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Identification of QTLs associated with resistance to soybean cyst nematode races 2, 3 and 5 in soybean PI 90763

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Abstract Soybean cyst nematode (SCN) is a major soybean pest throughout the soybean growing regions in the world, including the USA. Soybean PI 90763 is an important SCN resistance source. It is resistant to several SCN populations including races 2, 3 and 5. But its genetics of resistance is not well known. The objectives of this study were to: (1) confirm quantitative trait loci (QTLs) for resistance to SCN race 3 in PI 90763 and (2) identify QTLs for resistance to SCN races 2 and 5. QTLs were searched in Hamilton × PI 90763 $F_{2:3}$populations using 193 polymorphic simple sequence repeats (SSRs) covering 20 linkage groups (LGs). QTLs for resistance to SCN were identified on LGs A2, B1, E, G, J and L. The same QTL was suggested for resistance to different SCN races where their 1-LOD support intervals of QTL positions highly overlapped. The QTL on LG G was associated with resistance to races 2, 3 and 5. The QTL on LG B1 was associated with resistance to races 2 and 5. The QTL on LG J was associated with resistance to races 2 and 3. The QTLs on LGs A2 and L were associated with resistance to race 3. The QTL on LG E was associated with resistance to race 5. We conclude that LGs A2 and B1 may represent an important distinction between resistance to SCN race 3 and resistance to SCN races 2 and 5 in soybean.

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Introduction

Soybean cyst nematode (SCN) (*Heterodera glycines Ichinohe*) is the most important pest of soybean (*Glycine max (L.) Merr*) in the world and causes more yield losses than any other soybean disease (Wrather et al. 1995, 2001).

The use of resistant cultivars is the most effective way to control SCN damage (Wrather et al. 1995; Bradley and Duffy 1982). A total of 118 SCN-resistant accessions have been identified 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 PI 437654, PI 438489B, PI 90763, PI 89772, PI 404198A, PI 404166, and PI 438489.

Understanding of the genetic basis of resistance to SCN in soybean is important for the development of SCN-resistant varieties and germplasm. Mapping resistance to SCN using molecular markers provides a powerful tool for characterization of the genetic basis of soybean resistance to SCN. Quantitative trait loci (QTLs) have been identified using molecular markers for resistance to SCN races 1, 2, 3, 5, 6 and /or 14 in a total of 13 accessions (9 resistance sources). They are located on all linkage groups (LGs) except for D1b, K and O (Concibido et al. 2004). The QTLs for resistance to SCN on LGs G and A2 (*rhg1* and *Rhg4*, respectively) have been well studied and molecular markers have been saturated around them (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), but functional tests of candiadate genes are needed to demonstrate that they are the correct candidate genes (Glazier et al. 2002). LG G seems to be involved in all SCN races except for race 14, whereas LG A2 seems to play a distinct role in resistance to race 3 (Guo et al. unpublished). Additional studies are needed for a greater understanding of

resistance to SCN races except for race 3 and for confirmation of existence of the QTLs except for those on LGs G and A2. In the meantime, some QTLs may not have been detected in earlier studies in which only a limited number of RFLPs and a small mapping population size was used. Further genome scanning of QTLs, including more SCN races and more coverage of genome scanning, especially using simple sequence repeat (SSR) markers, is needed in soybean resistance sources.

Soybean PI 90763 is one of the few sources which can provide a greater spectrum of resistance to SCN. It is resistant to SCN races 1, 2, 3, 5 and 6 and moderately resistant to race 14 (Arelli et al. 1997, 2000; Concibido et al. 2000). This PI line has also begun to be used in USA soybean breeding programs. PI 90763 is also used for classifying SCN populations (Schmitt and Shannon 1992; Niblack et al. 2002). However, its genetics of resistance to SCN is not well known. Concibido et al. (1997) mapped the resistance of PI 90763 to SCN races 1, 3 and 6 using a small number of RFLPs and a small population size. QTLs for SCN resistance were identified on LGs G and J.

A new classification and designation system of SCN populations was recently published by Niblack et al. (2002). However, adoption of this new system may cause some confusion in comparing data to previously published studies. The SCN populations used for SCN-resistant QTL mapping in the past came largely from two sources, namely, Missouri and Minnesota (Concibido et al. 1994, 1996, 1997). The Missouri populations have been used by the Missouri soybean group (Qiu et al. 1999; Yue et al. 2001a, 2001b) and other research groups (Wang et al. 2001; Meksem et al. 2001; Heer et al. 1998; Webb et al. 1995). For convenience of comparison with earlier studies, we used Schmitt and Shannon's (1992) classification system in this study. But we also gave the HG types of the SCN populations which we used.

