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Genetic mapping of soybean cyst nematode race-3 resistance loci in the soybean PI 437.654

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Abstract Resistance to the soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) is difficult to evaluate in soybean [*Glycine max* (L.) Merr.] breeding. PI 437.654 has resistance to more SCN race isolates than any other known soybean. We screened 298 $F_{6:7}$ recombinant-inbred lines from a cross between PI 437.654 and 'BSR101' for SCN race-3 resistance, genetically mapped 355 RFLP markers and the *I* locus, and tested these markers for association with resistance loci. The Rhg_4 resistance locus was within 1 cM of the *I* locus on linkage group A. Two additional QTLs associated with SCN resistance were located within 3 cM of markers on groups G and M. These two loci were not independent because 91 of 96 lines that had a resistant-parent marker type on group G also had a resistant-parent marker type on group M. Rhg_4 and the QTL on G showed a significant interaction by together providing complete resistance to SCN race-3. Individually, the QTL on G had greater effect on resistance than did Rhg_4, but neither locus alone provided a degree of resistance much different from the susceptible parent. The nearest markers to the mapped QTLs on groups A and G had allele frequencies from the resistant parent indicating 52 resistant lines in this population, a number not significantly different from the 55 resistant lines found. Therefore, no QTLs from PI 437.654 other than those mapped here are expected to be required for resistance to SCN race-3. All 50 lines that had the PI 437.654 marker type at the nearest marker to each of the QTLs on groups A and G were resistant to SCN race-3. We believe markers near to these QTLs can be used effectively to select for SCN race-3 resistance, thereby improving the ability to breed SCN-resistant soybean varieties.

Key words Glycine max · Heterodera glycines · RFLP · Genetic mapping

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Introduction

The soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) causes substantial yield loss in North American soybean [*Glycine max* (L.) Merr.] (Mulrooney 1988). For example, susceptible soybean cultivars had 6–36% lower seed yields than did resistant cultivars on SCN race-3-infested sites in Iowa (Niblack and Norton 1992). Since SCN was discovered in North America in 1954 (Winstead et al. 1955), soybean breeding for resistance to SCN has mostly utilized genes from two plant-introduction lines, 'Peking' and PI 88788 (Hartwig 1985). Although these lines have resistance genes for several SCN races, including race-3, they do not provide resistance to all known races.

The plant introduction PI 437.654 is the only known soybean to have resistance to SCN races-3 (Anand 1984), -1, -2, -5, -14 (Anand 1985), -6, and -9 (Rao-Arelli et al. 1992b). However, PI 437.654 has a black seed coat, poor standability, seed shattering, and low yield, necessitating the introgression of its SCN resistance into elite germplasm with a minimum of linkage drag. Conventional breeding with PI 437.654 produced the variety 'Hartwig' (Anand 1991), which is more adapted to cultivation and can be used as an alternative source of SCN resistance in soybean breeding programs.

Resistance to SCN is multigenic and quantitative in soybean (Mansur et al. 1993), though complete resistance can be scored qualitatively. For complete resistance to SCN, PI 437.654 has two or three loci for race-3, two or four loci for race-5, and three or four loci for race-14 (Myers and Anand 1991). The multiple genes

and SCN races involved contribute to the difficulty breeders have in developing SCN-resistant soybean varieties.

Breeding programs for SCN resistance rely primarily on field evaluations where natural nematode populations occur. However, these populations can be mixtures of undetermined races (Young 1982) and the environment can affect the overwintering and infection capability of the nematode (Niblack and Norton 1992). Although evaluations using inbred nematode populations in controlled greenhouse environments are superior, they are prohibitively expensive and the nematodes are difficult to manage for large breeding programs (Rao-Arelli, personal communication). These deficiencies in each evaluation method make SCN resistance a difficult trait to manipulate in soybean improvement programs.

Genetic markers closely linked to important genes may be used to indirectly select for favorable alleles more efficiently than direct phenotypic selection (Lande and Thompson 1990). The *i* allele at the *I* locus, responsible for black or imperfect black seed-coat type, is closely linked in coupling to the SCN resistance allele, Rhg_4, in the variety Peking (Matson and Williams 1965). The *I* locus has been mapped to linkage group VII of the classical genetic map (Weiss 1970) and to linkage group A of a public RFLP map (Keim et al. 1990). SCN race-3 resistance loci are also associated with RFLP markers mapped to linkage groups A, G and K in the soybean PI 209.322 (Concibido et al. 1994).

