

Inheritance of Resistance to *Heterodera glycines* Race 3 in Soybean Accessions

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ABSTRACT

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Soybean cyst nematode (*Heterodera glycines*) is a major pest of soybean (*Glycine max*). Crop losses are primarily reduced by the use of host plant resistance. Soybean accessions PI 89772, PI 209332, PI 438489B, and PI 404166 were crossed with susceptible cv. Essex to determine inheritance of resistance to *H. glycines* race 3. The F₁, F₂, and F₃ plants from each cross were bioassayed in the greenhouse. The reactions of the parental lines and progenies to infection by *H. glycines* support the hypothesis that resistance in PI 89772 and PI 209332 is conditioned by one dominant and one recessive gene. Resistance in PI 438489B is conferred by two recessive genes. Two dominant genes and one recessive gene condition resistance in accession PI 404166. Knowledge of the inheritance of resistance to *H. glycines* race 3 in these accessions should enhance their usefulness in breeding programs.

Soybean cyst nematode (*Heterodera glycines* Ichinohe) was first reported in 1915 in Japan and soon was found on soybean (*Glycine max* (L.) Merr.) worldwide (14). In the United States, *H. glycines* is the primary cyst nematode and is a most serious pest of soybean (18). During 1988–1991, estimated annual yield losses were 3.08% in the southern soybean production area of the United States (16).

Crop losses due to *H. glycines* have been reduced primarily by the use of host plant resistance, but resistance has not been durable. The genetic base of host resistance in soybean cultivars is very narrow and is susceptible to virulent races of *H. glycines*. Nearly all resistant soybean cultivars in the United States have introgressed resistance genes from cv. Peking and/or PI 88788. Both sources have been well characterized, and the genetics of their resistance have been studied (2–4,6,8,9,17).

Soybean accession PI 437654, a recent introduction from Russia, has been reported to be resistant to seven of the 16 races of *H. glycines* (1,13). This accession has been well characterized and the

genetics of resistance also have been reported (5). Additional soybean accessions with resistance to several races of *H. glycines* are available (1,13,19). These accessions are an invaluable source of genetic variation to be exploited by soybean breeding programs for introgressing resistance genes into elite cultivars. Prior knowledge of the structure and distribution of genetic variation for resistance to *H. glycines* within these accessions is essential to breed cultivars for improved genetic diversity and gene pyramiding. Development of durable resistance to *H. glycines* will depend on genetic diversity and gene pyramiding in elite cultivars.

The objective of this research was to determine the inheritance of resistance in soybean accessions PI 89772, PI 209332, PI 438489B, and PI 404166 to *H. glycines* race 3. A brief summary of the results has been reported (11).

MATERIALS AND METHODS

A field population of *H. glycines* was obtained from the Ames Plantation located near Grand Junction, Tennessee (courtesy of L. D. Young, USDA-ARS, Jackson, TN), and maintained under isolation in a greenhouse. Females used for inoculum were produced on the roots of the susceptible cv. Essex for several generations to obtain a near-homogeneous population. This isolate averaged 3, 0, 2, 3, and 198 females per plant, respectively, on the four host differentials—cv.

Peking, PI 90763, PI 88788, and cv. Pickett 71—and the susceptible cv. Essex and was classified as race 3 (15). Seeds of the soybean accessions used in this study were obtained from R. L. Bernard and R. L. Nelson, USDA-ARS, University of Illinois, Urbana-Champaign, and E. E. Hartwig, USDA-ARS, Stoneville, Mississippi. Soybean plant introductions (PIs) 89772, 209332, 438489B, and 404166 are resistant to several isolates of races 6 and 9 of *H. glycines* (11). Essex is susceptible to all known races of *H. glycines*. The following crosses were made in the summer of 1989 at the University of Missouri Delta Center, Portageville: PI 89772 × Essex, PI 209332 × Essex, PI 438489B × Essex, and PI 404166 × Essex.

