Theor Appl Genet (2001) 102:921–928

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Molecular characterization of resistance to *Heterodera glycines* in soybean PI 438489B

Received: 25 March 2000 / Accepted: 4 August 2000

Abstract Soybean (*Glycine max* L. Merr.) plant introduction (PI) 438489B is a newly found germplasm source that has resistance to multiple soybean cyst nematode (*Heterodera glycines* Ichinohe, SCN) races. We studied the inheritance of resistance to SCN races 1, 2, 3, 5 and 14 in PI 438489B using F_2 and $F_{2:3}$ families, which were generated by crossing to the susceptible cultivar 'Hamilton.' The objectives of this study were to investigate the inheritance for resistance to SCN races in PI 438489B, to find molecular markers associated with resistances, and to study the allelic relationships among resistance loci for different SCN races. The results showed that the responses to SCN races were approximately normally distributed with large environmental effects, and were also highly correlated, which implied that genes giving resistance to different races were similar. The narrow-sense heritabilities of resistance to all five SCN races ranged from 0.55 to 0.88. Fifty one restriction fragment length polymorphism (RFLP) markers and 64 simple sequence repeat (SSR) markers were found to be polymorphic in the F_2 population. Quantitative trait loci (QTLs) associated with resistance to SCN races were anchored on soybean linkage groups (LGs) A1, A2, B1, B2, C1, C2, D1a, E and G. These QTLs explained 47.3%, 45.8%, 51.5%, 34.5% and 37.2% of the total phenotypic variances, respectively, for each race we investigated. Some QTLs for different races encompassed the same region of flanking markers; therefore, QTLs for multiple races may be linked or pleiotropic effects may be involved. Some loci provided resistance in a race-specific manner. Resistance to SCN race 14 had a different pattern compared to other races. Our results indicated that resistance to race 14 did not include loci on LGs A2 and G. These flanking markers associated with QTLs could be used to select for resistance to multiple SCN races in soybean breeding programs.

Keywords Soybean · *Heterodera glycines* · Marker-assisted selection · Molecular marker

Communicated by H.C. Becker

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Introduction

Soybean cyst nematode is a serious pest of soybean worldwide. In 1994, yield-losses due to SCN damage in the USA were estimated at 1.99 million tons with a value of more than $430 million (Wrather et al. 1997). Compared to previous data (2.12 million tons between 1990 to 1994; Wrather et al. 1995), this pest has increasingly become a more dominant disease in soybean producing states. Soybean cyst nematode was first found in Missouri in 1956 and in Illinois by 1959. It is now common in all soybean-producing states and is recognized as the most yield-limiting factor of soybean production in the Midwest of the USA (Doupnik 1993).

Resistant soybean cultivars are the most-effective means to control SCN. Soon after the detection of the pest in the USA, plant breeders included SCN resistance in their breeding programs. Many resistant germplasm sources have been identified. Some have been used in breeding programs successfully, such as the resistance derived from 'Peking' and PI 88788. However, the genetic base of host resistance in soybean is relatively narrow. Most resistant soybean cultivars in the USA have SCN resistance genes from Peking, PI 88788, or both (Hartwig 1985; Rao Arelli 1994). With a restricted genetic base, the pathogen easily overcomes the resistance in soybean cultivars due to adaptation to resistance or "race shifts" (Dong et al. 1997). It is important to collect and characterize genetically diverse sources of resistance and thereby broaden the range and source of SCN resistance genes in soybean breeding.

Soybean PI 438489B, introduced from China, is resistant to multiple SCN Races. This PI line showed resistance to SCN Races 1, 2, 3, 5, 6, 9 and 14 (Diers et al.

1997; Rao Arelli et al. 1992). Cluster analysis results based on molecular analysis indicated that PI 438489B is genetically distant from other known resistance sources, such as Peking, PI 88788, PI 437654 and PI 90763 (Diers et al. 1997). PI 438489B also has a favorable agronomic performance, which makes it an attractive source of SCN resistance for use in a soybean breeding program.

Our objectives were to investigate the inheritance of resistance to SCN Races 1, 2, 3, 5 and 14, to find molecular markers associated with resistance to these races, and to study the allelic relationships for resistance among loci in PI 438489B.

Materials and methods

Plant materials

The cross of PI 438489B by Hamilton was made in the summer of 1995 at the University of Missouri Agronomy Research Center located near Columbia, Mo. Hamilton, released by the Illinois Agricultural Experimental Station in 1989 (Nickell et al. 1990), is reported to be susceptible to all known SCN races. One-hundred and eighty four F_2 plants, along with both parents, were grown at the Agronomy Research Center, Columbia, Mo., in 1996. Leaves from individual F_2 plants were harvested and used for DNA isolation and molecular-marker analysis. Plants were allowed to set seed and these $F_{2:3}$ families were used for SCN bioassays in the greenhouse.

