A Retrospective DNA Marker Assessment of the Development of Insect Resistant Soybean

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ABSTRACT

There has been limited success over the past 30 yr in the development of superior soybean cultivars [*Glycine max* (L.) Merr] with insect resistance. Success may be hampered by the quantitative nature of resistance and by linkage drag from resistant plant introduction (PI) donor parents. Soybean insect resistance quantitative trait loci (SIR QTLs) have been identified from PI 229358 and PI 171451 by restriction fragment length polymorphism (RFLP) analysis. The objective of this study was to tag the SIR QTLs from PI 229358 with simple sequence repeat (SSR) markers and to determine the extent to which the SIR QTLs have been introgressed in registered cultivars, germplasm releases, or breeding lines that have resistance derived from this PI or from PI 171451. Marker analysis defined intervals by 5 centimorgans (cM) or less for a SIR QTL on linkage group D1b (SIR-D1b), and for SIR-G, SIR-H, and SIR-M. SIR QTLs were tracked through pedigrees by evaluating the inheritance of PI alleles at marker loci tightly linked to the QTLs during the phenotypic selection for insect resistance. It was inferred that at least 13 of the 15 SIR genotypes studied had introgressed SIR-M. PI genome introgression around SIR-M was measured to assess linkage drag. Some genotypes exhibited a dramatic reduction in the amount of linked PI genome, which likely occurred in response to phenotypic selection for agronomic performance as a means of reducing linkage drag. Only a few genotypes were inferred to possess SIR-G or SIR-H, and no genotypes possessed SIR-D1b. The results of this study indicate that marker-assisted selection for SIR QTLs is needed to introgress these loci into elite genetic backgrounds.

The development of soybean cultivars with insect resistance (soybean insect resistance, SIR) has been an objective of several public breeding programs in the USA over the past few decades (All et al., 1999). Most breeders have utilized Japanese PI 229358 or PI 171451 as the initial donor parent for SIR (Lambert and Tyler, 1999). In a comprehensive review on SIR, Boethel et al. (1999) reported that these PIs were initially identified as highly resistant to Mexican bean beetle [MBB; *Epilachna varivestis* (Mulsant)], and later were characterized as having resistance to several insect pests of soybean. They also indicated that SIR in both PIs is quantitatively inherited and that they exhibit antixenosis (nonpreference) and antibiosis (detrimental effect on insect development) resistance properties.

There have only been three cultivars released with SIR derived from a PI, and none of these cultivars has been widely accepted by growers because of inadequate resistance levels, inferior seed yield, or poor agronomic characteristics (Lambert and Tyler, 1999). The lack of development of superior SIR cultivars may be due to the quantitative nature of resistance and to the retention of undesirable PI donor alleles affecting any number of traits because of their tight linkage with the insect resistance alleles, or QTL. This condition is often associated with the use of nondomesticated germplasm for the introgression of novel alleles and is generally referred to as linkage drag.

The use of marker-assisted selection (MAS) could circumvent linkage drag by enabling concurrent selection for SIR QTLs and against undesired genomic regions from a resistant PI during backcrossing. MAS could also reduce the need for phenotypic selection that may be inefficient in identifying genotypic differences for SIR. Towards this end, Rector et al. (1998, 1999, 2000) used RFLP markers to map SIR QTLs from PI 229358 and from PI 171451. They detected a major SIR QTL conditioning antixenosis and antibiosis within a similar interval on linkage group (LG) M (SIR-M) from both PI 229358 and PI 171451. Another QTL associated with antixenosis in both PI 229358 and PI 171451 was detected on LG H (Rector et al., 1998; 1999). From PI 229358, SIR-D1b was detected for resistance on the basis of antixenosis and SIR-G for antibiosis. SIR-D1b was not detected from PI 171451, and a lack of polymorphic RFLP markers limited detection of SIR-G. The SIR QTLs accounted for much of the variation for resistance in the mapping population, and they were found to exhibit primarily additive gene action. While these RFLP-based maps provide a framework for MAS of SIR QTLs, the exact location of the SIR QTLs is not clear because of sparse RFLP marker coverage. Furthermore, the use of RFLP markers for MAS in a breeding program is difficult because of their low polymorphic content in soybean and their high technical demand. Mapping the SIR QTLs with SSR markers, which are abundant and polymorphic in soybean and practical for high-throughput analysis, would facilitate MAS.

Additional information on SIR QTLs could be obtained by determining the extent to which breeders using phenotypic selection for insect resistance have incorporated the SIR QTLs into breeding lines or cultivars. The availability of molecular linkage maps and pedigree records provides an opportunity to track genomic regions through the breeding process (Shoemaker et al., 1992). Young and Tanksley (1989) first used DNA marker-derived graphical genotypes of tomato (*Lyco-*

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Abbreviations: CEW, corn earworm; cM, centimorgan; LG, linkage group; MAS, marker-assisted selection; MBB, Mexican bean beetle; PI, plant introduction; QTL, quantitative trait loci; RFLP, restriction fragment length polymorphism; SSR, simple sequence repeat; SIR, soybean insect resistance; SBL, soybean looper; VBC, velvetbean caterpillar.

persicon esculentum Mill.) cultivars to compare the extent of introgression of donor [*Lycopersicon peruvianum* (L.) Mill.] parent genome from the introgression of *Tm-2* for *Tobacco mosaic virus*. Lorenzen et al. (1995) used graphical genotypes and pedigree records to track genomic regions from ancestral soybean genotypes to modern cultivars. King et al. (1999) tracked *Vf* for scab resistance [causal agent *Venturia inaequalis* (Cooke) G. Wint.] in apple (*Malus*) accessions and assessed linkage drag through a genome scan of regions flanking the introgression site. Narvel et al. (2001) used a molecular pedigree analysis to track rxp for bacterial pustule resistance [causal agent *Xanthomonas campestris* pv. *glycines* (Nakano) Dye] in elite North American soybean.

