Published online February 1, 2006

Glycine soja PI 468916 SCN Resistance Loci's Associated Effects on Soybean Seed Yield and Other Agronomic Traits

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ABSTRACT

The soybean cyst nematode (SCN, *Heterodera glycines* Riggs and Niblack) is the most important soybean [*Glycine max* (L.) Merr.] pathogen in the USA and its control relies on genetic resistance. When resistance genes from exotic sources are transferred into elite cultivars, deleterious alleles are frequently transferred with resistance through genetic linkages. Two SCN resistance loci have been identified in *G. soja* Sieb. and Zucc. PI 468916, and the effect these loci have on yield and other agronomic traits is not known. The objective of this study was to determine the effect of each *G. soja* SCN resistance gene on yield and other agronomic traits in elite soybean backgrounds. These effects were tested in two populations each segregating for SCN resistance derived from *G. soja*, with one population also segregating for the SCN resistance loci, *rhg*1, derived from *G. max* PI 88788. Each population was analyzed for genetic markers linked to the SCN resistance loci and field tested in multiple environments with low to high SCN infestations. The *G. soja* and *rhg1* SCN resistance alleles either had no effect or significantly ($P < 0.05$) enhanced yield compared with the susceptible alleles. The SCN resistance alleles were also associated with increasing days to maturity, plant height, and lodging scores. Deployment of the *G. soja* SCN resistance loci will increase the genetic diversity for SCN resistance and will provide breeders with an alternative source of SCN resistance that is not associated with reduced yield.

Soybean cyst nematode is the most important soybean pathogen in the USA and its control relies on genetic resistance (Niblack et al., 2004). Of the SCN resistant cultivars developed in the USA, resistance can be traced to *G. max* 'Peking', PI 88788, PI 90763, PI 437654, and PI 209332 (Diers et al., 1997). The predominant source of SCN resistance in the midwestern USA is PI 88788 (Diers and Arelli, 1999) with a few cultivars released with resistance from PI 90763 (Hartwig and Young, 1990), PI 437654 (Anand, 1992a), and PI 209332 (Anand, 1992b; Orf and MacDonald, 1995).

Molecular marker studies have shown that the *G. max* sources of SCN resistance have genes in common (Diers and Arelli, 1999; Concibido et al., 2004). Plant introduction 437654 (Webb et al., 1995), PI 209332, PI 88788, PI 90763, PI 89772, and Peking (Concibido et al., 1996, 1997; Chang et al., 1997; Yue et al., 2001a) all have the major SCN resistance gene, *rhg*1, on linkage group G (Cregan et al., 1999). This locus controls a large portion of the total variation for resistance and is effective against several HG Types of SCN (Concibido et al., 1996, 1997, 2004). In addition, Peking (Matson and Williams, 1965; Mahalingam and Skorupska, 1995; Chang et al., 1997), PI 209332 (Concibido et al., 1994), and PI 437654 (Webb et al., 1995) have the resistance gene *Rhg*4 that maps near the *I* locus (black seed-coat pigmentation) on linkage group A2 (Cregan et al., 1999).

It has been estimated that soybean cultivars selected for resistance to SCN, derived from *G. max*, yield 5 to 10% less than susceptible cultivars when grown in environments with low SCN pressure (Noel, 1992). This was noted by Chen et al. (1999) who reported that soybean cultivars with SCN resistance from PI 88788 yielded on average 161 kg ha^{-1} less than susceptible cultivars in noninfested field trials in Minnesota during 1999. The reduced yield associated with SCN resistance in noninfested, or low SCN pressure environments, can be attributed to pleiotropic effects of the SCN resistance gene(s) on yield or linkage and coinheritance of genes effecting yield.

Linkage between SCN resistance and reduced yield was reported by Mudge et al. (1996). In their study, populations segregating for SCN resistance derived from *G. max* PI 209332 revealed yield reducing quantitative trait loci (QTL) alleles in coupling linkage with the SCN resistance gene *rhg1*. These yield reducing alleles mapped approximately 10 cM from each other and a difference of 296 kg ha^{-1} for the QTL distal to *rhg*1 and 632 kg ha^{-1} for the QTL proximal to *rhg*1 was measured when homozygous resistant and susceptible lines were compared. This region was also associated with an increase in height and lodging, later maturity, and a decrease in seed protein and oil content. Kopisch-Obuch et al. (2005) tested for linkage between SCN resistance and reduced yield in near isogenic line (NIL) populations developed from soybean cultivars with resistance derived from *G. max* PI 88788. Five NIL populations segregated for resistance at *rhg*1 and two segregated for resistance at cqSCN-003 on LG J. In multiple field studies at locations with low SCN pressure, NILs carrying the SCN resistance allele yielded significantly ($P < 0.05$) less (118 kg ha^{-1}) than NILs carrying the susceptible alleles in one population segregating for *rhg*1 and in one population segregating for cqSCN-003 (76 kg ha^{-1}). Molecular marker analysis of the regions flanking the resistance genes suggested the presence of a yield reducing allele distal to *rhg*1 and possibly another yield reducing allele linked or pleiotropic to cqSCN-003. In several populations, an association between SCN resistance with maturity, height, and lodging

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Published in Crop Sci. 46:622–629 (2006).
Crop Breeding, Genetics & Cytology
doi:10.2135/cropsci2005.06-0131

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Abbreviations: ANOVA, analysis of variance; LG, linkage group; NIL, near isogenic line; PCR, polymerase chain reaction; PI, plant introduction; QTL, quantitative trait loci; SCN, soybean cyst nematode; SSR, simple sequence repeat.

was measured, but differences were small in magnitude. Brucker et al. (2005) tested two of the NIL populations that segregated for *rhg*1 in fields moderately to highly infested with SCN. They found that the resistance allele at *rhg*1 was associated with significantly greater yield (273 kg ha^{-1}) than the susceptible allele for one population. In both populations, they observed that the *rhg*1 resistance allele was associated with reduced SCN reproduction compared with the susceptible allele.