In the present study, we genetically mapped two loci in PI 437.654 to linkage group A and G which together gave complete resistance to the SCN race-3. We also mapped a locus to linkage group M that was involved with the resistance allele at the locus on group G. Markers linked to these loci may be used for marker-assisted selection during the introgression of SCN race-3 resistance from PI 437.654 into elite soybean germplasm.

Materials and methods

Germplasm development and characteristics

A population of 328 recombinant-inbred lines (RILs) was licensed by Pioneer Hi-Bred International, Inc. from Iowa State University and used in this study. This population originated from a cross between two soybean *max* lines, PI 437.654 and BSR101, and was developed by single-seed-descent inbreeding from the F_2 to the $F_{6:7}$ generation (Baltazar and Mansur 1992; Keim et al. 1994). PI 437.654 is a plant introduction from China in the USDA soybean germplasm collection received from the USSR in 1980 (Nelson et al. 1988). It is in Maturity Group III and is resistant to all known races of SCN. BSR101 was developed at Iowa State University and is in Maturity Group I and is susceptible to SCN (Tachibana et al. 1987). At the *I* locus, PI 437.654 carries the *i* allele for black or imperfect black seed, and BSR101 carries the i^i allele for yellow or green seed. We scored the RIL population for these alleles and mapped the *I* locus as a marker.

Soybean cyst-nematode screening

SCN race-3 resistance screening was conducted in a greenhouse using a homogeneous population of SCN race-3 obtained from soil at the Ames Plantation, near Grand Junction, Tenn. (courtesy of Dr. L. D. Young, USDA-ARS, Jackson, Tenn). This race-3 isolate was increased and maintained for approximately 60 generations on roots of the standard susceptible cultivar 'Essex' in a geenhouse at the Delta Center, University of Missouri, Portageville. In standardized host trials, it averaged 3, 1, 4, 4, and 186 white females (cysts) per plant on Peking, PI 90763, PI 88788, 'Picket 71', and Essex, respectively.

Ten plants from each of 298 $F_{6:7}$ RILs of the PI 437.654 × BSR101 population were evaluated against SCN race-3 in batches of 30 RILs and the five host differentials. The inoculation and evaluation methods were as previously described (Rao-Arelli and Anand 1988; Rao-Arelli et al. 1991b). Thirty days after inoculation, plant roots were washed and the dislodged white females were counted under a stereomicroscope. To minimize the environmentally caused variation in cyst counts among the different batches of lines, an index of parasitism (IP) was calculated for each RIL as a percentage of the cysts on plants of the susceptible variety Essex grown at the same time and under the same conditions.

$$\text{IP} = \frac{\text{Avg. no. of cysts per RIL}}{\text{Avg. no. of cysts per Essex}} \times 100$$

Laboratory methods

DNA of soybean material was extracted using a CTAB method (Murray and Thompson 1980; Keim et al. 1988), with the following modifications. Lyophilized tissue was powdered by adding 2.5 g of glass beads (Fisher cat. #11-312A) and 750 mg of tissue in a 50-ml tube and shaking in a paint-can shaker. The concentration of CTAB (hexadecyltrimethyl-ammonium bromide) in the extraction and precipitation buffers was reduced from 1% to 0.5%. After the DNA was precipitated with CTAB, the DNA pellet was dissolved in 2 ml 1 M NaCl with shaking at 65 °C, 200 rpm, for 2–3 h. The DNA was re-precipitated by adding 4.5 ml ice-cold 95% EtOH. The spooled DNA was washed with 1 ml of 65%, then 1 ml of 85% EtOH, to further remove salts. After the EtOH washes, the DNA was dissolved in 500–1000 µl TE (10, 1), diluted to 500 ng µl^{-1}, and stored at 4 °C until required.

Most RFLP markers used were from *Pst*I-cloned genomic libraries and were either public (Keim and Shoemaker 1988) or proprietary (prefixed php) to Pioneer Hi-Bred Int. Some RFLP markers used were from USDA-ARS (Beltsville, Maryland) cDNA clones (prefixed pBLT). The cloned inserts used as probes were amplified by the polymerase chain reaction. Oligonucleotides of the T_3 and T_7 promoter regions of the phagemid vector pBS$^{+/-}$ were used as amplification primers. The restriction enzymes *Eco*RI, *Hin*dIII, *Eco*RV, *Dra*I, *Taq*I, and *Hae*III were employed to digest the parental and population DNA. Approximately 900 RFLP markers were used against PI 437.654 and BSR101 to identify and map 355 RFLP markers segregating in the RIL population. The DNA digestions, electrophoresis, Southern transfers, and DNA hybridizations were all conducted as described previously (Keim et al. 1989).

