Near Isogenic Lines Confirm a Soybean Cyst Nematode Resistance Gene from PI 88788 on Linkage Group J

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ABSTRACT

Soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) is one of the most destructive soybean [*Glycine max* (L.) Merr.] pests worldwide. The most common source of SCN resistance used in soybean breeding in the northern USA is PI 88788. Previous research has shown that PI 88788 carries a major quantitative trait locus (QTL) conferring SCN resistance on linkage group (LG) G, which is believed to be *rhg1*. The objective of our research was to map and confirm additional SCN resistance QTL in Bell, a cultivar with resistance from PI 88788. One hundred four F_4–derived lines (F_4 population) developed from crossing the cultivars Bell and Colfax were tested for associations between 54 molecular markers and resistance to SCN populations PA3 (HG type 7, race 3) and PA14 (HG type 1.3.5.6.7, race 14). Three populations of near isogenic lines (NILs) were developed from F_4 plants heterozygous for a region on LG J where a significant QTL was identified in the F_4 population. The NIL populations were tested with genetic markers and also for resistance to both SCN populations. In the F_4 population, SCN resistance QTL were identified at both *rhg1* and on LG J. The LG J QTL was confirmed in NIL populations and was given the confirmed QTL designation cqSCN-003. The effect of cqSCN-003 was diminished in the NIL populations compared to the F_4 population. This was at least partially the result of segregation distortion in the F_4 population between the region containing *rhg1* and the region containing cqSCN-003. These results show the importance of verifying QTL in confirmation populations to estimate accurately their effects.

THE MOST WIDELY USED SOURCE of resistance to SCN in the northern USA is PI 88788. Diers and Arelli (1999) showed that 230 out of 247 SCN resistant cultivars available for planting in Illinois during 1998 received their resistance from PI 88788 alone. The diversity of resistance to SCN in the Midwest was narrowed further by the use of the cultivar Fayette as the primary source of PI 88788 resistance. Fayette has been widely used in crosses because it combines SCN resistance with high yield and desirable plant phenotype (R. Bernard, personal communication, 1998).

Genetic studies indicate that 'Peking', PI 90763, and PI 88788 have major genes in common that provide resistance to SCN Race 3 (Rao Arelli and Anand, 1988). Further research by Rao Arelli et al. (1992) indicates that Race 3 SCN resistance in PI 88788 is inherited by three genes, with one recessive and two dominant. The genetic evidence indicates that one of the dominant genes is at a previously unreported locus which was designated *Rhg*5 (Rao Arelli, 1994) the second gene is *Rhg4*, which maps close to the *i* gene (Matson and Williams, 1965), and the recessive gene is *rhg2*. Genetic mapping efforts have since shown that PI 88788 has a major QTL on LG G (Concibido et al., 1997), and a second minor QTL on LG C2 (Diers et al., 1997a). The QTL on LG G maps to the same region where a major resistance locus was mapped in PI 437654 (Webb et al., 1995), Peking, PI 90763, PI 89772, and PI 209332 (Concibido et al., 1997; Concibido et al., 1996; Yue et al., 2001). The resistance gene in this region has been designated *rhg1* in the literature and Cregan et al. (1999b) reported a linkage of 0.4 centimorgans (cM) between the simple sequence repeat (SSR) marker Satt309 and *rhg1* in crosses with Peking and PI 209332 as sources of SCN resistance.

Although many QTL have been mapped in soybean, few have been confirmed in additional populations in the same or different genetic backgrounds. The confirmation of QTL after initial mapping is a critical step before the selection of the QTL with markers in breeding programs. Near isogenic line populations are particularly useful for QTL confirmation because they are developed to segregate for QTL in an otherwise homogeneous background. Populations of NIL can be formed quickly by identifying lines in inbred mapping populations that were derived from plants that were heterozygous for the region containing the QTL. Plants from within these lines would be individually harvested to form populations of NIL (Haley et al., 1994).

There is great interest in conducting marker-assisted selection (MAS) for SCN resistance genes since screening with nematodes is tedious and expensive. Soybean PI 88788 continues to be a widely used source of SCN resistance, and mapping of additional SCN resistance genes from this source is necessary. The mapping of these genes will allow efficient MAS in germplasm developed with PI 88788 resistance. The objective of our research was to map and confirm SCN resistance QTL in Bell, a cultivar carrying resistance from PI 88788.

