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Quantitative Trait Loci underlying Resistance to Three Soybean Cyst Nematode Populations in Soybean PI 404198A

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ABSTRACT

Soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) is a major pest of soybean [G*lycine max* (L.) Merr.] in the USA. Soybean plant introduction (PI) 404198A is one of the newly identified sources that can provide a broad spectrum of resistance to SCN. The objective of this study was to identify quantitative trait loci (QTL) associated with resistance to SCN races 1, 2, and 5 in PI 404198A. Linkage groups (LGs) G and A2 were found to be associated with resistance to race 1. QTL on LG G was located on the Satt309-Satt688 region, and it explained a larger proportion of the total variation (20.2%). QTL on LG A2 was located at the Satt424-Satt632-Sat_406 region, and it accounted for a smaller proportion (9%). LGs G and B1 were shown to be associated with resistance to race 2. QTL on LG G was located on the Satt688-Satt309-Satt163 region, and it explained 12.5% of the total variation. Molecular marker Satt453 on LG B1 was found to be associated with resistance to race 2, and it explained 11% of the total variation. LGs G, B1, and N were found to be associated with resistance to race 5. QTL on LG G was located on the Satt688-Satt309-Satt163 region, and it explained 6.3% of the total variation. Molecular marker Satt453 on LG B1 was found to be associated with resistance to race 5, and it explained a larger proportion of the total variation (13%). QTL on LG N was mapped on the Satt584-Sat_280-Satt549, and it explained 9.5% of the total variation. In conclusion, soybean PI 404198A may carry *rhg1* on LG G, *Rhg4* on LG A2, and a QTL on LG B1. Further studies are needed to lend credibility for QTL on LG N.

Soybean cyst nematode is a major pest of soybean in the USA and causes more yield losses than any other soybean disease (Wrather et al., 1995, 2001). A 2 to 6% annual yield loss due to SCN has been estimated in U.S. soybean production (Wrather et al., 1995).

SCN populations are diverse. Variability of SCN populations is described in two ways. One is the race determination test (Schmitt and Shannon, 1992) that uses four soybean lines to categorize SCN into 16 "races". Recently, Niblack et al. (2002) published a new scheme that uses seven soybean lines to characterize and expand the diversity of SCN. "HG type" is used instead of race to describe SCN populations. They, however, also emphasized that the race determination test (Schmitt and Shannon, 1992) can sill be used for describing genetic studies. For convenience of comparison to earlier studies, the race determination test was used in this study. However, the HG types of the SCN populations were also given.

Resistant cultivars have been widely used for controlling SCN damage (Wrather et al., 1995; Bradley and Duffy, 1982). A total of 118 SCN resistant accessions have been reported in the USA (Arelli et al., 1997, 2000), but few are resistant to more than four different SCN races. These multiple-SCN resistant accessions include PIs 437654, 438489B, 90763, 89772, 404198A, 404166, and 438498. Very few resistance sources are currently used in USA soybean breeding programs. Resistance of most commercial cultivars comes from Peking and/or PI 88788 in the USA (Diers and Arelli, 1999) and has led to genetic vulnerability.

Quantitative trait loci (QTL) have been identified by molecular markers for resistance to SCN races 1, 2, 3, 5, 6 and/or 14 in a total of 13 soybean accessions (nine resistance sources). QTLs associated with SCN resistance have been located on all linkage groups (LG) except for D1b, K, and O (Concibido et al., 2004). The QTLs on LGs G and A2 (*rhg1* and *Rhg4* separately) have been well studied and molecular markers have been saturated around these two loci (Cregan et al., 1999a,1999b; Mudge et al., 1997; Weisemann et al., 1992; Matthews et al., 1998; Meksem et al., 2001). It is reported that *rhg1* and *Rhg4* have been cloned and sequenced (Hauge et al., 2001; Lightfoot and Meksem, 2002). Soybean SCN resistance gene *rhg1* seems to be involved in resistance to almost all SCN races studied, whereas *Rhg4* seems to play a distinct role in resistance to race 3 (Table 1 in Concibido et al., 2004). A QTL was identified on LG E in cultivated soybean (*G. max*) (Yue et al., 2001b) and wild soybean (*G. soja* Siebold & Zucc.) (Wang et al., 2001), but its resistance to SCN races is inconsistent. A QTL was detected on LG J (Concibido et al., 1994, 1996, 1997) and was recently confirmed by means of near isogenic lines (Glover et al., 2004). LG B1 has been found to be associated with resistance to SCN but the locations of the QTLs declared are significantly inconsistent (Yue et al., 2001a, 2001b; Vierling et al., 1996). QTLs identified on other LGs show inconsistent results for QTL location or are not supported by a second study.

Soybean PI 404198A is one of the few sources that can provide a broad spectrum of resistance to SCN. It is resistant to SCN races 1, 2, 3, and 5. PI 404198A was introduced from Russia into the USA and was found to be resistant to multiple races of SCN (Arelli et al., 1997). It has been demonstrated that this PI is distantly related with important resistance sources including Pe-

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Abbreviations: FI, female index; LG, linkage group; QTL, quantitative trait locus; SCN, soybean cyst nematode; SSR, simple sequence repeat.

Table 1. Reaction of soybean differential lines and two parents to SCN races.

SCN race	Race 1†		Race 2†		Race 5†	
replication	I	II	I	II	I	II
Pickett	1	4	60	65	50	57
Peking	2	2	53	45	7	2
PI 88788	51	32	36	33	52	56
PI 90763	0	0	4	4	0	0
PI 4044198A	0	1	12	7	1	0
Magellan	96	92	77	95	89	96

† Female index (FI) (%), expressed as percentage of susceptible control 'Hutcheson'.

king, PI 88788, PI 89772, PI 90763, and PI 209332 but relatively closely related to PI 437654 (Zhang et al., 1999). Genetics of resistance in PI 404198A to SCN is not known.

