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RESEARCH

Stacking Resistance Alleles from Wild and Domestic Soybean Sources Improves Soybean Cyst Nematode Resistance

Myungsik Kim, David L. Hyten, Terry L. Niblack, and Brian W. Diers*

ABSTRACT

The soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe) is the most economically important soybean [*Glycine max* (L.) Merr.] pathogen in the United States. Field SCN populations are adapting to the narrowly based SCN resistance currently deployed in soybean cultivars. The objective of our research was to measure the effects of combinations of SCN resistance genes or quantitative trait loci (QTL) from the wild soybean (*Glycine soja* Siebold & Zucc.) PI 468916 and the domesticated soybean accessions PI 88788 and PI 437654. Two populations were developed to test the combinations of QTL and genes. Both populations segregated for the *G. soja* resistance QTL cqSCN-006 and cqSCN-007. Population 1 also segregated for resistance from PI 88788 and Population 2 segregated for resistance from PI 437654. The populations were tested for resistance to three SCN isolates in a greenhouse and with single nucleotide polymorphism (SNP) and simple sequence repeat (SSR) markers. In both populations, the two *G. soja* resistance alleles significantly increased SCN resistance compared with the alternative alleles. The SCN resistance alleles *rhg1* and *Rhg4* from PI 437654 and *rhg1-b* from PI 88788 also significantly increased resistance compared with the alternative alleles. The two *G. soja* QTL alleles significantly enhanced the resistance derived from PI 88788. These results show that SCN resistance can be increased through stacking genes and QTL from multiple resistance sources.

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Abbreviations: CTAB, hexadecyltrimethylammonium bromide; EDTA, ethylenediaminetetraacetic acid; FI, female index; H^2 , broad-sense heritability; HG, *Heterodera glycines* Ichinohe; LG, linkage group; LOD, logarithm of the odds; MQM, multiple QTL model; PCR, polymerase chain reaction; PVC, polyvinyl chloride; QTL, quantitative trait loci; SCN, soybean cyst nematode; SNP, single nucleotide polymorphism; SSR, simple sequence repeat; USLP, Universal Soy Linkage Panel.

SOYBEAN CYST NEMATODE (SCN) is the most damaging pest of soybean in the United States based on yield loss estimates (Wrather and Koenning, 2006). Because of the importance of SCN, there has been focused research on host resistance to this pest. This research has led to the identification of at least 118 SCN resistant soybean plant introductions from the USDA-ARS soybean germplasm collection (Arelli et al., 2000). Some of these resistance sources have been used in breeding programs and resistant cultivars have been available in the United States since 1965 (Brim and Ross, 1966). Although many sources of resistance are available, more than 94% of the SCN resistant cultivars in Illinois trace their source of resistance to only PI 88788 (Shier, 2008). This wide use of cultivars with resistance from a single source is leading to an increased frequency of SCN populations that can overcome this resistance. In a survey of soil samples collected in fields from Illinois in 2005, it was found that 70% of the SCN positive samples had populations that could overcome PI 88788 resistance (Niblack et al., 2008).

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The genetic basis of resistance to SCN has been studied and many quantitative trait loci (QTL) controlling this resistance have been mapped (Concibido et al., 2004). In the majority of the sources of resistance studied, the resistance gene *rhg1* was mapped as a QTL onto chromosome 18 (formerly linkage group [LG] G). This gene was mapped in 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), PI 88287 (Bassuner et al., 2001), and PI 404198A (Guo et al., 2006). In addition, research showed that PI 437654 and PI 88788 have different alleles at *rhg1* (Brucker et al., 2005). The allele from PI 88788 was recently designated *rhg1-b* by the Soybean Genetics Committee (Kim et al., 2010). *rhg1* was detected in most studies with QTL mapping methods because SCN resistance typically segregates quantitatively in populations. Although SCN resistance is quantitatively inherited, *rhg1* has a gene designation because it often contributes a sufficiently large effect in populations to be mapped as a Mendelian trait. In this paper, we will use the term gene in reference to *rhg1* and a second named and mapped gene *Rhg4*, but the resistance genes or QTL from *Glycine soja* Siebold & Zucc., which are distinct loci from *rhg1* and *Rhg4*, will be referred to as QTL. However, in some cases the terms QTL and gene will be used interchangeably for ease in explanation.

Because of the wide use of PI 88788 as a resistance source, it has been the focus of a number of studies. These mapping studies have revealed the presence of only two resistance QTL that can be traced to this source (Concibido et al., 1997; Glover et al., 2004). This includes *rhg1-b* on chromosome 18, which also has the confirmed QTL (cq) designation cqSCN-001, and a second QTL on chromosome 16 (formerly LG J), which has the designation cqSCN-003 (Glover et al., 2004).

In contrast to the mapping of only two QTL from PI 88788, many resistance QTL have been mapped from PI 437654 (Webb et al., 1995; Wu et al., 2009). This may explain why PI 437654 is resistant to more HG (*Heterodera glycines* Ichinohe) types than PI 88788 (Anand et al., 1988; Diers et al., 1997; Niblack et al., 2002). In a recent study, Wu et al. (2009) detected QTL for SCN resistance from PI 437654 on chromosomes 1 (LG D1a), 4 (LG C1), 8 (LG A2), 9 (LG K), 11 (LG B1), 12 (LG H), 18 (LG G), and 20 (LG I) and epistatic interactions mapping to all chromosomes except chromosomes 2 (LG D1b), 9 (LG K), and 14 (LG B2). Consistent with previous studies, the most important resistance allele mapped from PI 437654 was *rhg1*. This allele was significantly associated with resistance to four of the five SCN isolates tested. In addition, the *Rhg4* resistance allele on chromosome 8 was identified as the second most important QTL for two of the five isolates.

