

Hybridization of Races of *Heterodera glycines*¹

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Abstract: Progeny from single females of four known races of *Heterodera glycines* Ichinohe were used to establish relatively uniform populations. Single females from these populations were mated with males of other races in all possible combinations to study compatibility and inheritance patterns. When race 1 or 3 was crossed with either race 2 or 4, there was a significant reduction in number of females and a greater number of eggless females than in crosses of races 1 x 3 and 2 x 4. More females matured and fewer were eggless when matings were of the same race. Parasitic capabilities of races 2 and 4 were dominant or partially dominant over those of races 1 and 3, based on parasitism of F₁ hybrids. Segregation patterns were generally similar for reciprocal crosses between races. There appeared to be either one or two major genes segregating for parasitism of 'Pickett' soybean in the different crosses. A hybrid isolate (race 3 x 4) that differed in parasitic capability from the four known races produced as many females on the resistant soybean genotype, PI 90,763, as on the susceptible Lee cultivar. Those data indicate that isolates of *H. glycines* with a different parasitic capability may develop from gene recombination.
Key Words: soybean cyst nematode, parasitic capability, compatibility, genetic recombination.

The soybean-cyst nematode (SCN), *Heterodera glycines* Ichinohe, was first found on soybeans in the United States in 1954 (13). Since the initial discovery, in North Carolina, infestations have been reported in more than a million hectares in 17 states. Seven cultivars resistant to SCN ('Custer,' 'Dyer,' 'Pickett,' 'Pickett 71,' 'Mack,' 'Forrest,' and 'Centennial') provided effective control for a few years until new races appeared. Four physiological races of SCN have been identified on the basis of ability to reproduce on a set of host differentials (5). Cultivars currently in production are resistant to races 1 and 3 but are susceptible to races 2 and 4.

Inheritance studies on resistance in soybean to races of SCN (2, 3, 6, 8, 10, 11) indicate a rather complex pattern. Five major genes have been identified for resistance to races 1, 3, and 4 with multiple alleles at one locus. Caldwell et al. (2) studied race 1; Matson and Williams (8), race 3; Hartwig and Epps (6), race 4; and Thomas and Caviness (3, 10, 11) worked mainly on race 4 but also studied the other 3 races.

Rapid development of new races of SCN under field conditions indicates that this organism possesses considerable genetic

variability. *Heterodera glycines* is a diploid (2n = 18) amphimitic organism. Koliopanos (7) showed that hybrids between races of this organism produce viable progeny. He also analyzed F₁ and F₂ progeny from mass crosses between different races and found that a nonrecessive type of inheritance conditioned for the ability of SCN to overcome resistance. Triantaphyllou (12) studied selection through the continuous stress of resistant plants with various populations of SCN. He also made mass crosses between two widely divergent populations. He concluded that three genes or groups of genes conditioned for ability to parasitize soybeans that had the major types of resistance to SCN.

No research studies were found in which controlled crosses had been made between single females and males of SCN races. However, limited studies were conducted by Andersen (1) on inheritance of races 1 and 2 of *Heterodera avenae*. He concluded from small F₁ and F₂ populations, obtained by mating single males and females, that race 2 possessed two dominant genes which conditioned for parasitic capability.

The objective of these investigations was to hybridize races of SCN and to: (1) determine compatibilities; (2) elucidate patterns of inheritance for parasitic capability; and (3) study the potential for new race development.

MATERIALS AND METHODS

These studies were conducted under controlled conditions in a greenhouse at the Main Experiment Station, Fayetteville,

Received for publication 25 July 1977.

¹Published with the approval of the Arkansas Agricultural Experiment Station Director. Submitted by the senior author in partial fulfillment of requirements for a Ph.D. degree. This research was supported in part by a grant (Coop. agreement no. 12-14-7001-36) from the Agricultural Research Service, United States Department of Agriculture.