A genetic linkage map of SIR QTLs consisting of highly informative markers could be used as a tool to track the QTLs in soybean germplasm and enable an assessment of the effectiveness of breeding techniques used to develop SIR. By evaluating genotypes developed by different programs, selection for SIR QTLs can, in retrospect, be assessed in diverse genetic backgrounds and environments. Such an analysis may identify the difficulties that have hampered the development of superior SIR cultivars and identify a useful MAS strategy. The objectives of this study were to develop a SSR-based linkage map of SIR QTLs from PI 229358 and to utilize the map for estimating the extent to which SIR QTLs have been introgressed in registered cultivars, germplasm releases, or experimental lines that have insect resistance derived from this PI or from PI 171451. The introgression process for SIR-M was studied by estimating the amount of linked PI genome to this QTL in the SIR genotypes.

MATERIALS AND METHODS

SSR-Based Mapping of SIR QTLs

The Cobb × PI 229358 mapping population and phenotypic data from Rector et al. (1998, 2000) were used in this study. The mapping population consisted of 100 of the 103 original $F_{2:3}$ lines, as DNA samples for the F_2 progenitors of three lines were not available. The methods used to evaluate resistance were described in detail by Rector et al. (1998; 2000). Briefly, antixenosis was measured as the average defoliation of a line from corn earworm [CEW; *Helicoverpa zea* (Boddie)] infestations in field cages across four replications in a single year. Antibiosis was measured as the average larval weight of CEW when reared on detached leaves from a line with the average determined from six leaves across four replications (24 total observations).

Using the integrated genetic linkage map of soybean (Cregan et al., 1999), we chose SSR markers that had the potential to map near the RFLP markers associated with the SIR QTLs. A total of eight SSR markers previously found to be polymorphic between Cobb and PI 229358 were selected near SIR-D1b, six near SIR-G, seven near SIR-H, and nine near SIR-M. The primer sequences for each SSR were obtained from Soybase, a USDA-sponsored genome database (http://129.186.26.94/ssr.html; verified June 6, 2001). Fluorescent-labeled forward primers and nonlabeled reverse primers were obtained from PE-ABI (Foster City, CA). PCR amplicons were analyzed on an ABI PRISM 377 DNA sequencer (AB-PEC, Foster City, CA). The PCR and gel protocols have been previously described (Mian et al., 1999). Data were collected with DNA Sequencing Collection software v. 2.5 and were analyzed with GENESCAN Prism software v.2.1 for allele size determination followed by analysis with GENOTYPER software v.2.5 for some markers. All software programs used for data collection and analysis were from PE/ABI.

Data from polymorphic SSR markers were combined with data from most of the RFLP markers. Marker orders and map distances were determined in MAPMAKER/EXP 2.0 (Lander et al., 1987) by the Kosambi map function. For grouping markers into linkage groups, a minimum LOD (logarithm of the odds) of 3.0 and a maximum distance of 37.2 cM were used. The positions of the SIR QTLs were estimated by interval analysis in MAPMAKER/QTL 3.0 (Lincoln et al., 1992). A minimum LOD score of 2.0 was used for determination of significance. The percentage of variation explained by each SIR QTL was estimated at the maximum likelihood QTL position. In cases where multiple peaks suggested the possibility of more than one QTL on a linkage group, composite interval mapping (CIM) was used (Zeng, 1994). We used the forward regression with backward elimination option in Windows QTL Cartographer V1.20, with window sizes from 2.0 to 10.0 cM, in an attempt to determine whether there was evidence for more than one QTL on linkage groups with multiple LOD peaks (Wang et al., 2001).

Introgression of SIR QTLs

Two criteria were used to select SIR genotypes for evaluation of introgression of SIR QTLs: (i) variation in coefficient of PI 229358 or PI 171451 parentage, and (ii) development by different breeding programs. A total of 12 genotypes with PI 229358 parentage and three genotypes with PI 171451 parentage was chosen for study (Table 1). The SIR genotypes were grouped into sets according to their program of origin and/or by their pedigree relationships. GatIR-81-296 from Set I is an experimental line that was characterized with resistance under field infestations (Beach and Todd, 1987). The remaining two SIR genotypes from Set I, G85-9853 and G92-18, had been characterized with SIR in the 1996 and 1997 Host Plant Resistance Uniform Tests coordinated by the USDA (Joe Burton, USDA-ARS, Raleigh, NC). The 12 genotypes from Sets II to VI were germplasm releases or registered cultivars that had been characterized with SIR, as indicated in their release articles. Seed for these genotypes and for their parents were obtained from the USDA Soybean Germplasm Collection maintained at the Univ. of Illinois (Urbana, IL). A coefficient of PI parentage was calculated under the assumptions that both parents contributed an equal number of genes to all progeny, the parents were completely inbred, and that the PIs and all noninsect resistant parents were unrelated.

The SIR genotypes and their progenitors were grown in the greenhouse to obtain leaf materials for DNA extraction. An equal number of newly developed trifoliolates from seven plants of each genotype was bulked and dried in a vacuum. DNA was extracted from the dried leaf material by a CTAB protocol (Keim et al., 1988). The SSR analysis protocol was as previously described.

