IDENTIFYING NOVEL SOURCES OF RESISTANCE TO THE SOYBEAN CYST NEMATODE

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Abstract

Soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) is the most serious yield-limiting pathogen on soybean [*Glycine max* (L.) Merr.]. Utilizing genetic resistance is an effective method to control SCN. Most commercial SCN-resistant cultivars in the North Central USA are developed from two sources of resistance, PI 88788 and Peking. However, frequent use of a limited number of resistance sources has shifted virulence phenotypes of SCN populations (HG Types) and the new types seem to overcome originally resistant cultivars. The main purpose of this study is to search for new sources of SCN resistance that are different from Peking or PI 88788 and to identify genetic regions that are associated with novel resistance loci. Since Peking is not resistant to HG Type 1- (race 14) and PI 88788 is not resistant to HG Type 2- (race 1), 17 soybean cultivars and accessions that were reported resistant to HG Type 1- or/and HG Type 2- were tested against 13 different nematode populations including race 1, race 2, race 3, race 4 and race 14. Most of the lines tested had high or moderate resistance to race 1, race 2 and race 3 populations and can serve as an alternative resistance sources to PI 88788. However, most of the lines were susceptible to race 4 and the two race 14 nematode populations. Only PI 633736 has a high level of resistance to all the nematode populations used. PI 417091, PI 404166, PI 567516C, PI 629013 have moderate or high resistance to race 4 and at least one of the two race 14 populations. The different resistance spectrums of those lines indicate that there should be novel genes in PI

633736, PI 417091, PI 404166, PI 567516C and PI 629013 that are different from Peking and PI 88788. QTLs conferring resistance to an HG Type 2.5.7 population (race 1) were sought with 92 MN0095 × PI 567516C F2:3 families from greenhouse (Experiment 1) and 92 F2:3 families from field (Experiment 2) using 1536 SNP markers. Altogether, 5 QTLs were declared for Experiment 1 and Experiment 2, including 2 significant QTLs (genome-wide type I error =0.05) and 3 suggestive QTLs (LOD > 3). The two significant QTLs were detected on chromosome 10 and chromosome 19 and the three suggestive QTLs were detected on chromosome 8, chromosome 18, and chromosome 20. The QTL with the highest LOD score, located on chromosome 10 was detected in both Experiment 1 and Experiment 2 and was recently reported by another group. This QTL has not been identified in other sources of SCN resistance. This QTL has significant additive effect, and explained 22.2% and 22.4% total variance in Experiment 1 and Experiment 2, respectively. The QTLs on chromosome 19 was detected only in Experiment 1. It had significant dominance effect, and explained 12.7% of total variance. The suggestive QTL mapped on chromosome 18 in Experiment 2 was at or near the *rhg1* locus. Haplotype analysis of *rhg1* and *Rhg4* genes for the 17 resistant soybean germplasm lines revealed that PI 567516C and Peking share the same *rhg1* allele. Markers closest to *rhg1* and the QTL on chromosome 10 might be considered for use in marker assisted selection.

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Chapter 1 Identification of novel resistance

1.1 Introduction

Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is estimated to be the most yield-limiting pathogen to soybean [*Glycine max* (L.) Merr.] among all the soybean pathogens (Wrather et al., 2001; Wrather et al., 2006). SCN can significantly reduce yield of susceptible cultivars without visually detectable symptoms (Wang et al., 2003). Using genetic resistance is an effective method to control SCN. However, there are only a few sources of SCN resistance used in SCN-resistant cultivars. From the 2011 list of Soybean Varieties with Soybean Cyst Nematode Resistance on the website of University of Illinois Extension, PI 88788, Peking, PI 437654, PI 438489B, PI 84946-2, PI 90363 and PI 209332 are the only sources of SCN resistance used in all the SCN-resistant cultivars developed by companies within Illinois and surrounding states. Of those 2000 listed resistant cultivars, 94% have their resistance derived from PI 88788 and 3% have their resistance derived from Peking (The cultivars that have unidentified source of resistance were not included in the calculation) (Shier et al., 2011). However, continuous cultivation of single source of resistance may shift the virulence phenotypes of SCN populations (HG Types) and the new types seem to overcome originally resistant cultivars. For example, 70% of the 2005 SCN samples in Illinois were able to overcome PI 88788 resistance while only 36% of 1989-1990 SCN samples from Illinois were able to overcome PI 88788 (Sikora et al., 1991; Niblack et al., 2008). In an SCN survey conducted in Missouri in 2005, 78% of the sampled nematode populations could

overcome PI 88788 and 30% of the nematode populations could overcome Peking (Mitchum et al., 2007). In contrast, only 58% of populations in 1998 could overcome PI 88788 (Niblack et al., 2003). In Minnesota, 11.9% and 15.3% of nematode populations sampled were able to overcome PI 88788 resistance in 1997-1998 and 2002, respectively (Zheng et al., 2006). A recent survey in Minnesota showed a dramatic increase of frequencies of SCN virulent phenotypes since 2002. Most of the populations (71.6%) collected in 2007-2008 reproduced well (FI > 10) on PI 88788, and 15.8% of the populations had FI > 10 on Peking (Chen et al., 2010).

New sources of resistance are needed to substitute for or rotate with the few sources of resistance especially for PI 88788 and Peking in the light of increasing virulent frequencies on these two sources of resistance. PI 437654 has been reported to be resistant to all the naturally occurring nematode populations (Anand et al., 1984; Anand et al., 1988; Arelli et al., 1997). However, it has black seed color and has low yield (Nelson et al., 1988). PI 633736 (Anand et al., 2004) and PI 629013 (Anand et al., 2002) are two germplasm lines that derived their SCN resistance from PI 437654 yet both have yellow seed color and high yield. Although PI 437654 is resistant to all naturally occurring nematode populations, it was reported to be susceptible to a synthetic nematode population LY1 that was developed from mass mating SCN Race 2 (HG Type 1.2.5-) females with SCN Race 5 (HG Type 1.2-) males (Arelli et al., 2009). Identifying soybean germplasms with novel SCN-resistance genes is also valuable to prepare for the situation when PI 437654 resistance might be broken down.

Since Peking is not resistant to HG Type 1- (race 14) and PI 88788 is not resistant to HG Type 2- (race 1), soybean lines that were reported resistant to HG Type 1- or/and HG Type 2- were tested against different nematode populations collected in Minnesota in order to identify soybean lines with broad spectrum of SCN resistance.

1.2 Materials and methods

1.2.1 Soybean lines

The SCN resistance levels of about 12,000 soybean accessions can be found on the website of USDA Agricultural Research Service Germplasm Resources Information Network (GRIN) (http://www.ars-grin.gov). Based on the information listed on GRIN and reviews (e.g., Shannon et al., 2004), 17 soybean lines that were reported to be resistant to race 1 and/or race 14 were selected for SCN resistance test.

1.2.2 SCN populations

Thirteen different nematode populations including race 1, race 2, race 3, race 4 and race 14 were originally collected from the fields in Minnesota and were maintained on several different soybean cultivars in the greenhouse. The origin of the nematode populations, year of collection and soybean cultivar used in culture are listed in Table 1.1.