Objective of this study was to identify QTLs associated with resistance to SCN races 1, 2, and 5 in soybean PI 404198A.

MATERIALS AND METHODS

Materials

Two hundred twenty-four $F_{2:3}$ families were developed from a cross between Magellan (Schapaugh et al., 1998) and PI 404198A. A cross was made in 2001 at Columbia, MO. F_2 and $F_{2:3}$ seeds were generated in Costa Rica in 2002 and 2003 separately. $F_{2:3}$ seeds were used for phenotyping and genotyping. PI 404198A is resistant to SCN races 1, 2, 3, and 5 and Magellan is reportedly susceptible to all known SCN races. PI 404198A and Magellan seeds were obtained from the National Soybean Research Laboratory, Urbana, IL.

SCN Bioassay

SCN races 1 (HG type 2.5.7, PA1), 2 (HG type 1.2.5.7, PA2), and 5 (HG type 2.5.7, PA5) maintained at the University of Missouri-Columbia were used. Origins and development of these SCN populations were described in detail by Arelli et al. (1997, 2000). These populations were believed to be near-homogeneous because of reproduction in a small population size for more than 30 generations (Arelli et al., 1997, 2000).

SCN bioassays were performed in the greenhouse at the University of Missouri-Columbia, as described by Arelli et al. (1997). Soybean seeds were germinated for 5 d and then transplanted into micropots (one plant in each micropot) filled with steam-pasteurized soil. Twenty micropots each were placed in plastic containers and maintained at 27 ± 1°C in a thermo-regulated waterbath (Forma Scientic Inc., Marietta, OH). Two days after transplanting, roots of each plant were inoculated with 2000 ± 50 SCN eggs with an automatic pipetter (Brewer Automatic pipetting Machine, Scientific Products, Baltimore, MD). Thirty days after transplanting, roots of individual plants were harvested and washed with pressurized water for collection of female nematodes. Nematode cysts were counted under a stereo-microscope. Two hundred twenty four-$F_{2:3}$ families (two replications, five plants for each replication in each family) and parents were evaluated for resistance to individual races 1, 2, and 5. SCN reaction of four differential soybean lines Peking, PI 88788, PI 90763, and Pickett and the susceptible soybean cultivar Hutcheson (Buss et al., 1988) (five plants for each differential line and 10 plants for Hutcheson) was also determined to monitor shifts of SCN races. No race shifts occurred (Table 1).

A female index (FI) was used to measure SCN reproduction on individual plants of $F_{2:3}$ families and SCN differentials (Schmitt and Shannon, 1992). Average of 10 plants was used to represent the response of each family to each race.

FI (%) = (number of female cyst nematodes on a given individual/average number of female nematodes on Hutcheson) × 100.

DNA Extraction and SSR Genotyping

Leaves from more than 16 plants in each $F_{2:3}$ family (20–30 plants in most families) were harvested and bulked in approximately equal amounts. DNA was extracted by the CTAB method (Keim et al., 1988). DNA from 224 $F_{2:3}$ families were used for simple sequence repeats (SSR) analysis and their genotypes were used to represent genotypes of their corresponding F_2 plants. SSRs described by Song et al. (2004) were used. They were either purchased from Research Genetics Inc. (Huntsville, AL, USA) or synthesized by Integrated DNA Technologies Inc. (Coralville, IA, USA). Polymerase chain reaction (PCR) was conducted in 96-well microplates with a final volume of 15 μL on the Eppendorf mastercycler gradient (Eppendorf AG, Germany). Each reaction included 50 ng genomic DNA, 0.25 μ*M* of each of the primers, 0.3 m*M* each of dNTPs, 2.5 m*M* of $MgCl_2$ and 0.3 unit of Taq DNA polymerase (Promega Corporation, Madison, WI). PCR reaction was performed at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 48.8°C for 30 s and 68.8°C for 45 s, with a final extension for 10 min at 72°C. Amplified products were separated on 3.5% (w/v) SFR agarose gels (Amresco Inc., USA) and were stained with ethidium bromide. Pictures were taken using an alphaImager 2200 (Alpha Innotech Corporation, San Leandro, CA) and bands were scored.

Data Analysis

The genetic linkage map was constructed by MAPMAKER/EXP version 3.0b (Whitehead Institute, Cambridge, MA). Haldane map function was used. Linkage was declared at LOD ≥ 3.0 and a maximum distance of 50 cM. The marker order of the highest LOD was chosen after checking the raw data if the foremost possible marker orders of one group given by MAPMAKER had close LOD values. This situation often occurred where some markers of a group were closely linked. Linkage groups were designated according to the soybean composite linkage map (Song et al., 2004).

Composite interval mapping (CIM) was used to detect QTL-marker associations by WINQTLCART v2.0 (Basten et al., 2002; Zeng, 1994). Model six was selected with control marker numbers (cofactors) of 5 and window size of 10 cM. The forward regression method was used for selecting the control markers. QTL was searched every 2 cM. The position of the highest LOD on a region of a group or a whole group was used to indicate the position of a QTL and its 1 − LOD confidence interval was obtained. Where multiple peaks occurred on a region and their 1 − LOD confidence intervals overlapped substantially, one QTL was declared for the peak with the highest LOD on this region.

