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MOLECULAR CHARACTERIZATION OF GENETIC RESISTANCE TO SOYBEAN CYST NEMATODE IN SOYBEAN LINE SS97-6946

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ABSTRACT

Breeding resistant cultivars is the most efficient means to control soybean cyst nematode (SCN) but the nematode overcomes the resistance. A study was conducted to identify Quantitative Trait Loci (QTL) conferring broad-spectrum SCN resistance in SS97-6946 at University of Missouri, USA. A set of 160 F_2 individuals from the cross PI 567476 X SS97-6946 was genotyped with 347 Single Sequence Repeat and evaluated against four races of SCN bioassay. Three markers mapped on linkage groups (LG) A2, E, and G, accounting for 33.8% of the total phenotypic variance for resistance to HG type 2.5.7. One resistant QTL, detected on LG A1 accounted for 18.8% of the total phenotypic variance of HG type 1.2.5.7. Three resistance QTL detected on LGs A2, G, and M that shared 24.9% of the total phenotypic variance HG type 0. Three QTL on LG G alone and another four on LGs A1, B2, M, and O were linked with SCN resistance to HG type 1.2.7 that accounted for 70.8% of the total phenotypic variance. Resistant QTL on LGs A1, M and O in SS97-6946, appear to be unique for SCN HG types 1.2.5.7, 0, and 1.2.7 that can potentially be used in marker assisted selection.

Key words: *Glycine max*; *Heterodera glycines*; HG type; linkage group; quantitative trait loci.

INTRODUCTION

Soybean cyst nematode (SCN) *Heterodera glycines* Ichinohe is the most yield reducing pest of soybean and estimated yield losses are 1.5 billion dollars annually in USA (Wrather *et al*., 2001, Wrather and Koenning, 2006). A wide range of variation is present in SCN in terms of size, color, shape and virulence. In the latest scheme, '*Heterodera glycines* (HG) type' was proposed instead of 'race'; and seven soybean indicator lines (Peking, PI88788, PI90763, PI437654, PI209332, PI89772 and Cloud) are currently being used in determining HG types (Niblack *et al*., 2002). Breeding and use of resistant cultivars along with other non-host crops in rotation is the primary and most effective management tool currently being employed to control SCN. Due to extremely variable populations of *H. glycines* (Ross, 1962; Miller, 1969; Riggs and Schmitt, 1988; Arelli *et al*., 1992), SCN can overcome resistance in cultivars and is capable of reproducing on resistant varieties and making them less effective in overall crop performance (Mitchum *et al*., 2007).

The existence of an ever changing nematode population dictates that research on resistant variety development using new resistant sources is needed to minimize yield losses by SCN. Several soybean scientists and breeders have devoted their efforts to discover resistant sources to populations of different HG types primarily 0, 1.2.7 and 1.3.6.7 (races 3, 5 and 14, respectively) by periodically evaluating the soybean germplasm since the detection of SCN in USA (Niblack *et al*., 2002). Arelli *et al*. (1992) identified a total of 118 resistant Plant Introduction (PI) lines with resistance to one or more of those HG types. Due to narrow genetic base, many SCN resistant varieties have been developed in the USA but almost all have resistance genes from Peking to HG types 2.5.7, and 0 (races 1 and 3, respectively) and/or PI88788 to HG types 0, and 1.3.6.7 (races 3 and 14, respectively). The pathogen has adapted and overcome resistance in soybean cultivars due to shifts to virulent HG types and to narrow genetic base (Dong *et al*., 1997). HG types 2.5.7, 1.2.5.7 and 1.2.7 (races 1, 2, and 5, respectively) have especially become increasingly more damaging to soybean in some states (Niblack *et al*., 2003, Mitchum *et al*., 2007). Thus, it is important to identify novel resistance genes from other sources for breeders to incorporate more effective genes into cultivars for resistance to predominant SCN HG types in soybean fields.

The heredity of SCN resistance is multigenic and quantitative (Concibido *et al*., 2004; Lu *et al*., 2006). Several soybean scientists and breeders reported their work on inheritance of SCN resistance in soybean. Three recessive genes designated as *rhg1*, *rhg2* and *rhg3* played a vital role in the degree of resistance in soybean cultivar Peking against SCN populations (Caldwell *et al*., 1960). Matson and Willium (1965) reported that one dominant gene *Rhg4* was associated with resistance to SCN in Peking. Arelli (1994) found that another dominant gene *Rhg5* was associated with SCN resistance in PI 88788. Finally, Arelli *et al*. (2009) investigated the genetic basis of SCN resistance in PI567516C and concluded that the resistance to the LY1 nematode in PI567516C was

conditioned by one dominant and two recessive genes, designated Rhg, rhg, rhg.

