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Identification of *Rotylenchulus reniformis* Resistant *Glycine* Lines

Salliana R. Stetina,[1] James R. Smith,[2] Jeffery D. Ray[2]

Abstract: Identification of resistance to reniform nematode (*Rotylenchulus reniformis*) is the first step in developing resistant soybean (*Glycine max*) cultivars that will benefit growers in the mid-South region of the United States. This study was conducted to identify soybean (*G. max* and *G. soja*) lines with resistance to this pathogen. Sixty-one wild and domestic soybean lines were evaluated in replicated growth chamber tests. Six previously untested soybean lines with useful levels of resistance to reniform nematode were identified in both initial screening and subsequent confirmation tests: released germplasm lines DS4-SCN05 (PI 656647) and DS-880 (PI 659348); accession PI 567516 C; and breeding lines DS97-84-1, 02011-126-1-1-2-1 and 02011-126-1-1-5-1. Eleven previously untested moderately susceptible or susceptible lines were also identified: released germplasm lines D68-0099 (PI 573285) and LG01-5087-5; accessions PI 200538, PI 416937, PI 423941, PI 437697, PI 467312, PI 468916, PI 594692, and PI 603751 A; and cultivar Stafford (PI 508269). Results of previously tested lines evaluated in the current study agreed with published reports 69.6% of the time for resistant lines and 87.5% of the time for susceptible lines. Soybean breeders may benefit from incorporating the newly identified resistant lines into their breeding programs.

Key words: Glycine, reniform nematode, resistance, *Rotylenchulus reniformis*, soybean.

The reniform nematode (*Rotylenchulus reniformis*) is found throughout the southern United States, from Texas to the East Coast. This nematode parasitizes more than 300 plant species including two major crops in the region, cotton (*Gossypium hirsutum*) and soybean (*Glycine max*) (Robinson et al., 1997). Based on nematode thresholds provided by the Mississippi State University Extension Service, losses are expected when susceptible soybean is planted in soil with population densities of 100 nematodes per 473-cm^3 soil, regardless of soil texture (http://msucares.com/lab/nematode-thresholds/soybean.pdf). Symptoms of infection by reniform nematode, which include stunting and incomplete pod filling (McGawley and Overstreet, 1999), are relatively uniform in field distribution and are often overlooked (Robinson, 2002).

In response to recent changes in commodity prices, production practices in Mississippi and other states in the mid-South region of the United States have shifted from cotton to increased acreage of other crops, primarily corn (*Zea mays*) or soybean. Most of the cotton acreage in Mississippi, the Delta region in particular, is infested with reniform nematode at levels that have caused economic losses in cotton (Robinson, 2007). Reniform nematodes have not been a problem in traditional mid-southern U.S. soybean production fields. However, the shift in acreage from cotton to soybean means that many soybean growers are now faced with the challenge of producing a profitable crop in reniform nematode–infested fields. As this is a relatively new challenge, little research has been conducted to identify reniform nematode resistant/tolerant soybean genotypes.

Host plant resistance would be highly advantageous to soybean growers because it is simple to deploy, environmentally friendly, cost-effective, and it persists throughout the entire growing season. However, because the geographic range of reniform nematode in the U.S. soybean production region and associated losses are limited compared with that of soybean cyst nematode (*Heterodera glycines*) (Pratt and Wrather, 1998; Wrather et al., 2003), breeding efforts by private industry have addressed the larger market and focused on incorporating resistance to the latter. Identification and transfer of resistance to reniform nematode into soybean breeding lines, and eventually into cultivars, would benefit growers in Mississippi and the mid-South where reniform nematode pressure is significant, especially in fields traditionally dedicated to cotton production.

In studies to identify resistance to reniform nematode and describe its inheritance in soybean, phenotype determination methods vary considerably. Field-based screening (Lim and Castillo, 1979) is not reported as frequently as evaluations done under controlled greenhouse or growth chamber conditions (Rebois et al., 1968; Williams et al., 1981; Harville et al., 1985; Robbins et al., 1994; Davis et al., 1996; Robbins and Rakes, 1996; Rodríguez-Kábana et al., 1998; Robbins et al., 1999,

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[1]Research Plant Pathologist USDA ARS Crop Genetics Research Unit, P.O. Box 345, Stoneville, MS 38776.

