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ORIGINAL PAPER

QTL associated with horizontal resistance to soybean cyst nematode in *Glycine soja* PI464925B

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Abstract Soybean cyst nematode (*Heterodera glycines* Ichinohe; SCN) is the primary disease responsible for yield loss of soybean [*Glycine max* (L.) Merr.]. Resistant cultivars are an effective management tool; however, the sources currently available have common resistant genes. *Glycine soja* Sieb. and Zucc., the wild ancestor of domesticated soybean, represents a diverse germplasm pool with known SCN resistance. The objectives of this research were to: (1) determine the genetic variation and inheritance of SCN resistance in a *G. max* ('S08-80') × *G. soja* (PI464925B) $F_{4:5}$ recombinant inbred line (RIL) population; and (2) identify and evaluate quantitative trait loci (QTL) associated with SCN resistance. Transgressive segregation for resistance was observed, although neither parent was resistant to the Chatham and Ruthven SCN isolates. Broad sense heritability was 0.81 for the Ruthven and 0.91 for the Chatham isolate. Root dry weight was a significant covariate that influenced cyst counts. One RIL [female index (FI) = 5.2 ± 1.11] was identified as resistant to the Chatham isolate (FI < 10). Seventeen and three RILs infected with Chatham and Ruthven isolates, respectively, had mean adjusted cyst counts of zero. Unique and novel QTL, which derived resistance from *G. soja*, were identified on linkage groups I, K, and O, and individually explained 8, 7 and 5% (LOD = 2.1–2.7) of the total phenotypic variation, respectively. Significant epistatic interactions were found between pairs of SSR markers that individually may or may not have been associated with SCN resistance, which explained between 10 and 15% of the total phenotypic variation. Best-fit regression models explained 21 and 31% of the total phenotypic variation in the RIL population to the Chatham and Ruthven isolates, respectively. The results of this study help to improve the understanding of the genetic control of SCN resistance in soybean caused by minor genes resulting in horizontal resistance. The incorporation of the novel resistance QTL from *G. soja* could increase the durability of SCN-resistance in soybean cultivars, especially if major gene resistance breaks down.

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Introduction

Soybean cyst nematode (*Heterodera glycines* Ichinohe; SCN) is the primary pest responsible for yield losses to the soybean (*Glycine max* [L.] Merr.) crop (Wrather et al. 2001). Annual losses due to SCN in the USA and Canada are estimated at $1.67 B (US) and $20.9 M (US), respectively. Management of SCN remains difficult in commercial production due to the length of the biological cycle (up to 12 years) (Riggs and Niblack 1999), frequent changes in population virulence (Colgrove et al. 2002), and the ease of spread via infested soil (Riggs and Niblack 1999). Effective management primarily relies on crop rotation in combination with resistant cultivars.

Large numbers of SCN-resistant accessions have been identified from *G. max* germplasm collections (Anand et al. 1988; Young 1990; Rao-Arelli et al. 1997), but only a few have been used to develop resistant cultivars (Diers and Arelli 1999; for a review, see Winter et al. 2006). Furthermore, breeding for SCN resistance has been difficult due to the quantitative nature of the trait (for a review, see Concibido et al. 2004), the genetic variation within cyst populations (Colgrove et al. 2002), the amount of time it takes to phenotype experimental soybean lines (Riggs and Niblack 1999), and the environmental factors that affect SCN reproduction (Young and Heatherly 1990; Johnson et al. 1993; Palmateer et al. 2000; Avendaño et al. 2004).

Recently, there has been considerable interest in quantitative trait loci (QTL) associated with SCN resistance. QTL have been identified on 17 of the 20 soybean linkage groups (LG), which explain 1–90% of the total phenotypic variation for resistance (for reviews, see Concibido et al. 2004; Winter et al. 2006). A major QTL, which was flagged using the RFLP marker C006V and designated as *rhg1*, is located on LG G (Concibido et al. 1994) and explains 54% of the total phenotypic variation in disease response in PI 209332 to Race 6, 50% to Race 3 and 35% to Race 1 (Concibido et al. 1996). Separate studies indicated that 'Peking' (Chang et al. 1997; Concibido et al. 1997; Meksem et al. 2001), PI 90763 (Concibido et al. 1997), PI 88788 (Concibido et al. 1997), PI 209332 (Concibido et al. 1994), PI 89772 (Yue et al. 2001), PI 437654 (Webb et al. 1995), 'Peking' + PI 437654 (Prabhu et al. 1999) and 'Peking' + PI 88788 + PI 90763 (Heer et al. 1998) have the major QTL on LG G near *rhg1*. The ability of the *rhg1* locus to yield resistance to multiple SCN races has resulted in extensive mapping of this region. For example, molecular marker Satt309 is only 0.4 cM from the *rhg1* gene (Cregan et al. 1999). A second major QTL for SCN resistance on LG A2 has been designated as the *Rhg4* gene. It is closely linked to the *i* locus depicting seed coat color (Matson and Williams 1965). This region was the first SCN resistant locus to be mapped using molecular markers (Weisemann et al. 1992). LG A2 explains 15% of the total phenotypic variation in PI 209332 to SCN Race 3 (Concibido et al. 1994) and a significant portion of the variation in 'Peking' (Mahalingam and Skorupska 1995; Chang et al. 1997; Meksem et al. 2001), PI 437654 (Webb et al. 1995) and 'Peking' + PI 88788 + PI 90763 (Heer et al. 1998). Many minor QTLs have been identified on LGs A1, B1, B2, C1, C2, D1a, D2, E, F, G, H, I, J, L, M and N (for a review, see Concibido et al. 2004). The variability associated with these QTLs matches that associated with the major QTLs. However, the cluster of current sources of SCN resistant germplasm into limited groups (Diers et al. 1997; Zhang et al. 1999) highlights the need to identify diverse SCN resistant germplasm.

