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ORIGINAL PAPER

QTL, additive and epistatic effects for SCN resistance in PI 437654

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Abstract PI 437654 is a unique accession because of its resistance to nearly all HG types (races) of soybean cyst nematode (*Heterodera glycines* Ichinohe; SCN). Objectives of this study were to confirm and refine the locations and gene action associated with SCN resistance previously discovered in PI 437654, and to identify new QTLs that may have been missed because of low coverage with genetic markers used in previous studies. Using 205 $F_{7:9}$ RILs and 276 SSR and AFLP molecular markers covering 2,406.5 cM of 20 linkage groups (LGs), we confirmed and refined the locations of major SCN resistance QTLs on LG-A2, -B1, and -G previously identified in PI 437654 or other resistant sources. We found that these major QTLs have epistatic effects among them or with other loci for SCN resistance. We also detected some new QTLs with additive or epistatic effects for SCN resistance to different HG types (races) on all LGs except LGs-B2 and -D1b. The QTL on LG-G was associated with resistance to HG types 2.5.7, 1.2.5.7, 0, and 2.7 (races 1, 2, 3, and 5), and it contributed a large proportion of the additive effects. The QTL on LG-A2 was associated with resistance to HG types 2.5.7 and 0 (races 1 and 3). The QTL on LG-B1, associated with resistance to HG types 2.5.7, 0, 2.7 (races 1, 3, and 5), was the similar QTL found in PI 90763 and PI 404198B. In addition to QTL on LGs-A2, -B1 and -G, a novel additive QTL associated with SCN resistance to HG types 0, 2.7, and 1.3.5.6.7 (race 3, 5, and 14) was identified on LG-I flanked by Sat_299 and Sat_189. Several minor QTLs on LGs-C1, D1a, H, and K were also found to be associated with SCN resistance. Confirmation of the new resistance QTL is underway by evaluating another RIL population with a different genetic background.

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Introduction

Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is the most damaging among yield-suppressing soybean diseases resulting in an annual loss of approximately $1.5 billion in the USA (Wrather et al. 2001, 2006). Utilizing cultivars resistant to SCN has been an effective and practical measure to reduce or eliminate yield losses due to nematode infestation.

Resistance to SCN has been well investigated in resistance sources 'Peking', PI 88788, PI 437654 and a few other Plant Introductions (PIs). Quantitative trait loci (QTL) conferring resistance to SCN have been identified in more than 18 accessions using molecular markers (Concibido et al. 2004; Guo et al. 2006; Lu et al. 2006), among which, PI 88788 is the predominant source of SCN resistance in US breeding programs (Diers and Arelli 1999; Concibido et al. 2004). Because of broad resistance to SCN HG types

(races), PI 437654 has been considered an excellent source from which to develop resistant cultivars. Anand et al. (1988) reported that PI 437654 was resistant to nearly all SCN races 1, 2, 3, 5, and 14, which was later confirmed by Diers et al. (1997). However, the introgression of PI 437654 SCN resistance into to elite lines has been hampered by its unfavorable traits such as black seed coat, poor lodging resistance, seed shattering, and low yield (Vierling et al. 2000). Compared to phenotypic selection, marker-assisted selection expedites gene introgression, minimizes linkage drag, and maximizes recovery of the target genome (Meksem et al. 2001).

SCN resistance is multigenic and quantitative (Mansur et al. 1993; Lu et al. 2006). Five major genes have been assigned for SCN resistance through conventional genetic analysis (Caldwell et al. 1960; Matson and Williams 1965; Rao-Arelli 1994). The major resistant gene, *rhg1*, has been found in most resistance sources (Concibido et al. 2004). With advances in molecular genetics, more than 60 loci located on 17 of the 20 chromosomes had been identified to be associated with SCN resistance in different PIs by 2007 (Concibido et al. 2004; Lee et al. 2007; Kassem et al. 2007). Myers and Anand (1991) estimated that in PI 437654 three genes were required for complete resistance to races 3 and 14, and four genes were needed for resistance to race 5.

Using restriction fragment length polymorphism (RFLP) markers, Webb et al. (1995, 2003) reported six QTLs for SCN resistance to races on LGs-A2, -C1, -G, -M and two unknown linkage groups (LG-25 and LG-26) in PI 437654, and that epistatic interactions were detected among these resistance loci (Webb et al. 1995). The two unknown LGs-25 and -26 were later assigned to LG-B1 and LG-J, respectively (Concibido et al. 2004). Because the loci on LG-M for SCN resistance were dependent on QTL on LG-G and their effect were not significant when the loci on M and G were tested simultaneously, QTL on LGs-A2, B1, C1, G, and J in PI 437654, therefore, gave complete resistance to SCN races 1, 2, 3, 5, and 14 (Webb et al. 2003). However, Vierling et al. (1996) identified different regions on LGs-A2, -B1 and -F associated with SCN resistance to race 3 in 'Hartwig' that was derived from PI 437654. Schuster et al. (2001) used bulk segregant analysis to identify a novel QTL for SCN resistance against race 14 on LG-D2 in Hartwig.

Although many SCN resistance QTLs have been discovered in different resistant PIs (Concibido et al. 2004), few QTLs were confirmed in these studies. This was due to limited coverage of linkage maps for QTL analysis, inadequate statistical procedures, small mapping populations, and ignoring of epistatic effects among resistance loci. Previous studies have shown that the SCN resistance locus, *rhg1*, located on LG-G, was generally necessary for resistance in genotypes derived from any resistance source when challenged by any SCN race (Cregan et al. 1999; Meksem et al. 1999). The major QTL on LG-G near *rhg1* had been identified from PIs or lines derived from resistant sources PI 209332 (Concibido et al. 1996), Peking (Chang et al. 1997; Concibido et al. 1997; Meksem et al. 1999, 2001), PI 90763 (Concibido et al. 1997; Guo et al. 2005), PI 88788 (Concibido et al. 1997), PI 89772 (Yue et al. 2001a), PI 404198A (Guo et al. 2006), PI 437654 (Webb et al. 1995), Peking + PI 437654 (Prabhu et al. 1999) and Peking + PI 88788 + PI 90763 (Heer et al. 1998). Later however, an SCN resistance source (PI 464925B) without *rhg1* on LG-G or *Rhg4* on LG-A2 was identified for resistance to race 3 (HG type 7) and race 2 (HG type 1.2.5.7) (Winter et al. 2006). This shows that *rhg1* and *Rhg4* may not be always present and necessary loci for SCN resistance in some resistant sources.

