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ORIGINAL PAPER

Novel quantitative trait loci for broad-based resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe) in soybean PI 567516C

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Abstract Soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) is the most destructive pest of soybean worldwide. Host plant resistance is an effective approach to control this pest. Plant introduction PI 567516C has been reported to be highly resistant to multiple-HG types of SCN. The objectives of this study were to identify and map novel quantitative trait loci (QTL) for SCN resistance to six HG types (also known as races 1, 2, 3, 5, 14, and LY1). Mapping was conducted using 250 $F_{2:3}$ progeny derived from a Magellan (susceptible) × PI 567516C (resistant) cross. $F_{6:7}$ recombinant inbred lines (RILs) developed from the $F_{2:3}$ progeny were employed to confirm the putative QTL identified. A total of 927 polymorphic simple sequence repeats (SSR) and single nucleotide polymorphism (SNP) markers were genotyped. Following the genetic linkage analysis, permutation tests and composite interval mapping were performed to identify and map QTL. Four QTL were associated with resistance to either multiple- or single-SCN HG types. Two QTL for resistance to multiple-SCN HG types were mapped to Chromosomes 10 and 18 and have not been reported in other SCN resistance sources. New QTL were confirmed by analysis of 250 $F_{6:7}$ RILs from the same population. SSR and SNP markers closely associated with these QTL can be useful for the development of near-isogenic lines for fine-mapping and positional cloning of candidate genes for SCN resistance.

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Introduction

Soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) is the most devastating pathogen of soybean [*Glycine max* (L.) Merr.], resulting in annual yield loss of approximately $1.5 billion in the United States alone (Wrather and Koenning 2006). Once established in a soybean field, it is very difficult to eradicate SCN infestation. Integrated management strategies of non-host crop rotation and resistant cultivars have been the best means of control.

Soybean cultivars resistant to SCN have been an effective and practical manner to control this pest; however, most SCN-resistant varieties have been derived from a few common resistance sources, such as plant introductions (PIs) 88788 and 548402 (Peking). Diers and Arelli (1999) reported that in the north-central USA, over 80% of public cultivars released during the 1990s with SCN-resistance were derived from PI 88788 alone. A similar trend was also observed for SCN-resistant cultivars developed by private industry. Thus, it has been shown that the continuous cultivation of the same source of resistance has resulted in genetic shifts of SCN populations, which could eventually overcome the plant resistance genes. Results of a survey recently showed that most of the SCN populations collected from Missouri soybean fields were virulent or could reproduce on indicator lines, like PI 88788, PI 209332, PI 548316, and PI 548402, used as resistance sources for soybean cultivars (Mitchum et al. 2007). Lack of diversity

for SCN resistance genes in soybean cultivars requires further investigation to identify new SCN genes from other sources of resistance.

Evaluation of most soybean (*Glycine max*) plant introductions in the USDA Soybean Germplasm Collection has been conducted to identify SCN resistance sources to SCN. Arelli et al. (1997) reported a total of 118 soybean accessions resistant to SCN races, or HG types (Niblack et al. 2002), but few were resistant to more than one SCN race. Arelli et al. (2000) evaluated an additional 32 soybean PIs and identified some yellow-coated seed PIs resistant to both races 1 and 2, such as PI 494182, PI 507354, and PI 507422. Recently, Chen et al. (2006) molecularly characterized over 120 SCN-resistant soybean accessions for races 3, 5, and 14 and reported several PIs with high resistance to multiple-races, including PI 567516C.

PI 567516C was first reported to be resistant to SCN races 1 and 2 (Arelli et al. 1997). Later, using restriction fragment length polymorphisms (RFLP), Xie et al. (1998) identified PI 567516C as a unique genotype with resistance to populations of SCN races 1, 2, and 3. Chen et al. (2006) used simple sequence repeat (SSR) markers to characterize genetic diversity among soybean accessions resistant to SCN and concluded PI 567516C was genetically unique from most other SCN resistance sources, including PI 88788 and Peking.

Over use of the same sources of SCN resistance has led to genetic vulnerability and the loss of resistance among many soybean cultivars. Young (1998) first reported a synthetic nematode population LY1, originating from a mass mating of races 2 and 3, which can reproduce on the broadly HG type resistant cultivar Hartwig, as well as its primary resistance source, PI 437654. In contrast, PI 567516C was found to be consistently resistant to this new population of SCN. Later, Arelli and Young (2005) confirmed the sustaining resistance of this PI to the LY1 nematode population. To further understand the genetic basis of its resistance to the LY1 nematode, Arelli et al. (2009) investigated the inheritance of PI 567516C using $F_{2:5}$ recombinant inbred lines derived from a PI 567516C × Hartwig cross and concluded that the resistance to the LY1 nematode in PI 567516C was conditioned by one dominant and two recessive genes, designated *Rhg, rhg, rhg*. The results indicated the multigenic and quantitative inheritance pattern of resistance to SCN, which was in agreement with various genetic studies of other sources of resistance (Anand and Rao Arelli 1989; Guo et al. 2005, 2006a; Mansur et al. 1993; Yue et al. 2001a).

Advances in molecular genetics and marker technologies have made it possible to efficiently identify and map quantitative trait loci (QTL) underlying resistance to SCN. Concibido et al. (1994) first reported three RFLP markers significantly associated with SCN resistance and mapped these to soybean molecular linkage groups (LGs) A2, K, and G (Song et al. 2004). Since then, additional SCN resistance QTL have been identified and mapped in a number of resistance sources of cultivated soybean (Guo et al. 2005, 2006b; Meksem et al. 2001; Qiu et al. 1999; Wu et al. 2009; Yue et al. 2001a, b), and wild soybean (*Glycine soja*) (Wang et al. 2001; Winter et al. 2007). Recently, Concibido et al. (2004) summarized 31 putative QTL associated with SCN resistance to various races, which were mapped to 17 of 20 soybean LGs. Of these, the QTL on LG G has been commonly found in various resistant PIs and has proven to be the most important QTL associated with the *rhg1* locus, which is involved in resistance to SCN races 1, 2, 3, 5, and 4 (Concibido et al. 1997; Guo et al. 2005, 2006b; Wu et al. 2009; Yue et al. 2001a, b). A second important QTL located on LG A2 was also identified in many resistant PIs and corresponds to the dominant locus *Rgh4*, which was reported to play a distinct role in resistance to SCN race 3 (Concibido et al. 1994; Heer et al. 1998; Webb et al. 1995).

By using a meta-analysis approach to evaluate evidence for SCN resistance QTL reported in various resistant sources, Guo et al. (2006a) showed that in addition to major QTL mapped on LGs G (*rhg1*) and A2 (*Rhg4*), three other QTL were mapped and confirmed on LGs B1, E, and J. Winter et al. (2007) identified three new QTL derived from a wild soybean accession, PI 464925B, which was mapped to LGs I, K, and O. Using a recombinant inbred line (RIL) population derived from PI 437654, Wu et al. (2009) confirmed known QTL on LGs G and A2 and reported a new QTL mapped to LG I for resistance to SCN races 3, 5, and 14. These studies supported that other than the *rhg1* and *Rhg4* loci, the comprehensive resistance to multiple-SCN populations can be conveyed by other genomic regions of the soybean.