Table 1.1 SCN populations selected for identifying novel sources of resistance

Nematode population	Race	HG Type	County of origin	Year of collection	Cultivar used in culture §
SY26	1	2.5.7	Martin	2007	Sturdy
F9001	1	2.5.7	Dodge	2008	Sturdy
SY131	1	2.5.7	Lincoln	2007	Sturdy
W5-07A1	1	2.5.7	Waseca	2007	Sturdy
W5-07A2	1	2.5.7	Waseca	2007	Freeborn
F9002	2	1.2.5.7	Houston	2008	Sturdy
SY-130	2	1.2.5.7	Meeker	2007	Sturdy
SY97	3	0	Swift	2007	Sturdy
W1-07A	3	7	Waseca	2007	Sturdy
SY53	3	0	Jackson	2007	Sturdy
SY87	4	1.2.3.5.6.7	Redwood	2007	Sturdy
MU24B	14	1.3.5.6.7	Murray	1998	91M90
SY133	14	1.3.5.6.7	Renville	2007	Sturdy

§ Cultivar Freeborn has SCN resistance derived from PI 88788, Cultivar 91M90 has SCN resistance derived from Peking. Cultivar Sturdy is susceptible to SCN.

1.2.3 Soybean cyst nematode bioassay

Before being used in the greenhouse bioassay, the females or cysts from the roots of the soybean cultivars used in culture of nematode inoculum were flushed through an 850-µm-aperture sieve onto a 250-µm-aperture sieve. Eggs were released from the female cysts with a mechanical tissue grinder (Faghihi et al., 2000), collected with a 25-µm-aperture sieve, and used as inoculum.

The 17 lines, 7 SCN HG Type indicator lines (Peking, PI 88788, PI 90763, PI 437654, PI 209332, PI 89772, PI 548316), and susceptible check Lee74 were included (Table 1.2). Six seeds from each soybean line (15 seeds from Lee74 control) for each SCN population were soaked over night. A cone-tainer (4-cm-diam. and 13.5-cm high) were filled with autoclaved soil (80% sands + 20% field clay loam soil without SCN infestation) to half and 2000 eggs in 2.5 ml of water were added. Additional soil was placed in the cone-tainer to approximately 2 cm from the top. A soybean seed was placed on the surface of

soil, another inoculum of 2000 eggs in 2.5 ml of water was added near the seed, and the seed was then covered with additional soil to about 1-cm depth. Six cone-tainers were set up for each soybean line. All of the cone-tainers were inserted into autoclaved sand in rectangle containers (35 × 31 × 15 cm) that had five holes at bottom for draining excess water. Each container contained one plant (cone-tainer) from each soybean line (2-3 plants for Lee74), and a total of six containers were used for each SCN population. The cone-tainers were maintained in the greenhouse with temperature set at 27 °C (20-30°C) and day light length at 16 hours. Starting 30 days after inoculation, one plant of Lee74 was examined for number of females. When the females had developed (generally between 35-40 days after planting) and number was approximately 100 or more, the experiment was terminated and all plants were examined for female number. The soybean plants with soil were removed from the cone-tainers. All plants of the same soybean lines were pooled, and soaked in water in 1-liter beakers for at least 30 min. If a line had fewer than 3 plants with good growth, the line was tested again. The soybean plants were gently removed from the beakers, and females were washed off the roots through an 850-µm-aperture sieve onto a 250-µm-aperture sieve. The females collected from the 250-µm-aperture sieve of all plants counted under a stereomicroscope. Female index were calculated based on the following formula: (Mean number of female nematodes on a given line) × 100 / (Mean number of female nematodes on Lee74).

Rating of SCN resistance was adopted from Schmitt et al. (1992): resistant (FI < 10), moderately resistant (FI = 10–29.9), and susceptible (FI ≥ 30).

1.3 Results

The greenhouse bioassay data is presented in Table 1.2. Most of the lines tested had high or moderate resistance to race 1, race 2 and race 3 populations and can serve as alternative sources of resistance to PI 88788. However, most of the lines were susceptible to race 4 and the two race 14 populations. Only PI 633736 had high resistance to all the nematode populations used. PI 417091, PI 404166, PI 567516C, PI 629013 had moderate or high resistance to race 4 and at least one of the two race 14 populations. PI 507471 was highly or moderately resistant to all the nematode populations that have been tested, however, no data is available for its resistance to race 4 and the two race 14 populations. The different resistance spectrums in those lines indicate that there may be novel genes in PI 633736, PI 417091, PI 404166, PI 567516C, PI 629013 that are different from Peking and PI 88788.

Table 1.2 Female indexes of 13 different nematode populations on different resistant soybean lines

Line	Population name	Nematode population												
		SY26	F9001	SY-131	W5-07A1	W5-07A2	F9002	SY-130	SY97	W1-07A	SY53	SY87	MU24B	SY133
	Race	1	1	1	1	1	2	2	3	3	3	4	14	14
	HG Type	2.5.7	2.5.7	2.5.7	2.5.7	2.5.7	1.2.5.7	1.2.5.7	0	7	0	1.2.3.5.6.7	1.3.5.6.7	1.3.5.6.7
Peking		0.3	0.7	0.0	1.6	6.6	20.1	28.1	0.2	0.4	5.6	52.9	58.1	53.2
PI 88788		17.4	24.6	46.2	57.8	61.7	23.5	44.9	4.7	7.7	0.9	12.3	4.7	4.8
PI 90763		0.1	0.1	0.0	0.3	0.0	0.0	0.1	0.0	0.2	2.6	22.6	38.4	25.9
PI 437654		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.1	0.0
PI 209332		26.9	-	53.7	23.4	155.3	33.8	65.4	4.1	-	5.6	40.7	-	10.7
PI 89772		0.2	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	3.1	35.8	25.6	15.4
PI 548316		26.0	25.7	70.0	29.5	28.8	69.1	55.3	3.7	19.5	3.2	42.9	35.2	56.8
Lee74		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
PI 494182		0.6	1.3	9.9	5.2	6.9	9.2	7.6	2.2	0.6	7.3	35.7	69.9	49.6
PI 507354		0.4	0.9	0.0	0.9	7.9	5.9	10.4	2.0	1.3	9.3	42.8	40.6	45.9
PI 467327		37.8	44.3	59.7	48.4	37.4	39.7	29.6	0.1	15.1	5.4	82.1	26.0	87.5
PI 468903		0.3	7.7	0.3	2.1	3.0	18.1	5.1	0.5	0.4	7.1	91.5	72.1	38.2
PI 416762		11.2	0.7	18.6	6.7	8.8	27.2	8.7	1.2	5.1	1.9	30.9	15.1	43.5
PI 417091		9.2	9.2	21.9	5.7	3.1	29.1	3.3	1.5	7.5	3.7	20.8	17.9	52.7
PI 468915		0.4	21.7	0.5	1.9	2.8	7.1	2.0	0.8	0.4	16.3	78.1	65.4	73.9
PI 567418A		67.5	56.0	47.2	101.8	98.4	88.1	44.1	54.0	84.4	11.9	70.3	67.5	138.8
PI 567507B		39.8	28.1	36.4	54.4	57.8	58.9	31.5	12.0	28.4	4.0	68.1	18.8	43.2
PI 404166		0.0	0.0	0.0	0.0	0.0	0.7	0.1	0.0	0.7	1.9	18.6	19.1	22.3
PI 437690		0.6	0.6	0.1	3.1	1.1	9.5	3.5	0.9	3.3	13.7	28.5	51.9	64.2
PI 507471		7.7	4.2	2.6	0.8	1.2	4.9	1.5	0.0	-	10.6	-	-	-
PI 404198A		0.1	0.0	0.0	0.0	0.5	3.0	5.8	0.0	2.8	14.6	52.4	65.1	75.1
PI 567516C		14.1	1.9	0.2	2.9	0.6	21.3	11.3	4.6	16.9	2.8	22.3	4.9	46.6
PI 629013		11.1	13.4	1.6	3.1	4.9	2.8	5.1	0.0	7.9	0.2	8.6	9.2	16.9
PI 633736		0.1	0.5	0.0	2.2	1.9	0.7	1.9	2.0	2.1	0.0	1.5	0.6	1.3
PI 567364		-	-	-	82.3	-	103.9	126.6	139.0	-	33.1	-	-	-