[2]Research Geneticist (Plants), USDA ARS Crop Genetics Research Unit, P.O. Box 345, Stoneville, MS 38776.

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E-mail: Sally.Stetina@ars.usda.gov

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2000, 2001, 2002, 2006; Ha et al., 2007; Robbins et al., 2007; Asmus, 2008; Robbins et al., 2008, 2009; Lawrence et al., 2011; Robbins et al., 2012). Under controlled conditions, test durations have ranged from 3 wk (Williams et al., 1981) to 15 wk (Robbins et al., 2006, 2008, 2012). In studies where genotypes were screened for resistance, the genotypes generally were replicated within a single test; however, the tests themselves may (Robbins et al., 1994; Robbins and Rakes, 1996) or may not (Rebois et al., 1968; Lim and Castillo, 1979; Davis et al., 1996; Rodríguez-Kábana et al., 1998; Robbins et al., 1999, 2000, 2001, 2002, 2006; Robbins et al., 2007; Asmus, 2008; Robbins et al., 2008, 2009; Lawrence et al., 2011; Robbins et al., 2012) have been repeated. In studies focused on describing inheritance of resistance to the reniform nematode (Williams et al., 1981; Harville et al., 1985) or identifying molecular markers associated with resistance (Ha et al., 2007), screenings typically were not repeated. The nematode life stage assessed also varies from test to test, with some researchers relying solely on root-associated females and egg masses (Lim and Castillo, 1979; Williams et al., 1981; Harville et al., 1985), others relying on counts of vermiform and egg stages found in the soil (Robbins et al., 2006, 2009; Lawrence et al., 2011), and still others using a combination of soil- and root-associated stages to make their determinations (Rebois et al., 1968; Robbins et al., 1994; Davis et al., 1996; Robbins and Rakes, 1996; Rodríguez-Kábana et al., 1998; Robbins et al., 1999, 2000, 2001, 2002; Ha et al., 2007; Robbins et al., 2007; Asmus, 2008; Robbins et al., 2008, 2012). Additional factors that varied from study to study included the genotypes selected as resistant and susceptible controls, inoculum level and method, volume of soil used, and geographic origin of the reniform nematode isolate(s) used in the evaluations.

Reniform nematode-resistant soybean lines have been identified (Rebois et al., 1968; Lim and Castillo, 1979; Robbins et al., 1994; Davis et al., 1996; Robbins and Rakes, 1996; Rodríguez-Kábana et al., 1998; Robbins et al., 1999, 2000, 2001, 2002, 2006; Robbins et al., 2007; Asmus, 2008; Robbins et al., 2008, 2009; Robbins et al., 2012), though in most studies, lines rated as susceptible greatly outnumber their resistant counterparts. It is not clear to what extent the lines share a common ancestor due to the lack of pedigree information in most cases.

Early studies (Rebois et al., 1968, 1970) suggested that genotypes with resistance to soybean cyst nematode also would be resistant to reniform nematode. However, this possible connection was not supported by subsequent work (Birchfield et al., 1971; Harville et al., 1985; Anand, 1992). More recently, soybean lines developed from soybean cyst nematode resistance sources cv. Peking, PI 90763, and PI 437654 have been reported to be resistant to reniform nematode (Robbins et al., 1994; Davis et al., 1996; Robbins and Rakes, 1996). Studies have reported various mechanisms governing resistance, including control by a single locus (Williams et al., 1981), two loci (Harville et al., 1985), or quantitative trait loci in two different linkage groups (Ha et al., 2007). If unique loci are involved, it may be possible to combine resistance from two or more sources.

