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QTLs Associated with Resistance to Soybean Cyst Nematode in Soybean: Meta-Analysis of QTL Locations

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ABSTRACT

Soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) is the most important pest of soybean [*Glycine max* (L.) Merr.] in the world. A total of 17 quantitative trait locus (QTL) mapping papers and 62 marker–QTL associations have been reported for resistance to soybean cyst nematode in soybean. Conflicting results often occurred. The objectives of this study were to: (i) evaluate evidence for reported marker–QTL associations for resistance to SCN in soybean and (ii) extract relatively reliable and useful information from the reported marker–QTL associations in soybean. A meta-analysis was conducted for QTL locations by comparing the 95% confidence intervals of the reported QTLs. QTLs for different races or different studies were classified into one cluster if their confidence intervals had a region in common. The QTLs of the same cluster may have a shared locus. QTLs for different races or different studies were classified into different clusters if their confidence regions had no region in common and were ≥ 20 cM away from each other. Different clusters may represent different loci. Reported SCN resistant QTLs were classified into three categories: suggestive, significant, and confirmed. Confirmed QTLs are credible and can be candidates for fine mapping and gene cloning. QTLs on linkage groups (LGs) G, A2, B1, E, and J were classified as confirmed. Two clusters of QTLs were identified on LG G. One of them is *rhg1*. One cluster of QTLs was identified near the end of LG B1, but one QTL may exist around the middle of LG B1. One cluster of QTLs was identified on LGs A2, E, and J, respectively. QTLs on LGs B2, C1, C2, D1a, D2, L, M, and N were classified into suggestive or significant. Confirmation studies are needed to lend credibility for these QTLs. A relationship between soybean QTLs and SCN races is discussed.

Soybean cyst nematode is the most important pest of soybean in the world and causes more yield losses than any other soybean disease (Wrather et al., 1995, 2001).

A total of 62 marker–quantitative trait locus (QTL) associations have been reported by 17 papers for resistance to SCN races 1, 2, 3, 5, 6, and/or 14 in a total of 13 soybean accessions (nine resistance sources) (Concibido et al., 2004; Glover et al., 2004). Conflicting results often occurred (Concibido et al., 2004). QTLs declared by different studies show a variation for QTL location that is sometimes large. A number of false positive QTLs may have been reported because of use of low threshold values and completion of a number of genome scans (studies). Nearly 3 false positives per genome scan [$\mu(T) = 20 + 2 \times 1.5 \times 25 \times 4.6 \times 2.5) \times 0.0032 = 2.8$] (Lander and Kruglyak, 1995) are expected when threshold LOD = 2.5 is used in soybean mapping. Chances of false positive QTLs are expected to increase with more genome scans even if stringent threshold values are used in single studies.

Usually, the position of a peak (a QTL) on a region or a chromosome does not necessarily coincide with the true position of a QTL in a particular experiment and QTLs detected by different studies is not necessarily mapped at the same exact location because of sampling error even if they are in fact located on the same locus. Sampling error comes mainly from phenotype evaluation and sampling of progeny individuals. Darvasi et al. (1993), Darvasi and Soller (1997), and Roberts et al. (1999) studied sampling distribution for QTL location using computer simulations. It was demonstrated that the confidence interval of QTL location was inversely proportional to population size and QTL effect. Large variation (even covering a whole chromosome) may occur when a QTL has a small gene effect and a small population size is used. A statistical method is needed to assess whether QTLs detected on a linkage group map by different studies are located on the same locus or linked. Recently, Goffinet and Gerber (2000) developed a maximum-likelihood-based meta-analysis for QTL locations among studies. It is called meta-analysis because it is involved in analyzing results from different studies and combining information from them. It requires more than 10 to 30 reported QTLs from independent studies on the same linkage group (LG) to be valid. One simple approach for analysis of QTL location is that a LG map is divided into regions of a length and QTLs declared by different studies are classified into a cluster if they fall on the same region (Concibido et al., 2004; Becker et al., 1998). The QTLs of the same cluster may share a locus. But its disadvantages are that the length of a region is arbitrary and it does not reflect the characteristics of experiments such as population size and type. In this study, a comparative analysis of QTL location among studies was developed which is based on the confidence interval of QTL location. This method is simple in computation and it reflects the characteristics of experiments such as population size and type as well as QTL itself.

An appropriate threshold level for declaring a QTL is an important issue because of an excessive number and dependence of test statistics obtained at a series of putative positions along the genome. QTL analysis involves multiple tests and the point-wise level should

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Abbreviations: LG, linkage group; PI, plant introductions; QTL, quantitative trait locus; SCN soybean cyst nematode.