1.4 Discussion

We can see from the results that even if the HG Types of two nematode populations are the same, they can have quite different reactions on different resistant lines. For example, MU24B and SY133 have very similar Female Index on the 7 indicator lines but very different female indexes for a few of the resistant lines tested. For example, the FI of MU-24B on PI 567516C was only 4.9, but the FI of SY133 on the line was high at 46.6. This indicates that the two SCN populations may contain different virulence genes that cannot be differentiated by the 7 indicator lines and that the PI 567516C and some other lines may contain genes that are different from Peking resistance.

PI 633736, also known as S97-1688, has its resistance derived from several sources of SCN resistance including PI 437654, PI 90763, Peking and PI 88788 (Anand et al., 2004). PI 629013 (S96-2692) has its SCN resistance derived from Hartwig, which itself was derived from PI 437654 (Anand et al., 2002). The pedigrees of PI 633736 and PI 629013 explain their broad-spectrum of resistance to SCN populations.

PI 417091 is an accession from Japan. Based on the literature, it is resistant or moderately resistant to race 1 and race 3 but susceptible to race 2 and race 4 (Anand et al., 1984; Anand et al., 1988; Arelli et al., 1997). However, in our study, it is resistant or moderately resistant to both race 2 and race 4 and only susceptible to one of the two race 14 populations.

PI 404166 is an accession collected from China. It was reported to be resistant or moderately resistant to race 1, race 2, race 3 and race 5 but susceptible to race 4 (Anand et al., 1984; Anand et al., 1988; Arelli et al., 1997). However, in our study, it is resistant or moderately resistant to all the nematode populations tested, and it might be useful to integrate the resistance to Minnesota cultivars.

PI 507471 is an accession collected from Japan. It was reported to be resistant to race 1, 2, 5 and 14 but not resistant to race 3 (Young, 1995; Arelli et al., 2000). However, in our study, it is resistant to all three populations of race 3 as well as other nematode

populations. The resistance information of PI 507471 to the most virulent SCN populations in our study were missing and need to be tested again.

PI 567516C is an accession collected from China. It was originally reported to be resistant or moderately resistant to race 1 and race 2 (Arelli et al., 1997). It was also reported to be resistant to a highly virulent nematode population LY1 (Arelli et al., 2009). In our study, PI 567516C is highly or moderately resistant to all the nematode populations except for a race 14 population SY133. The broad-spectrum resistance of PI 567516C makes it a valuable source of resistance.

The different results between our study and the previous work may be due to the fact that the nematode populations used are different even if they were identified as the same race. The results also indicate that the genetic diversity of SCN populations cannot be completely described by the current indicator lines.

In conclusion, PI 633736, PI 629013, PI 417091, PI 404166 and PI 567516C all have resistance spectrum different from Peking or PI 88788 and may all serve as valuable new sources of resistance.

Chapter 2 QTL mapping of PI 567516C and haplotype analysis of *rhg1* and *Rhg4* loci

2.1 Introduction

We have identified several possible sources of novel SCN resistance in the study above and then the aim here is to identify the quantitative trait loci that confer the novel resistance and do haplotype analysis of the major SCN-resistance genes to facilitate marker assisted selection.

Quantitative Trait Loci (QTL) mapping experiments have been performed during the past 20 years and the resulting QTLs positions on soybean genetic maps are available in SoyBase (Grant et al., 2010). A major QTL on chromosome 18 (linkage group (LG) G), which is located around 10 cM in the Consensus Genetic Map 4.0, was consistently mapped in several sources of SCN resistance including Peking, PI 88788, PI 437654, PI 89772, PI 90763, PI 209332 and PI 404198A (Guo et al., 2006b). This locus is known as the *rhg1* locus, which has been reported to be associated with resistance to race 1, 2, 3, 5, 6, 14 (Concibido et al., 2004). A second important QTL was frequently mapped around 50 cM on chromosome 8 (LG A2) on Consensus Map 4.0 with several sources of SCN resistance including Peking, PI 90763, PI 437654, PI 438389B and PI 404198A (Guo et al., 2006b). This locus is known as *Rhg4* gene and has only been detected resistant to race 1 and race 3 (Concibido et al., 2004).

Aside from these two major SCN-resistance loci, many loci have been mapped on other linkage groups (Concibido et al., 2004). SCN-resistance QTLs have been reported on 18 out of 20 linkage groups. No QTL at the *rhg1* locus was detected for PI 438489B, PI 468916 or PI 464925B. For PI 438489B, the major QTLs for race 1 and race 2 were mapped at around 36 cM on chromosome 18 (LG G), race 3 at around 45 cM at chromosome 18 and race 5 at around 60 cM of chromosome 18, and no QTL was detected on chromosome 18 for race 14 (Concibido et al., 2004). For PI 468916, the major resistance locus to race 3 was located at around 80 cM on consensus Genetic Map 4.0 on chromosome 18 (LG G) (Wang et al., 2001). For PI 464925B, no QTLs were mapped on chromosome 18 (LG G). The QTL in PI 464925B with the largest effect only accounts for 7% of the total variance which may indicate that the resistance in this germplasm is conveyed by many minor effect QTLs or the major QTL may be located in a genome region not well covered by existing molecular markers (Winter et al., 2007). No QTLs were detected at *Rhg4* locus for PI 88788, PI 89772 and PI 468916. Although Concibido et al. (1994) detected a QTL on chromosome 8 (LG A2) with the closest RFLP marker A085 for M85-1430 (PI 209332 source of resistance) resistant to a race 3 population, this marker is at 30 cM on Consensus Genetic Map 4.0 which is 20 cM away from the *Rhg4* locus mapped by others (Prabhu et al., 1999; Meksem et al., 2001; Yue et al., 2001a; Guo et al., 2006a, 2006b). Concibido et al. (1996) also mapped the resistance of PI 209332 to race 1, race 3 and race 6, none of the experiments have located a QTL on chromosome 8 (LG A2).

Although *rhg1* and *Rhg4* are the major SCN-resistance genes in many lines, they are not associated with SCN resistance in same of the SCN-resistant lines and many other loci may have great effect on SCN resistance. This makes QTL mapping of the novel SCN-resistant lines necessary.

PI 567516C is a germplasm line collected from China. It was originally reported to be resistant or moderately resistant to race 1 and race 2 (Arelli et al., 1997). It was also reported to be resistant to a highly virulent nematode population LY1 (Arelli et al., 2009) (LY1 was developed by mass mating of different nematode populations and is virulent on PI 437654 which is highly resistant to all the naturally occurring nematode populations). In our greenhouse test, PI 567516C is highly or moderately resistant to all SCN populations except for a race 14 population SY133. Chen et al. (2006) analyzed the genetic diversity of over 100 SCN-resistant lines with 85 SSR markers and reported PI 567516C to be in a different cluster with Peking and PI 88788. The broad spectrum of resistance in PI 567516C and the possible uniqueness of its genome make it a valuable source of resistance.