Glycine soja, the wild ancestor of soybean, has genetic diversity not present within *G. max* soybean germplasm (Keim et al., 1989; Maughan et al., 1995). In 2001, Wang et al. (2003) mapped two major loci that conferred resistance to PA3, a HG Type 0 (Niblack et al., 2002) (Race 3) SCN isolate, in a population of F_2–derived lines developed from a cross with *G. soja* PI 468916. One locus mapped in the interval between Satt598 and Satt491 on LG E and the second locus mapped in the interval between Satt288 and Satt472 on LG G. The locus on LG G mapped to a position where no SCN resistance loci has been reported and is over 72 cM from the major SCN resistance gene, *rhg*1, from *G. max* [http://soybase.ncgr.org (verified 20 November 2005; Concibido et al., 2004]. However, a SCN resistance locus has been reported from *G. max* PI 438489B at the same location as the LG E locus from *G. soja* (Yue et al., 2001b). Kabelka et al. (2005) recently confirmed the *G. soja* SCN resistance loci in a population of BC_4F_3–derived lines referred to as LDX01–1. *G. soja* PI 468916 was used as the donor parent and the experimental line A81–356022 was the recurrent parent during backcrossing. Using a PA3 HG Type 0 SCN isolate, they observed significant associations ($P < 0.05$) between a greenhouse SCN bioassay of the LDX01–1 population and markers linked to the SCN resistance loci on LGs E and G. Additional molecular markers were added within the regions containing the SCN resistance loci by using amplified fragment length polymorphism (AFLP) markers and bulked segregant analysis (BSA). The positions of the resistance loci also were better defined by developing and testing several backcross populations that segregated for different genetic regions where the resistance loci map.

While studies have confirmed the *G. soja* PI 468916 SCN resistance loci, the effect of each locus on seed yield has not been investigated. Therefore, the objective of this research was to determine the effect of each *G. soja* PI 468916 SCN resistance gene on seed yield and other agronomic traits. This association was tested in two populations that each segregated for SCN resistance derived from *G. soja* PI 468916.

MATERIALS AND METHODS

Plant Material

A population of 93 BC_4F_3–derived lines, referred to as LDX01–1, was developed with *G. soja* PI 468916 as the donor parent and the soybean experimental line A81–356022 as the recurrent parent during backcrossing. Genetic markers identifying the SCN resistance loci on LGs E and G were used to select the *G. soja* alleles during each cycle of backcrossing. A single BC_4F_1 plant that was heterozygous at the SCN resistance loci on LGs E and G was selected and a population was inbred to the BC_4F_3 generation through single-seed descent. The *G. max rhg*1 SCN resistance alleles, located on LG G and positioned at least 72 cM away from the *G. soja* SCN resistance locus (Cregan et al., 1999; Wang et al., 2001), are not present within this population. This genetic region is fixed for the *G. max* A81–356022 alleles. BC_4F_3 plants were sown and individually harvested to develop $BC_4F_{3:4}$ lines. Seed of each line was sown and bulk harvested for use in the 2003 ($BC_4F_{3:5}$) and 2004 ($BC_4F_{3:6}$) field tests.

A second population of 100 F_3–derived lines, referred to as LDX01–2, was developed by crossing the maturity group II cultivar Dwight with a BC_3F_1 plant carrying the *G. soja* SCN resistance QTL alleles. Dwight possesses the LG G *rhg*1 resistance allele from *G. max* PI 88788 (Nickell et al., 1998). The BC_3F_1 parent was developed by backcrossing the two G. *soja* SCN resistance alleles into the background of A81–356022 as part of the same backcrossing program that developed LDX01–1. F_1 plants produced through crossing the BC_3F_1 plant with Dwight were tested with markers and an F_1 that was heterozygous for all three resistance loci was selected. An F_3 population was developed from this selected plant through single-seed descent. F_3 plants were sown and individually harvested to develop $F_{3:4}$ lines. Seed of each line was sown and bulk harvested for use in the 2003 ($F_{3:5}$) and 2004 ($F_{3:6}$) field tests.

Field Trials

LDX01–1 was field tested at three locations in 2003, designated Numbers 1 (Crop Science Main Farm, Urbana, IL, planted 13 May), 2 (Ivesdale, IL, planted 17 May), and 3 (East Grein Tract, Urbana, IL, planted 16 May), and at two locations in 2004, designated Numbers 4 (Cruse Farm Tract, Urbana, IL, planted May 6) and 5 (Ivesdale, IL, planted 5 May). LDX01–2 was field tested at two locations in 2003, designated Numbers 1 (Ivesdale, IL, planted 14 May) and 2 (East Grein Tract, Urbana, IL, planted 16 May), and at two locations in 2004, designated Numbers 3 (Cruse Farm Tract, Urbana, IL, planted 6 May) and 4 (Ivesdale, IL, planted 5 May). The two populations were evaluated in separate tests by a randomized complete-block design with two replications at each location. In the LDX01–2 field trial Number 3, only one replication could be evaluated because of heavy rains and flooding. Cultivars Dwight (SCN resistant) and IA2052 (SCN susceptible) and experimental line A81–356022 (SCN susceptible) were included as checks. At all locations, 1.5- × 3.2-m two-row plots were planted at 395 000 seeds ha^{-1}. Conventional tillage practices were followed, fields were maintained weed-free, and recommended fertilization levels were applied at all locations.

Plots were evaluated for days to maturity, plant height, lodging, and seed yield. Days to maturity was recorded as the number of days after planting when approximately 95% of the pods had reached mature pod color (R8; Fehr et al., 1971). Plant height was measured at maturity in centimeters as the average distance from soil surface to the apex of the main stem. Lodging was scored at maturity on a scale of 1 to 5 with 1 designated as plants standing erect and 5 as plants prostrate. Seed yield expressed as kilograms per hectare was adjusted to 130 g kg^{-1} moisture.