MATERIALS AND METHODS

Plant Material and Soybean Cyst Nematode Evaluation

The mapping of QTL was done in a population developed from a cross between the cultivars Bell (Nickell et al., 1990)

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Abbreviations: cM, centimorgan; LG, linkage group; NIL, near isogenic line; QTL, quantitative trait locus; SCN, soybean cyst nematode; SSR, simple sequence repeat marker.

and Colfax (Graef et al., 1994). Bell has SCN resistance derived from PI 88788 and Colfax is susceptible. The parents were crossed in 1993, and two F_1 plants were grown in 1994 and confirmed as hybrids with morphological markers. F_2 and F_3 generations were advanced by single-seed descent in plantings in Belize during the winter of 1994–1995 and the F_4 seed was planted in the field in Michigan during the summer of 1995. Individual F_4 plants were harvested to form 104 F_4–derived lines (F_4 population).

Three populations that segregate for the region containing the LG J SCN resistance QTL were developed to confirm this QTL. Each population was developed from one of three lines from the original F_4 population. These three lines were selected because each was derived from a plant heterozygous for the region on LG J. These lines were segregating for the SSR markers Satt431, Satt244, and Satt547 on LG J. In addition, the allele at *rhg1* in each line was predicted to be fixed based on results from the SSR marker Satt309, which is closely linked to *rhg1*. The F_4–derived lines were advanced to the F_7 generation as bulks and the NIL populations were developed by threshing individual F_7 plants from the selected lines. There were 48 lines in both NIL populations 1 and 2 (NIL1, NIL2), and 56 lines in NIL population 3 (NIL3).

The lines in both the original F_4 population and the NIL populations were evaluated for resistance to SCN populations PA3 (HG type 7, race 3) and PA14 (HG type 1.3.5.6.7, race 14) in a greenhouse using five plants from each line according to Diers et al. (1997b). In addition, a second resistance test was done on the NIL populations with PA14. The cultivar Hutcheson was included as a susceptible control for each evaluation. A female index (FI) was calculated for each plant using the formula (Golden et al., 1970):

$$\text{FI} = (\text{Number of cysts and females per plant} / \text{Average number of cysts and females on Hutcheson}) \times 100$$

Hutcheson was used as the susceptible check because it provides a consistent susceptible reaction. Because the five plants from each line were not randomized in the SCN tests, the five plants were treated as subsamples in the data analysis. Pearson product-movement correlations calculated with PROC CORR (SAS, 1988) were used to compare the means of lines for resistance to the two SCN populations.

Molecular Marker and QTL Analysis

The lines in the F_4 population were initially tested with 21 restriction fragment length polymorphism (RFLP) and 18 simple sequence repeat (SSR) markers. These markers were selected based on their reported association with SCN resistance in other genetic backgrounds (Chang et al., 1997; Concibido et al., 1994; Concibido et al., 1997; Cregan et al., 1999b; Mahalingam and Skorupska, 1995; Mudge et al., 1997; Webb et al., 1995). An additional 672 SSR markers were used to screen four SCN resistant and four susceptible genotypes in an attempt to identify markers that are likely associated with new resistance QTL from PI 88788. This screening was done by testing PI 88788, the cultivars Fayette, Jack, and Bell, which have resistance from PI 88788, and the susceptible genotypes 'Williams', 'Hardin', Colfax, and LN80-10398. These genotypes were selected because Fayette was developed by backcrossing using Williams as a recurrent parent and PI 88788 as a donor parent; Fayette crossed with Hardin resulted in the development of Jack, and Bell was selected from a cross between LN80-10398 with Fayette. The entire F_4 population was tested with 15 markers that were identified as incorporated from PI 88788 into the SCN resistant cultivars. The NIL populations were tested with only the SSR markers Satt431, Satt244, and Satt547, which map near the LG J QTL.