Data analyses

Variability was assessed for IP among and within lines based on the linear model:

$$Y_{j(i)} = \mu + Ri + \varepsilon_{j(i)} \quad (1)$$

for $i = 1, 2, 3, \ldots$, 298 RILs; $j = 1, 2, 3, \ldots$, 10 plants per RIL; μ is the mean IP score; Ri is the effect on resistance of recombinant inbred line i; and $\varepsilon_j(i)$ is the variability among plants sampled from each RIL. Effects due to both RILs and plants within RILs are assumed to be random because they are sampled from a theoretically infinite population based on the cross of BSR101 and PI 437.654. An estimate of broad-sense heritability (H) was obtained from the analysis of variance based on (1):

$$\hat{H} = \frac{M\hat{S}_{RI} - M\hat{S}_{residual}}{M\hat{S}_{RI}}$$

where $\hat{MS}_{RI}$ is the estimated mean square of RILs and $\hat{MS}_{residual}$ is the estimated mean square of residual error and a measure of variability due to sampling plants within lines.

Genetic linkage and distances between markers were estimated by maximum-likelihood analysis of segregating RFLP-marker patterns in the RIL population, using the computer program MAPMAKER/EXP 3.0 (Lincoln et al. 1993) and a mapping protocol similar to one described by Landry et al. (1991). Centimorgan distances reported throughout this manuscipt were considered comparable to those that would be obtained using an F_2 population.

The genome was initially scanned for QTLs by calculating likelihood statistics (LOD scores) based on an additive genetic model at each marker locus using MAPMAKER/QTL (Lincoln and Lander 1990). Based upon Lander and Botstein's simulations (1989) and the genome size and density of marker loci used in the present experiment, we decided prior to analyses that a LOD score of 3.0 was an appropriate threshold for declaring linkage of a marker with a QTL.

Interval mapping (Lander and Botstein 1986) with MAPMAKER/QTL to estimate the positions of QTLs relative to their nearby markers was performed with maximum-likelihood tests at positions every 2 cM between adjacently linked markers. Because inbred populations show approximately twice the recombination found in their F_2 generation (Haldane and Waddington 1931), we considered the 2-cM distances used in this interval mapping to be equivalent to the 1-cM distances calculated for our map using the RIL function of MAPMAKER/EXP 3.0.

It is possible that segregation at unlinked markers might explain some of the same phenotypic variability. To decrease bias from multi-colinear or unbalanced data (Knapp et al. 1992), and to assess interaction effects (epistasis) among QTLs, we simultaneously evaluated the markers that had the highest LOD score in genomic regions that exceeded the 3.0 LOD threshold, using a linear model that accomodates multiple marker loci and their interactions:

$$Y_{i(g)} = \mu + M_g + l(M)_{i(g)}, \quad (2)$$

where $Y_{i(g)}$ is the IP score for recombinant inbred line i nested in genotype g, μ is the mean, $g = 1, 2, 3, \ldots G$ and is an index of the genotypic class for the marker loci and their interaction effects M_g, and $l(M)_{i(g)}$ are the random effects of RI line i within genotypic class g.

$$M_g = \sum_m q_g(m) + \prod_{m<m'} q_g(m)q_g(m') + \prod_{m<m'<m''} q_g(m)q_g(m')q_g(m'') + \cdots$$

for $m = 1, 2, 3, \ldots$ marker loci, where g is an index of the genotypic class at marker locus m arbitrarily designed as having zero or two alleles from PI 437.654, and $q_g(m)$ represents the genetic effects of the QTL detected at marker locus m.

Results

Phenotypic variation

The broad-sense heritability estimate for IP was 0.97, indicating the number of cysts per plant within inbred lines was relatively consistent and that sampling variation was low. Having high heritability and expecting few genetic loci contributing to the phenotypic variation, the population size of 298 lines should be adequate to detect the loci (Carbonell et al. 1993).

Fifty five of the two-hundred and ninety-eight lines had complete resistance (IP < 2%) to SCN race-3 (Fig. 1). Eleven lines had IP scores of 5–10%, but after we mapped the SCN race-3 resistance QTLs, examined the marker alleles flanking both sides of these QTLs, and found that these lines most likely had susceptible alleles at one or more of these QTLs, we did not consider these lines to be completely resistant. Our resistance threshold of IP less than 2% was contrary to the usual classification system where resistance is defined as an IP less than 10% (Golden et al. 1970).

