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Association between Soybean Cyst Nematode Resistance Loci and Yield in Soybean

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ABSTRACT

Breeding for resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe; SCN) is an important objective of soybean [*Glycine max* (L.) Merr.] breeders. There is concern, however, that SCN resistant soybean cultivars yield less than SCN susceptible cultivars under low SCN pressure. This is supported by the report that two quantitative trait loci (QTL) conferring yield depression are linked to the major SCN resistance gene *rhg*$_1$ on linkage group (LG) G in the resistant plant introduction (PI) 209332. The objective of our study was to test for the association of SCN resistance with yield and other agronomic traits in populations developed from soybean cultivars with resistance derived from PI 88788. We tested five populations of near isogenic lines (NIL) segregating for genetic markers Satt309 or CTA linked to *rhg*$_1$ and two populations of NILs segregating for Satt431 linked to the resistance QTL cqSCN-003 on LG J. Population sizes ranged from 23 to 44 NILs, and the populations were yield tested in two years across four locations that had low SCN incidence in previous years. Near isogenic lines predicted to carry the SCN resistance allele yielded significantly ($p < 0.05$) less than NILs predicted to carry the susceptibility allele in one out of five populations segregating for Satt309 (118 kg ha^{-1}) and in one out of two populations segregating for Satt431 (76 kg ha^{-1}). Significant differences between the two classes of NILs were also observed for plant maturity, plant lodging and plant height in some populations; however, these differences were small in magnitude. Molecular marker data from the NIL populations for the genetic regions flanking the segregating resistance genes suggest the presence of a yield depression QTL distal to *rhg*$_1$ on LG G and another yield depression QTL linked or pleiotropic to cqSCN-003 on LG J. Confirmation and refinement of our results will enable soybean breeders to use marker-assisted breeding to select against yield depressing regions and develop SCN resistant cultivars that compete with SCN susceptible cultivars even under low SCN pressure.

Soybean cyst nematode has been the most important soybean pathogen in the USA and worldwide (Wrather et al., 1997; Wrather et al., 2003). The pathogen was first observed in China and Japan in the 1880s (Hartman et al., 1999). In the USA, SCN was first reported in 1954 in North Carolina (Winstead et al., 1955) and has since become the most important soybean disease in the North Central states (Doupnik, 1993). For the 2002 growing season, yield losses in the USA due to SCN were estimated at 3.6 million megagrams or an equivalent of $783.8 million (Wrather et al., 2003).

Soon after the first discovery of SCN in the USA, resistance was studied as a potential means of control (Caldwell et al., 1960). Early inheritance studies suggested that SCN resistance in soybean is controlled by three recessive genes designated *rhg*$_1$, *rhg*$_2$, and *rhg*$_3$ (Caldwell et al., 1960) and one dominant gene designated as *Rhg*$_4$ (Matson and Williams, 1965). Breeding for resistance to SCN was initiated in the 1950s, and 'Pickett', the first SCN resistant cultivar developed in the USA, was released in 1966 (Brim and Ross, 1966). The resistance in Pickett was derived from 'Peking', a plant introduction from China. Plant introduction 88788 was used as an additional resistance source (Hartwig and Epps, 1977) and has become the predominant SCN resistance source in the midwestern USA (Diers and Arelli, 1999). Recently, a few cultivars were released with resistance from PI 90763 (Hartwig and Young, 1990), PI 437654 (Anand, 1992b), and PI 209332 (Anand, 1992a).

Quantitative trait loci mapping studies revealed that PI 88788 has a major SCN resistance QTL on linkage group G (Concibido et al., 1997; Diers et al., 1997). A major QTL was also mapped to this region in all the previously mentioned SCN resistance sources (Concibido et al., 1994, 1997; Webb et al., 1995), and this QTL was assigned the name *rhg*$_1$. An additional minor resistance QTL that mapped on LG J from PI 88788 was designated cqSCN-003 (Glover et al., 2004). This QTL maps to the same position as SCN resistance QTL previously identified in PI 209332 (Concibido et al., 1994, 1996) and PI 90763 (Concibido et al., 1997).

Despite outyielding SCN susceptible cultivars under high SCN pressure, SCN resistant soybean cultivars were estimated to yield 5 to 10% less than susceptible cultivars under low SCN pressure (Noel, 1992). A similar, but smaller, effect was reported by Chen et al. (2000) who estimated this yield difference to be at least 67 to 135 kg ha^{-1} in Minnesota variety trials from 1996 to 1998 where 34 out of 50 resistant cultivars had SCN resistance from PI 88788. This lower yield may be caused by yield drag, defined as yield suppression resulting either from pleiotropic effects of the resistance gene or from genes linked and coinherited with the resistance gene. Alternatively, the difference between resistant and nonrelated susceptible cultivars could be caused by yield lag, meaning that SCN resistance has not yet been introgressed into the best performing germplasm. While yield lag should be overcome after a few breeding cycles, the difficulties of developing high yielding SCN resistant cultivars suggest that yield drag plays an important role.

Evidence for yield drag related to SCN resistance was provided by Mudge et al. (1996). They field-tested two

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Abbreviations: AFLP, amplified fragment length polymorphism; ANOVA, analysis of variance; CAPS, cleaved amplified polymorphic sequence; LG, linkage group; MG, maturity group; NIL, near isogenic line; PCR, polymerase chain reaction; PI, plant introduction; QTL, quantitative trait loci; RF, SCN reproductive factor; SCAR, sequence characterized amplified region; SCN, soybean cyst nematode; SSR, simple sequence repeat.

independent breeding populations segregating for SCN resistance derived from PI 209332. In each population, they found coupling linkage between the SCN resistance allele at *rhg1* and an allele conferring reduced yield. The respective yield QTL in these two populations mapped about 10 cM from each other and on opposite sides of *rhg1*. The yield difference between lines homozygous for the high yield allele and lines homozygous for the low yield allele was 296 kg ha^{-1} for the QTL distal to *rhg1* and 632 kg ha^{-1} for the QTL proximal to *rhg1*. While Mudge et al. (1996) used nonelite and experimental breeding lines developed from a PI cross, it should be more informative to study the association between yield and SCN resistance in breeding lines with released SCN resistant cultivars in their pedigrees because these have already undergone repeated cycles of recombination and selection. Also, plant material derived from the widely used resistance source PI 88788 is more representative of the germplasm soybean breeders utilize than PI 209332, which has been used for cultivar development to a lesser extent.

The objective of our research was to test for the association between SCN resistance and yield as well as other agronomic traits in soybean cultivars with resistance derived from PI 88788. This association was tested in five NIL populations that segregate for SCN resistance at *rhg1* on LG G and two that segregate at cqSCN-003 on LG J.

