Genetic Diversity Among Isolates of *Heterodera glycines* and Sources of Resistance in Soybeans

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ABSTRACT

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Populations of the soybean cyst nematode (*Heterodera glycines*) (SCN) are extremely variable. A recent nematode classification system includes 11 new races, for a total of 16. Little is known about newly described races 4, 6–13, 15, and 16. We have attempted to examine the genetic diversity among isolates of *H. glycines*, isolate sources of resistance in soybeans, and identify soybean differentials for describing intrarace variation of SCN. Thirty-two field isolates of *H. glycines* collected from 10 states were evaluated in the greenhouse for female maturation on soybean (*Glycine max*) genotypes Peking, Pickett, Custer, Bedford, Lee, PI 209332, PI 404166, PI 404198B, PI 437654, PI 437690, PI 438489B, PI 438496B, PI 438503A, PI 88788, PI 89772, and PI 90763. Among the isolates tested, 3.1, 6.2, 43.7, 9.3, 25, 6.2, and 6.2% were races 1, 2, 3, 5, 6, 9, and 14, respectively, according to standard race determination scheme. Race 2 isolates were separated by their ability to multiply on PI 437690. Race 3 isolates were better characterized by their ability to mature on Custer and PI 438503A. Custer, Bedford, PI 209332, PI 404166, PI 404198B, PI 437690, and PI 438496B were useful in describing variation in race 6 field isolates. Race 14 isolates were separated according to their response to soybean lines PI 404166, PI 404198B, PI 438489B, PI 438503A, and PI 89772. None of the additional hosts further separated race 9 isolates. The additional hosts used in this study are genetically stable in reaction to nematode and, therefore, may be useful in describing variability among SCN isolates. PI 437654 is recommended as a resistant control of race determination. We found soybean lines PI 437654, PI 438489B, PI 438503A, and PI 89772 resistant to all isolates of newly designated SCN races 6 and 9. Additional resistance to isolates of race 9 was found in PI 209332 and PI 404166. Soybean line PI 89772 has agronomically desirable traits and may be used as an alternate source of SCN resistance in breeding soybean cultivars.

Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, was first observed in the United States in North Carolina in 1954 (16). SCN is now a major pest of soybean, *Glycine max* (L.) Merr., in the United States. Soon after SCN was identified as a soybean pest, scientists sought resistance. Ross and Brim (15) were first to report on soybean resistance to this pest. After planting resistant cultivars in a field for a few years, genetic variation in SCN was reported for plants in those fields (14). Subsequent reports demonstrated the presence of diversity among isolates of SCN (5,6,10,13). A procedure for designating populations of SCN using four soybean differentials, Peking, Pickett, PI 88788, and PI 90763, with Lee as a susceptible control, was established in 1970 (3). As a result of this procedure, four races were described. Later, a population of *H. glycines* from Japan was described as race 5 (4), and it was similar to a population found in Tennessee (17). Sixteen races can be designated with all combinations of four differentials and a +/− system; the remaining 11 races were described by Riggs and Schmitt in 1988 (12).

Soybean lines with resistance to newly designated races 4, 6, 7, 8, 9, 10, 11, 12, 13, 15, and 16 of SCN have not been reported. A few commercial cultivars carried resistance to races 4 and 6 (11). Identification of sources of resistance to the newly described races is desirable, and the use of an appropriate source of SCN resistance in different soybean cultivars could drastically minimize race shifts in nematodes. Because a newly expanded race scheme has been recently proposed, it is necessary to reexamine the genetic diversity among isolates of *H. glycines* and identify new host differentials.

This paper describes genetic diversity among isolates of SCN, identification of new soybean lines that could be used as new host differentials to further separate SCN populations, and isolation of sources of resistance in soybeans to newly designated races.