Breeding lines with homozygous resistant alleles *rhg1* and *Rhg4* can perform differently for reaction to SCN HG type 0 (race 3) populations. The genetic response of multiple alleles to SCN HG types consists of not only gene actions at single loci, but also the inter-locus interactions and gene-by-environment interactions. Complexity of gene action can be largely attributed to epistatic effects. Inclusion of gene-by-gene interactions (epistasis) and gene-by-environment interactions in QTL mapping is expected to increase discovery of more QTLs, improve accuracy and precision of genetic gains, and enhance our ability to understand the genetic basis of complex traits (Carlborg and Haley 2004). However, a major restriction to date has been the limited ability to detect and quantify QTLs influenced by effects of gene-by-gene and gene-by-environment interactions (Podlich et al. 2004). In QTL analysis for SCN resistance, phenotyping usually is performed in a controlled environment. In this case, the gene-by-environment interactions should not be considered in QTL analysis. Therefore, the inter-locus interactions should be addressed along with QTLs with additive effects for QTL mapping of SCN resistance.

The most popular QTL mapping methods—composite interval mapping (CIM) (Zeng 1993, 1994) or multiple QTL model (MQM) (Jansen and Stam 1994) are used to detect individual QTLs more precisely than ANOVA (analysis of variance) or interval mapping (IM). There have been few studies to address epistatic effects, which influence SCN resistance in soybean. Significant epistatic effects for SCN resistance were detected by a pair-wise comparison of markers instead of QTL regions (Winter et al. 2006).

A number of major and minor QTLs associated with SCN resistance to different races have been mapped, but only few loci including *rhg1* and *Rhg4* loci are extensively used in marker-assisted selection. In theory, genetic

information obtained from QTL mapping experiments can be utilized in genetic improvement of SCN resistance. Superior genotypes with SCN resistance can be predicted based on results of QTL mapping (Yang and Zhu 2005).

Although several SCN resistance QTL were discovered (Concibido et al. 2004) and patented (Vierling et al. 2000; Webb et al. 2003) in PI 437654, this source of resistance needs to be evaluated extensively to identify other SCN resistance QTLs that may have been missed in previous studies, and to study epistatic effects between QTL regions. Since PI 437654 shows resistance to most HG types, the purpose of this study is to dissect all QTLs conferring resistance to most SCN HG types and to reveal additive and epistatic relationships of QTLs to different SCN races with up to date analysis methods. Understanding genetic relationships among QTLs associated with resistance to various SCN HG types will enable soybean breeders to improve introgression of SCN resistance QTLs into elite soybean cultivars.

Materials and methods

Plant material

A population of 205 $F_{7:9}$ recombinant inbred lines (RILs) from a cross of Essex × PI 437654 was used in this study. PI 437654 is a plant introduction that originated from China but was contributed to the USDA Soybean Germplasm Collection via a Russian germplasm collection. PI 437654 is resistant to most known SCN HG types (Anand et al. 1988) while Essex is susceptible to all known SCN HG types or races (Chen et al. 2006). Soybean seeds from each RIL were planted for phenotyping to SCN populations and leaf tissue was collected for DNA isolation. Genomic DNA was extracted from a pooled sample of leaves of five plants of each RIL.

SCN bioassay

SCN bioassays were performed in the greenhouse at the University of Missouri-Columbia using established methods (Arelli et al. 1997). SCN races 1 (HG type 2.5.7), 2 (HG type 1.2.5.7), 3 (HG type 0), 5 (HG type 2.7), and 14 (HG type1.3.5.6.7) used in this experiment were inbred, near-homogeneous populations maintained for many generations at the University of Missouri-Columbia (Arelli et al. 1997, 2000). Population purity of each race and success of the phenotyping experiments were monitored and evaluated by SCN reaction to indictor lines, Pickett, Peking, PI 88788, PI 90763, PI 437654, PI 209332, PI 89772, PI 548316, and Hutcheson (susceptible control). Two independent experiments were conducted for all HG types (races). Based on the reactions of indictor lines to HG types, all experiments except one for HG type 1.3.5.6.7 (race 14) were successfully performed.

Germinated soybean seeds were transplanted into micropots (one plant in each micropot) filled with steam-pasteurized sandy soil. Five plants of each indictor line and five single-plants per RIL were randomly planted in micropots. Twenty-four micropots each were placed in plastic containers and maintained at 27 ± 1°C in a water bath. Two days after transplanting, each plant was inoculated with 2,000 ± 25 SCN eggs. Thirty days after transplanting, nematode cysts were washed from roots of each RIL and counted under a microscope. The female Index (FI) was used to evaluate SCN response of each individual seedling of all plants including 205 RILs, parental lines, and indicator lines. The FI was calculated as a percentage as follows:

$$\mathrm{FI}(\%) = \frac{\text{number of female cyst nematodes on a given individual}}{\text{average number of female nematodes on Hutcheson}} \times 100$$

Molecular marker analysis

DNA from leaf tissue of each RIL was extracted using the CTAB method (Keim et al. 1988). All SSR markers used in this study were already mapped in the soybean composite map (Song et al. 2004). A subset of SSRs was selected based on location of SSR markers on the composite map to test polymorphism between the two parents. SSR primers were synthesized by Illumina Inc. (San Diego, CA, USA) or Integrated DNA Technologies Inc. (Coralville, IA, USA) for regular oligos, and by Applied Biosystems (Foster City, CA, USA) for fluorescent labeled oligos. Polymerase chain reaction (PCR) was conducted with a final volume of 15 μL on the Eppendorf 96-well master cycler gradient (Eppendorf AG, Germany). For regular PCR, each reaction included 50 ng genomic DNA, 0.2 μM of each of the primers, 0.2 mM each of dNTPs, 2.5 mM of $MgCl_2$, and 1 unit of *Taq* DNA polymerase (Genscript Corporation, Piscataway, NJ, USA). For PCR with fluorescent labeled primers, forward primer (labeled) was used with a final concentration of 0.13 and 0.2 μM of reverse primer, no change for other components. PCR reaction was performed at 95°C for 5 min, followed by 35 cycles of 94°C for 30 s, 47°C for 30 s, and 72°C for 45 s, with a final extension for 10 min at 72°C. PCR products were separated on 3.5% SFR argarose gels (Amresco Inc., USA) stained with ethidium bromide for regular primers or run on ABI sequencer 3,100 or 3,730 for multiplex pooled samples amplified with labeled primers. The allele of each marker was determined by manually scoring the bands on

gel images or by analysis of ABI data with GeneMapper 3.7 software. A library of 700 SSR markers was initially screened against the two parents Essex and PI 437654 to determine polymorphic markers. From which, 343 polymorphic markers were identified. Of the 343 markers, 276 markers evenly distributed over the 20 linkage groups (Song et al. 2004) were selected and genotyped.