A well developed genetic map covering all linkage groups (LG) allows researchers to accurately assign specific SCN resistance loci in soybean. A total of more than 20 sources were reported for QTLs association with SCN resistance (Concibido *et al*., 2004; Guo *et al*., 2005, 2006a; Lu *et al*., 2006). Quantitative trait loci (QTL) for SCN resistance to HG types 2.5.7, 1.2.5.7, 0, 2.7 and 1.3.6.7 (race 1, 2, 3, 5 and 14, respectively) have been identified in 19 out of 20 soybean LGs except LG D1b from 13 resistance sources (Guo *et al*., 2006a, Winter *et al*., 2007a). Those QTL shared 1 to 91% of total phenotypic variation for SCN resistance (Concibido *et al*., 2004; Lu *et al*., 2006; Winter *et al*., 2007b). The common QTL designated as *rhg1* assigned on LG G effects 54% of the total phenotypic variation for resistance in PI 209332 to SCN race 6, 50% for race 3 and 35% for race 1 (Concibido *et al*., 1996). Another study confirmed the QTL on LG G near *rgh1* in soybean accessions Peking, PI90763, PI88788, PI209332, PI89772, PI437654 (Concibido *et al*., 2004), PI404198A, PI467312, PI468916 (*Glycine. soja*) (Guo *et al*., 2006b), PI 464925B (*G. soja*) (Winter *et al*., 2007a). A second major QTL designated as *Rhg4* was identified on LG A2 for SCN resistance, which affects 15% of total phenotypic variation in PI209332 for resistance to SCN races (Concibido *et al*., 1994). Additional studies confirmed the QTL on LG A2 near *Rhg4* locus in soybean accessions Peking, PI88788, PI90763, PI209332, PI437654 (Concibido *et al*., 2004). Wu *et al*. (2009) confirmed QTL on LGs G and A2 and identified a new QTL located on LG I for resistance to SCN races 3, 5, and 14 using a recombinant inbred line (RIL) population derived from PI 437654. Vuong *et al*. (2010) identified and confirmed two unique QTL mapped on LG O and G using a new resistance source PI567516C. Later Arelli *et al*. (2010) also reported same QTL located on LG O using same resistance source PI567576C. Although more than a dozen soybean accessions have been mapped for QTLs association with SCN resistance, most of the QTLs were assigned on the same LG regions or closely linked but few of them are confirmed (Concibido *et al*., 2004, Guo. *et al*., 2006b). Thus, it is important to identify and confirm novel resistance genes from other sources for breeders to incorporate more effective genes into cultivars for resistance to predominant SCN HG types in soybean fields.

Soybean germplasm line SS97-6946 has shown resistance to all major SCN HG types and has other desirable traits. It has a relative maturity of 4.3 and was developed from the cross between 'Essex' x PI438503A with yellow seed coat. Zhang *et al*. (1999) found one of the parents PI438503A of SS97-6946 showed resistant to all major SCN races and was a different SCN resistance source from known sources like Peking, PI437654, and PI 438489B. The seed of this line has high protein and oil content, which is valuable for food, feed and industrial applications. These desirable traits and resistance to multiple HG types is positive for using this in soybean breeding programs to introgress SCN resistance into productive varieties. However, identification and characterization of resistance genes for SCN resistance in this germplasm line has not been studied. The objectives of the present research was to investigate the inheritance of resistance to SCN populations of HG types 2.5.7, 1.2.5.7, 0 and 1.2.7 (corresponding to races 1, 2, 3, and 5, respectively) and identify the QTLs associated with SCN resistance in SS97-6946.

MATERIALS AND METHODS

Plant materials: A total 160 $F_{2:3}$ progenies derived from cross SS97-6946 × PI567476 were used for greenhouse bioassays of SCN HG types 2.5.7, 1.2.5.7, 0 and 1.2.7 (races 1, 2, 3 and 5, respectively). Soybean germplasm line SS97-6946 is resistant to multiple SCN races has purple flowers with grey pubescence and developed by the soybean breeding lab, University of Missouri, Columbia (D.A. Sleper, personal communication, 2008). PI567476 also has purple flowers with grey pubescence is moderately susceptible to SCN races and donated by China to USDA soybean germplasm collection. It is a low allergen soybean line. The cross between parents was made in the summer in 2006. The F_1 seeds were grown in Costa Rica in 2006 and F_2 seeds were grown at the Bradford Research and Extension Center near Columbia, Missouri, USA in summer 2007.

SCN bioassay: The SCN bioassay was performed in the greenhouse at the University of Missouri, Columbia, Missouri, USA following established methods (Arelli, 1994; Arelli *et al*., 1997) in summer 2008. Inbred or near homogeneous populations of SCN HG types 2.5.7, 1.2.5.7, 0 and 1.2.7 (races 1, 2, 3 and 5, respectively) were used in this study. Those SCN populations have been maintained for many generations. Resistance sources 'Pickett', 'Peking', PI 88788, PI 90763, and PI 437654, PI 209332, PI 89772 were used as indicator lines to monitor the purity of HG type populations and success of the phenotyping experiments (Niblack *et al*., 2002). 'Hutcheson' was used as the standard susceptible control. Four plants for each of the two biological replications were planted in the greenhouse from each of the 160 $F_{2:3}$ families and indicator lines for each of the tested HG types 2.5.7, 1.2.5.7, 0 and 1.2.7 (races 1, 2, 3 and 5, respectively) during April 2008 for SCN bioassays following established procedure described by Arelli *et al*. 1997. Individual plants of each parent and lines above were randomly transplanted into micro pots filled with steam-pasteurized Brosely fine sandy loam. Each of the plastic containers (20 cm diameter) contained twenty-five

micro pots and were partially submerged in a water-bath and maintained at 27±1^{0}C throughout the experiment. Three days after transplanting, each plant was inoculated with 2000±25 SCN eggs in 5 ml of suspension using an automatic pipette. Thirty days after inoculation, individual plant roots were collected and washed with a strong jet of water to dislodge nematode cysts and counted under a stereomicroscope. The female index (FI) based on the standard classification system (Schmitt and Shannon, 1992) was used to evaluate SCN reaction of each individual plants including 160 $F_{2:3}$ lines, parents and indicator lines. Soybean cyst nematode reaction was determined based on number of white female development on four plants from each $F_{2:3}$ families per replication. $F_{2:3}$ lines were classified into two categories resistant and susceptible based on female index. A female index (FI) of ≤ 10% is considered a resistant (R) reaction whereas a female index (FI) >10% is defined as susceptible (S). The female index was calculated as a percentage as follows:

$$FI\ (\%) = \frac{\text{Mean number of females on roots in a given subfamily}}{\text{Mean number of females on roots of Hutcheson}} \times 100$$

Plant DNA preparation and marker analysis: Young leaves (trifoliate) of F_2 plants from cross PI567476 X SS97-6946 along with both parents were collected from field during July 2007 and stored at -80^{o}C for future use. Stored leaves were fridge dried and fine powder was made from dried leaves by using automatic Geno grinder. DNA was extracted from soybean leaf powder of each F_2 individuals and parents following CTAB method (Keim *et al*., 1988) with minor modifications and used for SSR analysis.