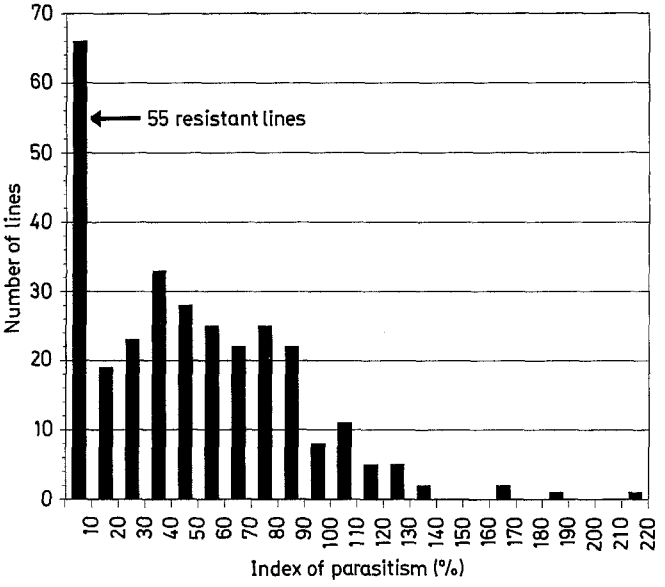

Fig. 1 Frequency distribution of the mean index-of-parasitism scores for SCN race-3 among 298 recombinant-inbred soybean lines from a cross between PI 437.654 and BSR101

Identification of SCN race-3 resistance loci

We identified three significant loci or QTLs associated with SCN race-3 resistance on the independent linkage groups A, G, and M, based on non-simultaneous QTL analyses of individual markers (Fig. 2 and Table 1). These linkage-group designations correspond to those of the June 1994 USDA-ISU RFLP linkage map and were confirmed by comparing band sizes from probe and enzyme combinations in common between the two maps (Randy Shoemaker and Lisa Lorenzen, personal communication). The markers *I*, php05354a, and php02301a had the highest LOD scores at marker positions within groups A, G, and M, respectively. Based on interval mapping, the highest LOD score on group A was at the *I* marker position and, consequently, placement of the QTL relative to the adjacent intervals could not be determined with these analyses. Also based on interval mapping, the most likely placement of the remaining QTLs were approximately 1 cM from php05354a on G and at an approximately equal distance (3 cM) from php02275a and php02301a on M (Fig. 2).

Simultaneous analyses without interactions using the markers *I*, php05354a, and php02301a showed *I* and php05354a were significant and php02301a was non-significant for association with race-3 QTLs (Table 1). Because php02301a was not significant by this test it was excluded from the simultaneous analysis with interac-