AFLP analysis was performed as described by Vos et al. (1995) with minor modifications (Wu et al. 2003). Briefly, primary template DNA was prepared using the restriction enzymes *Eco*RI and *Mse*I, followed by a ligation reaction with those enzyme-specific adapters:

*Eco*RI adapter forward, 5′-CTCGTAGACTGCGTACC-3′; reverse *Eco*RI adapter R 5′-AATTGGTACGCAG TCTAC-3′;
*Mse*I adapter forward, 5′- GACGATGAGTCCTGAG-3′; reverse 5′-TACTCAGGACTCAT-3′

The first PCR amplification of *Eco*RI/*Mse*I-digested ligated fragments was performed using two sets of primers with one selective nucleotide:

Pre-amplification primer *Eco*RI 5′-GACTGCGTACC AATTCA-3′
Pre-amplification primer *Mse*I 5′-GATGAGTCCTGA GTAAC-3′

The second PCR amplification of restriction fragments was performed using 32 pairs of selective primers with three extended nucleotides to test polymorphism between parents. We selected six primer pairs with more polymorphic AFLP markers to genotype the entire population to provide 90 AFLP markers. The primer pairs used are Eaac/Mcac, Eaac/Mctc, Eaag/Mcat, Eaca/Mctt, Eagg/Mcag, and Eagg/Mctt. The 5′nucleotide of the *EcoR*I (+3) selective amplification primers was labeled with 6-carboxyfluorescein (6-FAM). Fluorescent labeled DNA fragments were size fractionated using an ABI 3,100 genetic analyzer with 36-cm capillaries, POP-4 polymer, and rhodamine X (ROX)-labeled GS400HD internal size standards according to the manufacturer instructions (Applied Biosystems Inc., Foster City, CA). The size of fluorescent AFLP was analyzed with GeneMapper 3.7 software, the size was then transferred to binary format using a Macro script (Rinehart 2004).

Statistical analysis

A broad-sense heritability estimated based on variance components was calculated on an entry-mean basis with the following formula: $H^2 = \sigma^2_{\mathrm{RILs}} / \left[\sigma^2_{\mathrm{RILs}} + \left(\sigma^2_{\mathrm{e}}/r\right)\right]$ (Fehr 1987), where σ^2_{RILs} is the genotypic variance component for female index per plant among RILs, σ^2_{e} is the error variance, and r is the number of replications. The formula for skewness is:

$$\text{skewness} = \sum_{i-1}^{N} \left(Yi - \overline{Y}\right)^3 / \left[(N-1) \times s^3\right]$$

The formula for kurtosis is:

$$\text{kurtosis} = \sum_{i-1}^{N} \left(Yi - \overline{Y}\right)^4 / \left[(N-1) \times s^4\right]$$

where $\overline{Y}$ is the mean, s is the standard deviation, and N is the number of data points.

The genetic linkage map was constructed using Joinmap 3.0 (Van Ooijen and Voorrips 2001). Parameters were set as default, i.e. LOD grouping thresholds ≥2.0 and a maximum distance of 50 cM. Linkage groups were assigned according to Song et al. (2004).

Localization of SCN resistance QTL, estimation of QTL main effects and prediction of epistasis were conducted by QTL mapping software, QTLNetwork (Yang et al. 2007), using a mixed-model for the composite interval mapping method. Significance level of genome scan for candidate intervals, putative QTL detection, and QTL effects was set at 0.05, 0.001, and 0.001, respectively. Permutation tests were conducted 1,000 times to determine a critical *F* value for minimizing the experimental type I error rate. The QTL effects were estimated by Monte Carlo Markov Chain (MCMC) method (Yang et al. 2007). To distinguish the QTLs identified in this study from with existing QTLs (http://www.soybase.org), we denoted a QTL by the following rules: the first letter was given as 'S' and represented the abbreviation of SCN resistance trait, followed by the linkage group serial number, then by '-', the digit following the '-' was the ordinal number of the QTL according to its position on the corresponding LG starting from the top end. New identified loci that were less than 5 cM apart were considered as the same QTL.

Results

Phenotypic variation of SCN resistance and relation of different races

Statistical analysis of the FI of all RILs in the Essex × PI 437654 population (Table 1) showed that two independent phenotyping experiments of each race were very consistent for response of genotypes to HG types 2.5.7 (race 1), 0 (race 3), and 2.7 (race 5). But an insignificant genotype-by-environment-interaction was found for HG type 1.2.5.7 (race 2). Broad-sense heritability on an entry-mean basis for female index of different races ranged from 0.54 to 0.84, suggesting that there was a major genetic component conditioning SCN resistance in this population.

Table 1 Female index (FI) phenotypic variance, and heritability analyzed for 205 $F_{7:9}$ RILs from Essex × PI 437654 to HG types 2.5.7, 1.2.5.7, 0, 2.7, and 1.3.6.7 (races 1, 2, 3, 5, and 14)

Race	V(G)	V(E)	V(GE)	V(e)	h^2
1	1212.4	42.0	0	2228.3	0.731
2	479.9	42.9	106.8	1962.0	0.551
3	1250.4	0.0004	0	1409.4	0.816
5	804.9	17.3	0	746.5	0.844
14	188.9	–	–	801.8	0.541

V(*G*) variance of genetic main effects, *V*(*E*) variance of environmental effects, *V*(*GE*) variance of genotype-by-environment interaction effects, *V*(*e*) variance of residual effects, h^2 broad-sense heritability on entry-mean basis

The skewness and kurtosis of the distribution of FI across the entire RIL mapping population deviated slightly from zero (Table 2; Fig. 1). This indicated that the frequency distributions of RILs for FI were not normally distributed for HG types 2.5.7 (race 1), 1.2.5.7 (race 2), 0 (race 3), and 2.7 (race 5). However, the frequency distribution of FI was normal for RILs for reaction to HG type 1.3.5.6.7 (race 14). Even though distributions were skewed, original data were used for QTL analysis for all races. The effect of non-normality on QTL analysis should be significantly reduced due to use of cofactor and permutation testing for the determination of an empirical threshold value (Yang et al. 2007).

Molecular markers and genetic map

The SSR marker order in a linkage group was arranged according to the expected order of the genetic marker anchored physical map (Wu et al. 2008) aligned with the 7× whole genome shotgun sequence assembly Glyma0 (http://www.phytozome.net/soybean, Soybean Genome Project, DoE Joint Genome Institute). If the position of a marker conflicted with the fixed order, the marker was removed from the LG to minimize the impact of genotyping errors on QTL position. Finally, a linkage map spanning 2,406.5 cM across 26 LGs was constructed using 238 SSR, 35 AFLP markers, and a deletion marker (Rhg1-indel) developed from *rhg1* candidate gene (GenBank AF506517, Ruben et al. 2006) that mapped near Satt309 on LG-G. Compared to the soybean composite map (Song et al. 2004; Choi et al. 2007), the linkage map constructed with the 205 RILs was very consistent in marker order and relative distance between SSR markers except few regions (Fig. 2). Single gaps (>50 cM between neighboring markers) existed in LGs-C1, -C2, -D2, -F, -H, and -J, respectively. The genetic map included 26 linkage groups, covering 2,406.5 cM with an average distance of 8.7 cM (Fig. 1).