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 genome-wide level is the probability that an extreme test statistics (LOD) occurs by chance somewhere in a whole genome. At this time, permutation tests (Churchill and Doerge, 1994) are a general approach for the adjustment. But other methods are also available including computer simulation (Lander and Bostein, 1989; Ooijen, 1999) and mathematical formulas (Lander and Kruglyak, 1995). Too relaxed a threshold value creates a large number of false positives, but too stringent a threshold value will slow down discovery of QTLs. To resolve this paradox, Lander and Kruglyak (1995) classified statistical evidence for marker–QTL associations into four categories: (i) suggestive QTL—one false positive per genome scan (genome-wide type I error = 0.63), (ii) significant QTL—0.05 false positive per genome scan (genome-wide type I error = 0.05), (iii) highly significant QTL—0.001 false positive per genome scan (genome-wide type I error = 0.001), and (iv) confirmed QTL—significant QTL that has been confirmed (replicated) by another independent study. A QTL is usually declared at genome-wide type I error = 0.05 (Lander and Kruglyak, 1995; Members of the complex trait consortium, 2003). A suggestive level often gives false positive QTL but it is worth reporting if accompanied with an appropriate warning label (Lander and Kruglyak, 1995). To be credible, a QTL should be confirmed, and it would be better to confirm QTL before proceeding to fine mapping and cloning. A locus name is appropriate for a confirmed QTL but not for a suggestive QTL (Members of the Complex Trait Consortium, 2003).

When more than two studies are conducted for the same traits, meta-analysis can also be used for analyzing statistical evidence (test statistics or p value) from different studies, so that evidence from different studies, as a whole, are evaluated and the power of QTL detection may be increased (Lander and Kruglyak, 1995). Actually, meta-analysis was first suggested for analysis of statistical evidence in QTL mapping (Lander and Kruglyak, 1995), and a number of methods have been suggested or developed in human and animal QTL mapping (for example, Lander and Kruglyak, 1995; Li and Rao, 1996; Gu et al., 1998; Etzel and Guerra, 2002; Wise et al., 1999; Allison and Heo, 1998; Badner and Gershon, 2002; Belknap and Atkins, 2001). Some of them (Wise et al., 1999; Allison and Heo, 1998; Badner and Gershon, 2002; Belknap and Atkins, 2001) are applicable for experimental organisms including plant species. Wise et al. (1999) developed a non-parametric meta-analysis in which a genome is divided into different regions and these regions are ranked according to test statistics or p value and, then, a nonparametric statistical method is applied. Allison and Heo (1998), Badner and Gershon (2002), and Belknap and Atkins (2001) referred to combining p values from different studies using the fact that $-2\ln(p)$ is distributed as χ^2 (df = 2) and the additive nature of independent χ^2 values. But the first two made adjustment of p values before combining p values but the last one did not. The goal of adjustment of p values is to control type I error. We tend to agree on no adjustment of p values before combining p values, but genome-wide adjustment after combining p values, because the adjustment after combining p values can also be used to control type I error and adjustment before combining p values will complicate meta-analysis. Allison and Heo (1998) and Badner and Gershon (2002) adopted different adjustments. The former one can be regarded as chromosome-wide adjustment but the latter one as region-wide adjustment. The key issues in use of meta-analysis for statistical evidence are heterogeneity among mapping populations and appropriate threshold. Heterogeneity among populations (for example, different SCN resistant plant introductions) often makes it complicated to interpret the results of meta-analysis. In addition, incomplete and different information reported in various studies make it difficult to conduct a meta-analysis. In this study, we did not conduct meta-analysis for statistical evidence because most of SCN resistant QTL studies used different resistance sources and test statistics or p values are available for regions with declared QTLs only. If raw datasets are available, pooled analysis (Walling et al., 2000; Li et al., 2005; Guo et al., unpublished) would be a better method for analysis of QTLs among studies.

Objectives of this study were to: (i) evaluate evidence for reported marker–QTL associations for resistance to SCN in soybean and (ii) extract relatively reliable and useful information from a large number of reported marker–QTL associations.