Genomic DNA was extracted from 10 bulked seedlings per line using a modified CTAB method (Kisha et al., 1997). Genotypic data for RFLP markers were obtained following the protocol described by Diers and Osborn (1994). Simple sequence repeat marker analysis was done with DNA primers developed by Dr. Perry Cregan, USDA-ARS. Polymerase chain reactions (PCR) were performed according to Cregan and Quigley (1997). The PCR products were analyzed by electrophoresis in 3% metaphor (FMC BioProducts, Rockland, ME) agarose gels or 6% (w/v) nondenaturing polyacrylamide gels (Wang et al., 2003).

For the F_4 population, linkage and map distances among the selected markers were determined using the computer program JoinMap (Stam, 1993) with the Kosambi (1944) mapping function and a minimum likelihood of odds (LOD) score of 4.0. The composite interval mapping (CIM) method (Zeng, 1994) was applied to detect QTL with the computer program package QTL-CARTOGRAPHER (Basten et al., 1994, 1999). The CIM was run with model 6 of the Zmapqtl program and a window size of 10 cM for all analyses. The threshold of the LOD score for declaring a putative QTL significant was obtained using 1000 permutations, which is a way to determine experimentwise significance levels and comparisonwise probabilities (Churchill and Doerge, 1994; Doerge and Churchill, 1996). Estimates of the R^2 value and additive effects for each QTL at its peak LOD position were obtained from the output of QTL analysis using the program Zmapqtl in QTL-CARTOGRAPHER (Basten et al., 1999). These R^2 values were obtained by fitting a model including all putative QTL for the respective trait simultaneously.

Single-marker analysis was performed by analysis of variance with PROC GLM in SAS (SAS, 1988) for the NIL and F_4 populations. In addition, single-marker analysis was done for the F_4 population using linear regression with the LRmapqtl program of QTL-CARTOGRAPHER (Basten et al., 1999). The markers with the greatest R^2 values at each independent QTL in the F_4 population were tested in pairs with two-factor analysis of variance with PROC GLM to test for epistatic interactions. The marker with the greatest R^2 value from each independent QTL was included in a multivariate model with PROC GLM to estimate the total phenotypic variance (σ_p^2) explained by the QTL.

RESULTS AND DISCUSSION

F_4 Population

The mean FI of lines in the F_4 population was 53 for PA3 and 83 for PA14. The phenotypic correlation between the PA3 and PA14 FI of lines was 0.68, which suggests that common or linked genes control both traits. No F_4 lines were significantly ($P < 0.05$) more resistant to either SCN population than Bell, the resistant parent (Fig. 1). In contrast, one F_4 line had a significantly greater FI for PA3 and 22 lines had a significantly greater FI for PA14 than Colfax, the susceptible parent.

The lines in the population were evaluated with 39 genetic markers selected primarily on their reported association with SCN resistance in other studies (Chang et al., 1997; Concibido et al., 1994; Concibido et al., 1997; Cregan et al., 1999b; Mahalingam and Skorupska, 1995; Mudge et al., 1997; Webb et al., 1995). In addition, the population was tested with 15 SSR markers that