Table 2 Summary of statistics on Female Index (FI) value of the parents and 205 $F_{7:9}$ RILs from Essex × PI 437654 for reaction to SCN HG types 2.5.7, 1.2.5.7, 0, 2.7, and 1.3.6.7 (races 1, 2, 3, 5, and 14)

Race	Parents		RILs					
	Essex	PI 437654	Mean	Min.	Max.	SD	Skew	Kurt
1	136.0	0.3	97.9	1.3	164.0	39.0	−0.98	0.33
2	129.4	0.2	91.1	3.5	160.4	34.1	−0.38	−0.03
3	141.5	1.4	74.7	0.0	167.4	38.1	−0.45	−0.41
5	110.2	0.5	61.6	0.8	120.5	25.0	−0.85	0.44
14	98.2	9.0	48.9	3.8	130.2	22.6	0.37	−0.02

Min. minimum, *Max.* maximum, *SD* standard deviation, *Skew* skewness, *Kurt* kurtosis

Additive QTLs conditioning SCN resistance

QTLs with additive effects for SCN resistance to HG types 2.5.7, 1.2.5.7, 0, 2.7, 1.3.5.6.7 (races 1, 2, 3, 5, and 14), using software QTLnetwork 2.0, are shown in Table 3 and Fig. 2.

Race 1

Five QTLs with additive effects were mapped to soybean LGs-A2, -B1, -G, -H, and -K. Heritability values at QTL (S12-1) mapping to a 3-cM region around Rhg1_indel locus and Satt309 on LG-G showed the largest additive effect (Table 3). SSR marker Satt309 associated with *rhg1*, a major gene for SCN resistance, has been effectively used for marker-assisted selection for SCN resistance (Cregan et al. 1999). A QTL (S2-4) mapped to a 4.3-cM interval between Satt187 and AW132402 on LG-A2 and accounted for 5.1% of the additive variance associated with resistance. Satt187 was close to the *I* locus affecting seed coat color (Song et al. 2004). This QTL may be associated with *Rhg4*, a previously reported resistance QTL on LG-A2. Another major QTL accounting for 2.7% of the additive effect was detected between Satt665 to EacaMctt067 on LG-B1. In the same region, one cluster of QTLs for races 2 and 5 had been reported in PI 90763 and PI 404198A (Guo et al. 2005, 2006). A QTL associated with reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira) resistance was also identified from the same region in PI 437654 and confirmed in a different genetic background (Ha et al. 2007). In addition, two minor QTLs: S13-4 on LG-H and S16-5 on LG-K each provided 1% additive effects for SCN resistance. This is the first report of a QTL on linkage group K associated with SCN resistance. The QTL (S13-4) on LG-H was 65 cM away from a previously reported resistance QTL by Qiu et al. (1999) in Peking, indicting that QTL S13-4 is a new resistance QTL.

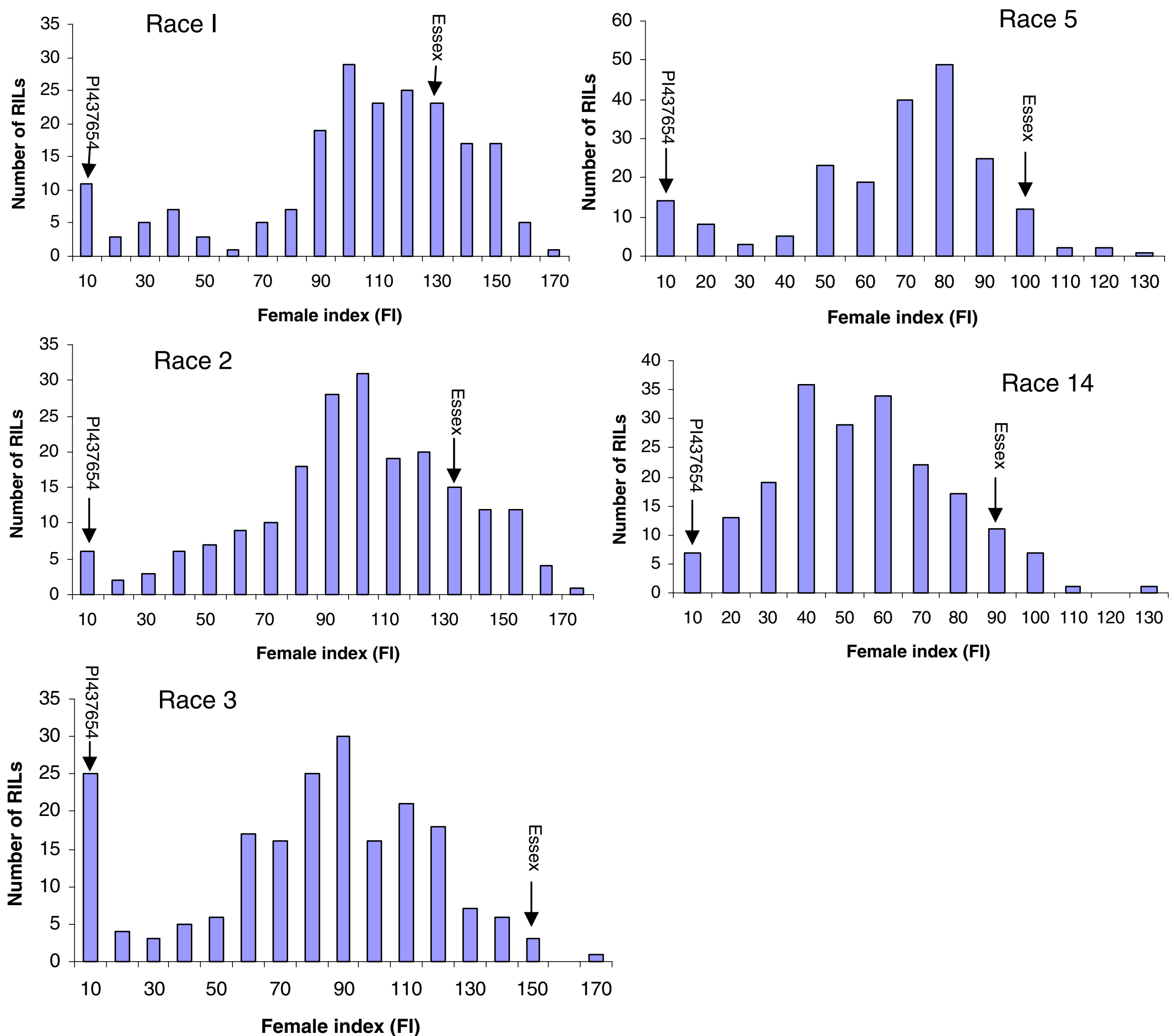

Fig. 1 Distribution of average Female Index (FI) of 205 $F_{7:9}$ RILs from Essex × PI 437654; PI 437654, SCN resistant parent; and Essex, SCN susceptible parent

Race 2

Two QTLs (S7-6 and S12-2) with additive effects, mapping to LGs- D1a and -G, respectively, showed a significant association with SCN resistance to Race 2 (Table 3; Fig. 2). As with race 1, the QTL S12-2 on LG-G was a major QTL that also mapped to the *rhg1* region. In this study, the QTL S7-6 mapped to a 13.0 cM region between AW781285 and Sat_305 on LG-D1a in which the QTL position was different from previously reported QTLs on the same LG (Yue et al. 2001a, b). The QTL S7-6 located on the 64–84 cM region in the soybean composite genetic map (Choi et al. 2007), may be different from the two reported QTLs that are located on regions of the 7.8–30.7 and 45.7–47.6 cM, identified in PI 89772 (Yue et al. 2001a) and PI 438489B (Yue et al. 2001b), respectively.