The objectives of this study were to: (1) confirm QTLs in PI 90763 identified by Concibido et al. (1997) and (2) identify QTLs for resistance to SCN races 2 and 5.

Materials and methods

Materials

Two hundred twenty-six $F_{2:3}$families were developed from a cross between Hamilton and PI 90763. PI 90763 is resistant to SCN races 1, 2, 3 and 5 and moderately resistant to race 14 (Arelli et al. 1997, 2000; Concibido et al. 2000). It was introduced into the USA from China in 1930. Hamilton was released by the Illinois Agricultural Experimental station in 1989 (Nickell et al. 1990) and is reportedly susceptible to all known SCN races. Leaves were harvested from each F_2 plant and used for DNA extraction and SSR genotyping. F_2 plants were allowed to set $F_{2:3}$seed. These $F_{2:3}$ were used for SCN phenotyping in the greenhouse.

Soybean cyst nematode bioassay

Soybean cyst nematode races 2 (HG type 1.2.5.7, PA 2), 3 (HG type 0, PA 3) and 5 (HG type 2.5.7, PA 5) maintained at the University of Missouri-Columbia were used. These races were believed to be near-homogeneous due to reproduction in a small population size for more than thirty generations (Arelli et al. 1997, 2000).

Soybean cyst nematode 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 days and then transplanted into the micropots filled with steam-pasteurized soil (1 plant in each micropot). Twenty micropots were placed in each plastic container and maintained at $27 \pm 1°$ 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 using an automatic pipetter (Brewer Automatic pipetting Machine, Scientific Products, Balltimore, MD). Thirty days after transplanting, roots of individual plants were harvested and washed using pressurized water for collection of female nematodes. Nematodes were counted under a stereo-microscope. Two hundred twenty-six $F_{2:3}$families (two replications, 5 plants for each replication in each family) and its parents were evaluated for individual races, together with four differential soybean lines (Peking, PI 88788, PI 90763 and Pickett) and the susceptible soybean cultivar 'Hutcheson' (control) (5 plants for each differential line and 10 plants for the control). Differentials and control were used to monitor the shift of SCN races. No race shifts occurred.

A female index (FI) was used to measure reaction of each individual plant to SCN (Schmitt and Shannon 1992). Averages of ten plants were used to represent the response of each family for each race.

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

DNA extraction and SSR genotyping

DNA was extracted from the parents and 226 F_2 plants using the CTAB method (Keim et al. 1988) and it was used for SSR genotyping. The SSR molecular markers described by Song et al. (2004) were used. They were 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 µm of each of the primers, 0.3 mm each of dNTPs, 2.5 mm of $MgCI_2$ and 0.3 U of Taq DNA polymerase (Promega Corporation, Madison, WI). The PCR reaction was performed at 94° C for