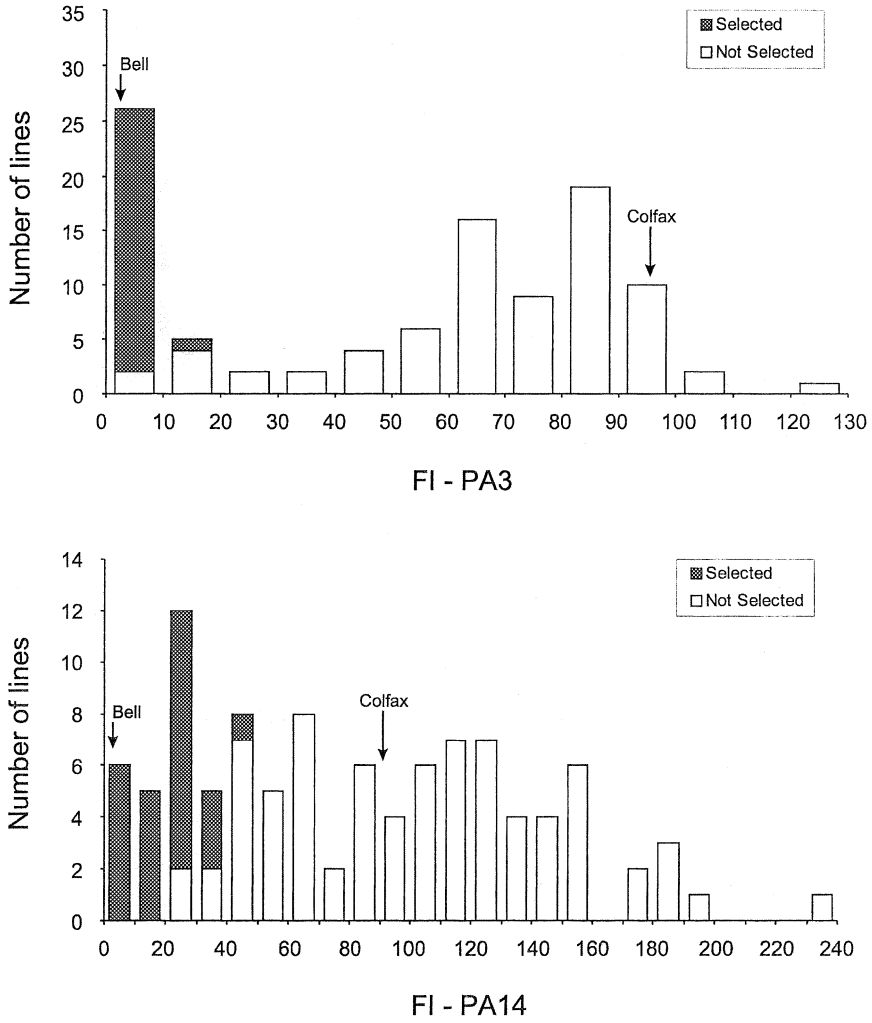

Fig. 1. Distribution of female index (FI) values for PA3 and PA14 in the F_4 population developed from crossing the cultivars Bell and Colfax. Means for the parents are shown by arrows. The shaded portion illustrates the number of lines that would be selected because they are predicted to be resistant because of homozygocity for alleles from the resistant parent Bell for the simple sequence repeat markers Satt309 and Satt431.

were identified in the screening of 672 SSR markers. These 15 SSR markers were selected based on our analysis showing that for these markers, alleles from PI 88788 were incorporated into the screened SCN resistant cultivars. When the marker data were analyzed by JoinMap, the marker order and relative distances were in general agreement with those reported by Cregan et al. (1999a).

The single-factor marker analysis using SAS and QTL Cartographer identified regions on LGs G, J, and C2 that were significantly ($P < 0.01$) associated with resistance to PA3 and PA14 (data not shown). The significant regions on LG G and J were also significant (experiment wise $P < 0.05$) with CIM for both PA3 and PA14 (Table 1). Of the 15 markers identified from the screening of resistant and susceptible genotypes, seven mapped near the QTL on LG J, two mapped near *rhg1*, and the remaining mapped to six other linkage groups. None of the markers on the other linkage groups were significantly associated with SCN. The regions on the other linkage groups were likely incorporated into the resistant lines by chance or because they conferred an agronomic advantage.

The most significant QTL for both SCN populations was *rhg1*, which was mapped near the marker Satt309 on LG G (Fig. 2, Table 1). This is consistent with the report by Concibido et al. (1997) that PI 88788 has a major SCN QTL near Satt309. The region near Satt309 also has been shown to harbor SCN resistance genes from other resistance sources including PI 209332, PI 90763, PI 437654, PI 89772, and Peking (Concibido et al., 1994; Concibido et al., 1997; Concibido et al., 1996; Cregan et al., 1999b; Webb et al., 1995; Yue et al., 2001).

The second QTL mapped to LG J between markers Satt547 and Satt431 (Fig. 2, Table 1). The LOD peaks for the two SCN populations are about 5 cM apart, however, our mapping resolution is insufficient to determine whether the same locus or two different loci control resistance to the SCN populations. Although a QTL providing SCN resistance from PI 88788 has not been previously reported on this linkage group, a QTL conferring Race 3 resistance from PI 90763 and PI 209332 has been mapped in this region (Concibido et al., 1997).