Soil Samples and Soybean Cyst Nematode Egg Densities

Soil samples were taken at the R1 soybean growth stage (Fehr et al., 1971) from 10 randomly chosen plots in each replication per location and population. Each 2.5-cm diameter soil sample core was taken at approximately 80 mm from the

plant row at a depth of 0.2 m and stored at 4°C until processed. Cyst extraction and staining to determine SCN egg density in a 100 cm^3 soil sample was performed according to Kopisch-Obuch et al. (2005).

Genetic Characterization of LDX01–1 and LDX01–2

Lines in LDX01–1 were tested with the simple sequence repeat (SSR) markers, Satt598 and Satt491 on LG E and Satt288 and Satt472 on LG G. These markers span the genetic regions conferring SCN resistance introgressed into LDX01–1 and LDX01–2 from *G. soja* PI 468916 (Fig. 1). Lines within LDX01–1 had been previously genotyped with the above SSR markers (Kabelka et al., 2005). Lines within LDX01–2 were genotyped with SSR markers Satt491, Satt288, Satt472, and Satt309. Satt309 maps approximately 0.4 cM from the *rhg*1 SCN resistance allele from Dwight (Cregan et al., 1999) (Fig. 1). Chi-square analyses revealed that each SSR marker segregated according to expected ratios within populations LDX01–1 and LDX01–2.

The SSR markers were developed by P.B. Cregan (USDA-ARS, Beltsville, MD). Genomic DNA used in the polymerase chain reaction (PCR) amplifications of the SSR markers was extracted from leaf tissue of eight greenhouse-grown seedlings from each line in each population according to Keim et al. (1988) with modifications. Specifically, leaf tissue was collected into 15-mL conical tubes, freeze-dried, and pulverized with five (4 mm each) glass beads. Pulverization was done for 4 min with a modified paint can shaker. Six milliliters of the previously described CTAB extraction buffer (Keim et al., 1988) was added to this macerated tissue and incubated for 1 h at 65°C. After cooling for 10 min, 6 mL of chloroform:isoamyl alcohol (24:1) was added to each tube, gently mixed, and then spun at 1861 *g* for 15 min. The resulting aqueous layer was transferred to a new 15-mL conical tube and treated with RNase before precipitation with 95% ethanol. The resulting pellet was resuspended in 1 mL of 0.1 × TE buffer and diluted 10 fold before use in genetic analysis. Polymerase chain reactions were performed according to Cregan and Quigley (1997). The PCR products were analyzed by electrophoresis in 6% (w/v) nondenaturing polyacrylamide gels (Sambrook et al., 1989; Wang et al., 2003), stained with 1 $\mu g\ mL^{-1}$ ethidium bromide, and viewed under ultraviolet light.

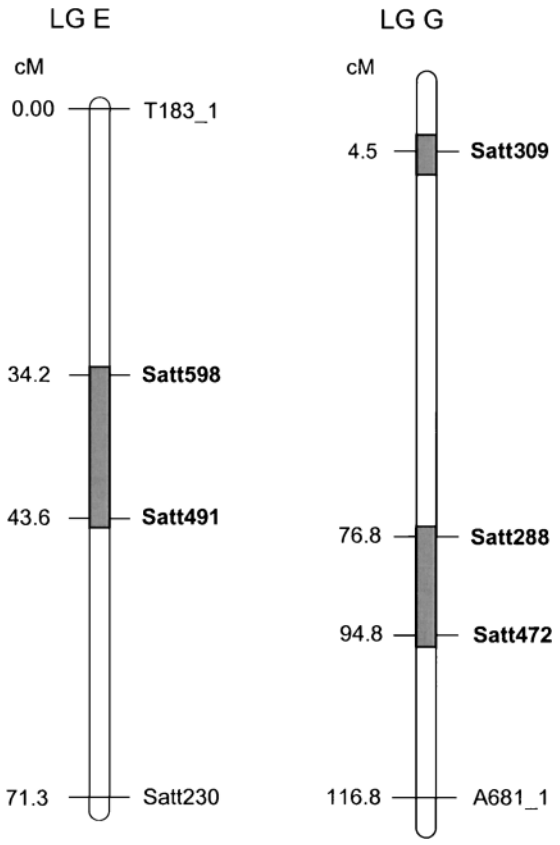

Fig. 1. Abbreviated LGs E and G maps of soybean (Cregan et al., 1999). Microsatellite markers, Satt598 and Satt491 on LG E and Satt288 and Satt472 on LG G, span the genetic regions conferring resistance to SCN introgressed from *G. soja* PI 468916 in both LDX01–1 and LDX01–2. Microsatellite marker Satt309 marks the SCN resistance *rhg*1 locus from 'Dwight' in LDX01–2. *G. max* A81–356022 alleles are present at Satt309 within LDX01–1.

Statistical Analysis

Agronomic traits were subject to analysis of variance (ANOVA) by the PROC GLM functions of SAS (Statistical Analysis System version 9.1, SAS Institute, Cary, NC). Lines within populations were considered as a fixed effect whereas environments, replications within environments, and environment × line interactions were considered as random effects. Each location–year combination was considered an environment in the analysis. Significance was determined by an *F* test with the numerator and denominator degrees of freedom approximated as described by Satterthwaite (1946). Least significant differences (LSD) between entry means were determined at a 5% significance level. Estimates of variance components were obtained using the REML method of PROC VARCOMP of SAS. Single-factor ANOVA was used to identify marker association with each agronomic trait using PROC GLM in SAS. Marker genotypes, used as class variables, were considered as fixed effects whereas environments, replications, and lines were considered random effects. Pair-wise comparisons of least square means were used to test the significance ($P < 0.05$) of each marker class. While all SSR markers were evaluated in this study, only the most significant markers associated with SCN resistance, across environments, were listed (Tables 1 and 2).