SCN bioassay

The SCN bioassays were performed in the greenhouse during the winter of 1996 using established methods (Rao Arelli et al. 1991). In brief, seeds of all F_2 and $F_{2:3}$ families, their parents and differentials, were germinated in 5×8-cm germination bags, and transplanted into micropots. Twenty three micropots were placed in plastic containers and were maintained at 27±1°C in a thermo-regulated waterbath (Forma Scientific Inc., Marietta, Ohio). Three to five days after transplanting, roots of each seedling were inoculated with 2000±50 SCN eggs by an automatic pipetter (Brewer Automatic Pipetting Machine, Scientific Products, Baltimore, Md.). Approximately 30 days after inoculation, plant roots were individually washed with pressurized water to dislodge the females and cysts. The females and cysts were then counted under a stereoscope. Five seeds in each $F_{2:3}$ family and approximately 120 F_2 seeds were used in each SCN race-screening. In total, 184 $F_{2:3}$ families were screened in this study. In each screening cycle, we also included 10–15 seedlings of each parent and differential lines, such as 'Peking', PI 88788, PI 90763 and 'Pickett-71', for the estimation of environmental variation and race identification. The cultivar 'Hutcheson' was also included as a susceptible control.

The Female Index (FI) was used to evaluate the SCN response of each individual seedling (Golden et al. 1970; Schmitt and Shannon 1992), and was calculated as follows:

$$\text{FI (\%)} = \frac{\text{number of females and cysts on a given individual}}{\text{average number of females and cysts on Hutcheson}} \times 100.$$

Plant DNA preparation and molecular marker analysis

DNA of soybean leaves was extracted using the CTAB method (Keim et al. 1988). For RFLP analysis, individual F_2-plant DNA samples were digested by five different restriction enzymes, *Dra*I, *Eco*RI, *Eco*RV, *Hind*III and *Taq*I. Hybond N^+ nylon membrane (Amersham Pharmacia Biotech Co. Ill., USA) was used as for Southern transfer. All RFLP probes used in this study were developed by R. C. Shoemaker's research group (Shoemaker and Specht 1995), and purchased from Biogenetic Services Inc., Brookings, S.D., USA. Inserts were obtained either directly from Biogenetic Services, Inc., or recovered from polymerase chain reaction (PCR) products. High Prime (Boeringher Mannheim Co. Indianapolis, Ind.) was used for labeling probes according to the manufacture's instructions. In total, 258 soybean probes were first used to screen for polymorphism between two parents. Polymorphic probes were then used to score with DNA from F_2 plants.

Polymerase chain reactions for SSRs were performed in a 96-well microplate at a final volume of 10 µl. 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, Ala.. Forward primer was labeled with ^{33}P-ATP (NEN Life Science, Boston, Mass.) using T_4 phosphate kinase. Each PCR reaction included 25 ng of genomic DNA, 0.1 µM of each primer pair, 20 µM of a dNTP mixture, 2.5 mM of $MgCl_2$, and one unit of *Taq* Polymerase. All chemicals and enzymes were purchased from Gibco BRL (Gaithersburg, Md.). The PCR was conducted on a Hybaid TouchDown thermocycler (Teddington, UK) using 35 cycles with 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 fragments were separated on a 5% (w/v) denatured polyacrylamide gel (5% acrylamide, 0.6% bisacrylamide, 8 M Urea, dissolved in 1× TBE buffer). After separation, the gel was placed in a 150°C oven for 30 min to 1 h to let the gel fully dry. The dried gel was used to expose X-ray film (Kodak BioMax, Eastman Kodak, Rochester, N.Y.) for 1 day at room temperature. The exposed film was then developed for marker scoring.

Statistical analysis

We did not transform the phenotypic data, even though it sometimes deviated from a normal distribution, because studies have suggested untransformed data rather than transformed data are best for QTL analysis (Mutschler et al. 1996; Bryne et al. 1998). A normal distribution is not expected when the trait is controlled by relatively few genes with moderate effects (Doerge and Churchill 1994; Mutschler et al. 1996). Furthermore, normalizing data through transformation may misrepresent differences among individuals by pulling skewed tails toward the center of the distribution (Mutschler et al. 1996).

Narrow-sense heritabilities and standard errors were calculated based on parent-offspring regression using parents, F_2 individuals, and $F_{2:3}$ families' data and assuming no environmental and major dominant effects following the method provided by Roff (1997).

Simple phenotypic correlation coefficients among different races were calculated using the SAS CORR procedure (SAS Institute 1990). Factor analysis was conducted using the SAS FACTOR procedure to compare the similarity of responses to different races in the $F_{2:3}$ families.