Reports of intraspecific variability in morphology, reproduction, and pathogenicity in reniform nematode (Agudelo et al., 2005; McGawley et al., 2010, 2011) raise the possibility of the pathogen adapting to one or more resistance sources, as has been observed with PI 88788-derived resistance and soybean cyst nematode (Niblack et al., 2008). Identification of new sources of resistance to reniform nematode and their incorporation into commercial cultivars, alone or in combination, will add to the arsenal of management tools available to combat this pathogen, and may ultimately allow rotation of various sources of resistance to prolong their utility.

The objective of this research was to evaluate a selection of *Glycine* lines for their reaction to a Mississippi population of the reniform nematode and to identify additional sources of host plant resistance that could be used against this pathogen. Preliminary reports have been published (Stetina et al., 2012a, 2012b).

Materials and Methods

Identification of resistant lines: Sixty-one wild and domestic soybean (*Glycine max* and *G. soja*) lines listed in Table 1 were evaluated for resistance to infection by reniform nematode in growth chamber tests. Lines for which reactions to reniform nematode were previously reported were included to assess their response to a Mississippi isolate of reniform nematode; the reactions reported in earlier studies are noted in Table 1. Seeds not already in the authors' research collections were obtained from the USDA, ARS Soybean Germplasm Collection, Urbana, IL.

Because of growth chamber space limitations, lines were divided into three tests, and most lines were evaluated in two separate screenings. However, four of the lines (02011-126-1-1-5-1, PI 468916, Lee 74 [PI 548658], and Terral TVX 48R018) had results from only one test because of poor seed germination. The day length was set at 16 hr and temperature was held constant at 28 °C. An automated watering system was used to maintain soil moisture with the timing adjusted as needed during the experiment to supply additional water as plants grew. The experimental design for each screening was a completely randomized design with five replications.

A single plant of each soybean line was established in a container (Ray Leach SL-10 Cone-tainer, Stuewe & Sons, Inc., Tangent, OR) filled with 120 cm^3 of a steam-sterilized soil mixture consisting of one part sandy loam soil mixed with two parts sand. Upon stand establishment (approximately 5 d after planting), 500 reniform nematodes (mixed vermiform life stages) suspended in 1 ml water were added to the soil in each container. A

TABLE 1. Infection of *Glycine* roots by *Rotylenchulus reniformis* females 28 d after inoculation in growth chamber tests. All soybean lines are *G. max* except for PI 468916, which is a wild *G. soja* accession.