RESULTS

Soybean Cyst Nematode Egg Densities

At the LDX01–1 test environments, SCN egg densities taken at the soybean R1 growth stage ranged from 86 eggs/100 cm^3 soil for Environment 4 in 2004 to 4280 eggs/100 cm^3 soil for Environment 2 in 2003 (Table 3). At the LDX01–2 test environments, SCN egg densities taken at the soybean R1 growth stage ranged from 112 eggs/100 cm^3 soil for Environment 4 in 2004 to 3480 eggs/100 cm^3 soil for environment 1 in 2003. To define which environments had low SCN pressure, we used a threshold of 240 eggs/100 cm^3 soil (Noel, 1986). This is a preseason threshold and because we took soil samples at R1, which would have provided time for one to two cycles of SCN reproduction after planting, sites we define as having moderate to high SCN infestations may have been below this threshold at planting. The environments we classified as having low SCN infestations were 4 (86 eggs/100 cm^3 soil) and 5 (168 eggs/100 cm^3 soil) of the LDX01–1 test sites and 4 (112 eggs/100 cm^3 soil) of the LDX01–2 test. All other test sites were considered to have moderate to high SCN infestations.

Table 1. Agronomic evaluation of the population LDX01–1, which segregates for loci conferring soybean cyst nematode resistance on LGs E and G derived from *G. soja* PI 468916. Only the most significant markers associated with SCN resistance, across environments, are listed. Data are arranged by individual environments and means across environments for seed yield, days to maturity, plant height and lodging score.

		SCN eggs	Seed yield (kg ha^{-1})				Days to maturity (R8)				Plant height (cm)				Lodging score (1–5)††			
Marker (LG)†	Env‡	(100 cm^3 soil)$^{-1}$	*P* value	Res§	Sus¶	Effect#	*P* value	Res	Sus	Effect	*P* value	Res	Sus	Effect	*P* value	Res	Sus	Effect
Satt491 (E)	1	3580	0.0006	2553	2459	+94	ns	–	–	–	ns	–	–	–	0.0068	2.1	1.9	+0.2
	2	4280	ns	–	–	–	ns	–	–	–	ns	–	–	–	ns	–	–	–
	3	785	0.0400	2681	2600	+81	ns	–	–	–	ns	–	–	–	0.0003	2.2	2.0	+0.2
	4	86	0.0277	3810	3756	+54	ns	–	–	–	ns	–	–	–	ns	–	–	–
	5	168	ns	–	–	–	0.0130	131	130	+1	ns	–	–	–	0.0025	2.4	2.1	+0.3
	Across	1780	0.0005	3131	3037	+94	0.0081	127	126	+1	ns	–	–	–	ns	–	–	–
Satt472 (G)	1	3580	0.0511	2553	2486	+67	ns	–	–	–	0.0042	93.0	88.4	+4.6	ns	–	–	–
	2	4280	ns	–	–	–	ns	–	–	–	ns	–	–	–	ns	–	–	–
	3	785	ns	–	–	–	ns	–	–	–	ns	–	–	–	ns	–	–	–
	4	86	ns	–	–	–	ns	–	–	–	ns	–	–	–	0.0294	2.9	2.5	+0.4
	5	168	0.0007	3803	3648	+155	ns	–	–	–	ns	–	–	–	ns	–	–	–
	Across	1780	0.0106	3131	3057	+74	ns	–	–	–	0.0466	106.7	104.1	+2.6	0.0357	2.4	2.1	+0.3

† LG, linkage group.
‡ Environments 4 and 5 represent low SCN infested environments (<240 eggs/100 cm^3 soil), whereas Environments 1, 2, and 3 represent moderate to high SCN infested environments in this study.
§ Mean of lines predicted to be homozygous for the SCN resistance allele on the basis of marker analysis.
¶ Mean of lines predicted to be homozygous for the SCN susceptible allele on the basis of marker analysis.
# Effect of resistant alleles on agronomic trait.
†† Lodging was scored at maturity on a scale of 1 (plants erect) to 5 (plants prostrate).

Table 2. Agronomic evaluation of the population LDX01–2, which segregates for loci conferring soybean cyst nematode resistance on LGs E and G derived from *G. soja* PI 468916 and *rhg*1 derived from *G. max* Dwight. Only the most significant markers associated with SCN resistance, across environments, are listed. Data are arranged by individual environments and means across environments for seed yield, days to maturity, plant height and lodging score.

		SCN eggs	Seed yield (kg ha^{-1})				Days to maturity (R8)				Plant height (cm)				Lodging score (1–5)††			
Marker (LG)†	Env‡	(100 cm^3 soil)$^{-1}$	*P* value	Res§	Sus¶	Effect#	*P* value	Res	Sus	Effect	*P* value	Res	Sus	Effect	*P* value	Res	Sus	Effect
Satt491 (E)	1	3480	ns	–	–	–	0.0493	119	117	+2	0.0402	88.1	85.6	+2.5	0.0357	1.7	1.5	+0.2
	2	700	ns	–	–	–	ns	–	–	–	ns	–	–	–	0.0107	1.5	1.3	+0.2
	3	272	ns	–	–	–	ns	–	–	–	ns	–	–	–	ns	–	–	–
	4	112	ns	–	–	–	ns	–	–	–	0.0377	93.5	90.0	+3.5	ns	–	–	–
	Across	1141	ns	–	–	–	ns	–	–	–	0.0160	85.3	82.8	+2.5	0.0166	1.5	1.4	+0.1
Satt309 (G)	1	3480	ns	–	–	–	ns	–	–	–	ns	–	–	–	0.0001	1.9	1.5	+0.4
rhg1	2	700	0.0045	2573	2426	+147	ns	–	–	–	ns	–	–	–	0.0120	1.6	1.3	+0.3
	3	272	0.0109	3507	3265	+242	ns	–	–	–	ns	–	–	–	0.0067	1.7	1.3	+0.4
	4	112	ns	–	–	–	ns	–	–	–	ns	–	–	–	0.0110	1.3	1.2	+0.1
	Across	1141	ns	–	–	–	ns	–	–	–	ns	–	–	–	0.0002	1.6	1.3	+0.3
Satt472 (G)	1	3480	0.0250	2943	2815	+128	ns	–	–	–	ns	–	–	–	0.0205	1.7	1.6	+0.1
	2	700	ns	–	–	–	ns	–	–	–	ns	–	–	–	ns	–	–	–
	3	272	0.0435	3561	3393	+168	ns	–	–	–	ns	–	–	–	ns	–	–	–
	4	112	0.0136	3447	3239	+208	0.0154	125	124	+1	ns	–	–	–	ns	–	–	–
	Across	1141	0.0066	3057	2909	+148	0.0424	121	120	+1	ns	–	–	–	0.0202	1.5	1.4	+0.1

