Fine Mapping of the SCN Resistance Locus *rhg1-b* from PI 88788

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Abstract

Soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) is the most economically damaging soybean [*Glycine max* (L.) Merr.] pest in the USA and genetic resistance is a key component for its control. Although SCN resistance is quantitative, the *rhg1* locus on chromosome 18 (formerly known as Linkage Group G) confers a high level of resistance. The objective of this study was to fine-map the *rhg1-b* allele that is derived from plant introduction (PI) 88788. F_2 and F_3 plants and $F_{3:4}$ lines from crosses between SCN resistant and susceptible genotypes were tested with genetic markers to identify recombination events close to *rhg1-b*. Lines developed from these recombinant plants were then tested for resistance to the SCN isolate PA3, which originally had an HG type 0 phenotype, and with genetic markers. Analysis of lines carrying key recombination events positioned *rhg1-b* between the simple sequence repeat (SSR) markers BARCSOYSSR_18_0090 and BARCSOYSSR_18_0094. This places *rhg1-b* to a 67-kb region of the 'Williams 82' genome sequence. The receptor-like kinase gene that has been previously identified as a candidate for the 'Peking'-derived SCN resistant *rhg1* gene is adjacent to, but outside of, the *rhg1-b* interval defined in the present study.

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SOYBEAN CYST NEMATODE is the pest that causes the most damage to soybean in the USA (Wrather and Koenning, 2006). The first report of SCN in the USA was in North Carolina during 1954 (Winstead et al., 1955). Since that time, SCN has spread throughout most soybean producing states (Niblack et al., 2008).

The USDA-ARS soybean germplasm collection has been screened to identify sources of SCN resistance and at least 118 soybean PIs with SCN resistance were identified (Arelli et al., 2000). Although these resistance sources are available to breeders, PI 88788 is the predominant resistance source for most commercially utilized SCN resistant cultivars in the northern USA. In a summary of soybean cultivars available for planting in Illinois during 2008, PI 88788 was the only SCN resistance source for 94% of the cultivars listed in maturity groups II through IV (Shier, 2008).

The genetic basis of SCN resistance was first studied through classical genetic experiments. The inheritance of SCN resistance from the SCN resistance source Peking fits a three recessive gene model, and the three genes were named *rhg1*, *rhg2*, and *rhg3* (Caldwell et al., 1960). A later study showed that Peking carried a fourth resistance gene designated *Rhg4* that conferred dominant resistance and this gene mapped near the *i* locus (Matson and Williams, 1965). An additional dominant gene designated *Rhg5* was later identified from PI 88788 (Rao-Arelli, 1994).

Many genes and quantitative trait loci (QTL) controlling SCN resistance have been mapped, and the results from these efforts were reviewed by Concibido

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Abbreviations: FI, female index; INDEL, insertion-deletion; LRR, leucine-rich repeat; QTL, quantitative trait locus (loci); SCN, soybean cyst nematode; SNP, single nucleotide polymorphism; SSR, simple sequence repeat; STS, sequence tagged site.

et al. (2004). This summary revealed that by 2004, 61 SCN resistance QTL or genes had been mapped onto 18 of 20 soybean linkage groups from eight resistance sources. Some of these QTL were found to confer resistance to multiple biotypes of SCN while others provided resistance to individual biotypes. Although this is a large number of mapped QTL, a few important trends emerged. One was that the SCN resistance locus *rhg1* was mapped as a major QTL in most resistance sources. A second was that in the sources in which *rhg1* was mapped, the locus was typically found to confer the greatest resistance of any of the resistance QTL.

The *rhg1* locus was mapped onto chromosome 18 (formerly linkage group G) from the SCN resistance sources PI 437654 (Webb et al., 1995), PI 209332 (Concibido et al., 1996), Peking, PI 90763, PI 88788 (Concibido et al., 1997; Glover et al., 2004), PI 89772 (Yue et al., 2001), and PI 404198A (Guo et al., 2006). A number of markers have been mapped close to this gene including the SSR marker Satt038, which was linked within 3 cM on the distal (telomeric) side of the *rhg1* locus (Mudge et al., 1997). Cregan et al. (1999) reported that the SSR marker Satt309 was mapped 0.4 cM on the proximal (centromeric) side of *rhg1*. In addition, Ruben et al. (2006) mapped *rhg1* from Peking to a 1.5-cM region near Satt309 using recombination events from four near isogenic populations.

rhg1 was originally described as a recessive resistance gene, and subsequent genetic marker-based mapping and inheritance studies have shown it to be recessive or partially recessive (Brucker et al., 2005; Concibido et al., 1997). In addition, Brucker et al. (2005) identified allelic diversity at *rhg1* by showing that when a population segregating for *rhg1* alleles from PI 88788 and PI 437654 was challenged with SCN in a greenhouse, the *rhg1* alleles from the two sources gave different resistant phenotypes. The resistance allele from PI 88788 was recently given the designation *rhg1-b* by the Soybean Genetics Committee.