Linkage maps were constructed employing MAPMAKER/EXP software (Version 3.0, Whitehead Institute, Cambridge, Mass.), using a minimum LOD ($\log_{10}$ of the likelihood odds ratio) score of 3.0 and a maximum distance of 50 cM to obtain linkage. Distances were estimated using the Haldane mapping function (Lander and Botstein 1989). Markers were assigned to linkage groups by referring to the recent soybean linkage map (Cregan et al. 1999a). Mapmaker/QTL software (Version 1.1b) was used to detect the association between markers and resistance loci. The association was considered significant if LOD was larger than 2.5. Some molecular markers failed to be assigned on the linkage map. We employed the SAS GLM procedure to analyze the association between these markers and possible resistance loci; Bonferroni adjustment was applied to set an experiment-wise error rate at 0.01 (Christensen 1996).

Results

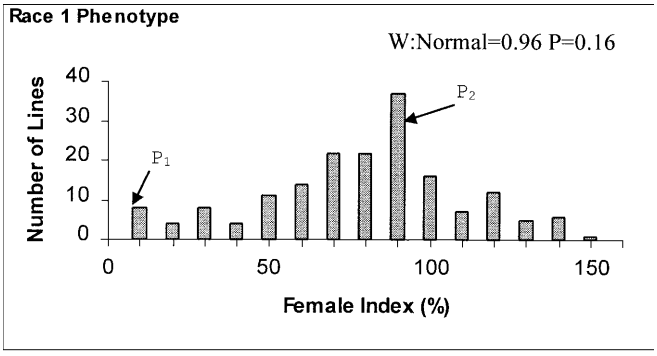
Phenotypic data analysis

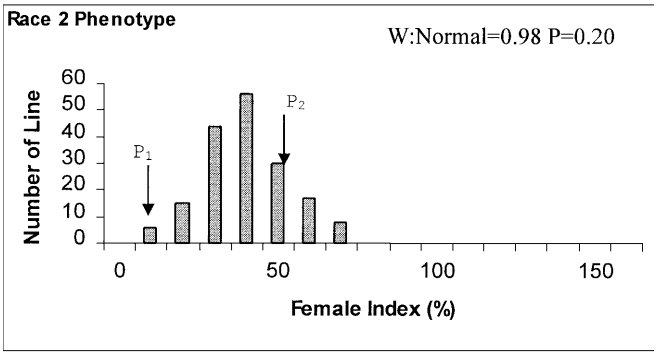
Our data indicated that response to SCN in this population was a quantitative trait, which supported the data of others (Mansur et al. 1993; Concibido et al. 1994; Webb et al. 1995). The responses to SCN races in the $F_{2:3}$ families showed continuous distributions (Fig. 1). We did not have a clear distinction between resistant and susceptible responses. The normality test indicated that responses to races 1 2, and 14 were normally distributed with Shapiro-Wilk's W=0.97, 0.98 and 0.98 (*P*-value=0.16, 0.20, and 0.21), respectively. Responses to races 3 and 5 were not normal distributions, based on statistical results. We noticed skewed trends in the low female index (FI) end in these two populations. In this study, we modelled the inheritance of resistance to SCN races as quantitative traits.

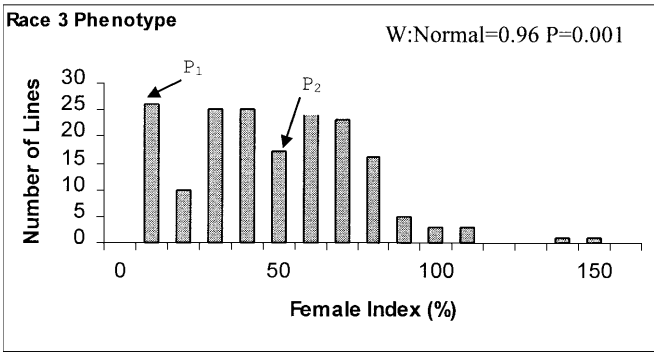
Means and standard deviations of parents and $F_{2:3}$ progenies are listed in Table 1. Transgressive segregation was observed within all five populations (Fig. 1, Table 1). The mean of the population was intermediate to the two parents for SCN races 1, 2, 3 and 5, but larger than the susceptible parent Hamilton for SCN race 14.

