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Loci underlying resistance to Race 3 of soybean cyst nematode in *Glycine soja* plant introduction 468916

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Abstract Soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) is an important soybean [*Glycine max* (L.) Merr.] pest in the U.S. and throughout the world. Genetic resistance is the primary method for controlling SCN and there is a need to identify new resistance genes. *Glycine soja* Sieb. and Zucc. is the wild ancestor of domesticated soybean and is a potential source of new SCN resistance genes. The goal of this research was to map quantitative trait loci (QTLs) that provide resistance to SCN Race 3 from the *G. soja* plant introduction (PI) 468916. Fifty seven F_2-derived lines from a cross between the *G. soja* PI 468916 and the *G. max* experimental line A81-356022 were tested for resistance to an SCN population with a Race-3 phenotype. These lines were also genotyped with 1,004 genetic markers and resistance genes were mapped by composite interval mapping with the computer program QTL-Cartographer. In the F_2 population, three significant (LOD > 3.0) QTLs were detected that explained from 5% to 27% of the variation for Race-3 resistance. The two most significant QTLs identified in the F_2 population were tested in a population of 100 $BC1F_2$ plants developed by crossing A81-356022 to a line from the F_2 population that carried the two resistance QTLs from *G. soja*. In the backcross population, both Race-3 resistance QTLs were significant, which confirms the existence of these QTLs. The QTLs identified in this experiment map to positions where SCN resistance genes have not been previously identified, suggesting that these are novel genes that could be useful for diversifying the resistance genes currently used in cultivar development.

Keywords *Glycine soja* · Soybean cyst nematode · Genetic mapping · Quantitative trait loci · SCN resistance

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Introduction

Soybean cyst nematode is the most economically damaging soybean pest in the U.S. (Wrather et al. 1999). Once established in a field, the infestation is difficult to eradicate, resulting in the need for long-term management strategies. Crop rotation and genetic resistance currently control SCN, but SCN populations are highly heterogeneous and may eventually overcome the resistance genes currently deployed by plant breeders.

Researchers have screened the *G. max* plant introductions (PIs) in the USDA soybean germplasm collection to identify those with resistance to SCN (Diers and Arelli 1999). Although many resistant PIs have been identified, only a few have led to the release of commercial cultivars. Diers and Arelli (1999) found that in north central USA, 13 out of 16 public cultivars released during the 1990s with SCN-resistance received their resistance from PI 88788 alone. A similar trend holds for SCN-resistant cultivars from private industry. Out of 247 SCN resistant cultivars available for planting in Illinois during 1998, 230 received their resistance from PI 88788 alone (Diers and Arelli 1999).

Quantitative trait loci that provide SCN resistance from a number of resistance sources have been identified. The QTL mapping studies indicate that several sources may have resistance genes in common. For example, PI 437654 (Webb et al. 1995), PI 209332, PI 88788, PI 90763 and Peking (Concibido et al. 1996, 1997; Chang et al. 1997) all had a major SCN resistance QTL on linkage group (LG) G (Cregan et al. 1999b)

where *rhg1* maps. Peking (Matson and Williams 1965; Mahalingam and Skorupska 1995; Chang et al. 1997) and PI 437654 (Webb et al. 1995) both had a resistance gene where *Rhg4* maps near the *I* locus on LG A2 (Cregan et al. 1999b). Additionally, PI 90763 and PI 209332 (Concibido et al. 1997) were found to have a resistance gene mapping to the same location on LG J.

The lack of diversity for SCN resistance genes in soybean cultivars highlights the need for identifying new resistance genes. A potential source of new genes is *G. soja*, an annual species that is generally interfertile with domesticated soybean and is believed to be the ancestor of domestic species (Hymowitz and Singh 1987). There is ample evidence that *G. soja* has genetic diversity not present in soybean (Keim et al. 1989; Maughan et al. 1995) and this diversity has been exploited in genetic mapping studies of soybean. A detailed genetic map was made in a population developed from a cross between the *G. soja* line PI 468916 and the soybean experimental line A81-356022. This map is composed primarily of restriction fragment length polymorphism (RFLP) (Shoemaker and Olson 1993) and simple sequence repeat (SSR) markers (Cregan et al. 1999b). Over 1,000 molecular markers have been placed on the genetic map developed from this population (Cregan et al. 1999b).

