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Mapping Resistance to Multiple Races of *Heterodera glycines* in Soybean PI 89772

Pin Yue, David A. Sleper,* and Prakash R. Arelli

ABSTRACT

Breeding soybean [*Glycine max* (L.) Merr.] cultivars for resistance to soybean cyst nematode (SCN) [*Heterodera glycines* Ichinohe] is the most efficient means to control this pest. Evaluating and developing new resistance germplasm sources for soybean breeding is very important to prevent SCN from race shifts and provide durable resistance in soybean. We evaluated a newly found germplasm, soybean plant introduction (PI) 89772, which is resistant to SCN Races 1, 2, 3, 5, and moderately resistant to Race 14. The objectives were to identify molecular markers associated with SCN resistance, investigate resistant allelic relationships, and evaluate marker-assisted selection efficiency. The F_2 and $F_{2:3}$ populations were produced by crossing PI 89772 with the susceptible soybean cultivar Hamilton. Thirty-nine restriction fragment length polymorphism (RFLP) and 54 simple sequence repeat (SSR) markers found to be polymorphic were used to anchor loci conferring resistance to SCN Races 1, 2, 3, and 5. Two to three loci were found to give resistance to each SCN race. We found that resistance loci for different races could be anchored within the same region on linkage group (LG) G. A region on LG B1 was also shared by loci providing resistance to SCN Races 1, 2, and 5. Our results indicated that no single locus could provide complete resistance to any one SCN race. The major loci combinations provided high levels of resistance. We conclude that these markers could be highly useful in marker-assisted selection.

Soybean is an important agricultural crop worldwide in terms of economic and nutritional value. SCN causes substantial yield loss in soybean. Yield losses due to SCN infection in the USA were estimated at more than $430 million in 1994 (Wrather et al., 1997). Many methods have been proposed to control this pest; however, breeding resistant cultivars has been proven to be the most effective means. Many resistant germplasm sources, such as 'Peking' and PI 88788, have been successfully used in breeding programs. However, the presence of multiple SCN race phenotypes in field populations makes controlling SCN more difficult (Riggs and Schmitt, 1988), and results in a need for soybean cultivars to carry wide ranges of resistance genes. It is necessary to understand the relationships of genes for resistance to multiple races. Three recessive resistance genes, *rhg1*, *rhg2*, and *rhg3*, were identified as controlling resistance in Peking against field populations of SCN found in North Carolina (Caldwell et al., 1960). A fourth gene designated as *Rhg4* was identified in Peking in 1965 (Matson and Williams, 1965). However, in a recent report only three genes, two recessive and one dominant, were verified in Peking conditioning resistance to SCN Race 3 phenotype (Rao Arelli et al., 1992a). Molecular marker analysis placed *rhg1* gene on linkage group (LG) G of the soybean linkage map (Cregan et al., 1999b; Mudge et al., 1997; Webb et al., 1995), and *Rhg4* gene on LG A2 (Matthews et al., 1998; Weisemann et al., 1992). Other resistance genes have not been anchored yet, partially because of lack of informative markers. Studies also indicated that neither the *rhg1* or *Rhg4* resistance alleles provide complete resistance to SCN Race 3 (Webb et al., 1995; Cregan et al., 1999b). Extensive reports have focused on mapping genes for resistance to SCN Race 3 (Concibido et al., 1994; Concibido et al., 1996a, 1996b; Cregan et al., 1999b; Matthews et al., 1998; Mudge et al., 1997; Qiu et al., 1999; Webb et al., 1995). However, very little is known about the resistance loci for other SCN races, and the genetic relationships among resistance genes for different races. Concibido et al. (1996a) reported that there were loci specific for different SCN races. Further understanding of relationships among these resistance genes will help breeders to pyramid these genes for multiple SCN races.

Molecular markers provide powerful tools to study problems in crop plants related to genetics and breeding. Markers are used successfully for gene cloning, marker-assisted selection, and identification of new resistant germplasm sources. Cregan et al. (1999a) reported that over 1000 molecular markers (RFLP, RAPD, AFLP, and SSR) have been developed that covered 20+ linkage groups of soybean. These markers could be very useful for interpreting the relationships among quantitative trait loci (QTL) for resistance to SCN races in soybean.