The genetic basis of SCN resistance in the *G. soja* accession PI 468916 has been studied and two resistance QTL were

mapped onto chromosomes 15 (LG E) and 18 (LG G) (Wang et al., 2001). These QTL were mapped to unique genetic regions that had not been previously associated with SCN resistance and the chromosome 15 QTL was given the confirmed QTL designation cqSCN-006 and the chromosome 18 QTL the designation cqSCN-007 (available at <http://soybase.org> [verified 20 Jan. 2011]). The resistance alleles were then backcrossed into a domestic soybean parent and their positions were further localized (Kabelka et al., 2005). Field studies later showed that the *G. soja* resistance alleles were not significantly associated with yield drag in fields with low to moderate SCN pressure (Kabelka et al., 2006).

Soybean breeders need new resistance sources, or combinations of resistance genes from different resistance sources, that can provide a more broadly based SCN resistance than is currently available from only PI 88788. The potential benefits of stacking SCN resistance genes from different sources to broaden host resistance to SCN have not been extensively studied. The objective of our study was to define the effects of SCN resistance genes or QTL from PI 468916, PI 88788, and PI 437654 when combined into common genetic backgrounds. This research specifically focused on cqSCN-006 and cqSCN-007 from *G. soja*, *rhg1-b* from PI 88788, and *rhg1* and *Rhg4* from PI 437654.

MATERIALS AND METHODS

Population Development

Population 1

Population 1 was developed to segregate for *rhg1-b* from PI 88788 and *G. soja* resistance QTL cqSCN-006 and cqSCN-007 from PI 468916. This population is the same as the LDX01-2 population in Kabelka et al. (2006). The population was developed by first backcrossing the two *G. soja* resistance QTL into the background of the Iowa State University experimental line A81-356022. During each cycle of backcrossing, markers linked to both QTL were used to select the *G. soja* SCN resistance alleles. An F₁ plant developed through three backcrosses (BC₃F₁) that carried both *G. soja* alleles was then crossed with the cultivar Dwight (Nickell et al., 1998), which carries the SCN resistance allele *rhg1-b* from PI 88788. F₁ plants produced through this cross were tested with markers linked to the *G. soja* resistance QTL and an F₁ plant heterozygous for both genes was selected. The population was then advanced through single-seed descent to the F₃ generation and then advanced to the F_{3:6} generation by growing each of 81 lines in bulk. When available seed became exhausted for some lines, a single F₆ plant was grown from each of the 81 lines and F_{6:7} lines from this population were developed.

Population 2

A second population of 100 F_{3:4} lines (Population 2) was developed from a cross of LD00-2817P with the germplasm line LDX01-1-65 (Diers et al., 2005). LD00-2817P is a high-yielding germplasm line with the SCN resistance sources PI 437654,

Peking, and PI 88788 in its pedigree (Diers et al., 2010). Marker analysis and the SCN resistance profile of LD00-2817P indicate that it carries *rhg1* from either Peking or PI 437654 and not *rhg1-b* from PI 88788. LDX01-1-65 was developed by backcrossing four times cqSCN-006 and cqSCN-007 from PI 468916 into the background of A81-356022. Population 2 was advanced to the F₃ generation through single-seed descent and each F₃ plant was bulk harvested to derive 100 F_{3,4} lines.

Soybean Cyst Nematode Bioassays

The SCN bioassays were performed individually for each soybean population and SCN isolate under greenhouse conditions from 2007 to 2009. The SCN bioassays were conducted as described by Niblack et al. (2009) using the SCN isolates PA1, PA3, and PA5 that were maintained on the susceptible cultivar Macon at the University of Illinois at Urbana-Champaign. These isolates were originally obtained from Dr. Prakash Arelli, USDA-ARS Mid South Area, Jackson, TN.

For Population 1, the bioassays with the isolates PA1 and PA3 were done by infesting F₆ plants from 81 F₃-derived lines. For the isolate PA5, the bioassays were done with F₇ plants from 81 F₆-derived lines. For Population 2, the bioassays for the three isolates were completed by infesting F₄ plants from 100 F₃-derived lines.

Soybean cyst nematode isolates were increased on Macon and the cysts were crushed to release eggs. The eggs were then collected, transferred to water, centrifuged at 300 g for 4 min and layered on the surface of a 45% sucrose solution. The egg solution was centrifuged at 300 g for 4 min and rinsed with water and a sample was counted to estimate the egg numbers.

Seed of the lines being tested were rolled in germination paper and germinated for 3 d in a growth chamber at 27°C. The seedlings were transplanted into polyvinyl chloride (PVC) tubes filled with steam-sterilized sandy soil packed into plastic crocks that were suspended in a thermo-regulated water bath. Before transplanting, each PVC tube was infested with approximately 1200 eggs in 1 mL distilled water with a micropipette. Each PVC tube contained one plant and was an experimental unit. The lines, SCN differentials, and parents were randomized and replicated three times for each isolate. The root systems of plants were maintained at 27 ±1°C and the plants were grown under a 16-h daylength for 30 d. The plants were then removed from the tubes by gentle soaking in water to loosen soil but not dislodge SCN females. The roots were placed on nested 850-µm aperture over 250-µm aperture sieves and females were removed from the roots with a water spray and washed into counting dishes. The cysts were counted under the stereomicroscope. Macon was used as the susceptible control to calculate a female index (FI) for each plant with the formula (Golden et al., 1970) FI (%) = (number of female cyst nematodes on a given individual/average number of female nematodes on Macon) × 100. Lines were classified as resistant (FI = 0–9), moderately resistant (FI = 10–30), moderately susceptible (FI = 31–60), and susceptible (FI > 60) according to Schmitt and Shannon (1992).