METHODOLOGY

Reported SCN-Resistant Marker–QTL Associations

Concibido et al. (2004) summarized 60 reported marker–QTL associations for resistance to SCN in soybean. Low threshold value may produce more false positives and easy by-chance replication of QTLs from a second study (Lander and Kruglyak, 1995). Lander and Kruglyak (1995) indicated that there would be many regions with point-wise p value of 0.05 in one genome scan by chance and some will appear again in a second study just by chance. To reduce false positives, only QTLs at LOD $\geq$ 3.0 (point-wise p value $\leq$ 0.001) were used in this study (Table 1), including QTLs detected by Glover et al. (2004), and our studies (Guo et al., unpublished; Lu et al., unpublished). It is noted that most studies used SCN populations maintained at the University of Missouri-Columbia.

Meta-Analysis of QTL Locations

In soybean SCN-resistant QTL mapping studies, fewer than 10 QTLs from independent studies were reported for each LG. Therefore, Goffinet and Gerber's (2000) method is not appropriate. Our approach for meta-analysis of QTL locations was based on the confidence intervals of QTLs. QTLs for different studies or different races were classified into one cluster if their confidence intervals overlapped. QTLs of the same cluster may have a shared locus.

Location of QTL

Molecular marker or position with the highest test statistics on a LG map or a region of a LG map was regarded as the estimated location of a QTL from a particular experiment.

Table 1. A summary of QTLs of LOD ≥ 3.0 reported for resistance to soybean cyst nematode by previous studies in soybean.

Resistance source	References	Linkage groups†	SCN races‡	Population type§	Data analysis¶
PI 438489B	Yue et al., 2001a	A2 B1 C1 C2 D1a E G	*1 2 3 5 14*	$F_{2:3}$	IM
PI 90763	Guo et al., unpublished	A2 B1 E G J L	*2 3 5*	$F_{2:3}$	CIM
	Concibido et al., 1997	G J	1 3 6	$F_{2:3}$	ANOVA
PI 40198A	Guo et al., unpublished	A2 B1 G N	*1 2 5*	$F_{2:3}$	CIM, ANOVA
PI 467312††	Lu et al., unpublished	C1 G E J	*3 5 14*	$F_{2:3}$	IM
Peking	Qiu et al., 1999	B2	*1 3 5*	$F_{2:3}$	ANOVA, IM
	Meksem et al., 2001	A2 G	*3*	RILs, NILs	IM, ANOVA
	Concibido et al., 1997	G N	136	$F_{2:3}$	ANOVA
	Mahalinggam and Skorupska, 1995	A2	3	$F_{2:3}$, $F_{2:4}$	ANOVA, IM
PI209332	Concibido et al., 1994	A2 G J	3	$F_{2:3}$	ANOVA, IM
	Concibido et al., 1996	D2 G	1 3 6	RILs, $F_{2:3}$	ANOVA, IM
PI89772	Yue et al., 2001b	B1 D2 E G	*1 2 3 5*	$F_{2:3}$	IM, ANOVA
PI88788	Glover et al., 2004	G J	*3 14*	RILs, NILs	CIM, ANOVA
	Concibido et al., 1997	G	1 3 6	$F_{2:3}$	ANOVA
PI437654	Webb et al., 1995	A2 G M	*3*	RILs	IM
Hartwig‡‡	Schuster et al., 2001	D2	14	$F_{2:3}$	ANOVA
	Vierling et al., 1996	B1	3	$F_{2:3}$	ANOVA
J87-233§§	Heer et al., 1998	A2 G	*1 3 5*	$F_{2:3}$	ANOVA, IM
PI468916 (*G. soja*)	Wang et al., 2001	C2 E G	*3*	RILs, BC_1F_2	CIM

† Linkage groups associated with resistance to SCN at LOD ≥ 3.0. Underlined linkage group was not used for meta-analysis because it is significantly different for marker order or map distance from the soybean composite linkage map or it has extreme R^2 (1% and 91%) which, we believe, need further study for confirmation.
‡ Italic type indicates SCN race populations maintained at University of Missouri-Columbia.
§ RILs: recombinant inbred lines; NILs: Near-isogenic lines.
¶ CIM: composite interval mapping; IM: interval mapping; ANOVA: analysis of variance.
†† Preliminary results. Study is still in progress.
‡‡ Hartwig is derived from PI437654 and Peking.
§§ J87–233 is derived from PI90763, PI88788, and Peking.

In reported SCN-resistant QTLs, the locations of QTLs were expressed on linkage maps constructed in particular experiments. For comparisons across different studies, the reported locations of QTLs need to be projected on a known common linkage map. We projected a reported QTL on the soybean composite linkage map (Song et al., 2004) based on its relative position between its flanking markers in the original studies. A reported QTL was not projected if its flanking markers in a particular experiment was not consistent with the soybean composite linkage map for LGs or if the LG map constructed in the particular experiment was significantly different from the soybean composite linkage map for marker orders.