Soybean plant introduction (PI), PI 89772, introduced

Dep. of Agronomy, 201 Waters Hall, Univ. of Missouri-Columbia, Columbia, MO 65211. Journal series number: 13,029 of Missouri Agric. Exp. Stn Received 5 May 2000. *Corresponding author (SleperD@missouri.edu).

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Abbreviations: AFLP, amplified fragment length polymorphism; RFLP, restriction fragment length polymorphism; RAPD, random amplified polymorphic DNA; LG, linkage group; SCN, soybean cyst nematode; SSR, simple sequence repeat.

into the USA from China in 1930, was recently identified as a useful source that provides resistance to SCN Races 1, 2, 3, 5, and moderate resistance to Race 14 (Diers et al. 1997). In an earlier report, PI 89772 was also found to be resistant to several isolates of Races 1, 2, 3, 5, 6, 9, and 14 (Rao Arelli et al., 1992b). Cluster analysis using RFLP markers indicated PI 89772 was closely grouped with Peking but distant from other known sources for SCN resistance, such as PI 90763, PI 88788, and PI 438489B (Diers et al., 1997; Zhang et al., 1999). This implies that PI 89772 potentially may have some unique SCN resistance loci. However, the genetics of resistance to SCN races in PI 89772 is unknown.

Our objectives were to (i) identify molecular markers associated with SCN resistance loci in a newly found resistance germplasm source, PI 89772, (ii) investigate the allelic relationships among loci controlling different SCN races, and (iii) evaluate the marker-assisted selection efficiency of these markers for use in soybean breeding.

MATERIALS AND METHODS

Plant Materials

PI 89772 is a maturity group IV soybean line with purple flowers, black seed coat and hilum color, and moderate lodging and shattering resistance. Cultivar Hamilton, released from the Illinois Agricultural Experimental Station in 1989 (Nickell et al. 1990), has white flowers, yellow seed coat and brown hilum color, and is reported to be susceptible to all known SCN races. The cross of PI 89772 by Hamilton was made in the summer of 1995 at the University of Missouri Agronomy Research Center located near Columbia, MO. The F_1 seeds were grown in Puerto Rico during the winter of 1995. F_2 seeds were grown at the Agronomy Research Center, Columbia, in 1997. F_2 Plants were thinned to 250 individuals. Young leaf tissues from individual F_2 plants were harvested and were used for DNA isolation and molecular marker analysis. Plants were allowed to set F_3 seeds and these were used for SCN bioassays in the greenhouse.

Seed coat and hilum color of $F_{2:3}$ families were also recorded. Seed coat color of the F_3 families ranged from yellow, brown to black. The hilum color was classified into two categories, brown and black. We tried to use these two traits as phenotypic markers because the *Rhg4* gene was reportedly associated with the *I*-locus, the locus controlling black seed coat color (Matson and Williams, 1965).

SCN Bioassays

The SCN bioassays were performed in the greenhouse during the winter of 1998 with established methods (Rao Arelli et al., 1991) except we germinated the seeds using germination bags, then transplanted seedlings to micropots in a waterbath (Arelli et al., 2000). The SCN race differential set and the susceptible control 'Hutcheson' were used in every cycle of SCN bioassay. The SCN races used in this study were the same as described by Diers et al., (1997), and were tested on the basis of the race classification schemes (Schmitt and Shannon, 1992). The female index (FI, previously called index of parasitism, Golden et al., 1970; Schmitt and Shannon, 1992) was used to evaluate SCN response of each individual seedling. For each SCN race screening, five seeds from each $F_{2:3}$ family were randomly transplanted to micropots within the same waterbath. The response of that $F_{2:3}$ family was expressed as the FI mean of these five individual seedlings.