The SIR genotypes were analyzed for SIR QTLs by tracking PI 229358 or PI 171451 alleles at tightly linked SSR markers, as determined from the mapping study. The ability to track a SIR QTL depended on whether the tightly linked markers were informative. An SSR marker was considered informative if the resistant parent possessed alleles different from those of all nonresistant parents in the pedigree of a SIR genotype. Allele data from informative markers were used to evaluate the extent of PI genome introgression in an 82-cM region

Table 1. Description of 15 soybean insect-resistant (SIR) genotypes including their origin of development, pedigree, and their coefficient of parentage for the SIR ancestor PI 229358 or PI 171451.

Set	SIR genotype	Pedigree†	Coefficient of PI parentage	Source
I	GatIR-81-296	GaSoy17 × PI 229358	0.50	Univ. of Georgia
	G85-9853	D77-6103 × GatIR-81-296	0.25	same
	G92-18	Coker 82-622 × G85-9853	0.13	same
II	L86K-96	Williams 82 × L76-0279 (Williams × PI 229358)	0.25	Elden et al. (1992)
	MBB80-133	Union × L76-0038 (Williams × PI 171451)	0.25	same
III	D75-10169	Govan × F_4 line (Bragg × PI 229358)	0.25	Hartwig et al. (1984)
	Lamar	F_4 line (Centennial × D75-10169) × Tracy M	0.06	Hartwig et al. (1990)
IV	N80-50232	Forrest (1)‡ × line 6 [D68-216 × sel.§ (Bragg × PI 229358)]	0.06	Burton et al. (1986)
	N80-53201	Forrest (2) × line 6 [D68-216 × sel. (Bragg × PI 229358)]	0.03	same
	N79-2282	Forrest (2) × line 4 [Govan × F_4 line (Bragg × PI 229358)]	0.03	same
V	HC83-123-9	Pixie × PI 229358	0.50	Cooper and Hammond (1988)
	HC95-15MB	Hobbit 87 × HC83-123-9	0.25	Cooper and Hammond (1999)
	HC95-24MB	Hobbit 87 × HC83-123-9	0.25	same
VI	Crockett	Hampton 266 A × PI 171451	0.50	Bowers (1990)
	D89-9121	Sharkey × T83-5367 (Hampton 266 A × PI 171451)	0.25	Kilen and Lambert (1994)

† Insect-resistant parents in a pedigree are underlined. If not indicated, the pedigree tracing each resistant parent to PI 229358 or PI 171451 can be determined from pedigree information within a set.
‡ The number in parenthesis indicates the total number of backcrosses made to Forrest.
§ Sel = resistant line selected from the corresponding cross.

harboring SIR-M. To compare the amount of PI genome retained among SIR genotypes, graphical genotypes for the 82-cM region were drawn in EXCEL (Microsoft Corp.) by scaling regions between markers according to their estimated genetic distance. In estimating the amount of introgressed PI genome, it was assumed that genomic regions defined by a single marker extended halfway to its flanking marker(s) and that there were no double or even-numbered crossovers between markers.

RESULTS

SSR-Based Mapping of SIR QTLs

The SIR QTLs mapped near the locations identified on the basis of RFLP analysis by Rector et al. (1998, 2000) (Fig. 1 and Table 2). The combined use of RFLP and SSR markers defined QTLs intervals to 5.0 cM or less. SIR- D1b mapped between Satt290 and markers Satt189 or Satt141, which mapped to the same location, with the maximum LOD peak closest to Satt290. SIR-G mapped between Satt472 and L002_2 with the highest LOD peak at Satt472. SIR-H mapped between Sat_122 and Satt541 with the maximum LOD peak near Sat_122. The R^2 estimate, the amount of variation accounted for by a QTL at its LOD peak, in this study was 10% for SIR-D1b, 14% for SIR-G, and 15% for SIR-H, which were similar to the original estimates (Table 2).

On the basis of RFLP marker analysis, SIR-M was previously found to condition both antixenosis and antibiosis (Rector et al. 1998, 2000). With the addition of SSRs, a maximum LOD peak for antixenosis was detected in an interval separate from, but very tightly linked to, an interval for antibiosis. The major peak for antixenosis occurred at A584−4 and was flanked by Satt463 (Table 2). The major peak for antibiosis was detected 0.6 cM from Satt536. The R^2 estimate for SIR-M for antixenosis was 37% and for antibiosis was 21%, which were similar to the original estimates.

The LOD trace for antixenosis and antibiosis on LG-M shows several peaks and valleys near the major peak, particularly for the antixenosis plot, suggesting that multiple SIR QTLs may exist in this region for one or both types of resistance. The LOD profiles obtained by CIM with window sizes of 2.0 to 10.0 cM (for the exclusion of nearby markers as background markers) did not suggest the presence of a QTL on LG M outside of the intervals flanking A584_4 or Satt536 (data not shown). Another explanation for the LOD trace patterns is genotyping errors. Lincoln et al. (1992) suggested that with tightly spaced markers in an interval containing a major QTL, a few genotyping errors could cause sharp peaks and valleys near the major peak. Using "error detection on" in MAPMAKER, we detected only a few candidate genotyping errors, so it seemed that this was not the cause of the ambiguous LOD trace patterns. On the basis of these results, the LG M QTL(s) appear closely linked to A584_4 and Satt536, but it is not clear which marker(s) should be used in MAS for SIR-M. The inheritance pattern of SSR markers covering the genomic region for the antixenosis and antibiosis intervals in insect breeding lines, germplasms, and cultivars was used in part to resolve this issue, as described in the following sections.