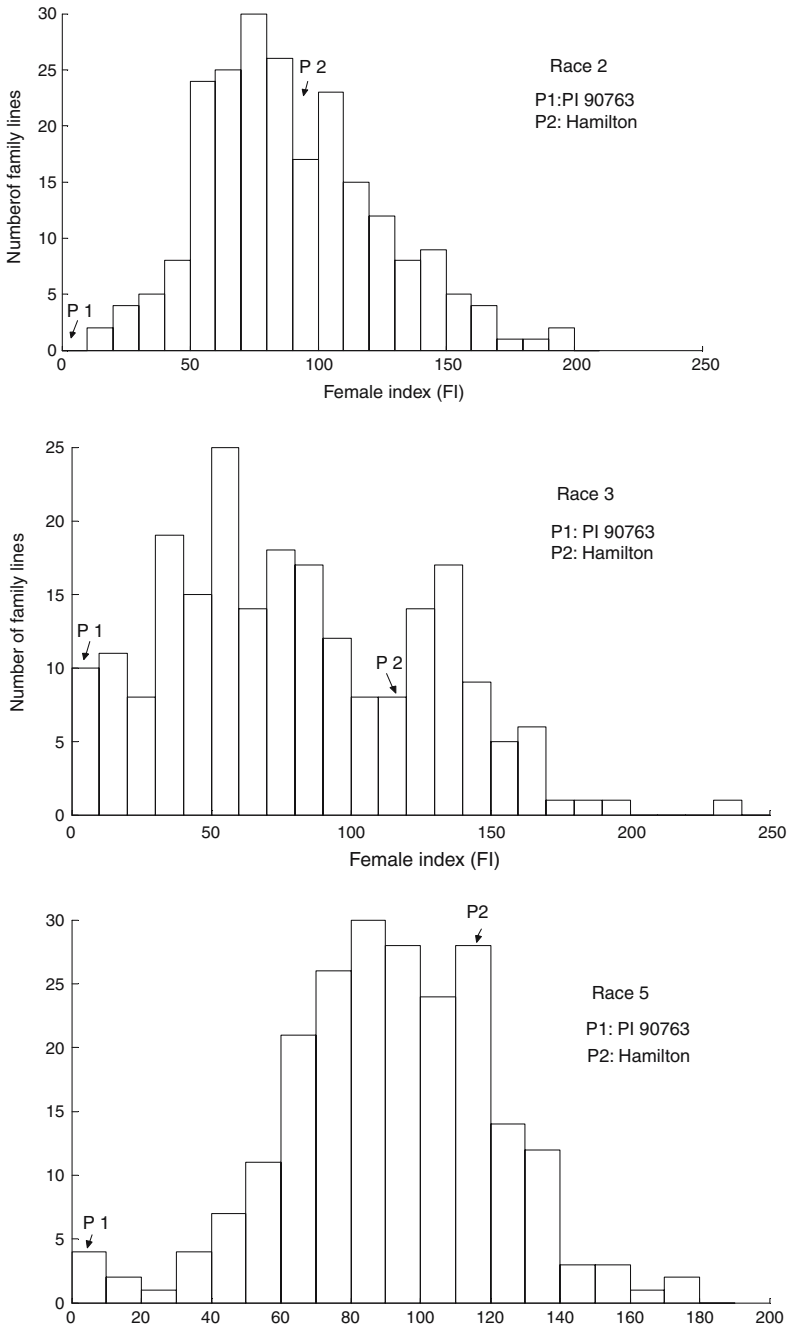

Fig. 1 Distribution of average female index (FI) of $F_{2:3}$ family lines from soybean cross Hamilton × PI 90763. PI 90763: SCN resistant parent. Hamilton: SCN susceptible parent

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% SFR argarose 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 using MAPMAKER/EXP version 3.0b (Whitehead Institute, Cambridge, MA). Haldane map function was used. Linkage was declared at LOD greater than or equal to 3.0 and a maximum distance of 50 cM. Linkage groups (LGs) were designated according to Song et al. (2004).

Composite interval mapping (CIM) was used to detect QTL-marker associations using 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. QTLs were searched for every 2 cM. The highest LOD on a chromosome or a region of the chromosome was used to indicate the position of a QTL and its 1-LOD support

interval was obtained. A suggestive QTL was declared at LOD = 3.0 and a significant QTL at LOD = 4.0. We determined LOD values of 2.9 at the suggestive level (1 false positive per genome scan, i.e., genome-wise type I error = 0.63) and 4.2 at the significant level (genome-wise type I error = 0.05) based on computer simulation tables (Ooijen 1999) (average soybean chromosome length = 125 cM, Song et al. 2004). We obtained similar thresholds at the significant levels using permutation tests (Churchill and Doerge 1994) (LOD = 3.7 to 3.9 for races 2, 3 and 5 at 1000 permutations each). On the basis of the formula by Lander and Kruglyak (1995), LOD values were 3.0 at the suggestive level and 4.5 at the significant level. We think that LOD = 3.0 at the suggestive level and LOD = 4.0 at the significant level were reasonable and convenient.

Results and discussion

Simple sequence repeats and linkage map

Approximately, 1000 SSR markers were surveyed between parents PI 90763 and Hamilton, and 341 polymorphic SSRs were obtained. One hundred ninety-two selected polymorphic SSRs were used for mapping. These SSRs produced 176 co-dominant and 18 dominant loci. Two SSRs had two polymorphic loci each.

A linkage map was constructed and shown in Fig. 2. One gap (≥50 cM between neighboring markers) occurred on LGs B1, B2, H, L, K, M, N and O, respectively, but two gaps occurred on LG F. Seven markers remained unassigned, but they were placed on the LGs and appropriate positions according to the soybean composite linkage map by Song et al. (2004). The Fig. 2 map was in good agreement for marker order and relative distance with the composite linkage map. A difference often occurred in marker order between the Fig. 2 map and the composite linkage map where markers were close (less than 5 cM). The correlations between the Fig. 2 map and the composite linkage map were high (r≥0.8) for map distance and the marker orders (Table 1). The Fig. 2 map had a linear relationship with the composite linkage map for map distance on all LGs except for D1b and J (data not shown).