Molecular Marker Analysis

DNA from leaf tissue of the F_2 population was extracted individually by the established CTAB method (Keim et al., 1988) with minor modifications. Two hundred fifty-eight RFLP probes and 167 SSR primer pairs were first screened for polymorphism between the two parents. Polymorphic probes and primers were then used to genotype the F_2 population. All RFLP probes used in this study were initially developed by P. Keim and R.C. Shoemaker (Iowa State University, USDA-ARS, Ames, IA), and either purchased from Biogenetic Services (Brookings, SD) or recovered from PCR by means of methods recommended by the company. RFLP procedure followed the method described by Qiu et al. (1999) with minor modifications. The SSR primers were developed by P.B. Cregan at USDA-ARS (Soybean and Alfalfa Research Lab, Beltsville, MD), and purchased from Research Genetics Inc. (Huntsville, AL). PCR was performed in 96-well microplates with a final volume of 10 μL. The forward primer was first labeled with ^{33}P-ATP (NEN Life Science, Boston, MA) by T_4 phosphate kinase (Gibco BRL, Gaithersburg, MD). Each reaction included 25 ng genomic DNA, 0.1 μ*M* of primer pair, 20 μ*M* of dNTP mixture, 2.5 m*M* of $MgCl_2$, and one unit of *Taq* Polymerase (Gibco BRL, Gaithersburg, MD). The PCR was conducted on a thermocycler (Hybaid TouchDown, Teddington, UK) using 35 cycles of the following steps: denaturing at 94°C for 30 s, annealing at 48.8°C for 30 s, and extending at 68.8°C for 45 s. After the last cycle, the program was designed to extend at 68.8°C for 5 min. The amplified products were then separated by 5% (w/v) denaturing polyacrylamide gel (5% arcylamide, 0.25% bisarcylamide, 8 *M* Urea, dissolved in 1× TBE buffer). The gel was then dried in a 150°C oven for 45 min. The dried gel was exposed to X-ray film (Kodak Biomax, EastMan Kodak, Rochester, NY) for one day at room temperature. The film was developed and bands were scored.

Statistical Analysis

The phenotypic responses of F_3 families to different races were tested for normality by Shapiro-Wilk's method (Shapiro and Wilk, 1965).

Polymorphic molecular markers were first tested for Mendelian segregation by SAS FREQ (SAS Institute, Gary, NC). A linkage map was constructed by MAPMAKER/EXP (Version 3.0b, Whitehead Institute, Cambridge, MA) with a minimum LOD ($\log_{10}$ of the likelihood odds ratio) score of 2.0 and a maximum distance of 50 centimorgans (cM). Distances were estimated using the Haldane mapping function (Lander and Botstein, 1989). Linkage groups were designated using some core markers corresponding to the published soybean linkage map (Cregan et al., 1999a).

QTL linkage analysis was conducted by both SAS GLM and MAPMAKER/QTL (Version1.1b). We did not transform the phenotypic data even though we observed that some phenotypic distributions deviated from normal distributions. Some studies have suggested nontransformed data for QTL analysis (Byrne et al., 1998; Mutschler et al., 1996). For interval mapping analysis, the association was considered significant if LOD was equal or greater than 2.5. For ANOVA analysis, SAS GLM procedure was used. Calculation of marker interactions was done in SAS. For overall *F*-test, we declared significance empirically if the *P*-value was less than 0.01, which was approximately equivalent to a LOD of 2.5 in MAPMAKER

in our results. To compare differences of FI among different marker combinations (markers were associated with QTL) for each race, we used the Bonferroni approach to make experimentwise error rate at 0.01 (Christensen, 1996). For example, if we compared the differences of FI means for two marker combinations, we set the $\alpha = 0.0011$ for each comparison because there were a total of nine means.

Degree of dominance of each locus was estimated following Edwards et al., (1987) and Stuber et al. (1987). The additive and dominance effects of the locus were given by MAPMAKER/QTL scan results. The MAPMAKER/QTL defaults to use the F_2 population, but our phenotypic data came from $F_{2:3}$ families. Thus, the dominance effect from MAPMAKER/QTL was underestimated. We corrected this problem by doubling the value of dominance effects from MAPMAKER/QTL results (Falconer and MacKay, 1996). The Dominance/Additive (D/A) ratio was then calculated. Gene action was considered as additive (A) if D/A ratio ranged from 0 to 0.20; partial-dominance (PD) if D/A ratio ranged from 0.21 to 0.80; dominance (D) if D/A ratio ranged from 0.81 to 1.20; and over-dominance (OD) if D/A ratio was larger than 1.20.