MATERIALS AND METHODS

Thirty-four isolates of SCN were collected during 1988 and 1989 from fields in Missouri, North Carolina, South Carolina, Tennessee, Arkansas, Georgia, Illinois, Florida, Ohio, and Iowa. These fields were planted to soybeans and had a history of infestation by SCN. Soil samples were taken in the soybean fields during the months of July and August, when high SCN numbers were expected to occur. Standard methods were used for sampling soil (1). A semiautomatic elutriator (2) was used to separate cysts from soil. Cysts were collected on a 60-mesh (250 μm) sieve and counted with a stereo microscope. Two samples contained less than 20 cysts per 225 cm^3 of soil and were excluded. Sand was mixed with each of the remaining 32 "original" soil samples, and the mix was placed into 80 clay pots (7.5 cm in diameter). Standard differentials, lines, and cultivars included for evaluation were Peking, Pickett, PI 88788, PI 90763, PI 209332, PI 404166, PI 404198B, PI 437654, PI 437690, PI 438489B, PI 438496B, PI 438503A, PI 89772, Custer, Bedford, and Lee. These lines were chosen for their genetic stability in reaction to SCN (7,8). Seeds were germinated in vermiculite, and when cotyledons opened, seedlings were transplanted singly into clay pots containing infested soil. There were five replications of each host, and the tests were repeated. Lee was the standard susceptible cultivar against which female maturation on each of 15 soybean lines and differentials was measured.

All plants were grown in a greenhouse at 27 ± 2 C. After 30 days, each pot was inverted and lightly tapped to release the plant and soil. Soil was removed from roots by gently crumbling the root ball. Cysts were washed from roots and counted under a stereo microscope (9).

The number of white females per root from all five replicates were averaged in each experiment, and an index of parasitism (IP) calculated (3). IP = average number of white females from differential/average number of white females from Lee × 100. The IP was rounded to the nearest whole number. An IP ≥10 was assigned a (+), and <10 was assigned a (−) (3).

RESULTS AND DISCUSSION

Of the 32 isolates tested on the four differentials used by Riggs and Schmitt (12), 3.1, 6.2, 43.7, 9.3, 25, 6.2, and 6.2% were races 1, 2, 3, 5, 6, 9, and 14, respectively (Table 1).

Some isolates were further separated into variants based on the reaction obtained on additional soybean lines. Two isolates, obtained from Missouri (isolate 1) and North Carolina (isolate 18), were characterized as race 2. Both isolates were further separated into variants

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based on their ability to mature on soybean PI 437690.

In this study 14 SCN isolates were characterized as race 3 reaction. One isolate from Tennessee (isolate 15) and one from Illinois (isolate 28) were rated (+) on both Custer and PI 438503A, and an isolate from South Carolina (isolate 20) was rated (+) on PI 438503A and (−) on Custer. Two isolates from North Carolina (isolates 7 and 9) and one from Illinois (isolate 29) were race 5. However, one isolate from North Carolina (isolate 9) was (+) on both PI 404198B and PI 438503A; isolate 29 from Illinois was (+) on PI 438503A and (−) on PI 404198B; and isolate 7 from North Carolina was (−) on both.

More variability was observed among SCN race 6 isolates than in other races in this study (Table 1). Of eight race 6 isolates, two (isolates 10 and 12) gave similar reactions on all soybean lines, a (+) on Custer and a (−) on the other additional hosts; two (isolates 6 and 18) were (+) on two additional lines, Bedford and Custer; and the remaining isolates (isolates 12, 22, 31, and 32) were more diverse. However, PI 209332, PI 404166, PI 404198B, PI 437690, PI 438496B, Custer, and Bedford appeared to be useful host differentials in dealing with race 6 classification.

One isolate from Tennessee (isolate 14) and one from South Carolina (isolate 19) were designated as race 14. These isolates differed in their development on PI 404166, PI 404198B, PI 438489B, PI 438503A or PI 89772. The isolates from Missouri (isolate 5) and Illinois (isolate 25) characterized as race 9 had similar ratings on the 11 additional lines and cultivars.

Each race isolate 1–32 produced a (+) reaction on a different number of additional hosts in this study. Race 1 isolates were (+) on two of the additional lines, race 2 on six, race 3 on two, race 5 on six, race 6 on five, race 9 on four, and race 14 on eight. All isolates were (−) on PI 437654, and apparently none of the isolates used in this study had a complete set of genes for parasitism of this soybean line.