Glycine soja Sieb. and Zucc., the wild ancestor of the domesticated soybean, represents a genetically diverse germplasm pool that may contain many desirable traits not found in *G. max*. *G. soja* grows in a range of micro environments throughout east Asia (Li 1994) and molecular studies have revealed large polymorphic rates across populations that reflect the high genetic diversity of the species (Chiang 1986; Dong et al. 2001). *G. soja* is significantly more genetically diverse than *G. max* (Chen and Nelson 2004) and has recently been used as a source of diverse resistance genes, such as those against soybean aphid [*Aphis glycines* Matsumura] (Hill et al. 2004), cucumber mosaic virus soybean strains (Hong et al. 2003) and SCN (Wang et al. 2001), which are not found in *G. max*. Previous studies have reported QTL that account for 27 and 23% of the total variation in resistance in *G. soja* to SCN Race 3 and map to LGs G and E, respectively (Wang et al. 2001). Since SCN resistance is associated with a large number of genes, it may be useful to identify germplasm with partial, or horizontal, resistance (Schmitt and Shannon 1992) to facilitate the stacking of resistance genes.

The objectives of this paper were: (1) to assess the genetic variation and inheritance of SCN resistance in a *G. max* × *G. soja* population; and (2) to identify and evaluate QTL associated with horizontal SCN resistance. To achieve these objectives a recombinant inbred line (RIL) population was generated from two parents that do not carry any known major resistance genes, such as *rhg1* or *Rhg4*.

Materials and methods

Plant materials

The population of 191 $F_{4:5}$ RILs was derived by single seed descent from 'S08-80' (*G. max*) × PI 464925B (*G. soja*). 'S08-80' seed was obtained from Syngenta Seeds (Arva, ON) and PI464925B seed was obtained from the USDA Soybean Germplasm Collection (Urbana-Champaign, IL). 'S08-80' and PI 464925B were chosen as parents for their early maturity and seed quality traits, and absence of known SCN resistance genes (e.g., *rhg1* or *Rhg4*). The parental lines, resistant controls 'INA', 'Jack' and 'Cyst X'^{TM}, and susceptible control 'Lee 74' were included in the bioassay. 'INA' has been reported as resistant to SCN Races 1, 2, 3 and

5, moderately resistant to Race 14 and moderately susceptible to Race 4 (Nickell et al. 1999). 'Jack' has been reported as resistant to SCN Race 3 and 4 (Nickell et al. 1990). 'Cyst X'™ is derived from 'Hartwig', which was derived from the cross 'Forrest' × PI 437654. SCN populations that develop on PI 437654 are HG Type 4 (Niblack et al. 2002; Dr. Terry Niblack, personal communication).

The SCN inoculation procedure was adopted from the Agriculture and Agri-Food Canada's (AAFC) Standard Operating Procedures for the Race Test used at the Greenhouse and Processing Crop Research Centre (GPCRC), AAFC, Harrow, ON, Canada. It is based on the method of Riggs and Schmitt (1991) with minor modifications as described below. The Chatham and Ruthven SCN isolate populations were obtained from AAFC, Harrow Research Station (Harrow, ON) and maintained under isolation on cultivar 'Westag 97' in greenhouse facilities at the University of Guelph (Guelph, ON). The Chatham isolate has been characterized as Race 3 and a HG Type 7, whereas the Ruthven isolate has been characterized as Race 2 and a HG Type 1.2.5.7.

Twelve seeds per line were planted 1 cm deep in standard 10 cm plastic pots filled with a fine vermiculite and perlite mixture (Therm-O-Rock East Inc., New Eagle, PA). The hilum was removed from each seed with a scalpel prior to being seeded to assist germination. The pots were kept on a bench in a greenhouse maintained at 27/23°C day/night temperature with a 16 h day length supplemented with a photosynthetic photon flux density of 100 mol m^{-2} s^{-1} at pot height (using high pressure sodium vapor lamps, Model SDN AGRO 430W 2/3, Philips Lighting Company, Somerset, NJ) for 3 days or until the radicle was 2–3 cm long.

Fox sandy loam soil (pH 6.5) from the Cambridge Research Station, University of Guelph (Cambridge, ON), was sterilized and sieved with a 1 mm wire mesh sieve. Plants were grown in SC-10 Super Cell conetainers (Stuewe and Sons, Corvallis, OR) arranged every other row in RL98 Trays (Stuewe and Sons, Corvallis, OR). RL98 trays were placed over greenhouse seedling trays filled to a height of 1 cm with sterilized soil and conetainers were filled with sterilized soil. The tips of the cones were immersed in the soil in the seedling trays. Three days prior to being seeded, trays were filled with water to allow capillary action to moisten the soil. Experimental units were spaced by one blank cell in the RL98 trays.

The experimental unit for the Chatham isolate consisted of three plants in individual conetainers arranged in a randomized complete block design with three replications. The experimental unit for the Ruthven isolate was one plant per cone arranged in a split plot design with three harvest dates as the main factor and entry number as the sub-plot factor, replicated three times. In both experiments, two seedlings were transplanted to each conetainer, just beneath the soil surface, and inoculated with 350 μl egg solution or approximately 2,600 SCN eggs directly onto the roots, then covered with sterilized soil and moistened with water. When one seedling reached the vegetative cotyledonary (VC) stage, the second seedling was pruned just above the soil surface.