A total of 547 SSR primer pairs were first screened against the two parents SS97-6946 and PI 567476 to identify polymorphic markers. The identified polymorphic primer pairs were used for genotyping the 160 F_2 individuals. All SSR markers used in this study have been assigned to the soybean composite genetic map (Song *et al*., 2004). Polymerase chain reaction (PCR) was conducted in both 96 and 384 well micro plates with a final volume of 15 µl on the eppendorf master cycler gradient (Eppendorf AG, Germany). Each reaction included 2.0 µl 10 x PCR buffer, 2.0 µl of 2µM dNTPs, 0.1 µl of 20 µM forward and 0.15 µl of 20 µM reverse SSR primer (labeled), 1 unit Taq polymerase (Genescript Corporation, Piscataway, NJ, USA), 1.5 µl PVP (10% w/v), 1 µl of 25 mM $MgCl_2$, 50 ng genomic DNA and 5.05 µl of sterile water. The PCR cycling program included a first cycle at 95^{o}C for 3 min for initial denaturation; 35 cycles at 94^{o}C for 30 sec for denaturing; 52^{o}C for 45 sec for annealing; and 72^{o}C for 1 min for extension. The reaction was terminated with a 7.0 min extension at 72^{o}C. The PCR products were run on an ABI 3100 sequencer.

Linkage map construction: The genetic linkage map was constructed using Joinmap 3.0 (Ooijen and Voorrips, 2001). Parameters were set as default, i.e. LOD grouping thresholds ≥ 3.0 and a maximum distance of 50 cM. Assignment of LG was based on similarity to the integrated soybean map (Song *et al*., 2004). All markers mapped to LGs were evaluated individually by the χ^2 test for goodness of fit against a 1:2:1 segregation ration at a 0.01 probability level.

QTL analysis: Composite interval mapping (CIM) was performed to localize and detect SCN resistance QTL using MapQTL version 5.0 (Ooijen and Voorrips, 2001). For CIM, forward and backward stepwise regressions were performed to select 10 markers as cofactors and the analysis was conducted using model 6 with a moving window size of 10 cM. At each interval, the significance of the QTL-trait association was tested by the likelihood ratio statistics. Permutation tests were conducted 1000 times to determine a critical LOD value to minimize the experimental type I error rate. For each trait, a significant threshold level was estimated by 1000 permutations at $p< 0.05$ using the MapQTL program. However a LOD score of 3.0 was used as a threshold to declare the presence of a putative QTL. The score was employed to identify all possible regions associated with SCN resistance. The putative QTL and LG figure was created using Mapchart 2.2 program (Voorrips, 2002).

Statistical analysis: A frequency distribution was determined for reaction of lines within individuals to each HG type. The Chi-square (χ^2) test using the Yates correction analysis to adjust for small population size was used to test the goodness of fit for the proposed gene models. In this study, each $F_{2:3}$ lines used in different HG type or race bioassays were derived from a given F_2 individual. Hence, a single correlation coefficient analysis was used to study the relationship of host plant resistance to different SCN HG types. The Shapiro-Wilk test was conducted to measure normality of the frequency distributions and suitability for detecting QTLs for SCN resistance in the 160 $F_{2:3}$ families to SCN races 1, 2, 3 and 5.

RESULTS

Phenotype evaluation: Reaction of the standard HG type indicator lines and control (Hutcheson) confirmed the purity of SCN populations (HG types) used in these studies. Results revealed that significant variation was observed for responses against all tested SCN HG types (races) among $F_{2:3}$ families, parents, controls and differentials (Table 1). This indicated that the reactions of individual plants to SCN HG types were significantly affected by environment. The frequency distribution of $F_{2:3}$ families response with SCN HG types 2.5.7, 1.2.5.7, 0 and 1.2.7 (race 1, 2, 3 and 5, respectively) showed a

normal or near normal distribution (Figure 1). But the normality test revealed that SCN HG types 2.5.7 and 0 (race 1 and 3, respectively) could be normally distributed due to higher p value and SCN HG types 1.2.5.7 and 1.2.7 (race 2 and 5, respectively) were not normally distributed (Table 1). However, the effect of non-normality on QTL mapping data analysis is reported to be significantly reduced because of use of cofactor markers in composite interval mapping (Jansen and Stam, 1993) and permutation testing for the determination of threshold values (Churchill and Doerge, 1994; Yang and Williams, 2007). Therefore, the data were used for further analysis without transformation. It was evident that a few $F_{2:3}$ families showed higher female index than the susceptible parent (data not presented). This could happen due to transgrassive segregation among families.

Genetics of SCN resistance: The reaction to SCN HG type 2.5.7 (race 1), types 1.2.5.7 (race 2), type 0 (race 3) and type 1.2.7 (race 5) showed susceptible: resistant reaction of $F_{2:3}$ individuals were 157:3, 158:2, 136:24 and 153:7, respectively (Table 2). Chi-square analysis showed that segregation ratios of susceptible and resistant families fit a three recessive gene (*rhg rhg rhg*) model for SCN HG types 2.5.7 and 1.2.5.7 (race 1 and 2, respectively) with the p value for $\chi^2 > 0.75$. Two dominant and one recessive gene model (*Rhg Rhg rhg*) was a good fit for reaction to SCN HG type 0 (race 3) with a p value for $\chi^2 > 0.73$. The proposed gene model for reaction to SCN HG type 1.2.7 (race 5), was a one dominant and two recessive gene model (*Rhg rhg rhg*) with the p value for $\chi^2 > 0.85$ (Table 2).