These data demonstrate that intrarace variation exists among the SCN isolates tested, and that a further study should be conducted to determine how these variants be considered. The isolates were further separated when PI 209332, PI 404166, PI 404198B, PI 437690, PI 438503A, Bedford, and Custer were included as hosts. Some of these lines and cultivars appeared to be genetically stable in reaction to SCN (7,8). Therefore, one or more of these lines and cultivars (specifically, PI 404198B, PI 437690, Bedford, and Custer) could be useful in a standardized test to further describe variability in SCN races 3, 5, 6, and 14. Soybean line PI 437654 should be recommended as a resistant differential.

Of the seven races characterized in this study, sources of resistance in soybeans have not been previously reported for races 6 and 9. PI lines 437654, 438489B, 438503A, and 89772 were resistant to isolates of both SCN races 6 and 9 (Table 2). Additional resistance to each of the two isolates of race 9 was found in soybean lines PI 209332 and PI 404166

Table 1. Means for cyst number (using Lee soybeans as standard) and ratings[a] on soybean lines, cultivars, and differentials inoculated with isolates of *Heterodera glycines*

State County	Isolate no.	Lee no.	Rating															Race class[b]
			Pickett	Peking	PI 88788	PI 90763	PI 438489B	PI 438496B	PI 404166	Bedford	Custer	PI 209332	PI 404198B	PI 437690	PI 89772	PI 438503A	PI 437654	
MO																		
Boone	1	140	(+)	(+)	(+)	(−)	(+)	(+)	(−)	(+)	(+)	(+)	(−)	(+)	(−)	(+)	(−)	2
Boone	2	288	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	3
Cape	3	188	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	3
Cape	4	125	(−)	(−)	(+)	(−)	(−)	(−)	(−)	(+)	(−)	(−)	(−)	(−)	(−)	(+)	(−)	1
New Madrid	5	102	(+)	(+)	(−)	(−)	(−)	(+)	(−)	(−)	(+)	(−)	(+)	(+)	(−)	(−)	(−)	9
NC																		
Washington	6	180	(+)	(−)	(−)	(−)	(−)	(−)	(−)	(+)	(+)	(−)	(−)	(−)	(−)	(−)	(−)	6
Beaufort	7	174	(+)	(−)	(+)	(−)	(−)	(+)	(−)	(+)	(−)	(+)	(−)	(−)	(−)	(−)	(−)	5
Beaufort	8	119	(+)	(+)	(+)	(−)	(−)	(+)	(−)	(+)	(+)	(+)	(−)	(−)	(−)	(+)	(−)	2
Wayne	9	178	(+)	(−)	(+)	(−)	(−)	(+)	(−)	(+)	(−)	(+)	(+)	(−)	(−)	(+)	(−)	5
Pasquotank	10	136	(+)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(+)	(−)	(−)	(−)	(−)	(−)	(−)	6
OH																		
Washington	11	214	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	3
TN																		
Dyer	12	156	(+)	(−)	(−)	(−)	(−)	(−)	(−)	(+)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	6
Dyer	13	152	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(+)	(−)	(−)	(−)	3
Obion	14	145	(+)	(+)	(−)	(+)	(+)	(+)	(+)	(−)	(+)	(−)	(−)	(−)	(+)	(−)	(−)	14
Jackson	15	531	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(+)	(−)	(−)	(−)	(−)	(+)	(−)	3
Lawrence	16	453	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	3
Dyer	17	138	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	3
AR																		
Lonoke	18	331	(+)	(−)	(−)	(−)	(−)	(−)	(−)	(+)	(+)	(−)	(−)	(+)	(−)	(−)	(−)	6
SC																		
Orangeburg	19	118	(+)	(+)	(−)	(+)	(−)	(+)	(−)	(−)	(+)	(−)	(+)	(−)	(−)	(+)	(−)	14
Orangeburg	20	153	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(+)	(−)	3
Orangeburg	21	124	(+)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(+)	(−)	(−)	(−)	(−)	(−)	(−)	6
GA																		
Screvens	22	217	(+)	(−)	(−)	(−)	(−)	(+)	(+)	(−)	(+)	(−)	(+)	(−)	(−)	(−)	(−)	6
IA																		
Kossuth	23	125	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	3
IL																		
Elksville	24	334	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	3
Sandridge	25	249	(+)	(+)	(−)	(−)	(−)	(+)	(−)	(−)	(+)	(−)	(+)	(+)	(−)	(−)	(−)	9
Effingham	26	146	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	3
Effingham	27	190	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	NA[c]	(−)	(−)	(−)	(−)	(−)	(−)	3
Effingham	28	189	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(+)	(−)	(−)	(−)	(−)	(+)	(−)	3
Pulaski	29	162	(+)	(−)	(+)	(−)	(−)	(+)	(−)	(+)	(+)	(+)	(−)	(−)	(−)	(+)	(−)	5
Pulaski	30	139	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	3
FL																		
Jackson	31	115	(+)	(−)	(−)	(−)	(−)	(+)	(−)	(−)	(+)	(+)	(+)	(+)	(−)	(−)	(−)	6
Jackson	32	132	(+)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	6