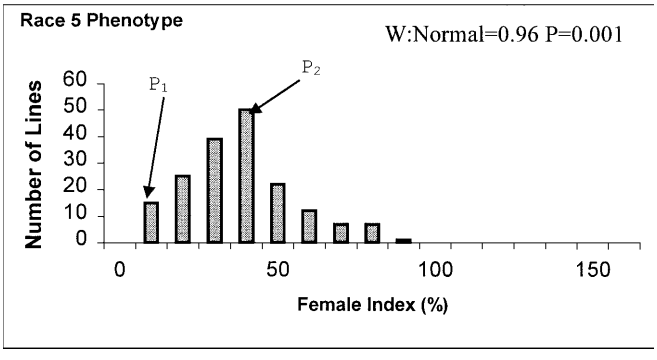
Narrow-sense heritability estimates for SCN resistance ranged from medium to high (Table 1), which agreed with previous results (Mansur et al. 1993). In this study, resistance to SCN races 5 and 14 had relatively higher narrow-sense heritabilities (0.88 and 0.73, respectively). The estimation of heritability for race 1 was the lowest (0.55). Higher heritabilities indicated that few major genes might be involved in controlling resistance to SCN in PI 438489B; however, the estimations were based on the assumption that no environmental and dominant effects were present. Our results indicated that there were some loci behaving as dominant (data not shown), hence some heritabilities may have been overestimated.

Because we used the same $F_{2:3}$ population to test for the responses to all five races, it was possible to compare the response to SCN in each $F_{2:3}$ family. First, we calculated the correlation among the responses to all five races in the $F_{2:3}$ families (Table 2). The data showed that responses to all five races were highly correlated ($P<0.001$), which indicated that some genes for different races may be shared, or at least some resistance genes may be linked and co-segregated during genetic recombination. Further, our marker data confirmed our hypothesis. Among 184 $F_{2:3}$ families, lines 27, 79, 151 and 161 showed complete resistance to all five races (FI<10%), which included approximately 50% of resistant families









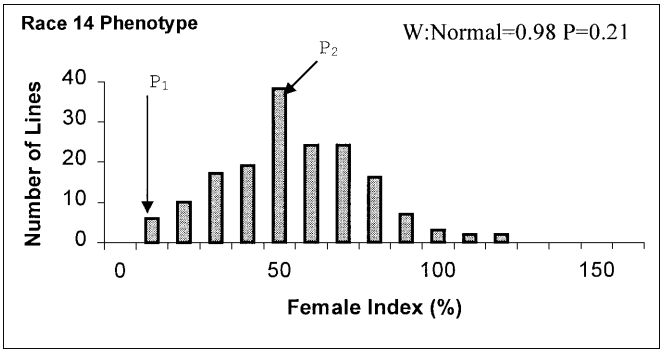


Fig. 1 Frequency distributions of the $F_{2:3}$ families response to five SCN races. The normality test was given at the upper corner of each figure. Responses to races 1, 2 and 14 were statistically normally distributed. Response to races 3 and 5 were skewed to the lower end of the FI. The FI means of both parents were listed in Table 1, and are indicated in the figure as an *arrow head*. Transgressive segregation was observed within all five populations

Table 1 Female index means, narrow-sense heritabilities, and standard errors of parents and $F_{2:3}$ families for SCN races 1, 2, 3, 5 and 14

Line	Race 1	Race 2	Race 3	Race 5	Race 14
PI 438489B	1.9±2.1	5.0±2.8	2.2±3.3	5.2±6.5	5.8±3.1
Hamilton	92.30±15.51	45.21±10.19	124.14±46.38	62.26±21.09	40.84±10.48
$F_{2:3}$ Families	74.4±31.6	34.6±13.5	44.0±27.6	33.0±17.6	50.0±22.5
Heritability	0.55±0.08	0.68±0.08	0.59±0.08	0.88±0.08	0.73±0.07

Table 2 Correlations among the female index for SCN races 1, 2, 3, 5 and 14 in the $F_{2:3}$ families

	Race 1	Race 2	Race 3	Race 5
Race 2	0.39*** 176[a]			
Race 3	0.42*** 176	0.33*** 179		
Race 5	0.34*** 174	0.42*** 177	0.31*** 177	
Race 14	0.30*** 163	0.34*** 165	0.30*** 165	0.39*** 164

*** Significant at the 0.0001 probability level
[a] Number of individuals

Table 3 Molecular markers associated with resistance loci to soybean cyst nematode races 1, 2, 3, 5 and 14

LG[a]	Interval[b]	Length (CM)[c]	QTL POS[d]	LOD Value	R^2 (%)
		Race 1			
B1	Satt583 – Sat_123	19.4	14.0	4.20	12.7
B2	Satt168 – A329	11.4	7.1	2.75	11.7
C2	Satt371 – Satt202	23.9	20.0	6.80	7.1
G	A096 – Satt130	11.4	6.0	9.08	15.8
		Race 2			
A1	A262 – Satt300	18.3	14.0	2.78	7.4
B1	Satt583 – Sat_123	19.4	12.9	2.79	7.4
C1	A463 – Satt396	40.3	10.0	2.56	10.2
E	A656 – Satt452	25.5	20.0	2.57	8.0
G	A096 – Satt130	11.4	2.0	7.52	12.8
		Race 3			
A2	K400 – T155	12.7	8.0	7.00	19.1
B2	Satt168 – A329	11.4	2.0	2.56	8.1
D1a	A398 – K478	21.7	4.0	5.47	10.7
G	Satt130 – Satt012	26.1	4.0	4.46	13.6
		Race 5			
B1	Satt583 – Sat_123	19.4	3.0	2.70	11.0
C2	Satt371 – Satt202	23.9	22.0	3.05	8.3
D1a	A398 – K487	21.7	14.0	4.17	9.4
G	Satt012 – Satt199	9.1	0.0	2.30	5.8
		Race 14			
C1	A059 – A463	22.8	2.0	3.61	11.1
D1a	A398 – K487	21.7	7.0	4.14	7.4
E	A656 – Satt452	25.5	16.0	5.01	18.7