There are reports that *rhg1* has been cloned and sequenced from the SCN-resistant source Peking (Hauge et al., 2001; Lightfoot and Meksem 2002). In both the Hauge et al. (2001) patent and the Lightfoot and Meksem (2002) patent application, a receptor-like kinase gene was identified as a candidate for the *rhg1* allele from Peking. The gene encodes a leucine-rich repeat (LRR) receptor-like kinase that carries similarity to the rice Xa21 LRR receptor kinase (Ruben et al., 2006; Song et al., 1995). This *rhg1* candidate gene from the Peking source was mapped between the markers SIUC-Sca13 and BARC-Satt309, and cosegregation was observed between SCN resistance and a 19-basepair insertion–deletion (SIUC-TMD1) contained within the gene (Ruben et al., 2006).

The objective of our study was to fine map *rhg1-b* from PI 88788. This work is needed because allelic variation exists for *rhg1* function from different SCN resistance sources (Brucker et al., 2005) and it is not known with certainty if the *rhg1-b* allele from PI 88788 and the *rhg1* allele from Peking are alleles of the same resistant gene or are two tightly linked genes. In addition, although DNA from the *rhg1* locus from Peking was cloned and described, there have been no reports of complementation or gene knock-down studies demonstrating that the candidate gene encoding the receptor-like kinase is *rhg1*.

MATERIALS AND METHODS

General Mapping Strategy

The *rhg1-b* allele was fine mapped by first identifying F_2 and F_3 plants and $F_{3:4}$ lines with recombination close to the gene using markers flanking the regions of interest. In all cases tested, the line developed from the recombinant plant or the selected line was homozygous on one side of the recombination point and was segregating on the other side. The positions of these recombinations were then mapped by testing the recombinant plants or lines with additional markers. Individual F_3 progeny from recombinant F_2 plants or F_4 progeny from recombinant F_3 plants were tested for SCN resistance and a marker from the segregating side of the recombination event. The resistance and marker data were then analyzed to test for a significant association. A significant association between segregation of the marker and segregation of the SCN resistance phenotype indicated that *rhg1-b* was on the segregating side of the recombination point, while a nonsignificant association indicated that *rhg1-b* was on the fixed side of the recombination point. Repetition of this process from multiple $F_{2:3}$ or $F_{3:4}$ populations carrying different recombination breakpoints established the genetic interval that encodes *rhg1-b*.

Development of Plant Material

Recombinant plants and lines were identified in populations developed from four crosses between susceptible genotypes and breeding lines carrying *rhg1-b* from PI 88788 (Table 1). The original crosses were made in 2005, and the F_1 plants were grown in the field at Urbana, IL, during 2006. Some F_2 seed from the populations were grown in a winter nursery in Puerto Rico during the winter of 2006–2007. At the winter nursery, a pod from each F_2 plant was harvested and pooled for each F_1 plant before threshing, and F_3 seed were planted at Urbana, IL, during the spring of 2007. Other F_2 seed were grown in the field at Urbana during 2009. The F_2 and F_3 plants grown in the field at Urbana were tagged and tested with markers flanking regions of interest to identify recombinant plants. Selected recombinant and nonrecombinant plants were harvested and individually threshed to form F_2– or F_3–derived lines in the F_3 or F_4 generation ($F_{2:3}$ or $F_{3:4}$).

DNA Extraction and Genotyping

DNA was extracted from plants by the quick extraction method described by Bell-Johnson et al. (1998) or through CTAB extractions according to Kabelka et al. (2006). To identify plants with recombinations near *rhg1-b*, the DNA samples were tested by SSR or insert-deletion (INDEL) markers flanking the gene. The SSR markers

had been developed and mapped previously by Song et al. (2004, 2010), using polymerase chain reactions (PCR) according to Cregan and Quigley (1997). The sequences of the SSR markers are available on Soybase (http://soybase.org/; verified 16 July 2010). The INDEL markers ss107914244 and ss107914431, which are located near Satt309 on chromosome 18 (Hyten et al., 2010), were converted to sequence tagged site (STS) markers with primers designed using the program Primer 3 (Rozen and Skaletsky, 2000). The forward and reverse primers for ss107914244 were 5′TTCGCATTGGTCTTCTTTGTAC3′ and 5′GATTGATTTGAAAGCCGTTGTG3′ and for ss107914431 were 5′GAGGTGACGTAAAATGGAATGTAAC3′ and 5′CAAACACGAGAAACTCTTTCCA3′. The PCR products for the SSR and INDEL markers were analyzed by electrophoresis in 6% (w/v) nondenaturing polyacrylamide gels (Wang et al., 2003). Individual plants in lines evaluated for SCN resistance in the greenhouse were tested with either a SSR or INDEL marker linked to *rhg1-b* and segregating in the line.