The development of cysts was monitored on the 'Lee 74' plants. Collection of cysts occurred approximately 35–40 days after inoculation. At harvest the above ground biomass was removed and individual conetainers were immersed in a water bath to remove the soil from the roots. The roots were placed in a 250 μm sieve, nested in a 600 μm sieve. The contents of the 600 μm sieve were collected in transparent 250 ml pill bottles and counted using either a RLM Series Magnifier (Sunnex Inc., Natick, MA) 3X magnifying glass with attached fluorescent lamp or aliquots taken from the bottle for examination using a stereomicroscope at 8X magnification. Plant phenotype was determined using a female index (FI), which was calculated as a percentage of the mean number of cysts on an individual line divided by the mean number of cysts on the susceptible host, 'Lee 74', where a $FI < 10\%$ was designated as resistant (Golden et al. 1970). RILs were also considered moderately resistant using Schmitt and Shannon's system (1992) of a $10 \leq FI < 30\%$. Roots were placed in a dryer for 10 days at 50°C and the dry root weight measured.

QTL mapping analysis

This procedure was adopted from MaizeGDB SSR protocols (http://www.maizegdb.org/documentation/maizemap/ssr_protocols.php) (verified 20 July 2006). Genomic DNA was extracted from leaf discs obtained from each line grown in the Chatham isolate SCN screen. Genomic DNA from the collected leaf material was extracted with a FastDNA® Kit (BIO101, Carlsbad, CA) and quantified with a DU-64 Spectrophotometer (Beckman Coulter, Fullerton, CA). PCR concentrations of DNA were prepared by adding 10 μl of the extracted DNA to 90 μl of distilled and deionized water.

PCR reactions consisted of approximately 60 ng of template DNA, 1.5 μl of 10X PCR buffer (Invitrogen Life Sciences, Carlsbad, CA), 0.75 μl of 50 mM $MgCl_2$ (Invitrogen Life Sciences), 0.6 μl of 5 mM dNTPs

solution (Invitrogen Life Sciences), 0.10 µl of 5 U/µl Platinum Taq Polymerase (Invitrogen Life Sciences), 2 µl of 2.25 pmol/µl of each forward and reverse primer (Lab Services, University of Guelph, Guelph, ON; AAFC, Harrow, ON; Eastern Cereal and Oilseeds Research Centre, AAFC, Ottawa, ON), and 5.05 µl of distilled, deionized water for a total volume of 15 µl. The PCR consisted of an initial 2 min denaturation step at 95°C, followed by 35 cycles of a 1 min denaturation step at 92°C, a 1.5 min annealing step at 47°C and a 1.5 min extension step at 68°C. Following cycle 35, the extension step was extended for 5 min at 72°C. PCR was carried out using a Robocycler® 96 Thermocycler (Stratagene Inc., La Jolla, CA), and products were separated on a 8% MetaPhor® Agarose (Mandel, Guelph, ON) gel or a 6% polyacylamide gel (PAGE). Ethidium bromide was used to stain the gels.

A library of 449 SSR markers was initially screened against 'S08-80' and PI464925B to determine polymorphic markers on Metaphor® Agarose and PAGE gels. The choice of markers was based on separation of at least 5 cM so that each LG was saturated.

Statistical analysis and QTL mapping

An analysis of variance (ANOVA) was performed to determine significant differences among fixed and random effects. For the Chatham isolate screen random effects were entry, blocks and block × entry, whereas for the Ruthven isolate fixed effects were harvest date and entry × harvest date and random effects were entry, block and block × harvest date. Adjusted means from the two variables, root weight and cyst count, were tested against a suspected covariate harvest date. A significant correlation between root weight and cyst counts was identified, which led to covariate analysis of the influence of root weights on cyst count. Since root size is a given in this population and genetically predetermined, we used it as a covariate in the model to calculate the adjusted means and adjusted FI for each entry (Dr. Brian Allen, Chair of Mathematics and Statistics Department, University of Guelph, personal communication). Adjusted means were also tested for significant differences from zero with a *t*-test. Residuals were used to confirm the assumptions of the ANOVA.

A single factor ANOVA, conducted with the use of a SAS v.8.2 (SAS Institute, Cary, NC) macro program obtained from Dr. Elizabeth Lee at the University of Guelph (Guelph, ON), identified markers significantly associated with SCN resistance. A Chi-square test was used to test markers for segregation distortion to determine if the populations' genotype followed the expected 1:1 (A:B) segregation ratio. Genotypic means were calculated from the means of the marker genotypes in combination with the adjusted RIL means from the SCN screen for each of the Chatham and Ruthven isolates. A contrast of the combined mean of each homozygous genotype for each significant marker associated with resistance was performed to determine significant differences between them. If significant differences were present, the homozygous class with the lowest cyst count determined the origin of the resistant allele. Analysis of variance was used to partition the total variance into genetic and environmental components. Broad sense heritability estimates (Hanson et al. 1956) were computed using the following forumula:

$$H^2 = \frac{(S_G)}{S_G + S_E/r}$$

where H^2 = heritability, S_G = genotypic variance, S_E = error and r = the number of reps for the trait.

Mapmaker 3.0 (Lander et al. 1987) was used to construct a linkage map from the genotypes of the RIL population. A minimum log-likelihood (LOD) score of 3.0 and a maximum distance of 50 cM were used to determine linkage. The linkage group mapping and the adjusted cyst count means of the 191 RILs from the Chatham and Ruthven screens was used in combination with Mapmaker/QTL v.1.1b to determine the presence of QTL for SCN resistance. Association was considered significant if LOD values were greater than 2.0.

Epistatic interactions between loci were tested with the macro program EPISTACY v. 2.0 (Holland 1998), which was run within SAS v. 8.2 . This program tests pairwise interactions of all the genetic markers. To reduce the experiment-wise error, the error term (α = 0.05) was divided by $g(g\text{-}1)/2$, where g is the number of soybean chromosomes (Holland 1998). The experiment-wise error was set at α = 0.0003 (g = 20) for all pair-wise comparisons. An R^2 statistic was calculated by EPISTACY as the interaction partial sum of squares divided by the total sum of squares, which represents the variation explained by the interaction after accounting for the main effects of the loci (Holland 1998).