[a] LG=linkage group
[b] The marker interval was given by listing the marker on the top of the linkage group first, then the bottom marker
[c] The distance was given by centiMorgans from Mapmaker/EXP version 3.0
[d] Expressed as the distance from the top marker

in the resistant category for each individual race (data not shown).

Secondly, to further interpret the relationships among responses to different SCN races in this $F_{2:3}$ population, we used factor analysis to detect the similarities of response to different races in these $F_{2:3}$ families. Results from factor analysis indicated that two factors were important. These two factors explained approximately 86% of the total variance (56% explained by factor 1 and 30% explained by factor 2). The linear combination of the two factors were listed as:

factor 1:
y=0.70(Race1)+0.72(Race2)+0.67(Race3)+0.72(Race5) +0.67(Race14)
factor 2:
y=0.43(Race1)–0.24(Race2)+0.54(Race3)–0.33(Race5)– 0.37(Race14).

The first factor can be explained as a common correlation factor. Each element had almost the same contribution to this factor. In the second factor, responses to SCN races 1 and 3 had a positive loading, and responses to races 2, 5 and 14 had a negative loading. This result indicated that resistance to SCN races 1 and 3 had related patterns, and resistance to races 2, 5 and 14 had relatively similar patterns.

Molecular markers associated with SCN resistance

Fifty four RFLP and 64 SSR markers were found to be polymorphic in this population. Among these, 13 RFLP markers and one SSR marker were observed to be dominant. Data from three RFLP markers were discarded because of severe deviation from the expected segregation ratio or because of too-much missing data. Only 51 RFLP and 64 SSR markers were used for further analysis. One hundred and six molecular markers were anchored on 16 LGs, with nine markers (six RFLP and three SSR markers) remaining unassigned. Most markers mapped to the same locations as previously published (Cregan et al. 1999a). However, some exceptions occurred, such as K070, which was mapped to LG B instead of LG M, and B124 which mapped to LG M instead of LG B. This discrepancy may be caused by the different plant populations used, the different enzyme digestions included in different studies, or by multiple alleles of these markers.

Mapmaker/QTL analysis indicated that three to five independent marker intervals were found tightly associated with resistance to each race (Table 3). Markers that failed to be assigned to any LG were analyzed using the SAS GLM procedure. However, no marker was found significantly linked with resistance to SCN (P<0.001).

Resistant QTLs for the race 1 were anchored on four LGs, namely, B1, B2, C2 and G (Table 3). By checking the phenotypic mean of marker classes and comparing the banding with the parent, we confirm that all resistant sources came from the resistant parent PI 438489B. Among these loci, the locus between RFLP marker A096 and SSR marker Satt130 on LG G explained 15.8% of the total phenotypic variation, which was the largest. The QTLs on LG B1 also showed a strong association with resistance as it explained 12.7% of the total variation. Overall, four loci explained approximately 47% of the total phenotypic variation.

Five loci were found significantly associated with resistance to race 2 (Table 3). These loci were located on LGs A1, B1, C1, E and G. The locus on LG G was the most important because it explained the largest proportion of the total phenotypic variation. One locus on LG C1 explained approximately 10% of the total phenotypic variation. Overall, these loci explained approximately 45.8% of the total phenotypic variation. Loci on LGs B1 and G were found located within the same marker intervals as the QTLs for resistance to race 1 (Fig. 2). Four loci were found tightly associated with resistance to race 3. A locus, located on LG A2 within the interval of two RFLP markers, K400 and T155, was the largest. It explained approximately 20% of the total phenotypic variation. This locus was also strongly associated with the *i_locus*, the gene controlling black seed coat color (Fig. 2). Another locus was located on LG G within the interval of two SSR markers, Satt130 and Satt012. Two other QTLs were anchored on LGs B2 and D1a. The QTL on LG D1a is the first report that showed an association with resistance to race 3. SSR marker Satt309 was

