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

**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

**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.
DNA of soybean leaves was extracted using the CTAB method (Keim et al. 1988). For RFLP analysis, individual F2 plant DNA samples were digested by five different restriction enzymes, DraI, EcoRl, EcoRV, HindIII and TaqI. Hybrid N+ nylon membrane (Amersham Pharmacia Biotech Co. III., 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 (Boehringer Mannheim Co. Indianapolis, Ind.) was used for labeling probes according to the manufacturer’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 F2 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 33P-ATP (NEN Life Science, Boston, Mass.) using T4 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 MgCl2, 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% Urea, dissolved in 1x 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.

When 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 (Mutchler 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; Mutchler et al. 1996). Furthermore, normalizing data through transformation may misrepresent differences among individuals by pulling skewed tails toward the center of the distribution (Mutchler et al. 1996).

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 F2.3 families.

Linkage maps were constructed employing MAPMAKER/EXP software (Version 3.0, Whitehead Institute, Cambridge, Mass.), using a minimum LOD (log10 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).

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 F2 plants, along with both parents, were grown at the Agronomy Research Center, Columbia, Mo., in 1996. Leaves from individual F2 plants were harvested and used for DNA isolation and molecular-marker analysis. Plants were allowed to set seed and these F2.3 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 F2 and F2.3 families, their parents and differentials, were germinated in 5×8-cm germination bags, and transplanted into microtots. Twenty three microtots 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 Automa tic 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 F2.3 family and approximately 120 F2 seeds were used in each SCN race-screening. In total, 184 F2.3 families were screened in this study. In each screening cycle, we averaged number of females and cysts on Hutcheson 100.

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.