Race 3

Six QTLs with additive effects were detected on LGs -A2, -B1, -C1, -D1a, -G, and -I. The QTL on LG-G provided a large proportion of the additive effects for SCN resistance to race 3 (Table 3; Fig. 2). The QTLs on LGs -A2 and -G identified in our study fell on the same regions associated with *rhg1* and *Rhg4* as the ones identified for resistance to

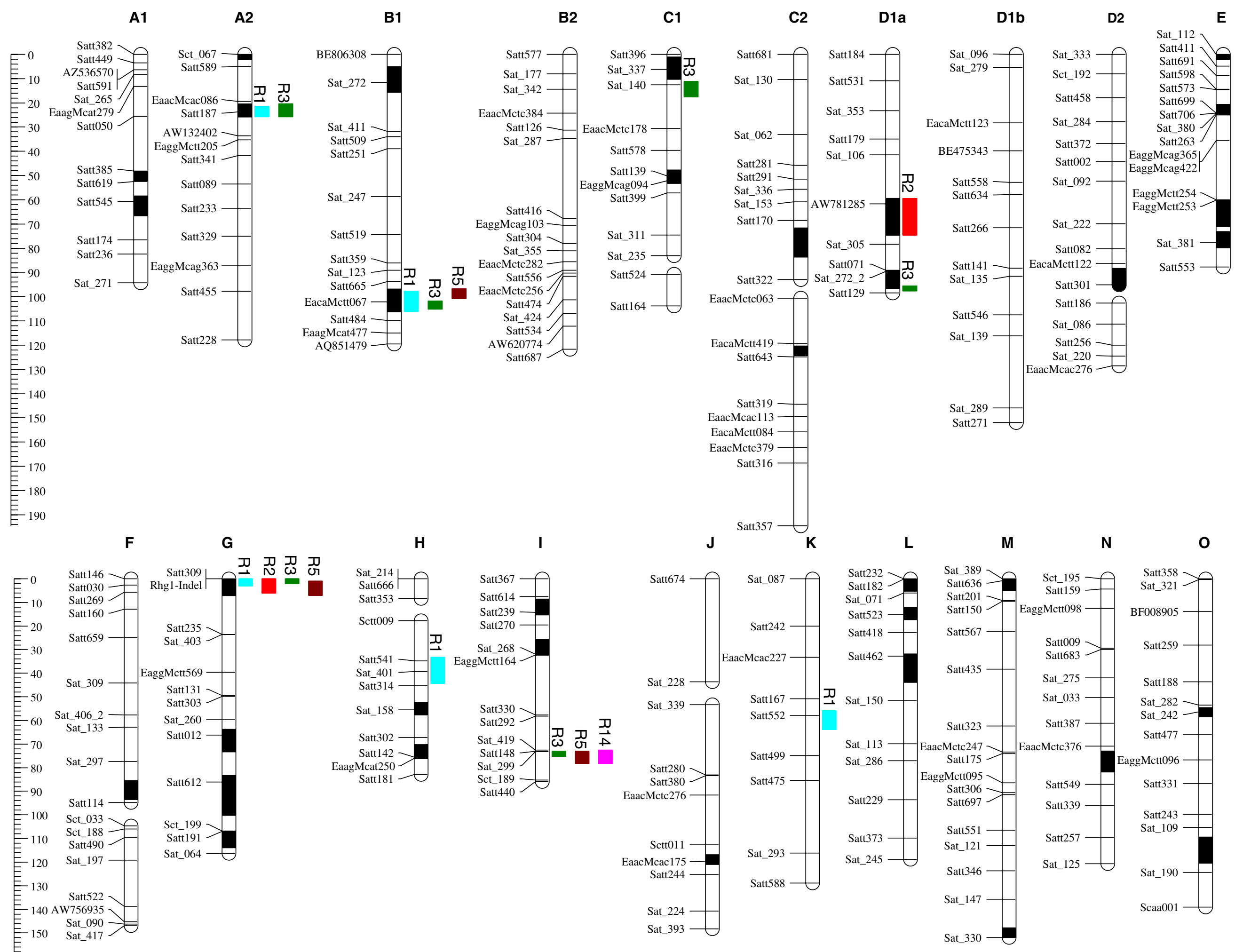

Fig. 2 Linkage map constructed using an $F_{7:9}$ RIL population from the cross Essex × PI 437654. Additive QTLs are indicated by a *bar* on the right of linkage group: *R1* race 1, *R2* race 2, *R3* race 3, *R5* race 5, and *R14* race 14. The *highlighted segments* in the linkage group bars represent epistatic QTLs

race 3 in other studies (Webb et al. 2003; Concibido et al. 2004; Yue et al. 2001a, b; Guo et al. 2005, 2006). Based on the soybean composite map (Choi et al. 2007), the QTL (S3-11) on LG-B1 (with 1.1% contribution to additive effect) identified in our study was different from the reported QTL on LG-B1 for race 3 identified in Hartwig, a derivative of PI 437654 (Vierling et al. 1996), and was also different from the patented QTLs associated with resistance to races 1, 2, 5, and 14 (Webb et al. 2003). The confidence interval of the QTL 3-11 for race 3 on LG-B1 overlapped with the one of QTL S3-10 for race 1. This QTL fell in the same region on LG-B1 as the one identified from PI 404198A and PI 90763 for resistance to races 2 and 5 (Guo et al. 2005, 2006). We also identified another new QTL, S14-11on LG-I with a 2.5% contribution of additive effect, located in a 2.2-cM region between Sat_299 and Sct_189. Associations between several markers on LG-I and SCN resistance to race 2 and 3 were observed in a *Glycine soja* PI 464925B (Winter et al. 2006). The QTL S14-11 is very close to these associated markers. The QTL S5-3 on LG-C1 is 17.3-cM away from a QTL identified from PI 438489B (Yue et al. 2001b) that has been confirmed in another population with different background (T. Vuong, University of Missouri, personal communication). Thus, the QTL on LG-C1 detected in this study may be the same QTL detected in PI 438489B. Although a QTL on LG-C1 was patented by Pioneer Hi-Bred International, Inc. (Webb et al. 2003), it is difficult to determine whether the QTL S5-3 and the patented QTL are located in the same region because the information for the location of the patented QTL in the composite map is unknown.