RESULTS

Phenotypic Variations

Our results indicated that phenotypic variations of FI against all four races within the two parents were relatively constant with small variations (Table 1). These results indicated that our SCN bioassays were applicable for further analyses. ANOVA results showed that there was significant phenotypic variability among F_3 families for all four races. The average FI of the F_3 population was skewed to the lower end (resistant) (Table 1). The overall responses of different SCN races showed approximately normal distributions. For Race 2, we failed to reject the null hypothesis that the response in the $F_{2:3}$ population was normally distributed (Table 1). However, the null hypothesis was rejected for Races 1, 3, and 5. We noticed that few families had higher FI than the susceptible parent line (more susceptible, data not presented), causing the FI to have a wide range, but the FI mean of the population was skewed to the resistant parent because most of these $F_{2:3}$ families had SCN responses ranging from moderately resistant to moderately susceptible.

Identification of SCN Resistance QTL

A total of 39 RFLP and 54 SSR markers tested were polymorphic in this population. Chi-square test results showed that segregation of one RFLP marker, E011, deviated statistically from 1:2:1 segregation (data not presented). This marker was not included in further analysis. Eighty-seven of these markers were anchored on 14 LGs with four RFLP and only one SSR markers remaining unlinked. Fourteen LGs were covered according to the linkage map published by Cregan et al. (1999a). Most of these markers were mapped to the similar locations as on the public map. However, the map distance between markers did not fit well with the previous map (Fig. 1). Seed coat and hilum colors were also used as markers in this study. However, linkage analysis showed that these two traits did not have strong linkages with any molecular markers.

The linkage map and phenotypic data were used to scan for loci controlling resistance to SCN races. Molecular markers that were found associated with SCN resistance using MAPMAKER/QTL are listed in Table 2. Unlinked markers were screened for possible association with resistance by SAS GLM procedure. However, no unlinked markers were found significantly associated with SCN resistance.

Three intervals were associated with resistance to Race 1. Among these, the QTL on LG G explained the largest proportion of the total phenotypic variation. This locus was anchored 2.0 cM away from SSR marker Satt309. Overall, three loci explained 47.8% of the total phenotypic variation (Table 4). The data indicated that the gene actions at these loci ranged from additive to over-dominant (Table 2).

Two intervals were associated with resistance to Race 2. These two intervals were located on LG B1 and G, respectively. However, these loci explained only 10% of the total phenotypic variation (Table 2). The D/A ratios suggested partial-dominance to over-dominance gene action for these two loci (Table 2).

Two intervals were also associated with resistance to Race 3. A QTL, located about 4 cM away from SSR marker Satt309 on LG G, explained 23% of the total phenotypic variation of the Race 3 response. These two loci were interpreted as having dominance and over-dominance effects, respectively.

Three intervals were found linked to resistance to Race 5. The locus on LG G maps to the same region as loci providing resistance to Races 1, 2, and 3. The locus on LG B1 was closely associated with loci giving resistance to Races 1, and 2. However, no markers explained large proportions of the total phenotypic variation for Race 5 response (Table 2).

Interactions among Marker Loci Associated with Resistance

We found that all resistance loci came from the resistant parent, PI 89772. Homozygous genotypes (A, same as PI 89772 banding pattern) had significant lower aver-

Table 1. Female index (FI) of the F_3 families responses to SCN races, and normality tests.

	Race 1		Race 2		Race 3		Race 5	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
PI 89772	6.3	0.0–9.7	7.3	2.0–12.4	0.9	0.0–1.1	1.7	0.0–4.0
Hamilton	145.5	111.0–209.0	91.8	76.0–166.0	177.9	133.4–193.3	132.7	107.5–180.5
$F_{2:3}$ Families	104.7	0.0–3.304	47.4	0.2–108.7	45.5	0.0–140.0	64.7	0.00–284.5
Shapiro-Wilk's W	0.9698		0.9792		0.9306		0.8538	
P-value	0.01		0.23		0.00		0.00	