Relationship of resistance to different SCN HG types: Phenotypic results revealed that some resistance genes might be common or are tightly linked in refining the reaction to different SCN HG types in the tested soybean populations. Soybean cyst nematode bioassay results showed that several $F_{2:3}$ families had broad-spectrum resistance to multiple races (data not shown). Two $F_{2:3}$ families showed complete resistant reaction to all tested SCN HG types 2.5.7, 1.2.5.7, 0 and 1.5.7 (race 1, 2, 3 and 5, respectively). One family had resistance to SCN HG types 2.5.7, 0 and 1.5.7 (race 1, 3 and 5, respectively) and another two families were resistant to SCN HG types 0 and 1.5.7 (race 3 and 5, respectively). Correlation analysis was conducted to evaluate the reactions to different SCN HG types for the same tested $F_{2:3}$ families. The response to all tested SCN HG types were significantly correlated (Table 3) to each other at the 0.1% level of significance.

DNA marker analysis and mapping: Three hundred forty-seven out of 547 (<63%) SSR primer pairs were found polymorphic between PI 567476 and SS97-6946. These 347 polymorphic primer pairs were selected covering all twenty LGs of soybean to screen 160 $F_{2:3}$ families derived from the cross PI 567476 x SS97-6946. Three-hundred forty-seven polymorphic primer pairs were nearly distributed among the lengths of the 20 LGs in the soybean composite linkage map (Song *et al*., 2004). Among 347 markers, 20 markers were discarded from further analysis because of missing data or did not amplify. Due to severe deviation from the expected segregation ratios, a total of 10 markers were not used for genetic mapping. Twenty-two SSR markers were observed to be dominant leaving 295 markers to possibly be placed on the linkage map. The software Joinmap 3.0 (Ooijen and Voorrips 2001) was used to construct LG using 295 good markers. Finally, 263 markers were positioned on the linkage map, which comprised 39 LGs. Compared to the soybean composite map (Song *et al*., 2004); the linkage map constructed with the 160 F_2's was very consistent in marker arrangement and relative distance between SSR markers with exception of a few regions. Eighteen markers were not assigned to the same order as the soybean composite map (Song *et al*., 2004) and these were not used. Fourteen markers were unassigned and not included. The linkage map spanning a total of 2550.1 cM across 39 LGs comprised 101% of the integrated soybean map (Song *et al*., 2004) and 99.99% of the soybean genetic map reported by Choi *et al*. (2007). The highest number of markers (26) was placed in LG G followed by LG O (18). Single gaps (≥50 cM between neighboring markers) occurred in LGs A2, B2, C1, C2, D1a, D2, E, J, K, L and M (chromosome no. 8, 14, 4, 6, 1, 17, 15, 16, 9, 19 and 7), respectively. Therefore eleven subgroups were formed, one in each respective LG in order according to the soybean composite map. Double gaps (≥50 cM between neighboring markers) existed in LG I (chromosome no. 20) according to soybean composite map. Two subgroups were formed in LG I. Triple gaps (≥50 cM between neighboring markers) existed in LGs D1b and F (chromosome no. 2 and 13), respectively. Three subgroups were formed each in LGs D1b and F in order according to soybean composite map. Markers assigned were uniformly disseminated along LGs except a few big gaps such as 45.7 cM gap between Sat_417 and Satt656 on LG F subgroup; 42.7 and 38.8 cM gap between Sat_158 - Satt142 and Sat_122 - Sat_158, respectively on LG H; 41.2 cM gap between Sat_289 and Satt271 on LG D1b subgroup. This occurred even though large numbers of SSR markers were screened for these LGs. This incomplete coverage reflected the low level of polymorphism in these gap regions between the parents.

QTLs conditioning to SCN resistance: Results revealed that SCN resistance is controlled by multiple, largely diverse loci that are distributed throughout the genome. Individual QTLs for SCN resistance accounted for 3.1 to 27.7 % of the total phenotypic variation (Table 4). A LOD score of 3.0 was considered to be the threshold for

declaring presence of a suggestive QTL, but permutation tests with 1000 permutations were run by MapQTL to determine a critical LOD value for minimizing the experimental type I error rate. Significant threshold LOD scores ($p<0.05$) were obtained (3.8 to 4.2 with an average of 4.05). Almost all previous reported studies used a LOD score 3.0 to declare their QTL as suggestive (Webb *et al*., 1995; Concibido *et al*., 1996; Heer *et al*., 1998; Qiu *et al*., 1999; Meksem *et al*., 2001; Wang *et al*., 2001; Yue *et al*., 2001a, b; Guo *et al*., 2005; 2006a). We chose a less stringent LOD score (3.0) to maximize the likelihood of identifying map locations associated with SCN resistance to keep consistent with earlier studies.

The result of identified QTL and their associate LOD with other values are showed in Fig 2 and Table 4. Two putative QTL associated with resistance to SCN HG types 1.2.5.7 and 1.2.7 were detected on LG A1 and located between SSR marker Satt684 and Satt382 and explained 18.8 and 27.7%, respectively of the total phenotypic variation with strong supporting statistical evidence LOD values = 5.19 and 13.15, respectively. Two resistant QTL associated with SCN HG types 2.5.7 and 0 detected on LG A2 mapped between marker Satt315 and Sat_157 with 5.3 cM interval and contributed 7.8 and 9.2%, respectively of the total phenotypic variation. The QTL located on LG E were assigned between markers Sat_107 and Satt483 and explained 7.2% of the total phenotypic variance associated with SCN HG type 2.5.7. Five putative resistant QTL were mapped on LG G related to SCN HG types 2.5.7, 0 and 1.2.7. Three QTL were located between markers Sat_315 and Sat_403 for all three SCN HG types and explained 18.8, 9.5 and 5.8%, respectively of the total phenotypic variance associated but QTL mapped between markers Sct_199-Satt191 and Sat_131-Sat_315 for only SCN HG type 1.2.7. Two QTL were identified on LG M associated with resistance to SCN HG types 0 and 1.2.7 located between markers Sat_121-Satt551 and Sat_147-Satt551, respectively. QTL on LGs B1, B2 and O flanked with markers Satt444 – Satt665, Sat_287 – Satt126 and Satt173 – Sat_282 explained 5.7%, 5.1% and 3.1%, respectively of the phenotypic effect for resistance to SCN HG type 1.2.7. The resistance QTL on LGs A1, A2, M, O and two QTLs (out of three) on LG G came from SS97-6946 but the remainder came from the other parent.