SNP Marker Analysis

All single nucleotide polymorphism (SNP) genotyping was performed by Sanger sequence analysis. PCR amplification and sequencing reactions were performed as described by Choi et al. (2007). Sequencing was performed on the ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA). SNPs identified between the parents were discovered in STSs as described by Matukumalli et al. (2006) and visually verified. If multiple SNPs were present in a STS, only one was used for recombinant screening since all SNPs within an STS were in complete linkage disequilibrium within each individual line. All STS tested markers had been developed by Hyten et al. (2007, 2010) and were shown to map near Satt309 or contained on the sequence AX196295 (Hyten et al., 2007, 2010). After the Glyma 1.01 soybean whole genome sequence (Schmutz et al., 2010) became available, all SNP markers used in this study, including markers previously mapped to chromosome 18, were positioned by the BLASTN (Altschul et al., 1997) available at www.phytozome.net; verified 16 July 2010(Table 2) using an E Threshold of 0.1. In each case, the highest similarity was to sequences on chromosome 18.

Soybean Cyst Nematode Greenhouse Test

The SCN resistance tests were done in a greenhouse in a thermo-regulated water bath system at the University of Illinois using procedures described by Arelli et al. (2000) and Niblack et al. (2002). Briefly, PVC tubes were filled with steam sterilized sandy soil and packed into plastic crocks that were suspended over a water bath maintained at a constant 27°C. Seeds were germinated, one plant was transplanted into each PVC tube, and infested with the SCN isolate PA3 (Table 3). This isolate was obtained from Dr. Prakash Arelli, USDA-ARS Mid South Area, Jackson, TN, and was maintained as greenhouse cultures on the susceptible soybean cultivar Macon. The plants were grown under a 16-h daylength and watered as needed. After the plants were established, a trifoliolate from each plant was sampled and DNA was extracted on a single-plant basis and used in a genetic marker analysis. Thirty days after transplanting, the cysts were collected by gently soaking each tube in a bucket of water to loosen soil but avoid dislodging females. Each root was placed on nested 850-μm aperture over 250−μm aperture sieves and females were dislodged from the roots with a water spray, and separated females were washed into counting dishes. The number of cysts on each root system was counted under a stereomicroscope, and a female index was calculated for each plant with the following formula (Golden et al., 1970): FI = (100 × Number of cysts per plant)/(Average number of cysts on susceptible host). The cultivar Macon was used as the susceptible control in the experiments.

Table 1. Populations segregating for soybean cyst nematode resistance tested with markers to identify genetic recombination events close to *rhg1-b*.

Cross no.	Female parent	Source of female parent	Male parent	Source of male parent
1	LD02–5320 (*R*)†	Univ. of Illinois	99805 (S)‡	Dairyland Seed
2	LD02–5025 (*R*)	Univ. of Illinois	LG03–1672 (S)	USDA-ARS
3	LD02–5025 (*R*)	Univ. of Illinois	LG00–3372 (S)	USDA-ARS
4	IA3023 (S)	Iowa State University	LD01–7323 (*R*)	Univ. of Illinois

†R, resistant parent in the cross.

‡S, susceptible parent in the cross.

Statistical Analysis

Associations between the segregation of markers and SCN phenotypes for each plant were analyzed by single-factor analysis of variance with PROC GLM of SAS (SAS Institute, 2002).

RESULTS

It was previously shown that Satt309 maps within 0.4 cM of the *rhg1* locus on chromosome 18 (Cregan et al., 1999). Recombinant screens were initially conducted to identify recombination events on either side of Satt309 because of uncertainty of which side of this marker *rhg1-b* is located. The first screen focused on the interval between Satt309 and ss107914244 (Table 2). Field grown F_3 plants were screened with the markers Satt309 and ss107914244 to identify recombinants in this estimated 219 kb/1.1-cM interval. A total of 1341 F_3 plants were screened, including 335, 326, 332, and 348 plants from crosses 1, 2, 3, and 4, respectively (Table 1). From these tests, 37 recombinant plants were selected and threshed to form $F_{3:4}$ lines. These lines were then tested with seven SNP markers between Satt309 and ss107914244 to narrow the approximate recombination point in each line (Table 4).