Single-marker analysis revealed that marker Satt277 on LG C2 was significantly associated with resistance

Table 1. Map locations and estimated genetic effects of quantitative trait loci providing resistance to soybean cyst nematode (SCN) in the F_4 population.

Trait†	Map interval	LG‡	Position (cM)§	LOD	*R2*	a¶
FI PA3	Satt309-Bng122	G	3.6	40.6**	0.68	−34.1
FI PA3	Satt547-Satt244	J	25.7	2.5*	0.02	−3.6
FI PA14	Satt309-Bng122	G	3.6	17.7**	0.44	−40.2
FI PA14	Satt244-Satt431	J	30.4	3.4*	0.07	−16.7

* Significant at the 0.05 experimentwise probability level.
** Significant at the 0.01 experimentwise probability level.
† FI PA3, female index for SCN population PA3; FI PA14, female index for SCN population PA14.
‡ LG, linkage group (Cregan et al., 1999a).
§ Position relative to the leftmost marker of the linkage group (See Fig. 2).
¶ Additive effect on FI of an allele from parent Bell.

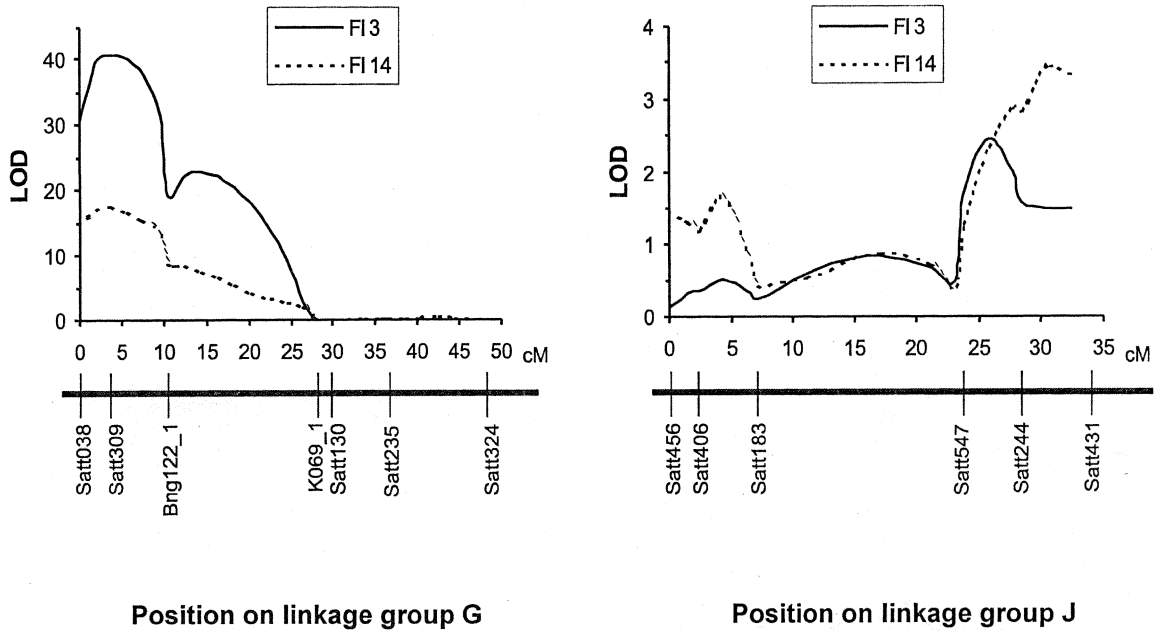

Fig. 2. Likelihood of odds (LOD) plots showing the locations of quantitative trait loci in the F_4 population developed from crossing the cultivars Bell and Colfax. The plots are based on the female index (FI) values for lines after testing with the soybean cyst nematode populations PA3 (FI 3) and PA14 (FI 14).

for PA3 and PA14. The CIM method, however, failed to detect any significant QTL at this location. The only previous report of a QTL providing SCN resistance in this region was by Diers et al. (1997a). This was a preliminary report of mapping SCN resistance QTL in the same F_4 population described in the current study.