† LG, linkage group.
‡ Environment 4 represents a low SCN infested environment (<240 eggs/100 cm^3 soil) whereas environments 1, 2, and 3 represent moderate to high SCN infested environments in this study.
§ Mean of lines predicted to be homozygous for the SCN resistance allele on the basis of marker analysis.
¶ Mean of lines predicted to be homozygous for the SCN susceptible allele on the basis of marker analysis.
# Effect of resistant alleles on agronomic trait.
†† Lodging was scored at maturity on a scale of 1 (plants erect) to 5 (plants prostrate).

Table 3. Average soybean cyst nematode egg densities (eggs/100 cm³ soil)⁻¹ taken at the soybean R1 growth stage within and across test environments of LDX01–1 and LDX01–2.

Population	Year	Environments	Average	SD
LDX01–1	2003	1	3580	1103
		2	4280	283
		3	785	205
	2004	4	86	99
		5	168	79
		Across	1780	
LDX01–2	2003	1	3480	509
		2	700	198
	2004	3	272	294
		4	112	91
		Across	1141	

Field Data Analysis

Significant ($P < 0.05$) effects for environments, lines, and environment × line interactions were detected in both the LDX01–1 and LDX01–2 studies for days to maturity, plant height, lodging score, and seed yield across environments. Variance component estimates revealed that the environment accounted for more of the variance compared with lines and environment × line interactions for all traits in both populations.

Across environments in the LDX01–1 field trials, the average seed yields of A81–356022 (3071 kg ha^{-1}) and lines in LDX01–1 (3097 kg ha^{-1}) were at least 6% less than the average seed yields of Dwight (3454 kg ha^{-1}) or IA2052 (3299 kg ha^{-1}) (Table 4). Within the moderate to high SCN infested environments (Numbers 1, 2, and 3), and across all environments, the highest yielding lines within LDX01–1 did not yield significantly different from either Dwight or IA2052. However, within the low SCN infested environments (Numbers 4 and 5), lines were present that yielded at least 11% higher than the checks. These high yielding lines tended to mature later than the checks, however. On average, Dwight and IA2052 matured 6 to 9 d earlier, were shorter in plant height, and lodged the least compared with either A81–356022 or the mean of lines in LDX01–1 (Table 5).

Across environments in the LDX01–2 field trials, the average seed yields of A81–356022 (2956 kg ha^{-1}) and lines in LDX01–2 (2936 kg ha^{-1}) were at least 7% less than the average seed yields of IA2052 (3192 kg ha^{-1}) and Dwight (3232 kg ha^{-1}) (Table 4). Within the moderate to high SCN infested environments (Numbers 1 and 2), and across all environments, lines were present within LDX01–2 that yielded at least 6% higher than either Dwight or IA2052, although these were later in maturity. Within the low SCN infested environment (Number 4), the highest yielding lines within LDX01–2 did not yield significantly different from either Dwight or IA2052. On average, Dwight and IA2052 matured 4 to 7 d earlier and lodged the least compared with either A81–356022 or LDX01–2 (Table 5). Dwight was the shortest in plant height, on average, next to IA2052 and LDX01–2, with A81–356022 being the tallest. Lines in LDX01–1 average 5 d later, 20.8 cm taller, and 0.7 greater lodging score than the average of lines in LDX01–2. This is consistent with the earlier maturity, shorter height, and less lodging of Dwight, one of the parents of LDX01–2, than A81–356022, the recurrent parent of LDX01–1.

Association between Soybean Cyst Nematode Resistance Loci and Agronomic Traits within LDX01–1

Across environments, the introgressed *G. soja* regions on LG E, marked by Satt491, and LG G, marked by Satt472, were associated with enhanced seed yield ranging from 74 to 94 kg ha^{-1} (Table 1). Within the low SCN infested environments (Numbers 4 and 5), the two regions either had no significant effect on seed yield or enhanced it up to 155 kg ha^{-1}. Within the moderate to high SCN infested environments, (Numbers 1, 2, and 3), the two regions either had no significant effect on seed yield or enhanced it up to 94 kg ha^{-1}. Across and within certain environments, the genetic regions on LGs E and G were also associated with either increasing days to

Table 4. Means and ranges for seed yield measured in populations LDX01–1 and LDX01–2, cultivars Dwight and IA2052, and experimental line A81–356022 within and across environments.

Environments†	1		2		3		4		5		Across	
SCN eggs (100 cm³ soil)⁻¹	3580		4280		785		86		168		1780	
	Seed yield (kg ha⁻¹)											
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
LDX01–1	2508	2127–2728	2844	2164–3413	2647	2153–2966	3802	3427–4307	3736	3131–4260	3097	2385–3366
A81–356022	2333		3121		2358		3877		3667		3071	
Dwight	2977		3578		3338		3638		3785		3454	
IA2052	2894		3743		2919		3428		3511		3299	
LSD (0.05)	275		673		387		447		409		204	

Environments	1		2		3‡		4		Across	
SCN eggs (100 cm³ soil)⁻¹	3480		700		272		112		1141	
	Seed yield (kg ha⁻¹)									
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
LDX01–2	2845	2174–3349	2503	1814–3017	3398	2454–4515	3280	2100–4015	2936	2197–3407
A81–356022	2678		1813		4102		3798		2956	
Dwight	3299		2416		3759		3712		3232	
IA2052	2792		2721		3716		3800		3192	
LSD (0.05)	329		404		–		376		199	

† Environments 4 and 5 of the LDX01–1 test sites and environment 4 of the LDX01–2 test sites represent low SCN infested environments. All other test sites represent moderate to high SCN infested environments.