MATERIALS AND METHODS

Plant Material

A total of seven NIL populations were developed that are segregating for either *rhg1* on LG G or cqSCN-003 on LG J. Four NIL populations were derived from a cross between Bell and Colfax. The maturity group (MG) I cultivar Bell has SCN resistance derived from PI 88788 (Fig. 1) (Nickell et al., 1990), while the MG II cultivar Colfax is susceptible to SCN (Graef et al., 1994). A population developed from this cross was inbred to the F_4 generation through single seed descent. F_4 plants were individually harvested, and lines were advanced to the $F_{4:7}$ generation in bulk. The $F_{4:7}$ lines were tested during the summer 2000 with genetic markers to first identify lines segregating for SCN resistance QTL and then to identify plants from within these lines that were homozygous for the markers linked to the QTL. The DNA used in marker testing was isolated by a quick extraction protocol developed by Bell-Johnson et al. (1998) and analyzed by simple sequence repeat (SSR) markers by methods described in Cregan and Quigley (1997). Polymerase chain reaction (PCR) products were analyzed in 3% (w/v) metaphor (FMC BioProducts, Rockland, ME) agarose gels or 6% (w/v) nondenaturing polyacrylamide gels (Wang et al., 2003). The lines were tested both with Satt309, which has been mapped 0.4 cM distal to *rhg1* (Cregan et al., 1999), and with Satt431, which is linked to cqSCN-003 (Glover et al., 2004). Both SSR markers were developed by P.B. Cregan (USDA-ARS, Beltsville, MD). Only plants predicted to be homozygous resistant or susceptible on the basis of these linked markers were kept to develop F_7–derived NILs. Populations BR-1 and BR-2 were segregating for *rhg1* with BR-1 consisting of eight resistant and 27 susceptible NILs, and BR-2 consisting of 22 resistant and 22 susceptible NILs. Populations BJ-1 and BJ-2 were segregating for cqSCN-003 with BJ-1 consisting of 10 resistant and 32 susceptible NILs, and BJ-2 consisting of 20 resistant and 21 susceptible NILs. Each population was developed from a different $F_{4:7}$ line. Seed used in the 2001 field tests was increased two generations in a winter nursery in Kekaha, HI.

Three additional NIL populations segregating for *rhg1* were derived from crossing Syngenta S22-C3 with Syngenta S42-M1. The MG II cultivar Syngenta S22-C3 is susceptible to *H. glycines* while the MG IV cultivar S42-M1 is SCN resistant and has the resistance sources Peking and PI 88788 in its ancestry (Fig. 1). A population developed from this cross was advanced to the F_6 generation in bulk, and F7-derived NILs were developed from $F_{6:7}$ lines as described above. The se-

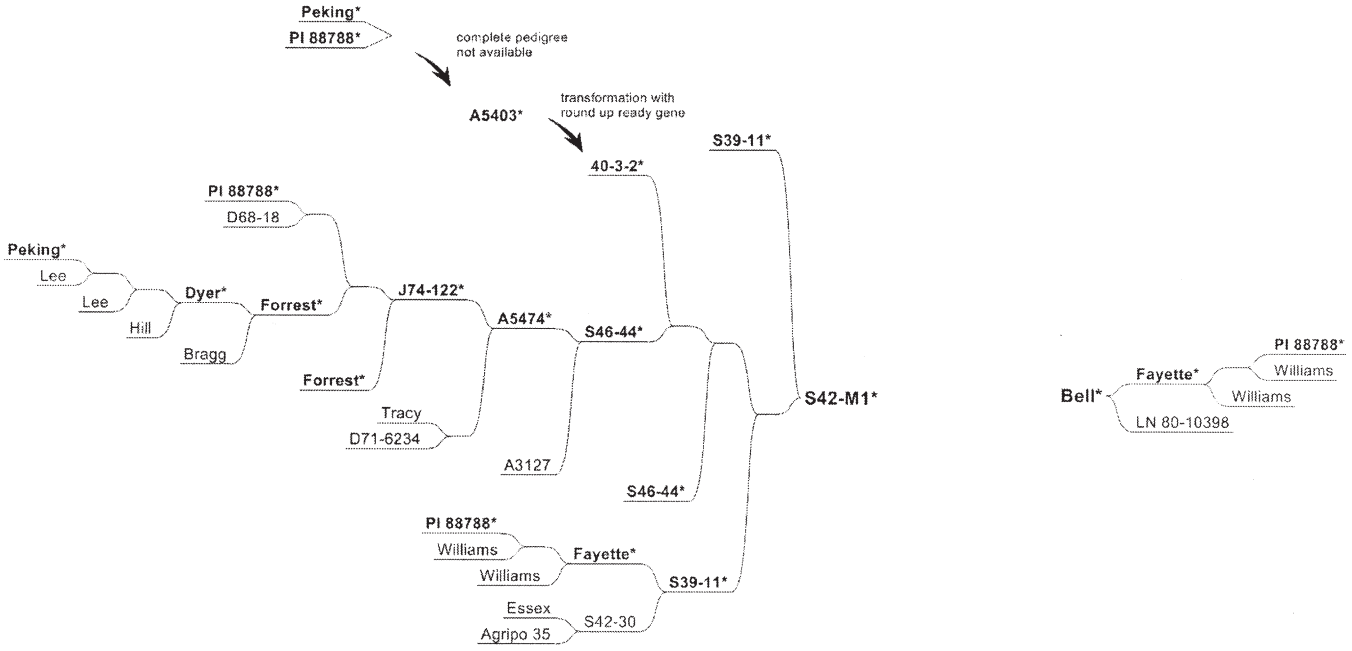

Fig. 1. Pedigree for the soybean cyst nematode (SCN) resistant parents S42-M1 (population SR-1, SR-2, and SR-3) and Bell (populations BR-1, BR-2, BJ-1, and BJ-2). Soybean cyst nematode resistant lines are marked with *. Lines D68-18, J74-122, D71-6234, and A5474 were not available for genotyping.

quence characterized amplified region (SCAR) marker CTA was used by methods described above to predict which lines were homozygous for *rhg$_1$* resistance or susceptibility alleles. This marker was developed from the amplified fragment length polymorphism (AFLP) marker $E_{CTA}M_{AGG}113$, which is 1 cM proximal to *rhg$_1$* (Meksem et al., 2001). These populations did not segregate for Satt431, which is linked to the SCN resistance QTL cqSCN-003 (Glover et al., 2004). Population SR-1 was comprised of 16 resistant and 10 susceptible NILs, SR-2 of 15 resistant and 17 susceptible NILs, and SR-3 of 12 resistant and 11 susceptible NILs.