Six recombinant lines were selected because they had recombination at breakpoints 1 through 5 that were spread across much of the interval between Satt309 and ss107914244 (Table 2). To position *rhg1-b* relative to these recombination breakpoints, 27 to 38 F_4 plants from each

Table 2. Positions of simple sequence repeat (SSR), insert-deletion (INDEL), and single nucleotide polymorphism (SNP) markers, marker data, and recombination points for plants or lines selected for having recombination near the *rhg1-b* gene on chromosome 18. The base pair (bp) positions are based on the Williams 82 whole genome sequence (Glyma 1.01 build) and the centimorgan (cM) positions are based on the published soybean genetic map (Hyten et al., 2010). The telomeric end is on top and the centromic end of the chromosome is on the bottom of the map. Note that "S", "H", and "R" refer to the marker genotype of the plant that the lines were derived from and not the SCN phenotype of the line noted at the top of the column. An arrow is placed in the genetic interval carrying the inferred recombination breakpoint; arrow points toward side of recombination breakpoint associated with the SCN resistance phenotype (inferred from data in Table 4).

			Line designation																						
Marker	Position on Chromosome 18	cM	2–124†	3–161	1–80	4–106	2–27	1–144	3–76	3–76–1	3–76–2	3–76–3	4–67	4–67–1	4–67–2	4–67–3	4–63	2–130	2–14	2–137	1–50	1–218	1–184	2–63
	bp																								
Satt038	1,343,760	7.9							S‡	S	S	S	H	S	H	H		S	H	R				
BARCSOYSSR_18_0066	1,374,039												H	S	H	H		S	H	R				
																		↓§	↓	↓				
BARCSOYSSR_18_0079	1,498,225												H	H	H	H		H	S	H				
BARCSOYSSR_18_0080	1,507,237												H	H	H	H		H	S	H				
BARCSOYSSR_18_0083	1,550,588												S	S	S	S	R	H	S	H	R	H	H	R
																					↓			
BARCSOYSSR_18_0085	1,572,977												H	H	H	H	R	H	S	H	H	H	H	R
																						↓		
BARC-73605	1,588,594												H	H	H	H	R	H	S	H	H	S	H	R
																							↓	
BARCSOYSSR_18_0090	1,609,101												H	H	H	H	R	H	S	H	H	S	R	R
																								↓
Sat_210	1621,238	9.4											H	H	H	H	R	H	S	H	H	S	R	H
																	↑							
BARCSOYSSR_18_0094	1,676,212																H							H
ss107914431	1,701,517												H	H	H	H		H	S	H				
Sat_168	1,706,033								S	S	S	S	H	H	H	H	H	H	S	H	H	S	R	H
													↑	↑	↑	↑								
ss107921416	1,709,681	10.0							S	S	S	S	S	S	S	S								H
BARC-037579	1,709,751								S	S	S	S	S	S	S	S								
ss107921590	1,712,701	10.0							S	S	S	S	S	S	S	S	H							H
ss107921591	1,712,691	10.0							S	S	S	S	S	S	S	S	H							H
ss107921597	1,712,948	10.0							S	S	S	S	S	S	S	S	H							H
									↑	↑	↑	↑												
BARC-037583–8	1,727,375								H	H	H	H	S	S	S	S								
Satt309	1,736,305	10.1	H	S	H	H	S	S	H	H	H	H	S	S	S	S		H	S	H				
BARC-037585–55	1,739,097		H	S	H	H	S	S																
								↑																
BARC-037589–19	1,749,867		H	S	H	H	S	H																
ss107914436	1,776,695	10.0	H	S	H	H	S	H																
							↑																	
BARC-037527–6	1,788,788		H	S	H	H	H	H																
					↑	↑																		
BARC-037537	1,819,729		H	S	S	R	H	H																
				↑																				
BARC-037549–3	1,879,787		H	H	S	R	H	H																
			↑																					
BARC-037557–1	1,912,635		S	H	S	R	H	H																
ss107914244	1,955,554	11.1	S	H	S	R	H	H																
Recombination point¶			1	2	3	3	4	5	6	6	6	6	7	7	7	7	8	9	9	9	10	11	12	13

†Designation of selected plants or lines.

‡S designates that the F_2, F_3, or F_4 plant that produced the $F_{2:3}$, $F_{3:4}$, or $F_{4:5}$ lines were homozygous for the allele from the susceptible parent, H designates that the plant was heterozygous, and R designates that the plant was homozygous for the allele from the resistant parent. An arrow is placed at the genetic interval containing the inferred recombination event.

§The arrows point toward the genomic region that carries *rhg1-b*, as indicated by the recombination breakpoint data of Table 2 and the phenotypic data of Table 4.

¶Numerical identification of the points of recombination referenced on Table 4.

Table 3. Response of differential soybean genotypes to the PA3 soybean cyst nematode isolate used in each experiment.