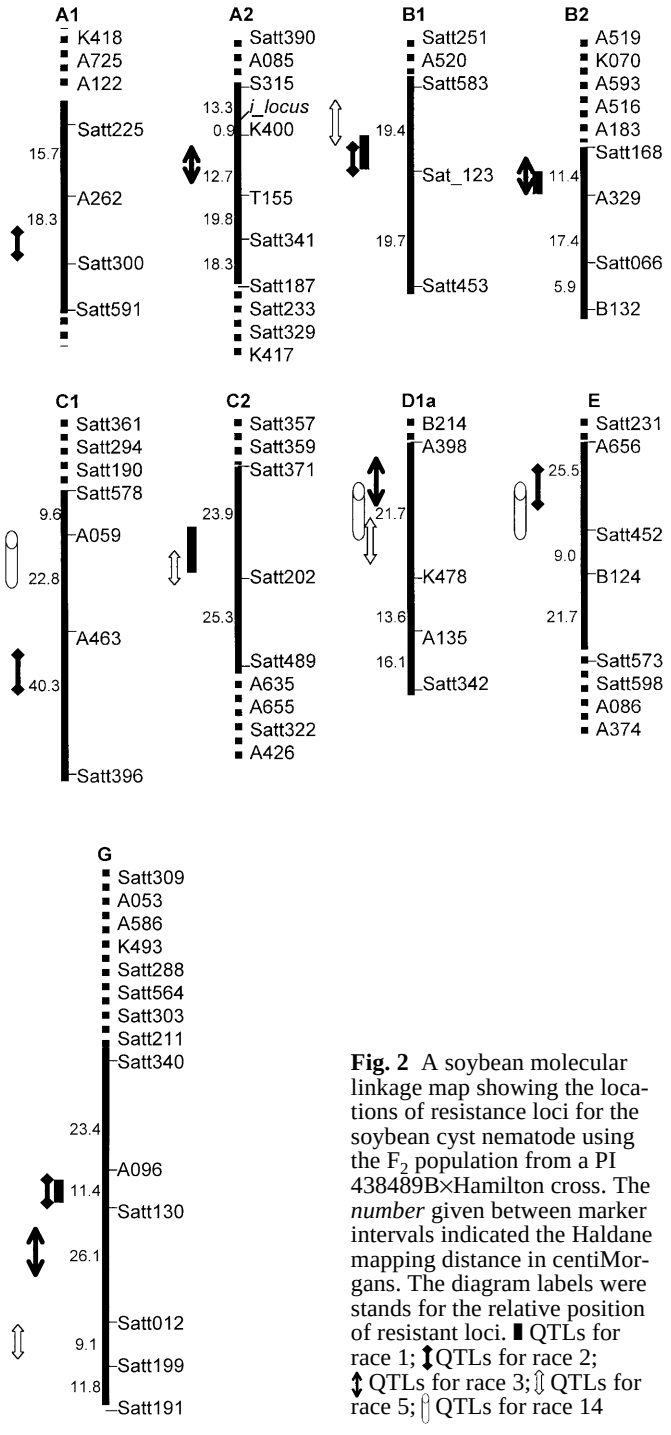


Fig. 2 A soybean molecular linkage map showing the locations of resistance loci for the soybean cyst nematode using the F_2 population from a PI 438489B×Hamilton cross. The *number* given between marker intervals indicated the Haldane mapping distance in centiMorgans. The diagram labels were stands for the relative position of resistant loci. ▮ QTLs for race 1; ↕ QTLs for race 2; ↕ QTLs for race 3; ⇕ QTLs for race 5; ⇕ QTLs for race 14

reported tightly associated with race 3 (Cregan et al. 1999b); however, this marker was weakly associated with resistance to SCN in our population. The locus on LG B2 was located within the same region with a locus for resistance to race 1. The locus on LG G was close to loci for resistance to races 1 and 2.

Four loci were found linked with loci controlling resistance to SCN race 5. These loci were located on LGs B1, C2, D1a and G. Compared to other races, the R^2 values of these loci were relatively low. The strongest locus was found located 3.0 cM away from SSR marker Satt583 on LG B1. Several QTLs were found located within the same marker interval as the QTLs controlling resistance to other races. The loci on LG B1 shared marker intervals with resistant QTLs for races 1 and 2. The QTL on LG D1a was located in the same region as the locus for resistance to race 3. The locus on LG C2 was anchored within the same region as a QTL for resistance to race 1. The QTL on LG G was located adjacent to these loci for resistance to races 1, 2 and 3.

We found three loci tightly linked with resistance to SCN race 14. A locus, located between RFLP marker A656 and SSR marker Satt452 on LG E, explained 18.7% of the total phenotypic variation. This locus is 5.0-cM away from A656. Another locus, located on LG C1, was 2.0-cM away from RFLP marker A059, and explained approximately 12% of the total phenotypic variation. This locus was adjacent to one locus for resistance to race 2. A locus on LG D1a shared the marker interval with resistant loci for resistance to races 3 and 5. We did not detect any markers on LG G that were significantly linked with loci giving resistance to race 14.