Field Experiment

Each NIL population was field tested separately as a randomized complete block design experiment with two replications at four locations each in 2001 and 2002. All locations had presumably low SCN disease pressure on the basis of low SCN infestations on roots in previous growing seasons and because soybean had been out of crop rotation for more than a year before we planted the experiment. We selected locations with low SCN disease pressure in previous years because our objective was to test for yield differences between resistant and susceptible lines due to their yield potential that is not related to differences in SCN damage. The field locations included DeKalb, IL, and Washington, IA, which are located in maturity zone II, and St. Joseph and Urbana, IL, which are in maturity zone III. At Washington and St. Joseph, 1.52- × 3.66-m two-row plots were planted at 395 000 seeds ha^{-1}, while in DeKalb and Urbana, 1.52- × 3.20-m two-row plots were planted at 565 000 seeds ha^{-1}. Because of their late maturity, populations SR-2 and SR-3 were not planted at the DeKalb location. In 2001, we planted F_9 and F_{10} seed harvested from the first and second generation of seed increase, respectively. Planting dates in 2001 were 16 May at DeKalb, 9 June at St. Joseph, 11 June at Urbana, and 8 June at Washington. F_{11} seed was planted in 2002 on 7 May at DeKalb, 24 May at St. Joseph, 21 May at Urbana, and 22 May at Washington.

Plots were evaluated for plant maturity, plant height, and plant lodging. Plant maturity (R8) was recorded as the number of days after 31 August when 95% of the pods had turned to their mature color (Fehr et al., 1971). Plant lodging was rated at R8 on a scale from 1 = all plants upright to 5 = all plants prostrate and plant height was measured at R8 as the distance from the ground to the uppermost node of an average plant. Plots were harvested to measure seed yield, which was expressed on a moisture basis of 130 g kg^{-1}. At Washington during 2001, the only data taken for BR-1, BR-2, BJ-1, and BJ-2 were yield measurements while for SR-1, SR-2, and SR-3, only yield measurements and ratings on plant lodging were taken. Plant height was not recorded for SR-3 at Urbana, 2002.

Soil Samples

Soil samples were taken at harvest from 10 plots in each replication per NIL population to confirm low SCN disease pressure. The sampled plots were chosen in a regular pattern across each replication, and 10 cores of about 0.2-m depth were taken from each sampled plot. The cores were taken about 80 mm from the plant rows and were evenly distributed over the sampled plots. Soil samples were pooled by replication and stored at 4°C for further analysis.

For cyst extraction, soil samples were dried at room temperature and manually pulverized with a rolling pin. The samples were mixed, and a 100-cm^3 subsample of soil from each replication was dissolved in 500 mL of water and run through a semiautomatic elutriator (Univ. of Georgia Science Instrument Shop, Athens, GA) (Byrd et al., 1976). Retrieved cysts were driven through a 150-μm opening mesh wire sieve with a rubber stopper to release eggs that were rinsed through a 75-μm sieve and caught by a 25-μm sieve. Egg solutions were stained with 3 mL of egg staining solution (0.35 g acid fusion, 250 mL lactic acid, 750 mL water), microwaved to boil for at least 30 s, and diluted to 100 mL. A 5-mL sample of the egg solution was taken to count eggs under a light microscope to determine the average egg density of each replication in eggs/100 cm^3 soil.

Genetic Characterization of the NIL Populations

We defined the genetic region introgressed from the resistance sources Peking or PI 88788 that is surrounding the SCN resistance QTL in each resistant parent. To do this, we traced the genetic regions surrounding *rhg$_1$* or cqSCN-003 from the resistance sources through the available lines in the pedigree of Bell and S42-M1 (Fig. 1). All available PCR-based markers from these regions were used in this analysis. To determine what part of the identified PI 88788 region is segregating within each NIL population, we genotyped six NILs in each population. Instead of taking a random sample of six NILs per population, we selected the three highest and the three lowest yielding NILs in each population. This was done because in no population did the three highest yielding NILs and the three lowest yielding NILs fall into distinct SCN marker classes and it allowed us to genotypically compare NILs not only across SCN marker classes but also across high and low yield classes.

The genotyping was done by the marker methods previously listed except that the DNA was isolated from a sample of five plants from each selected NIL and available lines in the pedigrees by a modified CTAB method (Kisha et al., 1997). In addition to SSR markers, we also analyzed the cleaved amplified polymorphic sequence (CAPS) markers 21E22.sp2 and 35E22.sp1 using methods outlined in Patzoldt et al. (2005). These CAPS markers map to LG J and were designed by Klos et al. (2000). According to the resistance locus of interest, genotyping was done in each NIL population only for LG G (BR and SR populations) or LG J (BJ populations). We used the map positions of the markers according to the soybean genetic linkage map by Song et al. (2004).

Statistical Analysis

Descriptive statistics for egg densities within and across environments were computed by the UNIVARIATE PROCEDURE of SAS (SAS Institute, 2000). An analysis of variance (ANOVA) for the genetic effects of NILs on yield, maturity, plant height, and lodging across environments was computed by the MIXED PROCEDURE of SAS (SAS Institute, 2000). Environments and NILs were treated as random factors and NIL by environment interactions were pooled into the error if not significant at $\alpha = 0.25$. Furthermore, an ANOVA was performed to test the genetic effects of the regions containing the SCN resistance genes, and preplanned contrasts were computed between NILs of the two different marker classes (resistant versus susceptible). The markers used in this analysis for the *rhg$_1$* region were Satt309 in the BR populations and CTA in the SR populations and marker class was considered a fixed factor. Satt431 was used to detect the cqSCN-003 region in the BJ populations. The NILs were nested in marker class, and marker class by environment as well as NIL by environment interactions were pooled into the errors if not significant at $\alpha = 0.25$. In addition, we conducted combined analyses across BR-1 and BR-2 (BR), BJ-1 and BJ-2 (BJ), and SR-1, SR-2, and SR-3 (SR) with populations as random factors.

RESULTS

Soil Samples

We detected a wide range of SCN egg densities within and across environments from soil samples taken at R8 (Table 1). Mean egg densities ranged from 60 eggs/100 cm^3 soil for DeKalb, 2002, to 4923 eggs/100 cm^3 soil for DeKalb, 2001. Medians were lower than means indicating that the distributions of egg densities were skewed to the higher egg densities. Ranges of egg densities within environments were as narrow as 280 egg/100 cm^3 soil at DeKalb and Washington, 2002, and as wide as 17 720 eggs/100 cm^3 soil at St. Joseph, 2002. Given the low SCN disease pressure observed in previous years, some of these end of season egg densities were higher than we expected suggesting that yield differences between resistant and susceptible NILs might have been masked by SCN damage on the susceptible NILs. However, damage thresholds have been established for preseason but not for end of season egg densities. Therefore, we selected half of the environments that had the least SCN pressure on the basis of mean egg densities for each environment. In a refined analysis of the agronomic data from the selected environments, we could reduce a potential impact of SCN damage masking yield differences between resistant and susceptible NILs. The lower SCN pressure environments included Washington in 2001, and DeKalb, Urbana, and Washington in 2002.