Table 1. Mean and range of Female Index (FI) of $F_{2:3}$ lines from population PI567476 x SS97-6946, parents and control to SCN HG types 2.5.7, 1.2.5.7, 0 and 1.2.7 (races 1, 2, 3 and 5, respectively) and normality test. Replicated experiment was conducted in greenhouse Missouri at 2008.

Lines	HG type 2.5.7[a]		HG type 1.2.5.7 [a]		HG type 0 [a]		HG type 1.2.7 [a]	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
SS97-6946 (%)	0.4	0.0-1.4	4.3	0.0-8.0	0.1	0.0-0.7	0.0	0.0-0.0
PI 567476 (%)	35.4	28.0-46.4	47.6	21.3-85.8	21.8	14.7-29.5	20.6	9.7-32.7
$F_{2:3}$ families (%)	36.1	0.9-70.9	36.8	9.1-78.4	21.3	0.2 -50.2	27.5	1.3-61.6
Hutcheson (%)[b]	211.0	191-231	187.5	155-208	135.8	78-174	143.8	122-180
Sharpiro-Wilk's W	0.987837		0.967722		0.985365		0.953866	
p-value	0.1798		0.0009		0.0906		0.0001	

[a]

$$FI(\%) = \frac{\text{Mean number of females on roots in a given subfamily}}{\text{Mean number of females on roots of Hutcheson}} \times 100$$

[b] Assign mean number of female (cyst).

Table 2. Genetic analysis and reaction of $F_{2:3}$ individuals of PI 567476 x SS97-6946 population to SCN HG types 2.5.7, 1.2.5.7, 0 and 1.2.7 (races 1, 2, 3 and 5, respectively). Replicated experiment was conducted in greenhouse Missouri at 2008.

HG types (races)	Number of lines			Hypothesized resistance genes	Expected genetic ratio[b]	χ^2	p-value
	Total	Observed ratio R:S[a]	Expected ratio R:S[a]				
2.5.7 (1)	160	3:157	2.5:157.5	rhg rhg rhg	1:63	0.102	0.750
1.2.5.7 (2)	160	2:158	2.5:157.5	rhg rhg rhg	1:63	0.102	0.750
0 (3)	160	24:136	22.5:137.5	Rhg Rhg rhg	9:55	0.116	0.733
1.2.7 (5)	160	7:153	7.5:152.5	Rhg rhg rhg	3:63	0.035	0.852

[a] R= resistant, S = Susceptible lines

b Expected genetic ration were obtained from Mendellian inheritance formula

Table 3. Correlation coefficients of reaction of $F_{2:3}$ individuals to the different tested SCN HG types (races). Replicated experiment was conducted in greenhouse Missouri at 2008.

SCN population	HG type 2.5.7	HG type 1.2.5.7	HG type 0	HG type 1.2.7
HG type 2.5.7	-			
HG type 1.2.5.7	0.367**	-		
HG type 0	0.547**	0.252**	-	
HG type 1.2.7	0.511**	0.365**	0.271**	-

** Significant at p value < 0.001

Table 4. QTLs associated with broad spectrum SCN resistance detected through composite interval mapping (CIM) in tested population. Replicated experiment was conducted in greenhouse Missouri at 2008.

HG types	LG[a]	Chr.[b]	Marker interval	Linked marker[c]	Length (cM)[d]	QTL position[e]	LOD	R^2 (%)[f]	Cum. R^2 (%)	A[g]
2.5.7	A2	8	Satt315-Sat_157	SUIC100-8K	5.3	4.0	3.2	7.8		-4.16
	E	15	Sat_107-Satt483	Satt268	7.8	4.0	3.3	7.2	29.1	1.99
	G	18	Sat_315-Sat_403	Satt610	23.5	12.0	7.0	18.8		-7.49
1.2.5.7	A1	5	Satt684-Satt382	Sat_368	30.9	20.0	5.2	18.8	18.8	-7.28
0	A2	8	Satt315-Sat_157	SUIC100-8K	5.3	5.3	4.6	9.2		-4.27
	G	18	Sat_315-Sat_403	Satt610	27.5	21.1	3.1	9.5	30.5	-4.36
	M	7	Sat_121-Satt551	Satt551	10.0	6.0	3.2	6.2		-3.85
1.2.7	A1	5	Satt684-Satt382	Sat_368	21.9	14.9	13.2	27.7		-10.32
	B1	11	Satt444-Satt665	Satt665	6.78	6.7	3.0	5.7		2.21
	B2	14	Sat_287-Satt126	Sat_287	13.0	5.0	3.7	5.1		2.68
	G	18	Sct_199-Satt191	Sct_187	16.6	11.6	10.2	12.9	58.9	-6.05
	G	18	Sat_131-Sat_315	Satt594	4.2	1.0	4.1	11.8		0.84
	G	18	Sat_315-Sat_403	Satt610	20.5	17.5	3.7	5.8		-4.46
	M	7	Sat_147-Satt551	Satt551	22.9	8.0	3.4	4.4		-3.86
	O	10	Satt173-Sat_282	Satt173	5.7	0.2	3.0	3.1		-3.02