The determination of threshold value for declaring a QTL is a challenge because of an excessive number and dependence of test statistics obtained at a series of putative positions along the whole genome. It involves multiple tests and the point-wise level should be adjusted to the genome-wide level. The point-wise level is the probability that an extreme test statistics (LOD) will occur at a specific locus only by chance whereas the genome-wide level is the probability that an extreme test statistics (LOD) occurs by chance somewhere in a whole ge-

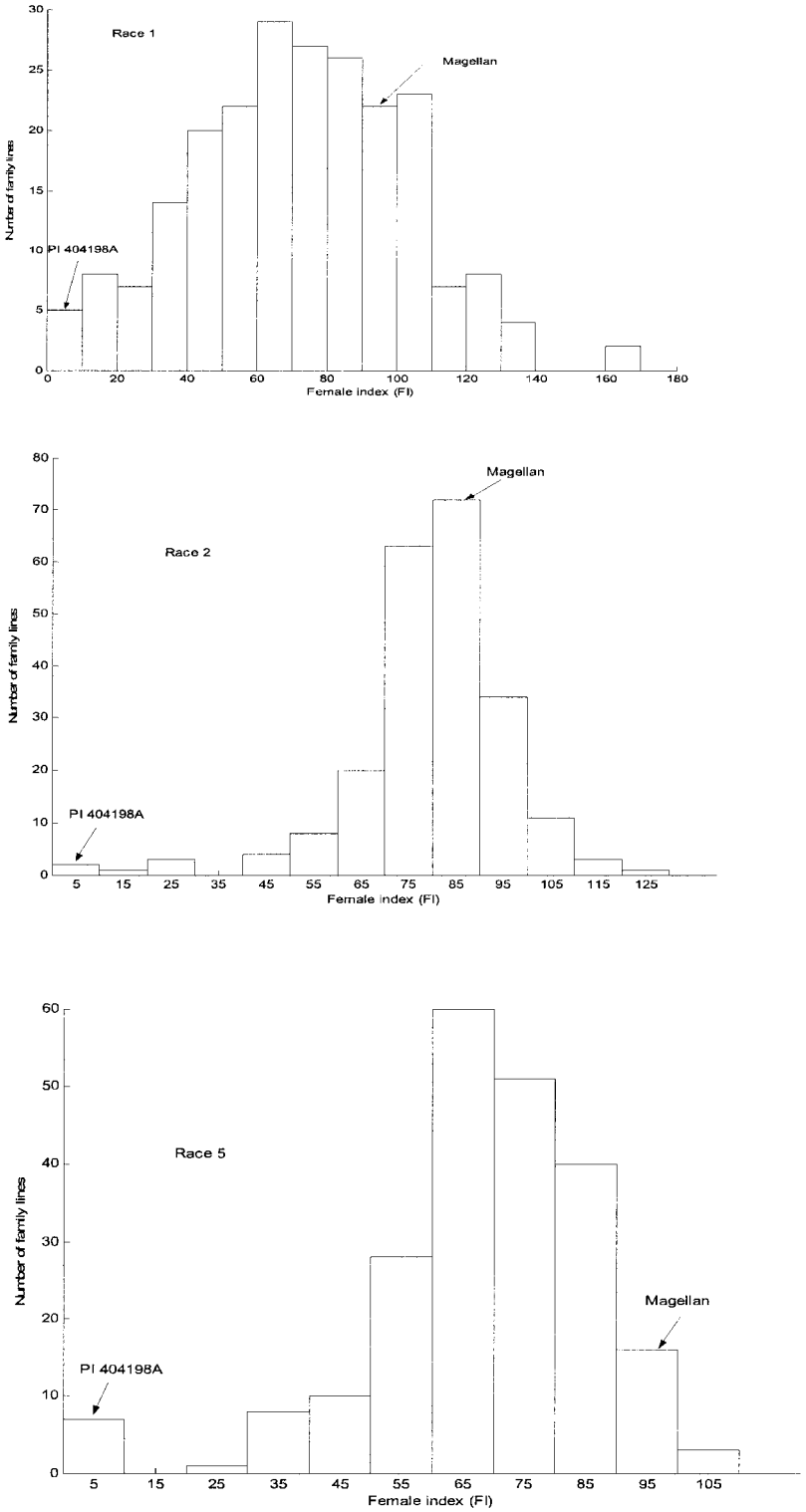

Fig. 1. Distribution of average female index (FI), expressed as percentage of susceptible control 'Hutcheson', of $F_{2:3}$ families from soybean cross PI 404198A × Magellan. The FI of parents are indicated by arrow. PI 404198A: SCN resistant parent. Magellan: SCN susceptible parent.

nome. A QTL is usually declared at genome-wide type I error = 0.05 (usually referred to as significant level) (Lander and Kruglyak, 1995; Members of the complex trait consortium, 2003). Permutation tests (Churchill and Doerge, 1994) are a general approach for the adjustment. We obtained significant threshold LODs of 3.76, 3.75, and 4.00 for races 1, 2, and 5, respectively, at genome-wide type I error = 0.05 using permutation tests (1000 permutations each race). In addition, we obtained significant threshold LOD of 4.2 using computer simulation table (Ooijen, 1999) and of 4.5 using the formula of Lander and Kruglyak (1995). Therefore, threshold LOD of 4.0 is approximate to the genome-wide type I error of 0.05 in soybean. A significant QTL was declared at LOD = 4.0 in this study. In the past, however, most of SCN QTL mapping

studies used threshold LOD = 3.0 (equivalently $p = 0.001$) for declaring a QTL (Webb et al., 1995; Heer et al., 1998; Qiu et al., 1999; Wang et al., 2001; Meksem et al., 2001). According to the formula of Lander and Kruglyak (1995), threshold LOD value of 3.0 was equivalent to genome-wide type I error = 0.63 in soybean (usually referred to as suggestive level) (Lander and Kruglyak, 1995; Members of the complex trait consortium, 2003). Suggestive level often gives false positive QTL but it is worth reporting if accompanied with an appropriate label, so that discovery of QTLs may not be delayed (Lander and Kruglyak, 1995; Members of the complex trait consortium, 2003). To keep consistent with earlier studies, a QTL was also declared at LOD = 3.0 in this study, but with label of "suggestive". A conclusive claim was made for suggestive QTL only if suggestive evidence was accompanied with significant evidence from other studies or other races.