Table 1. Descriptive statistics for end of season soybean cyst nematode (SCN) egg densities (eggs/100 cm^3 soil) in eight environments.

	Measures of central tendency		Measures of variability	
Environment	Mean	Median	Range	Interquartile range
DeKalb, 2001	4923	2856	16 800	4144
St. Joseph, 2001	3016	2912	7 952	1764
Urbana, 2001	2534	868	12 208	3192
Washington, 2001	1646	392	8 512	672
DeKalb, 2002	60	20	280	80
St. Joseph, 2002	4770	3480	17 720	4600
Urbana, 2002	1050	640	4 200	1400
Washington, 2002	145	120	280	160
All environments	2300	868	18 560	3040

Association between SCN Resistance and Agronomic Traits

We detected significant differences among NILs across environments for yield in populations BR-1, BR-2, and BJ-2 at a probability level of $p < 0.05$. Near isogenic line × environment interactions for yield were significant in BJ-1, BJ-2, and SR-2. Significant differences were observed among NILs for plant maturity in all populations; for plant lodging in all populations except BJ-1 and SR-1; and for plant height in all populations except SR-1 and SR-2. Significant NIL × environment interactions were detected for plant maturity in BR-1, BJ-2, and SR-3; for plant lodging in BR-1, BR-2, BJ-2, and SR-2; and for plant height in BR-2, BJ-2, and SR-1. Taking into consideration the wide range of average egg densities across environments (Table 1), we reanalyzed the experiment using data only from the least SCN infested environments as classified above. In this refined analysis, we detected significant differences among NILs for yield in BR-1, BR-2, and BJ-2 with significant NIL × environment interactions in BJ-2. Significant differences among NILs for plant maturity were detected in all populations except BJ-1 and SR-1; for plant lodging in BJ-2; and for plant height in BJ-2. Near isogenic line × environment interactions were detected for plant maturity in all populations except BJ-2 and SR-3; for plant lodging in BR-1, BR-2, BJ-2, and SR-1; and for plant height in BR-2.

Although significant differences among NILs were not observed in all populations, we computed the preplanned contrasts between the NILs with the resistance allele and those with the susceptibility allele on the basis of marker class. In all populations, the NILs predicted to be resistant yielded less across all environments than the NILs predicted to be susceptible (Table 2). However, this yield difference was statistically significant only in BR-1 (118 kg ha^{-1}), BJ-2 (76 kg ha^{-1}) and across BJ (70 kg ha^{-1}). Marker class × environment interactions were significant in BR-2, across BR, in SR-2 and across SR. In the refined analysis of the environments with the lowest SCN pressure, yield differences in BJ-2 and across BJ were no longer significant, although there was no decline in the magnitude of yield depression (Table 2). The most apparent change occurred in SR-2, where the yield difference increased threefold to 91 kg ha^{-1} ($p = 0.064$) in the refined analysis compared with the analysis across all environments. Marker class × environment interactions were significant in BJ-1 and across BJ.

Only small agronomic differences were observed between resistant and susceptible NILs (Table 3). Plant maturity differed by less than a day. In BR-2, across BR and in SR-2, resistant NILs were significantly later maturing whereas in BJ-1 and across BJ, susceptible NILs were significantly later. Plant lodging differed at most by 0.2 units and was significantly greater for resistant NILs in BR-1, BR-2, and across SR. Plant height differed at most by 2.5 cm and resistant NILs were significantly taller in BR-2 and SR-2. Marker class × environment interactions were significant for maturity in BR-1, BR-2, across BR, and across SR; and for plant lodging in BR-2, across BR, and in BJ-1. Dropping the environments with the greatest SCN infestation resulted in very little change in size for the difference between the resistant and susceptible NILs. Resistant NILs were no longer significantly later maturing in BR-2 and across BR, plant lodging remained significant only across SR, and plant height was significant only for SR-2 in the refined analysis. Marker class × environment interactions were significant for maturity in BR-2 and SR-3 and for plant lodging in BR-1.

Genetic Characterization of the Introgressed Region Segregating in the NIL Populations

Genotyping lines from the pedigree of the resistant parent Bell (Fig. 1) for the genetic area flanking *rhg*$_1$ on LG G resulted in the identification of a region in Bell that traces only to PI 88788. This insert from PI

Table 2. Yields for seven near isogenic line (NIL) populations segregating for loci conferring soybean cyst nematode (SCN) resistance. Yields were calculated across all environments and across the three or four least SCN infested environments.

			N†		Yield across all environments				Yield across least SCN infested environments			
Population	Locus	Marker	Res‡	Sus§	Env¶	Mean#	Contr.††	*p*	Env	Mean	Contr.	*p*
					kg ha^{-1}							
BR-1	*rhg$_1$*	Satt309	8	27	8	2753	−118**	0.004	4	2611	−132*	0.023
BR-2	*rhg$_1$*	Satt309	22	22	8	2726	−4	0.933	4	2577	12	0.806
Across BR	*rhg$_1$*	Satt309	30	49	8	2740	−62	0.097	4	2594	−60	0.089
BJ-1	cqSCN-003	Satt431	10	32	8	2666	−64	0.103	4	2445	−97	0.246
BJ-2	cqSCN-003	Satt431	20	21	8	2769	−76*	0.036	4	2641	−77	0.088
Across BJ	*rhg$_1$*	Satt431	30	51	8	2718	−70*	0.048	4	2543	−87	0.154
SR-1	*rhg$_1$*	CTA	16	10	8	3151	−22	0.371	4	3276	−46	0.243
SR-2	*rhg$_1$*	CTA	15	17	6	3638	−30	0.781	3	3735	−91	0.064
SR-3	*rhg$_1$*	CTA	12	11	6	3960	−42	0.343	3	3848	−4	0.939
Across SR	*rhg$_1$*	CTA	43	38	6 or 8	3614	−30	0.383	3 or 4	3678	−47	0.080

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
† N: Population size.
‡ Res: Lines predicted to be homozygous for the SCN resistance allele based on marker analysis.
§ Sus: Lines predicted to be homozygous for the SCN susceptible allele based on marker analysis.
¶ Env: Number of environments.
# Mean: Intraclass mean between NILs predicted to be homozygous SCN resistant and NILs predicted to be homozygous susceptible.
†† Contr.: Contrast of NILs predicted to be homozygous SCN resistant based on marker analysis- − NILs predicted to be homozygous susceptible.