Confidence Intervals of QTL Location

The 95% confidence interval (CI) of a QTL location was obtained by the below formula:

$$\text{CI} = 3000/(N\alpha^2) \text{ or } 530/(NR^2) \text{ for backcross} \quad [1]$$

$$= 1500/(N\alpha^2) \text{ or } 530/(NR^2) \text{ for } F_2 \text{ intercross} \quad [2]$$

$$= 250/(N\alpha^2) \text{or } 163/(NR^2) \text{ for recombinant inbred lines} \quad [3]$$

where α is the standardized phenotypic effect (expressed in residual standard deviation units) of a single allele substitution at a QTL, N the population size and R^2 a proportion of the total variation explained by a QTL. The R^2 provided by interval mapping, composite interval mapping or ANOVA was used for estimation of R^2 in the above formulae. Here, we assumed that interval mapping and composite interval mapping provided a good estimate of R^2 and ANOVA provided a reasonable estimate of R^2.

The formulae [1] and [2] were first derived by Darvasi and Soller (1997) in the case of dense molecular marker linkage maps using extensive simulations. They were independently proven by Visscher and Goddard (2004) and Weller and Soller (2004) using somewhat different mathematical methodologies. The formula [3] can easily be derived from the formula described by Weller and Soller (2004) (phenotyping five plants for each line). The above formulae can also apply to a moderate marker spacing (10–20 cM) (Darvasi and Soller, 1997). If an unbiased estimate of α or R^2 is used, an unbiased CI will be obtained (Darvasi and Soller, 1997). Use of threshold for declaring a QTL may cause overestimation of gene effect and underestimation of CI if a QTL has a small gene effect (i.e., low detection power). However, the CI can still be obtained with approximately the correct probability of containing the true map location of the QTL (Darvasi and Soller, 1997).

If the heterogeneous region of near-isolines was not clearly defined in the original study or it was larger than the CI determined by the above formula [1], [2], or [3], the above formula [1], [2], or [3] was used for obtaining the CI of a QTL.

The CI region of one QTL on the soybean composite linkage map was determined through placing the center of its estimated CI on its location. If one side of the QTL was beyond the end of a LG, the CI was cut off from the end of the LG.

Meta-Analysis of Reported Marker–QTL Associations

QTLs for different races or different studies were classified into one cluster if their estimated 95% CI regions had a region in common. QTLs from the same cluster may have a shared locus. To exclude or confirm that QTLs from the same cluster are closely linked genes, fine mapping is needed. QTLs for different races or different studies were classified into different clusters if their CI regions had no region in common and were ≥20 cM away from each other. Different clusters may represent different loci. Additional studies are needed if the CI regions were close but did not overlap. QTL detected in a particular experiment was excluded if its CI region covered a whole chromosome.

Classification of Statistical Evidence of QTLs

Thresholds for Declaring QTLs

In previous soybean SCN-resistant QTL mapping studies, the following threshold levels were used for declaring QTL:

(i) LOD = 2.5 (equivalently $p = 0.003$) (Yue et al., 2001a, 2001b), (kii) $p = 0.002$ (Concibido et al., 1994, 1996, 1997), and (iii) LOD = 3 (equivalently $p = 0.001$) (Webb et al., 1995; Heer et al., 1998; Qiu et al., 1999; Wang et al., 2001, Meksem et al., 2001). Few permutation tests were used to determine threshold levels (Glover et al., 2004). We used three methods to determine threshold value for declaring a QTL at the suggestive level (genome-wide type I error = 0.63) and at the significant level (genome-wide type I error = 0.05) for soybean F_2 mapping populations (used in the majority of studies). We obtained threshold LOD = 2.9 at the suggestive level and 4.2 at the significant level using Ooijen's (1999) computer simulation tables. Threshold LOD was 3.0 at the suggestive level and 4.5 at the significant level using Lander and Kruglyak's (1995) formula. Threshold LOD was 3.7 to 4.0 for different races at the significance level using permutation tests (Churchill and Doerge, 1994) based on our two mapping populations (1000 permutation tests each race for each population) (Guo et al., unpublished). In summary, threshold LOD of 3.0 is approximate to genome-wide type I error = 0.63 (suggestive level) and threshold LOD of 4.0 to genome-wide type I error = 0.05 (the significant level) in soybean. A QTL is usually declared at genome-wide type I error = 0.05. Suggestive level often gives false positive QTL, but it should be good evidence if accompanied with other evidence. Therefore, a suggestive QTL was declared at LOD $\geq$ 3.0 and a significant QTL at LOD $\geq$ 4.0 in this study.