A three-factor analysis was done with PROC GLM of SAS (SAS, 1988) using a model that included Satt309, Satt431, and Satt277, the most significant markers on LG G, J, and C2. For both PA3 and PA14, only the markers Satt309, and Satt431 were significant ($P < 0.05$) in the analysis. The two significant markers together explained 87% of the phenotypic variance for PA3 and 64% of the phenotypic variance for PA14. No significant resistance interactions were found between any pairs of markers in the two-way and three-way analyses of variance for either SCN population.

Rao-Arelli et al. (1992) reported that PI 88788 has the SCN resistance gene *Rhg4*, which is closely linked to the *i* gene (Matson and Williams, 1965). The *i* gene controls seed coat color and has been mapped to LG A2 (Shoemaker and Specht, 1995). We tested the markers A085I-1 and GMENOD2B that flank *i* and did not uncover evidence of a QTL conferring resistance to either SCN population in this region. It is possible that PI 88788 had a resistance allele near *i*, but the gene was not transferred in the development of Bell or its parent Fayette. However, during the development of Fayette, Bernard (per. com, 1999) did not observe evidence of linkage between SCN resistance and seed coat color. Rao Arelli et al. (1992) also did not find evidence of linkage between black seed color from PI 88788 and its resistance to SCN Race 3.

Near Isogenic Line Populations

The NIL populations were developed from lines derived from F_4 plants that were heterozygous for the region on LG J that carries the SCN resistance QTL. Because these F_4 plants are inbred, the NILs in each population should segregate on average for only 12.5% of the genome, allowing the effect of the LG J QTL to be tested in a relatively homogeneous background. Based on segregation of Satt309, lines in NIL1 and NIL3

Table 2. Mean female index values of the near isogenic line (NIL) populations tested with SCN populations PA3 and PA14.

	NIL1	NIL2	NIL3
	Female Index		
Predicted allele for *rhg1*†	Susc.	Res.	Susc.
SCN population PA3	57	12	52
SCN population PA14	91	54	71

† The NIL populations were predicted to be fixed for the resistance (Res) or susceptibility (Susc) allele for *rhg1*, a major SCN resistance gene on LG G, based on segregation of the linked marker Satt309.

populations are predicted to be homozygous for the susceptibility allele at *rhg1*, whereas lines in the NIL2 population are predicted to be homozygous for the resistance allele at *rhg1*. These predictions are consistent with the overall means of these populations, with NIL1 and NIL3 having greater mean FI for both SCN populations than NIL2 (Table 2).

The marker Satt431 showed the greatest association with resistance of the three markers on LG J used to test the populations. Across the three NIL populations, Satt431 was significantly associated with resistance in each of the two resistance tests with PA14 ($P < 0.05$) and for the mean of lines across the two PA14 tests ($P < 0.005$) (Table 3). Across the three populations and both SCN PA14 tests, Satt547 was significantly ($P < 0.05$) associated with resistance and Satt244 was not significant (data not shown). When resistance to PA14 was analyzed across both tests and separately for each population, Satt431 was significant at a threshold of $\alpha = 0.05$ for only NIL2 (Table 3), and both Satt244 and Satt547 were significant for NIL3 (data not shown). There was no significant association between any of the markers tested on LG J and resistance to PA3 across all three populations or in any of the individual populations.

These results confirm the presence of a QTL on LG J for PA14 resistance from Bell. Although Satt431 was not significant in all three NIL populations at $\alpha = 0.05$, lines homozygous for the Bell allele for Satt431 showed greater resistance than lines homozygous for the Colfax allele in all populations (Table 3). In the F_4 population, the effect of the LG J QTL was greater for PA14 than PA3 (Table 1), which is consistent with the NIL results. Based on these confirmation results, the SCN resistance QTL on LG J is designated as cqSCN-003 under the category of confirmed QTL at the Soybase website (http://soybase.ncgr.org/). The prefix cq designates that

Table 3. Mean female index values and significance probabilities for genotypic classes of Satt431 across and within near isogenic line (NIL) populations after inoculation with SCN populations PA3 and PA14.