88788 spans 10 SSR markers from at least cM position 2 (Satt038) on the LG G soybean genetic linkage map (Song et al., 2004) to at least cM position 23 (Satt130) (Fig. 2). The insert shows putative interruptions at cM positions 2 (Satt275) and 13 (Satt570) that would require double crossovers within intervals of 2 and 7 cM, respectively. The small likelihood of these events suggests that these markers might actually map to positions other than those reported previously. Therefore we disregarded the two markers for population BR-1 and BR-2. Marker data from the sampled NILs revealed a region spanning seven polymorphic SSR markers from at least cM position 2 (Satt038) to 11 (Satt610) is segregating for PI 88788 alleles in both BR populations. No informative marker was available above Satt038 and the next informative marker below Satt610 was at cM position 18 (Satt217). The populations are expected to have different breakpoints in the crossover intervals since they each trace back to different F_2 plants. To define these breakpoints, however, additional marker data in the crossover intervals are necessary.

Molecular marker data from the pedigree of the resistant parent Bell for the genetic region surrounding cqSCN-003 on LG J (Fig. 3) revealed a PI 88788 insert in Bell ranging from above Satt380 (cM position 43) to at least Sat_393 (cM position 91), below which no genetic markers were available. In BJ-1, we identified the PI88788 segregating fragment comprising eight polymorphic markers over 36 cM from at least cM position 59 (35E22.sp1) to 91 (Sat_393). A smaller segregating fragment was revealed in BJ-2 spanning three SSR markers over 11 cM from at least cM position 68 (Satt547) to cM position 79 (Satt431).

Since not all lines in the pedigree of the resistant parent S42-M1 were available for genotyping (Fig. 1), we cannot be as conclusive in describing the size of the region introgressed from the resistance source into S42-M1 as with the BR and BJ populations. Comparing marker data from the region surrounding *rhg$_1$* in S42-M1 and the two potential resistance sources Peking and PI 88788, revealed ten SSR markers on a 13-cM interval from cM position 0 (Satt163) to cM position 13 (Satt688)

Table 3. Agronomic performance of seven near isogenic line (NIL) populations segregating for loci conferring soybean cyst nematode (SCN) resistance. Traits were analyzed across all environments and across the three least SCN infested environments.

		Agronomic traits across all environments						Agronomic traits across least SCN infested environments					
		Plant maturity†		Plant lodging¶		Plant height#		Plant maturity		Plant lodging		Plant height	
Population	Locus	Mean‡	Contr.§	Mean	Contr.	Mean	Contr.	Mean	Contr.	Mean	Contr.	Mean	Contr.
BR-1	*rhg$_1$*	10.1	0.6	1.5	0.1*	60.8	0.7	6.4	0.4	1.3	0.1	54.3	0.2
BR-2	*rhg$_1$*	10.7	0.7*	1.5	0.2*	64.5	1.5*	7.3	0.7	1.3	0.1	57.7	0.7
Across BR	*rhg$_1$*	10.4	0.6*	1.5	0.1	62.6	1.1	6.2	0.6	1.3	0.1	56.0	0.5
BJ-1	cqSCN-003	13.3	−0.4**	2.2	0.0	84.5	−0.5	4.9	−0.6**	1.8	0.0	77.4	−1.7
BJ-2	cqSCN-003	9.4	−0.4	1.6	0.0	72.5	−2.5	4.9	−0.5	1.4	−0.1	68.4	−2.9
Across BJ	cqSCN-003	11.2	−0.4*	1.9	0.0	78.5	−1.5	4.9	−0.5*	1.6	−0.1	72.9	−2.3
SR-1	*rhg$_1$*	18.3	−0.2	1.4	0.0	76.1	−0.8	16.8	−0.2	1.4	0.0	72.1	−2.1*
SR-2	*rhg$_1$*	35.1	0.9**	2.4	0.1	96.1	2.0**	34.2	0.7*	2.1	0.1	96.0	1.8
SR-3	*rhg$_1$*	35.1	0	2.3	0.1	97.4	−0.5	34.7	−0.2	2.4	0.2	98.8	0.5
Across SR	*rhg$_1$*	30.2	0.2	1.9	0.1**	88.7	0.2	29.8	0.1	1.6	0.1*	85.2	0.1

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
† Plant maturity recorded as the number of days after August 31 when plants reached R8.
‡ Mean: Intraclass mean between NILs predicted to be homozygous SCN resistant and NILs predicted to be homozygous susceptible.
§ Contr.: Contrast of NILs predicted to be homozygous SCN resistant based on marker work- − NILs predicted to be homozygous SCN susceptible.
¶ Plant lodging rated on a scale from 1 = all plants upright to 5 = all plants prostrate.
# Plant height measured in cm.