Classification of QTLs

With reference to Lander and Kruglyak (1995), we classified soybean SCN-resistant QTLs into three categories: (i) suggestive QTL: LOD $\geq$ 3.0 (p value $\leq$ 0.001), (ii) significant QTL: LOD $\geq$ 4.0 (p value $\leq$ 0.0001), and (iii) confirmed (replicated) QTL. A confirmed QTL is defined by Lander and Kruglyak (1995) as being a significant QTL from one study that has subsequently been confirmed by a second study. Confirmation of a QTL includes two stages. The first stage is involved in searching for a QTL, usually, on a whole genome using one mapping population sample. The second stage just focuses on QTL detection using another mapping population sample on the QTL candidate region (typically, 20 cM) that has been established in the previous study. The second stage can be accomplished using near-isogenic lines, independent crosses, and breeding selection (Members of the complex trait consortium, 2003). Typical examples of confirmed SCN resistance QTLs are Meksem et al. (2001), Glover et al. (2004) and Wang et al. (2001). The first two studies used near-isogenic lines and the last one an independent cross in the second stage. The above definition of confirmed QTL was extended in this study to include the two following situations.

1. Two or more studies were independently conducted, and a QTL was detected on the same region in these studies. These studies referred to the same cross but different progeny individuals or the same source of SCN resistance (soybean plant introduction (PI) or its breeding line).
2. QTLs were frequently detected on the same region in studies where different SCN resistance sources (PIs) were used. QTLs identified by different studies were defined as falling on the same region if their 95% confidence intervals overlapped, i.e., QTLs for different races or different studies were classified into one cluster in meta-analysis described above. If the QTLs from the same cluster came from at least three independent studies, they were regarded as being confirmed in this study.

RESULTS

Data in Table 2 and Fig. 1 summarizes confirmed QTLs. They are located on LGs G, A2, B1, E, and J. We believe that these QTLs are credible and can be candidates for fine mapping and cloning. It is reported that QTLs on LG G and A2 (*rhg1* and *Rhg4*) have been cloned and sequenced (Hauge et al., 2001; Lightfoot and Meksem, 2002). QTLs on LGs B2, C1, C2, D1a, D2, L, M, and N were classified into suggestive or significant. We believe that confirmation (replication) studies (Lander and Kruglyak, 1995) are needed to lend credibility for these QTLs.

Two clusters of QTLs were identified on the ends of LG G. One cluster was located on the left end of the group (Table 2 and Fig. 1). QTLs of this cluster were identified in soybean PI 90763, PI 88788 (including 'Bell'), 'Peking' (including 'Forrest'), PI 89772, PI 404198A, PI 467312, PI 437654, and PI 209332. The CI

Table 2. Confirmed QTLs associated with resistance to soybean cyst nematode in soybean.

	Linkage groups					
Soybean PIs	**G (*rhg1*)**	**G (2rd locus)**	**A2 (*Rhg4*)**	**B1†**	**E**	**J (*CqSCN-003*)**
PI 438489B	+**‡		+**‡	+**(a)‡	+**	
PI 90763	+§		+**	+**(a)	+**	+§
PI 404198A	+**		+**	+**(a)		
PI 467312	+**				+**	+**
Peking	+§	+**	+§			
PI 209332	+§		+*			+§
PI 437654	+**		+**			
PI 89772	+**			+**(b) ‡	+*‡	
PI 88788	+§					+§
PI 468916 (*G. soja*)		+§			+§	

*** Significant QTL (LOD = 3.0 or point wise $p = 0.001$).**
**** Suggestive QTL (LOD = 4.0 or $p = 0.0001$).**
† Different letters (a, b) indicate no overlap for the confidence interval among QTLs. The same letter (a) indicates overlap for confidence interval among QTLs.
‡ Indicates that further studies are needed because of conflicting results or weak evidence.
§ QTL was confirmed using near isogenic lines or another independent cross or QTL was detected on the same region in two or more studies where the same SCN resistance source (PI) or its breeding line was used. SCN resistance source (PI) was inferred to carry the QTL if a QTL was identified in its breeding line.