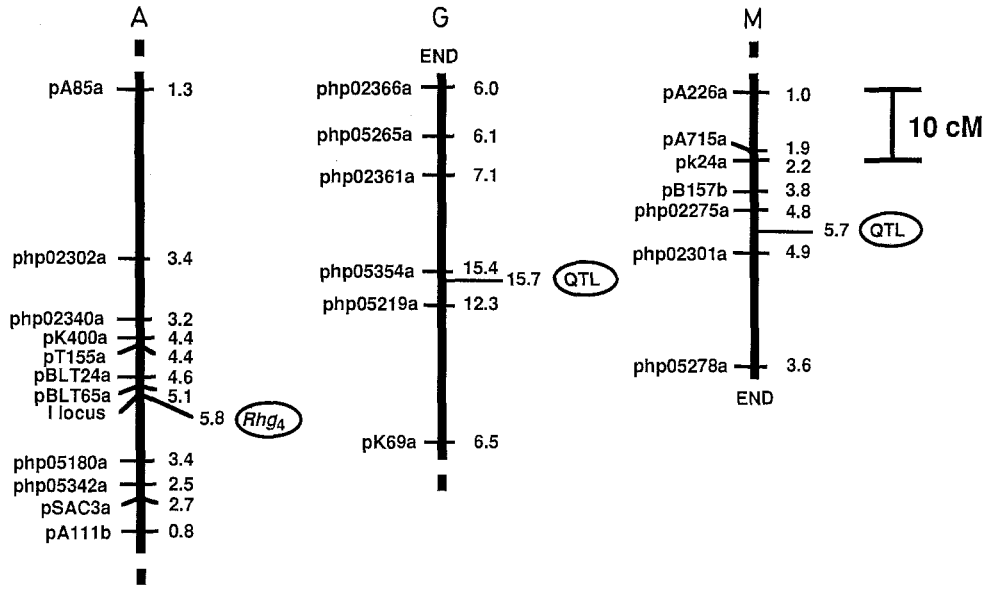

Fig. 2 Locations of RFLP markers and three QTLs, Rhg_4 and two others, associated with SCN race-3 resistance found in PI 437.654 on linkage groups A, G, and M, respectively. Marker names are on the left and marker-QTL LOD scores are on the right of each linkage group. LOD scores at markers were from single-locus analyses of additive gene effects using MAPMAKER/QTL. Genetic distances (cM) were from the recombinant-inbred function of MAPMAKER/EXP 3.0. A distance scale is shown beside group M. The *longest line* to the right of each linkage group shows the estimated QTL positions based on 2-cM interval mapping using MAPMAKER/QTL

Table 1 Test statistics from non-simultaneous analyses and simultaneous analyses without and with interaction for marker and QTL associations based on the index of parasitism for SCN race-3. The coefficient of determination (R^2) is the estimated proportion of phenotypic variation explained by each source

Source	Non-simultaneous estimates		Simulataneous estimates			
	F[a]	R^2	Without interaction		With interaction	
			F	R^2	F	R^2
I locus (A)	25.9	0.09	28.4	0.08	47.2	0.11
php05354a (G)	85.8	0.22	66.6	0.18	103.9	0.24
php02301a (M)	18.6	0.07	0.1	0.00	–	–
A × G	–	–	–	–	21.6	0.05

[a] Based on permutation tests, an $F \geqslant 10.5$ was associated with a 95% probability for marker and QTL association

tion. In the analysis with interaction, the F statistics increased substantially for *I* and php05354a and their interaction was significant (Table 1).

Distortion in marker classes

Of the 298 lines in this population, 252 were homozygous and without missing data for the markers *I*, php0534a, and php02301a on linkage groups A, G, and M, respectively. Having eight possible homozygous classes for three markers combined, we expected 32 lines per class. The actual number of lines in these marker classes are shown in Table 2. The two classes ABA and BBA that had the php05354a allele from PI 437.654 and the php02301a allele from BSR101 had five and zero lines, respectively; substantially fewer lines than occurred in the other classes. Considering only the marker classes for php05354a and php02301a (Table 2), it can be seen that of those lines that had php05354a from the parent BSR101 (A score), about half received php02301a from BSR101 and half from PI 437.654 (B score), as would be expected between independent loci. However, 91 of the 96 lines that had the PI 437.654 allele at php05354a also had the PI 437.654 allele at php02301a.

Apparently, the combination of alleles from PI 437.654 in the region of php05354a with alleles from BSR101 in the region of php02301a was deleterious to survival, and selection occurred during the development of the inbred lines. We cannot distinguish whether this distortion in allele frequencies and the association of these two regions with SCN resistance were a result of pleiotropy, linkage of other genes to the SCN-resistance QTL, or a combination of both situations. However, because this population was developed by single-seed descent withut conscious selection, we attribute the distortion in allele frequencies to natural selection asociated with particular genotypes on linkage groups G and M prior to the maturation of seed. It was not evident whether the loss of genotypes occurred in the gametophyte or after fertilization.

Table 2 Actual and expected number of lines in each of the homozygous classes for the marker combination *I*, php05354a, and php02301a, and for the marker combination php05354a and php02301a. The A allele came from BSR101 and the B allele came from PI 437.654

I/php05354a/php02301a			php05354a/php02301a		
Class	#Lines		Class	#Lines	
	Actual	Expected		Actual	Expected
AAA	46	32	-AA	81	63
BAA	35	32			
AAB	43	32	-AB	75	63
BAB	32	32			
ABA	5	32	-BA	5	63
BBA	0	32			
ABB	44	32	-BB	91	63
BBB	47	32			
Total	252	252		252	252