[a] Linkage group [b] Correspondent chromosome number of soybean genome according to Song *et al*. 2004
[c] Marker used as cofactor in CIM [d] Location of the QTL on the LG given in centiMorgans from Join Map and MapQTL
[e] QTL position expressed as the distance from the left marker of the respective QTL region
[f] Production of total phenotypic variation shared by QTL
[g] Additive effect. '-'indicates that resistance allele of QTL comes from parent SS97-6946

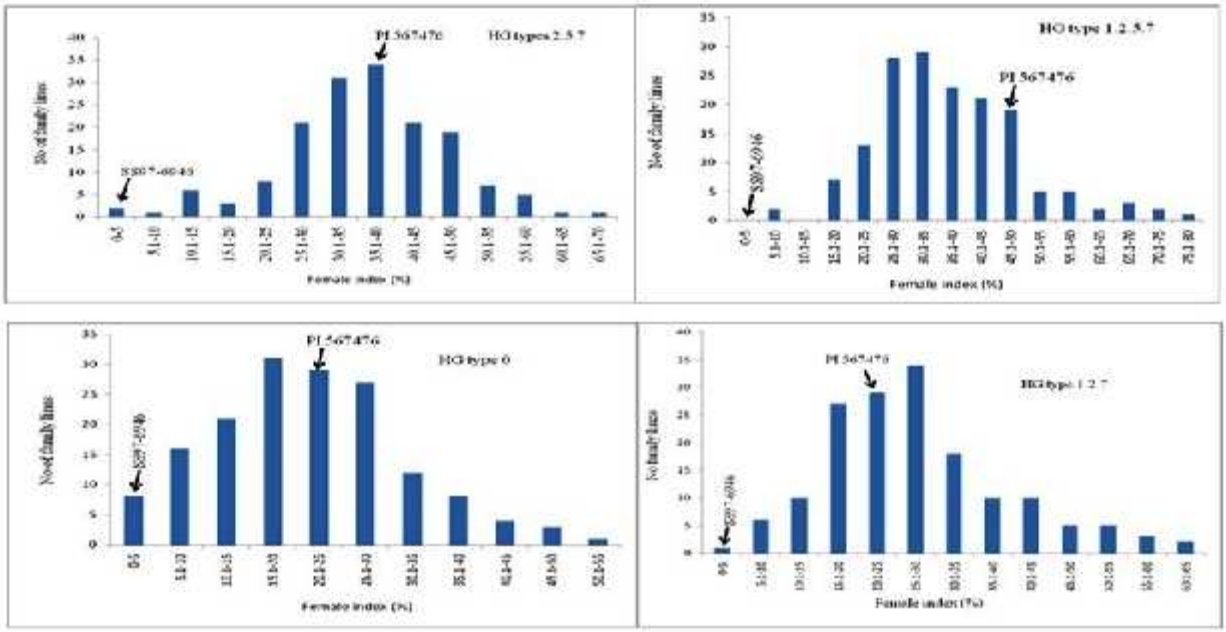

Figure 1. Frequency distribution of mean female index (FI%) of 160 $F_{2:3}$ family of PI 567476 X SS97-6946; PI 567476 and SS 97-6946, SCN resistant parent to different SCN HG types. Replicated experiment was conducted in greenhouse Missouri at 2008.