Although several resistant sources have been utilized for the genetic characterization of SCN resistance QTL, information is limited on molecular basis of broad-based resistance of PI 567516C, particularly the resistance to the synthetic population LY1. The objectives of this study were to detect and map QTL underlying the resistance to multiple-SCN races in PI 567516C using $F_{2:3}$ progenies of a Magellan × PI 567516C cross, and subsequently to confirm the novelty of identified QTL using advanced inbred lines developed from the same population.

Materials and methods

Genetic populations

A genetic population was developed from crossing a SCN-susceptible cultivar Magellan (Schapaugh et al. 1998), and

SCN-resistant accession PI 567516C (Arelli et al. 1997), in 2005 at the Bradford Education and Extension Center (BREC), University of Missouri-Columbia, MO, USA. Verified F_1 plants were grown to produce F_2 seeds in the winter nursery in Costa Rica. Genomic DNA was extracted from 250 individual F_2 plants and their parents and was used for molecular marker genotyping. $F_{2:3}$ progeny from the F_2 plants were harvested and used for SCN bioassay in a greenhouse. Primary QTL mapping was performed using molecular markers and phenotypic data generated from this population.

For a confirmation study, individual $F_{2:3}$ progeny were advanced to develop recombinant inbred lines (RIL) using the single-seed descent method. 250 $F_{6:7}$ RILs were then utilized to confirm QTL that had been identified and mapped in the $F_{2:3}$ mapping population.

DNA extraction

Genomic DNA samples were isolated from young leaf tissue of F_2 plants and $F_{6:7}$ RILs using the automated Autogen 960 system and the CTAB protocol of the manufacturer (AutoGen Inc., Holliston, MA, USA). Briefly, ground leaf tissue was mixed with CTAB extraction buffer, followed by an incubation period at 65°C for 1.5 h. Chloroform was added to the suspension, followed by agitation and centrifugation. The aqueous layer was collected and treated with RNase enzyme. Following the DNA precipitation, DNA pellets were washed with 70% ethanol and dissolved in TE (Tris–HCl-EDTA, pH 8.0) buffer. DNA concentration was quantified with a spectrophotometer (NanoDrop Technologies Inc., Centreville, DE, USA) and normalized at 20 ng/μl for polymerase chain reaction (PCR) amplification.

Simple sequence repeat (SSR) marker analysis

For fluorescently labeled SSR markers, PCR amplifications were performed in 12.5 μl final volumes containing 40–50 ng of template DNA, 0.13 μM of labeled forward primer (Applied BioSystems, Foster City, CA, USA) and 0.2 μM of reverse primer (IDT Inc., Coralville, IA, USA), 1× reaction buffer (20 mM Tris–HCl, pH 8.0, 50 mM KCl), 2.5 mM $MgCl_2$, 0.2 mM of each of the dNTPs, and 1 unit of *Taq* DNA polymerase (GenScript Corp., Piscataway, NJ, USA). The thermal cycler program was started at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 47 or 52°C for 45 s, and extension at 72°C for 1 min. A 7-min extension at 72°C followed the last cycle. The resulting PCR products of eight SSR markers were pooled and washed following the Whatman PCR cleanup procedure (Whatman Inc., Piscataway, NJ, USA) and using the automated Biomek FX system (Beckman Coulter Inc., Fullerton, CA, USA). The multiplex PCR samples were analyzed with the ABI 3100 or 3730 DNA sequencer (Applied BioSystems, Foster City, CA, USA). The allele size of each marker was then determined by fragment analysis using the GeneMapper 3.7 program (Applied BioSystems, Foster City, CA, USA).

A total of 570 SSR fluorescently labeled markers specially distributed throughout the 20 soybean chromosomes, corresponding to 20 genetic linkage groups (Grant et al. 2010), were selected to screen for DNA polymorphisms in the two parental lines. Polymorphic markers were subsequently used for population genotyping.

Single nucleotide polymorphism (SNP) analysis

The universal soybean linkage panel 1.0 (the USLP 1.0) containing 1,536 SNP loci (Hyten et al. 2008) was utilized to genotype the $F_{6:7}$ RIL mapping population using the Illumina GoldenGate assay (Fan et al. 2006). These SNP loci had been mapped onto the integrated molecular genetic linkage map (Hyten et al. 2010).

For the assay, 5 μl of 50 ng/μl of RNase-treated genomic DNA sample was activated by biotinylation, followed by the oligonucleotide/target annealing step, in which the SNP-specific oligonucleotide was annealed to the activated DNA by ramping up the temperature from 70 to 30°C over a 2 h period. Three oligonucleotide sequences, two allele-specific oligos (ASO) and one locus-specific oligo (LSO) designed for each SNP, contained regions of genomic complementary and universal PCR primer sites. The LSO also contained a unique IllumiCode sequence complementary to a particular Illumina bead type. Following oligonucleotide hybridization, excess and mishybridized oligos were removed by washing. Oligonucleotide extension and ligation assays were then implemented at 45°C for 15 min. Basically, DNA polymerase was used to extend the ASO and fill the gaps between the ASO and LSO; and a DNA ligase was employed to seal the nick between the extended ASO and LSO to form a PCR template that could be amplified with three universal PCR primers. These universal primers were 5′-labeled with Cy3, Cy5, and biotin. The PCR amplification was then performed using a thermal cycler program of 10 min at 37°C, followed by 3 min at 95°C, 34 cycles of denaturation at 95°C for 35 s, annealing at 56°C for 35 s, and extension at 72°C for 2 min. A 10-min extension at 72°C followed the last cycle.

The resulting double-stranded PCR products were immobilized onto paramagnetic particles, followed by washing and denaturing. The released single-stranded DNAs were then hybridized to their complementary bead type through their unique IllumiCode sequence of the Sentrix array matrix (SAM) under a temperature gradient program for at least 12 h. The hybridized SAM was rinsed

and dried for 20 min in the dark. Array imaging was performed using the Illumina BeadStation (Illumina, San Diego, CA, USA).

The allele calling for each SNP locus was conducted with the BeadStudio 3.0 software (Illumina, San Diego, CA, USA), based on the intensities detected from the two channels, Cy3 and Cy5. The clusters of homozygous and heterozygous genotypes for each SNP were manually checked for polymorphisms between the two parental lines. The polymorphic SNP loci were then employed for genetic linkage analysis and QTL mapping.

Soybean cyst nematode bioassay

Different SCN populations have been maintained at the University of Missouri-Columbia by reproduction in small population sizes for more than 30 generations; thus, these populations were believed to be near-homogeneous (Arelli et al. 2000). Recently, a new classification and designation system of SCN populations was published by Niblack et al. (2002) in which several soybean indicator lines were employed to characterize and expand the diversity of SCN, and the term "HG type" was adapted in place of "race" to describe SCN populations. However, for convenience of reference to earlier published studies, the race determination system (Schmitt and Shannon 1992) will be given along with the HG types of the SCN populations used in this study.