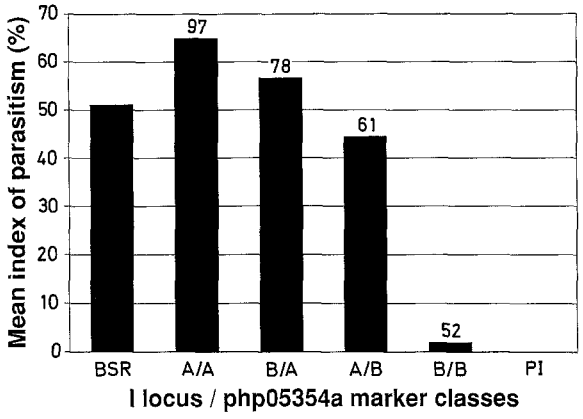

Fig. 3 Mean SCN race-3 index-of-parasitism scores for the parents, BSR101 (labeled *BSR*) and PI 437.654 (labeled *PI*), and their recombinant-inbred progeny for the homozygous classes of the markers *I* and php05354a on linkage groups A and G, respectively. "A" and "B" scores represent BSR101 and PI 437.654 homozygous marker types, respectively. The numbers of lines in the population contributing to each class mean are shown above the columns

QTL effects on SCN resistance

The region on group M near php02301a was involved with SCN resistance to the extent that it was needed in lines carrying the resistance QTL on group G. However, because this was a dependent relationship, these two regions contributed to much of the same variation in phenotypes and the locus on group M was not significant when the loci were tested simultaneously (Table 1).

The effects of the SCN race-3 resistance QTL located within 1 cM of *I* and php05354a could be estimated with minimal bias because of their tight linkage to markers. By several measures, the QTL on group G had a greater estimated effect on resistance than did the QTL on group A. The F statistic and coefficient of determination for php05354a were two-times greater than they were for *I* when tested simultaneously with interaction (Table 1), the difference in least-square means for IP between the homozygous marker classes was greater for php05354a than it was for *I* (Table 3), and lines that had the resistant-parent marker allele at php05354a and not at *I* had a mean IP of 44 which was noticeably lower than the mean IP of 57 for lines that had the resistant-parent marker allele at *I* and not at php05354a (Fig. 3).

Although the locus on G had greater effect on SCN resistance than did the locus on A, having a resistance allele at either of these loci resulted in less infestation of nematodes compared to lines that had the susceptible-parent marker allele at both loci (Fig. 3). However, the degree of resistance provided by each individual locus was similar to the susceptible parent BSR101 (Fig. 3). These data indicate that having a resistance allele at only one locus would not produce a noticeable level of resistance in a commercial soybean variety.

Resistance alleles at the QTLs on A and G were needed together for lines to have nearly complete resistance to SCN race-3. These two loci had a significant interaction (Table 1), the basis of which was that the lines homozygous for the PI 437.654 alleles at both loci had much less SCN infestation than expected from the additive affects of PI 437.654 alleles at the individual loci (Fig. 3).

Table 3 Index-of-parasitism least-square means for the homozygous classes (A and B) at markers *I* (group A), php05354a (group G), and the two markers combined. Class-A came from BSR101 and class-B came from PI 437.654. $\bar{A}$–$\bar{B}$ represents the estimated effect of each locus and the loci combined for the index of parasitism

Marker	$\bar{A}$	$\bar{B}$	$\bar{A}$–$\bar{B}$
I locus (A)	54.6	29.2	25.4
php05354a (G)	60.7	23.1	37.6
A + G	64.8	1.9	62.9

Number of SCN race-3 resistance loci

The 55 resistant lines in this 298-line population deviated significantly ($\chi^2 = 10.0$, $P < 0.01$) from the 37 we would expect when three independent QTLs are required for resistance and each QTL has a normal (1:1) segregation of alleles. The 55 resistant lines also deviated significantly ($\chi^2 = 7.1$, $P < 0.01$) from the 75 we would expect for a two-locus model with each locus having a normal segregation of alleles. We know that when the resistance allele occured on group G, another allele occurred at a locus on group M; violating the assumption of independent loci. Therefore, we did not include the locus on group M to estimate the number of resistant lines expected based on actual allele frequencies in this population. Using the allele frequencies of 0.45 and 0.39 that occurred for the PI 437.654 alleles at the markers *I* and php05354a, respectively, we would expect 52 resistant lines in this population. This was not significantly different ($\chi^2 = 0.2$, $P = 0.7$–0.5) from the actual number of 55 resistant lines. Therefore, we believe that no

segregating loci other than those identified here contributed to SCN race-3 resistance in this population.

Discussion

Number and nomenclature of SCN race-3 resistance loci

We genetically mapped three loci associated with resistance to SCN race-3 in the soybean PI 437.654. Because all progeny from crosses between Peking and PI 437.654 are resistant to SCN race-3 (Anand 1985; Myers and Anand 1991), the resistance loci mapped here should be in Peking. By the same logic, because all progeny from crosses between Peking and PI 90763 are resistant to SCN race-3 (Rao-Arelli and Anand 1988; Rao-Arelli et al. 1992 a), the resistance loci mapped here should also be in PI 90763.