[a] Based on index of parasitism, (+) = number of white females recovered was 10% or more of the number on Lee, and (−) = number of white females recovered was less than 10% of the number on Lee. Ratings for soybean lines were consistent in both the tests.
[b] Based on Riggs and Schmitt classification (1988).
[c] Not available.

Table 2. Reaction of soybean lines to isolates of *Heterodera glycines* races 6 and 9[a]

Line	*H. glycines* race, isolate	Number of mature females			Reaction[b]
		Mean	Range	Variance	
PI 438489B	6,6	0	0	0	(−)
	6,10	0	0	0	(−)
	6,12	0	0	0	(−)
	6,18	0	0–1	0.2	(−)
	6,21	5	1–7	3.8	(−)
	6,31	0	0	0	(−)
	6,32	0	0–1	0.2	(−)
PI 89772	6,6	0	0	0	(−)
	6,10	0	0	0	(−)
	6,12	1	0–4	2	(−)
	6,18	1	4–10	4.3	(−)
	6,21	5	0–3	0.7	(−)
	6,31	10	4–16	16.3	(−)
PI 438503A	6,6	0	0	0	(−)
	6,10	0	0	0	(−)
	6,12	0	0–2	0.4	(−)
	6,18	6	4–13	6.5	(−)
	6,21	5	1–7	3.8	(−)
	6,31	5	2–10	9.5	(−)
	6,32	3	0–7	4.9	(−)
PI 209332	6,6	3	0–6	4.3	(−)
	6,10	0	0	0	(−)
	6,12	13	4–16	6.8	(−)
	6,18	16	8–14	4.4	(−)
	6,21	1	0–4	2.2	(−)
	6,22	5	0–3	0.7	(−)
	6,32	5	0–6	0.9	(−)
PI 437654	6,6	0	0	0	(−)
	6,10	0	0	0	(−)
	6,12	0	0	0	(−)
	6,18	1	0–1	0	(−)
	6,31	0	0	0	(−)
	6,32	0	0	0	(−)
PI 438489B	9,5	2	0–5	2.3	(−)
	9,25	2	0–5	2.3	(−)
PI 89772	9,5	7	4–17	15.7	(−)
	9,25	5	0–11	10.0	(−)
PI 438503A	9,5	5	1.8	4.9	(−)
	9,25	3	1–5	1.6	(−)
PI 437654	9,5	0	0	0	(−)
	9,25	3	1–6	1.8	(−)
PI 404166	9,5	8	5–10	3.1	(−)
	9,25	16	11–28	26.6	(−)
PI 209332	9,5	1	0–6	3.3	(−)
	9,25	3	1–6	1.8	(−)
Lee[c]	Race 6	171	139–204	323.1	(+)
Lee[c]	Race 9	189	136–240	799.5	(+)

[a]Ten plants were tested for each *H. glycines* race isolate.
[b]− = Numbers of white females recovered was less than 10% of the number on Lee (i.e., resistant); + = numbers of white females recovered was 10% or more of the number on Lee (i.e., susceptible).
[c]Susceptible control.