We recently found that PI 468916, the *G. soja* parent used in forming the genetic map discussed above, is resistant to SCN Race 3. The goal of our research was to map QTLs from PI 468916 that confer resistance to this SCN race.

Materials and methods

F_2 population

The population consists of 57 F_2-derived lines from a cross between the Iowa State University experimental line A81-356022 and the *G. soja* accession PI 468916. Population development and RFLP marker testing were described in detail by Shoemaker and Specht (1995) and Shoemaker and Olson (1993). Testing of this population with SSR markers was described by Cregan et al. (1999b). The population has been used to map QTLs controlling a number of traits including hard seededness (Keim et al. 1990a), plant morphology, date of flowering and maturity (Keim et al. 1990b), protein and oil concentration (Diers et al. 1992) and fatty acid content (Diers and Shoemaker 1992).

Five F_4 plants from each F_2-derived line were inoculated with an SCN population with a Race-3 phenotype. The SCN inoculation procedure was described in detail by Diers et al. (1997). A female index (FI) was calculated for each plant using the formula (Golden et al. 1970):

$$\mathrm{FI} = \frac{100 \times \text{number of cysts and females per plant}}{\text{Average number of cyst and females on 'Hutcheson'}}$$

Hutcheson also was included as a susceptible check. Lines with a FI less than 10 were classified as resistant (Golden et al. 1970). In each inoculation test, the set of SCN host differentials were included (Riggs and Schmitt 1988) and our SCN population had a Race-3 phenotype in each test. Estimates of variance components and broad-sense heritabilities for the FI values were calculated from mean squares (Fehr 1987) obtained from the PROC GLM of SAS (SAS 1988).

Backcross population

A backcross population was developed by crossing A81-356022 with a line from the F_2 population that, based on genetic markers, was homozygous for the two QTL alleles from *G. soja* that provide SCN resistance. This cross was made in the field in Urbana, Ill., during the summer of 1999. A $BC1F_1$ plant was grown in a greenhouse from September of 1999 to January of 2000. One hundred $BC1F_2$ plants from the cross were planted, tested with genetic markers and inoculated with an SCN population with a Race-3 phenotype. The plants were tested with SSR markers using DNA extracted from young leaf tissue according to Bell-Johnson et al. (1998). Polymerase chain reactions (PCRs) were carried out according to Cregan and Quigley (1997). The PCR products were analyzed by electrophoresis in 6% non-denaturing polyacrylamide gels (Sambrook et al. 1989) and by staining with 1 μg ml^{-1} of ethidium bromide.

Molecular marker and QTL analysis

Genotypic data for a total of 1,004 genetic markers, including 501 RFLP, 486 SSR, 10 RAPD, 4 isozyme and 3 classical markers, were collected previously for the F_2 population (Shoemaker and Specht 1995; Cregan et al. 1999b). The computer program MAPMAKER/EXP 3.0 (Lander et al. 1987) was used to construct the genetic linkage map. A minimum LOD score of 3.0 and the Kosambi (1944) mapping function were used for the map construction.

Simple linear regression was performed to identify markers significantly associated with resistance to SCN Race 3 using the LRmapqtl program of QTL-CARTOGRAPHER (Basten et al. 1999). *F*-statistics were used to test the significance of the regression. A significance level of $P < 0.01$ was used to declare the regression significant. The LOD score was used to show the likelihood ratio of the marker being linked, versus unlinked, to the resistance. The LOD score is defined as $\log_{10}(L1/L0)$, where L1 is the likelihood that the marker is associated with the resistance and L0 is the likelihood that the marker is not associated with the resistance.