Introgression of SIR QTLs

The SIR genotypes have coefficients of PI parentage ranging from 0.5 to a low of 0.03 (Table 1). This served to strengthen the inference that a SIR QTL was introgressed on the basis of the presence of a PI allele at the SSR marker locus tightly linked to the SIR QTL by limiting the probability that the PI allele was inherited by chance. The SSR marker linkages for SIR-H and SIR-M described in Table 2 for PI 229358 were assumed to be similar in PI 171451 because these QTLs were originally detected from each of these PIs by the same RFLP markers. It was recently found by analysis of the PI 171451 × Cobb mapping population for Satt472

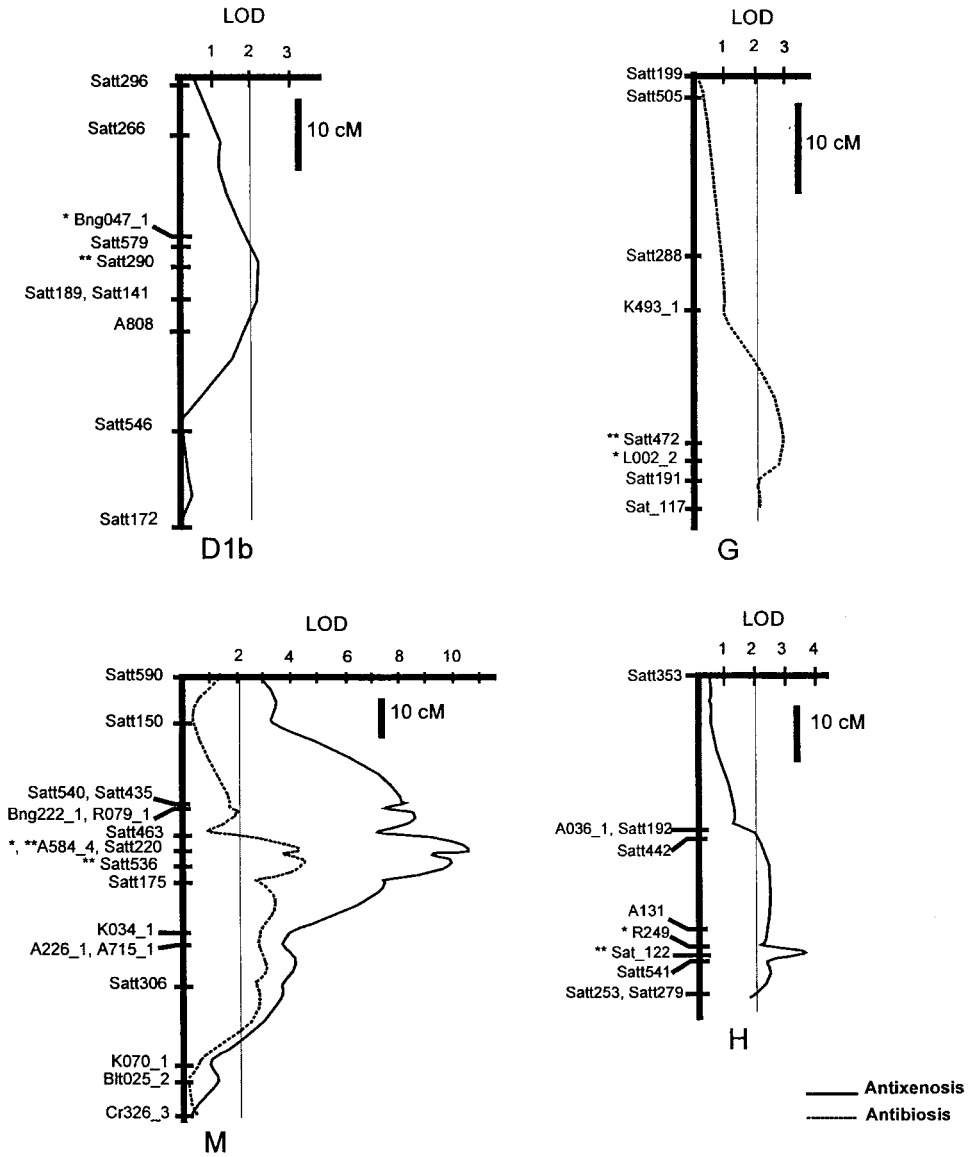

Fig. 1. QTL-likelihood traces for SIR QTLs found to condition resistance to corn earworm in the Cobb × PI 229358 mapping population LGs D1b, G, H, and M. RFLP markers that were found most tightly linked to the SIR QTLs by Rector et al. (1998, 2000) are indicated by a single asterisk. Markers found most tightly linked to the SIR QTLs in this study are indicated by two asterisks. The significance threshold is indicated by a line at LOD = 2.0. Map scales are indicated by bars representing 10 cM. Markers positioned at the same location on a LG mapped less than 0.05 cM apart.

flanking SIR-G that PI 171451 lacks this QTL (unpublished data). Similarly, analysis for Satt290 showed that PI 171451 lacks SIR-D1b, which was consistent with the results from RFLP analysis by Rector et al. (1999).

The SIR genotypes were developed by one of several soybean improvement programs that utilized various breeding strategies in diverse environments. This served to increase the scope of inference as to the robustness of SIR QTL effects. In a few cases, resistance evaluation targeted antixenosis or antibiosis and are noted accordingly. For all other selection schemes, it was assumed that no particular resistance mechanism was targeted. Breeding and selection procedures are summarized to show differences among programs. Complete descriptions for the procedures are provided in the release articles indicated in Table 1 or referenced herein.