The F₁ plants were grown in Puerto Rico (off-season nursery) and from them F₂ plants were grown at the Delta Center to generate approximately 100 F₃ families for each of the four crosses. A sample of F₁ and F₂ plants and randomly chosen F₃ families (because bioassay destroyed F₂ plants, F_{2,3} families were not used) from each cross were bioassayed for their reaction to *H. glycines* race 3 in the greenhouse during 1990, 1991, and 1992. Each cross included 10 plants of each parent, 10 F₁ plants, 288–339 F₂ plants, and 24–32 F₃ plants from each of 48–65 F₃ families. A set of host differentials consisting of Peking, PI 90763, PI 88788, Pickett 71, PI 437654, and Essex were standards in each evaluation. Essex was substituted for cv. Lee as the susceptible control.

The plants were grouped in six experiments and were bioassayed consecutively. The F₁ and F₂ plants from PI 89772 × Essex and PI 209332 × Essex were included in a single experiment, and similar plants from PI 438489B × Essex and PI 404166 × Essex formed the next experiment (Table 1). The F₃ families from each of the four crosses were individually evaluated in four separate experiments. The plants were grown in 200 × 25 mm polypropylene micropots filled with steam-pasteurized Broseley fine sandy soil adjusted to pH 6.8 with

Ca(OH₂) (7). A single 4-day-old seedling with a 15- to 20-mm long radicle was transplanted into each micropot. Approximately 20 of these micropots were placed in a polypropylene container (20 cm diameter) and maintained at 27 ± 1 C in a water bath. One plant each of five host differentials and one susceptible control were also included in every container. The transplanted seedlings were allowed to establish for 5 days prior to inoculation with 1,000 ± 28 eggs and juveniles of *H. glycines* race 3 (10).

Approximately 30 days after inoculation, plant roots were individually washed with a strong jet of water to dislodge the females and cysts. The

females and cysts were counted under a stereomicroscope.

The F₁, F₂, and F₃ plants that were parental-type in reaction (in terms of the number of females and cysts on the individual plant roots) for resistance to *H. glycines* were defined as resistant for the purpose of our genetic analyses (3,11). The overall reaction of an individual F₃ family was determined by the range of females and cysts found on the plants in each family. On the basis of the reaction of 24–32 individual F₃ plants, all F₃ families were included in three categories (Table 2). A family was categorized as resistant when all the plants were nonsegregating and uni-

formly resistant. Plants in these families essentially had the same infection levels (range of females and cysts) as those of their respective resistant parents. A F₃ family with uniformly susceptible plants (nonsegregating) was categorized as susceptible. A family with a combination of resistant and susceptible plants was categorized as segregating. Chi-square tests (Yates correction term applied) were used to test goodness of fit of observed to appropriate genetic hypothesis in the F₂ and F₃ populations.

RESULTS AND DISCUSSION

The female and cyst means for F₁ plants of all crosses were between the means for the susceptible and resistant parents, indicating incomplete dominance (Table 1). Means of infection levels were slightly higher for resistant F₂ and F₃ plants than those of their respective resistant parents for all crosses (Table 1). These differences for infection levels in segregates were presumably due to transgressive variation and modifying genes.

The F₁ hybrids from PI 89772 × Essex and PI 209332 × Essex were susceptible to *H. glycines* race 3 (Table 2). The F₂ plants from each of the two crosses segregated closely to 3R:13S, the expected dihybrid ratio for two gene pairs with dominant and recessive epistasis (indicated by *Rhg*, *rhg* in Table 2). In the F₃ generation, the segregations were consistent with the expected ratio of 1R:8Seg:7S (Table 2).

The F₁ plants from the cross PI 438489B × Essex were susceptible. The F₂ population fit closely a ratio of 1R:15S, indicating that resistance to *H. glycines* in the resistant parent is conditioned by two recessive genes (*rhg*, *rhg*). The F₃ families for this cross were classified 4 resistant, 34 segregating, and 24 susceptible, which is a close fit to the expected 1:8:7 ($P = 0.7-0.8$).