The composite interval mapping (CIM) method (Jansen and Stam 1994; Zeng 1994) was applied to determine the locations of QTLs. The CIM was carried out with the computer program package QTL-CARTOGRAPHER (Basten et al. 1999). The CIM was run with model 6 of the Zmapqtl program and a window size of 10 cM for all analyses. The number of markers for the background control was set to 5, meaning that the five most-significant markers outside the interval under analysis were fitted to the model. The markers used for the background control were detected through forward and backward stepwise regression using the program SRmapqtl. The likelihood value of the presence of a QTL was expressed as LOD score $\log_{10}(L1/L0)$, where L1 is the likelihood of the model with the putative QTL and L0 is the likelihood of the model without the QTL. The threshold of the LOD score for declaring a putative QTL significant was chosen to be 3.0. The estimate of the QTL position is the point of maximum LOD score in the region under consideration.

The phenotypic variance explained by a single QTL was estimated by the square of the partial correlation coefficient (R^2). 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 Zmapqtl program in QTL Cartographer. The marker with the greatest R^2 value from each significant QTL was included in a multivariate model with PROC GLM in SAS to estimate the total phenotypic variance (σ^2_p) explained by the QTL.

Eight SSR markers were chosen from regions where QTLs were detected in the F_2 population to genotype the $BC1F_2$ population to confirm these QTLs. Map distances among the selected markers in the $BC1F_2$ population were determined using Mapmaker with a minimum LOD score of 4.0. Analysis of QTLs for the $BC1F_2$ population was carried out using the program QTL-Cartographer with the same parameters used for the F_2 population.

Results

F_2 population

There was significant genetic variation in the F_2 population for resistance to our SCN population with a Race-3 phenotype. The line means for FI ranged from 2.3 to 90.9 with a population mean of 26.2. There were no transgressive segregants with significantly greater resistance than PI 468916, the resistant parent (Fig. 1). The broad-sense heritability for resistance was 0.75.

Simple linear regression analysis resulted in the identification of 42 markers significantly ($P < 0.01$) associated with resistance to SCN (Table 1). Twenty three of these markers mapped to a region spanning 115 cM on LG G and 17 were located in a region covering 101 cM on LG E. The remaining two markers mapped 243-cM apart on LG C2. The CIM method located four significant LOD peaks for SCN resistance, two on LG G, and one each on LG E and C2 (Fig. 2). Three of the four LOD peaks were within the regions where markers significantly ($P < 0.01$) associated with resistance were located (Fig. 2; Table 2), indicating the presence of QTLs. The LOD peak close to Satt394 on LG G was determined to be an artifact based on results from simple

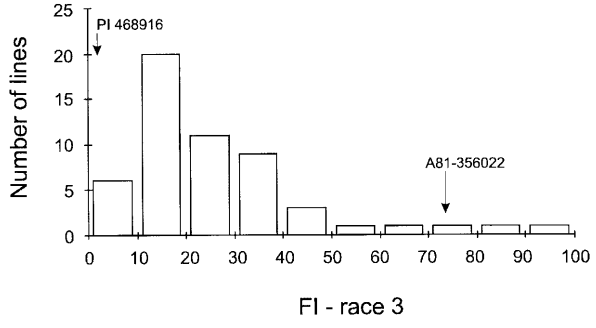

Fig. 1 Distribution of line means for the Race-3 female index (*FI*) values in the PI 468916 by A81-356022 population of $F_{2:4}$ lines. Means of the parents are shown by *arrows*

Table 1 Markers within 30 cM of QTLs that are significant ($P < 0.01$) in the population of $F_{2:4}$ lines for resistance to soybean cyst nematode Race 3 based on simple linear regression analysis