Introgression of SIR-M

On the basis of the results from interval mapping, SSR markers Satt463, Satt220, and Satt536, which span the antixenosis and antibiosis intervals of SIR-M, were used to estimate the frequency of introgression for this QTL. Graphical genotypes for the genomic regions flanking SIR-M were drawn on the basis of allele data from eight of the nine SSR markers surveyed on LG-M; Satt540 was not used because it was found to be monomorphic. The graphical genotypes were used to monitor introgression of SIR-M and to assess linkage drag (Fig. 2). Linkage drag was assessed under the assumption that in a cross with a PI, the elite parent contributed alleles for traits other than for SIR that were superior to those from the PI. Given this assumption, selection for agronomic performance was expected to lead to

Table 2. Summary of MAPMAKER QTL interval analysis for SIR QTLs conditioning resistance to corn earworm with resistance alleles contributed by PI 229358 in a cross with Cobb.

QTL	Resistance	Interval	Length	QTL position†	LOD	R^2‡
			cM	cM	score	%
SIR-D1b	Antixenosis	Satt141–Satt290	3.7	2.0	2.3	10 (10)
SIR-G	Antibiosis	Satt472–Satt191§	4.4	0.0	3.0	14 (19)
SIR-H	Antixenosis	Sat_122–Satt541	0.5	0.0	3.6	15 (16)
SIR-M	Antixenosis	Satt463–A584_4	5.0	5.0	9.8	37 (37)
SIR-M	Antibiosis	Satt220–Satt536	2.5	2.0	4.6	21 (22)

† Position of LOD peak given as distance from the first marker listed in interval.

‡ Numbers indicated in parentheses are the R^2 values reported by Rector et al. (1998; 2000). The estimate for the heritability of antixenosis was 64% (Rector et al. 1998), and for antibiosis was 62% (Rector et al., 2000). SIR-J conditioning antibiosis (R^2 = 19%), with the resistance allele contributed by Cobb, was not evaluated in this study.

fixation of non-PI alleles at SSR loci loosely linked to SIR-M and that additional hybridization and selection events would further reduce the amount of introgressed PI genome.

The SIR genotypes from Set I were developed by the Univ. of Georgia Soybean Breeding Program over the course of 20 yr. GatIR-81-296 was selected for resistance to defoliation in the field under natural infestations, primarily consisting of soybean looper [SBL; *Pseudoplusia includens* (Walker)], and velvetbean caterpillar [VBC; *Anticarsia gemmatalis* (Hübner)] (Beach and Todd, 1987). GatIR-81-296 is homozygous for PI 229358 alleles at Satt463, Satt536, and Satt220 and for all other markers analyzed on LG-M, except that it is heterogeneous for Satt590 and Satt150. G85-9853 and its progeny G92-18 were selected for resistance to defoliation by CEW, VBC, and by SBL in a greenhouse assay designed primarily to assess antixenosis, as described by All et al. (1989). A final selection for resistance to CEW was conducted in artificially infested field cages, followed by selection for seed yield and agronomic characteristics. G85-9853 and G92-18 both have the PI allele for Satt536 and each lacks the PI allele for the seven other markers spanning the 82-cM window around SIR-M. Although it is possible that recombination between Satt536 and flanking genomic regions in the development G85-9853 could have changed the linkage phase with SIR-M, the probability is low given that the PI allele was retained by G92-18. Moreover, these data likely reflect a tight linkage relationship between Satt536 and SIR-M. The graphical genotypes show that crossover events flanking Satt536/SIR-M led to a reduction of introgressed PI genome from 80% in GatIR-81-296 to 5% in G85-9853 in a single breeding cycle. This reduction suggests elimination of linkage drag as opposed to random selection of recombination events, i.e., selection for seed yield and agronomic performance fixed non-PI alleles near SIR-M.

The SIR genotypes in Set II were developed by a combination of bulk and pedigree selection for resistance to MBB feeding in the field and in the laboratory. L86K-96 does not have the PI 229358 allele for Satt463 or Satt536, and Satt220 is not informative, so it could not be inferred whether this genotype has SIR-M. MBB80-133 has its resistance derived from PI 171451, and has the alleles of this PI for Satt463, Satt220, and Satt536. Because selections were made primarily on the basis of resistance to MBB, detection of SIR-M in MBB80-133 indicates that SIR-M conditions resistance to a coleopteran. There were two markers out of the eight analyzed on LG-M that were not informative in MBB80-133, which limited an adequate assessment of the amount of PI genome retained by this genotype.

The resistant line derived from 'Bragg' × PI 229358 that was crossed to 'Govan' in the development D75-10169 (Set III) was selected for resistance to defoliation under natural field infestations of MBB. D75-10169 was selected for resistance to defoliation by SBL in field cages, followed by selection for resistance to VBC from evaluations made in Brazil. 'Lamar' was selected for resistance to SBL as described for D75-10169. D75-10169 has the PI allele for markers Satt463, Satt220, and Satt536, while Lamar only has Satt536. These results show that the PI allele for Satt536 in Lamar was retained across four cycles of hybridization, generation advancement, and selection indicating a tight linkage of Satt536 to SIR-M. Lamar has approximately 57% less PI genome than D75-10169 within the region studied. As was indicated for G85-9853, this suggests that selection among SIR lines for seed yield and agronomic performance reduced linkage drag.

