

Inheritance of Reaction to Race 4 of Soybean-cyst Nematode¹

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ABSTRACT

Five soybean (*Glycine max* L. Merr.) crosses involving parents that differed in reaction to race 4 of soybean-cyst nematode (SCN) (*Heterodera glycines* Ichinohe) were studied in F₁, F₂, and backcross populations. Reaction of the parents to race 4 was: PI 88788, resistant; PI 90763, moderately resistant; 'Peking,' susceptible; 'Mack' and 'Hill,' very susceptible.

Frequency distributions from three crosses involving parents that were resistant, moderately resistant, and susceptible indicated that three alleles at a single locus conditioned reaction to SCN. Resistance possessed by PI 88788 was recessive to PI 90763 and the moderately resistant reaction of PI 90763 was recessive to the susceptible Peking cultivar. Backcross data also appeared to confirm this inheritance pattern. Segregation in the F₂ generation gave a good fit for a ratio of three resistant and 61 susceptible plants from two crosses; Hill × PI 90763 and Mack × PI 90763 which involved very susceptible and moderately resistant parents. This ratio indicated one dominant and two recessive genes conditioned resistance in these crosses. Apparently two additional loci with two alleles at each locus, also are necessary for resistance to race 4.

Additional index words: *Glycine max* L. Merr., Inheritance, Resistance, *Heterodera glycines*.

SOYBEAN (*Glycine max* L. Merr.) yields may be seriously reduced by a destructive disease incited by the soybean-cyst nematode (SCN) (*Heterodera glycines* Ichinohe) feeding on roots of the plants. This

pest was first identified on soybeans in the United States at Castle Hayne, N. C. in 1954 by Winstead, Skotland, and Sasser (7). It has been suggested that SCN may have been introduced from Japan, where it was identified on soybeans as early as 1915. In 1973, there were 13 states in the U.S. that reported infestations of SCN.

Resistance to SCN was identified by Ross and Brim (6), and inheritance was reported by Caldwell, Brim, and Ross (1) to be controlled by three independently inherited recessive genes. Matson and Williams (5) later found that in addition to the three recessive genes already reported, a dominant allele closely linked with the *I* locus for seed-coat color was also necessary for resistance. Golden et al. (2) in 1970 described a group of host differentials to identify four infraspecific forms which they designated as races 1, 2, 3, and 4. An additional recessive gene was reported by Hartwig and Epps (3) to be necessary for resistance to a variant biotype from Virginia now designated race 2. No studies have been reported on inheritance of reaction to race 4 (a new biotype that is causing considerable damage to soybeans in parts of the southern production area). The objectives of this study were: i) to identify levels of resistance possessed by selected cultivars to races 1, 2, 3, and 4 of SCN and ii) to determine inheritance of reaction to race 4.

MATERIALS AND METHODS

We selected the cultivars PI 88788, PI 90763, 'Peking,' 'Mack,' and 'Hill' as parents for this study. It was necessary to use several parents because varying levels of reaction were present in different cultivars as shown in Table 1. Crosses were made in 1971 and the F₁ plants were grown in the greenhouse during the following winter. Backcrosses were made and parental, F₁, F₂, F₃, and backcross populations were evaluated for SCN reaction during the following 2 years.

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Plants were evaluated for SCN reaction in the greenhouse by growing them in sand infested with eggs and larvae of *Heterodera glycines*. The initial cultures of the four races of SCN were obtained from the following areas: i) race 1 was obtained from K. R. Barker, N. C. State U., who collected it from a field near Castle Hayne, N. C.; ii) race 2 was obtained from L. I. Miller, V. P. I., Blacksburg, Va., who collected it near Holland, Va.; and iii) races 3 and 4 were collected by R. D. Riggs, U. of Ark, from a field near Blytheville, Ark. Races 1 and 3 were reproduced on 'Lee;' race 4 on Pickett; and race 2 on PI 79693.

Seed were planted approximately 1.2-cm deep in flats filled with vermiculite. After 2 to 3 days, radicles of germinating seed were approximately 4.0 cm in length and near optimum for transplanting. Seedlings were transplanted in 7.6-cm clay pots filled with sterilized sand and inoculated with larvae and eggs of SCN when one trifoliate leaf was fully expanded and the second partially expanded.

The procedure used to obtain larvae and eggs for inoculation was as follows: plants and sand were placed in water and cysts rubbed from roots; the water was roiled and the suspension was poured through nested 20 and 60-mesh sieves; cysts caught on the 60-mesh sieve were broken by forcing them through an 80-mesh sieve; debris was removed by screening through a 200-mesh sieve and eggs and larvae were collected on a 400-mesh sieve. Approximately the same number of larvae and eggs were added to each pot in an experiment. This was accomplished by adding inoculum with an automatic syringe which delivered 2.3 cc/aliquot and three aliquots were added to each pot. A minimum of 700 larvae and eggs were added to each pot which generally produced about 150 larvae on each plant.