The aim of this study is to identify the genomic regions associated with SCN-resistance in PI 567516C using a MN0095 × PI 567516C population. Before we finished our study, Vuong et al. (2010) published their work in mapping the resistance loci in PI 567516C using a Magellan × PI 567516C F2:3 population. We continued our experiment to confirm the QTL effect in the genetic background of our susceptible parent MN0095.

Cregan et al. (1999) reported the SSR marker satt309 to be 0.4 cM from *rhg1* gene. Two groups have filed patent applications claiming identified reporter like kinase genes for *rhg1* and *Rhg4* and released sequences supporting the claim to Genbank (Hauge et al., 2001; Lightfoot et al., 2001). Ruben et al. (2006) summarized the process of identifying the *rhg1* gene by fine mapping and substitution mapping, and characterized the protein encoded by the candidate *rhg1* gene as a leucine-rich repeat serine-threonine kinase. However, no information was available on how the *Rhg4* gene was cloned. To date, several groups have released the sequences of *rhg1* and/or *Rhg4* to Genbank (Hauge et al., 2001; Ruben et al., 2006; Li et al., 2009; Takeuchi et al., 2009). To obtain further understanding about the putative resistance genes in different soybean collections and to help decide what molecular markers to use in marker assisted selection, these two major SCN-resistance genes were included in our haplotype analysis of the SCN-resistance genes. Although the evidence of the sequence of *Rhg4* gene is not as concrete as that of the *rhg1* gene, we still included it into our haplotype analysis.

2.2 Materials and Methods

2.2.1 QTL mapping

Soybean materials

A F2:3 population was developed by crossing the SCN-resistant line PI 567516C and an adapted SCN susceptible line MN0095. 1000 F2 plants were planted in the field in

summer 2010 in St. Paul, MN as part of the ongoing soybean-breeding program at the University of Minnesota. Since PI 567516C is in maturity group IV, which matures so late in the season that many lines fail to mature in time as far north as St. Paul, it is possible that we could not obtain a truly randomly selected population of F2:3 families, thus artificially introducing segregation distortion. To make sure we get randomly selected F2:3 families, 120 randomly chosen F2 seeds were planted in the greenhouse in the hope that we could harvest randomly selected F3 families for our QTL mapping experiment. In the end, 92 F2:3 families from the greenhouse population were used in QTL mapping Experiment 1 and 92 F2:3 families from the field population were used in QTL mapping Experiment 2 to confirm the results of QTL mapping Experiment 1.

DNA extraction and genotyping

One young leaf was collected from each F2 plant and the two parents. DNA was extracted using DNeasy 96 Plant Kit manufactured by QIAGEN. The 1536 SNP markers in Universal Soy Linkage Panel (USLP 1.0) designed by Hyten et al. (2010) were used for genotyping the F2 plants and the two parents. Genotyping was done by Illumina GoldenGate Assay in two 96 well plates in BioMedical Genomics Center, University of Minnesota.

Soybean cyst nematode bioassay

In Experiment 1, nematode inocula were prepared from a population of HG Type 2.5.7 that was originally collected from a field in Minnesota and maintained on soybean

'Freeborn' (PI 88788 resistance) for two years before it was cultured on the susceptible soybean 'Sturdy' for inoculation. In Experiment 2, part of the nematode population (around 60-70 %) was harvested from the roots of Experiment 1 mapping population and the other part from the greenhouse culture of the same nematode population on Sturdy. Cysts were collected and stored at 4 °C and used within 60 days for inoculation. At the time of inoculation, cysts were recovered from sand, ground in a mechanical tissue grinder (Faghihi et al., 2000) to release eggs and the inocula were counted with a hemocytometer to make a solution with 2000 eggs/ml. Nine replicates were set up for each experiment. Each replicate included one F3 plant from each F2:3 family, the two parents (MN0095 and PI 567516C), 7 SCN HG-Type indicator lines (Peking, PI 88788, PI 90763, PI 437654, PI 209332, PI 89772, PI 548316), and the susceptible check Lee74. Two soybean seeds were planted in sand-filled Ray Leach cone-tainers (164 ml) (Stuewe\&Sons, Inc., Portland, OR) which were placed in sand-filled 8-L buckets (Experiment 1) or 11.4-L rectangle white plastic containers (Experiment 2). In Experiment 1, each replicate was fitted into six buckets with each containing 19-20 cone-tainers. In Experiment 2, each replicate was fitted into two plastic containers with each containing 52-53 cone-tainers. Seven to ten days after planting, soybean seedlings were thinned to 1 plant/cone-tainer by pulling the extra seedling out of the sand and inoculated 5000 eggs with a 5ml pipette. The experimental plants were maintained in the greenhouse with temperature set at 28 °C and light of 16 hour. Experiment 1 was conducted in January 2011 and Experiment 2 in April 2011. Thirty days after inoculation, cone-tainers were immersed in water and soybean roots were pulled out from

the cone-tainer and flushed with water to wash the females (cysts) from roots through an 850-μm-aperture sieve onto a 250-μm-aperture sieve. The females were collected from the 250-μm-aperture sieve. Females were counted for individual F3 plants in each F2:3 family. Female index was calculated for each individual F3 plant using the following formula:

$$FI = \frac{Number\ of\ \ female\ nematodes\ on\ a\ given\ individual}{Mean\ number\ of\ \ female\ nematodes\ on\ Lee74} \times 100$$

The mean FI of all nine F3 plants in a F2:3 family was obtained and used to represent the FI of the F2.

Data Analysis

There were 780 markers that were segregating between the two parents MN0095 and PI 567516C. Of the 780 markers, the markers with significant segregation distortion based on the chi-square test with p-value $\leq 10^{-7}$, or markers with similarity of 1 to other markers were removed from analysis. Linkage maps were built according to the marker genotypes of the 184 F2 plants from both Experiment 1 and Experiment 2. Linkage groups were created by JoinMap 4 (Van Ooijen, 2006) with a LOD threshold of 5 and maximum genetic distance of 50 cM for initial grouping of markers. A LOD threshold of 5 was chosen to ensure that all the markers in the same linkage group were truly linked. Marker orders were estimated with JoinMap 4 (Van Ooijen, 2006) using the Maximum likelihood mapping algorithm. Genetic distances were estimated using Kosambi mapping function (Kosambi, 1944). The new assignments of linkage groups and chromosome numbers were based on soybean Consensus Map 4.0 (Hyten et al., 2010).

Composite interval mapping (CIM) method (Jansen, 1993; Zeng, 1994) was used for QTL analysis. Appropriate cofactors were chosen by forward selection and composite interval mapping were subsequently performed using the code from 'cim' function in R/qtl (Broman et al., 2003). A permutation test was performed with 1,000 runs to determine the P=0.05 genome-wide significance level for declaring a significant QTL (Churchill et al., 1994). The highest LOD was used to indicate the position of the QTL and its 1-LOD support interval was obtained. The proportion of the phenotypic variance explained by each significant QTL was estimated by the 'fitqtl' function in R/qtl (Broman et al., 2003). Additive (A) and dominant (D) effects of a given QTL were estimated by fitting a linear model in R. Since there were only 92 individual F3 families in each mapping experiment, no epistatic interactions were estimated for the QTLs. The genetic maps of chromosomes with QTL positions were created using the MapChart 2.2 program (Voorrips, 2002) based on the outputs from JoinMap 4 (Van Ooijen, 2006) and R/qtl (Broman et al., 2003).