The SCN bioassays to identify reactions to six HG types of SCN [2.5.7 (race 1), 1.2.5.7 (race 2), 0 (race 3), 2.7 (race 5), 1.3.5.6.7 (race 14), and LY1] were performed in a greenhouse following the established procedure described by Arelli et al. (1997). Briefly, soybean seeds were gently sanded to scarify seed coats, if necessary, and were germinated in germination paper pouches for 3–4 days. Each seedling was then transplanted into single micropots filled with steam-pasteurized sandy soil. Five plants of each $F_{2:3}$ family and $F_{6:7}$ RIL and soybean indicator lines, including 'Hutcheson', were used for root inoculation. Two independent assays were conducted together in a random way. Seedling-containing micropots were placed in water bath tanks with the temperature maintained at 27 ± 1°C. 2 days after transplanting, roots of individual seedlings were inoculated with 2,000 ± 25 SCN eggs using an automatic pipetting machine (Scientific Products, Baltimore, MD, USA), and were watered daily to maintain soil moisture. Root samples were harvested at 30 days post inoculation and were washed to collect female cysts, which were counted under a stereomicroscope. A female index (FI%) was estimated to evaluate the response of each plant to each HG type of SCN using the following formula:

FI (%) = (Number of female cyst nematodes on a given individual/Average number of female nematodes on the susceptible 'Hutcheson') × 100.

Data analysis

Female index (%) among $F_{2:3}$ progeny and $F_{6:7}$ RILs assayed with each HG type were tested for normality using the PROC UNIVARIATE procedure of SAS 9.1 (SAS Institute, Cary, NY, USA). The Shapiro–Wilk (w) statistic was used to test the null hypothesis that FI (%) data were normally distributed (Elliott 1999). Correlation coefficients among the FI of the six HG types were estimated using the PROC CORR procedure of SAS. The broad-sense heritability for FI (%) for each HG type was estimated based upon the expected means squares (EMS) derived from an analysis of variance (ANOVA) with the PROC GLM procedure of SAS (Nyquist 1991).

Linkage analysis was performed with the computer program JoinMap 3.0 (van Ooijen and Voorrips 2001) to construct a genetic linkage map using the Kosambi mapping function. A likelihood of odds (LOD) threshold score of 3.0 and a maximum genetic distance of 50 cM were used for initial linkage grouping of markers. The new assignments of chromosome numbers (Chr.) (Grant et al. 2010) were correspondingly designated to the soybean genetic linkage groups (LGs) (Song et al. 2004).

Interval mapping (IM) method was initially conducted for QTL prediction. Composite interval mapping (CIM) was subsequently performed using the multi-QTL method (MQM) with the program MapQTL 5.0 and the appropriate cofactor (van Ooijen and Voorrips 2001). A permutation test (Churchill and Doerge 1994) was performed with 1,000 runs to determine the $P = 0.05$ genome-wide significance level for declaring a QTL significant. The proportion of the phenotypic variance explained by the QTL effects was estimated by CIM at the QTL peaks. Additive (A) and dominant (D) effects of significant QTL were estimated from an output of the program MapQTL 5.0. The total phenotypic variance explained by the significant QTL was determined using a multivariate ANOVA model in SAS.

For the prediction of epistatic interactions between QTL, the computer program QTLNetwork 2.0 (Yang et al. 2007) was used with a mixed-model. Significance levels for the genome scans for candidate intervals, QTL detection and effects were set at 0.05, 0.001, and 0.001, respectively. The chromosomes with LOD plots were subsequently created using the MapChart 2.2 program (Voorrips 2002) based on the outputs from JoinMap 3.0 and MapQTL 5.0.

Results

Primary QTL mapping in $F_{2:3}$ population

Phenotypic variation

The FI data of parents and $F_{2:3}$ progeny showed large genetic variation among the 250 $F_{2:3}$ progeny when assayed with each of six HG types of SCN (Table 1). For instance, the FI mean for HG type 0 was 98.0 and the extremes ranged from 1.1 to 263.2. In comparison, the FI data for LY1 had a mean of 103.1 and ranged from 3.8 to 232.2 (Table 1). The plots of FI distribution displayed continuous variations, and there was no clear distinction between resistant and susceptible responses among the progeny (Fig. 1). The normality tests using the Shapiro–Wilk (w) statistic indicated that the responses to HG types 2.5.7, 1.3.5.6.7, and LY1 were normally distributed, with respective w values of 0.99, 0.99, and 0.93 (P values = 0.15, 0.21, and 0.16; data not shown). In contrast, the responses to HG types 1.2.5.7, 0, and 2.7 were not normally distributed according to the estimations of the Shapiro–Wilk (w) statistic and the skewness and kurtosis of the FI distribution, which deviated slightly from zero (Table 1). Skewed trends were toward the lower FI for these HG types.

Broad-sense heritability for the FI of each HG type was calculated based upon the analysis of variance of family means. These values ranged from 0.46 to 0.55 (Table 1). Because the same $F_{2:3}$ progeny were used for phenotyping of different HG types of SCN, the correlation between the FI values was also calculated to compare the responses of these progenies. Pearson correlation coefficients of the FI ranged from 0.10 ($P < 0.093$) between HG types 1.2.5.7 and 0 to 0.55 ($P < 0.0001$) between HG types 0 and 1.3.5.6.7 (Table 2).

Genetic linkage analysis

Five hundred and seventy SSR markers were initially surveyed for DNA polymorphisms between parental lines, Magellan and PI 567516C. Of these, 252 markers (44.2%) were polymorphic and were utilized for genotyping the $F_{2:3}$ mapping population. A molecular linkage map was constructed, spanning approximately 2,200 cM across 20 chromosomes (Chr.) and linkage fragments (Fig. 2). The shortest linkage fragment had two markers, Satt009 and Satt683, on Chr. 3 (LG N) and the longest linkage group had 15 markers on Chr. 8 (LG A2), covering 176.1 cM from Satt390 to Satt409. The average genetic distance between markers was 12.5 cM, varying from 0.4 to 52.1 cM. A few genetic gaps (≥50.0 cM) between neighboring marker loci were also observed on Chrs. 1, 8, and 11 (LGs D1a, A2, and B1, respectively). Overall, most of the polymorphic SSR markers were successfully mapped on 20 chromosomes or linkage fragments, covering 95.8% of the soybean genome.

QTL for SCN resistance

Preliminary QTL mapping was initially conducted using $F_{2:3}$ progeny. Based on a genome-wide permutation test, an LOD threshold of 3.4 ($P = 0.05$) was used to identify significant QTL. Four QTL significantly associated with resistance to multiple- or single-HG types were identified. These QTL were mapped to Chrs. 10, 18, and 8, corresponding to LGs O, G, and A2 (Grant et al. 2010), respectively.