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Arkansas, during 1973-1976. The temperature was maintained at about 26-32 C during the day and 26 at night.

Uniform populations of races 1, 2, 3, and 4 were established by selecting single females or cysts and culturing their progeny. The initial cultures of the races used in these studies were obtained from several sources. Race 1 was obtained from K. R. Barker, Plant Pathology Department, North Carolina State University, Raleigh. L. I. Miller, Plant Pathology and Physiology Department, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, furnished a race 2 population. Races 3 and 4 were isolated by R. D. Riggs, Plant Pathology Department, University of Arkansas, Fayetteville. Races 1 and 3 were maintained on the Lee cultivar, and races 2 and 4 on Pickett. Host response to races 1, 2, 3, and 4 was determined by inoculating the cultivars 'Lee,' 'Pickett,' 'Peking,' 'PI 88,788,' and 'PI 90,763' with eggs and larvae from each of the populations. Average reactions from a number of inoculations are given in Table 1. These ratings differ somewhat from ratings given by Golden et al. (5), particularly race 4 against PI 88,788 and PI 90,763. However, the race 4 population used was taken from the population designated by Golden et al. as race 4, and it was tested several times on the five lines to obtain the ratings reported.

Procedure for culturing nematodes: Soybean seeds were germinated in flats filled with vermiculite. A single seedling was

transplanted into a 7.6-cm clay pot filled with sterilized fine river sand when roots were 4 to 5 cm long. Eggs and larvae of SCN were added when plants had reached the V3 stage (4).

Twenty-eight days after inoculation, females with eggs were recovered by the roiling and sieving method. Female nematodes were rubbed from roots into a container of water along with the sand, suspended by roiling and poured through nested sieves of 0.85-mm and 0.25-mm mesh. Material collected on the 0.25-mm-mesh sieve was washed into a 10-cm-square petri dish with a grid on the bottom, and females were counted at 15x magnification.

Inoculum consisting of eggs and larvae was prepared by forcing the mature females and cysts through a 0.18-mm-mesh sieve to break them, and eggs and larvae were collected in a beaker. Inoculum was applied to each pot with an automatic pipette adjusted to deliver a given volume of standardized egg and larval suspension.

Nematode mating techniques: Metal flats of 40.6 x 61 x 10 cm were used to grow the plants used in the matings. The plants were grown in mounded sand around the outer wall of the flat to provide accessibility to the lateral roots. Lee soybean plants were grown to the V3 stage, and then two lateral roots were directed into individual glass vials (1.5 cm diam x 5 cm deep) at the sides of the mounds. Forty vials were used for each cross, and 20 for intraracial matings. Sterilized sand was placed around the roots in each vial, and a single second-stage larva was selected from the appropriate race and placed in each vial near a root. Fifteen days later, 40 males were placed in each vial. The large number of males increased the probability that a male would mate with the single female in the vials in which females developed. Vials were covered with six layers of cheesecloth and watered daily. Twenty days after the males were added, a 2-ml pipette attached to a water source by a rubber hose was used to wash the roots and sand from the vials onto nested sieves of 0.85-mm and 0.25-mm mesh. The number of females recovered and the number of eggs per female were recorded.

TABLE 1. Reaction of soybean cultivars to four races of *Heterodera glycines*.

Cultivar	Reaction on race*			
	1	3	2	4
Lee	VS	VS	VS	VS
Pickett	VR	R	VS	VS
Peking	VR	VR	S	S
PI 88,788	MR	R	S	R
PI 90,763	VR	VR	MR	MR

*Reaction to SCN was designated as follows: VS, very susceptible; S, susceptible; MR, moderately resistant; R, resistant; and VR, very resistant.

Ratings were based on the following index values. VR = 0-7; R = 8-24; MR = 25-41; S = 42-58; VS = above 58. The index was determined by dividing the square root of the number of cysts recovered from each plant by the square root of the highest number recovered from a Lee plant.