	PA3†		PA14		
Class	Across NIL populations		NIL1	NIL2	NIL3
P > *F*	0.72	0.005	0.11	0.05	0.25
			Female index		
Bell	41	65	84	49	63
Colfax	41	78	101	59	75
Heterozygous	52	71	94	45	74

† PA3, results from one test with SCN population PA3; PA14, results from two tests with SCN population PA14.

Table 4. Observed and expected number of lines in homozygous marker classes for Satt309 and Satt431 in the F_4 population developed from crossing the cultivars Bell and Colfax.

	Satt 309†	
Satt 431	Res	Susc
Res	25 (22)‡	21 (22)
Sucs	7 (22)	35 (22)

† Segregation of the alleles for each marker associated with resistance (Res) and susceptibility (Susc) to SCN.
‡ Number of lines observed and expected (in parentheses).

this QTL has been confirmed according to the rules developed by the Soybean Genetics Committee. Based on this convention, *rhg1* has been given the designation cqSCN-001 and *Rhg4* has been given the designation cqSCN-002.

In the analysis across NIL populations, there was no significant interaction between marker classes for Satt431 and NIL population for either PA3 or PA14. This indicates that the effect of this QTL is independent of whether *rhg1* is present in the background. The difference in average FI between the NIL1 and 3, and the relatively high FI value for NIL2 with PA14, suggests that there are still SCN resistance QTL in the Bell that have not been mapped.

Comparison of Results from F_4 and Near Isogenic Line Populations

We have observed a reduced effect of cqSCN-003 in the NIL populations compared to the effect observed in the F_4 population. It is common to observe inflated QTL effects in mapping studies when relatively small populations are used (Melchinger et al., 1998; Beavis, 1994). A factor that may have inflated the effect of cqSCN-003 was segregation distortion between the regions containing *rhg1* and cqSCN-003 (Table 4). If only lines homozygous for each region were considered, the combined segregation of markers from both regions did not fit expectations ($\chi^2 = 18.4$, $P < 0.01$) in the F_4 population. The distorted segregation was largely the result of fewer lines than expected carrying the resistance allele for Satt309 (Satt309-R) and the susceptibility allele for Satt431 (Satt431-S). The fewer lines than expected in the Satt309-R Satt431-S class would have inflated the difference between the homozygous classes for Satt431 because most Satt431-S lines also carried the susceptibility allele for Satt309 (Satt309-S). This distortion would have made the Satt431-S lines more susceptible than expected based only on the segregation of cqSCN-003. When each marker was evaluated independently, Satt309 deviated from a 1:1 segregation ($\chi^2 = 6.5$, $P < 0.05$) with fewer lines than expected carrying the resistance allele from Bell. In contrast, segregation of Satt431 did not significantly differ from expected ($\chi^2 = 0.18$, $P < 0.5$).

Some of the effects of segregation distortion on cqSCN-003 were controlled by CIM. The proportion of the variability (R^2) for PA3 resistance in the population explained by the LG J QTL dropped from 0.20 in the single-marker analysis to 0.02 with the CIM method (Table 2). A similar, though not as drastic, trend was

observed for PA14 resistance. For PA14, the R^2 value dropped from 0.20 in the single-marker analysis to 0.07 for CIM.

Webb et al. (1995) observed a strikingly similar segregation distortion in a soybean population developed from crossing the SCN resistant PI 437654 with the cultivar BSR101. They found that segregation of the region containing *rhg1*, where Satt309 maps, did not segregate independently from a region on LG M, where a second SCN QTL mapped in their population. Similar to our finding, they observed fewer than expected lines carrying both the resistance allele at *rhg1* and the allele from the susceptible parent at the second region. Further study is needed to uncover possible mechanisms causing this segregation distortion.

In our study, we have confirmed that cqSCN-003 provides resistance to PA14. Marker assisted selection for cqSCN-003 and *rhg1* can be useful for breeders when selecting for SCN resistance with the PI 88788 background since cqSCN-003 can provide greater resistance than is obtained with *rhg1* alone. Further work is need to determine the prevalence of cqSCN-003 in elite breeding material and to determine whether there is sufficient polymorphism among elite lines for markers linked to this gene.

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