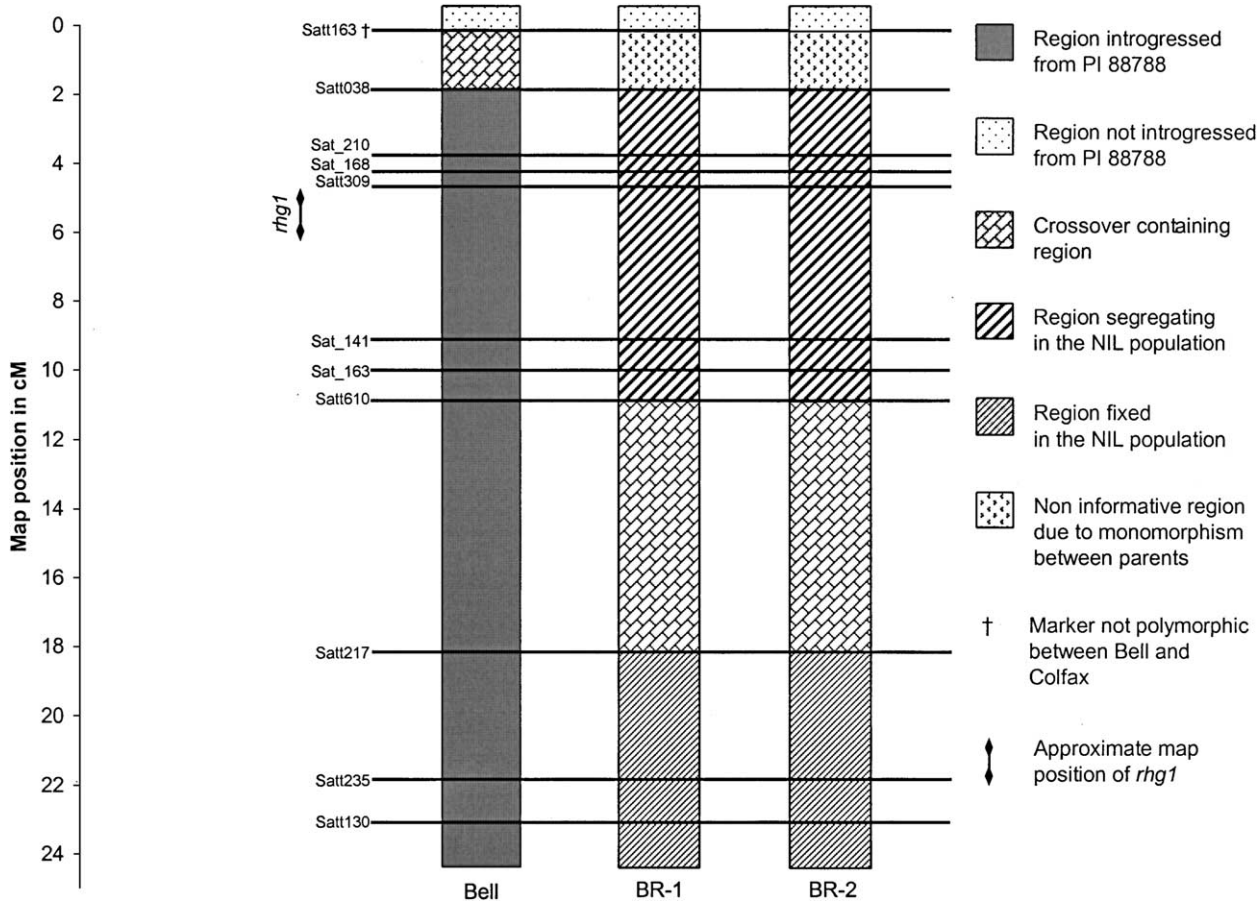

Fig. 2. Approximate size of the PI 88788 introgression in Bell and the PI 88788 derived fragments segregating on linkage group G in near isogenic line (NIL) populations BR-1 and BR-2 developed from crossing Bell and Colfax. Map positions are according to Song et al. (2004). Approximate position of *rhg*$_1$ according to Cregan et al. (1999).

that were common for both S42-M1 and PI 88788. Six of these markers that were distributed throughout that interval were polymorphic between S42-M1 and Peking, suggesting that SCN resistance in this region of S42-M1 is not derived from Peking but from PI 88788. No marker data were available for the region above the 13-cM interval, and the next three available markers below it, Satt217 (cM position 18) to Satt130 (cM position 23), were polymorphic between S42-M1 and PI 88788. This indicates that the PI 88788 insert in S42-M1 is likely from at least Satt163 to Satt688. To further investigate which branch in the pedigree this insert was derived from, we compared molecular marker data for the lines S42-M1, S46-44, 40-3-2, and S39-11. The longest interval of markers monomorphic with S42-M1 was detected in S46-44 spanning from cM position 0 (Satt163) to cM position 23 (Satt130). This suggests that *rhg*$_1$ resistance was most likely inherited through S46-44. The molecular marker data for these lines also indicated the misplacement of Satt275 and Satt570 that we noticed in the Bell pedigree. Because of the complexity of the pedigree (Fig. 1) and the unavailability of the lines D68-18, J74-122, D71-6234, and A5474, we were not able to further narrow down the size of the PI 88788 insert in S42-M1. We identified a segregating fragment in SR-1 which spanned five SSR markers over 6 cM from at least cM position 5 (Satt309) to cM position 11 (Satt610) on LG G (Fig. 4). The fragment is separated by the fixed markers Sat_168 and Sat_210 from an additional segregating fragment above, which was revealed by Satt038 (cM position 2). To test whether that additional segregating fragment had a yield effect, we computed an extra ANOVA for Satt038. The ANOVA, however, revealed no significant ($p = 0.274$) effect on yield for this fragment (data not shown). In SR-2, a fragment was revealed containing an interval of five SSR markers over 5 cM from at least cM position 2 (Satt038) to cM position 7 (CTA). In SR-3, a segregating fragment was identified spanning four SSR markers on a 4 cM interval from at least cM position 7 (CTA) to cM position 11 (Satt610).

DISCUSSION

In this study, we were able to detect yield depression associated with SCN resistance in environments with low to moderate SCN pressure. Across all test environments, we could detect significant yield drag ($p < 0.05$) in two out of seven NIL populations segregating for SCN resistance. While both of these populations had the same genetic background (Bell × Colfax), they were segregating for different SCN resistance loci, *rhg*$_1$ on LG G in BR-1 and cqSCN-003 on LG J in BJ-2. The