‡ Only one replication could be evaluated because of heavy rains and flooding. No LSD is available.

Table 5. Means and ranges for days to maturity, plant height, and lodging score measured in populations LDX01–1 and LDX01–2, cultivars Dwight and IA2052, and experimental line A81–356022 across environments.

	Days to maturity (R8)		Plant height		Lodging score	
	Mean†	Range	Mean	Range	Mean	Range
			cm		1–5	
LDX01–1	126	120–129	105.2	95.8–112.0	2.2	1.7–3.1
A81–356022	126		106.7		1.8	
Dwight	120		80.5		1.0	
IA2052	117		91.9		1.2	
LSD (0.05)	1.0		4.8		0.4	
	Days to maturity (R8)		Plant height		Lodging score	
	Mean†	Range	Mean	Range	Mean	Range
			cm		1–5	
LDX01–2	121	114–127	84.3	73.7–92.5	1.5	1.0–2.6
A81–356022	120		101.6		1.7	
Dwight	116		73.7		1.0	
IA2052	113		87.4		1.0	
LSD (0.05)	1.2		5.8		0.3	

† Days to maturity from planting, plant height, and lodging score averaged across five environments.
‡ Days to maturity from planting, plant height, and lodging score averaged across four environments.

maturity by up to 1 d, plant height by up to 4.6 cm, or lodging scores by up to 0.4 units.

Lines within LDX01–1 that carried both *G. soja* SCN resistance loci averaged, across environments, 3163 kg ha^{-1} in yield, matured at 127 d, were 106 cm tall, and had a lodging score of 2.6. In comparison, lines that carried both the *G. max* SCN susceptible alleles at these loci averaged, across environments, 2936 kg ha^{-1}, matured at 125 d, were 105 cm tall, and had a lodging score of 2.1. A line recently released from LDX01–1, designated LDX01–1–65 (Diers et al., 2005), averaged, across environments, 3265 kg ha^{-1} in yield, matured at 128 d, was 107 cm tall, and had a lodging score of 2.9.

Association between Soybean Cyst Nematode Resistance Loci and Agronomic Traits within LDX01–2

Within and across environments, the genetic region introgressed from *G. soja* on LG E, marked by Satt491, did not have a significant effect on seed yield (Table 2). However, this region, across and within certain environments, was associated with increasing days to maturity by up to 2 d, plant height by up to 3.5 cm and lodging scores by up to 0.2 units. The introgressed *G. soja* region on LG G, marked by Satt472, within the low SCN infested environment (Number 4), and across all environments, was significantly associated with enhancing seed yield by up to 208 kg ha^{-1}. Within the moderate to high SCN infested environments (Numbers 1, 2, and 3), this region either had no significant effect on seed yield or enhanced it up to 168 kg ha^{-1}. The introgressed *G. soja* LG G region, across and within certain environments, was also associated with increasing days to maturity by 1 d and lodging scores by 0.1 units. Segregation at the *G. max rhg*1 SCN locus on LG G marked by Satt309, across environments and within the low (Number 4) and high (Number 5) SCN infested environments, did not have a significant association with seed yield. However within the environments considered to have moderate SCN infestation (Numbers 2 and 3), the resistance allele was associated with a seed yield increase of up to 242 kg ha^{-1}. This resistance allele was also associated with increasing lodging scores by up to 0.4 units within and across all environments.

Lines within LDX01–2 carrying all three SCN resistance loci averaged, across environments, 3051 kg ha^{-1} in yield, matured at 121 d, were 84 cm tall, and had a lodging score of 1.5. Lines within LDX01–2 that carried the SCN susceptible alleles at these loci averaged, across environments, 2853 kg ha^{-1}, matured at 120 d, were 86 cm tall, and had a lodging score of 1.4.

DISCUSSION

This study reveals that the *G. soja* PI 468916 SCN resistance loci, located on LGs E and G, either had no effect on seed yield or enhanced it by up to 6% in environments with SCN infestations ranging from low to high. This yield enhancement was significant over environments for both genetic backgrounds with LG G but only one for LG E. The two *G. soja* SCN resistance loci were also found, across and within certain environments, to be associated with increasing days to maturity by up to 2 d, plant height by up to 4.6 cm, and lodging score by up to 0.4 units.

The positive yield response observed for the *G. soja* LG E locus in the backcross population is consistent with results reported by Wang et al. (2004) who also used PI 468916 as a donor parent of backcross populations. They tested BC_2 populations for yield and other agronomic traits across four environments and found that the *G. soja* allele at Satt491 was associated with 53 kg ha^{-1} greater seed yield than the allele from 'IA2008', the recurrent parent. Although SCN infestation levels were not quantified for their field environments, high infestation levels were not observed at any location.

The results from our study indicate that the *G. soja* SCN resistance alleles can be used in soybean improvement without major concerns of linkage drag reducing yield. When genes are introgressed from exotic sources, linked drag often hinders breeding progress (Tanksley and McCouch, 1997). The introgression of the two *G.*

soja resistance genes had a nondetectable to a positive effect on yield, suggesting that negative yield effects were not linked to these genes. We cannot resolve with certainty whether the positive yield effects were the result of SCN resistance, or linked genes that have a positive impact on yield. This is especially difficult to determine because there was no clear pattern between SCN infestation levels and the effect of these regions on yield. To resolve this, the populations would need to be tested in fields that have no SCN infestations; which are extremely rare in Illinois.