In addition, ANOVA were used for detecting QTL-marker associations for unassigned SSR markers using Window SAS version 8.2. Statistical evidence (F value) from ANOVA was transformed into LOD by the formulae $-2 \log (p)/4.6$ (where p is the P value corresponding to the observed F value). LOD obtained through transformation is comparable to the LOD (df = 2) that is used in composite interval mapping, because 4.6 LOD (equal to likelihood ratio) obtained by composite interval mapping and $-2 \log (p)$ both follow chi square distribution with degree freedom (df) of 2. The same threshold values (suggestive QTL at LOD = 3.0 and significant QTL at LOD = 4.0) were used for declaring a QTL in ANOVA. Additive effect (A) and dominant effect (D) were obtained in ANOVA using A-M and H-M separately, where A is average FI of PI 404198A allele-homozygous genotypes, H is average FI of heterozygous genotypes, B is average of Magellan allele homozygous genotype, and M is average of A and B.

Two-locus epistatic interactions between QTLs were detected by two-way ANOVA of pair-wise combinations of molecular markers tightly linked with resistance to SCN. The markers used for detection of epistatic interactions were selected after looking over the QTL-marker association data from all molecular markers. Therefore, adjustment of pair-wise level should be based on all possible epistatic interactions. Holland and Ingle (1998) recommended an adjustment for detecting all possible epistatic interactions by dividing the genome-wide level by $g\ (g - 1)$, where g is the number of linkage groups or chromosomes. For soybean, g is 20. Epistatic interactions were declared at genome-wise type I error = 0.63 (suggestive level) and genome-wide type I error = 0.05 (significant level). Similarly, statistical evidence (F value) of two-way ANOVA can also be transformed into LOD. Suggestive threshold value for detecting epistatic interactions is pair-wise p value = 0.0016 (LOD = 2.8) and significant threshold value pair-wise p value = 0.0001 (LOD = 4.0). These levels are close to the above threshold levels for declaring a QTL.

RESULTS AND DISCUSSION

Phenotype Variation

The $F_{2:3}$ families showed a large variation for FI for races 1, 2, and 5 (Fig. 1). The FI mean is 72.4 with a range of 0.8 to 168.3 for race 1, 79.9 with a range of 7.1 to 122.8 for race 2, and 68.2 with a range of 0.5 to 106.4. Five of 224, 2 of 222 (two missing), and 7 of the 224 $F_{2:3}$ families showed FI of less than 10% for races 1, 2, and 5 separately (Table 2). FI of $F_{2:3}$ families showed a normal distribution for race 1 (Shapiro-Wilk's $w = 0.99$, p value = 0.4536; skewness = 0.021; kurtosis = 0.008) but non-normal distributions for races 2 (Shapiro-Wilk's $w = 0.896$, p value < 0.0001; skewness = -1.419; kurtosis = 4.662) and 5 (Shapiro-Wilk's $w = 0.915$, p value < 0.0001; skewness = -1.268; kurtosis = 2.692) (Fig. 1). Correlations for responses of $F_{2:3}$ families to SCN between races 1 and 2 and between races 1 and 5 were low ($r = 0.336$, p value < 0.0001 and $r = 0.323$, p value < 0.0001, respectively) but high between races 2 and 5 ($r = 0.586$, p value < 0.0001).

SSR Markers and Linkage Group Maps

Nearly 1000 SSR markers were surveyed between parents PI 404198A and Magellan and 377 polymorphic SSRs were obtained. One hundred ninety-four selected polymorphic SSRs covering the 20 soybean LGs were used for mapping. These SSRs produced 182 codominant and 12 dominant loci. It is noted that dominant loci frequently occurred on LG K (Fig. 2), which was also observed in our other mapping population Hamilton × PI 90763 (Guo et al., unpublished).

A linkage map was constructed using Magellan × PI 404198A and shown in Fig. 2. LGs were designated according to the soybean composite linkage map (Song et al., 2004). One gap (≥50 cM between neighboring markers) occurred on LGs B1, D1a and L. Subgroups of these groups each were arranged in order according to the soybean composite linkage map. Two markers remained unassigned, but they were placed on LGs B1 and B2 according to the soybean composite linkage map. The correlations between the map in Fig. 2 and the soybean composite linkage map were highly significant ($r > 0.8$) for LG marker orders and LG map distances except for those of LGs B2 and K (Table 3). The order of Sat_342 and Sat_177 was reversed on LG B2 compared with the soybean composite linkage map (Fig. 2). Relative distance of markers on LG K was in good agreement with the soybean composite linkage map, but the order of markers was in poor agreement because a number of closely linked markers were used on this group. A difference often occurred in marker order between the map in Fig. 2 and the soybean composite linkage map if adjacent markers were less than 5 cM. The map in Fig. 2 had a linear relationship with the soybean composite linkage map for LG map distance of all LGs except for LGs E and K (data not shown). A good agreement was also observed in our other mapping population Hamilton × PI 90763 (Guo et al., unpublished).

QTLs Associated with Resistance to SCN

Original phenotypic data were used for QTL analysis. Transformation failed using square root and log. The effect of non-normality on QTL mapping data analysis is expected to be significantly reduced when the composite interval mapping method and permutation tests are used (Zeng 1993, 1994; Jansen, 1993; Churchill and Doerge, 1994).