2.2.2 Haplotype analysis of *rhg1* and *Rhg4* loci

Soybean lines

The same 17 soybean lines that were used in the greenhouse bioassay were used for haplotype analysis at *rhg1* and *Rhg4* loci. In addition, the 7 SCN indicator lines, Lee74

and 3 adapted lines with no SCN resistance (MN0095, IA2073, MN1410) were also used in the analysis.

DNA extraction

One young leaf was collected from each of the 28 lines. DNA was extracted using DNeasy Plant Mini Kit manufactured by QIAGEN.

SNP markers for *rhg1* and *Rhg4* loci

Cregan et al. (1999) reported an SSR marker Satt309 on chromosome 18 (LG G) to be in 0.4 cM of *rhg1*. In previous lab work, SNPs located within 250 kb from Satt309 were selected based on Hyten et al. (Hyten et al., 2007; Hyten et al., 2010) and tested on several SCN-resistant lines using iPLEX MassARRAY technology. Among these SNPs, 6 SNPs were able to differentiate between Peking, PI 88788, PI 209332 and were used in the haplotype analysis for this experiment. Genotypes of the 28 lines for the 6 SNPs were also obtained by iPLEX MassARRAY system. The primers for the 6 SNPs are listed in Table 2.1.

Table 2.1 Primers for 6 SNPs used in haplotype analysis

Marker	SNP marker name	Left primer	Right primer
SNP1	BARC_015377_01829	ACGTTGGATGGTTGGGCGATCAA TTTCAAT	
SNP2	AF506516_1	ACGTTGGATGAGCATCAAACCTC TTAGCAC	ACGTTGGATGATAGCCACTGTCA TTCCAGC
SNP3	BARC_G01477_00243	ACGTTGGATGACTGGGTTCTGAG ACACTTG	ACGTTGGATGATATCCAGAGAAC CAGACCC
SNP4	AY618857_2	ACGTTGGATGAAAGAAGCAGGA ACGTCACC	ACGTTGGATGGTGGGAATTCCAA GAATGGC

SNP5	BARC_G01475_00237	ACGTTGGATGGCATGCAAACACA AACACAG	ACGTTGGATGGCTTTTTTACACT AACACCCC
SNP6	BI970984	ACGTTGGATGTGTGCTAAGGAGA CCATACC	ACGTTGGATGGTCACATTGTTGG CAACCAC

Since SNPs that can cause amino acid substitution (non-synonymous SNPs) will have more information in telling whether there might be functional differences in different alleles, non-synonymous SNPs were found by aligning coding sequences and protein sequences of *rhg1* and *Rhg4*. Released sequences of *rhg1* (Hauge et al., 2001; Ruben et al., 2006; Li et al., 2009; Takeuchi et al., 2009) and *Rhg4* (Hauge et al., 2001; Takeuchi et al., 2009) genes were extracted from GenBank and aligned with ClustalW (Thompson et al., 1994).

Based on the alignment of *rhg1* and *Rhg4* coding sequences and protein sequences, 9 SNPs were identified able to cause amino acid substitution in *rhg1* while 3 SNPs were identified able to cause amino acid substitution in *Rhg4*. Primers were designed to amplify the gene fragments that contain most of the SNPs and the amplified fragments from the 28 soybean lines were sequenced. Eight of the 9 SNPs in *rhg1* were covered by 2 DNA fragments while 2 of the 3 SNPs in *Rhg4* were covered by 1 DNA fragment. Sequencing was performed both forward and reverse using the forward primers and reverse primers by ABI PRISM^{TM} 3730xl DNA Analyzer. The primer sequences, their position in the CDS and their product size are listed in Table 2.2. Sequences were aligned by ClustalW (Thompson et al., 1994) and the genotypes for the non-synonymous SNPs were called from the aligned sequences.

Table 2.2 Primers for sequencing of *rhg1* and *Rhg4*

Name	Seq	Position	Product size	Length
rhg1_1_F	TATGTGTGTGGAGCCTTGTTG	131	731	21
rhg1_1_R	GCTACCCAAAGAAGCAGGAAC	861		21
rhg1_2_F	TCAGCGCAGTCTAAATCTCTTC	1221	656	22
rhg1_2_R	CCAAAACATTGGGGTGTCTA	1876		20
rhg4_F	GGTCTGAAACAACCCCATTCT	44	657	21
rhg4_R	TGAGCTGCAAGTCAGACAAAG	700		21

2.3 Results

2.3.1 Phenotypic variation

The reactions of soybean indicator lines and the two parents to SCN are listed in table 2.3. The phenotypic variation is large even for the genetically homozygous lines such as MN0095 and PI 88788. The SCN population used in Experiment 1 was HG Type 2.5.7. Although there was no data of FI on Peking in Experiment 2, the population must be HG Type 2.5.7 also, because there was no change of Female Index in PI 567516C and the other two indicator lines PI 90763 and PI 89772 of which the FI is generally correlated with FI of Peking.

Table 2.3 Reaction of soybean indicator lines and two parents to SCN

	Experiment 1				Experiment 2			
Lines	Mean	SD	Min	Max	Mean	SD	Min	Max
				Number of female nematodes				
Lee74	233	129	36	426	147	86	55	376
				Female Index				
MN0095	144	59	86	228	98	50	26	169
PI 567516C	2	2	0	4	1	1	0	3
1 (Peking)	5	4	1	12	ND	ND	ND	ND†
2 (PI 88788)	37	27	8	92	34	15	7	54
3 (PI 90763)	1	1	0	3	1	1	0	2
4 (PI 437654)	0	1	0	2	0	1	0	1
5 (PI 209332)	65	40	11	126	35	16	8	63
6 (PI 89772)	1	2	0	5	0	1	0	1
7 (PI 548316)	35	25	9	63	21	10	6	34

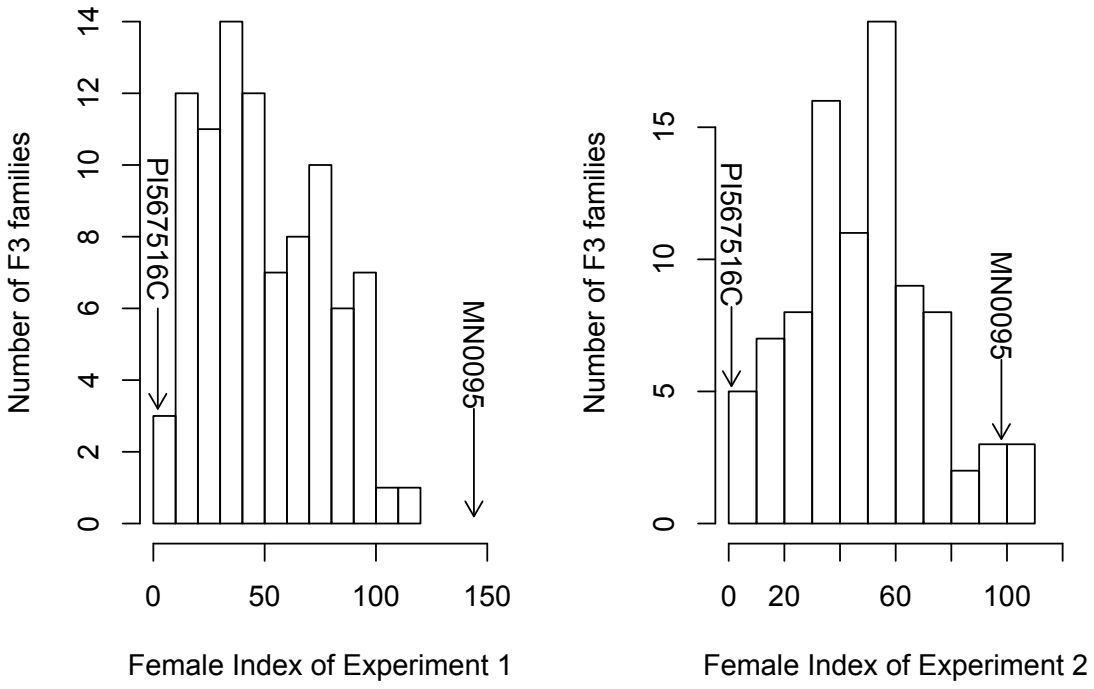
† ND, no data.