(Table 2). PI 89772 was nearly as resistant as PI 437654 for SCN reaction in this study and also had agronomically desirable traits (Rao-Arelli, Wrather, and Anand, *unpublished*). This line should be useful as an alternate source of SCN resistance in soybean breeding programs.

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LITERATURE CITED

1. Barker, K. R., Townsend, J. L., Bird, G. W., Thomason, I. J., and Dickson, D. W. 1986. Determining nematode population responses to control diseases. Pages 283-296 in: Methods for Evaluating Pesticides for Control of Plant Pathogens. K. Hickey, ed. American Phytopathological Society, St. Paul, MN.
2. Byrd, D. W., Barker, K. R., Ferris, H., Nusbaum, C. J., Griffin, W. E., Small, R. H., and Stone, C. A. 1976. Two semiautomatic elutriators for extracting nematodes and certain fungi from soil. J. Nematol. 8:206-212.
3. Golden, A. M., Epps, J. M., Riggs, R. D., Duclos, L. A., Fox, J. A., and Bernard, R. L. 1970. Terminology and identity of infra-specific forms of the soybean cyst nematode (*Heterodera glycines*). Plant Dis. Rep. 54:544-546.
4. Inagaki, H. 1979. Race status of five Japanese populations of *Heterodera glycines*. Jpn. J. Nematol. 9:1-4.
5. Miller, L. I. 1969. Physiologic variation of six isolates of *Heterodera glycines*. (Abstr.). Phytopathology 59:1558.
6. Miller, L. I. 1969. Physiologic variation of five isolates of the soybean cyst nematode. (Abstr.). Virginia J. Sci. 29(3):99.
7. Rao-Arelli, A. P., and Anand, S. C. 1988. Genetic relationships among soybean plant introductions for resistance to race 3 of soybean cyst nematode. Crop Sci. 28:650-652.
8. Rao-Arelli, A. P., Anand, S. C., and Myers, G. O. 1989. Partial dominance of susceptibility in soybean to soybean cyst nematode races 3, 4, and 5. Crop Sci. 29:1562-1564.
9. Rao-Arelli, A. P., Matson, K. W., and Anand, S. C. 1991. A rapid method for inoculating soybean seedlings with *Heterodera glycines*. Plant Dis. 75:594-595.
10. Riggs, R. D., Hamblen, M. L., and Rakes, L. 1981. Infra-species variation in reaction to hosts in *Heterodera glycines* populations. J. Nematol. 13:171-179.
11. Riggs, R. D., Hamblen, M. L., and Rakes, L. 1988. Resistance in commercial soybean cultivars to six races of *Heterodera glycines* and to *Meloidogyne incognita*. Ann. Appl. Nematol. 2:70-76.
12. Riggs, R. D., and Schmitt, D. P. 1988. Complete characterization of the race scheme for *Heterodera glycines*. J. Nematol. 20:392-395.
13. Riggs, R. D., Slack, D. A., and Hamblen, M. L. 1968. New biotype of soybean cyst nematode. Arkansas Farm Res. 17(5):11.
14. Ross, J. P. 1961. Physiological strains of *Heterodera glycines*. Plant Dis. Rep. 46:766-769.
15. Ross, J. P., and Brim, C. A. 1957. Resistance of soybeans to the soybean cyst nematode as determined by double-row method. Plant Dis. Rep. 41:923-924.
16. Winstead, N. N., Skotland, G. B., and Sasser, J. N. 1955. Soybean-cyst nematode in North Carolina. Plant Dis. Rep. 39:9-11.
17. Young, L. D. 1982. Reproduction of Tennessee soybean cyst nematode population on cultivars resistant to race 4. Plant Dis. Rep. 66:251-252.