Soybean line	Reported rating(s)[a]	Test 1				Test 2				Test 3			
		Count[b]		Index[c]	Rating[d]	Count[b]		Index[c]	Rating[d]	Count[b]		Index[c]	Rating[d]
Delta King DK4968[b]	S (11)	56.0	a	124.1	S	103.2	a-c	100.0	S	76.7	a-d	48.3	MS
Morsoy RTS4706N[b]	S (10)	34.1	a-c	75.6	S					240.8	a	151.6	S
02011-126-1-1-2-1		9.7	cd	21.6	MR					1.8	jk	1.2	R
02011-126-1-1-5-1						15.3	b-f	14.8	MR				
Asgrow AG4605	R (11), S (13)	33.1	a-c	73.4	S	154.8	a	150.0	S				
Delta Grow 4970RR	R (11)	39.6	ab	87.8	S	110.6	a-c	107.2	S				
DS97-84-1		1.9	e	4.3	R					22.7	d-i	14.3	MR
DT97-4290	S (8)	22.3	a-c	49.5	MS					66.7	a-e	42.0	MS
LG01-5087-5		34.0	a-c	75.4	S					54.4	a-e	34.3	MS
PI 200538						87.5	a-d	84.8	S	68.4	a-d	43.1	MS
PI 209332	MS (6), R (12)					218.9	a	212.1	S	67.0	a-d	42.2	MS
PI 230977						18.5	b-f	18.0	MR	22.6	d-i	14.2	MR
PI 303652	R (6)					28.4	a-e	27.5	MR	37.6	b-g	23.7	MR
PI 339868 B	R (6)					7.2	d-f	7.0	R	49.8	a-f	31.4	MS
PI 404166	R (6)					4.2	ef	4.1	R	36.1	c-g	22.7	MR
PI 404198 A	R (6)					19.2	b-f	18.6	MR	32.6	c-h	20.6	MR
PI 404198 B	R (6)					34.3	a-d	33.2	MS	137.7	ab	86.7	S
PI 416937						85.7	a-d	83.1	S	56.5	a-e	35.5	MS
PI 417050		25.7	a-c	57.1	MS					22.8	d-i	14.4	MR
PI 417274		35.7	a-c	79.3	S					36.5	b-g	23.0	MR
PI 417321		50.4	a	111.8	S					29.2	c-h	18.4	MR
PI 423941		30.7	a-c	68.1	S					70.3	a-d	44.2	MS
PI 437654	R (5, 6)					13.6	b-f	13.2	MR	40.7	a-g	25.7	MR
PI 437679	R (6)					27.5	a-e	26.6	MR	31.5	c-h	19.8	MR
PI 437690	R (6)					8.3	d-f	8.1	R	26.0	d-h	16.4	MR
PI 437697						75.4	a-d	73.1	S	134.4	a-c	84.6	S
PI 437725	R (6)					20.3	a-f	19.6	MR	6.8	g-j	4.3	R
PI 438489 B	R (6)					240.9	a	233.5	S	89.0	a-d	56.0	MS
PI 438497	R (6)					7.3	d-f	7.1	R	8.0	f-j	5.1	R
PI 438498	R (6)					10.6	b-f	10.3	MR	80.1	a-d	50.4	MS
PI 467312						67.8	a-d	65.7	S	78.4	a-d	49.4	MS
PI 468916						127.3	ab	123.3	S				
PI 507354						29.4	a-d	28.5	MR	0.5	k	0.3	R
PI 508269 (Stafford)						81.1	a-d	78.5	S	74.2	a-d	46.7	MS
PI 518671 (Williams 82)	S (12)	28.3	a-c	62.8	S					59.2	a-e	37.3	MS
PI 543795 (Hartwig)	R (5)					13.2	b-f	12.8	MR	24.4	d-i	15.4	MR
PI 547419 (L63-1889)		30.7	a-c	68.0	S					36.9	b-g	23.2	MR
PI 548316 (Cloud)	MS (6), R (12)					50.8	a-d	49.3	MS	28.9	c-h	18.2	MR
PI 548402 (Peking)	R (6)					10.1	c-f	9.8	R	47.8	a-f	30.1	MS
PI 548533 (Clark)		29.4	a-c	65.3	S					35.5	c-h	22.4	MR
PI 548655 (Forrest)	R (2)					14.3	b-f	13.9	MR	4.0	h-k	2.5	R
PI 548657 (Jackson)	S (1)					66.0	a-d	64.0	S	66.9	a-d	42.1	MS
PI 548658 (Lee 74)	S (4)									80.9	a-d	50.9	MS
PI 548659 (Braxton)	S (4)					100.5	a-c	97.3	S	130.8	a-c	82.4	S
PI 548982 (Pickett 71)	R (3)					16.4	b-f	15.9	MR	6.9	g-j	4.4	R
PI 553039 (Davis)	S (3)					120.4	a-c	116.7	S	18.5	d-i	11.6	MR
PI 567516 C						8.7	d-f	8.5	R	1.1	jk	0.7	R
PI 573285 (D68-0099)						116.3	a-c	112.7	S	99.5	a-d	62.7	S
PI 587982 A		44.4	ab	98.5	S					43.3	a-f	27.3	MR
PI 594692		34.5	a-c	76.4	S					56.5	a-e	35.5	MS
PI 595081 (KS4895)	R (7)	14.5	bc	32.1	MS					52.9	a-e	33.3	MS
PI 603751 A		32.5	a-c	72.1	S					170.0	ab	107.1	S
PI 614732 (Anand)	R (9)					58.1	a-d	56.3	MS	1.6	jk	1.0	R
PI 634193 (5002T)	S (13)	28.5	a-c	63.2	S					98.2	a-d	61.9	S
PI 656647 (DS4-SCN05)		3.8	de	8.3	R					35.4	c-h	22.3	MR
PI 659348 (DS-880)		2.6	de	5.7	R					14.9	e-i	9.4	R
PI 84751	R (6)					29.3	a-e	28.4	MR	29.8	c-h	18.8	MR
PI 88788	S (6)					133.3	a	129.2	S	195.4	ab	123.0	S
PI 89772	R (6)					7.9	d-f	7.6	R	4.0	i-k	2.5	R
PI 90763	R (6)					2.4	f	2.3	R	26.2	d-h	16.5	MR
PI 96354						56.1	a-d	54.4	MS	34.8	c-h	21.9	MR
SS93-6181		49.0	a	108.7	S					23.2	d-i	14.6	MR
Terral TVX48R018	R (11)	28.8	a-c	63.8	S								