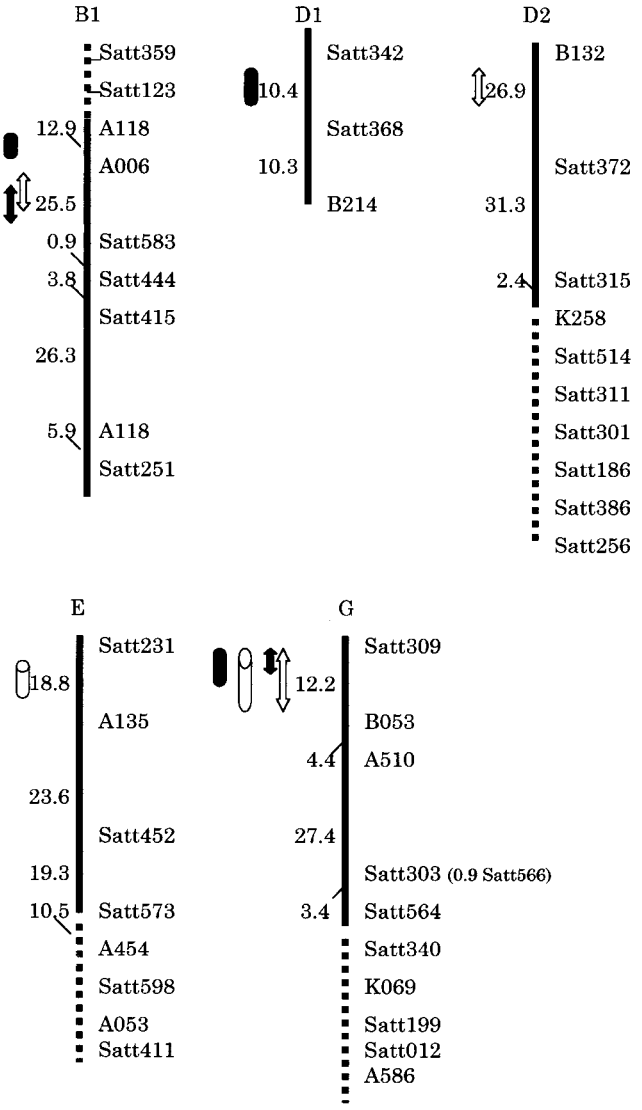

Fig. 1. Soybean molecular linkage map showing positions of loci conditioning resistance to SCN Races 1, 2, 3, and 5. The number given between marker interval indicated the Haldane mapping distance in centimorgans. The square labels indicated relative position of these QTL. QTL position for Race 1 (double-headed open arrow); QTL position for Race 2 (double-headed closed arrow); QTL position for Race 3 (open cylinder); QTL position for Race 5 (closed cylkinder).

age FI ($P < 0.001$) than homozygous genotype B (same as Hamilton banding pattern) (Table 3, Fig. 2). For most loci, heterozygous genotype H had larger FI than the homologous genotype A, indicating that resistances to SCN in this population is controlled mostly by recessive genes.

Our results indicated that some interactions among these marker loci occurred (Table 4). Marker loci giving resistance to Race 1, located on LG B1 and G, respectively, had significant interactions. Two loci controlling resistance to Race 5 also had interactions, these loci were located within the same marker regions as the loci for Race 1. We did not detect significant interactions among marker loci for Races 2 and 3.

We noticed that any single locus failed to provide high levels of resistance to SCN races, and QTL combinations could provide complete resistance. Marker A006 and Satt309 were two major marker loci for Race 1. The average FI means of these $F_{2:3}$ families with marker genotype similar to the resistant parent (we designated as A genotype), PI 89772, were 84.6 and 69.3%, respectively, which could still be classified as susceptible on the basis of the criteria of Golden et al. (1970), and Schmitt and Shannon (1992). However, combinations of resistance QTL gave a higher level of resistance than any single QTL. These $F_{2:3}$ families with marker loci combination of A006 and Satt309 (we designated as A/A genotype) had FI means of 22.2% (Table 4, Fig. 2). This observation was consistent with results from other races in this study.

Table 2. Molecular markers associated with loci giving resistance to soybean cyst nematode Races 1, 2, 3, and 5.

LG†	Interval‡	Length§	QTL-POS¶	LOD value	R^2 (%)	D/A Ratio#
		cM				
		Race 1				
B1	A006–Satt583	25.5	12.0	6.83	16.6	0.18
D2	B132–Satt372	26.9	6.0	4.59	9.7	1.10
G	B053–Satt309	12.2	10.0	13.67	26.6	0.08
		Race 2				
B1	A006–Satt583	25.5	14.0	2.78	6.8	0.17
G	B053–Satt309	12.2	12.0	2.53	4.6	0.87
		Race 3				
E	A135–Satt231	18.8	14.0	3.56	15.7	−0.87
G	B053–Satt309	12.2	12.0	12.65	23.0	−0.44
		Race 5				
B1	A118–A006	12.9	8.0	3.71	9.5	0.75
D1	Satt342–Satt368	10.4	6.0	3.30	7.8	1.87
G	B053–Satt309	12.2	12.0	5.02	10.0	0.70

† LG = linkage group.
‡ The marker interval was given by listing the marker on the top of the linkage group first, then the bottom marker.
§ The distance was given in centimorgans from Mapmaker/EXP version 3.0.
¶ Expressed as the distance from the top marker.
# The D/A ratio = (dominance effect)/(additive effect).