The first putative QTL was concurrently associated with resistance to five HG types: 2.5.7, 0, 2.7, 1.3.5.6.7 (corresponding to races 1, 3, 5, and 14, respectively) and LY1. Based on the 1-LOD confidence intervals, the QTL positions overlapped in the genomic region flanked by Sat_038

Table 1 Summary of statistics on female index (FI%) of parental lines and 250 $F_{2:3}$ families developed from a Magellan × PI 567516C cross for their response to six soybean cyst nematode HG types 2.5.7, 1.2.5.7, 0, 2.7, 1.3.5.6.7, and LY1, in greenhouse assays. The normality of the FI values are shown by Shapiro–Wilk (w), skewness, and kurtosis. Broad-sense heritability of each HG types is also estimated based on the ANOVA

HG type	FI (%)						Shapiro–Wilk (w)	Skewness	Kurtosis	Heritability (h_b^2)
	Parents		250 $F_{2:3}$ families							
	Magellan	PI 567516C	Mean	Min	Max	SD				
2.5.7	81.7	8.7	60.6	12.7	126.2	20.5	0.99	0.38	0.29	0.47
1.2.5.7	88.4	6.4	67.8	10.8	185.6	30.0	0.91	1.31	2.43	0.55
0	95.2	5.8	98.0	1.1	263.2	51.7	0.97	0.59	−0.01	0.48
2.7	74.9	3.1	43.7	0.0	176.0	25.0	0.96	0.89	2.47	0.47
1.3.5.6.7	115.8	7.8	68.9	5.1	145.0	30.2	0.99	0.12	−0.35	0.47
LY1	59.7	9.9	103.1	3.8	232.2	44.2	0.93	0.32	−0.24	0.54

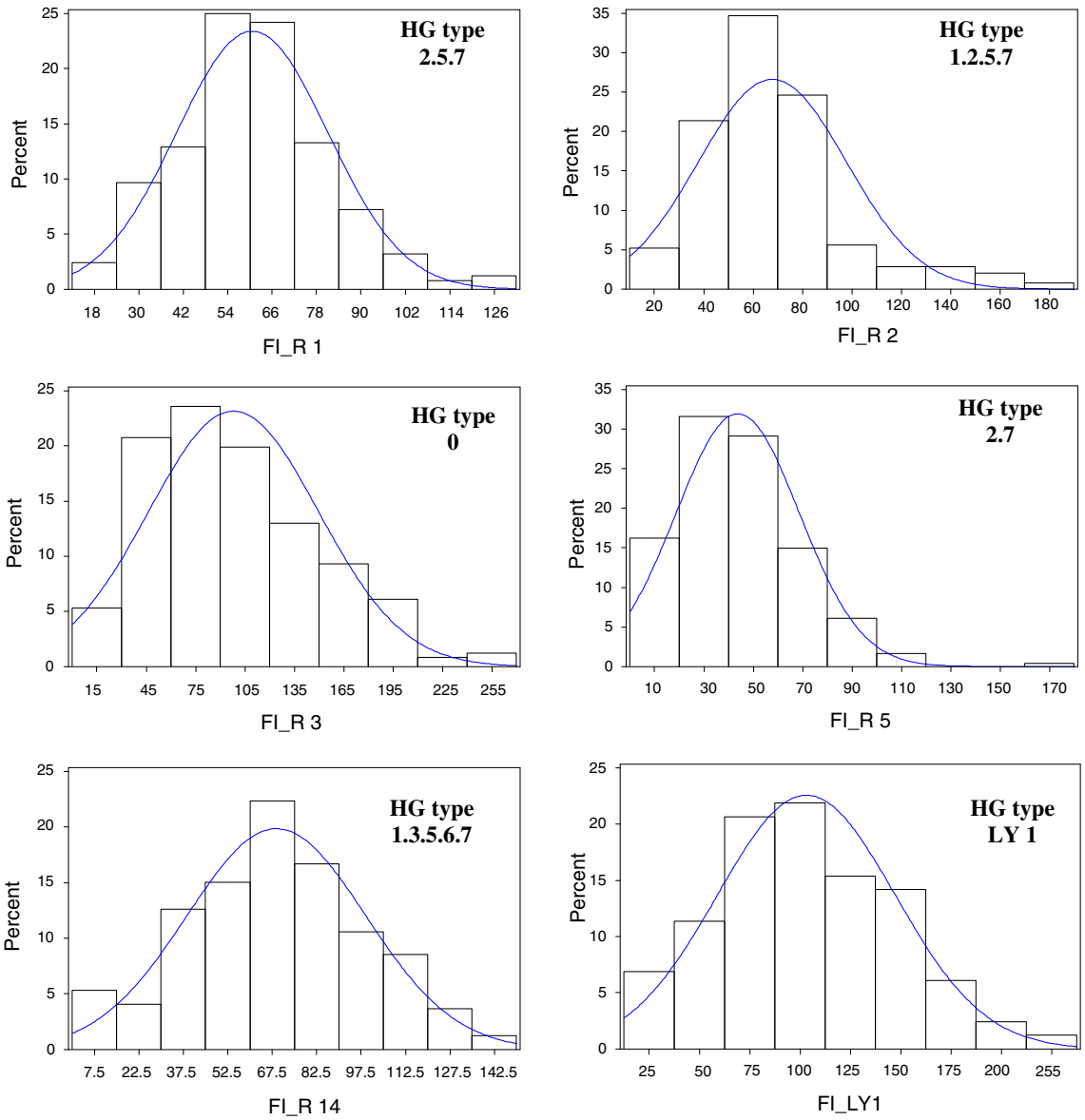

Fig. 1 Distribution of female index (FI%) among $F_{2:3}$ families for six HG types (races) of soybean cyst nematode (SCN, *Heterodera glycines*). The $F_{2:3}$ mapping population was derived from a cross between a SCN susceptible cultivar 'Magellan' and SCN resistant parent, PI 567516C

Table 2 Pearson correlation coefficients and their probabilities (in parenthesis) among female index (FI %) of $F_{2:3}$ families when assayed for reaction to six HG types (races) of soybean cyst nematode

HG type	1.2.5.7	0	2.7	1.3.5.6.7	LY1
2.5.7	0.23 (0.0003)	0.32 (<0.0001)	0.31 (<0.0001)	0.36 (<0.0001)	0.33 (<0.0001)
1.2.5.7		−0.07 (0.228)	0.11 (0.093)	−0.23 (0.0002)	−0.03 (0.581)
0			0.13 (0.042)	0.48 (<0.0001)	0.55 (<0.0001)
2.7				0.33 (<0.0001)	0.23 (0.0002)
1.3.5.6.7					0.53 (<0.0001)

and Satt592 on Chr. 10. The marker Sat_274, which is located between the flanking markers, was closely associated with the QTL peak (Fig. 3), with LOD scores of 6.6, 9.7, 5.2, 9.8, and 13.2 for HG types 2.5.7, 0, 2.7, 1.3.5.6.7, and LY1, respectively (Table 3). The total FI phenotypic variation explained by these QTL varied from 7.9% for HG