Linkage groups G and A2 were found to be associated with resistance to race 1 in soybean PI 404198A (Table 4, Fig. 2). QTL on LG G was located on Satt309-Satt688

Table 2. Marker genotypes in F_2 generation of family lines of ≤25% female index (FI) for molecular markers closely linked with resistance to soybean cyst nematode.

Line	Satt632†	Satt453†	Satt163†	Satt309†	Sat_208†	Race 1‡	Race 2‡	Race 5‡
810	A	A	A	A	H	1.2	21.3	2.8
775	A	A	A	A	H	1.2	24.2	3.2
721	H	A	A	A	H	14.3	7.2	0.6
619	H	A	A	A	H	25.0	7.1	0.5
731	H	A	A	A	A	63.2	14.1	1.8
688	H	A	A	A	H	32.7	20.0	0.7
645	A	A	A	A	B	0.8	43.7	7.5
698	A	H	A	A	A	5.5	60.2	43.5
814	H	B	A	A	A	8.4	58.8	91.0
805	A	H	A	A	H	10.2	52.7	78.4
602	A	H	A	A	A	10.7	76.3	65.4
668	A	B	A	A	A	11.7	80.2	74.0
741	A	H	A	A	H	11.9	80.0	45.7
724	A	H	A	A	A	13.9	68.2	55.1
740	A	H	A	A	H	14	77.6	86.3
708	H	H	A	A	H	19.8	80.5	59.6
713	A	B	A	A	H	21.6	103.1	62.0
811	B	H	A	A	H	21.8	72.3	61.5

† A: both alleles come from resistance parent PI 404198A. B: both alleles come from susceptible parent Magellan. H: one allele comes from PI 404198A and the other allele comes from Magellan.

‡ Female index (FI) (%), expressed as percentage of control 'Hutcheson'. Underscored number with FI ≤ 15 for race 1, ≤ 25 for races 2 and 5 was in agreement with the genotypes of molecular markers associated with resistance to races, respectively. It is noted that no family line shows FI of between 10 and 25 for race 5.

region (Fig. 2), and it explained a larger proportion of the total variation (20.2%) (Table 4). Two close peaks occurred on the Satt688-Satt309-Satt163 region, but their 1 − LOD confidence intervals overlapped substantially (data not shown). One QTL was declared for the larger peak on this region (Table 4). Soybean SCN resistance gene *rhg1* has been located 0.4 to 1.25 cM from molecular marker Satt309 (Cregan et al., 1999a,1999b; Meksem et al., 2001). It is within the 1 − LOD confidence interval of the QTL on LG G in PI 404198A. Therefore, it is concluded that PI 404198A may carry *rhg1*. QTL on LG A2 was located at Satt424-Satt632-Sat_406 region and it accounted for a smaller proportion of the total variation (9%) (Table 4, Fig. 2). Soybean SCN resistance gene *Rhg4* has been mapped close to molecular markers Satt632 and pBlt65 and I locus (Cregan et al., 1999b; Meksem et al., 2001). Satt632 and pBlt65 and I locus are close together (Song et al., 2004; Matthews et al., 1998). *Rhg4* is within the 1 − LOD confidence interval of the QTL on A2 in PI 404198A. It is concluded that PI 404198A may carry *Rhg4*. It has been shown that *Rhg4* was frequently associated with resistance to race 3 (Webb et al., 1995; Concibido et al., 1994; Mahalingam and Skorupska et al., 1995; Meksem et al., 2001; Heer et al., 1998). But Heer et al. (1998) also showed that LG A2 was associated with resistance to race 1. They used J87–233 (derived from Peking, PI 88788, and PI 90763) as SCN resistant parent and the same SCN race 1 population as the one used in this study. All 12 families having FI ≤ 15% for race 1 carried both alleles from resistant parent PI 404198A at markers Satt163 and Satt309 on LG G and 10 of them both alleles from PI 404198A at marker Satt632 on LG A2 (Table 2). This is consistent with the result that QTLs for resistance to race 1 was located around Satt309 and Satt632 separately.

Linkage groups G and B1 were shown to be associated with resistance to race 2 in PI 404198A (Table 4, Fig. 2). QTL on LG G for resistance to race 2 was located on the Satt688-Satt309-Satt163 region (Fig. 2), and it explained 12.5% of the total variation (Table 4). Like QTL for resistance to race 1 on LG G, two close peaks occurred on this region, but their 1 − LOD confidence intervals were the same (data not shown). One QTL was declared for the peak with largest LOD (Table 4). Molecular marker Satt453 was found to be associated with resistance to race 2 (Table 4), and it explained 11% of the total variation. Satt453 was not linked with the other molecular markers of LG B1 used in this study because no polymorphic markers were found between it and molecular marker Satt415 (Fig. 2). However, this marker has been placed on LG B1 and it is distant from molecular marker Satt415 on the soybean composite linkage map (Song et al., 2004). Satt453 was also mapped on LG B1 in our other study where the Hamilton × PI 90763 population was used (Guo et al., unpublished). All of the six families having FI ≤ 25% carried both alleles from resistant parent PI 404198A at markers Satt163 and Satt309 on LG G and Satt453 on LG B1 (Table 2), which is consistent with the result that QTLs for resistance to race 2 were mapped close to Satt163 and Satt453 separately.