DISCUSSION

A QTL for resistance to SCN Race 3 was found located at the top of LG G, which is consistent with results from other studies (Chang et al., 1997; Cregan et al., 1999b; Webb et al., 1995). This locus was confirmed to be *rhg1* (Concibido et al., 1996, 1997; Cregan et al., 1999), and the SSR marker, Satt309, mapped very close to the gene (Cregan et al., 1999b). Our data indicated that Satt309 was also tightly associated with loci giving resistance to SCN Races 1, 2, and 5 (Table 2, Fig. 1). However, we did not have evidence to declare this to be the same *rhg1* gene. Another important locus for resistance to Race 3 in this study was found located on LG E. Qiu et al., (1999) reported that a resistance locus for Race 3 in Peking was placed on this linkage group. However, no locus was found on LG A2, where the locus was designated as the *Rhg4* gene (Matthews et al., 1998; Weisemann et al., 1992) and tightly linked to the *I*-locus. In our study, even though we had multiple markers on both LGs A1 and A2 (12 and 14 markers,

Table 3. Female index means and standard deviations (SD) of these genotypes with marker A006 in combination with Satt309 for SCN Races 1, 2, 3, and 5.

Markers type		Race 1			Race 2			Race 3			Race 5		
A006	Satt309	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD
A†	A	14	22.2‡§	26.5	14	32.2	16.6	14	13.7‡	17.3	14	24.1‡	27.0
A	H	16	115.2	5.8	21	43.9	19.0	21	38.4	24.4	20	58.1	22.8
A	B	10	123.0	30.2	12	48.8	20.0	11	65.9	16.2	11	83.2	36.1
H	A	20	77.5‡	69.5	24	42.8	16.8	23	38.3	35.2	24	58.6	42.9
H	H	61	99.9	39.9	68	50.7	19.2	64	44.0	31.9	66	65.7	27.7
H	B	25	136.7	37.8	24	42.8	16.8	27	67.6	29.5	26	74.9	57.2
B	A	16	100.3	62.4	16	49.1	18.8	15	30.6	27.0	15	74.5	50.2
B	H	20	105.1	47.9	21	50.6	15.9	21	30.0	20.7	20	72.9	55.9
B	B	17	141.4	54.0	18	51.8	19.8	18	74.0	34.7	15	74.1	25.8
Total		199			218			214			197		

* Significant at $\alpha = 0.0625$ when compared with mean FI of genotype B/B.
† A: genotypes with the same banding pattern as PI 89772; H: the genotypes with heterozygous banding pattern; and B: genotypes with similar banding pattern as Hamilton.
§ Each mean was compared with the mean of B/B marker type with a significant level at 0.00625.

respectively), we did not find any significant association between resistance and these markers. Also the seed phenotypic trait, seed coat color, failed to be assigned to any linkage group when we classified segregating seed coat color as yellow, brown, and black categories. These observations implied that resistance to SCN Race 3 in PI 89772 might not require resistance at *Rhg4*, or the *Rhg4* resistance allele had only a small effect that we failed to detect in this study. This is an important finding for utilizing resistance provided by other than *Rhg4* gene sources.

Very few studies have focused on resistance to SCN Races 1, 2, and 5. RFLP markers on LGs B (B1 or B2), E, H and A2 were found associated with resistance to SCN Race 1 (Heer et al., 1998; Qiu et al., 1999). Vierling et al. (1996) reported that the RFLP marker A006 was associated with one resistance allele and explained 91% of the total variation. We found that the marker A006 on LG B1 was associated with alleles giving resistance to SCN Races 1, 2, and 5 (Table 2). However, neither of these alleles explained large proportions of the total variation. Locus on LG D2 was a newly found locus in this population controlling resistance to Race 1.

