using the number of recombinants divided by the total number of meioses (3822), multiplied by 100.

interval. Furthermore, if QTL-M were located in the Satt220–Sat_258 interval, RSL 30 should not be susceptible to CEW and SBL. At this level of resolution, it should be possible to saturate the interval with additional markers and build a bacterial artificial chromosome contig across it, thus setting the stage to clone QTL-M.

Differences in marker order and map distances were noticed when the vicinity of QTL-M was compared with the region in the integrated map published by Song et al. (2004). The order Satt220–Satt323–Satt626 was more parsimonious with the marker data from this study (Fig. 2). The order Satt220–Satt626–Satt323 in the integrated map could only be explained if all 22 of the recombinations between Satt323 and Satt626 identified from the 1911 $BC_7F_{1:2}$ individuals had resulted from double crossovers, which is highly unlikely (Fig. 3). The map distance between Satt220 and Satt536 in the integrated map (5.85 cM) was greater than the estimated length for the interval in this study (2.46 cM). However, the distance for the subinterval from Sat_258 to Satt702 carrying QTL-M was about 0.5 cM in both studies (Fig. 2; Song et al., 2004).

Main Effect of QTL-M and Its Interactions with QTL-H and QTL-G

The eight NILs with all combinations of homozygous Benning or PI 229358 alleles at QTL-M, QTL-H, and QTL-G were evaluated for both antixenosis and antibiosis with CEW (Table 1). Although the main effect of QTL-M on reducing CEW defoliation was not evident in the pair-wise comparison of NIL 1 and NIL 2 (Table 1), it was apparent when the average defoliations of lines with and without QTL-M were compared (Table 2). The main effect of QTL-M on decreasing CEW larval weight was obvious when the NILs with and without QTL-M were compared (Table 1). Analyses of variance for both antixenosis and antibiosis indicated that the main effect of QTL-M was significant when the QTL was transferred into a Benning genetic background (Table 2). The main effect of QTL-H for antixenosis, and the main effect of QTL-G for antibiosis

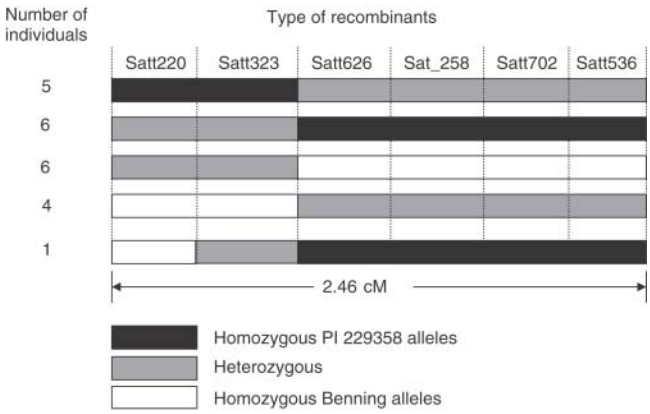

Fig. 3. Schematic diagram of a segment of linkage group M showing all the 22 recombinants between markers Satt323 and Satt626 identified from 1911 $BC_7F_{1:2}$ individuals of Benning soybean.

Table 2. Main effects of QTLs, and epistatic effects between QTL-M and QTL-H averaged across QTL-G for antixenosis, and between QTL-M and QTL-G averaged across QTL-H for antibiosis to CEW in a Benning soybean background.

		QTL-M absent	QTL-M present	Mean
Defoliation, %	QTL-H absent	31.4a†	23.4b	27.4ns‡
	QTL-H present	30.1a	19.2c	24.7
	Mean	30.8***	21.3	
Larval weight, mg	QTL-G absent	131.3a	80.0b	105.7ns
	QTL-G present	128.4a	49.4c	88.9
	Mean	129.9***	64.7	

*** Significantly different from its counterpart at the 0.001 probability level based on ANOVA.
† Numbers followed by the same letters are not significantly different at the 0.05 probability level.
‡ ns, not significantly different at the 0.05 probability level.

were not significant ($P > 0.05$) when the QTLs were introgressed into the Benning genetic background. However, both of the QTLs were involved in significant epistatic interactions with QTL-M (Table 2). Lines carrying QTL-M were more resistant if PI 229358 alleles were also present at QTL-H for antixenosis or at QTL-G for antibiosis (Tables 1 and 2).

The NILs with the PI 229358 allele at QTL-M averaged 9.5% less CEW defoliation and 65.2 mg less weight than the NILs homozygous for the Benning allele at the QTL (Table 2). The QTL-M was previously detected for both antixenosis and antibiosis in Cobb × PI 229358 and Cobb × PI 171451 mapping populations (Rector et al., 1998, 1999, 2000), and has been confirmed in a Jack genetic background (Walker et al., 2002a, 2004), and now in a Benning background. Walker et al. (2004) were also unable to confirm the main effect of QTL-H in a Jack background. Although the main effects of QTL-H and QTL-G were not significant in this study, the combination of PI 229358 alleles at QTL-M and QTL-H resulted in the least CEW defoliation, and the combination of PI 229358 alleles at QTL-M and QTL-G led to the least CEW larval growth among the eight NILs (Tables 1 and 2).

The NILs homozygous for the PI 229358 allele at QTL-H averaged 4.2% less CEW defoliation when the NILs were fixed for the PI 229358 allele at QTL-M, but QTL-H showed no effect when the NILs were fixed for the Benning allele at QTL-M (Table 2). Similarly, the NILs homozygous for the PI 229358 allele at QTL-G averaged 30.6 mg less weight when the NILs were fixed for the PI 229358 allele at QTL-M, but QTL-G did not affect the CEW larval weight when QTL-M was fixed for the Benning allele. The MAPMAKER/QTL program used in the original mapping studies limited the ability to detect QTL interactions (Rector et al., 1998, 1999, 2000). Although the QLT-M × QTL-H interaction for antixenosis was not significant for Jack BC_2F_3–derived lines in field-cage tests with CEW and SBL, it did approach significance with CEW (Walker et al., 2004). The PI 229358 allele at QTL-M was required for the PI 229358 allele at either the antixenosis QTL-H or the antibiosis QTL-G to provide insect resistance in a Benning background. Further research on this may help our understanding of different resistance mechanisms exhibited by soybean plants to most insect herbivores.

In summary, QTLs for insect resistance were introgressed from PI 229358 into a Benning genetic background using marker-assisted backcrossing to produce NILs. On the basis of the results from the NILs for QTL-M, QTL-H, and QTL-G, it is clear that QTL-M has a major effect on both CEW antixenosis and antibiosis in a Benning genetic background. Given this is the fourth genetic background in which the QTL-M effect has been detected, QTL-M would probably be expressed in a wide range of genotypes. The QTL-H effect on CEW antixenosis and the QTL-G effect on CEW antibiosis are dependent on the presence of the PI 229358 allele at QTL-M. After preliminary substitution mapping, QTL-M has been mapped to a 0.52-cM segment on LG M. These results should increase the feasibility of cloning QTL-M and should provide guidance in further formulating a strategy for the effective development of an insect resistant soybean cultivar.

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