A linear model was built for the reaction of both SCN isolates to identify the total amount of variation that could explained from previous ANOVA and EPISTACY results. Significant markers identified from single factor ANOVA were initially used to develop a linear model (Holland et al. 1997). The best linear

model used a combination of significant markers and epistatic terms to identify the highest R^2 value without regard to number of loci.

All statistical procedures were performed using CORR, GLM, REG, FREQ, MEANS, MIXED and UNIVARIATE procedures of SAS v. 8.2. Adjusted means were derived using the LSmeans command to generate mean values that account for experimental variation. Unless otherwise specified, the Type One error rate (α) was 0.05 for all analysis.

Results

Genetic variation of SCN resistance

Chatham isolate

The hybridization of ‘S08-80’ and PI464925B produced a population that segregated for FI (Fig. 1) and mean root dry weight (data not shown). A significant correlation existed among the adjusted means for cyst count and root weight (correlation coefficient = –0.209). Root weight was not a significant covariate in the recalculated ANOVA and was left out of the analysis.

Adjusted FI, as calcuated from the adjusted means of cyst count from the ANOVA, revealed that ‘S08-80’ and PI464925B were not significantly different. RILs that differed significantly in cyst count from both parents were found, indicating that transgressive segregation occurred in the RIL population (Fig. 1). The parental means were close to the mean of the RIL population and three RILs had adjusted means greater than the susceptible cultivar, ‘Lee 74’. One RIL (FI = 5.2 ± 1.1) was identified as resistant (FI < 10), but an additional ten RILs were partially resistant (10 < FI < 30%) under the criterion of Schmitt and Shannon (1992). A *t*-test identified 16 more RILs with adjusted means not significantly different from zero. Broad sense heritability for the Chatham isolate was 0.81, which confirmed that the SCN resistance was primarily controlled by the genotype.

Ruthven isolate

Hybridization of ‘S08-80’ and PI464925B resulted in a population with means that segregated for FI (Fig. 2) and mean root dry weight (data not shown). A significant correlation existed between the adjusted means for cyst count and root weight (correlation coefficient = 0.142). Reanalysis of the ANOVA for cyst count showed significant differences between entries and the covariate, root weight.

A female index (FI) was calculated from the adjusted means of cyst count from the ANOVA. The adjusted means for cyst count revealed that ‘S08-80’ and PI464925B were not significantly different. The parental cyst count means were similar to the mean of the RIL population and a large number of RILs had adjusted means that were larger than that for the susceptible cultivar, ‘Lee 74’ (Fig. 2). Cyst count (Fig. 2) and root dry weight in several RILs differed significantly from that in the parents, indicating that transgressive segregation occurred No RILs were deemed resistant (FI < 10%) or partially resistant by Schmitt and Shannon’s criterion (10 < FI < 30%). A *t*-test identified three RILs with means not significantly different from zero. Only one RIL was resistant to both SCN isolates under the criteria of means not significantly different from zero. Broad sense heritability for the Ruthven isolate was 0.81, which confirmed that the SCN resistance was primarily controlled by the genotype.

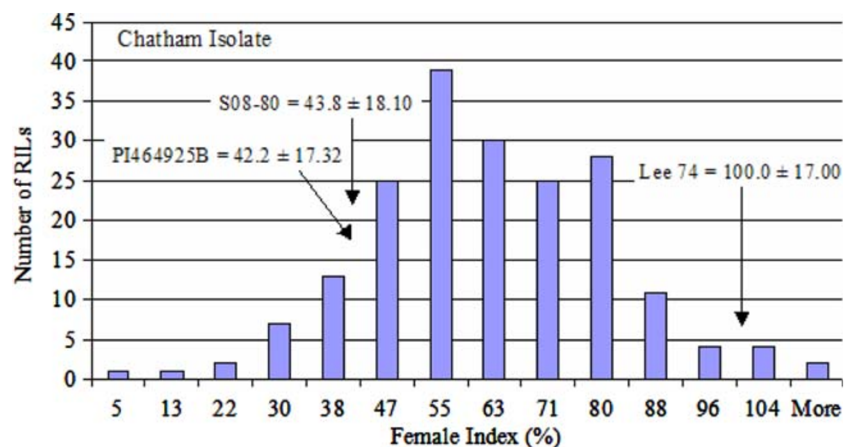

Fig. 1 Segregation of female index (*FI*) for the RIL population derived from ‘S08-80’ × PI464925B inoculated with 2,600 eggs of the SCN Chatham isolate. Parental and susceptible control (‘Lee 74’) values are shown. FI was derived from the adjusted data

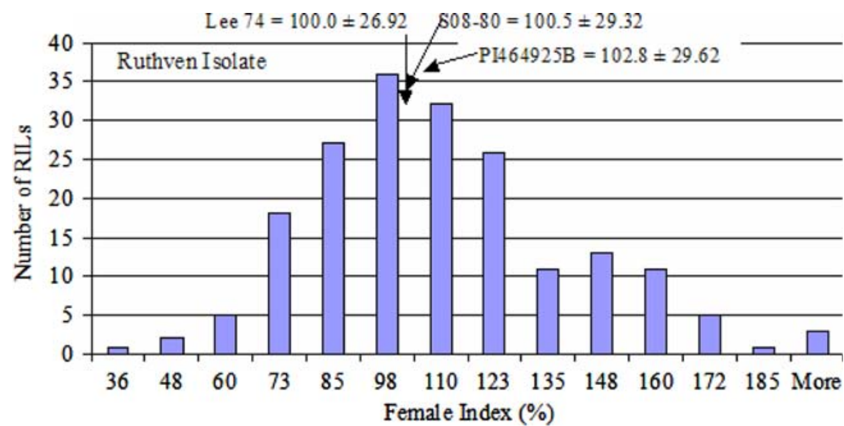

Fig. 2 Segregation of female index (*FI*) for the RIL population derived from 'S08-80' × PI464925B inoculated with 2,600 eggs of the SCN Ruthven isolate. Parental and susceptible control ('Lee 74') values are shown. FI was derived from the adjusted data