(*Continued*)

TABLE 1. Continued.

Soybean line	Reported rating(s)[a]	Test 1			Test 2			Test 3		
		Count[b]	Index[c]	Rating[d]	Count[b]	Index[c]	Rating[d]	Count[b]	Index[c]	Rating[d]
F		4.13			3.10			3.89		
P > *F*		<0.0001			<0.0001			<0.0001		
$df_{num/den}$		23/83			40/77			57/177		

Values are means of five replications; means followed by the same letter are not significantly different based on differences of least squares means ($P \leq 0.05$).

[a] Methods to determine susceptibility or resistance vary among reports, as do the control genotypes used to make these determinations. Numbers following the response designation indicate the published study in which the response was first reported as follows: (1) Rebois et al., 1968; (2) Williams et al., 1981; (3) Harville et al., 1985; (4) Robbins et al., 1994; (5) Davis et al., 1996; (6) Robbins and Rakes, 1996; (7) Robbins et al., 1999; (8) Robbins et al., 2000; (9) Robbins et al., 2006; (10) Robbins et al., 2007; (11) Robbins et al., 2008; (12) Lawrence et al., 2011; (13) Robbins et al., 2012.

[b] Number of females per g of fresh root tissue.

[c] Percentage of females per g of fresh root tissue as compared with the average observed for the susceptible soybean cultivars Delta King DK4968 (Tests 1, 2, and 3) and Morsoy RTS4706N (Tests 1 and 3)

[d] Rating follows the index described by Schmitt and Shannon (1992) for soybean cyst nematode, where an index < 10 is resistant (R), 10-30 is moderately resistant (MR), 31-60 is moderately susceptible (MS), and > 60 is susceptible (S).

second inoculation was conducted 1 wk later resulting in a total inoculum level of 1,000 nematodes per container. Mississippi reniform nematode population MSRR04 (Arias et al., 2009) was used for all experiments. This population was derived from a single egg mass removed from a cotton plant in 2003 and has been maintained in a greenhouse on tomato (*Solanum lycopersicon* cv. Rutgers). Root infection was measured 4 wk after the second inoculation. At harvest, plant shoots were removed at the soil line and discarded. Plant roots were separated from soil, stained with red food coloring using standard protocols (Thies et al. 2002), and the number of swollen females attached to the roots were counted. After counting, roots were allowed to drain briefly on paper towels to remove excess water and fresh weights were recorded. To compensate for differences in root sizes, counts were expressed as females per g of fresh root tissue.

In addition to statistically comparing root infection levels, lines within each test were classified based on a nematode index, following that described by Schmitt and Shannon (1992) for soybean cyst nematode. Infection on a soybean line is expressed as a percentage of the average number of females that developed on the susceptible cultivars Morsoy RTS4706N (Tests 1 and 3) and Delta King DK4968 (Tests 1, 2, and 3); the cultivar Morsoy RTS4706N did not germinate in Test 2. Based on the nematode index, lines were classified as resistant (nematode index <10%), moderately resistant (10% to 30%), moderately susceptible (31% to 60%), or susceptible (>60%). Both relative infection and consistency of phenotype across tests contributed to identification of the best materials.