Table 1. Mean number and range of females or cysts per plant obtained for parent, F₁, F₂, and F₃ plants inoculated with *Heterodera glycines* race 3 in a greenhouse during 1990, 1991, and 1992^a

Cross	Population	Number of females or cysts					
		Resistant		Segregating		Susceptible	
		Mean	Range	Mean	Range	Mean	Range
PI 89772 × Essex	F ₁					91.0	88-98
	F ₂	2.0	0-5			115.0	6-346
	F ₃	4.1	0-5	61	0-290	110.0	6-240
PI 209332 × Essex	F ₁					91.2	82-99
	F ₂	3.0	0-7			73.9	8-340
	F ₃	3.8	0-7	52	0-281	59.1	8-290
PI 438489B × Essex	F ₁					62.2	58-68
	F ₂	0.2	0-1			32.6	2-130
	F ₃	0.5	0-1	42	0-102	58.0	28-118
PI 404166 × Essex	F ₁					57.4	48-64
	F ₂	0.3	0-1			39.2	2-191
	F ₃	0.8	0-1	59	0-151	49.0	2-171
PI 89772 (P ₁)		1.5	0-5			140.0	128-320
Essex (P ₂)						152.0	141-301
PI 209332 (P ₁)		2.9	0-7			118.0	91-136
Essex (P ₂)						182.0	156-194
PI 438489B (P ₁)		0.3	0-1				
Essex (P ₂)							
PI 404166 (P ₁)		0.2	0-1				
Essex (P ₂)							

^aEach cross included 10 plants of each parent, 10 F₁ plants, 288–399 F₂ plants, and 24–32 F₃ plants in each of 48–65 F₃ families.

Table 2. Segregation and χ^2 tests for *Heterodera glycines* race 3 reaction in F₁, F₂, and F₃ populations from resistant × susceptible soybean crosses

Cross	Generation	Number of plants or families ^a						Hypothesized resistance genes	Genetic ratio ^b	χ^2	P value
		Observed			Expected						
		R	Seg	S	R	Seg	S				
PI 89772 × Essex	F ₁	0		10	0		10		0:1		
	F ₂	65		274	63.5		275.5	<i>Rhg</i> , <i>rhg</i>	3:13	0.04	0.8-0.9
	F ₃	3	28	22	3.3	26.5	23.2		1:8:7	0.173	0.9-0.95
PI 209332 × Essex	F ₁	0		10	0		10		0:1		
	F ₂	64		224	54		234	<i>Rhg</i> , <i>rhg</i>	3:13	2.05	0.1-0.2
	F ₃	3	25	20	3	24	21		1:8:7	0.10	>0.95
PI 438489B × Essex	F ₁	0		10	0		10		0:1		
	F ₂	20		280	18.75		281.25	<i>rhg</i> , <i>rhg</i>	1:15	0.03	0.7-0.8
	F ₃	4	34	24	3.87	31	27.13		1:8:7	0.49	0.7-0.8
PI 404166 × Essex	F ₁	0		10	0		10		0:1		
	F ₂	45		258	42.6		260.4	<i>Rhg</i> , <i>Rhg</i> , <i>rhg</i>	9:55	0.09	0.7-0.8
	F ₃	1	30	34	1.00	26.8	38.2		1:26:37	0.88	0.5-0.7

^aR = resistant, Seg = segregating, S = susceptible.

^bResistant:susceptible, resistant:segregating:susceptible.

Of the 303 F₂ plants tested from the cross PI 404166 × Essex, 45 were resistant and 258 were susceptible (Table 2). The segregation in the F₂ was very close to 9R:5S ($P = 0.7-0.8$). The F₃ families for this cross were classified 1 resistant, 30 segregating, and 34 susceptible, which is a good fit to the expected 1:26:37 ($P = 0.5-0.7$) (Table 2). This suggested that resistance in PI 404166 is conferred by a combination of two dominant (*Rhg*, *Rhg*) and one recessive (*rhg*) resistance genes.

Caldwell et al (2) reported three recessive genes (*rhg*₁, *rhg*₂, *rhg*₃) and Matson and Williams (4) reported a dominant gene (*Rhg*₄) conditioning resistance to *H. glycines* in Peking. Rao-Arelli et al (9) reported an additional dominant gene (*Rhg*₅) in PI 88788 that is at a locus other than those previously reported. It is not known if any of those resistance genes are the same as those found in this report. A preliminary study based on restriction fragment length polymorphisms indicated that PI 209332 is genetically unrelated to parental lines PI 438489B and PI 89772 but is in the same cluster with PI 404166 (12). This may make PI 209332 an additional source of resistance to *H. glycines* by having unique gene(s). Further studies are needed to establish the uniqueness of the resistance genes identified in this report.

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