Marker	LG[a]	Position	$P > F$	LOD[b]
A121_1	C2	0	0.008	1.6
A458_1	E	108	0.004	1.9
A455_1	E	108	0.005	1.8
R051_1	E	108	0.004	1.9
A646_1	E	108	0.006	1.7
Satt573	E	117	0.002	2.1
Satt598	E	121	0.000	3.5
Bng107_1	E	143	0.002	2.1
Satt288	G	212	0.000	3.6
A885_1	G	221	0.000	2.9
K493_1	G	222	0.002	2.2
bac1F11R	G	229	0.003	2.0
p28_13_2	G	230	0.001	2.4
A245_2	G	232	0.000	4.8
Satt472	G	236	0.004	1.9

[a] Linkage group

[b] The LOD score is defined as $\log_{10}(L1/L0)$, where L1 is the likelihood that the marker is associated with the resistance and L0 is the likelihood that the marker is not associated with the resistance

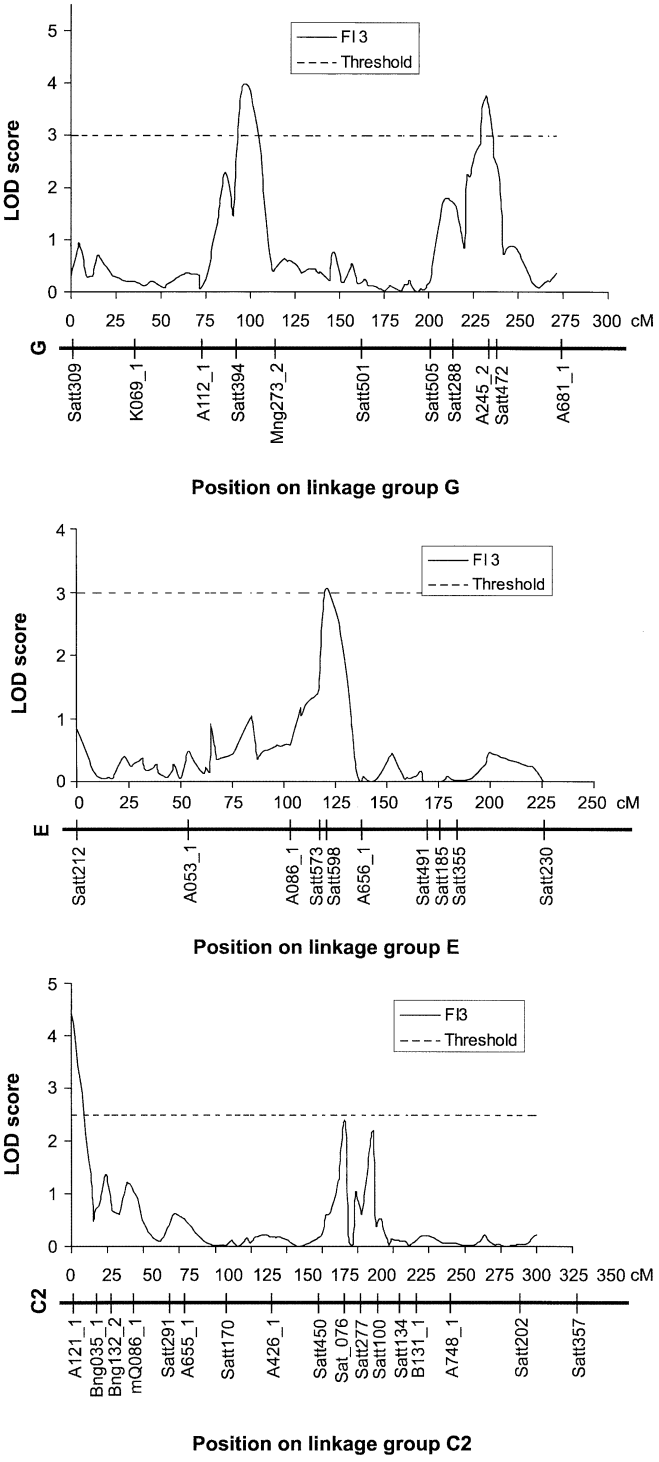

Fig. 2 Plots of LOD scores from the PI 468916 by A81-356022 population of $F_{2:4}$ lines. The plots show the locations of quantitative trait loci identified for the Race-3 female index (*FI 3*) on linkage groups G, E and C2. The *markers* shown are a subset of markers tested from the linkage groups. See Cregan et al. (1999b) for the relative positions of the remaining markers on the linkage groups