Over all, it seemed that soybean LGs A1, A2, B1, B2, C1, C2, D1a, E, M and G contained major resistance QTLs for SCN races in this population. Further analysis showed that all these resistant QTLs came from the resistant parent PI 438489B.

Relationship among resistance QTLs controlling different races

The phenotypic data analysis presented (Tables 1 and 2) in the first part of our results indicated that resistance to different SCN races might have genes in common. The results of our factor analysis indicated that resistant patterns for races 1 and 3 were closer, as were resistant patterns for SCN races 2, 5 and 14.

Molecular marker data gave further evidence to suggest that pleiotropic effects may have occurred in providing resistance to different SCN races. We found markers on LG G that were associated with resistance to races 1, 2, 3 and 5, even though these loci were anchored by different markers (Fig. 2). A marker interval, Satt583 to Sat_123 on LG B1, was shared by resistance loci to races 1, 2 and 5 (Table 3, Fig. 2). Resistance loci for races 3, 5 and 14 were anchored within the same marker interval, A398 to K478, on LG D1a. Resistance loci for races 2 and 14 were anchored within the same interval as markers A656 and Satt452 on LG E. Loci for resistance to races 1 and 3 shared more common marker intervals, and explained a larger portion of the total phenotypic variation, such as the loci on LGs B2 and G.

We noticed that there were several loci associated with resistance to particular races. A locus on linkage group A1 (between markers A262 and Satt300) only provided resistance to race 2. A locus on linkage group A2, which was located 4.7-cM away from RFLP marker T155 and 8.9 cM away from the *i-locus*, was found to be associated only with resistance to race 3.

Discussions

Molecular characterization of resistance to SCN race 3 has been extensively studied by different groups using different soybean populations (Concibido et al. 1994, 1996, 1997; Webb et al. 1995; Mudge et al. 1997; Cregan etal. 1999a). Linkage groups A2 and G have been established as the most-important location of major resistance loci for SCN resistance. Some minor loci were also found on LGs F, J, K, M and N (Heer et al. 1998). Our study showed that QTLs on LGs G and A2 are the most-important loci for resistance to race 3 in PI 438489B. Both QTLs explained relatively large proportions of the total phenotypic variation. We found two other QTLs on LGs B2 and D1a, which were also important considering the magnitude of the R^2 values presented (Table 3).

Linkage groups B, E, H (Qiu et al. 1999) and A2 (Heer et al. 1998) are major regions where QTLs for resistance to SCN race 1 were found. In our studies, three additional QTLs were located on LGs A1, C1 and G. No loci were found to be associated with resistance to race 1 on LGs E and H, even though we had 11 markers that covered approximately 110 cM on LG E. However, only one marker could be assigned to LG H in this study, and we did not detect any association between this marker and resistance QTLs.

Qiu et al. (1999) reported that three RFLP makers were associated with resistance to race 5, and were located on LGs B and H. In our study, resistant QTLs in PI 438489B for race 5 were located on LGs B1, C2, D1a and G; however, each locus had only small effects. It is possible that there might be major QTLs that we have not detected, or perhaps our markers may not be close enough to the loci we identified.

There is lack of information on molecular markers associated with resistance to races 2 and 14. In our study, we found a unique locus on LG A1 that only provided resistance to race 2. Loci only associated with resistance to races 5 or 14 were not found. All resistant loci for races 5 and 14 in our study were found to share intervals with loci for other races (Fig. 2). Resistant loci for race 14 were unique. Several studies reported that resistance gene *rhg*1, located on LG G (Webb et al. 1995; Concibido et al. 1996, 1997; Cregan et al. 1999b), and *Rhg*4, located on LG A2 and close to the *i-locus* (Weisemann et

Table 4 The efficiency of marker-assisted selection based on flanking markers associated with QTLs for SCN race 3

Flanking marker pair combination				No. of plants selected	Female index mean (%) of selected individuals				
1[a]	2	3	4		Race 1	Race 2	Race 3	Race 5	Race 14
+	–	–	–	16	58.7[b]	51.0	17.5	28.8	46.5
–	+	–	–	11	53.0	27.2	27.1	24.1	37.2
–	–	+	–	19	75.4	53.5	28.0	32.4	54.2
–	–	–	+	12	80.1	47.7	40.0	28.2	48.1
+	+	–	–	2	1.3	5.6	0.7	4.6	2.1
+	–	+	–	7	11.0	25.4	6.5	14.4	27.8
+	–	–	+	4	29.7	29.1	5.3	35.5	34.0
–	+	+	–	1	4.34	29.7	0.4	31.1	30.9
–	+	–	+	2	23.7	17.4	16.5	26.1	61.4
–	–	+	+	0	N/A[c]	N/A	N/A	N/A	N/A
+	+	–	+	1	0.41	7.43	1.13	1.27	43.64
Female Index Mean (%) and SD of the Population					74.4±31.6	34.6±13.5	44.0±27.6	33.0±17.6	50.0±22.5