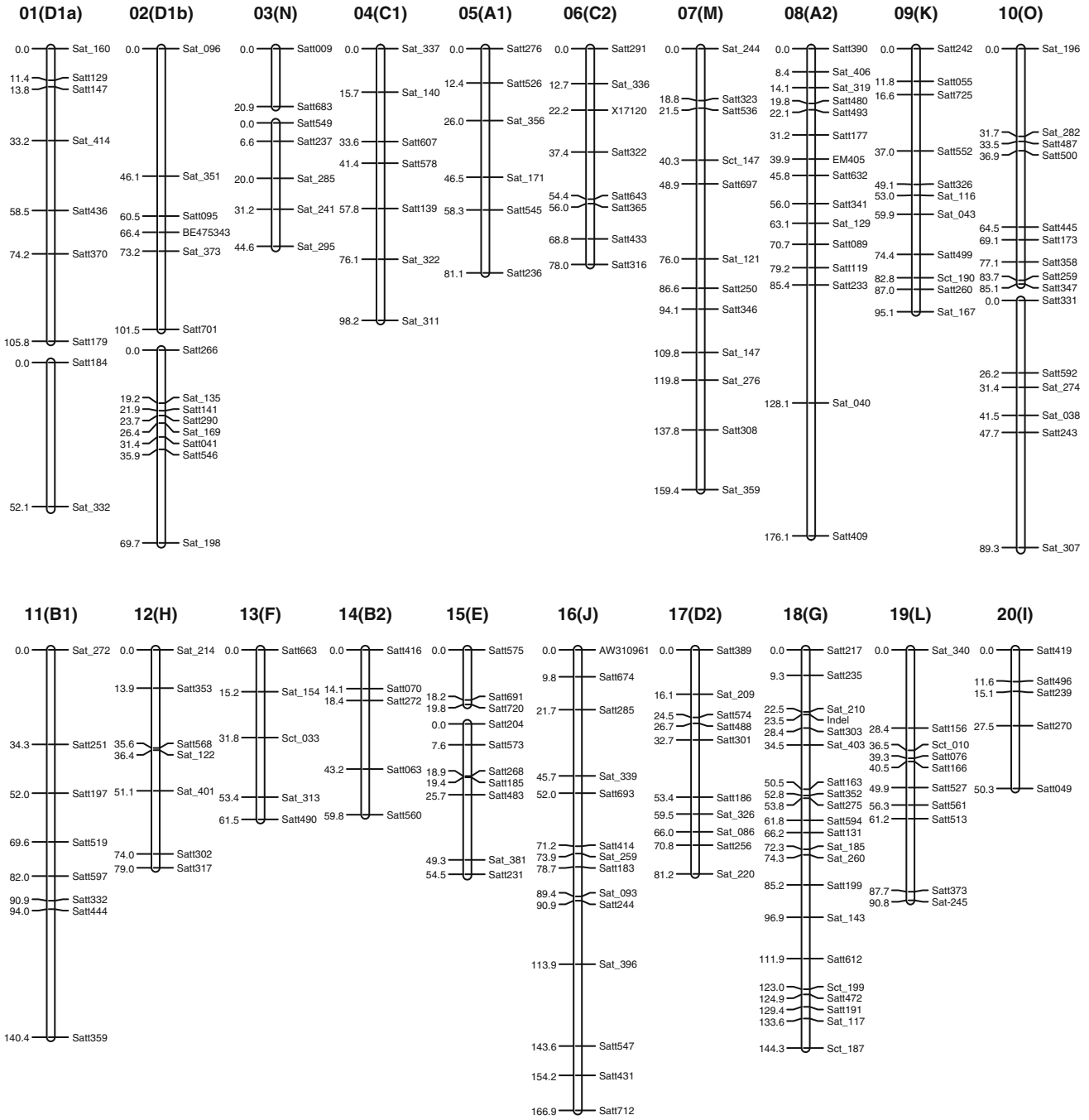

Fig. 2 A genetic linkage map constructed using a $F_{2:3}$ mapping population derived from a Magellan × PI 567516C cross. 252 polymorphic simple sequence repeat (SSR) markers were mapped to the 20 soybean chromosomes or linkage fragments, covering 95.8% of the soybean genome. The molecular genetic map was employed for primary QTL mapping

type 0 to 21.7% for LY1 (Table 3). The favorable allele on Chr. 10 from the resistant parent, PI 567516C, conferred greater resistance and had an additive effect on reducing the FI of five HG types (Table 3).

The second putative QTL was also concurrently associated with resistance to five HG types: 2.5.7, 0, 2.7, 1.3.5.6.7, and LY1. The QTL positions overlapped in the genomic region flanked by the markers Satt612 and Satt191 on Chr. 18 based on the 1-LOD confidence intervals. The marker Satt472, located between the flanking markers, was closely associated with the QTL peak (Fig. 3), with LOD scores of 6.5, 4.0, 11.7, 3.5, and 5.6 for HG types 2.5.7, 0, 2.7, 1.3.5.6.7, and LY1, respectively (Table 3). Overall, the QTL on Chr. 18 accounted for a smaller proportion of the total FI phenotypic variation relative to the QTL mapped to Chr. 10 (Table 3). The

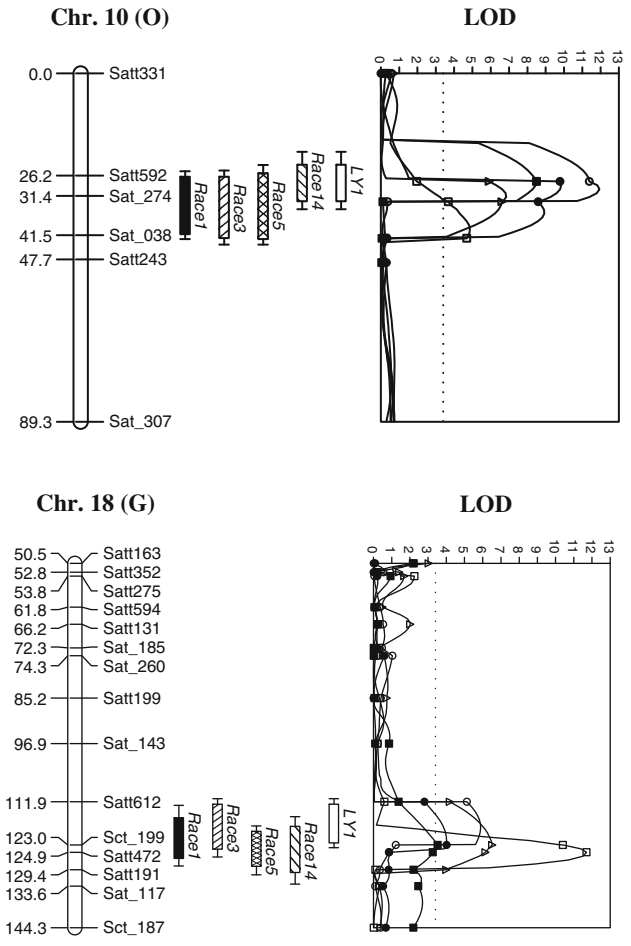

Fig. 3 Likelihood of odds (LOD) plots for Chrs. 10 and 18 showing the locations of quantitative trait loci (QTL) providing resistance to multiple-SCN HG types (races) using a LOD threshold of 3.4. These QTL were mapped in a $F_{2:3}$ population derived from a Magellan x PI 567516C cross. The relative positions of the markers are given in centiMorgan (cM). Line style codes: *arrow head* HG type 2.5.7 (race 1), *filled circle* HG type 0 (race 3), *open square* HG type 2.7 (race 5), *filled square* HG type 1.3.5.6.7 (race 14), and *open circle* HG type LY1 (race LY1)

favorable allele was also inherited from the resistant parent, PI 567516C, and had an additive effect on reducing the FI of five HG types. A multivariate model analysis that included the two markers most closely linked to the QTL, Sat_274 and Satt472, showed that each marker was significant at $P < 0.001$. Altogether they explained 19.7, 21.1, 25.7, 20.8, and 26.9%, of the total phenotypic variation in FI of HG types 2.5.7, 0, 2.7, 1.3.5.6.7, and LY1, respectively (Table 3).