Table 2 Map locations and estimated genetic effects of quantitative trait loci providing resistance to soybean cyst nematode Race 3 in the population of $F_{2:4}$ lines based on composite interval mapping

LG[a]	Pos.[b]	Interval length[c]	LOD	R^2	a[d]
G	232	10	3.8	0.27	–11.5
E	121	12	3.1	0.23	–16.3
C2	0	6	4.4	0.05	9.64

[a] Linkage group
[b] Position (cM) relative to the left-most marker on the linkage group (Fig. 2)
[c] One-LOD support interval length (cM)
[d] Estimated additive effect of substituting one allele of A81-356022 with an allele from PI 468916

Table 3 Markers tested for association with resistance to Race-3 soybean cyst nematode in the $BC1F_2$ population based on simple regression analysis

Marker	LG[a]	*P* > *F*	LOD[b]
Satt573	E	0.007	1.6
Satt598	E	0.001	2.3
Satt491	E	0.114	0.6
Satt185	E	0.169	0.4
Satt501	G	0.001	2.6
Satt505	G	0.004	1.8
Satt288	G	0.001	2.3
Satt472	G	0.000	3.0

[a] Linkage group
[b] The LOD score is defined as $\log_{10}(L1/L0)$, where L1 is the likelihood that the marker is associated with the resistance and L0 is the likelihood that the marker is not associated with the resistance

Table 4 Map locations and estimated genetic effects of quantitative trait loci providing resistance to soybean cyst nematode Race 3 in the $BC1F_2$ population based on composite interval mapping

LG[a]	Position[b]	LOD	R^2	a[c]
G	37	3.8	0.36	–14.4
E	18	4.8	0.26	–6.0

[a] Linkage group
[b] Position (cM) relative to the left-most marker tested on the linkage group (Fig. 3)
[c] Estimated additive effect of substituting one allele of A81-356022 with one allele of PI 468916

linear regression analysis (see discussion). The resistant alleles for the QTLs on both LG E and G originated from PI 468916, whereas the resistant allele for the QTL on LG C2 originated from A81-356022 (Table 2). Of the total phenotypic variance for SCN resistance, the QTLs on LG G explained 27%, E explained 23%, and C2 5%.

When the markers nearest to the QTLs on LG G, E, and C2 were included in a three-factor analysis resistance with the PROC GLM of SAS, only A245_1 on LG G, and Satt598 on LG E were significant ($P < 0.01$). The two significant markers together explained 66% of the phenotypic variance and there was a significant ($P < 0.01$) interaction between them.

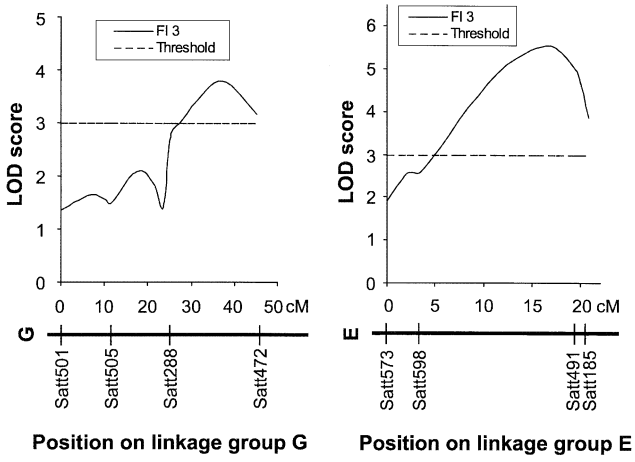

Fig. 3 Plots of LOD scores from the $BC1F_2$ population developed using PI468916 as the donor parent and A81-356022 as the recurrent parent. The plots show the locations of quantitative trait loci for the Race-3 female index (*FI 3*) on linkage groups G and E that were mapped in this population