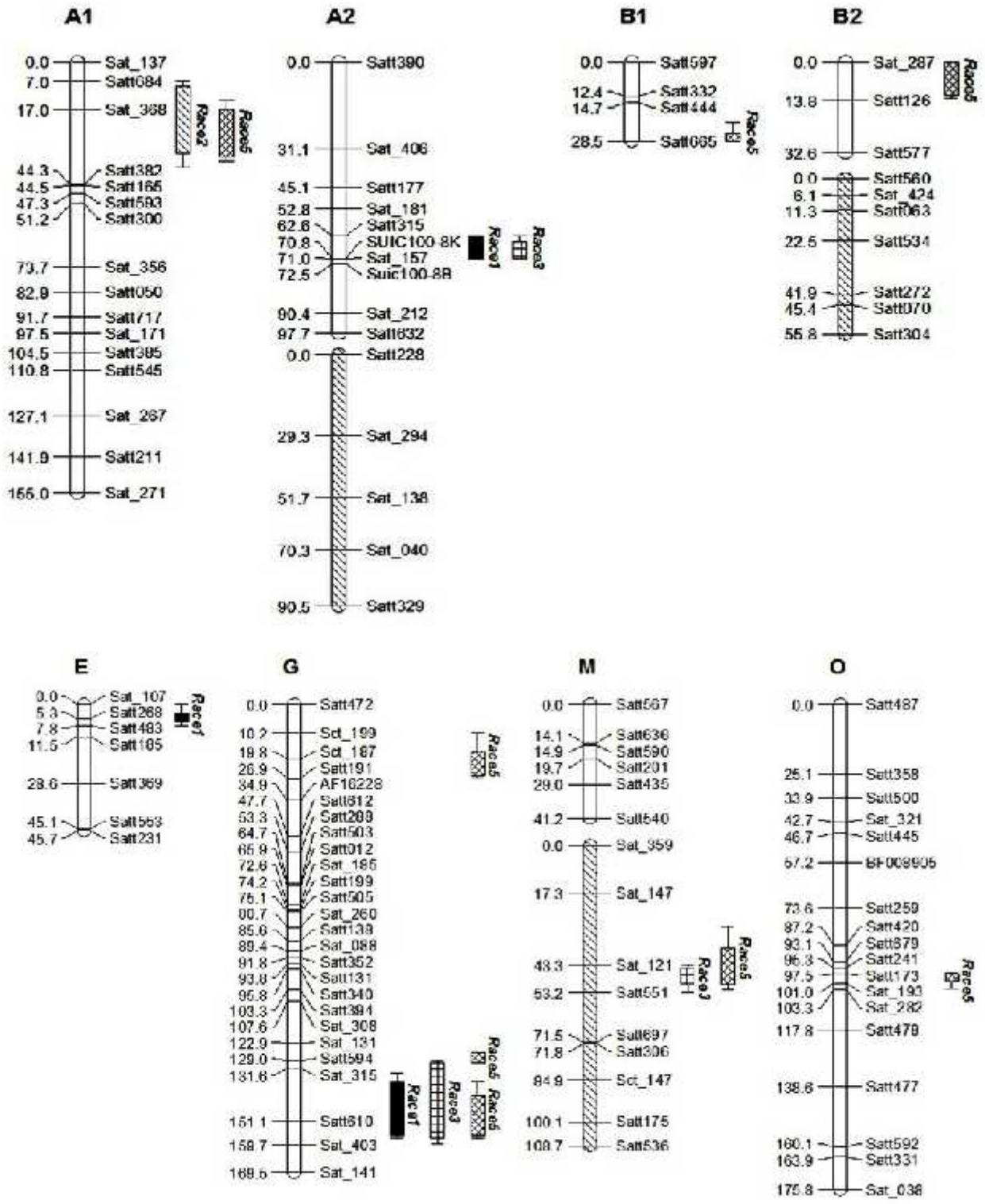

Figure 2: Linkage groups and QTL locations associated with SCN resistance to HG types 2.5.7, 1.2.5.7, 0 and 1.2.7 (race 1, 2, 3 and 5, respectively) in 160 F_2 line of PI 567476 X SS97-6946 population. Linkage was declared at LOD ≥ 3.0 with a maximum distance of 50 cM. Detected QTL are designated by the bars with respective race designation on the right of the LG. The open bar of the LG represents the main group and crossed bars represent the sub group of the respective LGs. Replicated experiment was conducted in greenhouse Missouri at 2008.

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DISCUSSION

Phenotypic evaluation: The purity of SCN HG types were tested using standard differential and result revealed that the reaction in all inoculations of differentials, parents and control against different SCN HG types were consistent with other reports (Chang *et al*., 1997; Cregan *et al*., 1999a; Yue *et al*., 2000; Lu *et al*., 2006). Inheritance of SCN resistance in the $F_{2:3}$ families showed three gene models with all tested SCN HG types which are similar to several previous studies. Caldwell *et al*. (1960) first reported the inheritance of SCN resistance in Peking and reported that three recessive genes (*rhg1 rhg2 rhg3*) were involved in resistance to SCN. *Rhg4*, a dominant gene responsible for SCN resistance was additionally reported in Peking (Matson and Williams, 1965). Another dominant gene *Rhg5* was identified in PI 88788 by Arelli *et al*. (1992). Yue *et al*. (2000) found two dominant and one recessive genes (*Rhg Rhg rhg*) in PI 438489B for resistance to SCN HG type 0 (race 3) and one dominant and two recessives (*Rhg rhg rhg*) for resistance to SCN HG types 1.2.7 (race 5). In another study a three gene model (*Rhg rhg rhg*) for SCN HG types 1.2.7 (race 5) were reported for resistance (Qiu *et al*., 1997). Earlier studies reported that there were common genes in several PIs for different SCN HG types. A major SCN resistance gene located in the same region of LG G were found in Peking, PI 88788, PI 437654 and PI 90763, PI 438489B for HG type 0 (race 3) (Concibido *et al*., 2004). Several reports showed that some of these PIs had QTLs in common for resistance to HG types 2.5.7, 1.2.5.7, 0, 1.5.7 (race 1, 2, 3 and 5, respectively) (Heer *et al*., 1998; Yue *et al*., 2001a, b; Webb, 2003; Concibido *et al*., 2004). Some germplasm were shown to be resistant to multiple races in studies conducted to evaluate responses of soybean germplasm against different SCN HG types (Young, 1995; Arelli *et al*., 2000). Inheritance of SCN resistance in SS97-6946 is complex like inheritance in other resistance sources have shown and could contain major genes for resistance. A reported QTL located on LG G was associated with resistant to HG types 2.5.7, 0 and 1.7 (races 1, 3 and 6, respectively) (Concibido *et al*., 1996). Previous studies reported that the responses to different SCN populations were highly correlated (Yue *et al*., 2000; Lu *et al*., 2006). Some common molecular markers in different linkage groups were closely linked and responsible for resistance to multiple SCN HG types (Yue *et al*., 2001a, b; Guo *et al*. 2006a).