Quantitative trait loci associated with resistance to SCN

Female index of $F_{2:3}$families showed non-normal distributions for race 2 (Shapiro-wilk's $w = 0.98$, P-value = 0.005; skewwness = 0.485, kurtosis = 0.031) and race 3 (Shapiro-wilk's $w = 0.97$, P-value = 0.0003; skewness = 0.379; kurtosis = −0.454) separately, but a normal distribution for race 5 (Shapiro-wilk's $w = 0.99$,

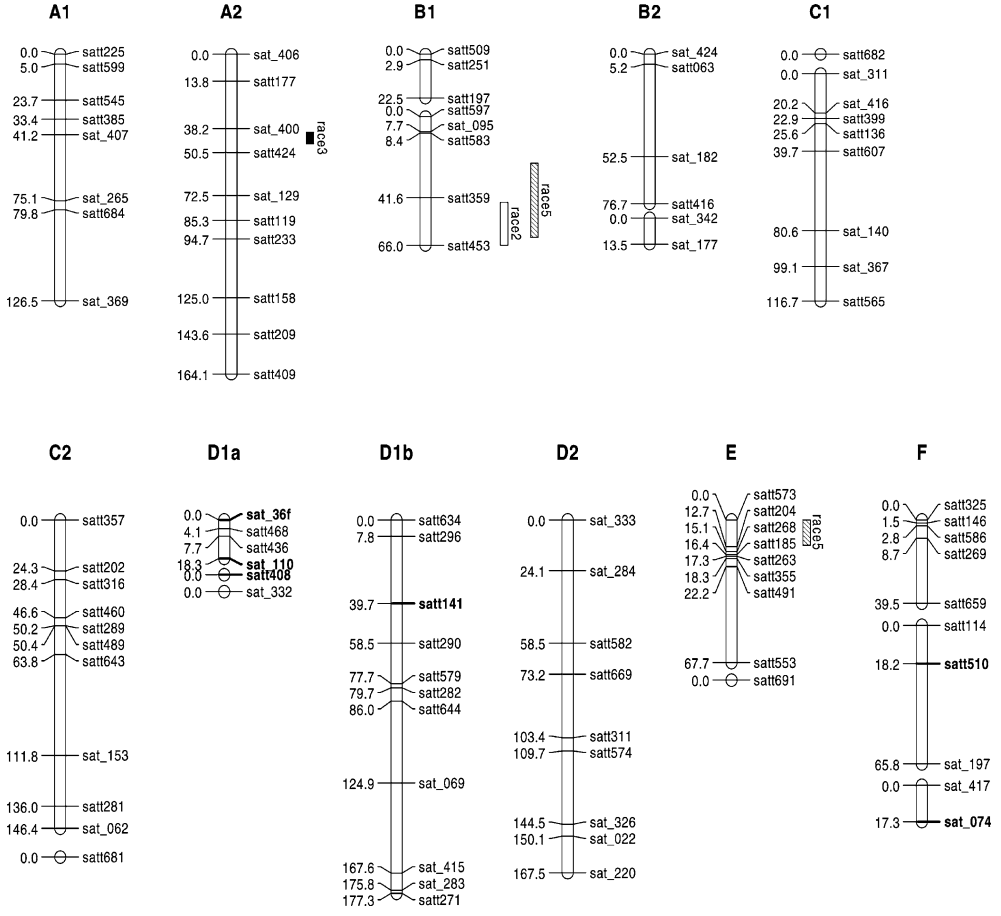

Fig. 2 Linkage map constructed from the cross Hamilton × PI 90763. QTLs are indicated by a bar on the right of linkage group and its 1-LOD support interval was given by the length of the bar. Bold SSR markers are dominant. Molecular markers Sat_036 and Satt244 produced two loci each. Faster bands (locus) are suffixed by f and slower bands by s. The two loci of Satt244 are mapped on nearly the same position. One locus of Sat_036 is mapped on the same position (D1a) as the soybean composite linkage map but the other on LG L

P-value = 0.1889; skewness = −0.222; kurtosis = 0.488) (Fig. 1). Original data were used for the QTL mapping data analysis (Fig. 2 and Table 2). The effect of non-normality on QTL mapping data analysis is expected to be significantly reduced because of use of cofactor markers in composite interval mapping (Zeng 1993; Jansen 1993) and permutation tests for the determination of threshold values (Churchill and Doerge 1994). Correlations between races were low to moderate at the significant level ($r = 0.206$ between races 2 and 3; $r = 0.30$ between races 3 and 5; $r = 0.41$ between races 2 and 5).