[a] The flanking marker pairs are: 1 K400 – T155, located on LG A2; 2 Satt130 – Satt012, located on LG G; 3 Satt168 – A329, located on LG B2; and 4 A398 – K478, located on LG D1a

[b] The Female Index values listed here are averages of the $F_{2:3}$ families selected. If only one individual was selected, the value was the average of that $F_{2:3}$ family

[c] N/A: not available

al. 1992; Matthews et al. 1998), are necessary to provide complete resistance to race 3. We did not find markers that were associated with resistance to race 14 on LGs A2 and G. Our markers had good coverage for LGs A2 and G (11 and 14 markers, respectively). There is a small chance that we failed to detect possible associations between markers and QTLs in these two regions because we detected markers for race 14 on LGs C1, D1a and E. The locus on LG E made a large contribution to the total phenotypic variation of response to race 14. One explanation is that resistance to race 14 does not need the combination of *rhg*1 and *Rhg*4, or perhaps *rhg*1 and *Rhg*4 do not function as strongly as they do for providing resistance to race 3. However, three loci discovered for resistance to race 14 explained only 37.2% of the total phenotypic variation. Other QTLs might be present, but have not yet been identified.

Clustering of resistance genes in soybean were observed by several authors (Concibido et al. 1996; Kanazin et al. 1996). On LG G, loci controlling resistance to different soybean diseases, such as the sudden death syndrome (SDS) caused by the fungus *Fusarium solani* f. sp. *glycine* (Njiti et al. 1998), SCN (Webb et al. 1995; Concibido et al. 1996, 1997; Chang et al. 1997; Cregan et al. 1999b), soybean root-knot nematode (*Meliodogyne incognita*, Mi) (Tamulonis et al. 1997), were found located within this region. Concibido et al. (1996) reported a locus on LG G giving resistance to SCN races 1, 3 and 6. Resistance loci for different SCN races were also found to be either linked (Heer et al. 1998) or pleiotropic (Qiu et al. 1999). In our study, we found that responses to different races were highly associated with resistant QTLs for different races, and were mapped to several common marker intervals. Our study also indicated that responses to races 1 and 3 seemed more similar, and that response to races 2, 5 and 14 were also more similar. These results imply that genes for resistance to races 1 and 3 may have closer relationships than genes for races 2, 5 and 14. However, we could not distinguish between linked loci and pleiotropic effects using these markers. Considering that these loci control resistance to the same pest with different phenotypic variations, it is more reasonable to believe that these loci are pleiotropic and that different gene combinations are responsible for the specificity of the races.

The consistency among loci for different SCN races provides an opportunity to select for resistance to multiple races using one set of molecular markers. To test this hypothesis, we evaluated the efficiency of marker-assisted selection by checking the FI mean of $F_{2:3}$ families selected by markers associated with resistance QTLs to race 3 (Table 4). Because all resistance loci came from PI 438489B, we selected F_2 individuals which had homozygous PI 438489B banding patterns for the marker pairs. We failed to find an individual with more than three pairs of selection markers. The selection based on a single marker pair failed to choose resistant individuals in all four cases (Table 4), which agreed with the conclusions of Webb et al. (1995) that neither locus alone provided a degree of resistance much different from that of the susceptible parent. We noticed that selection using two marker pairs had a better result than single marker pairs. When we employed these two pairs of markers on LGs A2 and G (K400 and T155 on LG A2, Satt130 and Satt012 on LG G), only two individuals were found to have all markers with the PI 438489B banding pattern. Both of these individuals were highly resistant to all five races. We obtained certain levels of improvement of the FI of other races if we selected for the marker for resistance to race 3, especially when we applied two pairs of markers. When we selected for resistance to the race 3, we noticed that the FI means for other races were lower than the population average in most cases. This result implied that we could use these markers to select for SCN resistance in PI 438489B, and it is possible that we could select for resistance to multiple races using fewer

markers. Flanking markers are better than single markers; however, the more markers we use, the bigger the plant population has to be to find genotypes with the desired marker combinations. Due to recombination, it is very difficult to apply many markers on a small population simultaneously and, in addition, the distance of the map interval is also a consideration.

Acknowledgements The authors thank Mr. John A. Wilcox for his assistance in preparing greenhouse SCN bioassays. This study was supported by a grant from the Missouri Soybean Merchandising Council. Contribution from the Missouric Agriculture Experimental Station Journal Serial No. 13013

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