DNA Extraction

Genomic DNA was extracted from a bulk sample of fresh leaf tissue from 10 seedlings of each F₃-derived line in both Populations 1 and 2. DNA was also isolated from the F₆ plants used to

develop the F_{6,7} lines in Population 1. DNA was extracted using the hexadecyltrimethylammonium bromide (CTAB) method as described by Saghai Maroof et al. (1984) with slight modifications. Soybean leaf tissue was freeze dried for 48 h and powdered with a paint shaker for 2 min, and 5 mL of 1x CTAB DNA extraction buffer (50 mM Tris, pH 8.0, 0.7 M NaCl, 10 mM ethylenediaminetetraacetic acid [EDTA], 1% [w/v] CTAB, and 0.1% [v/v] 2-mercaptoethanol) was added to the powdered tissue. The solution was incubated for 60 min at 60°C and, after incubation, 5 mL of chloroform-octanol (24:1) was added and mixed by inversion and centrifuged at 3400 rpm for 10 min. The aqueous phase was removed and the DNA was precipitated by adding 2/3 volume of isopropanol. Precipitated DNA was spooled on a micropipette, transferred to a new tube, washed with 5 mL of a 70% ethanol, and then dried at room temperature overnight. Dried DNA was dissolved in 0.1x Tris-EDTA (TE) buffer and used as templates in polymerase chain reactions (PCR).

Genetic Marker Analysis

The F₃- and F₆-derived lines in Population 1 and the F₃-derived lines in Population 2 were evaluated according to Cregan and Quigley (1997) with simple sequence repeat (SSR) markers linked to SCN resistance genes or QTL known to be segregating in the populations. Primer sequences were obtained from the Soybase website (<http://soybase.org> [verified 10 Jan. 2011]) and the PCR products were sized via electrophoresis on 6% nondenaturing polyacrylamide gels at 340 V for 2 h and 20 min (Wang et al., 2003). The products were stained with a 1 µg mL⁻¹ ethidium bromide staining solution for 25 min and visualized under ultraviolet (UV) light.

The F₆-derived lines from Population 1 and the F₃-derived lines from Population 2 were evaluated with the Illumina GoldenGate 1536 Universal Soy Linkage Panel 1.0 (USLP 1.0) (Hyten et al., 2010). The GoldenGate assay was performed as per the manufacture's protocol and as described by Hyten et al. (2008) with the sentrix array matrix read on the Illumina BeadStation 500G (Illumina Inc., San Diego, CA). Scoring of the GoldenGate data was accomplished with the Illumina software BeadStudio v.3.2 and visually inspected to ensure that homozygous and heterozygous clusters were properly assigned.

Statistical Analysis

Broad-sense heritability (H²) was calculated for resistance to each isolate in both populations using variance components as described by Fehr (1987). Confidence intervals for the heritability estimates were calculated according to Knapp et al. (1985). The mapping of QTL controlling SCN resistance was completed separately for each population and SCN isolate with the program MapQTL 4.0 (Van Ooijen, 2000; Van Ooijen and Maliapaard, 1996) using the map position of these markers from Hyten et al. (2010). The data were then analyzed with the restricted multiple QTL model (MQM) of MapQTL 4.0, which uses markers as cofactors in a multiple-QTL model. Cofactor markers were selected by the automatic cofactor selection option ($\alpha = 0.010$) to identify a set of cofactors. A marker was used as a cofactor if it was associated with a logarithm of the odds (LOD) score of at least 1.5. If the multifactor analysis decreased the LOD score to below 1.5, that marker was dropped as a cofactor and the analysis was repeated until there was no further change in cofactors. For those QTL known to be

Table 1. The reaction of three soybean cyst nematode (SCN) isolates to the *Heterodera glycines* Ichinohe (HG) type differentials and the susceptible cultivar Macon in tests of Population 1 and Population 2.

Population test	Isolate	Avg. no. of females on Macon	Female index [†]							HG type
			1 Peking	2 PI 88788	3 PI 90763	4 PI 437654	5 PI 209332	6 PI 89772	7 PI 548316	
1	PA1	78	1	7	0	0	14	0	27	5.7
1	PA3	63	1	2	1	3	9	0	6	0
1	PA5	408	4	83	0	0	94	ND [‡]	87	2.5.7
2	PA1	101	0	44	0	0	20	0	31	2.5.7
2	PA3	136	0	28	0	0	35	0	28	2.5.7
2	PA5	42	0	65	0	0	67	0	81	2.5.7

[†]Female Index = $(N_1/N_2) \times 100$, where N_1 is the average number of females per entry and N_2 is the average number of females on Macon.

[‡]ND, not determined.

segregating in a population, including *rhg1*, *rhg1-b*, *Rhg4*, cqSCN-006, and cqSCN-007, a significance LOD threshold of 2.0 was used. For any other QTL detected in this study, the significance threshold was set to 2.8 for Population 1 and 2.9 for Population 2. These higher thresholds correspond to a genome-wide probability of 0.05 as determined by permutation tests in MapQTL 4.0.

The QTL mapping with MapQTL 4.0 was done for all three SCN isolates in Population 1 with the SSR and single nucleotide polymorphism (SNP) marker data set from the F_6 -derived lines. These data were used in the mapping although the SCN phenotypes were obtained from F_3 -derived lines for the SCN isolates PA1 and PA3 while the F_6 -derived lines were tested with PA5. Analyzing phenotypes collected on F_3 -derived lines with markers collected on F_6 -derived lines would have reduced our ability to detect QTL because it is predicted that only one-eighth of the F_3 -derived lines that were expected to be segregating for any given marker would likely still be segregating in the F_6 -derived lines. Although this is a concern, we used the F_6 marker data in this analysis because SNP marker data were only available for this generation.