In addition to the major QTL described above, two additional QTL were identified to be significantly associated with resistance to HG type 1.2.5.7 (race 2). One QTL was mapped to the Sat_210-Sat_403 interval of Chr. 18, with an LOD score of 4.5. The second QTL was mapped to the Satt233-Sat_040 interval of Chr. 8, with an LOD value of 4.3. These QTL individually explained relatively small proportions (6.2 and 7.0%, respectively) of the total FI variation. A multivariate model analysis was conducted that included the QTL-associated SSR markers, and this provided an estimate of the total FI phenotypic variation of the HG type 1.2.5.7; however, altogether these two QTL only explained 8.1% of the observed variation. No significant epistatic interactions (additive × additive or additive × dominant) were detected among either the two multiple-HG type resistance QTL mapped to Chrs. 10 and 18 or the other single-HG type resistance QTL mapped to Chrs. 8 and 18 (data not shown).

QTL Confirmation in a $F_{6:7}$ RIL population

A $F_{6:7}$ RIL population derived from the same cross, Magellan × PI 567516C, was used to confirm QTL identified in the $F_{2:3}$ generation. The FI data estimated for each HG type indicated a large genetic variation among RILs studied and a normal distribution based on the Shapiro–Wilk (w) statistic (data not shown).

In addition to the SSR markers mapped earlier, the 1,536 soybean SNP panel (the USLP 1.0) was also used to genotype the $F_{6:7}$ RIL mapping population. Out of these, 698 SNP loci (45.5%) were polymorphic, and these were incorporated into the molecular linkage analysis. Altogether, a total of 927 SSR and SNP markers were mapped to 20 soybean chromosomes and linkage fragments, generating a high-density marker linkage map (Fig. 4). This newly constructed genetic linkage map covered 2,170 cM (94.5%) of the soybean genome with an average genetic distance between markers of 2.6 cM, varying from 0.0 to 22.6 cM. A large number of SNPs were mapped between SSR markers, resulting in several marker clusters in many chromosomal regions, such as on Chrs. 8, 10, and 18. Overall, marker orders among loci in our constructed genetic map were consistent with those of the soybean composite linkage map and the soybean physical map (Grant et al. 2010), except for slight marker order rearrangements in some chromosomal regions.

Results of QTL analysis showed that the two major QTL associated with resistance to multiple-HG types of SCN in $F_{2:3}$ progeny were also detected and consistently mapped to the same intervals on Chrs. 10 and 18 (Fig. 4) in the $F_{6:7}$ RIL population. Besides the SSR markers, many SNP markers were also associated with these QTL. For instance, BARC-008021-00209, BARC-043247-08565, and BARC-015925-02017 mapped within the QTL interval on Chr. 10. Among these, BARC-008021-00209 was positioned 0.1 cM from the marker Sat_274 located at the peak of the QTL (Fig. 4). Similarly, many SNPs were mapped within the QTL interval on Chr. 18, including BARC-017681-03105, BARC-038873-07372, and BARC-048095-10484.

Table 3 Marker intervals, significant LOD scores, R^2 values, and explanation of total phenotypic variation of QTL mapped on Chrs. 10, 18, and 8. These QTL are responsible for resistance to multiple- or single-HG types of soybean cyst nematode (SCN)

HG type	Chr. 10 (O)			Chr. 18 (G second locus)			Chr. 8 (A2)			Total FI variation
	Interval	LOD	R^2	Interval	LOD	R^2	Interval	LOD	R^2	Explained (%)
2.5.7	Sat_038–Satt592	6.6	11.2	Satt612–Satt191	6.5	10.2				19.7
1.2.5.7				Sat_210–Sat_403	4.5	6.2	Satt233–Sat_040	4.3	7.0	8.1
0	Sat_038–Satt592	9.7	16.6	Satt612–Satt191	4.0	6.3				21.1
2.7	Sat_038–Satt592	5.2	7.9	Satt612–Satt191	11.7	10.4				25.7
1.3.5.6.7	Sat_274–Satt592	9.8	16.0	Satt612–Satt191	3.5	5.2				20.8
LY1	Sat_274–Satt592	13.2	21.7	Satt612–Satt191	5.6	8.3				26.9

Among these, BARC-038873-07372 was positioned 0.7 cM (Fig. 4) from Satt472, which was located at the QTL peak (Fig. 4).

The two minor QTL for resistance to HG type 1.2.5.7 mapped to Chrs. 18 and 8 in $F_{2:3}$ progeny were not confirmed in the $F_{6:7}$ RIL population, although many SSR and SNP markers were significantly correlated to the FI values based on single marker-trait association analysis (data not shown). In the $F_{2:3}$ progenies LOD values of 1.0 and 1.5 showed a poor association of these QTL for resistance in the $F_{6:7}$ RILs. In contrast, a significant QTL for resistance to this HG type with a LOD score of 7.0 was detected in the same genomic region as the previously mapped QTL on Chr. 10, in the interval flanked by Sat_038 and Satt592 (data not shown). No epistatic interactions were found between the two major QTL on Chrs. 10 and 18 in the $F_{6:7}$ RIL population either.

Discussion

In many earlier studies to map QTL associated with SCN resistance, FI data were normally distributed, a lack of normality has occasionally been also reported (Guo et al. 2005, 2006b; Wu et al. 2009; Yue et al. 2001a). For reliable QTL detection in such occasions, the effect of non-normality can be significantly reduced by performing genome-wide permutation tests to determine empirically derived threshold values for LOD score significance (Churchill and Doerge 1994), together with CIM and cofactor marker selection (Zeng 1993, 1994). In our study, we utilized original phenotypic data without data transformation, followed by appropriate permutation tests and the CIM method. Results showed a similar trend to that of previous studies, and indicated that significant QTL associated with resistance to several SCN HG types were detected in spite of the non-normality of some of the FI data.

Broad-sense heritability estimated in this study was not as high as expected for $F_{2:3}$ progenies; however, they showed a similar trend to that of many previous reports (Guo et al. 2005, 2006b; Yue et al. 2001a). This indicated that besides a genetic component, the response to SCN was significantly affected by environmental conditions. Moreover, it shows that environment controlled bioassays are warranted to obtain improved estimates of genetic variation.

In an effort to discover and map QTL that were concurrently resistant to multiple SCN HG types, the same $F_{2:3}$ progeny were phenotyped for resistance to different HG types of SCN. Correlations between the FI values were calculated to compare the responses of these progenies to different HG types. Correlation coefficients shown in Table 2 ranged from non-significant to highly significant, suggesting that the resistance to different HG types can be concurrently conditioned by the same genes or QTL (Guo et al. 2006b; Yue et al. 2001b). Results of QTL analysis showing one QTL responsible for resistance to multiple SCN HG types in the present study confirmed those observations.