Table 3 Additive QTLs for SCN resistance in 205 $F_{7:9}$ RILs from Essex × PI 437654 for reaction to HG types 2.5.7, 1.2.5.7, 0, 2.7, and 1.3.6.7 (races 1, 2, 3, 5, and 14 detected by mixed-model based composite interval mapping method)

LG	QTL	Interval	Confidence interval (cM)	*a*	*P* value	h_a^2
Race 1						
A2	S2-4	Satt187-AW132402	21.4–25.7	13.2263	0.000000	0.0508
B1	S3-10	Satt665-EacaMctt067	97.8–106.2	9.6159	0.000000	0.0269
G	S12-1	Satt309-Rhg1-indel	0.0–3.0	25.0819	0.000000	0.1828
H	S13-4	Sctt009-Satt541	14.5–26.6	5.4355	0.000062	0.0086
K	S16-5	Satt552-Satt499	55.8–63.8	5.6047	0.000001	0.0091
Race 2						
D1a	S7-6	AW781285-Sat_305	59.4–74.6	5.6625	0.000028	0.0126
G	S12-2	Rhg1-indel-Satt235	0.0–6.0	12.7443	0.000000	0.0637
Race 3						
A2	S2-3	EaacMcac086-Satt187	20.4–25.7	10.6135	0.000000	0.0424
B1	S3-11	EacaMctt067-Satt484	101.8–105.2	5.3639	0.000000	0.0108
C1	S5-3	Sat_140-EaacMctc178	11.2–17.6	4.3267	0.000000	0.007
D1a	S7-9	Sat_272_2-Satt129	95.6–97.6	5.3501	0.000000	0.0108
G	S12-2	Rhg1-indel-Satt235	0.0–2.0	24.1888	0.000000	0.22
I	S14-11	Sat_299-Sct_189	73.0–75.2	8.1915	0.000000	0.0252
Race 5						
B1	S3-10	Satt665-EacaMctt067	96.8–100.8	14.1926	0.000000	0.1298
G	S12-2	Rhg1-indel-Satt235	1.0–7.0	15.9775	0.000000	0.1645
I	S14-11	Sat_299-Sct_189	73.0–78.2	7.1189	0.000000	0.0327
Race 14						
I	S14-11	Sat_299-Sct_189	72.5–78.2	5.4274	0.000000	0.0312

QTLs were designated as "S LG serial number-serial numberof intervals". Confidence interval denotes the region of a QTL measured as the genetic distance in centi-Morgan. *a* denotes additive effect of QTL. h_a^2 represents the contribution ratio of QTL effect. All estimated values were significant at 0.005 probability level

Race 5

Single QTLs from PI 437654 were identified on LGs-G, -B1, and -I, and the QTLs on LG-G and LG-B1 were predominate, providing 16.5, 13.0, and 3.3% of the additive effects for resistance to race 5, respectively (Table 3; Fig. 2). These three QTLs regions were often associated with SCN resistance to multiple races in this study (Table 3; Fig. 2).

Race 14

Only one QTL on LG-I from PI 437654 was associated with SCN resistance to race 14 (Table 3; Fig. 2). The QTL was in a similar position those identified for races 3 and 5. Thus, they may be the same resistance gene(s).

In conclusion, based on our results, the major gene *rhg1* on LG-G indeed plays an important role for SCN resistance to races 1, 2, 3, and 5 but not to 14. The major gene *Rhg4* on LG-A2 seems be effective only for races 1 and 3; one QTL region on LG-B1 flanked by Satt665 and Satt484 is an important QTL for resistance to race 1, 3, and 5. A novel QTL on LG-I is associated with SCN resistance to race 3, 5, and 14. Therefore, in addition to the two well-studied loci on LGs-G and -A2 that are associated with *rhg1* and *Rhg4*, respectively, QTLs on LG-B1 and LG-I are also important for resistance to multiple races.

QTL epistasis

In the present study, a total of 24 pairs of epistatic interactions were detected for SCN resistance to races 1, 2, 3, 5, and 14 (Table 4). The negative epistatic effects of additive × additive (AA) indicated that the two epistatic loci (i and j) with homozygous alleles from the resistant parent PI 437654 could decrease FI and enhance SCN resistance. Meanwhile, the positive epistatic effect (AA) indicated that the two epistatic loci (i and j) have different alleles, one from the resistant parent PI 437654 and another from susceptible parent Essex.

Race 1

Three pairs of epistatic interactions were detected (Table 4) and involved five loci distributed on different linkage groups. Among the detected epistatic effects, three loci with additive effects located on A2, B1, and G play an important role because they were involved in multiple pairs of epistatic interactions. The epistatic interaction occurring between the loci Rhg1-indel (LG-G, *rhg1*) and Satt187 (LG-A2, close to *Rhg4*), had the largest effect and accounted for 4.6% of the epistatic effects. We also found one epistatic interaction involved the Essex (susceptible) allele of locus Satt396 on LG-C1 with the Satt385 allele on LG A1.

Table 4 Epistatic interactions between different QTLs analyzed for 205 $F_{7:9}$ RILs from Essex × PI 437654 for reaction to SCN HG types 2.5.7, 1.2.5.7, 0, 2.7, and 1.3.6.7 (races 1, 2, 3, 5, and 14)