The SIR genotypes in Set IV were derived from backcrossing with selections made for resistance to defoliation by MBB or CEW. On the basis of their coefficients of PI parentage alone, the probability that N80-50232 would possess a PI allele by chance is about 6% and for N80-53201 is 3%. Both genotypes have the PI allele for Satt463 and Satt536, and Satt220 is not informative in these genotypes or in N79-2282. N79-2282 has the genotype of its nonresistant recurrent parent ('Forrest') for Satt463 and Satt536, suggesting that this line lacks SIR-M. N80-50232 and N80-53201 have relatively large segments of PI genome within the 82-cM region of LG-M. From the registration notice for these SIR genotypes, there is no indication that replicated progeny testing for yield or other agronomic characteristics was conducted at any stage during backcrossing or if selections were made among lines after backcrossing. A lack of selection based on replicated tests may explain the extent of PI genome retained by N80-50232 and N80-53201 despite their low coefficients of PI parentage.

The SIR genotypes in Set V were developed by pedigree selection involving a MBB larval antibiosis assay in the laboratory with leaf tissue obtained from greenhouse or field-grown plants, as described by Rufener et al. (1987). HC 83-123-9 has the PI allele for Satt463, Satt220, and Satt536, as do HC95-15MB and HC95-24MB, except that Satt220 is not informative in these genotypes. As was indicated for MBB80-133, introgression of SIR-M in these genotypes demonstrates that the QTL has resistance properties towards a coleopteran. HC95-15MB and HC95-24MB show a reduction of PI genome flanking Satt536/SIR-M when compared with their resistant parent HC83-123-9.

'Crockett' was the only cultivar analyzed that was released as possessing PI 171451 parentage, and the resistant parent of D89-9121, T83-5367, was derived

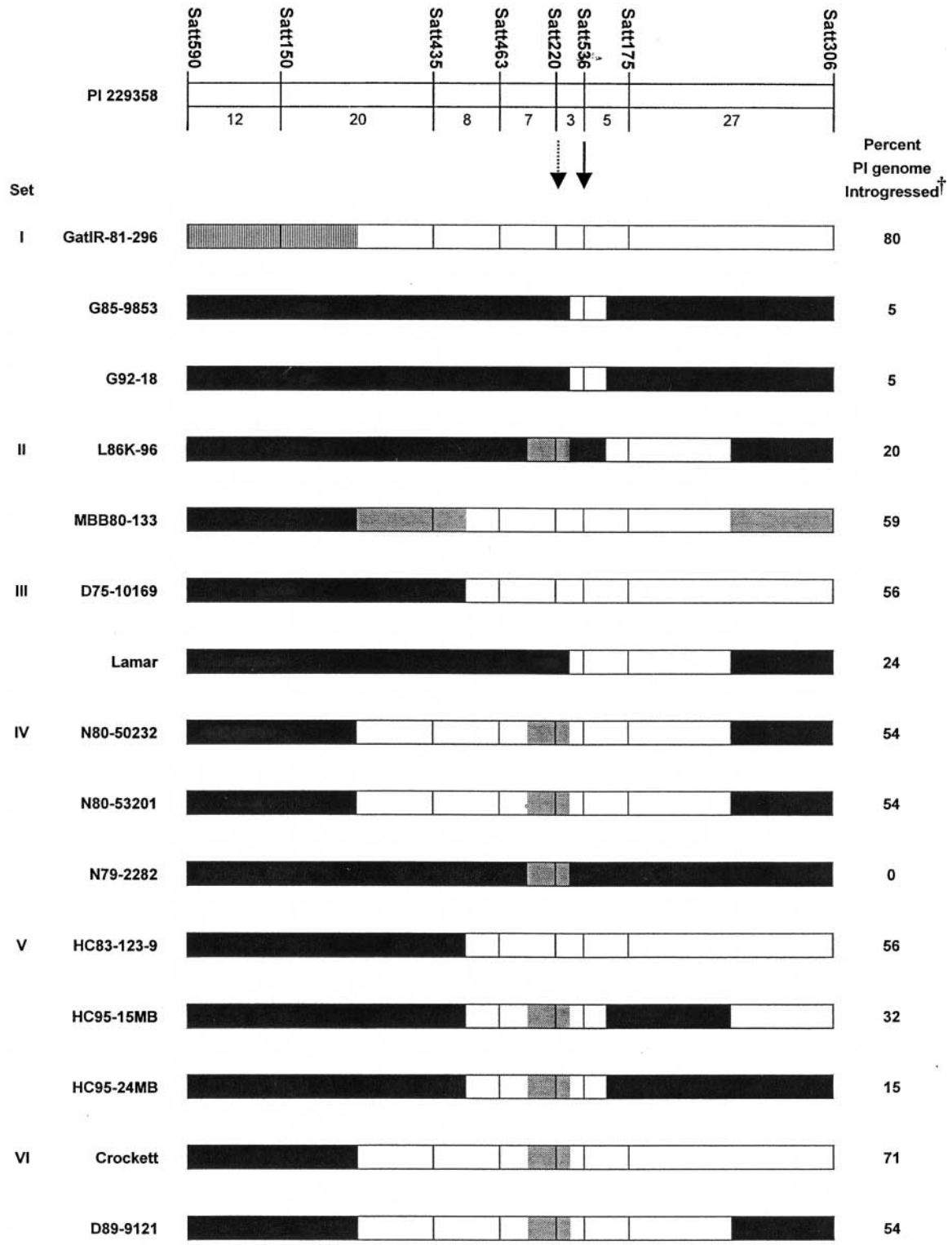

Fig. 2. Graphical genotypes of an 82-cM segment of LG M containing SIR-M for PI 229358 and for the 15 SIR genotypes described in Table 1. Numbers shown between markers in PI 229358 are estimated genetic differences in centimorgans. As described in Table 2, maximum LOD scores occurred in different, but adjacent intervals for different resistance effects. The most likely position for the antixenosis QTL is indicated by a dotted arrow and for the antibiosis QTL by a solid arrow. Genomic segments were coded according to the origin of the SSR allele. White segments indicate PI 229358 (or PI 171451) origin, black segments indicate non-PI origin, vertical-striped segments indicate that a genotype was heterogeneous for a locus (found only in GatIR-81-296 for Satt590 and Satt150), and gray segments indicate that a locus was not informative at that genotype. † The percentage of PI 229358 or PI 171451 genome introgressed was estimated from informative markers (e.g., the estimate of zero for N79-2282 does not include the region defined by Satt220).