For primary QTL analysis, a molecular linkage map was constructed using SSR markers. In addition to successfully mapped chromosomes, such as Chrs. 7, 8, 16, and 18, five linkage fragments remained unattached to their corresponding chromosomes, such as Chrs. 2, 3, 10 (Fig. 2). This may have been due to the lack of marker polymorphisms in some genomic regions of the parental lines studied. In comparison of marker order between loci, overall our constructed genetic map was consistent with those of the soybean composite linkage map (Choi et al. 2007). However, reversed marker orders were also observed in some regions of Chrs. 8 and 16. This inconsistency in marker order has been observed in previous mapping studies (Guo et al. 2005, 2006b; Wu et al. 2009). It could be the result of one or a combination of the following conditions: different genetic background, population size and type, and mapping methods (Concibido et al. 2004). Moreover, accurate detection and estimation of QTL positions also depends on the number of molecular

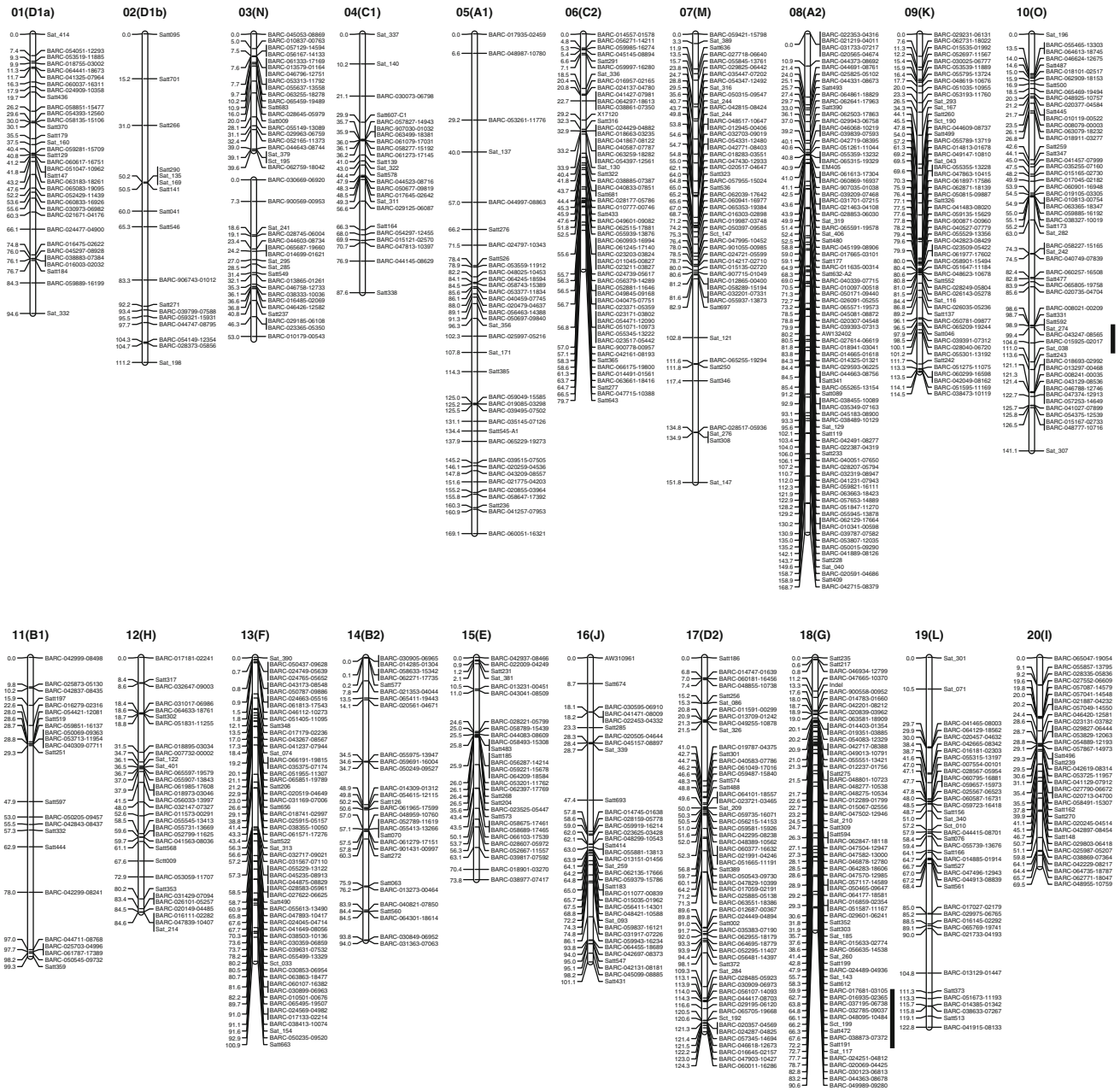

Fig. 4 An integrated genetic linkage map constructed using a $F_{6:7}$ RIL confirmation population derived from a Magellan × PI 567516C cross. A total of 927 polymorphic simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers were mapped to the 20 soybean chromosomes or linkage fragments, covering 94.5% of the soybean genome. The novel QTL associated with resistance to multiple-HG types of SCN earlier identified in the $F_{2:3}$ progenies were confirmed in the Sat_038-Satt592 interval on chromosome 10 (LG 0) and in the Satt612-Satt191 interval on chromosome 18 (LG G) shown by *solid bars*

markers used, their distribution across chromosomes (Wu et al. 2009), and the accuracy of genotyping data. Overall, although a few genetic gaps were observed, most of the polymorphic SSR markers were successfully mapped on 20 chromosomes or linkage fragments. In particular, a large number of markers was mapped on Chrs. 8 and 18 (LGs A2 and G), where two known QTL regions associated with the *rhg1* and *Rhg4* loci have been commonly reported in many resistance sources (Concibido et al. 2004).

Concibido et al. (2004) reviewed several previous studies conducted to identify SCN resistance QTL and summarized 31 putative QTL intervals mapped on 17 molecular LGs. Since 2004, efforts have continued to map QTL from additional SCN-resistant soybean accessions,

including PI 468916 (Kabelka et al. 2005), PI 90763 and PI 404198A (Guo et al. 2005, 2006b), and PI 437654 (Wu et al. 2009), but no QTL mapped to Chr. 10 (LG O). Recently, Winter et al. (2007) analyzed a $F_{4:5}$ RIL population derived from PI 464925B (*G. soja*), and reported a QTL with a LOD score of 2.0 located between Satt173 and Satt466 on Chr. 10. This QTL was responsible for resistance to the Ruthven isolate of *H. glycines*, which was collected at the Agriculture and Agri-Food Canada (AAFC) Harrow Research Station in Ontario, and characterized as HG type 1.2.5.7 (race 2). On basis of the soybean genetic and physical maps (Grant et al. 2010), however, the Chr.10 QTL in our study and the QTL reported by Winter et al. (2007) are separated by a genetic distance of approximately 53.9 cM, and were physically clustered in two different supercontigs, #124 (Gm10:43054285..45118377) and #98 (Gm10:27984938..30603250), respectively. Moreover, the QTL detected in PI 567516C was shown to be concurrently associated with resistance to five HG types of SCN, but not with resistance to HG type 1.2.5.7. Our data, therefore, indicated that the SCN resistance QTL mapped to Chr. 10 in our study was a novel QTL that has not been reported in other SCN resistance sources, including wild soybean.

Regarding previously reported QTL on Chr. 18 (LG G), independent studies identified four QTL responsible for resistance to several SCN HG types in different regions on this chromosome (Concibido et al. 2004). Besides the major QTL at the *rhg1* locus, which has been consistently reported in a number of SCN-resistant accessions (Concibido et al. 1997; Guo et al. 2005, 2006b; Kabelka et al. 2005; Winter et al. 2007; Wu et al. 2009), many additional QTL have been identified in various resistant sources, such as Peking (Concibido et al. 1997), PI 438489B (Yue et al. 2001a), and PI 468916 (Wang et al. 2001), but they mapped to regions distant from the *rhg1* locus. Since each of these QTL explained a percentage of resistance variation, it was believed to be a minor QTL requiring further confirmation (Concibido et al. 2004).