QTL_i	LG	Interval_i	Range_i	QTL_j	LG	Interval_j	Range_j	AA	*P* value	h^2_{aa}
Race 1										
S2-4	A2	Satt187-AW132402	21.4–25.7	S12-1	G	Satt309-Rhg1-indel	0.0–3.0	−12.63	0.000000	0.0463
S3-10	B1	Satt665-EacaMctt067	97.8–106.2	S12-1	G	Satt309-Rhg1-indel	0.0–3.0	−6.51	0.000000	0.0123
S1-8	A1	Satt385-Satt619	48.1–52.1	S5-1	C1	Satt396-Sat_337	1.0–9.2	4.48	0.000195	0.0058
Race 2										
S5-6	C1	Satt139-EaggMcag094	47.6–52.2	S12-12	G	Satt191-Sat_064	106.8–113.9	−8.66	0.000000	0.0294
S14-2	I	Satt614-Satt239	8.5–15.2	S17-6	L	Satt462-Sat_150	31.8–43.8	−7.17	0.000001	0.0202
S7-6	D1a	AW781285-Sat_305	59.4–74.6	S12-2	G	Rhg1-indel-Satt235	0.0–6.0	−5.88	0.000019	0.0136
S1-10	A1	Satt545-Satt174	58.4–66.6	S9-10	D2	EacaMctt122-Satt301	88.2–95.2	7.92	0.000000	0.0246
S14-4	I	Satt270-Sat_268	25.6–32.2	S18-2	M	Satt636-Satt201	0.0–4.8	5.06	0.000011	0.0101
Race 3										
S2-3	A2	EaacMcac086-Satt187	20.4–25.7	S12-2	G	Rhg1-indel-Satt235	0.0–2.0	−10.55	0.000000	0.0419
S5-6	C1	Satt139-EaggMcag094	50.2–53.2	S6-11	C2	EacaMctt419-Satt643	112.6–115.6	−9.77	0.000000	0.0359
S10-14	E	Sat_381-Satt553	73.1–79.8	S13-9	H	Satt302-Satt142	60.9–66.9	−4.90	0.000000	0.0090
S3-11	B1	EacaMctt067-Satt484	101.8–105.2	S12-2	G	Rhg1-indel-Satt235	0.0–2.0	−4.83	0.000000	0.0088
S5-7	C1	EaggMcag094-Satt399	50.2–53.2	S17-4	L	Satt523-Satt418	12.0–17.2	−3.82	0.000001	0.0055
S11-9	F	Sat_297-Satt114	85.4–93.4	S13-8	H	Sat_158-Satt302	43.0–48.3	7.07	0.000000	0.0188
S10-7	E	Satt706-Sat_380	20.6–24.9	S20-7	O	Sat_242-Satt477	54.5–58.3	6.34	0.000000	0.0151
S2-1	A2	Sct_067-Satt589	0.0–2.0	S12-10	G	Satt612-Sct_199	83.2–88.1	4.73	0.000000	0.0084
S14-5	I	Sat_268-EaggMctt164	29.6–32.0	S17-2	L	Satt182-Sat_071	0.2–5.2	3.53	0.000253	0.0047
Race 5										
S3-10	B1	Satt665-EacaMctt067	96.8–100.8	S12-2	G	Rhg1-indel-Satt235	1.0–7.0	−11.39	0.000000	0.0836
S5-2	C1	Sat_337-Sat_140	3.0–10.2	S18-16	M	Sat_147-Sat_330	147.7–151.7	−5.88	0.000000	0.0223
S10-13	E	EaggMctt253-Sat_381	60.0–71.1	S20-12	O	Sat_109-Sat_190	109.3–120.3	7.66	0.000000	0.0377
S6-8	C2	Satt170-Satt322	71.5–83.5	S19-9	N	EaacMctc376-Satt549	72.8–81.8	6.99	0.000000	0.0315
S12-10	G	Satt612-Sct_199	87.1–100.1	S15-8	J	EaacMcac175-Satt244	107.2–111.4	5.09	0.000000	0.0167
Race 14										
S3-2	B1	Sat_272-Sat_411	5.0–15.6	S10-1	E	Sat_112-Satt411	0.0–2.0	−3.97	0.000082	0.0167
S7-9	D1a	Sat_272_2-Satt129	89.1–96.6	S12-9	G	Satt012-Satt612	63.6–73.2	5.95	0.000000	0.0374

QTLs were designated as "S LG serial number-serial numberof intervals", *QTL_i and QTL_j* the two QTL involved in epistatic interaction, *interval_i* the flanking markers of QTL_i, *LG* linkage group, *range_i* the position of support interval of QTL_i in cM, *interval_j* the flanking markers of QTL_j, *range_j* the position support interval of QTL_j in cM, *AA* the estimated additive by additive effect, h^2_{aa} represents the contribution ratio of QTL effect

Race 2

Five pairs of epistatic interactions were detected (Table 4) involving 10 loci distributed on eight linkage groups. Among the detected epistatic effects, two pairs of interactions involved Essex alleles at five loci with Satt301 (LG-D2) and Satt636 (LG-M). One pair of interactions occurred between the two QTLs with additive effects, the QTL S7-6 on LG-D1a and the QTL S12-2 on LG-G close to *rhg1*.

Race 3

Nine pairs of epistatic interactions were detected (Table 4) and involved 14 loci distributed on 11 linkage groups. Among the detected epistatic effects, three QTLs with additive effects on LGs-A2, -B1, and -G were involved in two pairs of interactions among these loci. The interaction between the loci Rhg1-indel (LG-G) and Satt187 (LG-A2, close to *Rhg4*) had the largest effect and contributed to 4.2% of the epistatic effects. We also found that four pairs of epistatic interactions involve the Essex (susceptible) allele at loci Sat_297 (LG-F), Satt612 (LG-G), Satt182 (LG-L), and Sat_242 (LG-O).

Race 5

Two pairs of epistatic interactions between loci with resistant alleles were detected (Table 4). Satt665 (LG-B1)

locus interacts strongly with Rhg1_indel (LG-G) locus so that the interaction of the two loci significantly improve SCN above the level of resistance to race 5 of the two QTLs acting additively. In addition, three pairs of epistatic interactions between loci with resistant alleles and loci with susceptible alleles were also detected. The epistatic loci involving the Essex allele are Satt244 (LG-J), Satt549 (LG-N), and Sat_109 (LG-O).

Race 14

Two pairs of epistatic interactions involving four non-additive loci were detected for race 14. One locus Satt012 on LG-G carrying the Essex allele contributed to a decrease in nematode number (Table 4).

Totally, we found 24 pair-wise epistatic interactions and 25% of interactions were involved by additive QTLs. But not all additive QTLs involved in epistatsis, only 10 of 17 additive loci involved. In fact, the additive QTLs involving in epistatic interactions were only four loci located on LGs-A2, -B1, -D1a, and -G across different races. In another words, most of epistatic loci were non-additive QTLs.

Discussion

QTL discovery and refinement

QTLs have been identified using molecular markers for SCN resistance to different races in more than 18 soybean accessions (Concibido et al. 2004; Guo et al. 2006). Although many QTLs have been mapped on 17 of the 20 chromosomes by 2007 (Concibido et al. 2004; Lee et al. 2007; Kassem et al. 2007), few have been confirmed in additional populations in the same or different genetic backgrounds (Glover et al. 2004). For example, Webb et al. (1995) reported three QTLs for SCN resistance against race 3 on LGs-A2, -G, and -M in PI 437654. However, Vierling et al. (1996) found different regions (LGs -A1, -B1, and -F) along with LG-G associated with SCN race 3 resistance in a ‘Williams 82’ × ‘Hartwig’ (resistance derived from PI 437654 and Peking) population. In addition, Webb et al. (2003) found some minor QTLs on LG-C1, L25, and L26 were effective against multiple races of SCN in PI 437654. Schuster et al. (2001) also identified a novel QTL for SCN resistance against race 14 on LG-D2 in a cross between ‘Hartwig’ and a susceptible line, ‘BR-92-31980’.