QTLs for resistance to race 5 were identified on LGs G, B1, and N in PI 404198A (Table 4, Fig. 2). QTL on LG G was located on the Satt688-Satt309-Satt163 region (Fig. 2), and it explained 6.3% of the total variation

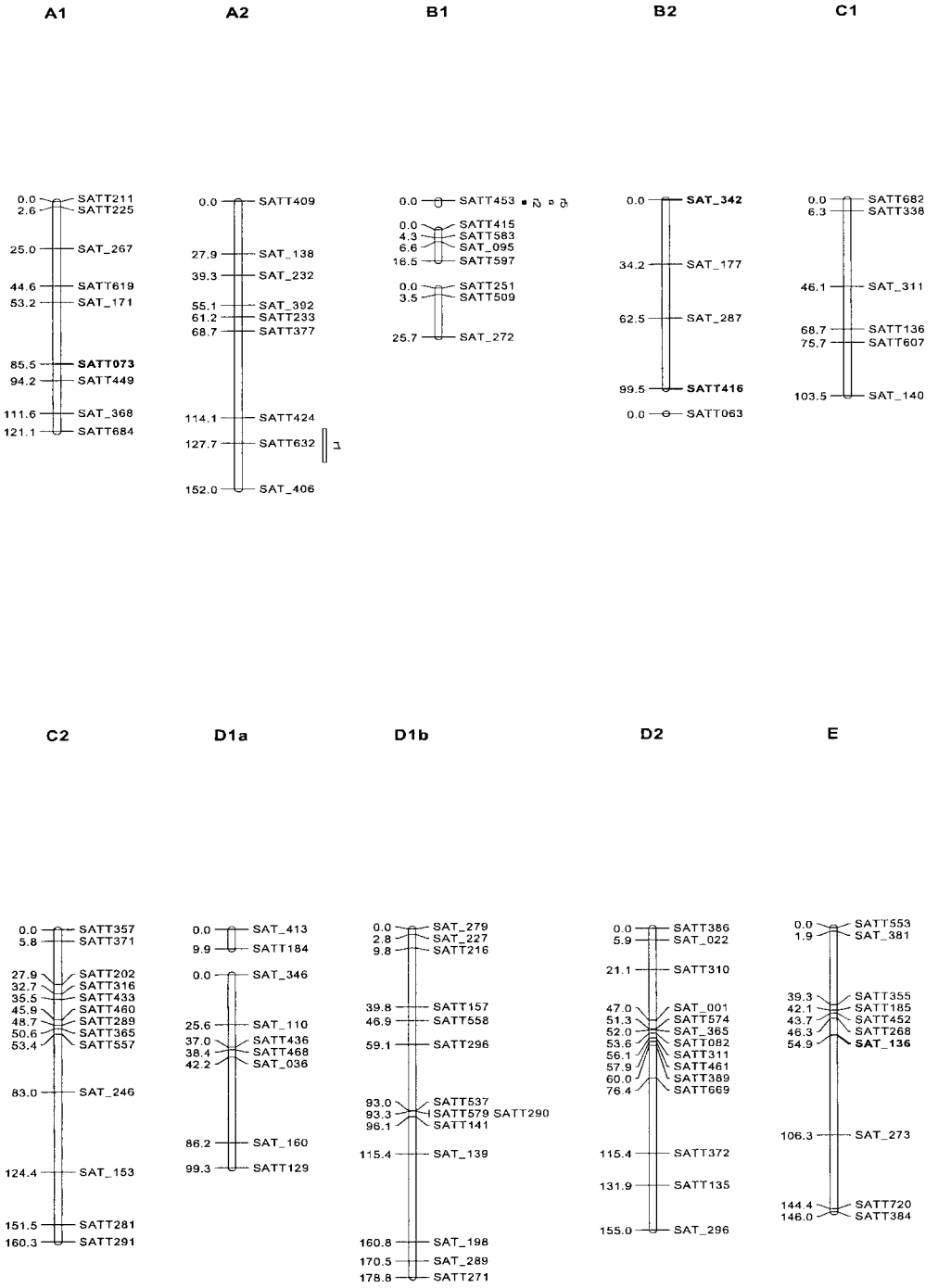

Fig. 2. Continued on next page.

(Table 4). Its statistical evidence reached the suggestive level only. But as stated above, this region was associated with resistance to races 1 and 2. In our other study where Hamilton × PI 90763 was used, this region showed considerable evidence (LOD = 7.1) for resistance to race 5 (Guo et al., unpublished). It is concluded that this region may be associated with resistance to race 5 in PI 404198A. Molecular marker Satt453 on LG B1 was also found to be associated with resistance to race 5, and it explained a larger proportion of the total variation (13%) (Table 4, Fig. 2). There was weak statistical evidence (LOD = 3.0) demonstrating that LG N was associated with resistance to race 5, and it explained 9.5% of the total variation (Table 4, Fig. 2). It is interesting to note that QTL on LG N had a lower FI when it was heterozygous than when it was homozygous (Table 4). Concibido et al. (1997) showed that LG N was associated with resistance to race 3, but its QTL location was somewhat distant from the QTL identified in this study. To be credible for this QTL, further studies are needed. All of the seven families with a FI ≤ 25% for race 5 (no families with FI of 10–25% for race 5) carried both

