Soybean Germplasm Resistant to Races 1 and 2 of *Heterodera glycines*

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ABSTRACT

Heterodera glycines **Ichinohe is a serious pest of soybean [*Glycine max* (L.) Merr.] in the USA. Several Races of *H. glycines* occur in the soybean growing areas. Soybean accessions with resistance to Races 3, 5, and 14 have been identified. Data on reaction of these accessions for Races 1 and 2 are not available. Our objective was to bioassay these accessions for resistance to *H. glycines* Races 1 and 2. Eighty-six accessions, host differentials, and susceptible control were bioassayed during 1995 to 1996 for each of the two Races in thermoregulated water baths in the greenhouse. The results indicated that 52 accessions had resistance to *H. glycines* Race 1, and 24 accessions had resistance to Race 2, respectively. Soybean PIs 89772, 90763, 404166, 404198A, 437654, 437690, 438489B, 567491A, and 567516C were either resistant or moderately resistant to both Races. These are potential resistance sources to *H. glycines* Races 1, and 2 for developing resistant soybean cultivars. These PI lines are being fingerprinted by means of molecular markers to identify the unique lines to allow broadening the diversity of resistance gene utilization.**

IN THE USA, *H. glycines* is the primary cyst nematode and is a serious pest of soybean (Wrather and Rao Arelli, 1992). The current classification system has designated 16 different Races for *H. glycines* based on their ability to parasitize a set of host differentials (Riggs and Schmitt, 1988). Soybean resistance to *H. glycines* was initially identified in North Carolina (Ross and Brim, 1957). Resistant sources were introduced from China and included 'Peking,' PI90763, and PI84751. The *H. glycines* population from North Carolina was later classified as Race 1 (Golden et al., 1970). A Race 2 population was reported in Virginia, and the soybean line PI90763 was found resistant (Hartwig and Epps, 1970). Recently, resistance to Races 3, 5, and 14 was identified in soybean line PI437654 (Anand et al., 1985) and it was found additionally resistant to several isolates of Races 1 and 2 (Rao Arelli et al., 1992).

Several accessions from soybean germplasm collections were evaluated for resistance to this pest (Young, 1990, 1995; Nelson et al., 1994). As of today, 118 resistant accessions are identified, and these are primarily resistant to Races 3, 5, and 14 of *H. glycines*. We have very little information on their reaction to Races 1 and 2. The objective of this research was to bioassay 86 accessions with known resistance to Race 3, 5, or 14 for reaction to isolates of *H. glycines* Races 1 and 2.

MATERIALS AND METHODS

Collection, and preparation of near-homogeneous Race isolates of *H. glycines* used in this research include the following two race isolates. Near-homogeneous isolates were developed and used as in previous genetic studies for stable reactions (Rao Arelli et al., 1989).

Race 1 Isolate

A field population of *H. glycines* was obtained from Cape Girardeau County, MO. Females and cysts used for inoculum in this study were first cultured on the roots of susceptible 'Hutcheson' for over 24 generations to obtain a near-homogeneous population. This population was categorized as a Race 1 according to the classification system of Riggs and Schmitt (1988), and was maintained on the roots of Hutcheson. In a typical greenhouse analysis, the mean number of females produced by this isolate on Peking, PI90763, PI88788, 'Pickett-71,' and Hutcheson were 4, 2, 82, 13, and 158, respectively.

Race 2 Isolate

The methods described for the Race 1 isolate were also used to develop a near-homogeneous population of *H. glycines* Race 2. A field population of Race 2 was collected from Beaufort County, NC. This was cultured and maintained on roots of Pickett-71 approximately for 31 generations before it was used in this study for inoculum. In a typical greenhouse test, the four differentials (Peking, Pickett-71, PI90763, and PI88788) had an average of 36, 59, 3, and 61 females, respectively, by this Race. The susceptible control, Hutcheson produced an average of 167 females.

Eighty-six accessions with resistance to Race 3, 5, or 14 (PI54571–PI567660B) were bioassayed. These were in maturity groups 0 to VI, and were collected from China, Japan, Russia, or South Korea. These accessions plus the four host differentials (Peking, PI90763, PI88788, and Pickett-71), and Hutcheson, a susceptible control, were included in each bioassay. Seeds of PI lines used in this study were obtained from R.L. Nelson, Curator, USDA-ARS, National Soybean Research Laboratory, Urbana, IL.

Bioassays were performed in the greenhouse during 1995 to 1996 for each of the two *H. glycines* races according to the procedures described by Rao Arelli (1994). Ten seedlings were included for each of the 86 accessions, susceptible control, and differentials. Within entry each seedling represented a single replication, and the test was completely randomized. In brief, the techniques involved growing plants in 200- by 25-mm plastic micropots filled with steam-pasteurized Brosely fine sandy soil (loamy, mixed thermic Arenic Hapludalf). Approximately 20 of these micropots were placed in a polypropylene container (20-cm diam.), and maintained at 27 ± 1°C in thermoregulated water baths (Forma Scientific Inc., Marietta, OH). Two seeds were planted in each micropot and thinned to one seedling per pot after germination. The seedlings were allowed to grow for 4 to 5 d prior to their inoculation with *H. glycines*.