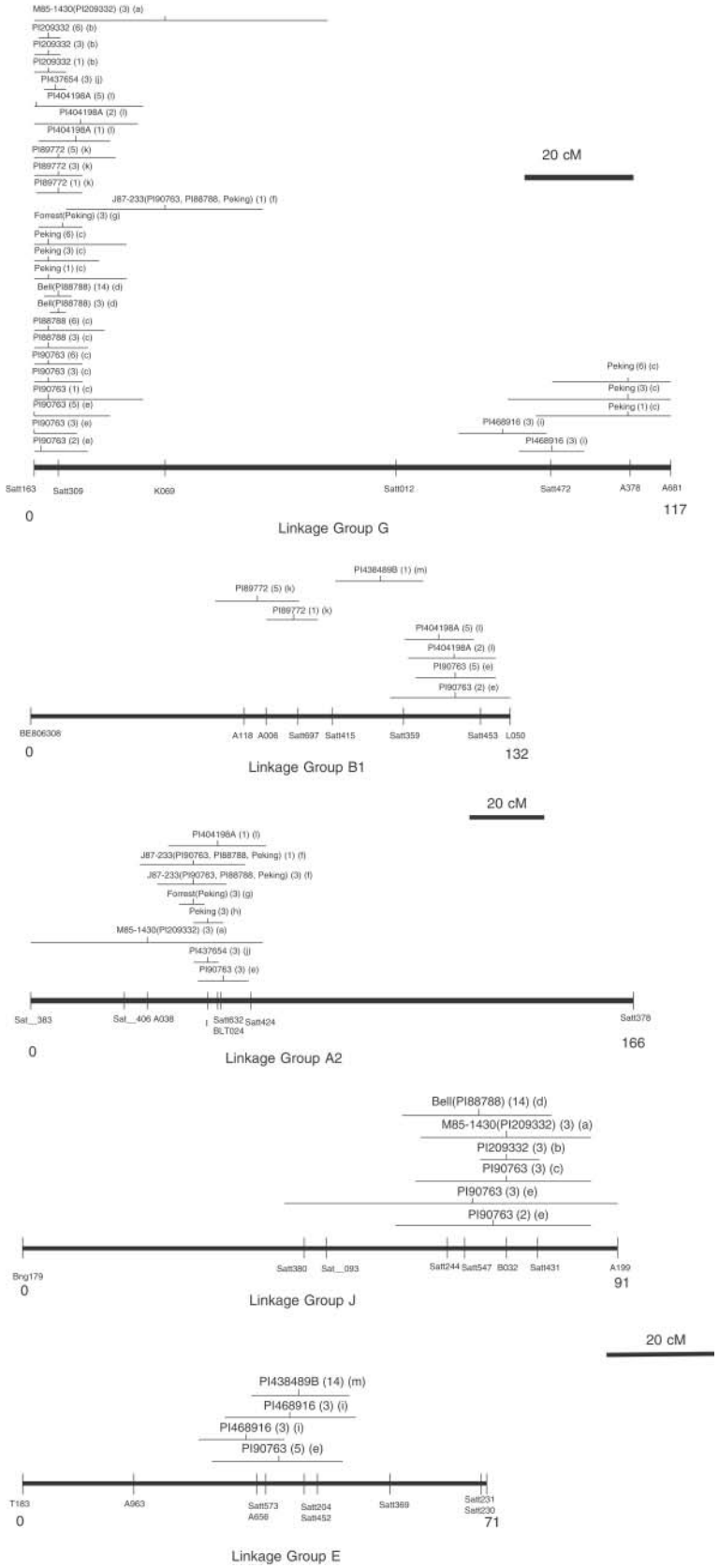

Fig. 1. Confirmed QTLs were projected on the soybean composite linkage map. The confidence interval region of a QTL is indicated by the length of a thin line above the linkage group map. The position of a QTL is indicated by the vertical thin line. The soybean accession on each thin line indicates that a QTL was reported in this accession. The number in parentheses following the accession indicates races—1, race 1; 2, race 2; 3, race 3; 5, race 5; 6, race 6; and 14, race 14. The letters in parentheses following the race indicates studies where QTLs were reported (a) Concibido et al., 1994; (b) Concibido et al., 1996; (c) Concibido et al., 1997; (d) Glover et al., 2004; (e) Guo et al., unpublished (PI 90763); (f) Heer et al., 1998; (g) Meksem et al., 2001; (h) Mahalingam and Skorupska et al., 1995; (i) Wang et al., 2001; (j) Webb et al., 1995; (k) Yue et al., 2001a; (l) Guo et al., unpublished (PI 404198A); (m) Yue et al. (2001b). QTLs detected in PI 4376122 (Lu et al., unpublished) were not projected because the study is in progress.