Identification and evaluation of QTL associated with inherited SCN resistance

Molecular marker polymorphism

Four hundred forty nine SSR markers were screened, and of these 207 markers exhibited clear polymorphic bands in a parental comparison. From the 207 markers, 118 markers that were evenly distributed cross the 20 chromosomes were chosen to screen the RIL population. Genotypic data generated from the marker screen putatively anchored 97 markers onto 28 LGs and 21 markers were unlinked. The 97 markers, except Satt102 and Satt230 loci, were putatively anchored to LGs defined by the consensus map (Song et al. 2004). Satt102 locus was putatively anchored to LG I instead of LG K, and Satt230 was putatively anchored to LG M instead of LG E. Satt512 locus was putatively anchored to LG E in our study; however, it was absent from the consensus map. A Chi-square test revealed 28 SSR markers with significant segregation distortion from the expected 1:1 segregation ratio (data not shown).

QTL for SCN resistance

On the basis of RIL means, single factor ANOVA identified 22 markers/QTL that were significantly associated with resistance to SCN. The resistance variation to Chatham and Ruthven SCN isolates could be attributed to five and three markers, respectively, with loci distributed over four LGs (Table 1). Each significant marker explained 4.7–5.8% of the total phenotypic variation observed.

Mean analysis of the Chatham isolate identified four markers that derived resistant alleles from *G. soja* and one marker from *G. max* (Table 1). The means analysis of the Ruthven isolate identified two markers that

Table 1 The coefficient of determination, linkage map location and cyst count genotypic mean ± SE of significant markers (α = 0.05) associated with SCN resistance for the $F_{4:5}$ RIL population derived from 'S08-80' × PI464925B and screened with Chatham or Ruthven SCN isolates

Marker	$P > F$	R^2 (%)	Linkage group	Allelic means[a]			Contrast between parental homozygotes ($P > F$)
				'S08-80' (AA)	Heterozygous (AB)	PI464925B (BB)	
Chatham							
Satt102	0.006	5.2	I[b]	42.1 ± 1.36	40.4 ± 2.16	40.0 ± 1.38	0.002
Satt162	0.010	4.9	I	41.5 ± 1.33	41.3 ± 2.17	35.8 ± 1.44	0.004
Satt260	0.008	5.1	K	42.2 ± 1.28	38.2 ± 2.61	36.2 ± 1.39	0.002
Satt330	0.004	5.8	I	41.7 ± 1.29	41.7 ± 2.40	35.5 ± 1.43	0.002
Satt517	0.011	4.7	G	37.2 ± 1.34	32.8 ± 3.88	41.8 ± 1.25	0.013
Ruthven							
Satt147	0.013	4.7	–[c]	10.8 ± 0.35	9.7 ± 0.50	11.5 ± 0.36	0.158
Satt148	0.005	5.7	I	11.5 ± 0.31	10.6 ± 0.64	9.9 ± 0.38	0.001
Satt173	0.012	4.7	O	11.7 ± 0.36	10.4 ± 0.51	10.3 ± 0.34	0.005

[a] Means calculated using adjusted cyst counts

[b] Marker found on a different LG than the integrated linkage map (Song et al. 2004)

[c] Unlinked marker

derived resistant alleles (i.e., lower cyst number for the parental homozygote) from the *G. soja* parent and one marker (Satt147) from *G. max* (Table 1). Contrasts between parental homozygous classes were significant for all, but one (Satt147) of these eight markers (Satt147).

Mapmaker/QTL revealed that five QTL intervals were associated with resistance to either the Chatham or Ruthven SCN isolate in the RIL population. For the Chatham isolate, QTL for resistance were identified on LGs G, I and K, and explained 7.1–10.1% of the total resistance variation (Table 2, Figs. 3, 4). QTL on LGs I and K derived resistance alleles from *G. soja*, whereas QTL on LG G derived the resistance allele from *G. max* (Table 2). For the Ruthven isolate, QTL for resistance were identified on LGs I and O, and explained 6.7 and 5.1% of the total phenotypic variation, respectively (Table 2, Fig. 4); the resistant alleles were derived from *G. soja* (Table 2).

Marker interaction and best-fit linear models

A pairwise comparison of all markers against RIL response to both isolates revealed significant interactions among three pairs of markers. Not all markers that contributed to the interaction were associated with the identified QTL. However, one marker (Satt173) individually associated with the Ruthven isolate (Tables 1, 2) was part of an epistatic pair for the Chatham isolate (Table 3). Epistatic interactions accounted for 10.6 to 15.9% of the total variation after the main effects were considered (Table 3). Stepwise regression in combination with the significant epistatic interaction derived best-fit linear models that explained up to 31.2% of the total variation (Table 4). Only significant epistatic interactions were included in the derived best-fit linear models. Satt102 was significant in the best-fit linear models for resistance to both Chatham and Ruthven isolates.