One objective of our study was to investigate the relationships among loci providing resistance to different SCN races. Our marker information showed that some loci giving resistance to different races were anchored at a few common marker intervals (Fig. 1, Table 2). This evidence suggested that some genes might be shared for resistance to different races. However, there were always unique loci that were only associated with resistance to certain races. We also noticed that only two to three loci were found associated with resistance to each race, implying that there were only a few major

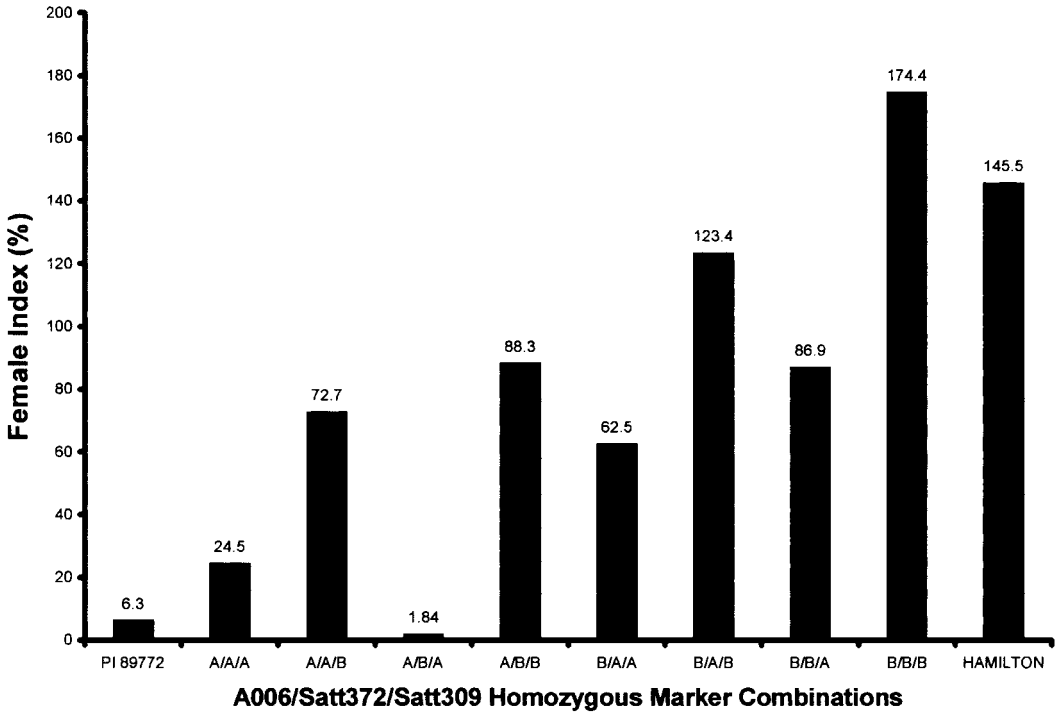

Fig. 2. Female index mean for two parents, and F_3 lines with combinations of homozygous classes of the markers A006, Satt372, and Satt309 for Race 1. A: the genotypes with the same banding pattern as PI 89772; H: the genotypes with heterozygous banding pattern; and B: genotypes with the same banding pattern as Hamilton.

Table 4. Test statistics of markers associated with loci providing resistance to SCN Races 1, 2, 3, and 5.

Markers and interactions†	*F*-value‡	*P*-value	R^2§	Markers and interactions	*F*-value	*P*-value	R^2
	Race 1				Race 5		
A006 (B1)	17.41	0.0001	47.8	A006 (B1)	7.10	0.0011	33.7
Satt372 (D2)	6.75	0.0014		Satt368 (D1)	6.76	0.0015	
Satt309 (G)	33.50	0.0001		Satt309 (G)	14.18	0.0001	
A006*Satt372	0.33	0.8601		A006*Satt368	2.28	0.0620	
A006*Satt309	11.02	0.0001		A006*Satt309	8.36	0.0001	
Satt372*Satt309	0.30	0.8786		Satt368*Satt309	3.01	0.0194	
	Race 2				Race 3		
Satt583 (B1)	5.79	0.0035	9.8	Satt231 (E)	5.38	0.0053	39.4
Satt309 (G)	4.76	0.0095		Satt309 (G)	36.38	0.0001	
Satt583*Satt309	0.73	0.699		Satt231*Satt309	0.59	0.6709	

† The interactions were calculated by SAS PROC GLM. The closest markers to the resistance loci were chosen (as shown in Table 3) for the analysis. Satt372, instead of B132 for Race 1, was chosen because B132 was a dominant marker in this study.