from the same population that produced Crockett. Extensive resistance evaluation was used to develop Crockett, including selection against MBB defoliation and against VBC defoliation from separate evaluations made in Brazil, Puerto Rico, and in Texas. D89-9121 was selected for its resistance to SBL in field cages. The analysis for SIR-M and its flanking region indicated that PI 171451 was not the parent of Crockett or D89-9121, but instead was most likely PI 229358. Crockett has the allele that is unique to PI 229358 for Satt435, Satt463, Satt175, and Satt306, and D89-9121 has likewise for most of these markers. On the basis of these results, Crockett and D89-9121 contain SIR-M from PI 229358. Crockett has approximately 71% of PI genome flanking SIR-M, the second largest amount among all SIR genotypes studied.

Introgression of Minor SIR QTLs

SIR-D1b, SIR-G, and SIR-H were considered minor QTLs because they individually accounted for less variation than did SIR-M (Table 2). On the basis of the results from interval mapping, Satt290 was used to estimate the frequency of introgression for SIR-D1b, Satt472 for SIR-G, and Sat_122 for SIR-H. An additional SSR marker defining the interval for each of these QTLs was used to monitor recombination, or in some cases, replace the most significant SSR marker if it was not informative. Satt290 and Satt141 were informative in all analyses, but none of the SIR genotypes have the PI-derived allele for either locus, indicating that no genotypes possess SIR-D1b. Satt472 was informative only in Crockett and in D89-9121 and both have the PI 229358 allele for Satt472 and for Satt191, indicating that they possess SIR-G, which also supports their PI 229358 parentage. Satt191 was informative in all other SIR genotypes, except in L86K-96, but none of the genotypes has the PI allele for this marker. GatIR-81-296 and G85-9853 have the PI alleles for Sat_122 and Satt541 flanking SIR-H. Sat_122 and Satt541 were informative in all other SIR genotypes, but none has the PI allele for either marker locus.

DISCUSSION

An SSR-based map of SIR QTLs was developed that improved map resolution over the original RFLP-based maps by Rector et al (1998, 2000). The SSR-based map was used as a tool to track the SIR QTLs through pedigrees and to evaluate linkage drag. One factor limiting this type of analysis is the possibility of recombination between a flanking marker locus and the QTL; however, with the tight linkage relationships shown in Table 2, this possibility seems remote. Another limitation is that a PI allele had some probability of being inherited by chance. The detection of PI alleles in genotypes with low coefficients of PI parentage reduced the probability. Tracking the SIR QTLs through diverse pedigrees provided an opportunity to identify the difficulties that have limited successful development of superior SIR cultivars. It also provided an opportunity to assess the robustness of QTL effects and to identify markers that would be most useful in MAS for SIR.

Tracking the SIR QTLs revealed that 13 of the 15 SIR genotypes apparently have SIR-M, the two exceptions being L86K-96 and N79-2282. Several conclusions can be drawn from these results. The exclusive retention of the PI allele for Satt536 by G85-9853 and transmission of the allele to G92-18 and, similarly, transmission of the PI allele from D75-10169 to Lamar indicates that Satt536 is very tightly linked to SIR-M. It does not clearly determine, however, whether SIR-M is a single QTL or a cluster of tightly linked QTLs with individual or multiple resistance effects. Our results demonstrate that Satt536 is the best marker to use in MAS for SIR-M. Satt536 should be polymorphic in most PI 229358, PI 171451, or PI-derived resistant parent × nonresistant parent crosses because both PIs had an allele that was unique among all genotypes analyzed in this study. If selection based on flanking SSR markers is desired to eliminate the rare possibility of misclassification from a crossover between Satt536 and SIR-M, Satt220 would be the best additional marker to use.

The high introgression frequency of SIR-M indicates that expression of this QTL is not limited by environment or by genetic background; thus, it has complete (or high) penetrance and expressivity. It also can be inferred that SIR-M conditions resistance to multiple lepidopteran species (CEW, SBL, and VBC) and to a coleopteran (MBB). Although the PIs used in this study are known to have resistance to multiple insects, the effect of SIR-M on multiple insects could not be drawn from the mapping study, because only CEW was used to detect the QTL. The effect of SIR-M against insects from different taxonomic orders or genera seems to be rare among insect-resistance QTLs that have been identified in other crops. In a comprehensive review on mapping insect resistance loci, Yencho et al. (2000) listed 233 QTLs that have been mapped in six crop species. From the information they provided, it appears that no single QTL has been reported with effects against insects belonging to different orders or genera. Of the 30 major-single insect resistance genes they reviewed, 29 of them have been reported to confer resistance to a single insect species or to closely related species within the same genus.