Linkage groups G, J and B1 were shown to be associated with resistance to race 2 in soybean PI 90763 (Fig. 2 and Table 2). The QTL on LG G explained a larger proportion of the total variation (approximately 15%), whereas the QTLs on J and B1 accounted for a smaller proportion (approximately 7 to 8%). Linkage groups G and B1 have been shown to be associated with resistance to race 2 in other accessions (Yue et al. 2001a, 2001b). The QTL on LG J was first shown to be associated with resistance to race 2. It has been shown to be associated with resistance to races 3 and 14 in other accessions (Concibido et al. 1997; Glover et al. 2004).

Quantitative trait loci for resistance to race 3 were found on LGs G, A2, J and L in PI 90763 (Fig. 2 and Table 2). The QTLs on LG G and A2 explained 22.1 and 17.7% of the total variation, respectively. However, the QTLs on LGs J and L accounted for only 4% separately. In Concibido's (1997) study where this same line was mapped, LGs G and J have been shown to be associated with resistance to race 3, which explained 44.8 and 18.8% of the total variation, respectively. The QTL on LG A2 identified in our study falls on the same region as the ones identified in other accessions (Webb et al. 1995; Concibido et al. 1994; Yue et al. 2001a, 2001b), which have also been shown to be associated with resistance to race 3 (Note: QTLs identified by different studies were defined as falling on the same region if their confidence intervals overlapped, where QTLs from different studies were projected on the soybean composite linkage map and their 95% confidence intervals were estimated based on the formulae by Darvasi and Soller (1997), Guo et al. unpublished). The fact that Concibido et al. (1997) did not report any QTL on LG A2 may be attributable to the use of fewer molecular markers.

Quantitative trait loci for resistance to race 5 were identified on LGs G, E and B1 in PI 90763 (Fig. 2 and Table 2). They explained a similar proportion of the total variation (about 12%). Linakge groups G and B1 have also been shown to be associated with resistance to race 5 in other accessions (Yue et al. 2001a, 2001b). The QTL on LG E identified by our study was mapped on the same region (Guo et al. unpublished) as the ones identified in other accessions (Yue et al. 2001a, 2001b; Wang et al. 2001), which have been shown to be frequently associated with resistance to race 3 and also with resistance to races 1, 2 and 14. Qiu et al. (1999) identified

Table 1 Comparison of the linkage map constructed in Hamilton × PI 90763 with the soybean composite linkage map

Linkage groups	No.of markers used	Coverage (%)[a]	Correlation[b]	
			Map distance	Marker order
A1	8	90	0.872**	0.965**
A2	10	72	0.996**	1.000**
B1	8	69	0.997**	1.000**
B2	6	47	0.996**	1.000**
C1	9	67	0.992**	1.000**
C2	11	58	0.979**	0.981**
D1a	6	11	0.980**	0.943**
D1b	11	65	0.984**	1.000**
D2	9	92	0.998**	1.000**
E	9	68	0.989**	0.817**
F	10	25	0.990**	0.988**
G	13	92	0.994**	1.000**
H	8	38	0.987**	0.976**
I	9	75	0.996**	0.983**
J	15	82	0.941**	0.991**
K	15	88	0.982**	0.961**
L	9	47	0.980**	0.975**
M	11	95	0.998**	1.000**
N	9	85	0.988**	1.000**
O	9	88	0.995**	0.983**

[a] 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 (Song et al. 2004)

[b] Unassigned markers or unlinked subgroups on the same linkage group are placed on appropriate order and positions according to the composite linkage map. Fifty cM was given between unassigned or unlinked makers and their neighboring markers. Correlations were performed using Window SAS version 8.2