‡ The *F*-values given here were from simultaneous test including interactions. Three-way interactions in the cases for Races 1 and 5 were not significant at 0.01 level; we did not include these two items here.

§ The coefficient of determination (R^2) was the overall estimated proportion of the total phenotypic variation in the simultaneous test.

QTL controlling resistance to each race. This hypothesis was supported by Webb et al. (1995) and Concibido et al. (1996b, 1997). However, these loci explained only a small proportion of the total phenotypic variation, especially for Races 2 and 5. Several reasons could account for the low R^2 value. First, genetic constitutions of SCN race populations are complex. Field populations of SCN are mixtures of many genotypes. Race designation of a field population is based upon the prevalent phenotype in the population (Dong et al., 1997). In our study, the environmental variation caused by variance from bioassay accounted for about 15 to 30% of the total phenotypic variation (variance was estimated on the basis of the phenotypic variance of both parental lines). It is difficult to obtain an accurate estimation of the genetic variance unless one has a much larger sample size, which is very difficult in practice. Second, the experimental design in this study may not be sensitive enough to detect minor-effect QTL. Because the greenhouse bioassay destroys the plant, we used $F_{2:3}$ families to estimate the SCN responses of corresponding F_2 individuals, which is an extensive time and labor consuming procedure. We used five randomly chosen $F_{2:3}$ individuals to estimate the average performance of the F_2 individual, which might have large experimental errors because of the small sample size and segregating characteristics of the $F_{2:3}$ families. Third, our markers may not have been close enough to the QTL. Greater marker saturation may help to find these minor QTL.

The markers identified here could be useful for marker-assisted selection in breeding programs. On the basis of our information, we calculated the changes of FI means resulting from the selection of lines based upon combinations of markers tightly linked with desirable alleles. For example, we chose two independent markers, A006 (B1) and Satt309 (G), which were associated with loci giving resistance to Race 1 (Fig. 1). The efficiency of direct selection for resistance to Race 1 and indirect selection for other races based on these two markers is listed in Table 3. We compared the means of these marker combinations with the mean of marker type B/B using the Bonferroni approach with an experimentwise error rate as 0.05 ($\alpha = 0.00625$ for each comparison, Christensen 1996). When we chose F_3 lines with markers A006 and Satt309, the average FI for Race 1 was significantly lower than for the other genotypes (Table 3). When we selected based on a single marker, none of the genotypes had significantly lower FIs compared with the population mean (Table 3). When we selected based on marker combinations of A006, Satt372, and Satt309 for Race 1, we had similar patterns as selection based on two markers, the A/A/A genotypes had an average FI of 24.5 (Fig. 2). The A/B/A genotype combination had only one line available and gave a resistant response, which we thought was an exception, and allele on LG D2 had a small contribution to the total phenotypic variation (Fig. 2). These results implied that no single SCN resistance gene in PI 89772 could provide complete resistance to a given race. A combination of several major resistance QTL is needed to maximize resistance in PI 89772.

Some markers might be used to select resistance to multiple races. By using markers A006 and Satt309 for Race 1, we noticed improvements of FI means for responses to other races, especially for Races 3 and 5 (Table 3). This observation is reasonable because our results showed associations of resistance loci for different races. Satt309 was found linked with resistance to all four SCN races in this study. Even though A006 was not significantly linked to resistance loci for Races 2, 3, and 5, we found significant improvement of FI for these races by using marker combinations that used A006 in combination with Satt309. Perhaps this may have been a coincidence or there may be small-effects loci associated with A006 that were not detected in this study. Overall, we conclude that major QTL found in this study, for example QTL on LG G and B1, could be used for marker-assisted selection for resistance to SCN from PI 89772, and indirect improvement of resistance to other races by using major markers associated with these QTL for one race is possible. However, it is unclear whether these markers would be useful for identifying resistant germplasm other than PI 89772.

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