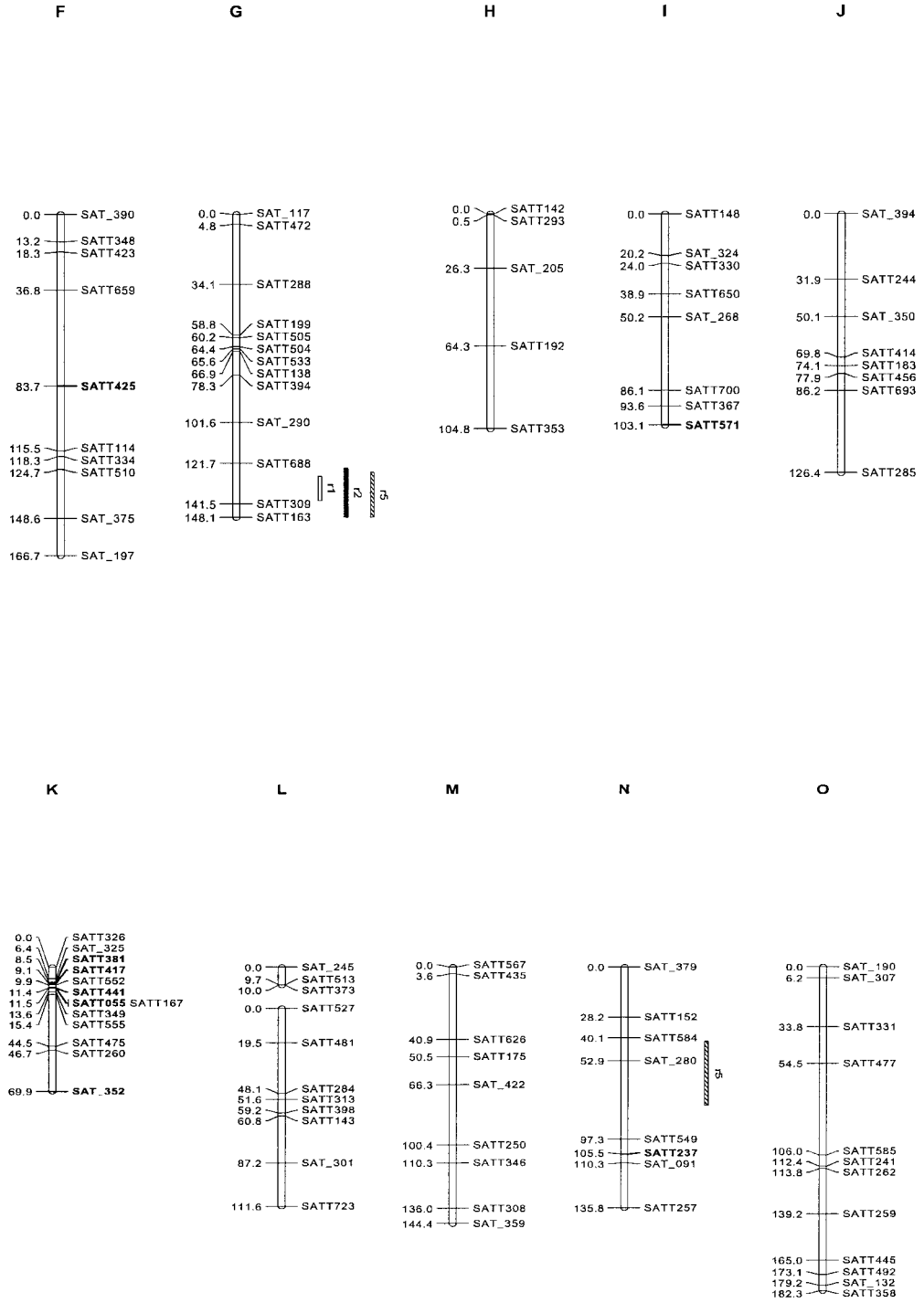

Fig. 2. Linkage map constructed using MAPMAKER/EXP from soybean cross Magellan × PI 404198A. Haldane map function was used. Linkage was declared at LOD ≥ 3.0 and a maximum distance of 50 cM. PI 404198A: SCN resistant parent. Magellan: SCN susceptible parent. Unassigned SSR markers were placed on the appropriate positions according to the soybean composite linkage map. QTLs are indicated by the bars on the right of linkage groups and their 1 − LOD confidence intervals are given by the length of bars (the location and confidence interval of QTL on B1 are undetermined because Satt453 was unlinked with other molecular markers): r1—Race 1, r2—Race 2, r5—Race 5. Bold SSR markers are dominant.

alleles from resistant parent PI 404198A at molecular markers Satt163 and Satt309 on LG G and Satt453 on LG B1 (Table 2), which is consistent with the result that QTLs for resistance to race 5 was mapped close to Satt163 on LG G and Satt453 on LG B1 separately. However, five of them are heterozygous at molecular marker Sat_208 on LG N. This is consistent with the result that heterozygous genotypes show smaller FI at QTL on LG N (Table 4).

The 1 − LOD confidence intervals for resistance to races 1, 2, and 5 overlapped substantially on LG G (Fig. 2). Molecular marker Satt453 on LG B1 was associ-

Table 3. Comparison of the soybean linkage map constructed using Magellan × PI 404198A with the soybean composite linkage map.

Linkage groups	No. of markers	Coverage (%)†	Correlation‡	
			Map distance	Marker order
A1	9	90	0.998**	1.000**
A2	9	72	0.998**	1.000**
B1	8	25	0.999**	0.976**
B2	5	41	0.826	0.800
C1	6	72	0.999**	1.000**
C2	13	71	0.996**	0.967**
D1a	9	56	0.997**	0.983**
D1b	14	97	0.998**	0.953**
D2	14	89	0.999**	1.000**
E	10	68	0.895**	0.806**
F	11	67	0.989**	0.988**
G	13	86	0.997**	0.995**
H	5	36	0.998**	1.000**
I	8	66	0.994**	1.000**
J	8	70	0.967**	0.929**
K	13	52	0.920**	0.258
L	11	68	0.998**	0.991**
M	9	75	0.998**	1.000**
N	8	76	0.993**	0.976**
O	12	64	0.993**	0.979**

** p value < 0.01.
† The distance of coverage by used markers excluding the interval of ≥ 30 cM between neighboring markers divided by the total group map length on the soybean composite linkage map.
‡ Unassigned markers or unlinked subgroups on the same linkage group are placed on appropriate order and positions according to the soybean composite linkage map. 50 cM was given between unassigned or unlinked markers and their neighboring markers. Correlations were performed using Window SAS version 8.2.

Table 5. ANOVA of molecular markers associated with resistance to soybean cyst nematode.