Confirmation of reaction to reniform nematode: A subset of 13 of the lines initially screened was further evaluated in a longer-duration test that measured reniform nematode reproduction. The cultivar Braxton (PI 548659) and accession PI 88788 were susceptible controls, the cultivar Hartwig (PI 543795) and accession PI 437654 were resistant controls, and the nine lines tested were 02011-126-1-1-2-1, 02011-126-1-1-5-1, DS97-84-1, PI 230977, PI 417050, PI 567516 C, DS4-SCN05 (PI 656647), DS-880 (PI 659348), and PI 90763. A fallow treatment (nematodes added to the soil in containers that were not seeded) was included to monitor survival of the nematode with no plant roots present.

Test establishment and inoculation procedures were the same as described for the infection-based screening. The experimental design was a completely randomized design with five replications. The test duration was extended to 8 wk. At the end of the test, vermiform stages of nematodes were extracted from all of the soil in each container using standard elutriation (Byrd et al., 1976) and sucrose centrifugation (Jenkins, 1964) protocols. In addition, eggs were extracted from the root system by cutting the roots into 2.5-cm segments, stirring for 10 min in a 0.6% NaOCl solution (Hussey and Barker, 1973), and collecting eggs on a standard 25-μm-pore sieve. This experiment was conducted three times; results are presented separately because preliminary analyses indicated significant test-by-treatment interactions in some cases.

Statistical analysis: To normalize data, nematode counts were subjected to $\log_{10}(x+1)$ transformation before analysis of variance (ANOVA). Backtransformed means are presented. Where ANOVA indicated significant differences among genotypes, differences of least squares means ($P \leq 0.05$) were used to compare infection levels among the *Glycine* lines. SAS statistical software (PROC MIXED; SAS Institute, Cary, NC) was used for analysis.

RESULTS

The reactions to reniform nematode for all 61 lines evaluated are presented in Table 1. This report is the first to document the responses of 27 of these lines to reniform nematode. Of these previously untested lines, eight were identified as being resistant or moderately resistant: released germplasm lines DS4-SCN05 (PI 656647) and DS-880 (PI 659348); accessions PI 230977, PI 507354, and PI 567516 C; and breeding lines DS97-84-1, 02011-126-1-1-2-1 and 02011-126-1-1-5-1. A total of 11 previously untested lines were classified as moderately

susceptible or susceptible: released germplasm lines D68-0099 (PI 573285) and LG01-5087-5; accessions PI 200538, PI 416937, PI 423941, PI 437697, PI 467312, PI 468916, PI 594692, and PI 603751 A; and cultivar Stafford (PI 508269). Inconsistent results between tests were obtained for the remaining eight lines, though in general they were considered to fall toward the susceptible end of the resistance spectrum.

In these screenings, 16 of 23 lines previously reported as resistant (Table 1) were considered resistant or moderately resistant: cultivars Hartwig (PI 543795), Peking (PI 548402), Forrest (PI 548655), and Pickett 71 (PI 548982); and accessions PI 303652, PI 339868 B, PI 404166, PI 404198 A, PI 437654, PI 437679, PI 437690, PI 437725, PI 438497, PI 84751, PI 89772, and PI 90763. However, five lines previously reported as resistant appeared to be moderately susceptible to susceptible when challenged with the Mississippi isolate of reniform nematode: cultivars Delta Grow 4970RR, KS4895 (PI 595081), and Terral TVX48R018; and accessions PI 404198 B and PI 438489 B. Results for the remaining two reportedly resistant lines were inconsistent in these tests. Of the eight lines previously reported as susceptible (Table 1), seven were rated as susceptible or moderately susceptible in these screenings: the registered germplasm line DT97-4290; accession PI 88788; and cultivars Williams 82 (PI 518671), Jackson (PI 548657), Lee 74 (PI 548658), Braxton (PI 548659), and 5002T (PI 634193). Results were inconclusive for the reportedly susceptible cultivar Davis (PI 553039). Conflicting reports of the reactions of two lines, cultivar Asgrow AG4605 and accession PI 209332, were reported in the literature, though both were susceptible in these screenings. Inconsistencies in responses were noted in both published reports and screening results for the cultivar Cloud (PI 548316).