A factor that may have contributed to the lack of association between yield and SCN infestation levels is diversity in the pathogenicity of SCN populations in the field locations. The SCN populations in some locations may be poorly controlled by the *G. soja* resistance loci, which would have resulted in little or no yield benefit from these genes. A line carrying both resistance loci that was released from the backcross LDX01–1 population, designated LDX01–1–65, was evaluated in the greenhouse with three SCN isolates (Diers et al., 2005). The female index of this line was 14 for PA 3, a HG type 0 SCN isolate; 57 for PA2, a HG type 1.2.5.7 isolate; and 3 for PA5, a HG type 2.5.7 isolate. If we had an SCN population similar to the HG type 1.2.5.7 isolate in our field, little yield benefit may have resulted from the resistance gene(s). In retrospect, we should have greenhouse-tested lines from the populations with SCN samples from each field environment to quantify the control we achieved with the resistance.

This study also revealed that the SCN resistance allele *rhg*1 derived from *G. max* PI 88788 within LDX01–2 either had no effect on seed yield or enhanced it by up to 7% in environments with moderate SCN infestations. The *rhg*1 locus was also found, within and across environments, to be associated with increasing lodging score by up to 0.4 units. Why *rhg*1 was not effective under the high SCN infestation environment in this study is not entirely clear. In fact, *rhg*1 has been shown to be effective in environments with even greater SCN infestation levels than observed in our Environment 1 (Brucker et al., 2005). The most likely explanation for the lack of association observed in Environment 1 is that, as SCN populations can differ for their virulence genes, it is possible the SCN population and its elevated levels at this site overcame the PI 88788 *rhg*1 allele that segregated in the LDX01–2 population. However, it is also possible that *rhg*1 alone could have had a positive effect on yield but because of the two *G. soja* resistance genes, also segregating in this population, the effect of *rhg*1 may have been diluted enough so that its influence on yield was not significant.

Comparison of our *rhg*1 findings with those of others (Mudge et al., 1996; Chen et al., 1999; Kopisch-Obuch et al., 2005) continues to suggest that the association between the *G. max* SCN resistance locus *rhg*1 and reduced seed yield in environments without high SCN infestations can be broken. In Kopisch-Obuch et al. (2005), the presence of a yield depression locus at least 3 cM distal to *G. max rhg*1 on LG G was identified. Their data also revealed possible breakage of the linkage between *rhg*1 and this yield depression locus in two out of three populations evaluated that segregated at this genetic region. Possibly in our LDX01–2 population, linkage between *rhg*1 and a yield reduction locus has been broken. Additional genetic analysis within and around *rhg*1 in LDX01–2 would be necessary to confirm this.

We detected an unfavorable association between all three SCN resistance alleles and greater lodging, which is a concern in cultivar development programs. This association was notable in LDX01–1, where the marker alleles from the resistance sources were associated with 0.2 (LG E) to 0.4 (LG G) greater lodging scores, across and within certain environments, than the susceptible alleles. The increased lodging score association with *rhg*1 (LG G) in LDX01–2 ranged from 0.1 to 0.4 units within and across all environments. This unfavorable lodging association may need to be resolved before or during cultivar development efforts.

ACKNOWLEDGMENTS

This research was supported by USDA/NRI Grant No. 2002-01225.

REFERENCES

Anand, S.C. 1992a. Registration of 'Hartwig' soybean. Crop Sci. 32: 1069–1070.

Anand, S.C. 1992b. Registration of 'Delsoy 4710' soybean. Crop Sci. 32:1294.

Brucker, E., T. Niblack, F.J. Kopisch-Obuch, and B.W. Diers. 2005. The effect of *rhg*1 on reproduction of *Heterodera glycines* in the field and greenhouse and associated effects on agronomic traits. Crop Sci. 45:1721–1727.

Chang, S.J.C., T.W. Doubler, V.Y. Kilo, J. Abu Thredeih, R. Prabhu, V. Freire, R. Suttner, J. Klein, M.E. Schmidt, P.T. Gibson, and D.A. Lightfoot. 1997. Association of loci underlying field resistance to soybean sudden death syndrome (SDS) and cyst nematode (SCN) race 3. Crop Sci. 37:965–971.

Chen, S.Y., P.M. Porter, J.H. Orf, C.D. Reese, W.C. Steinstra, N.D. Young, D.D. Walgenbach, P.J. Schaus, T.J. Arlt, and F.R. Breitenbach. 1999. Performance of soybean cyst nematode resistant varieties from 1996 to 1999 in Minnesota. University of Minnesota Southern Research and Outreach Center. Waseca, MN. Available at http://sroc.coafes.umn.edu/Nematology/%20Home.htm (Verified 14 January 2005).

Concibido, V.C., R.L. Denny, S.R. Boutin, R. Hautea, J.H. Orf, and N.D. Young. 1994. DNA marker analysis of loci underlying resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe). Crop Sci. 34:240–246.

Concibido, V.C., B.W. Diers, and P.R. Arelli. 2004. A decade of QTL mapping for cyst nematode resistance in soybean. Crop Sci. 44: 1121–1131.

Concibido, V.C., D.A. Lange, R.L. Denny, J. Orf, and N.D. Young. 1997. Genome mapping of soybean cyst nematode resistance genes in Peking, PI90763, and PI88788 using DNA markers. Crop Sci. 37: 258–264.

Concibido, V.C., N.D. Young, D.A. Lange, R.L. Denny, D. Danesh, and J.H. Orf. 1996. Targeted comparative genome analysis and qualitative mapping of a major partial-resistance gene to the soybean cyst nematode. Theor. Appl. Genet. 93:234–241.

Cregan, P.B., T. Jarvik, A.L. Bush, R.C. Shoemaker, K.G. Lark, A.L. Kahler, N. Kaya, T.T. VanToai, D.G. Lohnes, L. Chung, and J.E. Specht. 1999. An integrated genetic linkage map of the soybean genome. Crop Sci. 39:1464–1490.