The FI data for the F3 families showed large genetic variation (Table 2.4, Figure 2.1). The FI of Experiment 1 ranged from 4 to 117 while the FI of Experiment 2 ranged from 6 to 107. From the distribution of FI in Figure 2.1 we can see that the trait is continuously distributed and should be controlled by quantitative genes.

Table 2.4 Summary statistics of Female indexes (FI) of parental lines and F3 families

	Experiment 1	Experiment 2
	Mean FI (Parents)	
MN0095	144	98
PI567516C	2	1
	FI (F3 Families)	
Mean	49	49
Min	4	6
Max	117	107
SD	27	24

Figure 2.1 Distribution of Female Index of 92 F2:3 families from each of the two QTL mapping experiments



2.3.2 Linkage analysis

Using the LOD threshold of 5, 20 linkage groups were built by JoinMap 4 and the grouping of markers were the same as the grouping of markers in consensus Genetic Map 4.0 (Hyten et al., 2010).

2.3.3 QTLs for SCN resistance

Based on the genome-wide permutation test (genome-wide type I error = 0.05), LOD thresholds of 4.06 and 3.94 were used for significant QTL declaration in Experiment 1

and Experiment 2 respectively. Since in the previous QTL mapping publications, a LOD threshold of 3 was often used for QTL declaration, QTLs with LOD larger than 3 were also reported as suggestive QTLs. Altogether, 5 QTLs were declared for Experiment 1 and Experiment 2, including 2 significant QTLs and 3 suggestive QTLs (Table 2.5). For 4 QTLs, the resistance alleles were from parent PI 567516C, only one QTL on chromosome 20 with its resistance allele from parent MN0095. The three QTLs on chromosomes 8, 10 and 18 have significant additive effect and non-significant dominance effect. The QTL on chromosome 20 has both significant additive effect and dominance effect. The QTL on chromosome 19 has significant dominance effect and non-significant additive effect (Table 2.5).

Table 2.5 SNP Markers associated with loci giving resistance to the soybean cyst nematode

LG	Chr	LOD	Left Marker	Right Marker	R^2(%)	A	D	Detected in
A2	8	3.3‡	BARC-028207-05794†	BARC-032319-08947	9.6	-12.9∗	-9.9	Exp. 1
O	10	8.5	BARC-020735-04704	BARC-008021-00209†	22.2	-22.4∗	-6.5	Exp. 1
L	19	5.6	BARC-055107-13809†	BARC-039977-07624	12.7	-4.7	-25.1∗	Exp. 1
O	10	6.9	BARC-020735-04704	BARC-008021-00209†	22.4	-19.2∗	-0.1	Exp. 2
G	18	3.1‡	BARC-014403-01354	BARC-019351-03885†	8.8	-13.9∗	3.9	Exp. 2
I	20	3.1‡	BARC-039753-07565	BARC-042897-08454†	8.8	9.0*	15.1*	Exp. 2

Linkage Group (LG) is designated according to soybean Consenus Genetic Map 4.0. A is additive effect of PI 567516C allele. D is dominance effect.
‡Suggestive QTL with LOD >3
†Closest marker to the QTL peak
∗Additive or Dominance effect significant at 0.01 level tested using linear regression
R^2(%) was estimated by the fitqtl function in R/qtl

The QTL with the largest effect is on chromosome 10 (LG O) and was detected in both Experiment 1 and Experiment 2. The largest effect QTL has significant additive effect and non-significant dominance effect. The total variance explained by the QTL was 22.2% and 22.4% in Experiment 1 and Experiment 2, respectively.

Another significant QTL was detected on chromosome 19 (LG L) in Experiment 1 but not in Experiment 2. This QTL had significant dominance effect but not significant additive effect. The variance explained by this QTL in Experiment 1 was 12.7%.

The three suggestive QTLs were located on chromosome 8 (LG A2), chromosome 18 (LG G) and chromosome 20 (LG I) and were only detected in Experiment 1 or Experiment 2, respectively. All these three QTLs can explain around ten percent of the total variance, respectively.

2.3.4 Haplotype analysis of *rhg1* and *Rhg4* loci

In the 8 SNPs discovered by sequencing of *rhg1*, 3 turned out not polymorphic in the soybean lines in our study. One SNP on *rhg1* had poor sequencing quality by forward sequencing and was not included in the analysis. Therefore, only 4 of the sequence-based SNPs were kept in the final data set of *rhg1*. The 2 sequence-based SNPs of *Rhg4* were all included in the final data set. The haplotypes of these two loci can be seen in Figure 2.3.

There are 4 distinctive haplotypes using the 4 sequence-based SNPs for *rhg1* gene and 10 distinctive haplotypes using the 6 sequence-based SNPs for *rhg1* and *Rhg4* genes. The 2 MassArray SNPs located approximately 200kb from the *rhg1* gene resolved 3 more haplotypes beyond the original 10 haplotypes while the other 4 MassArray SNPs that are very close to the gene do not add more haplotypes. Since the 2 MassArray SNPs located

200kb from *rhg1* (SNP1 and SNP6) are relatively far from the gene, they are excluded from analysis when we talk about the *rhg1* gene haplotypes in the rest part of this paper.