regions of these soybean lines were narrow (3–20 cM) with a 2-cM region in common (the CI region of PI 467312 was undetermined because the study is still in progress). *Rhg1* has been located 0.4 to 1.25 from molecular marker Satt309 (Cregan et al., 1999a, 1999b; Meksem et al., 2001). *Rhg1* is within the CIs of QTLs identified in PI 90763, PI 88788 (including Bell), Peking, Forrest, PI 89772, PI 404198A, PI 467312, PI 437654, and PI 209332 (Fig. 1). It is concluded that these PIs may carry *rhg1*. The CI regions of soybean J87–233 and M85–1430 were wide (36 and 54 cM, respectively) (Fig. 1). J87–233 was derived from PI 90763, PI 88788 and Peking (Heer et al., 1998). Its SCN resistance genes came from one of them. Its CI region overlapped with the CI regions of PI 90763, PI 88788, and Peking (Fig. 1), as expected. Soybean M85–1430 was derived from PI 209332 (Concibido et al., 1994). Its SCN resistance genes came from PI 209332. Its CI region overlapped with that of PI 209332 (Fig. 1), as expected. Therefore, J87–233 and M85–1403 may also carry *rhg1*. The second cluster was located near the right end of the group (Table 2 and Fig. 1). QTLs of the cluster were identified in wild soybean (*G. soja*) PI 468916 and cultivated soybean Peking. The CI regions have a 2-cM region in common except for that of Peking for resistance to SCN race 6.

One cluster of QTLs were identified near the I locus on LG A2 in soybean PI 90763, PI 437654, Peking (including Forrest), PI 404198A, J87–233, and M85–1430 (Table 2 and Fig. 1). The CI regions of the first five soybean lines were 4 to 29 cM. The CI region of the last one was wide (64 cM). The CI regions have a 2-cM region in common. *Rhg4* has been mapped close to molecular marker Satt632 and the I locus (Cregan et al., 1999b; Meksem et al., 2001). Satt632 and the I locus were within the CIs of QTLs identified in PI 90763, PI 437654, Peking, Forrest, PI 404198A, J87–233, and M85–1430 (Fig. 1). It is concluded that these PIs may carry *Rhg4*. It must be noted that *Rhg 4* may be detected in one study, but it might not be detected in others, although a high detection power is expected because of its large effect. For example, it was detected in soybean breeding line M85–1430 (Concibido et al., 1994) but not in PI 209332 from which M85–1430 was derived (Concibido et al., 1996). One possible explanation is that the QTL on LG A2 may be modified by other genes.

One cluster of QTLs was identified near one end of LG B1 in soybean PI 90763, PI 404198A, and PI 438489B (Table 2 and Fig. 1). The CI regions have a 2 cM region in common. Another QTL was identified near the middle of LG B1 in soybean PI 89772 (Fig. 1). Its CI region was close to that of soybean PI 438489B but ≥20 cM away from those of soybean PI 90763 and PI 404198A. There are two possible explanations for the QTL on LG B1 in PI 89772. One is that the QTL peak obtained in PI 89772 is a local peak because of sampling error not a global peak on LG B1 because the end region (from Satt359 to Satt451) of LG B1 was not searched for a QTL (Yue et al., 2001b). The other one is that one QTL truly exists near the middle of LG B1. Additional studies are needed to resolve this paradox.

One cluster of QTLs was identified near the middle of LG E in soybean PI 90763, PI 468916, PI 438489B, and PI 467312 (Table 2 and Fig. 1). Their CI regions had a 5-cM region in common (the CI region of PI 467312 was undetermined). One QTL on LG E was identified from the original study in soybean PI 89772 but the distance between its flanking markers, A135 and Satt231, in the original study (Yue et al., 2001b) was significantly different from that on the soybean composite linkage map. Because of this, it was not shown in Fig. 1. Further study is needed.

One cluster of QTLs was identified near the end of LG J in soybean PI 90763, PI 209332 (including M85–1430), Bell (PI 88788), and PI 467312 (Table 2 and Fig. 1). Their CI regions had a 9 cM region in common (the confidence region of PI 467312 was undetermined). The QTL in Bell has been confirmed and designated as *cqSCN-003* (Glover et al., 2004). It is concluded that these PIs may carry *cqSCN-003*.