RESULTS AND DISCUSSION

Mature females or cysts containing eggs

were recovered from all mating combinations (Table 2). Results from reciprocal crosses were generally not significantly different; therefore, data were combined. Intraspecific matings produced the lowest percentage of eggless females and the greatest number of F_1 eggs per female (Table 2). Intra-race-3 matings produced significantly more eggs than other intraspecific matings. This fact may explain why race 3 was a better competitor than other races in controlled experiments in the greenhouse (9). Race 3 also appeared to be a better competitor than race 4 under field conditions. Crosses between races 1 x 3 and races 2 x 4 produced significantly more eggs per female than crosses of races 1 x 2, 1 x 4, 3 x 2, or 3 x 4. A higher percentage of eggless females resulted from crosses of races 1 x 2 or 1 x 4 than from the crosses 3 x 2 or 3 x 4.

Maturation of F_1 progeny on Lee and Pickett cultivars varied with the mating (Table 3). All four races of SCN reproduce on Lee, whereas only races 2 and 4 reproduce on Pickett. Reciprocal crosses of races 1 x 2, 2 x 3, 2 x 4, and 3 x 4 produced about the same number of females on both Lee and Pickett. Apparently slightly fewer individual females matured on Pickett than

TABLE 2. Reproductive capacity of F_1 females from crosses of four races of *Heterodera glycines*.

Cross [†]	Total no. of females	No. of females containing eggs	Mean no. of eggs per female [‡]	Percent eggless females
1 x 1	8	7	133 b	12.5
2 x 2	7	6	123 b	14.3
3 x 3	9	9	163 a	0.0
4 x 4	7	6	114 b	14.3
1 x 3	20	17	73 c	15.0
2 x 4	21	16	64 c	24.0
1 x 2	17	4	27 d	76.4
1 x 4	14	7	37 d	50.0
2 x 3	20	12	31 d	40.0
3 x 4	15	10	33 d	33.0

[†]There were no differences between reciprocal crosses, so the data are combined. There were only half as many attempts with intraspecific crosses as interracial crosses.

[‡]Means in the column having the same letter are not significantly different at the 5% level of probability by Duncan's Multiple Range Test.

TABLE 3. Maturation of F_1 females from intra- and interracial matings of *Heterodera glycines* on Lee and Pickett cultivars.

Cross	Initial no. of eggs and larvae [†]		Percent maturation (no. of females)	
	Lee	Pickett	Lee [‡]	Pickett [‡]
1 x 1	460	464	41	0
2 x 2	368	365	44	42
3 x 3	700	700	49	0
4 x 4	300	300	46	47
1 x 2	30	25	40	36
2 x 1	31	20	45	35
1 x 3	280	276	36	0
3 x 1	340	330	43	1
1 x 4	74	74	45	35
4 x 1	60	53	47	19
2 x 3	180	111	43	41
3 x 2	100	89	41	37
2 x 4	251	251	48	49
4 x 2	182	180	44	46
3 x 4	100	69	45	44
4 x 3	145	120	35	29

[†]An attempt was made in dividing the inoculum to place the same number of eggs and larvae on the Lee and Pickett cultivars.

[‡]All four races reproduce on the Lee cultivar, whereas only races 2 and 4 reproduce on Pickett.

on Lee. Reciprocal crosses between races 1 and 4 appeared to differ slightly, though that was not apparent in the other crosses.

These data indicate that races 2 and 4 express dominance or partial dominance for host specificity when crossed with the other races because hybrids parasitized both Lee and Pickett in approximately equal numbers. Despite this genetic dominance expressed in hybrids, races 1 and 3 have been shown to be better competitors when mixed populations of all 4 races were evaluated for a number of generations (9).

When males and females of the same race were mated, the reaction of progeny females was consistent for the race (Table 3). This response indicated that the nematode populations in the study were homogeneous for race characteristics. However, the testing procedure probably would not demonstrate the presence of heterozygosity in races 2 and 4, which appear to have the dominant genes for host specificity.