** indicates P-value < 0.01

Table 2 The QTLs associated with resistance to SCN in soybean PI 90763

Race	Linkage group	Flanking markers	Distance[a]	QTL position[b]	LOD	R^2 (%)
II	G	Satt163—Satt688	18.5	2.0	7.9**	14.7
	J	SatT547—Sat_224	4.7	2.7	4.6**	7.8
	B1	Satt453—Satt359	24.0	8.0	3.0*	6.7
III	G	Satt163—Satt688	18.5	0.0	22.1**	28.1
	A2	Sat_400—Satt424	12.3	4.0	14.5**	17.7
	J	Satt547—Sat_224	4.7	0.7	3.9*	4.2
	L	Sat-286—Satt229	18.0	8.0	3.0*	4.0
V	G	Satt163—Satt688	18.5	4.0	7.1**	13.0
	E	Satt573—Satt204	12.7	6.0	7.2**	12.5
	B1	Satt453—satt359	24.0	8.0	6.0**	11.2

[a] The distance between flanking markers
[b] The distance from the left flanking marker
* Suggestive at LOD = 3.0 (one false positive per genome scan or genome-wise type I error = 0.63)
** Significant at LOD = 4.0 (genome-wise type I error = 0.05)

one QTL on LG E for resistance to race 5, but it is somewhat distant from the one identified in this study.

The 1-LOD support intervals of QTL positions for resistance to different races highly overlapped on LG G, B1 and J, respectively (Fig. 2). We regarded QTLs for resistance to different races as being the same if their support intervals overlapped highly. The QTL on LG G was associated with resistance to SCN races 2, 3 and 5, which falls on the same region (Guo et al. unpublished) as the ones in other accessions (Concibido et al. 2004) except for PI 438489B (Yue et al. 2001a) and PI 468916 (*G. soja*) (Wang et al. 2001). It had a larger effect on race 3 than on races 2 and 5 (Table 2). The QTL on B1 was associated with resistance to races 2 and 5, which is somewhat distant from the ones identified in other accessions (Yue et al. 2001a, 2001b). The QTL on J was associated with resistance to races 2 and 3, which falls on the same regions (Guo et al. unpublished) as those identified in other accessions (Concibido et al. 1994, 1996; Glover et al. 2004).

It has been demonstrated that LGs G and A2 are associated with resistance to race 3 in most of SCN resistance accessions studied to date (Concibido et al. 2004). In contrast, however, LGs G and B1 are identified for resistance to races 2 and 5 in all three accessions studied (our study and Yue et al. 2001a, 2001b). Therefore, we concluded that LGs G and A2 are important for resistance to SCN race 3, whereas LGs G and B1 are important for resistance to races 2 and 5. The QTLs on LG A2 and B1 may reflect an important distinction between resistance to race 3 and resistance to race 2 and 5 in soybean.

A QTL was detected on LG L at the suggestive level in this study. It is not supported by a second study. Most of the reported SCN-resistant QTLs are not confirmed by a second study (Concibido et al. 2004). Additional studies are needed to lend credibility to these QTLs. These additional studies include confirmation (replication) studies and extension studies (Lander and Kruglyak 1995; Members of the complex trait consortium 2003). Confirmation studies are used for the confirmation of reported significant marker-QTL associations, whereas extension studies are needed for confirmation of reported QTLs which do not reach significant levels. In confirmation studies, QTL scanning is targeted on a region of about 20 cM around a reported QTL. We can also determine the scanning region using Darvasi's formula $CI = 530 / (N \times R^2)$ for backcross or F_2 intercross, where N is population size and R^2 is a proportion of the total variation explained by the QTL (Darvasi and Soller 1997; Visscher and Goddard 2004; Weller and Soller 2004). Confirmed QTL is declared at an interval (scanning region)-wise significance level. Thresholds can be determined using Lander and Krugyak's formula (1995). For example, P = 0.01 can be used to declare confirmed QTLs if the scanning should region is 20 cM. In extension studies, we suggest that a chromosome-wise scanning be conducted because the effect of the gene that does not reach a significant level is usually small. An increased population size is needed and the initial study population can be included. Populations size can be determined using $N = 530 / (CI \times R^2)$, where CI is the 95% confidence interval length that you expect in your experiment. If a significance level is reached, then a confirmation study is needed.

In summary, QTLs for SCN resistance were identified on LGs A2, B1, E, G, J and L in soybean PI 90763. The QTL on LG G was associated with resistance to races 2, 3 and 5. The QTL on LG B1 was associated with resistance to races 2 and 5. The QTL on J was associated with resistance to races 2 and 3. The QTL on LGs A2 and L were associated with resistance to race 3. The QTL on LG E was associated with resistance to race 5. Additional studies are needed to lend credibility for the QTL on LG L.

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