Backcross population

The $BC1F_2$ population had a mean Race-3 FI of 28.3 with a range of 1.8 to 78.0. Eight SSR markers, four each from the regions on LG G and E where QTLs mapped in the F_2 population, were chosen to genotype the $BC1F_2$ population (Figs. 2, 3). Six of the eight markers were significantly ($P < 0.01$) associated with SCN resistance in the $BC1F_2$ population based on simple regression analysis (Table 3). The CIM method confirmed the presence of the QTLs on LG G and E within the regions covered by the selected SSR markers (Fig. 3; Table 4). The QTLs on LG G explained 36%, and E explained 26%, of the total phenotypic variance for resistance in the $BC1F_2$ population. The resistant alleles for both QTLs originated from PI 468916, which is consistent with the F_2 population analysis.

Discussion

Two major QTLs that confer resistance to SCN Race 3 were mapped in a population of F_2-derived lines and these QTLs were confirmed in a backcross population. The presence of these QTLs in the confirmation population provides strong evidence that the mapped QTLs are real and not artifacts of the analysis. The QTL on linkage group G was placed in the interval between Satt288 and Satt472 in both populations (Figs. 2 and 3) and was more than 230-cM from the *rhg1* locus. The QTL on linkage group E shifted from mapping near Satt598 in the F_2 population to the interval between Satt598 and Satt491 in the backcross population (Figs. 2 and 3). Although the marker order is consistent, the LG E map is greatly expanded in the F_2 population compared to the backcross population, which may contribute to the inconsistent map positioning. The distance between Satt573 and Satt491 is 58-cM in the F_2 population, 20-cM in the

backcross population, and only 7-cM apart in the University of Utah population (Cregan et al. 1999b). Additional mapping using larger populations will be needed to more-precisely map the locations of these QTLs.

The simple linear regression analysis suggests that the LOD peak close to Satt394 on LG G was most likely an artifact. The LOD peak was detected in a 21-cM interval on LG G where the mean interval was 4 cM. None of the markers within 30 cM of the LOD peak were significantly ($P < 0.01$) associated with SCN resistance in the simple linear regression analysis. This LOD peak might have resulted from ghost effects as discussed by Martinez and Curnow (1992). The nearest marker that was significantly associated with SCN resistance was 38 cM from the LOD peak and there were four more markers that were significantly associated with SCN resistance within 12 cM of this marker. However, no QTLs were found in this region by the CIM method.

The *G. soja* parent was also found to be resistant to SCN Races 5 and 14. The F_2 population was tested with SCN populations with Race-5 and -14 phenotypes to map QTL conferring resistance to these races. A Race-5 resistance QTL was located to the same position as the Race-3 resistance QTL on LG G near marker A245_2 (data not shown), which suggests that the resistance to both races may be controlled by the same locus. No QTLs were detected for Race 14 resistance in the F_2 population.

The SCN resistance QTLs mapped to LG E and G may be novel resistance genes that can broaden the genetic diversity for SCN resistance in elite germplasm. These genes may be novel because there are no other reports of SCN resistance genes mapping to LG E and to the regions on G where our QTLs mapped (Diers and Arelli 1999). One gene previously mapped to LG G is *rhg*1, which maps near Satt309 (Concibido et al. 1996; Danesh et al. 1998; Cregan et al. 1999a; Meksem et al. 1999; Prabhu et al. 1999) and is more than 230-cM from the QTL peak in the F_2 population (Cregan et al. 1999a). The second report on LG G is a QTL from 'Peking' that maps to the restriction fragment length polymorphism (RFLP) marker A378 (Concibido et al. 1997). This RFLP marker was not significantly associated with SCN resistance in our population and maps 35-cM from the QTL peak in the F_2 population.

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