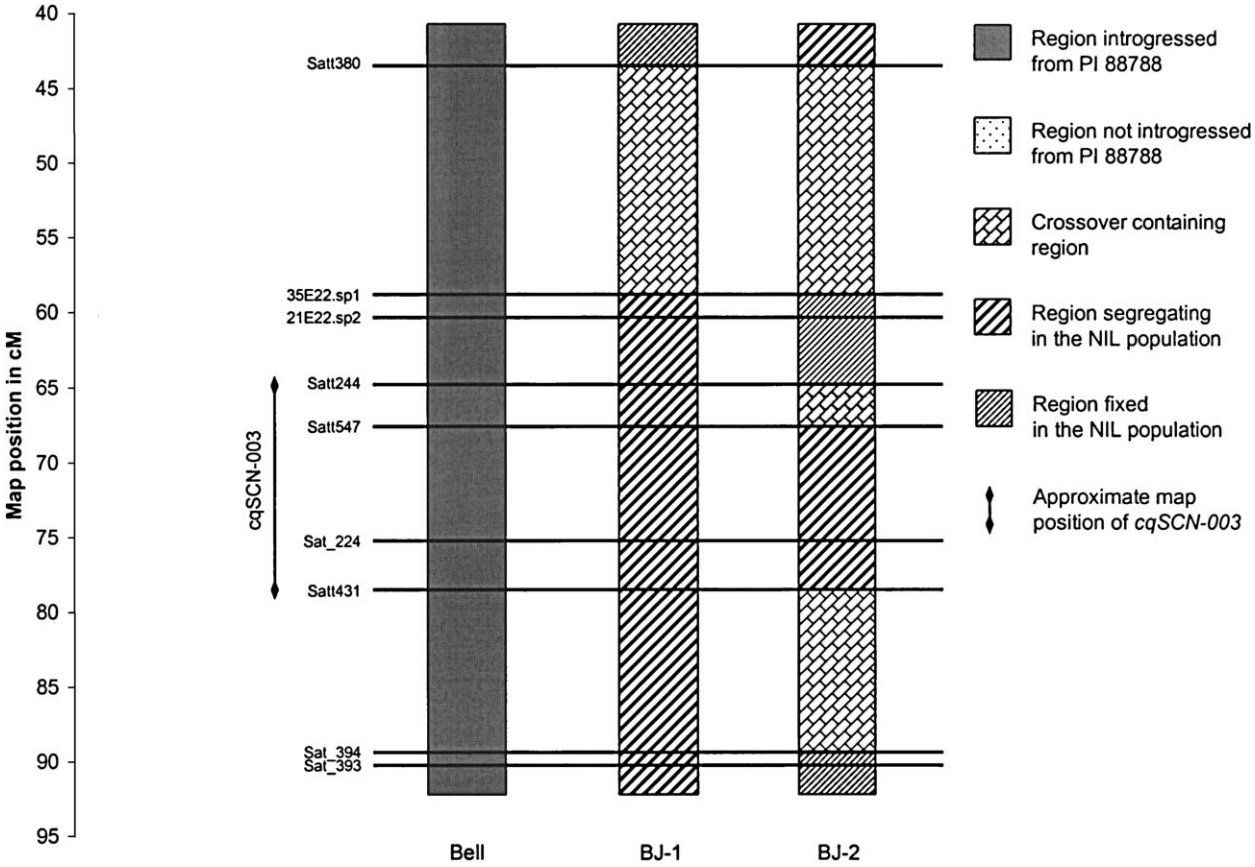

Fig. 3. Approximate size of the PI 88788 introgression in Bell and the PI 88788 derived fragments segregating on linkage group J in near isogenic line (NIL) populations BJ-1 and BJ-2 developed from crossing Bell and Colfax. Map positions are according to Klos et al. (2000) and Song et al. (2004). Approximate position of cqSCN-003 according to Concibido et al. (1994, 1997) and Glover et al. (2004) is between markers Sat_224 and Satt431.

yield effect associated with cqSCN-003 was also significant when data were combined across both BJ populations. Although we detected statistically significant differences between resistant and susceptible NILs for plant maturity, plant lodging, and plant height (Table 3), these differences were small in magnitude and at most 0.9 d for maturity, 0.2 units for plant lodging, and 2.9 cm for plant height.

We conducted this study in presumably low SCN pressure environments to avoid masking of yield effects through nematode damage; however, the only available data to confirm low SCN disease pressure were end-of-season egg densities from soil samples taken at harvest. To determine whether the SCN pressure in our tests was at the damage threshold when yield effects are expected, preseason SCN egg densities are required. However, SCN reproductive factors (RF = end of season egg density/pre-season egg density) that are needed to calculate preseason egg densities, depend on several host, environmental, and pathogen factors such as soybean cultivar, plant maturity, planting date, preseason egg density, and HG type and are therefore highly variable (Riggs et al., 2000; Wang et al., 2000; Chen et al., 2001; Niblack et al., 2002). Wheeler et al. (1997) reported RFs ranging from 0.1 to 5.5 for SCN resistant cultivars and from 0.4 to 112.2 for SCN susceptible cultivars grown in Ohio. In a study on SCN reproduction in the North Central USA (Wang et al., 2000), RFs ranged from 0.3 to 2.6 for resistant cultivars and from 1.3 to 70.8 for susceptible cultivars. Chen et al. (2001) estimated RFs from 0.3 to 1.7 for resistant cultivars and from 1.0 to 10.6 for susceptible cultivars in Minnesota. Also, in an independent study with populations BR-2 and SR-2 conducted in Illinois environments different than ours, Brucker (2004) reported RF ranges from 0.4 to 4.3 for resistant NILs, and from 0.5 to 18.9 for susceptible NILs.

Preseason egg densities not exceeding Noel's (1986) SCN damage threshold of 240 eggs/100 cm^3, would require a RF of at least 20.5 to obtain the end of season egg density mean of 4923 eggs/100 cm^3 soil observed at DeKalb, 2001 or a RF of at least 19.9 at St. Joseph; 2002. These numbers are above the RF ranges reported by Chen et al. (2001) and Brucker (2004), but still in the ranges reported by Wheeler et al. (1997) and Wang et al. (2000). We therefore cannot be certain whether all test environments had preseason egg densities below the damage threshold. At the same time, the data indicate that at least at DeKalb, 2002, SCN disease pressure was low, since high preseason egg densities above the damage threshold would require a small RF below 0.25 to obtain an end of season egg density of only 60 eggs/100 cm^3. Therefore, if SCN damage in environments with higher end of season egg densities masked yield