Linkage drag is often regarded as a limitation to the use nondomesticated germplasm for the introgression of novel alleles. The extent of linkage drag depends on numerous variables, such as the population size, the number of meiotic generations before selection is applied, and the genomic location of the locus of interest (Hanson, 1959; Stam and Zeven, 1981). These variables were not controlled in this study. Selection for other traits would also have an impact on linkage drag if undesirable alleles from PI 229358 or PI 171451 were linked to SIR-M. This could explain the reduction of PI genome that was observed in HC95-24MB, Lamar, and, in particular, in G85-9853. Although linkage relationships with SIR-M in PI 171451 or PI 229358 are not known, a recent survey in SoyBase, the USDA-ARS Genome Database (http://129.186.26.94/; verified June

6, 2001), revealed that 20 QTLs conditioning an array of traits including seed yield, seed weight, plant height, canopy height, pod maturity, leaf area, leaf width, seed fill period, reproductive period, and flowering have been mapped to (or near) the region of LG-M surveyed in this study. Given the putative importance of this genomic region, it seems quite possible that these Japanese PIs could possess alleles with unfavorable effects under the growing environment of North America.

In the study by Young and Tanksley (1989), wide variations for the size of introgressed donor segments within a 72-cM region surrounding *Tm-2* were detected among tomato cultivars, but in most cases, large donor segments were retained even with extensive backcrossing. For example, a cultivar derived from 21 backcrosses retained a PI segment estimated at 51 cM in length. They concluded that traditional backcross breeding with selection mainly conducted for the trait being introgressed is largely ineffective in reducing the size of linked DNA around an introgressed gene. The analysis of N80-50232 and N80-53201 in this study revealed that backcross breeding was ineffective at reducing PI genome linked to SIR-M. With tight linkages between Satt536 and its flanking markers, linkage drag during backcrossing can be minimized by selecting those backcross progenies that possess the PI allele only for Satt536.

On the basis of a codescent of PI alleles at marker loci tightly linked to the minor SIR QTLs, it was inferred that Crockett and D89-9121 possess SIR QTL-G and that GatIR-81-296 and G85-9853 possess SIR QTL-H. Because CEW was not solely used to select for resistance in these genotypes, it is likely that either SIR QTL-G, or -H confer resistance to multiple insects, although not as convincingly as shown for SIR-M. Limited introgression for the minor SIR QTLs may have been due to the inability of the resistance screening procedures to differentiate between lines possessing SIR-M and any of the minor SIR QTLs. On the basis of the strong effect for SIR-M, it could conceivably mask the effect of any other QTL in a given resistance screening procedure. It is also possible that the expression of SIR QTL-D1b, -G or -H is environment specific or genetic-background dependent. Another potential cause is a masking effect of insect resistance QTL(s) contributed by the parents that were considered to be susceptible to insect feeding. Variation for measurable levels of resistance among conventional soybean cultivars has been reported (Rowan et al., 1991). Lack of apparent introgression of SIR-D1b suggests that it may not be real. Beavis et al. (1998) indicated that detection of QTLs with minor effects in small populations may be false positives. The population size used for mapping was approximately 100 individuals, and SIR-D1b did have a relatively low LOD score compared with the other SIR QTLs, and it explained only about 10% of the variation for antixenosis.

The highly informative level that was observed for Satt141 and Satt290 flanking SIR-D1b and for Sat−122 and Satt541 flanking SIR-H demonstrates that these markers should be polymorphic in most crosses to a nonresistant parent. MAS for either SIR QTL could be achieved with one of the flanking markers given the short QTL interval length or, as was indicated for SIR-M, both flanking markers could be used to minimize misclassification from recombination. As shown on the integrated genetic map of soybean (Cregan et al., 1999), the genomic regions harboring SIR-D1b and SIR-H are rich in SSR markers. This will enable a reduction of linkage drag through selection for non-PI alleles at SSR loci neighboring these QTLs, as was described for SIR-M. Selection for SIR-G will not be as easy based on the limited informativeness that was observed for Satt472. In addition, the genomic region harboring SIR-G contains few publicly available SSR markers on the integrated map. Additional SSRs or other types of markers, such as single nucleotide polymorphisms (SNPs), will need to be added to this region to facilitate MAS for SIR-G. MAS will enable SIR QTL pyramiding because it will be easier to determine when a plant carries multiple QTL by detecting molecular markers than by using insect bioassays, for which the environment can strongly affect resistance and because other QTLs can have masking effects.

The SSR marker assessment of SIR breeding lines and cultivars conducted in this study provides some insight into the lack of success in the development of productive SIR cultivars. In their review, Lambert and Tyler (1998) indicated that virtually none of the SIR germplasm releases possess insect resistance levels equal to those of PI 229358 or PI 171451. Their observation is supported by our results. Although breeders and entomologists seem to have been successful in transferring SIR-M into soybean lines, their success for the minor SIR QTLs appears limited. On the basis of the SSR marker data of the germplasm analyzed in this study, which represents a large proportion of the SIR genotypes released to date, the development of a cultivar with the four SIR QTLs would require crosses between Crockett or D89-9121 and G85-9853 or GatIR-81-296. We have used MAS to develop near-isogenic lines of 'Benning' by selecting for the PI 229358 allele at polymorphic SSR loci nearest to SIR-D1b, SIR-G, SIR-H, and SIR-M and for the Benning allele at several SSR loci flanking these SIR QTLs and at loci distributed throughout the genome (unpublished data). These lines will seemingly represent the only soybean germplasm with stacked insect resistance from a PI. The use of MAS for SIR is more appealing considering the potential to pyramid resistance from multiple sources. For example, unique SIR QTLs have been identified from PI 227687 (Rector et al., 1999, 2000). MAS pyramiding of SIR QTLs from this PI along with those from PI 229358 or PI 171451 might lead to transgressive segregates with resistance levels that have not yet been observed. When our findings are considered in the context of the limited success of over 30 yr of traditional breeding for SIR, MAS for insect resistance in soybean is justified.

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