Cregan, P.B., and C. Quigley. 1997. Simple sequence repeat DNA marker analysis. p. 173–185. *In* G. Caetano-Anolles, and P.M. Gresshoff (ed.) DNA markers: Protocols, applications and overview. J. Wiley & Sons, New York.

Diers, B.W., and P.R. Arelli. 1999. Management of parasitic nematodes of soybean through genetic resistance. p. 300–306. *In* H.E.

Kauffman (ed.) Proc. World Soybean Res. Conf. VI. Superior Printing, Champaign, IL.
Diers, B.W., P.R. Arelli, S.R. Carlson, W.R. Fehr, E.A. Kabelka, R.C. Shoemaker, and D. Wang. 2005. Registration of 'LDX01–1-65' soybean. Crop Sci. 45:1671–1672.
Diers, B.W., H.T. Skorupska, A.P. Rao-Arelli, and S.R. Cianzio. 1997. Genetic relationships among soybean plant introductions with resistance to soybean cyst nematodes. Crop Sci. 37:1966–1972.
Fehr, W.R., C.E. Caviness, D.T. Burmood, and J.S. Pennington. 1971. Stage of development descriptions for soybeans, *Glycine max* (L.). Merrill. Crop Sci. 11:929–931.
Hartwig, E.E., and L.D. Young. 1990. Registration of 'Cordell' soybean. Crop Sci. 30:231–232.
Kabelka, E.A., S.R. Carlson, and B.W. Diers. 2005. Localization of two loci that confer resistance to soybean cyst nematode from *Glycine soja* PI 468916. Crop Sci. *In* press.
Keim, P., T.C. Olson, and R.C. Shoemaker. 1988. A rapid protocol for isolating soybean DNA. Soybean Genet. Newsl. 15:150–152.
Keim, P., R.C. Shoemaker, and R.C. Palmer. 1989. Restriction fragment length polymorphism diversity in soybean. Theor. Appl. Genet. 77: 786–792.
Kopisch-Obuch, F.J., R.L. McBroom, and B.W. Diers. 2005. Association between SCN resistance loci and yield in soybean. Crop Sci. 45: 956–965.
Mahalingam, R., and H.T. Skorupska. 1995. DNA markers for resistance to *Heterodera glycines* I. Race 3 in soybean cultivar Peking. Breed. Sci. 45:435–443.
Matson, A.L., and L.F. Williams. 1965. Evidence of a fourth gene for resistance to the soybean cyst nematode. Crop Sci. 5:477.
Maughan, P.J., M.A. Saghai Maroof, and G.R. Buss. 1995. Microsatellite and amplified sequence length polymorphism in cultivated and wild soybean. Genome 38:715–723.
Mudge, J., V. Concibido, R. Denny, N. Young, and J. Orf. 1996. Genetic mapping of a yield depression locus near a major gene for soybean cyst nematode resistance. Soybean Genet. Newslett. 23: 175–178.
Niblack, T.L., P.R. Arelli, G.R. Noel, C.H. Opperman, J. Orf, D.P. Schmitt, J.G. Shannon, and G.L. Tylka. 2002. A revised classification scheme for genetically diverse populations of *Heterodera glycines*. J. Nematol. 34:279–288.
Niblack, T.L., G.L. Tylka, and R.D. Riggs. 2004. Nematode pathogens of soybean. p. 821–851. *In* H.R. Boerma and J.E. Specht (ed.) Soybeans: Improvement, production, and uses. 3rd ed. Agron. Monogr. 16. ASA and SSSA, Madison, WI.
Nickell, C.D., G.R. Noel, and T.R. Cary. 1998. Registration of 'Dwight' soybean. Crop Sci. 38:1398.
Noel, G.R. 1986. The soybean cyst nematode, p. 257–268. *In* F. Lamberti and C.E. Taylor (ed.) Cyst nematodes. NATO ASI Series, Series A. Life Sciences, Plenum Press, New York.
Noel, G.R. 1992. History, distribution, and economics. p. 1–13. *In* R.D. Riggs and J.A. Wrather (ed.) Biology and management of the soybean cyst nematode. APS Press, St. Paul, Minn.
Orf, J.H., and D.H. MacDonald. 1995. Registration of 'Faribault' soybean. Crop Sci. 35:1227.
Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular cloning, a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press. Plainview, New York.
Satterthwaite, F.E. 1946. An approximate distribution of estimates of variance components. Biometrics Bull. 2:110–114.
Tanksley, S.D., and S.R. McCouch. 1997. Seed banks and molecular maps: Unlocking genetic potential from the wild. Science (Washington, DC) 277:1063–1066.
Wang, D., G.L. Graef, A.M. Procopiuk, and B.W. Diers. 2004. Identification of putative QTL that underlie yield in interspecific soybean backcross populations. Crop Sci. 108:458–467.
Wang, D., P.R. Rao-Arelli, R.C. Shoemaker, and B.W. Diers. 2001. Loci underlying resistance to race 3 of soybean cyst nematode in *Glycine soja* plant introduction 468916. Theor. Appl. Genet. 103:561–566.
Wang, D., J. Shi, S.R. Carlson, P.B. Cregan, R.W. Ward, and B.W. Diers. 2003. A low-cost, high-throughput polyacrylamide gel electrophoresis system for genotyping with microsatellite DNA markers. Crop Sci. 43:1828–1832.
Webb, D.M., B.M. Baltazar, A.P. Rao-Arelli, J. Schupp, K. Clayton, P. Keim, and W.D. Beavis. 1995. Genetic mapping of soybean cyst nematode race-3 resistance loci in the soybean PI 437.654. Theor. Appl. Genet. 91:574–581.
Yue, P., D.A. Sleper, and P.R. Arelli. 2001a. Mapping resistance to multiple races of *Heterodera glycines* in soybean PI 89772. Crop Sci. 41:1589–1598.
Yue, P., P.R. Arelli, and D.A. Sleper. 2001b. Molecular characterization of resistance to *Heterodera glycines* in soybean PI 438489B. Theor. Appl. Genet. 102:921–928.