Although we mapped three loci individually associated with SCN resistance, only the two loci on groups A and G accounted for the genetic variation needed for SCN race-3 resistance. Also, the number of resistance lines found in this population was approximately the number expected based on the allele frequencies at the nearest markers to the QTLs of groups A and G. Though these two resistant loci should be in Peking and PI 90763, it is not clear whether or not those two soybean varieties require a third locus for resistance. Rao-Arelli et al. (1992a) used phenotypic segregation ratios of resistant and susceptible progeny to estimate the number of SCN race-3 resistance loci segregating in F_2 and F_3 populations of Peking × Essex and PI 90763 × Essex. Their F_2 data did not allow them to reject either of their two hypotheses that there are one dominant and two recessive resistance loci, or only two recessive resistance loci, in Peking and PI 90763. Their F_3 data allowed them to reject both these hypotheses, not because of the number of resistance lines but because of the greater than expected number of segregating lines. This leaves open the possibility that Peking and PI 90763 require only the two loci we found in PI 437.654 for SCN race-3 resistance.

Matson and Williams (1965) reported a dominant SCN resistance locus, which they named Rhg_4, which had about 0.35% recombination from the *I* locus in Peking. In our study, the *I* locus was the closest marker associated with a resistance locus on linkage group A (LOD score 5.8, Fig. 2). We assigned Rhg_4 to this resistance locus on our map because its position relative to the *I* locus was approximately the same as found with Peking. We could not confirm the gene action of any resistance locus because the population we used was inbred.

Caldwell et al. (1960) identified three recessive loci, rhg_1, rhg_2, and rhg_3, in Peking for SCN race-1 resistance [race identified after publication (Rao-Arelli et al. 1991a)]. Later, Rao-Arelli et al. (1992a) assigned rhg_1 and rhg_2 to two recessive loci for SCN race-3 resistance which they concluded were in Peking, and selected rhg_2 to be the recessive resistance locus also found in the soybean PI 88788. Because different SCN races were used and no common reference markers existed, the rhg_1 and rhg_2 designations may have been assigned to different loci in each study. It is unknown whether the same loci govern both SCN race-1 and race-3 resistance. We found a race-3 resistance locus in PI 437.654 (also in Peking) on linkage group G, but we cannot distinguish between rhg_1 and rhg_2 based on the assignment of Rao-Arelli et al. (1992a) without knowing which one is in PI 88788. Therefore we did not assign a locus name to the QTL on group G.

Comparison to previous RFLP mapping studies

Some to the RFLP markers we used have been associated by others with the *I* locus and SCN race-3 resistance. Keim et al. (1990) placed pT153 (pT153 equals pT155 in band pattern and two linkage-map locations; P. Keim, personal communication), *I*, and pA111 in this order with distances of 14 and 22 map units, respectively. Our order of these three markers was the same with distance of 7 and 15 cM, respectively. Weisemann et al. (1992) placed pBLT24, *I*, and pBLT65 in this order with distances of 4.4 and 4.0% recombination, respectively. We ordered these markers as pBLT24, pBLT65, and *I* at distances of 1.5 and 0.6 cM, respectively. We expect distances between markers to vary according to population, number of markers, and method of calculation; however, the order of markers is expected to be the same among different populations of the same species. The different order found may be due to marker-scoring errors in one or both of these experiments or to a short chromosomal inversion in one or the other population.

Using PI 209.332 as the source of SCN resistance, Concibido et al. (1994) found pA85 on group A to be significantly associated with SCN race-3 resistance. Additionally, they noted that pA111 on group A was not associated with SCN resistance. They also found that the *I* locus on group A showed some association with resistance but at a level that was not statistically significant. Our results with these three markers clearly placed *I* as the nearest marker to Rhg_4, while pA85a and pA111a were not associated with resistance (Fig. 2). Our linkage of *I* with Rhg_4 at less than 1 cM was similar to the 0.35% recombination estimated by Matson and Williams (1965). We placed six markers between pA85a and the *I* locus for a total distance of 42.4 cM. Concibido et al. (1994) had 10.9 cM between pA85 and the *I* locus with no additional markers between them. For a more direct comparison, we removed the markers between pA85a and the *I* locus from our data and then estimated the distance to be 30.1 cM. The greater recombination between these two markers in our experiment may have contributed to pA85a not being associated with a QTL. However, given the non-significant QTL association of

the *I* locus found by Concibido et al. (1994), it may be the QTL found by them in PI 209.332 on group A is a different locus from Rhg_4 found by Matson and Williams (1965) in Peking and found by us in PI 437.654.