The SNP or SSR marker closest to the peak for each significant QTL from the MQM analysis was tested in a multivariate ANOVA with PROC GLM in SAS (SAS Institute, 2001). All two- and three-way interactions between significant markers were tested and those markers that were not significant at $p = 0.05$ were removed from the model and the analysis was repeated. An exception to this analysis was the data from PA1 and PA3 on Population 1. For these isolates, single-marker analysis was done in SAS using SSR markers collected from the F_3 -derived lines and the interactions were tested using SSR markers closely linked to the significant QTL. The R^2 values from the multivariate models are estimates of the total phenotypic variance explained by the genes or QTL and their interactions. These R^2 values were used to estimate the proportion of the genotypic variance explained by the QTL using the ratio R^2/H^2 (Schön et al., 1994).

RESULTS

Soybean Cyst Nematode Bioassays

The FI values in the population evaluations and HG type tests were calculated using Macon as a susceptible control instead of the recommended 'Lee 74' (Niblack et al., 2002, 2009). Macon was used because Lee 74 did not germinate in some experiments. Soybean cyst nematode reproduction on Macon varied from a low of 42 cysts plant⁻¹ in the test

with PA5 in Population 2 to a high of 408 cysts for PA5 in Population 1 (Table 1). The biotypes of the SCN isolates did not respond as expected in the HG type tests. The isolate PA1 was originally defined as a Race 1 isolate (HG type 2-), PA3 was a Race 3 (HG type 0-), and PA5 was a Race 5 (HG type 2-). In our tests, PA1 was an HG type 5.7 (equivalent to a Race 3 or 6) or 2.5.7 (Race 1 or 5), PA3 was an HG type 0 (Race 3 or 6) or 2.5.7 (Race 1 or 5), and PA5 was an HG type 2.5.7 (Race 1 or 5) (Table 1). It is not unusual to observe changes in SCN HG types as these populations are heterogeneous mixtures of nematodes that can shift to different phenotypes over time (Colgrove et al., 2002).

Results from Soybean Cyst Nematode Tests of Populations

Population 1

The FI of lines in both populations segregated quantitatively with a continuous distribution of FI values (Fig. 1). Population 1 was developed by crossing Dwight, which carries *rhg1-b* from PI 88788, to a BC_3F_1 plant carrying cqSCN-006 and cqSCN-007. LDX01-1-65, which carries cqSCN-006 and cqSCN-007, was included in the PA1 and PA3 tests of Population 1 and it was found to be moderately susceptible to both isolates (Fig. 1; Table 2). A81-356022, the susceptible recurrent parent used in developing LDX01-1-65 and the BC_3F_1 parent of Population 1, was susceptible to both isolates with FI values >100. Dwight was resistant to PA3 but susceptible to PA1 (FI = 62 for PA1). The FI of parents was not determined for PA5 because these genotypes germinated poorly in this test. Dwight would likely have been susceptible to PA5 because PI 88788, its resistance source, was susceptible to the isolate (FI = 83) (Table 1). The range of FI for lines in Population 1 was 15 to 121 for PA1, 3 to 120 for PA3, and 13 to 114 for PA5 (Fig. 1; Table 2). The H^2 of FI ranged from 0.45 to 0.91 (Table 2).

Population 2

Population 2 was developed by crossing LDX01-1-65 with LD00-2817P. The line LD00-2817P was shown to likely contain *rhg1* from PI 437654 or Peking (Diers et al., 2010) and probably *Rhg4*. In tests with all three isolates, LD00-2817P