DISCUSSION

QTLs Associated with Resistance to SCN in Soybean PI 438489B

One QTL on LG G was declared in PI 438489B from the original study (Yue et al., 2001b), but it is distant from *rhg1*. It is noted that the linkage map constructed in the original study was significantly different in marker order from the soybean composite linkage map. We speculated that this line could probably have *rhg1* because all other cultivated PIs studied carried *rhg1* (Table 2). One QTL on LG A2 was declared in soybean PI 438489B from its original study (Yue et al., 2004b). However, the LG of its flanking markers, K400 and T155, were not consistent with the soybean composite linkage map and therefore it was not shown in Fig. 1. However, Webb et al. (1995) also located marker K400 on LG A2; therefore, we believe that PI 438489B would likely carry *Rhg 4* (Table 2).

Specific Association of QTLs with SCN Races

QTLs for resistance to different races fall on the same regions on LGs G, A2, B1, E or J (Fig. 1). QTLs for resistance to different races were regarded as the same if they fell on the same region. Data in Table 3 summarizes the relationship of soybean QTLs with resistance to SCN populations maintained at the University of Missouri-Columbia (which are designated as races 1, 2, 3, 5, and 14). These races are believed to be near-homogeneous because of reproduction in a small population size for more than thirty generations (Arelli et al., 1997, 2000). QTL on LG G (*rhg1*) is associated with resistance to races 1, 2, 3, and 5 in all the involved PIs (Table 3). But, it may be less frequently associated with resistance to race 14 (it might have a small effect on race 14 and races more virulent to *rhg1* might exist). QTL on LG A2 (*Rhg4*) is frequently associated with resistance to race 3; however, it is less frequently associated with resistance to races 2, 5, and 14. In contrast to QTL on LG A2, QTL on LG B1 is frequently associated

Table 3. Specific Association of soybean QTLs with SCN races.

	SCN races†				
QTL (linkage group)	**1**	**2**	**3**	**5**	**14**
G (*rhg1*)	**3/3‡**	**4/4**	**8/8**	**5/5**	**1/3**
A2 (*Rhg4*)	**1/3**	**0/4**	**4/8**	**0/5**	**0/3**
B1§	**1/2**	**2 + (1¶)/3**	**0/5**	**2 + (1¶)/4**	**0/1**
E	**0/3**	**1/4**	**3/5**	**1/5**	**2/2**
J (*cqSCN-003*)	**0/1**	**1/2**	**3/5**	**0/3**	**2/2**

† SCN populations maintained at University of Missouri-Columbia.

‡ The number below the slash is the number of soybean PIs in which the QTL candidate region was searched for a QTL. The number above the slash is the number of soybean PIs in which a QTL were detected on the candidate region. Qiu et al's (1999) and Heer's (1998) studies were not included because fewer markers or mixed SCN resistance sources were used.

§ Region around the end of LG B1. PI 89772 was not included because the end region of LG B1 was not searched for a QTL in this PI (Yue et al., 2001b).

¶ QTL with 2.5 < LOD < 3.0 in PI 838489B (Yue et al., 2001a).

with resistance to races 2 and 5, but it is less frequently associated with resistance to race 3. QTLs on LGs E and J are frequently associated with resistance to races 14 and 3. LG E may be less frequently associated with resistance to race 1, 2, and 5. LG J is less frequently associated with resistance to race 5. In conclusion, there seems to be a specific relationship between soybean QTLs and SCN populations.

An accurate and unbiased estimation of QTL location and its CI region could be obtained using Darvasi's formulae described earlier if an evenly and densely distributed molecular marker LG is used. Unfortunately, however, in practice, it is not easy to construct an evenly and densely distributed molecular marker LG. In previous soybean SCN resistant QTL mapping studies, fewer molecular markers were used and LG maps with poor coverage were constructed and used in particular experiments. Therefore, CIs estimated in this study were approximate. The CI of a QTL for QTL location can also be estimated by 1 − LOD confidence interval (Lander and Bostein, 1989), bootstrap (Lebreton and Visscher, 1998; Visscher et al., 1996), and others (Mangin et al., 1994) in addition to the method used in this study. Unfortunately, they were not reported or estimated in previous SCN-resistant QTL mapping studies. In addition, this study did not evaluate, as a whole, statistical evidence from different studies. In soybean, information on reported SCN-resistant QTLs is very limited and non-standardized. This limited information makes it difficult to conduct accurate analysis for QTL locations among studies and meta-analysis for statistical evidence (test statistics or p-value).

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