Eggs and larvae from F_1 females were placed on Lee and Pickett cultivars and allowed to reproduce (Table 4). Segregation for host reaction occurred in the F_2

TABLE 4. Maturation of F₂ progeny of interracial crosses of *Heterodera glycines* on roots of Lee and Pickett soybean.

Cross	Host cultivars [†]		Race		Expected ratio	Chi-square	P-value
	Lee	Pickett	1 or 3	2 or 4			
	No. of females		No. of females				
1 x 2	599	447	152	447	1:3	0.05	.75-.90
1 x 4	700	539	161	539	1:3	1.49	.25-.10
1 x 3	3664	231	3433	231	15:1	0.02	.75-.90
2 x 3	1085	639	446	639	7:9	3.08	.10-.05
3 x 4	898	532	366	532	7:9	3.27	.10-.05

[†]All races reproduce on Lee, whereas only races 2 and 4 reproduce on Pickett. About the same number of eggs and larvae were placed on Lee and Pickett.

[‡]Races 1 and 3 estimated by subtracting number of females on Pickett from number of females on Lee.

generation. However, inheritance patterns could not be fully elucidated because the reaction for all races could not be determined from the Lee and Pickett cultivars. A single gene pair conditioned parasitic ability in the crosses races 1 x 2 and 1 x 4, as indicated by low chi-square values for an expected 1:3 ratio. There appeared to be a minimum of two major genes segregating for host specificity in the crosses races 2 x 3 and 3 x 4 (Table 4). These results are consistent with those of Triantaphyllou (12). Chi-square values were relatively high, 3.08 and 3.27, for these two crosses, but both had probability values between 0.10 and 0.05.

In one large F₂ population from a cross between races 1 and 3, neither of which can reproduce on Pickett, 6.5% of the individuals reproduced on Pickett. This percentage would fit a 15:1 ratio, which would indicate a factor controlled by

two recessive genes. The parasitic reaction of an isolate obtained from a cross between races 3 and 4 is shown in Table 5. In the F₂ generation, this isolate appeared to possess a different parasitic capability from that of other known races. It was maintained for two additional generations and still gave a similar parasitic reaction on host differentials (Table 5). The reaction of this isolate was considerably different from that of any of the known races since it reproduced as well on PI 90,763 as on either Lee or Pickett. Also, its reproductive capability was much higher on PI 88,788 and Peking than other known races. These data showed that recombinant types may result from crosses of races of SCN.

It appeared that relatively few major genes conditioned parasitic capability in SCN, but the data were not adequate to propose specific inheritance patterns. Only Lee and Pickett were used as hosts; there-

TABLE 5. Females recovered and cyst indices of races 3 and 4 and an F₄ isolate selected from the cross 3 x 4 of *Heterodera glycines*.

Race or isolate	Number of females and cyst index on the host cultivar [†]				
	Lee	Pickett	Peking	PI 88,788	PI 90,763
3	340 (100)	0 (0)	0 (0)	0 (0)	0 (0)
4 [*]	223 (100)	230 (103)	16 (7)	18 (8)	4 (2)
37-90	232 (100)	300 (129)	194 (84)	162 (70)	280 (121)

[†]Cyst index is given as a percent of the females recovered from Lee soybean.

^{*}The cyst index for "race 4" does not correspond to the characterization published for race 4. However, the isolate designated race 4 is the one described as race 4 by Golden et al. (5), and the indices reported are the averages of several tests.

fore, races could not be distinguished. Although inheritance patterns could not be fully elucidated, the data clearly showed that genetic segregation occurred following hybridization of different races of this organism.

More research is needed to verify proposed inheritance patterns of races of SCN. Additional research is also needed to determine the potential for new biotype development through gene recombination and to check for the existence of these biotypes in soybean fields.

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