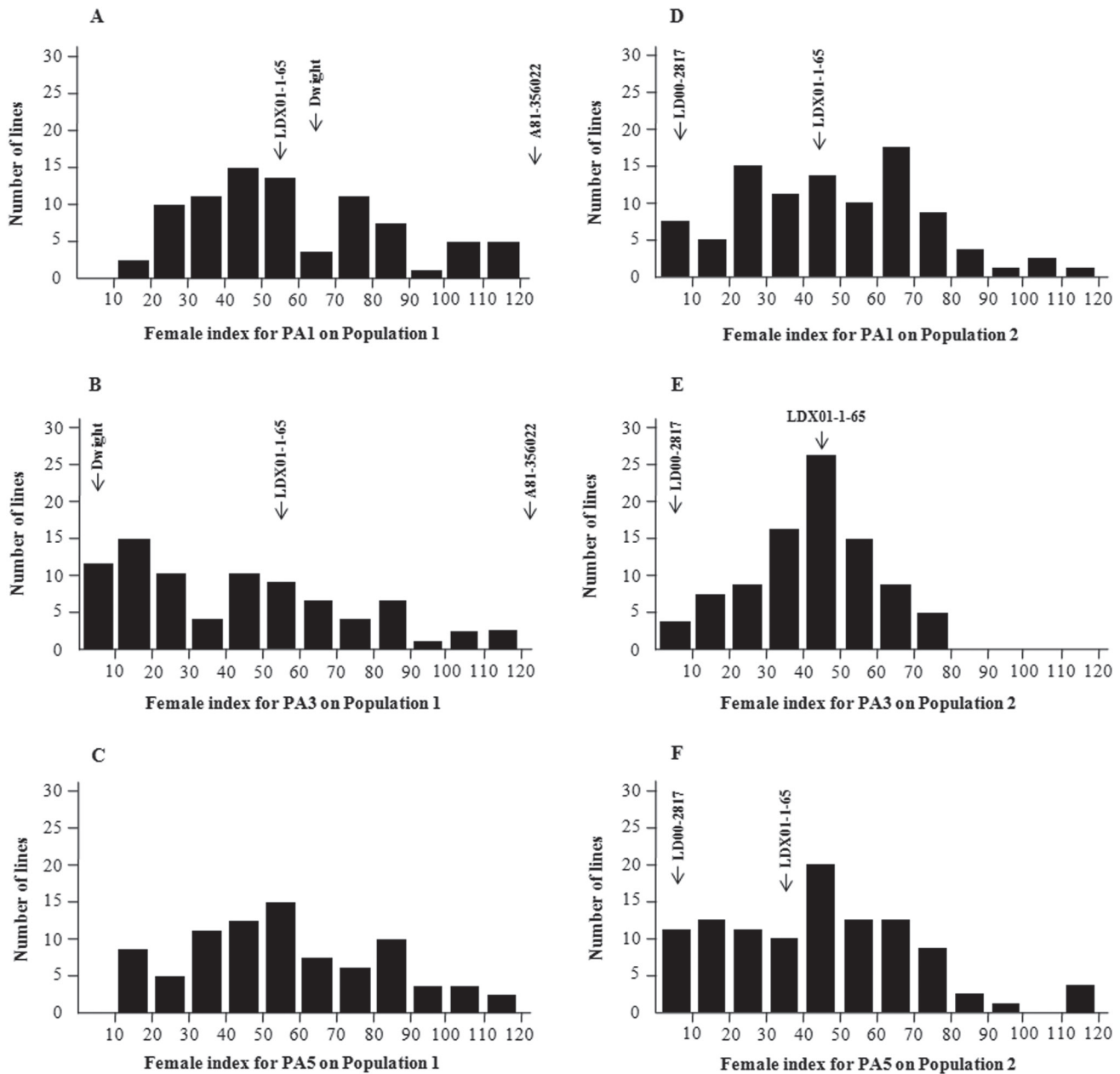


Figure 1. Distribution of female index (FI) for soybean cyst nematode (SCN) isolates PA1, PA3, and PA5 in Population 1, which segregates for resistance from *Glycine soja* PI 468916 and PI 88788 (A–C), and Population 2, which segregates for resistance from *G. soja* PI 468916, PI 437654, and Peking (D–F). Parental female index values are indicated by arrow in each distribution except for the PA5 test of Population 1. In this test, the parents germinated poorly.

was rated as resistant and LDX01-1-65 was rated as moderately susceptible (Fig. 1; Table 2). The means of lines in the populations ranged from 0 to 125 for PA1, 4 to 77 for PA3, and 0 to 119 for PA5. The H^2 for FI ranged from 0.64 to 0.77 (Table 2).

Detection of Soybean Cyst Nematode Resistance Quantitative Trait Loci

Population 1

Quantitative trait loci in Population 1 were mapped with 252 polymorphic SNP markers from the USLP 1.0 and three SSR markers. The population was expected to segregate for the resistance allele *rhg1-b* from Dwight and

resistance alleles at cqSCN-006 and cqSCN-007 originating from *G. soja* PI 468916. These QTL were detected in different combinations with the MQM analysis for the SCN isolates and no other QTL were detected in the population (Table 3). When QTL were significant, alleles tracing to *G. soja* were associated with greater resistance than the alleles from Dwight for cqSCN-006 and cqSCN-007 and the Dwight allele for *rhg1-b* was associated with greater resistance than the allele from the recurrent parent A81-356022. This distribution of effects fit expectations based on the source of the resistance alleles. Although the LOD peaks were not in the same position in the tests for

Table 2. Estimates of means, ranges, broad-sense heritabilities (H^2), proportion of the phenotypic variance explained in multiple quantitative trait loci (QTL) models (R^2) with and without the interaction effect, and proportion of genetic variance explained by the QTL model and interactions in Population 1, which segregates for resistance from *Glycine soja* PI 468916 and PI 88788, and Population 2, which segregates for resistance from *G. soja* PI 468916, PI 437654, and Peking.

SCN† isolate	Population range	Mean				H^2 ‡	R^2 without interaction	R^2 with interaction	R^2/H^2 §
		Population	A81-356022	Dwight	LDX01-1-65 LD00-2817P				
Female Index									
Population 1									
PA1	15–121	57.6	152.8	61.8	53.7	0.45 (0.25, 0.58)¶	0.35	NS#	0.78
PA3	3–120	40.7	147.2	9.6	51.5	0.82 (0.74, 0.87)	0.53	NS	0.64
PA5	13–114	56.3				0.91 (0.87, 0.93)	0.74	0.75	0.82
Population 2									
PA1	0–125	47.7			40.3	0.77 (0.68, 0.82)	0.63	0.73	0.95
PA3	4–77	43.7			49.6	0.64 (0.51, 0.75)	0.57	0.71	1.11
PA5	0–119	46.7			34.7	0.73 (0.63, 0.79)	0.59	0.76	1.04

†SCN, soybean cyst nematode.

‡ H^2 , broad-sense heritability.

§Proportion of genetic variation explained with the QTL model and interactions.

¶Lower and upper 90% confidence limits in parentheses.

#NS, no significant interaction.

Table 3. Quantitative trait loci (QTL) for resistance to soybean cyst nematode (SCN) isolates PA1, PA3, and PA5 based on restricted multiple QTL mapping in both Population 1, which segregates for resistance from *Glycine soja* PI 468916 and PI 88788, and Population 2, which segregates for resistance from *G. soja* PI 468916, PI 437654, and Peking.