QTL analysis: The most common QTL for across the three HG types (2.5.7, 0, 1.2.7) were detected on LG G and linked marker Satt610 is placed 6.3 cM downstream of Marker Satt309 (according to soybean composite map), which was tightly linked with the SCN resistance gene *rhg1* (Cregan *et al*., 1999a, b; Meksem *et al*., 2001). QTL identified in our study fell in the same region as ones identified by other studies on LG G, resistant to SCN race 1 and 3 in PI 209332, and Peking (Concibido *et al*., 1994 and Heer *et al*., 1998). Therefore, soybean line SS97-6946 could contain SCN resistance gene *rhg1*. Another two QTL were also identified on the same linkage group were associated with HG type 1.2.7 (race 5). The QTL linked with Satt594 could be the same QTL due to markers associated with QTLs located in the same region in the soybean composite map (Song *et al*., 2004) and identified as associated with SCN resistance to multiple races. The other QTL on this LG was separated and no QTL has been reported for resistance to SCN HG type 1.2.7 (race 5) in the region of marker interval Sct_199 and Satt191; however, resistance for SCN race 3 was mapped in the same region (Wang *et al*., 2001 and Yue *et al*., 2001b). Earlier studies showed that the gene *Rhg4* was mapped in the region of Satt632-pBlt65-I and associated with resistance to SCN HG types 2.5.7 and 0 (races 1 and 3) (Heer *et al*., 1998; Cregan *et al*., 1999b; Meksem *et al*., 2001). Another common QTL across two HG types 2.5.7 and 0 (races 1 and 3) were identified in our study located on marker interval Satt315-Sat_157 that overlaps with Satt632-pBlt65-I. Thus, it is likely that the detected QTL carries *Rhg4* for SCN HG type 2.5.7 (race 1) resistance. The marker interval of the common QTL on LG A1 associated with resistance to HG types 1.2.5.7 and 1.2.7 (races 2 and 5) was close to the same interval that was identified QTL associated with resistance to SCN race 2 in LG A1 in PI439489B (Yue *et al*., 2001a). According to soybean composite map the end of the marker interval identified in our study is 4.5 cM upstream from the beginning of the marker interval found by Yue *et al*. (2001a) for QTL resistance to SCN race 2 in LG A1 in PI439489B. But Vierling *et al*. (1996) reported QTL resistant to SCN race 3 in the same region as reported here. This QTL region appears to be a new resistance source against SCN HG type 1.2.7 (race 5). The final common QTL related to HG types 0 and 1.2.7 (races 3 and 5) located on LG M also believe to be unique in SS97-6946. Heer *et al*. (1998) and Webb *et al*. (1995 and 2003), studied with single marker analysis and assigned minor QTLs linked with markers A131 and php020301a, respectively on LG M associated with SCN HG types 2.5.7, 0 and 1.2.7 (races 1, 3 and 5, respectively) resistances. The interval associated with HG type 0 (race 3) resistances detected in this study was distant from markers reported in J87-233 and PI 437654. Thus, the reported soybean accessions may not contribute to the common resistance genes with SS97-6946 on LG M to race 3 and 5. So far no QTL resistant to SCN HG type 2.5.7 (race 1) on LG E has been reported in any previous study but QTL resistant to SCN HG type 1.2.5.7, 0 and 1.2.7 (race 2, 3 and 5, respectively) on LG E were reported by others (Yue *et al*., 2001a, b; Wang *et al*., 2001; Guo *et al*., 2006a) in the same region of the

soybean composite map. Another three QTL were identified in this study located on LGs B1, B2, O and all of those were associated with resistance to HG type 1.2.7 (race 5). Yue *et al*. (2001a) mapped resistance QTL for SCN races 1, 2 and 5 on LG B1 in soybean PI 438489B which overlapped the QTL region identified in this study. In other studies the QTL region mapped close to the QTL resistance for race 5 on LG B1 in this study (Webb, 2003; Guo *et al*., 2006a). Thus, QTL identified in the tested accession could be the same as previously detected in LG B1. No QTL associated with resistance to SCN race 5 has been reported on LG B2. Two QTL for resistance to race 1 and race 3, however, mapped to a different region on LG B2 (Qiu *et al*., 1999 and Yue *et al*., 2001a). One QTL associated with resistance to SCN race 2 was identified but not confirmed on LG O with a minimum LOD value 2.1 which matched the resistance QTL to race 5 in this study (Winter *et al*., 2007a). Recently, Vuong *et al*. (2010) and Arelli *et al*. (2009) reported a same QTL on LG O linked with marker Satt592. However, on the basis of physical map (Grant *et al*., 2010) that QTL was mapped approximately 53 cM away from QTL indentified in this study. Therefore, QTL identified on LGs B2, M and O could be a novel putative resistance locus for SCN race 5.

QTLs for SCN resistance for different races were classified into three categories including confirmed QTLs, suggestive QTLs and significant QTLs (Guo *et al*., 2006b). QTL associated with SCN resistance have been confirmed on LGs A2, B1, E, G and J (Concibido *et al*., 2004; Guo *et al*., 2006b), but only QTLs on G (*rhg1*) and A2 (*Rhg4*) are reported to be cloned and sequenced (Meskem *et al*., 2001; Ruben *et al*., 2006). In this study, QTLs on LGs A2, G, E and B1 were also associated with resistance to HG types 2.5.7, 0, and 1.2.7 (races 1, 3 and 5, respectively). The QTL in the same region on LG G is likely to be *rhg1* and was associated with resistance to SCN HG types 2.5.7, 0, and 1.2.7 (races 1, 3 and 5, respectively). The QTL on LG E was located in the same region as reported in other mapping studies (Concibido *et al*., 2004, Guo *et al*., 2006b) and was associated with SCN resistance to HG type 2.5.7 (race 1), but not with HG type 0, and 1.2.7 (races 3 and 5, respectively). The QTL in the same region on LG A2 was associated with SCN resistance to HG types 2.5.7 and 0 (races 1 and 3, respectively) but not with HG type 1.2.7 (race 5) (Concibido *et al*., 2004; Guo *et al*., 2006b). The QTL located in the same region on LG B1 as reported in other mapping studies (Concibido *et al*., 2004; Guo *et al*., 2006b) was associated with SCN resistance to HG type 1.2.7 (race5) but not with HG type 2.5.7 and 0 (races 1 and 3, respectively). These results are consistent with previous reports (Guo *et al*., 2006b). However, QTL on LGs A1, M and O associated with HG types 1.2.5.7, 0, and 1.2.7 (races 2, 3 and 5, respectively) in SS97-6946, have not been reported, and could be a novel putative locus for SCN resistance for respective races. Another possible novel QTL has identified on LG B2 but the resistance gene associated with HG type 1.2.7 (SCN race 5) could be come from other parent. A confirmation study is needed to verify these results.

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