Discussion

Significant variation was created in the RIL population even though the FIs of ‘S08-80’ and PI464925B were not significantly different from each other and the parents did not show major gene resistance. The population segregated transgressively for cyst count in bioassays with both Chatham and Ruthven SCN isolates. The lack of clustering of genotypes within the population suggests that the resistance trait is multigenic, resulting from many minor genes with combined effects, i.e., horizontal resistance. Many previous studies used highly resistant germplasm to isolate resistant QTL, resulting in clustered population distributions due to contribution of only a few major genes. Neither ‘S08-80’ nor PI464925B possessed strong resistance as individual genotypes; however, when combined they had SCN resistance genes that complemented each other to produce RILs with extreme values. Since *G. max* and *G. soja* are genetically diverse as indicated by molecular marker analysis (Maughan et al. 1995; Dong et al. 2001; Chen and Nelson 2004), *G. soja* could contribute new alleles for SCN resistance (current study), which complement the limited allelic diversity currently available within *G. max*. Here, we have chosen not to backcross G. soja to *G. max* in order to increase the genetic contribution and allelic diversity of the *G. soja* parent to the RIL population. As a result and due to the relatively large population size, the allelic diversity reported here is perhaps somewhat greater than in other studies that used *G. soja* as source of resistance (e.g., Wang et al. 2001).

Table 2 QTL location, interval, length, position, likelihood value and variance explained from a $F_{4:5}$ RIL population derived from ‘S08-80’ × PI464925B and screened with Chatham and Ruthven SCN isolates

LG[a]	Interval	Length[b] (cM)	QTL POS[c]	LOD value	R^2 (%)
Chatham					
G	Satt533–Satt517$_{(max)}$[d]	26.3	16.4	2.46	10.1
I	Satt162–Satt330$_{(soja)}$[e]	11.2	5.6	2.76	7.6
K	Satt260–Satt196$_{(soja)}$[e]	38.3	11.1	2.08	7.1
Ruthven					
I	Satt102–Satt148$_{(soja)}$[e]	18.3	12.7	2.57	6.7
O	Satt173–Satt466$_{(soja)}$[e]	10.9	7.1	2.02	5.1

[a] Linkage group

[b] Distance of the QTL on the LG given in centiMorgans from Mapmaker/QTL

[c] QTL position expressed as the distance from the first marker

[d] Max denotes that the resistant alleles in this QTL were derived from ‘S08-80’

[e] Soja denotes that the resistant alleles were derived from PI464925B

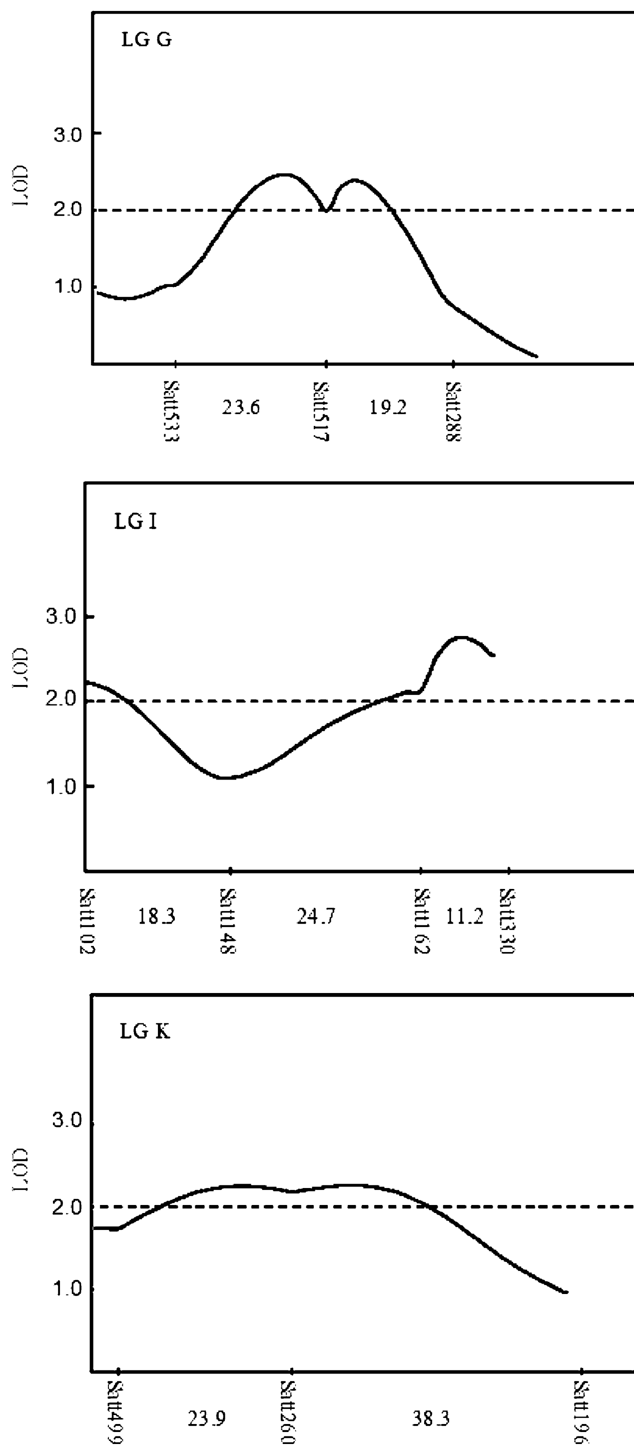

Fig. 3 LOD scores and QTL map of LG G, I and K for soybean cyst nematode (*SCN*) resistance to the Chatham isolate of SCN in an $F_{4:5}$ RIL population derived from 'S08-80' × PI464925B. The map distance between markers is expressed in Haldane cM