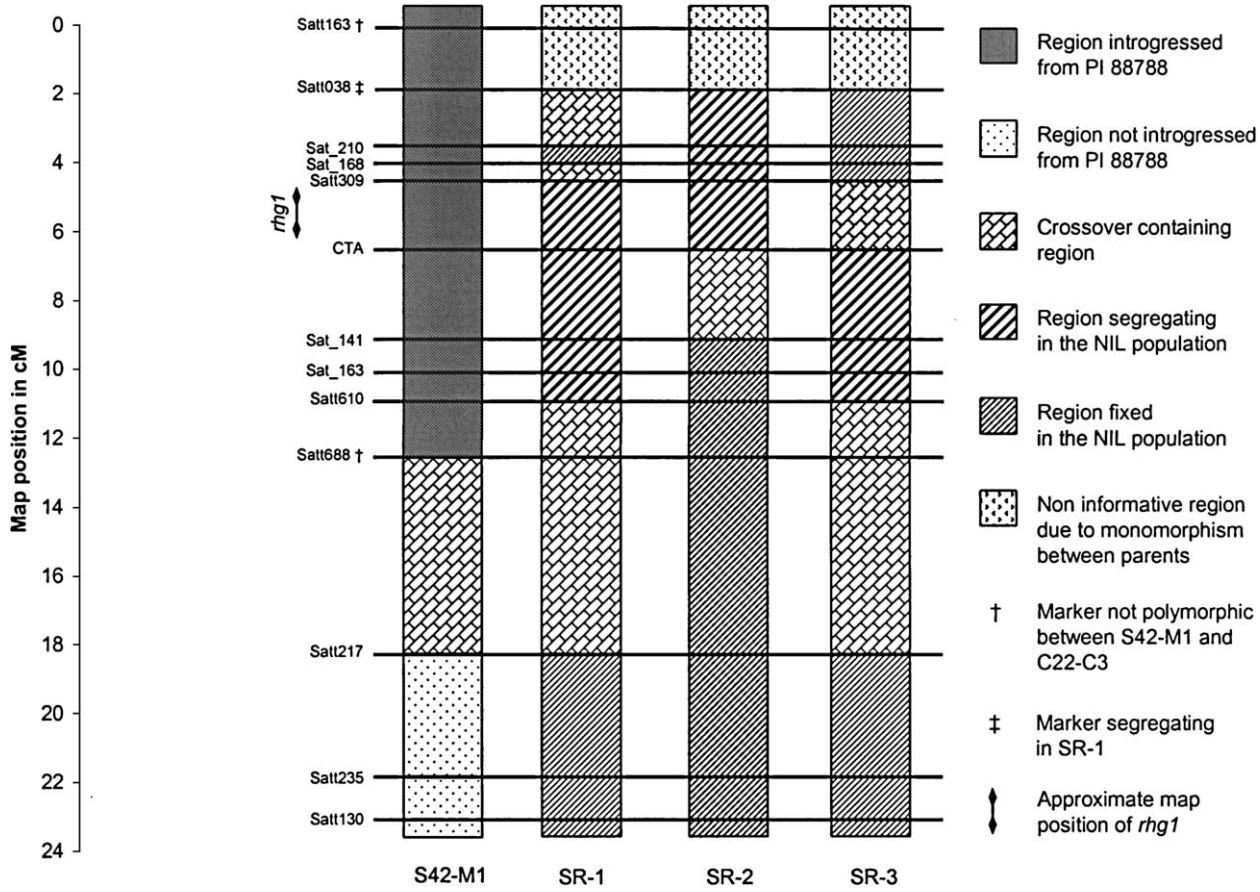

Fig. 4. Approximate size of the PI 88788 introgression in S42-M1 and the PI 88788 derived fragments segregating on linkage group G in near isogenic line (NIL) populations SR-1, SR-2, and SR-3 developed from crossing S42-M1 and C22-C3. Map positions are according to Meksem et al. (2001) and Song et al. (2004). Approximate position of *rhg1* according to Cregan et al. (1999) and Meksem et al. (2001).

differences between resistant and susceptible NILs, removing these environments from the analysis should lead to the detection of greater yield differences.

However, reanalyzing the data for only half of the environments with the lowest SCN pressure did not drastically change the magnitude of the yield effects for the Bell × Colfax derived populations (BR and BJ). The only remarkable change occurred in SR-2, where yield depression increased threefold to 91 kg ha^{-1} (p = 0.064). This change in magnitude and p value suggests that partial masking of yield effects through SCN damage may have occurred in this population. One reason why masking was not observed more widely is the small effect on resistance of the minor SCN resistance QTL cqSCN-003 in the BJ populations. In addition, the SCN pressure in general might have been too low to cause masking in our study.

Near insogenic lines were developed by testing indirectly for SCN resistance with one genetic marker in each population. Further genotyping of a subset of six NILs from each population revealed that both markers flanking the SCN QTL were cosegregating in all populations except SR-3 (Fig. 2–4). Since the map positions of both SCN QTL have been reported repeatedly (Concibido et al., 1994, 1996, 1997; Webb et al., 1995; Diers et al., 1997; Cregan et al., 1999; Meksem et al., 2001; Glover et al., 2004), we are confident that populations BR-1, BR-2, BJ-1, BJ-2, SR-1, and SR-2 are segregating for the SCN resistance QTL. In a related study on the effect of *rhg1* on SCN reproduction, Brucker (2004) tested populations BR-2 and SR-2 and verified the SCN phenotypes of all NILs in these two populations. However, for population SR-3, we cannot conclude with certainty that NILs are segregating for *rhg1* because only one of the flanking markers (CTA) was segregating (Fig. 4) and recombination between *rhg1* and CTA could have occurred.

Soybean cyst nematode resistance was significantly associated with lower yield only in two populations across all environments and one population across the least SCN infected environments. This suggests coupling linkage between SCN resistance and a putative yield reducing QTL allele was broken in at least some of the populations that had no significant yield effects. To break that linkage was not unlikely given the fact that the NIL populations were derived from a heterozygous F_4 (BR and BJ) or F_6 plant (SR) allowing three (BR and BJ) or five generations (SR) of recombination at the heterozygous region encompassing SCN resistance. These generations of recombination might also explain the high cross over density detected in population SR-1 (Fig. 4).

The association of SCN resistance with yield depression that we detected in BR-1, but not in BR-2, suggests that only in BR-1 the segregating PI 88788 fragment on

LG G encompasses a putative yield reducing QTL allele in coupling linkage with SCN resistance. Such a yield QTL might be located in the two crossover regions on LG G, the 2-cM interval bounded by Satt163 and Satt038 or the 7-cM interval bounded by Satt610 and Satt217 (Fig. 2). Comparing the genotypic data of SR-2, the only SR population we detected a yield effect ($p = 0.064$) with SR-1 and SR-3, suggests the existence of a putative yield QTL between Satt309 and the upper end of LG G regardless of whether SR-3 is indeed segregating for *rhg*$_1$ or not. Combining the information from the BR and SR populations suggests that there is a putative yield QTL in the 2-cM interval between Satt163 and Satt038. This falls into the same genetic region where the 296 kg ha^{-1} yield QTL was reported by Mudge et al. (1996). While yield depression on LG G was estimated at most 132 kg ha^{-1} (Table 2) in our study, the larger estimate in the study of Mudge et al. (1996) can be explained by the different genetic background they used. The association between SCN resistance and lower yield across BJ populations indicates a putative yield QTL on the segregating PI 88788 fragment that is common to BJ-1 and BJ-2, which is on the 25-cM interval between Satt244 and Sat_394 on LG J (Fig. 3).

CONCLUSIONS

The findings of this study show that a cause for SCN resistant cultivars yielding less than SCN susceptible cultivars under low SCN disease pressure is potentially yield drag associated with SCN resistance. While yield lag should be overcome quite easily in cultivar development, to overcome yield drag depends on whether the association between resistance and yield depression is caused by pleiotropy or linkage. Our data suggest that a putative yield QTL is located at least 3 cM above Satt309, which should make it feasible to break coupling linkage between SCN resistance and yield depression on LG G. Our data indicate that this already occurred in populations BR-2 and SR-1. At this point, however, no conclusions can be made on whether or not yield depression on LG J is pleiotropic to resistance at the SCN resistance QTL cqSCN-003. To confirm and refine our findings, experiments with plant material of similar genetic constitution need be performed, preferably in environments that lack SCN pressure.

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