SCN isolate	Gene or QTL	Chromosome	Marker nearest to the QTL peak	Map position (cM)†	LOD‡	R^2	Add.§
Population 1							
PA1	cqQTL-006	15	ss107926496	78.6	3.1	12.5	9.5
	<i>rhg1-b</i>	18	ss107914462	11.1	3.8	15.6	-10.6
PA3	<i>rhg1-b</i>	18	ss107927739	1.6	8.6	33.5	-17.3
PA5	cqQTL-006	15	ss107926496	78.6	15.6	34.2	15.3
	cqQTL-007	18	ss107912619	78.3	17.3	40.0	17.4
Population 2							
PA1	<i>Rhg4</i>	8	ss107915471	46.4	5.4	10.1	-17.2
	cqQTL-006	15	Satt491	75.6	5.0	9.1	9.4
	<i>rhg1</i>	18	ss107921425	9.9	9.4	19.2	-15.3
	cqQTL-007	18	ss107912912	78.5	9.8	20.3	13.7
PA3		16	ss107920706	25.3	3.3	5.8	7.7
	<i>Rhg4</i>	8	ss107925847	49.3	2.1	6.6	-4.2
	cqQTL-006	15	ss107913808	77.7	3.2	10.1	5.6
	<i>rhg1</i>	18	ss107914420	10.4	4.4	14.6	-8.0
PA5	cqQTL-007	18	ss107913107	73.3	7.5	28.2	9.3
	cqQTL-006	15	ss107913808	77.7	9.6	23.1	16.1
	<i>rhg1</i>	18	Satt309	10.1	3.2	6.5	-9.2
	cqQTL-007	18	ss107912912	78.5	11.6	29.4	17.6

†Position of the marker nearest the QTL peak on the single nucleotide polymorphism (SNP) map (Hyten et al., 2010).

‡LOD, logarithm of the odds.

§Additive effect of an allele substitution, which is equivalent to the difference between the means of the two homozygous classes divided by two. Alleles are coded such that in Population 1, the positive additive effects occur when resistance alleles are from *G. soja* PI 468916 and negative effects occur when resistance alleles are from the cultivar Dwight (resistance from PI 88788). In Population 2, the positive additive effects occur when resistance alleles are from LDX01-1-65 (resistance from *G. soja*) and negative effects occur when resistance alleles are from the cultivar LD00-2817P (resistance from PI 437654, Peking, and PI88788).

each isolate (Table 3), the peaks were sufficiently close to indicate that the same QTL were likely conferring resistance to each SCN isolate for which they were significant.

For PA1, markers linked to cqSCN-006 and *rhg1-b* were significant in the MQM analysis (Table 3) using the SNP and SSR marker data from the F_6 -derived lines and the SCN FI from the F_3 -derived lines. When SSR marker data from the F_3 -derived lines linked to the three known

SCN resistance genes segregating in the population were used in a single-marker analysis with SAS, cqSCN-007 was also significant ($p < 0.006$). In the multivariate model, no significant interaction ($p = 0.05$) was detected between these three significant loci and together they explained 35% of the total variance for resistance (Table 2). Lines in the population homozygous for the three susceptible-

ity alleles had a FI of 74.5 compared to a mean of 28.1 for lines homozygous for the three resistance alleles.

Only *rhg1-b* was significant for PA3 in the MQM analysis with the SNP data from the F₆-derived lines and the FI values from the F₃-derived lines. *rhg1-b* was also the only QTL or gene significant when the single-marker analysis was done in SAS using the SSR markers from the F₃-derived lines that were used to collect the FI values. This QTL was associated with 53% of the total variance for resistance in this population when the marker data from the F₃-derived lines were used (Table 2). Those lines homozygous for the resistance allele had a FI of 15.8 compared to 64.4 for homozygous susceptible lines.

The F₆-derived lines were tested for resistance to PA5 and both cqSCN-006 and cqSCN-007 were significant in the MQM analysis for this isolate, but *rhg1-b* was not. It was expected that *rhg1-b* would not be significant given that PI 88788 was susceptible to the isolate. When the two significant QTL were included in a multivariate model, a significant interaction was detected. The two QTL and their interaction explained 75% of the total variation for resistance to the isolate (Table 2). Those lines homozygous for the two resistance alleles had a FI of 24.0 compared with a mean FI of 88.7 for the homozygous susceptible lines.

Population 2

Population 2 segregated for 390 SNP markers from the USLP 1.0 and the data from these SNP markers and three SSR markers were used to map QTL. The population was expected to segregate for cqSCN-006, cqSCN-007, *rhg1*, and *Rhg4* based on parentage. In contrast to Population 1, the two *G. soja* QTL and *rhg1* were significantly associated with resistance to each of the three isolates in the MQM analysis (Table 3). Consistent with expectations, the allele conferring increased resistance was from LD00-2817P for *rhg1* and *Rhg4* and from LDX01-1-65 for cqSCN-006 and cqSCN-007.

For PA1, cqSCN-006, cqSCN-007, *rhg1*, *Rhg4*, and a new QTL on chromosome 16 (formerly LG J) were significant in the MQM analysis (Table 3). The chromosome 16 resistance allele was derived from LDX01-1-65 and it maps to a region where no other SCN resistance QTL have been reported in the literature (<http://soybase.org> [verified 10 Jan. 2011]). To determine the origin of the chromosome 16 resistance allele, the recurrent parent A81-356022 and the donor parent PI 468916 were compared to LDX01-1-65 with the 1536 markers in USLP 1.0. There was no evidence of introgression of regions on chromosome 16 from PI 468916 into LDX01-1-65, indicating that the resistance allele on this chromosome originated from A81-356022 and not PI 468916.

The R² values for the PA1 resistance genes and QTL ranged from 20.3 (cqSCN-007) to 5.8 (chromosome 16 QTL). When markers linked to these genes and QTL and all possible two-way interactions were added to a

multivariate model (Table 2), significant interactions between *Rhg4* and *rhg1* and between *Rhg4* and cqSCN-006 were detected. The significant markers and the two significant interactions explained 73% of the total variation for resistance in the multivariate model (Table 2). There were no lines in the population that carried all five alleles conferring resistance or susceptibility; however, those lines that carried four resistance alleles had FI averaging 4.9 and those with four susceptibility alleles averaged 79.0.