Based on the soybean genetic and physical maps (Grant et al. 2010), the *rhg1* locus and the QTL on Chr. 18 in our study are approximately 90.3 cM apart and they physically clustered into two different supercontigs, #110 (Gm18:1..2280793) and #8 (Gm18:52424675..61937939), respectively. In a separate study, Yue et al. (2001a) detected other QTL underlying SCN resistance to races 1, 2, 3, and 5 (HG types 2.5.7, 1.2.5.7, 0, and 2.7, respectively), and mapped these QTL in different intervals between the markers A096 and Satt199 located approximately 57.6 cM from the *rhg1* locus (Grant et al. 2010). The genetic and physical maps of these markers showed that they were distant from the Chr. 18 QTL in our study, and were clustered in different supercontigs, #128 (Gm18:3347440..5366262) and #8 (Gm18:52424675..61937939), respectively. In another study, Concibido et al. (1997) used cv. Peking and mapped QTL associated with the marker A378H for SCN resistance to race 3 (HG type 0) at the opposite end of Chr. 18. Despite the fact that the physical map showed both the RFLP marker A378H and Satt472 clustered in the supercontig #8, these two markers were located at a distance of 18.6 cM from the end of the genetic map (Grant et al. 2010). Wang et al. (2001) also reported a different QTL on Chr. 18 associated with resistance to race 3 in a wild soybean accession, PI 468916, and was designated SCN22-2 in the Soybean Genome Database (Grant et al. 2010). Our study indicated that the Chr.-18 QTL in PI 567516C was associated with broad-based resistance to multiple-HG types of SCN, which was different from other SCN resistance sources and also confirmed the previous findings by Wang et al. (2001). This QTL can be efficiently introgressed into elite soybean lines using SSR and/or SNP markers.

While analyzing $F_{2:3}$ populations derived from PI 90763 and PI 404198A, Guo et al. (2005; 2006b) mapped a QTL responsible for resistance to race 2 (HG type 1.2.5.7) to the Satt163–Satt309–Satt688 region of Chr. 18, in which Satt309 was known to be closely linked to the *rhg1* locus (Cregan et al. 1999; Meksem et al. 2001). Recently, Wu et al. (2009) also identified a QTL in the same region with the markers Satt309 and *rhg1*-Indel. This QTL was associated with resistance to several HG types, including HG type 1.2.5.7. In this study, the QTL associated with resistance to HG type 1.2.5.7 was mapped in the Sat_210-Sat_403 region that overlapped with the Satt309-*rhg1* region, as previously reported. It suggested that PI 567516C carried the single-HG type resistance QTL, which was detected in the same location as in earlier studies (Guo et al. 2005, 2006b; Wu et al. 2009).

In addition to the *rhg1* locus-associated QTL on Chr. 18, resistance to HG type 1.2.5.7 in several germplasm accessions has been shown to be conditioned by QTL or single genes on Chrs. 4, 5, 11, and 15 (Yue et al. 2001a), Chrs. 11 and 16 (Guo et al. 2005, 2006b), Chr. 10 (Winter et al. 2007), and Chr. 1 (Wu et al. 2009). In this study, we report a putative QTL associated with resistance to this HG type that was physically mapped to Chr. 8 (LG A2) in the supercontig #110 (Gm18:1621167..6169649). This QTL has not been detected in other SCN-resistant accessions, and due to the low LOD score and small proportion of FI variation explained, it requires further study for confirmation.

The absence of significant epistasis was not consistent with the findings of previous studies of SCN resistance in which epistatic interaction was demonstrated to be an important genetic component of SCN resistance (Meksem et al. 2001; Webb et al. 1995; Wu et al. 2009). Performing a two-way analysis, Meksem et al. (2001) detected a

significant interaction between the LG-A2 and -G QTL controlling resistance to race 3 (HG type 0) in a RIL population derived from a Forrest x Essex cross. Webb et al. (1995) also reported an epistatic interaction between resistant alleles at the QTL on LGs A2 and G in PI 437654, which was subsequently confirmed by Prabhu et al. (1999). Employing a genetic population derived from the same soybean accession, PI 437654, Wu et al. (2009) also reported a number of epistatic interactions among QTL for resistance to many SCN races. Unlike the conclusions of previous studies, no additive × additive interaction between these two QTL was detected. It is possible that these two multiple-HG type resistant QTL have unique mechanisms of resistance that are able to function independently. Moreover, different genetic backgrounds and the genetic complexity of SCN populations used in our study may affect detection and quantification of epistatic interactions (Wu et al. 2009). Further investigation is needed to clarify the speculation.

To confirm putative QTL earlier identified, we used advanced inbred lines developed from the same cross. The FI data estimated for each HG type indicated a large genetic variation among RILs and a normal distribution of phenotypes based on the Shapiro–Wilk (*w*) statistic (data not shown). A large number of polymorphic SNP markers were used along with SSR markers to construct a high-density linkage map (Fig. 4). By doing this, several unmerged linkage fragments were successfully incorporated into their corresponding chromosomes.

According to the standard proposed by Lander and Kruglyak (1995), a confirmed QTL is defined as being significant from an initial study that has subsequently been confirmed by further studies that can be accomplished by using either near-isogenic lines (NILs), independent crosses, or breeding selection. NILs (Glover et al. 2004; Meksem et al. 2001) and independent crosses (Wang et al. 2001; Wu et al. 2009) have previously been used to confirm SCN QTL. Using $F_{6:7}$ recombinant inbreds we were able to confirm two major QTL that had been detected and mapped to Chrs. 10 and 18 using phenotypic data from $F_{2:3}$ families. Moreover, the soybean physical and genetic maps (Grant et al. 2010) in conjunction with the soybean genome sequence information (Goodstein, personal communication) were used to confirm the novelty of these QTL. Two other minor putative QTL mapped to Chrs. 18 and 8 in $F_{2:3}$ families could not be confirmed in the $F_{6:7}$ RIL population. It was possible that genetic segregation of heterozygous progenies in conjunction with generation advancement have resulted in the loss of SCN resistant alleles for these two QTL in advanced inbred lines. In contrast, a new significant QTL for resistance to HG type 1.2.5.7 was mapped in the same genomic region on Chr. 10. This QTL was flanked by the markers Sat_038 and Satt592; however, since this QTL was not detected in the $F_{2:3}$ progenies, it requires further study with a different genetic background for confirmation.

In conclusion, we report the discovery and confirmation of QTL associated with resistance to multiple-SCN HG types in soybean PI 567516C. These QTL were consistently mapped to genomic regions on Chrs. 10 and 18 and were confirmed in a $F_{6:7}$ RIL mapping population derived from the same cross. The comprehensive information of physical and genetic maps and the soybean genome sequence provided further evidence of the novelty and reality of these QTL. The SSR and SNP molecular markers closely associated with the QTL regions can be efficiently employed in marker-assisted breeding programs to develop new soybean germplasm and cultivars with broad-based resistance to SCN. Furthermore, these markers would facilitate the development of NILs using the marker-assisted backcrossing (MAB) approach leading to fine-mapping and positional cloning of genes for SCN resistance.

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