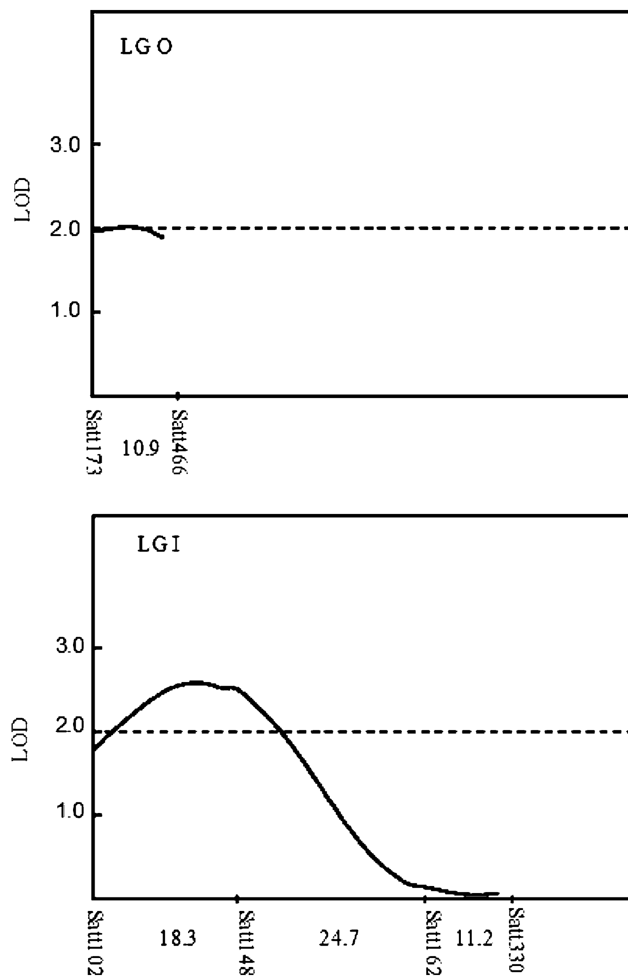

Fig. 4 LOD scores and QTL map of LG O and I for soybean cyst nematode (*SCN*) resistance to the Ruthven isolate of SCN in an $F_{4:5}$ RIL population derived from 'S08-80' × PI464925B. The map distance between markers is expressed in Haldane cM

Molecular marker analysis confirmed that SCN resistance is a quantitative trait in our population. The high broad sense heritability values of 0.81 for the Ruthven isolate and 0.91 for the Chatham isolate suggest that the horizontal resistance is primarily under genetic rather than environmental control. Furthermore, the high heritability values provide us with confidence that the QTLs found in this study are real, even if their individual effects might be smaller than those previously reported. The latter finding is a reflection of our objective to target horizontal resistance, which is controlled by polygenes rather than major genes, to further improve our understanding of SCN resistance in soybean.

Of the 449 SSR markers screened, 46% were polymorphic. Previous studies of populations derived from *G. max* cultivars × *G. max* PIs reported polymorphic values of 54.3% (Concibido et al. 1996) and 36.2–40.3% (Concibido et al. 1997). Weir (2002) reported a polymorphic rate of 63% in a *G. max* ('S08-80') × *G. soja* (PI 458536) population. The high rates of polymorphism observed may be caused by the amount

Table 3 Molecular markers with significant epistatic interaction for resistance to the Chatham and Ruthven isolates of SCN in a $F_{4:5}$ RIL population derived from 'S08-80' × PI464925B, as identified using the SAS macro program EPISTACY

Locus 1		Locus 2		Interaction			
L G	Name	LG	Name	*df*[a]	*F*-value[a]	$P > F$[ac]	R^2[b]
Chatham							
O	Satt173	H	Satt469	1	15.97	0.00011	10.7
Ruthven							
A2	Satt158	D2	Satt528	1	18.31	0.00004	15.9
M	Satt245	D1a	Satt507	1	14.06	0.00028	10.6

[a] Values for the interaction without main effects

[b] The coefficient of determination as a percent for the interaction after the main effects were considered

[c] $\alpha = 0.05$

Table 4 Significant markers and epistatic interaction as determined by best-fit linear models for resistance to the Chatham and Ruthven isolates of SCN in a $F_{4:5}$ RIL population derived from 'S08-80' × PI464925B

Marker	*df*	*F*-value	$P > F$[a]	LG	
				Song et al. (2004)	Current study
Chatham					
Satt102	2	4.76	0.0199	K	I[b]
Satt562	2	5.25	0.01	I	–
Satt173 × Satt469	8	3.20	0.002	O × H	O × H
Model R^2 = 21.4%	14	3.69	<0.0001	–	–
Ruthven					
Satt102	2	8.01	0.0006	I	I
Satt154	2	6.32	0.0026	D2	–
Satt466	2	5.67	0.0046	O	O
Satt573	2	6.52	0.0022	E	E
Model R^2 = 31.2%	8	5.71	<0.0001	–	–

[a] $\alpha = 0.01$

[b] Marker found on a different linkage group (LG) than the integrated linkage map (Song et al. 2004)

of diversity and unrelatedness between the *G. soja* PI and the elite *G. max* parent.

SCN resistance was significantly associated with eight markers (Table 1). The large sample size (191 RILs) and large amount of *G. soja* germplasm in the RILs enabled identification of markers associated with SCN resistance. All SCN resistance markers identified here for *G. soja*, with the exception of Satt517, were different from those previously reported (Wang et al. 2001) (Table 1), which result may reflect the very different germplasm source used.

QTL associated with SCN resistance

Three QTL were associated with the Chatham SCN isolate and mapped to LGs G, I and K (Table 2). It is known that the major SCN resistance gene, *rhg1*, and three additional QTL for resistance to SCN are putatively anchored to LG G (Concibido et al. 2004). Satt309 and Satt235, which map close to the *rhg1* gene (Cregan et al. 1999)were polymorphic between the parents in this study *t*; however, they were not significant for SCN resistance in our population.

The QTL on LG G overlapped a previously reported QTL for resistance to SCN derived from *G. soja* PI 468916, which explained 27% of the phenotypic resistance to Race 3 (Wang et al. 2001). The QTL identified on LG G derived resistance from *G. max* and explained 10% of the variation associated with the Chatham isolate (Table 2). The parental *G. max* germplasm was not documented nor expected to be SCN resistant; however, it exhibited partial resistance in both SCN evaluations (Figs. 1, 2). Previous research has shown that in molecular analysis, the use of parental germplasm that lacks large QTL is more likely to identify minor QTL that are associated with traits of interest (Asins 2002). The present study suggests that the resistant QTL allele on LG G may not be unique to *G. soja*. Further analysis is required to determine if this QTL carries the same or different functional alleles (Concibido et al. 2004).