Rhg4, *rhg1*, cqSCN-006, and cqSCN-007 were all significant for PA3 in the MQM analysis (Table 3). The multivariate SAS model that included the genes and QTL and all two-way interactions revealed only one significant interaction: between *rhg1* and cqSCN-006. The genes and QTL together with the significant interaction explained 71% of the total variation for resistance (Table 2). Those lines that carried three or four resistance alleles had a FI averaging 23.3 and those with three or four susceptibility alleles had a FI of 57.6.

For PA5, cqSCN-006 and cqSCN-007 and *rhg1* were significant in addition to two-way interactions between cqSCN-006 and cqSCN-007 and between *rhg1* and cqSCN-007 and a three-way interaction between the three loci. The combination of the two QTL, *rhg1*, and their interactions explained 76% of the total variation for resistance. Those lines with three resistance alleles had an FI of 14.3 and those with three susceptibility alleles had an FI of 92.1.

DISCUSSION

The populations used in these experiments were developed to test the effects of cqSCN-006 and cqSCN-007 in combination with *rhg1-b* from PI88788 and with *rhg1* and *Rhg4* from PI 437654 or Peking. These QTL and genes were all detected in the current study showing the effectiveness of cqSCN-006 and cqSCN-007 in combination with genes from these other resistance sources.

Because the main objective of this study was to test these new combinations of known QTL that have fairly large effects, we used relatively small populations. The employment of these small populations may have resulted in the inability to detect some small-effect QTL segregating in these populations.

The locations of *rhg1*, *rhg1-b*, *Rhg4*, cqSCN-006, and cqSCN-007 were mapped in the current study and matched their positions reported in previous studies. Cregan et al. (1999) and Kim et al. (2010) reported that the SSR marker Satt309 mapped within 1 cM on the proximal (centromeric) side of *rhg1* and both *rhg1* and *rhg1-b* mapped within 8.5 cM of Satt309 in the current study. *Rhg4* was previously mapped near the *i* locus (Matson and Williams, 1965; Webb et al., 1995), which is at cM position 48 on the 2003 composite soybean map (<http://soybase.org> [verified 10 Jan. 2011]). In Population 2, *Rhg4* was mapped within

2 cM of this position. Both cqSCN-006 and cqSCN-007 also mapped close to the regions where they were positioned by Kabelka et al. (2005).

A previously undetected QTL was mapped in the current study to chromosome 16 in Population 2. There are previous reports of a SCN resistance QTL on chromosome 16, which includes the confirmed resistance QTL cqSCN-003 originating from PI 88788 (Glover et al., 2004). The region where cqSCN-003 maps was previously identified to have resistance QTL from PI 90763 (Guo et al., 2005; Concibido et al., 1997) and PI 209332 (Concibido et al., 1994). The QTL detected in Population 2 maps more than 40 cM from cqSCN-003 to a region where there are no previously mapped SCN resistance QTL listed on the Soybase website (<http://soybase.org> [verified 10 Jan. 2011]), suggesting that this is a newly identified SCN resistance QTL.

Both Population 1 and 2 were developed using backcross lines that have A81-356022 as the recurrent parent; however, the chromosome 16 QTL was mapped in Population 2 but not in Population 1. The marker analysis showed that the resistance allele for this QTL originated from A81-356022 in Population 2, so this resistance QTL should have been present in both populations. These results suggest that there is background specificity in the effect of the QTL. Alternatively, although the chromosome 16 QTL is significant with a genome-wide threshold of 0.05, it is also possible that the detection of this QTL was a type 1 error.

In contrast to detecting only the effects of *rhg1* and *Rhg4* from LD00-2817P, our parent with resistance tracing to PI 437654, Wu et al. (2009) detected SCN resistance genes and QTL at nine locations in the genome from PI 437654 using five SCN isolates. Of these genes and QTL, generally *rhg1* and *Rhg4* had the largest effects although a QTL on chromosome 11 (LG B1) had a large effect for the Race 5 isolate used in the study. In contrast to Wu et al. (2009), who developed their population by directly crossing PI 437654 to a susceptible line, we developed our population using the elite germplasm line LD00-2817P as a parent. Minor SCN resistance QTL in PI 437654 were especially likely to be lost during the breeding process resulting in LD00-2817P not carrying the full complement of genes and QTL detected by Wu et al. (2009). Evidence of this loss is that Ina, the parent LD00-2817 inherited its resistance from PI 437654, was reported to not have full resistance to SCN Race 14 (Nickell et al., 1999). In contrast, PI 437654 was shown to have complete resistance to this race (Diers et al., 1997). In addition, Diers et al. (2010) found that LD00-2817P generally had greater FI values than PI 437654 in a test with 12 SCN isolates. Another possible reason for not identifying minor QTL from PI 437654 is that Population 2 was composed of 100 F₃-derived lines compared with 205 lines in the population used by Wu et al. (2009). The small size of

our population would have reduced our ability to detect minor QTL if they were present in LD00-2817P.

Significant interactions were observed between significant QTL and genes. The interaction observed between *Rhg4* and *rhg1* was previously reported by Wu et al. (2009), Brucker et al. (2005), and Webb et al. (1995) for these genes from PI 437654 and by Meksem et al. (2001) for these genes from Peking. The interaction we observed between *Rhg4* and *rhg1* was SCN isolate specific as it was detected for PA1 but not PA3 or PA5. The SCN isolate specificity of this interaction was also observed by Brucker et al. (2005) and Wu et al. (2009). Brucker et al. (2005) observed a significant interaction between *rhg1* and *Rhg4* for SCN isolate PA3, which was shown to have a HG Type 0 or 7 in that study, but not the isolate TN13, which had a HG Type 1.2.5.7. Wu et al. (2009) detected a significant interaction between *Rhg4* and *rhg1* for Race 1 (HG type 2.5.7) and Race 3 (HG type 0) isolates but not for Race 2 (HG type 1.2.5.7), 5 (HG type 2.7), and 14 (HG type 1.3.5.6.7) isolates.