Lines tested	Number of cysts of the susceptible cultivar Macon	Female index						
		1 Peking	2 PI 88788	3 PI 90763	4 PI 437654	5 PI 209332	6 PI 89772	7 PI 548316
2–027, 2–124, 3–161	32	2	5	1	0	22	0	0
1–080, 1–144, 4–106	112	1	4	0	0	10	1	11
3–76, 4–67, 2–116, 2–130, 2–14, 2–137	153	0	20	0	0	30	0	37
3–76–1, 4–67–1	21	5	ND†	1	ND	ND	0	ND
3–76–2, 4–67–2	612	2	19	0	0	32	1	33
3–76–3, 4–67–3	146	3	32	0	0	51	1	39
4–63, 1–50, 1–218, 1–184, 2–63	330	1	26	0	0	39	0	31

†ND, Not determined because the differential plants did not germinate in the test.

of the six lines were then tested for their SCN resistance and for their genotype at a marker located on the segregating side of the recombination breakpoint (Table 4). A significant ($P < 0.001$) association was observed between segregating markers and SCN resistance in lines 1–80, 2–124, and 4–106 and no association was observed in lines 1–144, 2–27, and 3–161 (Table 4). The test results from these six lines are consistent, indicating that *rhg1-b* from PI 88788 is located on the telomeric side of the SNP marker BARC-037589–19 or above this marker as the results are presented in Table 2.

An example of how these different recombinant lines were used to position *rhg1-b* can be demonstrated by examining the results of lines 4–106 and 1–144. Line 4–106 is fixed for the marker allele from the resistant parent for BARC-037537 and the region below (see Table 2), and is segregating for markers above BARC-037537 such as Satt309 (Table 2). A highly significant ($P < 0.0001$) association between Satt309 and SCN resistance was found among plants in this line (Table 4), showing that *rhg1-b* must be in the segregating interval and therefore above BARC-037537. The position of *rhg1-b* was further delineated with line 1–144. This line was

Table 4. Marker and soybean cyst nematode resistance results for $F_{2:3}$, $F_{3:4}$, or $F_{4:5}$ lines used to position *rhg1-b*.

Line	Recombination point‡	No. of plants tested§	Marker used in F test	Female index†			$P > F$¶	R^2#
				R	H	S		
2–124	1	33	Satt309	22	53	73	0.0010	0.37
3–161	2	30	ss107914244	93	73	25	0.14	0.13
1–80	3	38	Satt309	5	31	81	< 0.0001	0.82
4–106	3	38	Satt309	8	29	62	< 0.0001	0.72
2–27	4	27	ss107914244	60	58	47	0.52	0.05
1–144	5	37	ss107914244	61	49	50	0.15	0.10
3–76	6	22	Satt309	91	67	94	0.22	0.14
3–76–1	6	36	Satt309	36	49	43	0.24	0.08
3–76–2	6	28	Satt309	72	80	83	0.40	0.07
3–76–3	6	39	Satt309	93	91	109	0.13	0.11
4–67	7	34	Sat_168	36	65	95	< 0.0001	0.58
4–67–1	7	35	Sat_168	26	52	74	< 0.0001	0.49
4–67–2	7	20	Sat_168	34	60	77	< 0.0001	0.73
4–67–3	7	36	Sat_168	38	62	91	< 0.0001	0.70
4–63	8	37	Sat_168	44	39	44	0.68	0.02
2–130	9	40	Satt309	38	57	70	0.02	0.19
2–14	9	40	Satt038	62	66	74	0.52	0.03
2–137	9	40	Satt309	29	44	69	< 0.0001	0.38
1–50	10	40	Sat_168	34	66	103	< 0.0001	0.46
1–218	11	17	BARCSOYSSR_18_0083	76	81	89	0.77	0.04
1–184	12	31	BARCSOYSSR_18_0083	71	79	84	0.88	0.02
2–63	13	37	Sat_168	48	84	102	< 0.0001	0.55

†Mean female index for the plants in each line that were predicted on the basis of the genetic markers Satt309, Sat_168, Satt038, or ss107914244, as noted in previous column, to be homozygous for allele from resistant parent (*R*), heterozygous (H), or homozygous for allele from susceptible parent (S).

‡Position of the recombination on Table 2.

§Number of plants in the line tested in the greenhouse for SCN resistance.

¶Significance level of the marker association.

#R^2 value of the marker association.

segregating for BARC-037589–19 and the region below this marker (including ss107914244) and was fixed for the alleles from the susceptible parent for the region above BARC-037589–19 (Table 2). No significant association was observed between ss107914244 and resistance (Table 4), indicating that *rhg1-b* is in the nonsegregating interval and therefore above BARC-037589–19.