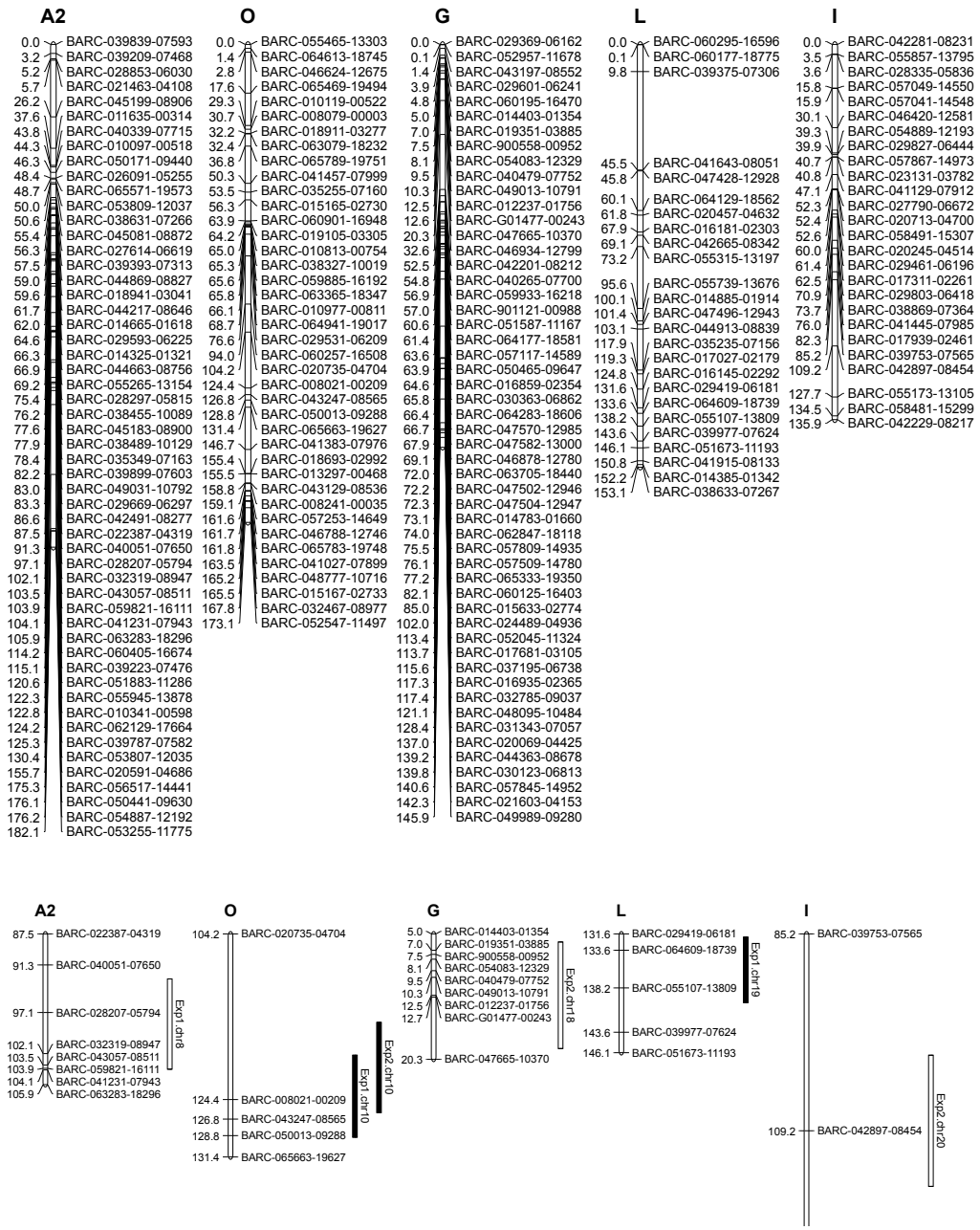


Figure 2.2 Linkage maps with QTLs

Linkage map constructed using an F2 population from cross MN0095 × PI 567516C. The region contains the 1 LOD interval of the QTL peak is demonstrated in the zoom-in version of the linkage map. Significant QTLs are indicated as solid squares. Suggestive QTLs are indicated as open squares. QTLs are named as Exp1.chr or Exp2.chr according to in which experiment the QTL is discovered and the chromosome assignment of the QTL.

						rhg1						*Rhg4*	
Position on gene	Reaction to race 3	SNP1	SNP2	SNP3	SNP4	Sequencing				SNP5	SNP6	Sequencing	
		-172kb	-1.8kb	-1.6kb	-644	260	343	820	1616	+23kb	+237kb	129	261
position on chrosome		1 5 3 9 4 5 7	1 7 0 9 8 8 4	1 7 1 0 0 7 4	1 7 1 1 0 4 8	1 7 1 1 9 5 2	1 7 1 2 0 3 5	1 7 1 2 5 1 2	1 7 1 3 3 0 8	1 7 3 7 7 2 7	1 9 5 1 4 2 4	8 2 8 0 7 6 9	8 2 8 0 9 0 1
PI 567418A	S	CC	TT	TT	GG	T	A	C	G	AA	TT	T	A
MN0095	S	AA	TT	TT	GG	T	A	C	G	AA	TT	T	A
IA2073	S	AA	TT	TT	GG	T	A	C	G	AA	TT	T	A
PI 567364	S	AA	TT	TT	GG	T	A	C	G	AA	TT	T	A
MN1410	S	AA	TT	TT	GG	T	A	-	G	AA	TT	T	A
PI 629013	R	CC	CC	GG	GG	C	C	C	G	AA	TT	T	A
PI 567516C	R	CC	CC	GG	GG	C	C	C	G	AA	CC	G	A
Peking	R	CC	CC	GG	GG	C	C	C	G	AA	TT	G	C
PI 437654	R	CC	CC	GG	GG	C	C	C	G	AA	TT	G	C
PI 89772	R	CC	CC	GG	GG	C	C	C	G	AA	TT	G	C
PI 437690	R	CC	CC	GG	GG	C	C	C	G	AA	TT	G	C
PI 404198A	R	CC	CC	GG	GG	C	C	C	G	AA	TT	G	C
PI 633736	R	CC	CC	GG	GG	C	C	C	G	AA	TT	G	C
PI 494182	R	CC	CC	GG	GG	C	C	C	G	AA	TT	G	C
PI 90763	R	CC	CC	GG	GG	C	C	C	G	AA	TT	G	C
PI 507354	R	CC	CC	GG	GG	C	C	C	G	AA	TT	G	C
PI 404166	R	CC	CC	GG	GG	C	C	-	G	AA	TT	G	C
PI 417091	R	AA	TT	TT	TT	T	C	-	C	CC	CC	G	C
PI 88788	R	AA	TT	TT	TT	T	C	A	C	CC	CC	G	C
PI 548316	R	AA	TT	TT	TT	T	C	A	C	CC	CC	G	C
PI 416762	R	AA	TT	TT	TT	T	C	A	C	CC	CC	G	C
PI 567507B	MR	AA	TT	TT	TT	T	C	A	C	CC	CC	G	A
PI 209332	R	AA	TT	TT	TT	T	C	A	C	CC	CC	T	A
Lee74	S	AA	TT	TT	TT	T	C	A	C	CC	CC	T	A
PI 468903	R	CC	TT	TT	GG	T	C	C	-	CC	CC	G	C
PI 467327	R	CC	TT	TT	GG	T	C	C	G	CC	TT	G	C
PI 468915	R	CC	TT	TT	GG	T	C	C	G	CC	CC	G	C
PI 507471	R	CC	TT	TT	GG	T	C	C	G	CC	CC	G	A

Figure 2.3 Haplotype analysis of *rhg1* and *Rhg4* loci

Peking, PI 437654 and PI 88788 are shaded in Yellow. The five lines with broad SCN resistance in the greenhouse SCN bioassay are colored in red. The 4 distinctive patterns based on the sequencing result of *rhg1* gene are bordered by bold solid lines. The 10 distinctive patterns based on sequencing result of *rhg1* and *Rhg4* genes are bordered by bold dash lines. For the 6 MassArray SNPs, positions on gene are relative to the start and end of the gene. The two MassArray SNPS around 200kb from the gene are shaded in Tan color, which added 3 more patterns to the original 10 patterns and the borders are marked by thin dash lines. The reaction type of these lines to a SCN HG Type 0 (race 3) population SY97 is listed: R, resistant; MR, moderately resistant; S, susceptible.