Concibido et al. (1994) reported pK69 on linkage group G associated with SCN race-3 resistance in PI 209.332. We also found this marker associated with a resistance locus on group G (Fig. 2). pK69 had been an end markerof group G (formerly linkage group D, Diers et al. 1992), and we found the resistance locus outside this linkage group of markers. We placed five new RFLP markers beyond pK69, two of which were approximately 5 cM apart and flanked the resistance locus (Fig. 2).

Concibido et al. (1994) also reported the marker pB32 on linkage group K associated with SCN race-3 resistance in PI 209.332. We believe pB32 can hybridize to four loci, two of which we mapped to linkage groups J and K and were not associated with SCN race-3 resistance in PI 437.654. They may have used one of the other two possible marker-loci for this probe. Their pB32 marker was linked to a pK417 marker, which was less significantly associated with SCN resistance. pK417 markers have been mapped to linkage groups A, K, and M on the USDA/Iowa State University public RFLP map (Randy Shoemaker, personal communication). We did not use pK417, but comparing our map with the USDA/ISU public map, the pK417 marker of group A may be near enough to detect linkage to the SCN resistance locus on that group.

PI 209.332 may have a different mode of SCN race-3 resistance than PI 437.654. Rao-Arelli et al. (1993) reported that the SCN race-3 resistance in PI 209.332 is most likely controlled by two loci, one dominant and one recessive. If so, evidence from Concibido et al. (1994) indicates those two loci are on linkage groups A and G, and the pB32 marker used by them may therefore go to group A. If PI 209.332 has three SCN race-3 resistance loci and the pB32 marker used by Concibido et al. is on linkage group K, then PI 209.332 and PI 437.654 may differ, not only by the position of the QTL on group A, but also by PI 209.332 having a race-3 resistance locus on K.

These differences between PI 209.332 and PI 437.654 may be due to different loci for SCN race-3 resistance or to differences in the SCN race isolates used in these studies. Although both isolates were classified as race-3 by their behavior on the standard soybean differentials, they may have been sufficiently different to induce responses from different resistance loci.

Marker-assisted selection

We believe these markers (Fig. 2), or similarly placed markers on groups A, G, and M, can be used for marker-assisted selection of SCN race-3 resistance in soybean breeding. However, because there were few lines in this population that had the susceptible allele on M and the resistance allele on G, markers may only be needed to select for the resistance loci on A and G. The allele on M associated with resistance was naturally selected in lines with the resistance allele on G. This dependent relationship between the loci on G and M should be confirmed in other genetic backgrounds before relying on this marker strategy.

Selecting for resistance based on two markers that flank each QTL should be more reliable than selections based on one marker linked to each QTL. Flanking-marker selection reduces the possibility of not detecting recombination between a marker and the QTL, and consequently, reduces the probability of making a Type-I error (selecting a line that is susceptible). However, when markers are closely linked to the QTL, as were found in our experiment, single-marker selections at each locus may have an acceptable Type-I error rate, substantially reduce the amount of laboratory work, and also reduce the Type-II error rate (not selecting resistant lines).

In this mapping population, all 44 lines with the PI 437.654 marker type at the four nearest RFLP markers flanking the resistance loci on A and G were resistant to SCN race-3. By comparison, all 50 lines with the PI 437.654 marker type at the individual markers nearest the QTLs on A and G were resistant to SCN race-3. If we conducted marker-assisted selection for SCN race-3 resistance in this population using two (single) markers instead of four (flanking), less laboratory work would be needed, no Type-I error would occur, and fewer Type-II errors would occur with the section of six additional resistant lines. Of 55 resistant lines in this population, five would be missed using single-marker selection and eleven would be missed using flanking-marker selection. No Type-I error in selection would be made by either method.

Given that PI 437.654 and Peking have the same resistance loci for SCN race-3, markers linked to these loci should be useful for marker-assisted selection in germplasm related to either source. However, PI 88788, another common source of SCN race-3 resistance, lacks a resistance allele at either A, G, or M and has a resistance allele at a different locus than does Peking (Rao-Arelli et al. 1992a) and PI 437.654. The unique locus in PI 88788 needs to be genetically mapped to identify the necessary markers for marker-assisted selection of all three SCN race-3 resistance loci in populations related to PI 88788.

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