The second interval tested was between Satt309 and ss107914431 (Table 2). A total of 590 $F_{3:4}$ lines from crosses 1 through 4 were tested with the markers Satt309 and ss107914431 to identify and select lines with a recombinant haplotype. Five recombinant lines were identified and tested with additional SNP markers from the interval. On the basis of the positions of these recombination events, two recombinant lines were selected and F_4 individuals from these lines were tested for SCN resistance phenotypes and marker genotypes. No association was found between the segregation of Satt309 and SCN resistance in line 3–76, which has breakpoint 6 (Table 4). This indicates that *rhg1-b* is located above BARC-037583–8 (1.727 Mb) (Table 2). The gene was further positioned by testing line 4–67, which was found to carry a recombination between Sat_168 (1.706 Mb) and ss107921416 (1.710 Mb), at breakpoint 7 (Table 2). This line was segregating for Sat_168, but not ss107921416, and a significant association was found between the segregation of Sat_168 and SCN resistance (Table 4). This result indicates that *rhg1-b* is located above ss107921416 (Table 2).

Because of the importance of the 3–76 and 4–67 recombinants in determining the position of *rhg1-b*, three F_4 plants from line 3–76 that were heterozygous for Satt309 and therefore had breakpoint 6, and three F_4 plants from 4–67 that were heterozygous for Sat_168 and had breakpoint 7, were selected and grown to maturity to develop independent confirmation lines. These selected heterozygous plants were tested with the same set of SSR and SNP markers in the Sat_168 to Satt309 interval as 3–76 and 4–67. The marker testing of the selected F_4 plants was consistent with the results from the $F_{3:4}$ lines, confirming the position of the recombination events. These six $F_{4:5}$ lines developed from 3–76 and 4–67 were then tested for SCN resistance and a segregating marker (Table 4). The results from the SCN resistance tests for these $F_{4:5}$ lines were in agreement with the results from the $F_{3:4}$ lines, indicating that *rhg1-b* is positioned above ss107921416 (Table 2).

To further delineate the position of *rhg1-b*, 1069 F_2 plants and 326 $F_{3:4}$ lines from crosses 1, 2, and 4 (Table 1) were tested with Satt038 and Satt309 to identify plants with recombination events (or lines derived from plants with recombination events) in the interval. These markers were chosen both for their map position and their relative ease of reliable use. Recombinant plants or lines were tested with additional markers and from this screening, three $F_{3:4}$ lines derived from recombinant F_3 plants (2–130, 2–14, and 2–137) and five recombinant F_2 plants (1–50, 1–218, 1–184, 2–63, and 4–63) were selected. The location of the recombination breakpoints were mapped to six different positions (breakpoints 8–13) between BARCSOYSSR_18_0066 (1.374 Mb) and BARCSOYSSR_18_0094 (1.676 Mb) (Table 2). A significant ($P < 0.02$) association was observed between a segregating marker and SCN resistance among plants in lines 2–130, 2–137, 1–50, and 2–63 but not in lines 2–14, 1–218, 1–184, and 4–63 (Tables 2 and 4). These results position *rhg1-b* to a 67 kb interval between BARCSOYSSR_18_0090 and BARCSOYSSR_18_0094. The two critical recombinants that position *rhg1-b* to this interval are the $F_{2:3}$ lines 2–63 (breakpoint 13) and 4–63 (breakpoint 8). The telomeric (upper) end of the genetic interval carrying *rhg1-b* was determined with line 2–63, which is fixed for BARCSOYSSR_18_0090 and the region above it and is segregating for the marker Sat_210 and the region below it (Table 2). A significant association between SCN resistance and Sat_168 was observed in this population, showing that *rhg1-b* is below BARCSOYSSR_18_0090 (Table 4). The centromeric (lower) end of the genetic interval carrying *rhg1-b* was defined by line 4–63, which is segregating for BARCSOYSSR_18_0094 and the interval below it and is fixed for Sat_210 and the region above this marker (Table 2). In this line, no association between SCN resistance and Sat_168 was observed, showing that *rhg1-b* is above BARCSOYSSR_18_0094 (Table 4).