The heritabilities for resistance varied from a low of 0.45 for PA1 in Population 1 to a high of 0.91 for PA5 in Population 1. Although the PA5 test for Population 1 did have the greatest number of females on the susceptible cultivar Macon for any of the tests, there was no clear association between reproduction on Macon and the heritability of the test. For example, the PA5 test for Population 2 had the least Macon reproduction but had a heritability of 0.73.

The proportion of the genetic variance explained by the QTL and interactions ranged from 0.77 to greater than 1, showing that the mapped genes control most of the genetic variance for resistance (Table 2). The values of greater than 1 obtained for PA3 and PA5 in Population 2 may be the result of our small population sizes, which can lead to an overestimate of R^2 values (Beavis, 1994). A second explanation is that the heritability values were underestimated, which is possible given the fairly large standard errors of the heritability estimates (Table 2).

In general, there was more value in combining cqSCN-006 and cqSCN-007 with PI 88788 resistance than with the PI 437654 or Peking resistance in Population 2. The usefulness of the *G. soja* QTL alleles in the PI 88788 background is shown by Dwight not being resistant to PA1 and PI 88788 not being resistant to PA5 (Table 1; Fig. 1). The deficiencies in the PI 88788 resistance to these isolates were at least partially offset by the addition of cqSCN-006 and cqSCN-007. We observed in Population 2 a significant effect of the two *G. soja* QTL; however, LD00-2817P, the parent with resistance tracing to PI 437654 and Peking, was already resistant to the three SCN isolates used in the study (Fig. 1; Table 2). When lines with different gene and QTL combinations in Population 2 were compared after inoculation with PA1, lines with the resistance alleles at *rhg1* and *Rhg4* and the susceptibility alleles at both *G. soja* alleles had a FI of 2.6,

whereas lines with the resistance allele for the four genes and QTL had a FI of 0.0, showing little advantage of the *G. soja* alleles. In contrast, there was an advantage of combining the *G. soja* alleles with *rhg1* for resistance to PA5 (*Rhg4* was not significant for this isolate). Lines that had only the resistance allele at *rhg1* had a FI of 55.1, whereas lines with *rhg1* and the two *G. soja* alleles had a FI of 14.4.

As stated previously, most SCN resistant cultivars in the midwestern United States have their SCN resistance from PI 88788. The widespread use of this resistance source is at least partially the result of the ability of breeders to combine high yield with this source of resistance. A strategy that breeders may want to consider is combining the PI 88788 resistance with cqSCN-006 and cqSCN-007. This will provide a unique resistance source with a broad diversity of resistance alleles. Although this combination does not give as high a level of resistance as PI 437654, it would give broad-based partial resistance that could help protect yields.

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Stacking Resistance Alleles from Wild and Domestic Soybean Sources Improves Soybean Cyst Nematode Resistance

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In Volume 51, Number 3, pp. 934–943, seven values in the eighth column of Table 3 were incorrect. The corrected table appears below.

Table 3. Quantitative trait loci (QTL) for resistance to soybean cyst nematode (SCN) isolates PA1, PA3, and PA5 based on restricted multiple QTL mapping in both Population 1, which segregates for resistance from *Glycine soja* PI 468916 and PI 88788, and Population 2, which segregates for resistance from *G. soja* PI 468916, PI 437654, and Peking.

SCN isolate	Gene or QTL	Chromosome	Marker nearest to the QTL peak	Map position (cM) [†]	LOD [‡]	R ²	Add. [§]
Population 1							
PA1	cqQTL-006	15	ss107926496	78.6	3.1	12.5	9.5
	<i>rhg1-b</i>	18	ss107914462	11.1	3.8	15.6	-10.6
PA3	<i>rhg1-b</i>	18	ss107927739	1.6	8.6	33.5	-17.3
PA5	cqQTL-006	15	ss107926496	78.6	15.6	34.2	15.3
	cqQTL-007	18	ss107912619	78.3	17.3	40.0	17.4
Population 2							
PA1	<i>Rhg4</i>	8	ss107915471	46.4	5.4	10.1	-17.2
	cqQTL-006	15	Satt491	75.6	5.0	9.1	9.4
	<i>rhg1</i>	18	ss107921425	9.9	9.4	19.2	-15.3
	cqQTL-007	18	ss107912912	78.5	9.8	20.3	13.7
PA3		16	ss107920706	25.3	3.3	5.8	7.7
	<i>Rhg4</i>	8	ss107925847	49.3	2.1	6.6	-4.2
	cqQTL-006	15	ss107913808	77.7	3.2	10.1	5.6
	<i>rhg1</i>	18	ss107914420	10.4	4.4	14.6	-8.0
	cqQTL-007	18	ss107913107	73.3	7.5	28.2	9.3
PA5	cqQTL-006	15	ss107913808	77.7	9.6	23.1	16.1
	<i>rhg1</i>	18	Satt309	10.1	3.2	6.5	-9.2
	cqQTL-007	18	ss107912912	78.5	11.6	29.4	17.6

[†]Position of the marker nearest the QTL peak on the single nucleotide polymorphism (SNP) map (Hyten et al., 2010).

[‡]LOD, logarithm of the odds.

[§]Additive effect of an allele substitution, which is equivalent to the difference between the means of the two homozygous classes divided by two. Alleles are coded such that in Population 1, the positive additive effects occur when resistance alleles are from *G. soja* PI 468916 and negative effects occur when resistance alleles are from the cultivar Dwight (resistance from PI 88788). In Population 2, the positive additive effects occur when resistance alleles are from LDX01-1-65 (resistance from *G. soja*) and negative effects occur when resistance alleles are from the cultivar LD00-2817P (resistance from PI 437654, Peking, and PI88788).

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