The discrepancies of SCN QTL mapping from different studies could be a result of genetic background, nematode population, the number of markers, population size and type, and QTL mapping methods (Concibido et al. 2004). The number of molecular markers and their distribution across linkage groups used for QTL analysis affect the number of QTLs detected and the estimation of their positions. If a small number of markers are used, some QTLs may be missed because of low coverage. Resistant accessions (such as Peking) used in different studies may have responded differently to SCN due to use of different seed sources rather than a common origin. Also, the original race scheme for designating SCN populations (Golden et al. 1970) was not broad enough to accurately classify or identify the variability in nematode populations used in different studies. Early mapping studies, where mapping discrepancies exist, were based on the race scheme rather than the new classification scheme for describing genetically diverse populations of SCN (Niblack et al. 2002).

As mentioned above, unique loci responding to a specific SCN population may exist in the soybean genome. In the present study, the QTL from PI 437654 on LG-I for resistance to SCN HG types 0, 2.7, and 1.2.6.7 (race 3, 5 and 14) mapped to an interval flanked by SSR markers Sat_299 and Sct_189 (Fig. 2). The two-point map distance between these SSRs was 11 cM in this population of 205 RILs, corresponding to a ∼4 Mb region on the Williams 82 physical map (unpublished data). RFLP marker K011_1 associated with SCN resistance to HG type 2.7 (race 5) was identified on LG-I in Peking (Qiu et al. 1999), but the map position was at 34.2 cM whereas Sat_299 was at 98.4 cM (Choi et al. 2007). Recently, Winter et al. (2006) reported that SSR markers Satt162 and Satt148 had a strong association for resistance to HG type 0 (race 3) and HG type 1.2.5.7 (race 2), respectively in *G. soja* accession PI 464925B. Satt162 mapped to 13.2 cM above Sat_299, but based on the soybean composite map (Choi et al. 2007), Satt148 was located within the QTL S14-11 region (Sat_299–Sct_189) on LG-I found in this study. In addition, two new QTLs with small effects in PI 437654 were associated with resistance to races 1 (Table 3). They were located on LGs -H and -K with flanking markers Sctt009-Satt541 and Satt552-Satt499, respectively. Recently, a QTL on LG-K flanked by SSR markers Satt269 and Satt196 mapped to 5.3 cM below Satt499 (Winter et al. 2006). The QTLs on LGs -I, -H, and -K discovered in this study may be novel QTLs but they need to be confirmed in additional populations with a different genetic background.

Using an appropriate mapping approach for QTL analysis is also important. There are several mapping approaches used in QTL mapping studies: ANOVA, interval mapping, composite interval mapping, multiple interval mapping, and a mixed-model based composite interval mapping. Most of markers or QTLs associated with SCN resistance that were identified in prior studies used analysis of variance (Concibido et al. 1996, 1997; Qiu et al. 1999; Winter et al. 2006) and/or the interval mapping method in MAPMAKER/QTL (Qiu et al. 1999; Yue et al. 2001a, b;

Schuster et al. 2001) to evaluate the associations between markers and SCN resistance or to estimate the position and the effect of single QTL. Because interval mapping was used, the existence of multiple QTLs in the linkage group can distort the QTL positions and effects. Therefore, the composite QTL mapping method adapted by QTL Cartographer software was commonly used in recent SCN QTL mapping investigations (Guo et al. 2005, 2006) because it was more powerful in detecting multiple QTLs. QTLNetwork 2.0 (Yang et al. 2007) is an alternative approach to dissect quantitative traits. It is based on QTLmapper 2.0 and integrates the effects of multiple QTLs, epistasis and QTL-by-environment interactions into one mapping system, so called mixed-model based composite interval mapping. This method has been used in rice (Liu et al. 2007) and identifies loci with additive effects and can detect epistatic loci. Despite many SCN resistance QTLs have been detected in previous studies, the role of epistasis among QTLs or non-QTL loci is not well established. Results indicate that epistasis is a very important genetic component for SCN resistance.

Epistatic interactions

Importance of epistatic gene action in expression of complex traits has been demonstrated in recent QTL mapping studies (Ohno et al. 2000; Yang et al. 2007). Some studies showed that the epistatic variance can account for a large proportion of the genetic variation of quantitative traits in a mapping population (Carlborg et al. 2005; Malmberg et al. 2005).

SCN resistance is controlled by multiple genes (Caldwell et al. 1960; Matson and Williams 1965; Rao-Arelli 1994; Lu et al. 2006). Although a number of major and minor QTLs had been mapped on most of the 20 chromosomes (Concibido et al. 2004), to date none has been cloned and characterized. The QTLs on LGs-G and -A2 (*rhg1* and *Rhg4*, respectively) have been well-studied and molecular markers have been saturated around these two loci (Cregan et al. 1999; Mudge et al. 1997; Weismann et al. 1992; Meksem et al. 2001; Lewers et al. 2002; Ruben et al. 2006). A candidate gene for *rhg1*was identified but its functions in SCN resistance is still unknown (Ruben et al. 2006). Evidence of epistasis and its importance in SCN resistance in soybean has not been well documented. Webb et al. (1995) reported that resistance alleles at the QTLs on LG-A2 and LG-G in PI 437654 had a significant epistatic interaction. Prabhu et al. (1999) in a study with Hartwig as the resistance source, confirmed the importance of the epistasis between QTLs on LGs-A2 and -G against SCN HG type 0 (race 3).

In most of previous studies on QTL mapping of SCN resistance, epistasis has been ignored. This is because either $F_{2:3}$ mapping populations were used, or QTL analysis programs used were unable to detect epistasis or statistical limitations exist. In animals and plants, epistatic interactions, explaining a majority of the effects, play an important role for parasite resistance (Wilfert and Schmid-Hempel 2008). The analysis of epistatic interactions can lead to the detection of a QTL that by itself have no impact on a trait, but has a significant effect when interacting epistatically with another QTL (Purcell and Sham 2004). Neglecting of epistatic interactions also leads to underestimation of genetic variance and to overestimation of individual QTL effects (Carlborg and Haley 2004). This neglect could result in considerable loss in genetic response to marker-assisted selection, especially at late generations (Liu et al. 2004). Ignoring epistasis involving a SCN QTL on LG-J may have diminished SCN resistance in populations using marker-assisted selection in late generations compared to the early generations (Glover et al. 2004). Our study showed that genetic interactions or epistasis are important and should be considered in selecting for resistance to SCN. Understanding the existence of systematic synergistic epistasis can guide the choice of loci to manipulate and predict the final outcomes of assembling a large number of favorable alleles in marker-assisted selection or introgression (Jannink et al. 2008). In addition, recovery rate of additive QTLs was significantly higher when the same host-parasite pair was tested in different environments than when different parasite isolates or host lines were used (Wilfert and Schmid-Hempel 2008). Therefore, the genetic architecture of quantitative variation in SCN resistance includes additive and epistatic loci. Some of them may be dependent on the genetic background and SCN HG types (races).

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