From the results of the first tests, 13 *Glycine* lines were selected for further evaluation. Reniform nematode development on these lines and in fallow pots in 60-d tests is summarized in Table 2. Accessions PI 567516 C and PI 90763, released germplasm lines DS4-SCN05 (PI 656647) and DS-880 (PI 659348), and breeding lines DS97-84-1, 02011-126-1-1-2-1, and 02011-126-1-1-5-1 consistently suppressed reniform nematode populations to levels comparable with those that developed on the resistant controls. Furthermore, in two of the three tests, the reniform nematode populations on all of these lines were equivalent to or smaller than the population that persisted in the fallow pots. Reniform nematode populations that developed on accessions PI 230977 and PI 417050 were equivalent to those that developed on the susceptible controls.

TABLE 2. Comparison of reniform nematode population development on 13 *Glycine* lines and one fallow treatment in three growth chamber experiments.

Soybean line	Nematodes per container[a] Test 1		Test 2		Test 3	
PI 548659 (Braxton)[b]	26,570	a	28,516	a	169,862	a
PI 88788[b]	7,673	a	30,185	a	29,915	a
PI 437654[c]	–		110	b-d	390	bc
PI 543795 (Hartwig)[c]	306	b	858	b	767	b
02011-126-1-1-2-1	–		285	b-d	344	b-d
02011-126-1-1-5-1	110	bc	274	b-d	36	de
DS97-84-1	274	b	42	d	125	b-d
PI 230977	9,657	a	25,579	a	98,650	a
PI 417050	55,411	a	103,228	a	78,450	a
PI 567516 C	29	c	120	b-d	137	b-d
PI 656647 (DS4-SCN05)	365	b	307	bc	1,208	b
PI 659348 (DS-880)	583	b	574	b	1,004	b
PI 90763	28	c	81	cd	70	c-e
Fallow	460	b	419	bc	7	e
F	9.49		13.91		15.08	
$P > F$	<0.0001		<0.0001		<0.0001	
$df_{num/den}$	11/34		13/46		13/52	

Values are means of five replications; means followed by the same letter are not significantly different based on differences of least squares means ($P \leq 0.05$).

[a] Vermiform stages in 120-cm^3 soil plus root-associated eggs extracted 8 wk after inoculation with 1,000 reniform nematodes.

[b] Susceptible controls are PI 548659 (Braxton) and PI 88788.

[c] Resistant controls are PI 437654 and PI 543795 (Hartwig).

Discussion

Six previously untested soybean lines were identified as having resistance to reniform nematode in both initial screening and subsequent confirmation tests: released germplasm lines DS4-SCN05 (PI 656647) and DS-880 (PI 659348); PI 567516 C; and breeding lines DS97-84-1, 02011-126-1-1-2-1, and 02011-126-1-1-5-1. The parentage of both DS4-SCN05 and DS97-84-1 consists of 3/16 PI 437654 and 1/2 'Hartwig' (http://www.ars-grin.gov/cgi-bin/npgs/acc/search.pl?accid=PI+656647), whereas the parentage of DS-880 is very similar, having 1/4 PI 437654 and 1/2 'Hartwig' (http://www.ars-grin.gov/cgi-bin/npgs/acc/search.pl?accid=PI+659348). 'Hartwig's' reniform nematode resistance is likely derived from PI 437654 and possibly 'Peking' (Hartwig and Epps, 1968; Hartwig and Epps, 1973; Anand, 1992; Ha et al., 2007). However, the reniform nematode resistance in the 02011 lines is derived from PI 567516 C ('Bolivar' × PI 567516 C), whose resistance is likely different from that in PI 437654 and 'Peking' (Arelli et al., 2009). Although PI 567516 C has greenish brown seed coat color, is viny, and lodges, the two 02011 lines both have yellow seed coat and upright plant architecture and the three lines derived from PI 437654 and 'Hartwig' are also yellow seeded and upright. Hence, these five lines may provide useful diversity to germplasm improvement programs that target development of reniform nematode-resistant soybean lines. Accession PI 507354 was scored moderately resistant in the screening test but was not included in the confirmation test.