2.4 Discussion

Segregation distortion can affect the estimate of recombination frequency and the correct estimate of mapping distance (Lorieux et al., 1995; Zhu et al., 2007). However, the estimation of recombination frequency between codominant markers is less affected by segregation distortion than for dominant markers (Lorieux et al., 1995). In our study, the numbers of extremely distorted markers ($p \leq 10^{-7}$) are approximately the same in the field population and greenhouse population, each with around 15 distorted markers. Since there is no evidence of more severe distortion in the filed population than in the greenhouse population, after removing those extremely distorted markers, the F3 families of Experiment 1 and Experiment 2 were combined for building genetic maps to take advantage of the larger population size.

Concibido et al. (2004) reviewed all the previous SCN QTL mapping efforts before 2004. After that, there has been additional mapping of SCN QTLs. Before we finished our mapping experiment with population of MN0095 × PI 567516C, Vuong et al. (2010) published their mapping data on Magellan (susceptible) × PI 567516C (resistant) in June 2010 and reported two novel loci in PI 567516C that have never been mapped by other QTL mapping experiments. In our study, we confirmed one major QTL reported by Vuong et al. on chromosome 10 (LG O), but were not able to confirm the other major QTL reported by Vuong et al. on Chromosome 18 (LG G). The closest SNP marker to the QTL on chromosome 10 (LG O) in Vuong's mapping experiment is “BARC-008021-

00209" which is exactly the same closest marker in our study in both Experiment 1 and Experiment 2. This locus has the highest LOD score and explained the largest fraction of genetic variance in both experiments in our study and in 4 of 6 QTL mapping experiments using different HG Types conducted by Vuong et al. The QTL on chromosome 10 was detected in 5 of 6 QTL mapping experiments (including HG Types 0, 2.5.7, 2.7, 1.3.5.6.7, LY1) conducted by Vuong et al. but not detected in HG Type 1.2.5.7 in the F2:3 mapping population. However, they were able to confirm the QTL on chromosome 10 for HG Type 1.2.5.7 in the RILs developed for QTL validation.

We were not able to confirm the major QTL on chromosome 18 that was detected by Vuong et al. (2010) in 5 of his 6 QTL mapping experiments. The closest marker to that QTL is Satt472 or BARC-038873-07372 that is located at 87.3cM on Soybean Consensus Genetic Map 4.0 and that is 80 cM away from the known *rhg1* gene. The marker BARC-038873-07372 is not polymorphic between PI 567516C and MN0095, although there is another close marker BARC-048095-10484 that is within 1 cM of BARC-038873-07372 and is polymorphic between MN0095 and PI 567516C. The failure to detect this QTL should therefore not be due to the lack of marker coverage in this region. Potentially, the reason why no QTLs were detected in this region in our population might be due to the genetic background and QTL interaction, the difference in nematode population used, or sampling error.

Another significant QTL was detected on chromosome 19 (LG L) in Experiment 1 but not in Experiment 2. This locus has significant dominance effect but not significant additive effect. Loci with dominance effect have also been detected in other SCN QTL mapping experiments (Yue et al., 2001b; Guo et al., 2006b). Although this locus has been mapped in PI 90763 with LOD=7.2 for a race 5 nematode population by Guo et al. (2005), it has not been reported in the other QTL mapping literatures. This locus is not detected in Experiment 2 and not in the experiments of Vuong et al. This might be due to environment and QTL interaction, differences in SCN populations or statistical error. Further investigation is needed to confirm the effect of this QTL.

PI 567516C seems to have the same haplotype as Peking at *rhg1* so it may have the same functional allele with Peking and PI 437654 at *rhg1*. A suggested QTL on chromosome 18 (LG G) was mapped in QTL mapping Experiment 2 with LOD=3.1 and the closest flanking SNP marker BARC-019351-03885. This marker is located at 9.1 cM in soybean Consensus Genetic Map 4.0 while the SSR marker satt309 is located at 10.1 cM in soybean Consensus Genetic Map 4.0. Satt309 was reported to be the closest marker to *rhg1* locus (Cregan et al., 1999). Although the *rhg1* gene in Peking or PI 437654 is a major resistance locus (Concibido et al., 2004), the LOD score for this locus in our mapping population is much lower than the locus on chromosome 10 (LG O). Potentially there might be some form of gene interaction going on which makes this allele less effective in PI 567516C than in Peking. Although we were not able to detect a QTL in *rhg1* region in Experiment 1, the LOD score is 2.75, which is close to 3. And this

suggests that it might be valuable to use the marker surround *rhg1* in marker assisted selection. The lower LOD score in experiment 1 might be due to the slight differences in the nematode populations in the two experiments, environmental effect and sampling error.

A suggestive QTL on chromosome 8 (LG A2) was mapped in Experiment 1 with the closest marker BARC-028207-05794 that is at 87.1 cM on Consensus Genetic Map 4.0. This QTL is clearly different from the *Rhg4* locus since *Rhg4* has been reported to be close to the marker Satt187 (Wu et al., 2009), which is at 49.9 cM on chromosome 8 in Consensus Genetic Map 4.0 (Webb et al., 1995; Yue et al., 2001a; Guo et al., 2006a; Wu et al., 2009). Vuong et al. (2010) also mapped a minor QTL on LG A2 at Satt233--Sat_040 interval in his mapping experiment for HG Type 1.2.5.7. The genetic position for this interval is 85.8 cM -103.4 cM on consensus Genetic Map 4.0 which is the same region as the QTL mapped by our study. Since this QTL was only observed in Experiment 1 and mapped only in 1 of the 6 experiments conducted by Voung et al. (2010), more investigation is needed to confirm the effect of this marker.

Another suggestive QTL on chromosome 20 (LG I) was mapped in Experiment 2 with the closest marker BARC-042897-08454 that is at 85.7 cM on Consensus Genetic Map 4.0. The resistance allele is from the susceptible parent MN0095 and it has both significant additive effect and dominance effect. MN0095 was not documented to be SCN resistance, however, it has lower FI compared to Lee74 (Table 2.3) and to the most

susceptible offspring in Experiment 2 (Figure 2.1). Winter et al. (2007) also reported having identified SCN resistant QTLs in the susceptible parent of a cross. The QTL on chromosome 20 was mapped in PI 437654 (Wu et al., 2009) and PI 464925B (Winter et al., 2007). However, it was not mapped in Experiment 1 of our study. More investigation is needed to confirm the effect of this QTL.

The inconsistence of QTLs mapped in different studies or different SCN populations are common in SCN QTL mapping experiments. Many QTLs mapped in the first study cannot be mapped in the second study (Concibido et al., 2004). For example, Webb et al. (2003) found QTLs on chromosome 8 (LG A2), chromosome 18 (LG G), chromosome 7 (LG M) for race 3 resistance in PI 437654 while Verling et al. (1996) reported a single locus on chromosome 11 (LG B1) for an inbred race 3 resistance in Hartwig (resistance from PI 437654) that could explain 91% of the total variance. Wu et al. (2009) located QTLs on chromosome 8 (LG A2), 11 (LG B1), 18 (LG G), 12 (LG H) and 9 (LG K) in PI 437654. Nematode populations with different HG Types would almost always have different sets of QTLs in the same study (Yue et al., 2001a; Guo et al., 2006a; Wu et al., 2009). Thus it is better to map the resistance to several nematode populations or do a validation experiment to confirm the QTL effect.

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