Race	Source of variation	F	P value (LOD)†	R^2 (%)‡
I	Satt309 G	17.45	9×10^{-8} (7.1)**	32.4
	Satt632 A2	16.53	2×10^{-7} (6.7)**	
	Satt309* Satt632	4.57	0.0015 (2.8)*	
II	Satt163 G	11.36	2×10^{-5} (4.7)**	29.3
	Satt453 B1	18.17	5×10^{-8} (7.3)**	
	Satt163* Satt453	6.44	7×10^{-5} (4.2)**	
V	Satt163 G	6.45	0.0019 (2.7)	35.7 (30.3)§
	Satt453 B1	11.02	3×10^{-5} (4.5)*	
	Sat_280 N	8.43	3×10^{-4} (3.5)*	
	Satt163* Satt453	4.57	0.0015 (2.8)*	
	Satt163* Sat_280	0.34	0.8507 (0.07)	
	Satt453* Sat_280	3.68	0.0065 (2.2)	

* Suggestive QTL (genome-wise type I error = 0.63).
** Significant QTL (genome-wide type I error = 0.05). See data analysis for suggestive and significant threshold LODs of single QTLs and epistatic interactions.
† See data analysis for computation of LOD.
‡ Proportion of the total variation explained by the model fitted (single markers plus interactions between markers).
§ Number inside parenthesis is obtained through fit of single markers plus suggestive interaction.

Table 4. QTLs associated with resistance to SCN in soybean PI 404198A.

SCN races	LG	Marker interval	Distance†	QTL position‡	LOD§	R^2 (%)¶	A#	D††
I	G	Satt309—Satt688	19.8	7.8	10.0**	20.2	−20.3	4.2
	A2	Satt632—Sat_406	24.3	0.0	5.8**	9.0	−13.8	0.2
II	G	Satt163—Satt309	6.6	0.0	7.1**	12.5	−8.2	3.5
	B1	Satt453‡‡			5.5**‡‡	11.1	−7.8‡‡	4.8‡‡
V	G	Satt163—Satt309	6.6	0.0	3.3*	6.3	−6.8	1.9
	B1	Satt453‡‡	44.4	8.0	6.7**‡‡	13.0	−8.8‡‡	8.2‡‡
	N	Sat_280—Satt549			3.0*	9.5	−1.2	−11.9

* Suggestive QTL (threshold LOD = 3.0, equivalent to genome-wide type I error = 0.63).
** Significant QTL (threshold LOD = 4.0, equivalent to genome-wide type I error = 0.05).
† The distance between the markers of marker interval.
‡ The position of QTL (peak) from the left flanking marker of marker interval.
§ The LOD corresponding to peak.
¶ Proportion of the total variation explained by QTL.
# A: additive effect. '- ' indicates that resistance allele of QTL comes from parent PI 404198A.
†† D: dominant effect. '- ' indicates that heterozygous genotype has smaller FI than mid-parent value.
‡‡ ANOVA was used for detecting this QTL, because Satt453 was not linked with other markers on this group. See Material and Methods for computation of LOD, additive effect, and dominant effect.

been found to be associated with resistance to SCN in soybean PI 89772 (Yue et al., 2001b), PI 438489B (Yue et al., 2001a), and Hartwig (Vierling et al., 1996). However, QTL on LG B1 identified in PI 404198A and PI 90763 was close to QTL identified in PI 438489B but distant from the QTL detected in PI 89772. QTLs identified in PI 89772 and PI 438489B have been found to be associated with resistance to races 1, 2, and 5. But QTL on LG B1 was not demonstrated to be associated with resistance to race 1 in PI 404198A. The same SCN populations were used in Yue et al.'s (2001a, 2001b) studies as in this study. Vierling et al. (1996) reported two QTLs on LG B1 (originally LGs B and S), but the R^2 of these two QTLs are so extreme. One is 91% and the other 1% only. To resolve these inconsistencies, confirmation studies and fine mapping are needed.

A suggestive interaction was detected between QTL-linked marker Satt309 on LG G and QTL-linked marker Satt632 on LG A2 for resistance to race 1 (Table 2). Single markers Satt309 and Satt632 plus the interaction between them explained 32.4% of the total variation. A significant interaction was found between QTL on

ated with resistance to races 2 and 5. QTLs for resistance to different races are not necessarily mapped on the same exact location even if they are the same gene because of sampling error. This sampling error may include variation caused by the SCN bioassay procedure and sampling of seeds from the $F_{2:3}$ which were genetically heterogeneous. QTLs for resistance to different races were regarded as the same if their confidence intervals overlapped substantially in this study. To exclude or confirm that closely linked genes are responsible for SCN resistance, fine mapping is needed.

In our other study where Hamilton × PI 90763 was used, QTL on LG B1 was detected 8 cM from Satt453 for resistance to races 2 and 5 (Guo et al., unpublished), and it seems to be located on the same region as the QTL identified in PI 404198A. Linkage group B1 has

LG G (Satt163) and QTL on LG B1 (Satt453) for race 2 and a suggestive interaction between them for resistance to race 5 (Tables 2 and 5). Single markers Satt163 and Satt453 plus the interaction between them explained 29.3% of the total variation for resistance to races 2. Single markers Satt163, Satt453, and Sat_280 plus interaction between the first two explained 30.3% of the total variation for resistance to race 5.

In summary, soybean PI 404198A may carry *rhg1* and *Rhg4*. It was shown that *rhg1* was resistant to all races studied (races 1, 2, and 5), whereas *Rhg4* was associated with resistance to race 1 but not races 2 and 5. Molecular marker Satt453 on LG B1 was found to be associated with resistance to SCN races 2 and 5 but not race 1. LG N may be associated with resistance to SCN race 5, but further studies are needed to lend credibility for this QTL.

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