Approximately 30 days after inoculation, cysts on plant roots were recovered on a 60-mesh sieve by the procedure described above. Material collected on the 60-mesh sieve was washed into a 10 cm² petri dish with a grid on the bottom and cysts were counted with the aid of a low power (15 \times) microscope. Individual plants were screened and the number of cysts/plant was recorded. Any cysts found would have developed on the test plants because the inoculum contained only eggs and larvae.

Square root transformations were made on the cyst-number data in an attempt to reduce apparent correlations of means and variances. Square roots were calculated on individual counts and these values were indexed. This was done by dividing the square root of the number of cysts on each plant by the square root of highest number found on a single plant of the Lee check out of a group of 10 plants. It appeared that this method of presenting data made comparisons among experiments more meaningful.

Reaction of cultivars to soybean-cyst nematode was based on the following average cyst values: 1) very resistant, 0 to 7; 2) resistant, 8 to 23; 3) moderately resistant, 24 to 39; 4) susceptible, 40 to 55; and 5) very susceptible, above 55. Cyst-nematode reactions given in Table 1 are mean values for the cultivars. The range of cyst values for plants of a cultivar occasionally exceeded the numerical limits designated for a reaction class; therefore individual plant values for parental or segregating populations did not fit the numerical limits for the reaction class in some cases.

RESULTS AND DISCUSSION

Reaction to Soybean-Cyst Nematode

Reactions of five soybean cultivars used for parents in these studies to races of soybean-cyst nematode are shown in Table 1. Golden et al. (2) previously described the reaction of a set of host differentials to four races of SCN but did not completely identify the degree of development on each cultivar. It is clearly evident that cultivars differ considerably in reaction to SCN (Table 1). It was necessary to identify the relative reaction of each cultivars before attempting to conduct an inheritance study. Race designation was based on the chronological discovery of each race but does not give any indication of how these races evolved.

Pathogenicity of races 1 and 3 appears to be similar and only PI 88788 reacted differently to the two races.

Also, races 2 and 4 are similar with all the cultivars having the same reaction to these races except PI 88788. These data clearly show the existence of four distinct races based on host differentials, but a similarity in pathogenicity of races 1 and 3 and races 2 and 4 is apparent.

Inheritance of Reaction to Race 4

We placed emphasis on determining inheritance of reaction to race 4, but also attempted to elucidate the interrelationship of race 4 and other known races of SCN. Data on SCN reaction for parental, F₁, F₂, and backcross populations for five crosses are given in Tables 2 and 3. Most of the data appeared to be quantitative; however uncontrolled variability, rather than polygenic inheritance, appeared to be the major reason for the apparent continuous variation in the F₂ population of most crosses. Therefore we attempted to analyze the data, using both quantitative and qualitative procedures. This large amount of uncontrolled variability was expected, because we grew two variable organisms (soybean plants and nematodes) in a host-parasite relationship for a 30-day period before classifying for degree of resistance or susceptibility.

Even though the data were variable, broad-sense heritability estimates for the five crosses reported in

Table 1. Reaction of soybean cultivars to four races of soybean-cyst nematode.

Origin	Cultivar	Race*			
		1	3	2	4
D632-15 \times D49-2525 [†]	Hill	VS	VS	VS	VS
[(NC55(3) \times S62-5-16-12) \times RA63-19-2] \times Lee 68 [‡]	Mack	R	R	VS	VS
Originally PI 17852 B (introduced from China)	Peking	VR	VR	S	S
Introduced from China	PI 90763	VR	VR	MR	MR
Introduced from China	PI 88788	MR	R	S	R

* Reaction to the soybean-cyst nematode was designated as follows: VS = very susceptible; S = susceptible; MR = moderately resistant; R = resistant; and VR = very resistant. Reaction of the cultivars are average values for several tests conducted during a 3-year period. [†] Developed in Mississippi. [‡] Developed in Arkansas.

Table 2. Reaction of parents, F₁, and segregating populations from three crosses to race 4 of soybean-cyst nematode.

Population	Cyst value*			No. of plants [†]		Expected ratio	χ^2	P range
	Mean	Range	Variance	R	MR			
PI 88788	20	14-24	10	25				
PI 90763	33	21-45	35	25				
F ₁	32	24-38	20	9				
F ₂	33	14-66	112	24	65	1:3	0.19	0.50-0.70
F ₁ \times PI 88788	25	14-38	104	5	7	1:1	0.33	0.50-0.70
F ₁ \times PI 90763	34	21-42	72	10				
				<u>R</u>	<u>S</u>			
PI 88788	16	8-24	22	24				
Peking	41	30-51	49	15				
F ₁	31	27-33	5	5				
F ₂	29	8-54	143	21	65	1:3	0.02	0.90-0.95
				<u>MR</u>	<u>S</u>			
Peking	41	26-50	36	25				
PI 90763	26	11-30	23	25				
F ₁	34	23-42	43	10 [‡]				
F ₂	37	7-65	78	93	300	1:3	0.37	0.50-0.70
F ₁ \times PI 90763	41	22-50	91	9				

* Cyst value = $\frac{\text{No. of cysts recovered/plant}}{\sqrt{\text{Highest no. of cysts/plant on Lee check}}} \times 100$.