DISCUSSION

Our results show that the SCN resistance-determining *rhg1-b* from PI 88788 is within a 67-kb region between the markers BARCSOYSSR_18_0090 and BARCSOYSSR_18_0094 (Table 2). This places the *rhg1-b* allele from PI 88788 in a genetic interval that does not include the receptor-like kinase gene candidate for *rhg1* from Peking that is described by Ruben et al. (2006) and emphasized in two patenting efforts (Hauge et al., 2001; Lightfoot and Meksem 2002). The candidate receptor-like kinase gene is positioned between 1.711 and 1.715 Mb on the Glyma 1.01 build Williams 82 sequence, yet we showed that *rhg1-b* is above 1.676 Mb (on the telomeric side of BARCSOYSSR_18_0094). Two independent recombination events from this study separated *rhg1-b* from the receptor-like kinase gene. The first recombination identified between *rhg1-b* and this candidate was in 4–67. In this line and the confirmation lines 4–67–1, 4–67–2, and 4–67–3, which were each developed from heterozygous plants from 4–67, a significant association was observed between Sat_168 and SCN resistance. This shows that *rhg1-b* is above ss107921416 at 1.710. The second recombination identified between *rhg1-b* and the candidate receptor-like kinase gene was in line 4–63. This line had a recombination between BARCSOYSSR_18_0094 and Sat_210 and the lack of association between resistance and BARCSOYSSR_18_0094 in the line positioned the gene above (telomeric to) BARCSOYSSR_18_0094, which is positioned at 1.676 Mb. These results indicate that there is at least a 35-kb interval between the candidate gene in the patent and *rhg1-b* from PI 88788.

As further evidence that the receptor-like kinase candidate gene from the patents is not the *rhg1-b* locus determinant of SCN resistance from PI 88788, three SNP markers (ss107921590, ss107921591, and ss107921597) within this receptor-like kinase gene are polymorphic between the parents of 4–67, and they were not segregating in 4–67 or the confirmation lines 4–67–1, 4–67–2, and 4–67–3, while there was significant association between SCN resistance segregation and Sat_168 in the population. In addition, the three SNP markers within the receptor-like kinase gene were segregating in 4–63, but there was no significant association between SCN resistance and Sat_168 in 4–63.

It is theoretically possible that the recombination breakpoint in 4–67 could cause segregation of a transcription enhancer element for the receptor-like kinase gene that is located greater than 2.0 kb upstream of the receptor-like kinase gene, which might be sufficient to cause phenotypically significant segregation of expression of the receptor-like kinase. However, if this element were in the interval between ss107921416 and BARCSOYSSR_18_0094, we would have expected to observe a significant association between SCN resistance and Sat_168 in 4–63, which we did not. If such an enhancer element exists, it would have to be above (telomeric to) BARCSOYSSR_18_0094, over 35 kb away from the receptor-like kinase open reading frame. We also cannot rule out more subtle contributions of the receptor-like kinase gene to defense processes, as has been reported for rice *Xa21* (Li et al., 2001). A more likely explanation for the present results, however, is that the primary SCN resistance contribution from the *rhg1-b* locus is encoded by one or more genes located above BARCSOYSSR_18_0094.

The genes encoding a predicted laccase and a predicted ion antiporter, which are adjacent to the receptor-like kinase gene at the Peking *rhg1* locus and were mentioned as candidate contributors to SCN resistance in recent preliminary finding/review articles (Afzal et al., 2008; Iqbal et al., 2009), also are not strong candidates to encode PI 88788 *rhg1-b* activity. They are both located below the receptor-like kinase gene (their position on chromosome 18 is > 1,723,000 bp; see Table 2) and hence they also are not segregating in the lines 4–67, 4–67–1, 4–67–2, and 4–67–3 and would be segregating in 4–63. However, the present study did not investigate the location of *rhg1* in Peking-derived material, and it is possible that the position and arrangement of the *rhg1* locus differs between Peking and PI 88788, the source of SCN resistance in the germplasm evaluated in our study.

Ruben et al. (2006) reported individual lines with multiple recombination events in the interval surrounding *rhg1* from Peking. In general, we did not observe more than one recombination event near *rhg1-b* per generation in the lines we selected. The line 4–67 and its progeny 4–67–1, 4–67–2, and 4–67–3 had an unexpected susceptible genotype for BARCSOYSRR_18_0083 which would have required two recombinations in a 66-kb interval, and this marker was consistent in repeated tests (Table 2). Although possible, this would be a very rare recombination. Another explanation is that there was some residual heterogeneity in LD01–7323, the resistant parent of the line, and the particular plant of LD01–7323 that was used as a parent to produce 4–67 had the alternative genotype for BARCSOYSRR_18_0083. We don't observe the susceptible genotype in 4–63, which had the same parents as 4–67. This can be explained by the fact that this selected plant was developed from a different F_1, which would have been produced from a different plant of LD01–7323.

The only line with two likely recombination events was 4–67–1, which was derived from an F_4 plant from the $F_{3:4}$ line 4–67 that was heterozygous for Sat_168. Because 4–67 had only one recombination, the second recombination would have occurred in the F_4 plant used to develop 4–67–1. One potential reason that we observed few double recombinations in the *rhg1-b* interval compared to Ruben et al. (2006) is that we selected plants and lines with recombinations based on markers flanking the intervals. This would not have resulted in the selection of double recombinant plants. Another potential reason is that the *rhg1-b* interval from PI 88788 may have a lower tendency to recombine than the interval from Peking.