Accession PI 230977 was scored moderately resistant in the screening test but grouped with the susceptible genotypes in the subsequent confirmation test. The reason for the inconsistency between the tests is unknown. Males may have outnumbered females in the

aliquots of inoculum used in the initial screening test, thus limiting the number of infections. Perhaps more eggs are produced by each female on this accession as compared with other lines, so that the population builds up quickly even if fewer initial infections occur. But, because the data to assess these scenarios were not collected, the contribution of these or other factor(s) that resulted in the apparent shift from a resistant to a susceptible classification remain undetermined. The inconsistency in reaction measured for accession PI 230977 illustrates the importance of repeating tests and utilizing different methods (e.g., durations, life stages assessed) to build a more complete picture of the level of resistance being expressed in a genotype.

The current study also identified 11 previously untested moderately susceptible or susceptible lines: released germplasm lines LG01-5087-5 and D68-0099 (PI 573285); accessions PI 200538, PI 416937, PI 423941, PI 437697, PI 467312, PI 468916, PI 594692, and PI 603751A; and cultivar Stafford (PI 508269). Although these lines are not useful sources of resistance to reniform nematode, they could serve as susceptible parents in crosses with resistant lines to help determine how the resistance is inherited, to identify molecular markers for resistance, and to map the location of the gene(s) conferring resistance.

Screening tests that are not repeated are used when the goal is rapid dissemination of information to end users. For example, information on reaction of current soybean cultivars to reniform nematode could be included in extension information distributed to growers to facilitate selection of cultivars for inclusion in crop rotations to manage reniform nematode (Robbins et al., 2001, 2002, 2006, 2007, 2008, 2009, 2012) and for limiting crop losses when soybean is planted in fields infested with this pathogen (Lim and Castillo, 1979; Asmus, 2008).

Documenting resistance is more difficult than documenting susceptibility. Missed pots during the inoculation process, poor viability of nematodes, and suboptimal environmental conditions all may contribute to low levels of infection that are not necessarily the result of host plant resistance. When selecting lines to use in germplasm development programs, greater emphasis should be placed on results from tests with multiple replications that have been repeated. In the absence of these types of data, the reaction of a line from multiple, nonrepeated experiments should be evaluated. The lack of a standardized screening method for reniform nematode resistance in soybean makes it challenging to compare results from diverse tests.

In the current study, soybean lines for which reactions to reniform nematode had already been reported were included to assess the consistency between the screening method used in this study and other published reports. For lines previously reported as resistant, results from this study agreed in 69.6% of the cases. For lines previously reported as susceptible, results from this study agreed in 87.5% of the cases. Given the wide range of screening methods in use, these levels of agreement suggest that the methods used in the current study were valid and comparable.

It is possible that differences in reniform nematode response between this study and other published reports may be a result of the soybean lines responding differently to the Mississippi population of reniform nematode used in this study. The current study is the first report of reaction of soybean lines to the MSRR04 reniform nematode population. In a previous study on cotton, the number of infections resulting from MSRR04 was intermediate between those resulting from populations originating in Georgia and Texas (Arias et al., 2009). Other researchers have reported cotton (Agudelo et al., 2005; McGawley et al., 2010) and soybean (Agudelo et al., 2005; McGawley et al., 2011) lines responding differently to unique geographic populations of reniform nematode.

In summary, this research provides information on the reaction of 27 previously untested soybean lines to reniform nematode, including six lines with useful levels of reniform nematode resistance that may be valuable additions to public and private soybean breeding programs. Inconsistencies in reactions to the reniform nematode among tests underscore the importance of repeating experiments and utilizing multiple levels of testing to identify resistant germplasm.

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