[†] R = resistant, MR = moderately resistant, and S = susceptible. [‡] Means were slightly higher than midparent values, therefore were placed in the susceptible class.

Table 3. Reaction of parents, F_1 , and segregating populations from two crosses to race 4 of soybean-cyst nematode.

Population	Cyst value*			No. of plants†		Expected ratio	χ^2	P range
	Mean	Range	Variance	MR	VS			
PI 90763	28	11- 39	58	17				
Mack	98	67-123	94		22			
F_1	101	85-104	77		7			
F_2	63	20-123	181	18	443	3:61	0.61	0.30-0.50
$F_1 \times$ Mack	61	39- 67	101		11			
Hill	85	78- 87	9		18			
PI 90763	28	21- 30	12	16				
F_1	63	61- 64	4		3			
F_2	55	21- 87	151	21	446	3:61	0.04	0.80-0.90

* See Table 2 footnote for explanation.

† MR = moderately resistant and VS = very susceptible.

this study ranged from 55 to 80%. The correlation coefficient was high ($r=0.81^{**}$) for F_2 plant values and means of F_3 families for the cross, PI 88788 \times PI 90763. This indicated that a relatively good measure of nematode reaction was obtained from individual plant measurements. Reaction of plants from F_3 families also was used in some cases to verify the genotype of F_2 plants.

Variances for segregating populations from three crosses (PI 88788 \times PI 90763, PI 88788 \times Peking, and Peking \times PI 90763) were considerably larger than for either parental or F_1 populations (Table 2) which indicated that genetic segregation occurred for reaction to race 4 of SCN. Data from these crosses also indicated that susceptibility was either dominant or partially dominant and parental types were recovered in rather small F_2 populations. Inheritance patterns were similar from reciprocal crosses, therefore we ruled out the possibility of maternal or cytoplasmic influence. PI 88788 was classified as resistant; PI 90763 as moderately resistant; and Peking as susceptible (Table 1).

A partitioning method of genetic analysis suggested by Leonard, Mann, and Powers (4) was used to estimate the number of plants in the susceptible class in the F_2 generation. A good fit was obtained for a ratio of 1 resistant to 3 moderately resistant plants as indicated by a high probability value ($P = 0.50$ to 0.70) in the cross PI 88788 \times PI 90763. The population from the backcross to the resistant parent (PI 88788) segregated into two distinct classes and gave a good fit to a 1:1 ratio. Also, plants from the backcross to the moderately resistant parent (PI 90763) were all moderately resistant which gave additional evidence that a single gene pair conditioned resistance in this cross.

A single gene pair also appeared to control resistance to race 4 in the cross PI 88788 \times Peking. F_1 plants were nearly as susceptible as the susceptible parent and the F_2 generation appeared to segregate in a 1:3 ratio for resistance and susceptibility as indicated by a low χ^2 value.

In a third cross to study inheritance of resistance to race 4, a moderately resistant cultivar (PI 90763) was crossed with Peking (a susceptible type). Again, a good fit was obtained for a ratio of 1 moderately resistant to 3 susceptible plants in the F_2 generation as shown in Table 2. Plants from a backcross to the susceptible parent were susceptible.

Data from three crosses involving parents that were resistant, moderately resistant, and susceptible strongly indicated that three alleles at a single locus conditioned resistance to race 4 of SCN. Resistance possessed by PI 88788 was recessive to PI 90763 and the moderate resistant reaction of PI 90763 was recessive to susceptible Peking.

Populations also were evaluated from crosses of moderately resistant and very susceptible cultivars (Table 3). Hill is very susceptible to all races of SCN and Mack is very susceptible to races 2 and 4 but is resistant to races 1 and 3. Data from two crosses (PI 90763 \times Mack and Hill \times PI 90763) showed similar inheritance patterns. F_1 plants from both crosses exhibited a high degree of dominance for the susceptible reaction. Also, F_2 populations from both crosses gave a good fit for a ratio of 3 moderately resistant to 61 susceptible as indicated by high probability values, Table 3. This ratio occurs where one dominant and two recessive genes are segregating. It is obvious that a 61:3 ratio is similar to a 60:4 (15:1) where only two recessive genes are segregating, but χ^2 values for the observed and expected for a 15:1 ratio differed significantly for both crosses. Even though results from our data strongly suggest this type inheritance, lines should be extracted from F_2 or backcross populations that differ only by the dominant allele to verify its existence.

Inheritance of resistance to race 4 of SCN appears to be conditioned by one dominant and two recessive genes. Three alleles appear to be present at one locus and two alleles at the other two loci; however additional data are needed to verify this genetic hypothesis.

It appeared significant that inheritance patterns for PI 90763 \times Mack and Hill \times PI 90763, were similar and χ^2 analyses indicated that genes at three separate loci conditioned resistance. Both Mack and Hill are very susceptible to races 2 and 4, but Mack is resistant to races 1 and 3 whereas Hill is highly susceptible to all races. Therefore it appears that genes possessed by Mack, which are necessary for resistance to races 1 and 3, apparently have no influence on resistance to race 4.

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