The SCN reproduction on Macon, the susceptible control in the tests, varied from 21 cysts plant^{-1} in the test of the lines 3–76–1 and 4–67–1 to a high of 612 in the test of the lines 3–76–2 and 4–67–2 (Table 3). Niblack et al. (2002) recommended only accepting results from SCN greenhouse tests when reproduction results in at least 100 cysts on each susceptible plant. We found, however, that the conclusions that we reached from the two tests with these high and low reproduction rates were the same. In both lines 3–76–1 and 3–76–2, no association was observed between resistance and segregating markers and there was a highly significant association observed in both lines 4–67–1 and 4–67–2. In 4–67–2, which had the greatest reproduction, we did observe a higher r^2 value for the association between markers and resistance then in 4–67–1, but the trends in the mean female index of the lines in each genotypic class were similar.

The PA3 isolate was used in these experiments because it was previously shown to give an HG type 0 phenotype. Technically, we did not conduct HG type tests in our experiments because we used Macon as our susceptible genotype instead of 'Lee 74', which is the susceptible standard in the HG type test protocol (Niblack et al., 2002). Macon was substituted for Lee 74 in these tests because of problems with emergence and root growth for Lee 74. If the experiments are interpreted using Macon as a susceptible standard, our results indicate that the HG type of our PA3 isolate had shifted so that in our experiments, the HG type of the isolate was 5, 5.7, or 2.5.7 (Table 3). A 2 in the HG type designation means that the SCN isolate was able to reproduce on PI 88788 plants at a rate greater than 10% of its reproduction on the susceptible plant genotype in that experiment

(Niblack et al., 2002). The highest female index on PI 88788 was 32 in the test of lines 3–76–3 and 4–67–3. This female index of 32 means that the PI 88788 still provided partial resistance to the isolate in that experiment, and the significant association between *rhg1-b* and resistance in these tests demonstrates that *rhg1-b* remained effective in providing partial control to the isolate.

It was previously shown that an interaction exists between *rhg1* and *Rhg4* when these genes are derived from Peking. Because of this interaction, the presence or absence of *Rhg4* is an important consideration when testing the effects of *rhg1* (Meksem et al., 2001; Brucker et al., 2005). However, for genotypes carrying *rhg1-b* from PI 88788, *Rhg4* has not been detected as a relevant QTL (Glover et al., 2004) nor has it been shown to interact with *rhg1-b* from PI 88788 when these genes are combined (Brucker et al., 2005). Therefore, the *Rhg4* should not have affected our research.

Previous work showed that resistance at the *rhg1* locus was recessive or partially recessive (Brucker et al., 2005; Concibido et al., 1997; Meksem et al., 2001). Although studying the gene action of *rhg1-b* was not the objective of this study, our testing of individual plants for both SCN resistance and molecular marker genotypes allowed us to evaluate the action of this gene. Across all SCN resistance tests in this study in which a significant association was detected between markers and SCN resistance, the mean FI of those plants that were predicted to be homozygous resistant was 28.9, the mean FI of the heterozygotes was 54.8, and the mean FI of the homozygous susceptibles was 81.5. The mean of the homozygous resistant and susceptible groups was 55.2, which is very close to the mean of the heterozygotes. This shows that *rhg1-b* from PI 88788 has additive or incomplete dominance gene action rather than being purely recessive.

Our mapping of *rhg1-b* to a 67-kb genetic region and the identification of SNP markers within this interval will provide additional marker resources that can be used in marker-assisted selection for this gene. In addition, our narrowing the interval that contains the gene should aid in efforts to clone it. The Williams 82 soybean genome sequence corresponding with the *rhg1-b* region defined in this study contains 11 predicted protein-coding genes on Glyma 1.01 (www.phytozome.net; Schmutz et al., 2010). Of these candidate genes, none encode nucleotide binding (NB)-LRR proteins or other proteins resembling LRR-containing disease resistance proteins (Jones and Dangl, 2006). The annotations associated with the putative proteins encoded in this region include a cation/hydrogen exchanger, a wound-induced protein, a SNAP (vesicle trafficking) protein, and an amino acid transporter. Other genes at this locus remain equally valid candidates for *rhg1-b*; agriculturally important genes that contribute to plant disease resistance include an intriguing array of proteins other than LRR proteins (e.g., Buschges et al., 1997; Fu et al., 2009; Krattinger et al., 2009). In addition, the above prediction of 11 genes is based on the Williams 82 sequence and PI88788 may have insertions, deletions, and other rearrangements that could result in PI88788 carrying genes different than the SCN susceptible Williams 82. How the *rhg1-b* region in PI 88788 and Williams 82 compare is currently not known and sequencing this region from PI 88788 will be an important step in the identification of *rhg1-b*.

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