The QTL on LG K and I have not previously been associated with SCN resistance, but accounted for 7.1 and 7.6% of the identified total phenotypic variation for the Chatham isolate in *G. soja*, respectively (Table 2). The QTL previously identified on LG I, which is derived from Peking, explains 11% of the variation to Race 5 SCN (Qui et al. 1999). Two QTL were associated with resistance to the Ruthven isolate of SCN, neither of which was previously associated with SCN resistance. The QTL on LG O and I explained 5.1 and 6.7% of the total phenotypic variation, respectively (Table 2). The QTL on LG I in the interval Satt102–Satt148 was 24.7 cM away from the QTL interval

associated with resistance to the Chatham isolate (Fig. 4). Previous race characterization by one of us (Welacky TW, unpublished data) in 2003 had classified both Chatham and Ruthven isolates as HG Type 7, which suggests that the two QTL could in fact be one. SCN resistance loci that appear at proximal locations may be a cluster of genes within which new resistant genes are created by DNA rearrangement (Heer et al. 1998). Yue et al. (2001) found QTL for resistance to different SCN races, which mapped to several common marker intervals and could result from linkage (Heer et al. 1998) or pleiotropy (Qui et al. 1999). Pleiotropy seemed a likely explanation as the loci control resistance to the same pest with different phenotypic variations (Yue et al. 2001). A similar explanation could be offered for our results.

The results presented in this paper demonstrated that neither of the parents of the RIL population was highly SCN resistant, substantiating our assumption that major genes for SCN resistance are absent. With only minor genes responsible for the SCN resistance, R^2 values were much smaller than the majority reported so far (Concibido et al. 2004). Minor QTL associated with SCN resistance in several genetic backgrounds have explained as little as 1% of the total phenotypic variation (Vierling et al. 1996; for a review, see Concibido et al. 2004). The R^2 values reported here are relatively small (Table 2), but are believed to be accurate and real as the mapped population was highly homozygous, large and extremely genetically diverse (Concibido et al. 1997; Asins 2002).

Epistatic interactions

A pair-wise comparison of all molecular markers identified significant epistatic interactions in response to the Chatham and Ruthven isolates, which explained up to 16% of the phenotypic variation after the main effects (Table 3). One interaction for resistance to the Chatham isolate was found between Satt173 and Satt469 on LGs O and H, respectively. Satt173 was also associated with a QTL for resistance to the Ruthven isolate (Table 1), which suggests that this region may not be isolate specific. The marker Satt469 was not significantly associated with resistance as determined by single factor ANOVA. Two epistatic interactions were evident between markers that were on an individual basis, significantly associated with resistance to the Ruthven isolate (Table 3).

Epistatic interactions between markers on LGs A2, F, G and M and significantly associated with SCN resistance have been previously reported (Heer et al. 1998; Meksem et al. 2001; Yue et al. 2001). The results presented here suggest that unlinked marker pairs could account for large amounts of variation despite the absence of individual association with SCN resistance, as indicated by single factor ANOVA. The variation explained by epistatic interactions was greater than any individual QTL and two to three times larger than any individual marker alone. Holland et al. (1997) reported several pairwise epistatic interactions between combinations of significant and not significant markers, and suggested that the incorporation of QTL with unknown epistatic interactions could significantly alter marker effects when incorporated into different genetic backgrounds.

Best-fit linear models

Best-fit regression models explained 21 and 31% of the total phenotypic variation in the RIL population to the Chatham and Ruthven isolates, respectively (Table 4). The Chatham isolate model included markers from QTL on LG I and K, but not LG G. Single factor ANOVA identified two markers associated with both QTL on LGs I and K (Tables 1, 2). However, only a single marker was associated with the QTL on LG G (Table 1), despite polymorphic markers flanking it at a 20 cM distance. Further investigation and perhaps marker saturation of this region is needed to validate the QTL on LG G in this population and to compare it to the QTL on LG G reported by Wang et al. (2001).

The best-fit model for the Ruthven isolate consisted of the identified QTL and individual markers. Unlinked marker Satt154 and Satt573 on LG E were significant in the linear model (Table 4). Wang et al. (2001) reported a QTL 37 cM away from Satt573, which accounts for 23% of the total phenotypic variation to SCN Race 3 and derives resistance from *G. soja* (PI468916). Satt573 was associated with SCN resistance, suggesting that PI464925B could react to Race 3 (or in our case, HG Type 7) of SCN in a similar manner.

Best-fit models provided further support for the presence of real QTL on LGs I, K and O, which derived resistance from *G. soja* (Table 4). Satt102 was significantly associated with resistance against both Chatham and Ruthven isolates, which supports the occurrence of one QTL region rather than two separate ones on LG I. Satt573 was confirmed on LG E as a QTL for SCN resistance (Table 4) derived from *G. soja* (Wang et al. 2001).

In conclusion, we report new and unique QTL derived from *G. soja*, which may be attributed to differences in genetic diversity of the *G. soja* sources used. The present study suggests that in highly resistant

germplasm QTL of lesser importance are often masked by those of major importance, and that the former can best be studied if major alleles for resistance are absent in parents. Indeed, our results suggest that moderately resistant germplasm could provide an important source of minor QTL in breeding for SCN resistance in soybean. This would represent a major shift in the industry from reliance on major gene resistance only to targeting a more stable, horizontal resistance. Thus, *G. soja* could provide an invaluable germplasm pool that leads to the development of elite commercial cultivars with increased durability against SCN.

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