Each seedling was inoculated with 1200 ± 25 eggs in 5 mL of suspension with an automatic pipetter (Brewer automatic pipetting machine, Scientific Equipment Products, Baltimore, MD) (Rao Arelli et al., 1991).

Approximately 30 d after inoculation, plant roots were individually washed with a strong jet of water to dislodge females and cysts. These were counted under a stereo microscope, and an index of parasitism (IP) or female index was calculated for

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Abbreviations: IP, index of parasitism; PI, plant introduction.

Table 1. Maturity group, seed color, and mean index of parasitism for 86 soybean accessions, host differentials and susceptible control bioassayed with *H. glycines* Races 1 and 2.

Strain designation	Maturity group	Seed color	Index of parasitism† Race 1	Index of parasitism† Race 2
PI54591	III	yellow	67	130
PI79609	II	black	74	155
PI79693	III	brown	28	61
PI84751	IV	black	3	50
PI87631-1	III	black	37	118
PI88788	III	black	38	118
PI89008	II	black	35	116
PI89014	II	yellow	35	131
PI89772	IV	black	2	7
PI90763	IV	black	1	3
PI91138	II	yellow	44	195
PI92720	III	black	74	140
PI200495	IV	black	28	81
PI209332	IV	black	47	126
PI303652	V	black	2	84
PI339868-B	IV	black	2	35
PI398680	IV	green	56	140
PI398682	IV	black	71	97
PI399061	VI	yellow	38	77
PI404166	III	black	1	10
PI404198-A	IV	black	1	9
PI404198-B	IV	black	2	79
PI407729	IV	black	11	82
PI407944	V	yellow	61	148
PI408192-2	V	green	45	146
PI416762	II	black	17	83
PI417091	II	black	19	101
PI417094	III	black	33	56
PI424137-B	V	yellow	61	148
PI424595	VI	black	59	80
PI437090	0	yellow	64	189
PI437379	I	yellow	85	179
PI437488	II	black	55	153
PI437654	III	black	0	2
PI437655	III	black	1	126
PI437679	IV	black	22	27
PI437690	III	black	1	13
PI437725	IV	black	1	31
PI437770	III	black	31	107
PI437908	II	black	74	143
PI438183	II	brown	50	167
PI438342	VI	black	38	37
PI438489-B	IV	black	0	7
PI438496-B	III	black	30	158
PI438497	III	black	5	30
PI438498	IV	black	1	30
PI438503-A	II	black	44	99
Cloud 16790 (Sel.)	III	black	51	99
Columbia	III	green	18	63
Ilsoy 6387 (Sel.)	III	brown	29	85
Patoka 70218–2-19–3	IV	yellow	134	168
Sooty	IV	black	37	146
PI17852B (Sel.)	IV	black	2	104
PI567285	IV	yellow	100	183
PI567286	IV	yellow	116	N/A‡
PI567303A	IV	black	114	N/A
PI567325B	V	yellow	128	139
PI567328	V	yellow	115	140
PI567336A	IV	black	116	12
PI567336B	IV	black	32	16
PI567432	V	green	20	8
PI567363B	III	yellow	81	141
PI567364	II	yellow	115	73
PI567365	III	green	94	170
PI567373A	IV	yellow	93	94
PI567373B	V	yellow	60	60
PI567400	V	yellow	77	156
PI567415A	IV	yellow	29	43
PI567418A	II	yellow	91	70
PI567421	IV	yellow	94	61
PI567445	IV	yellow	15	47
PI567491A	III	black	0	11
PI567492	IV	yellow	103	115
PI567507B	II	yellow	66	115
PI567510A	III	yellow	107	124
PI567512B	II	yellow	95	150
PI567516C	IV	yellow	9	13
PI567535A	IV	yellow	100	77
PI567562A	IV	yellow	114	90
PI567568A	IV	yellow	43	105
PI567577	IV	yellow	91	117
PI567581	IV	yellow	62	70
PI567583C	IV	yellow	37	98
PI567583C	IV	yellow	91	118
PI567636	IV	yellow	93	85
PI567660B	V	yellow	65	59
	Differentials and susceptible control			
Peking	IV	black	7	132
PI88788	III	black	39	118
PI90763	IV	black	2	1
Pickett-71	VI	yellow	8	116
Hutcheson	V	yellow	121§	177§
LSD (P = 0.05)			2	9

† Index of Parasitism (IP) is the number of white, yellow and brownish-colored *H. glycines* females occurring on a soybean 30 ± 1 d after inoculation, expressed as the percentage of mean number of females on Hutcheson: mean of three separate tests (total of 10 replications). Four levels of resistance indicated by IP values include: resistant = 0–9, moderately resistant = 10–30, moderately susceptible = 31–60, and susceptible = >60 (Schmitt & Shannon, 1992).

‡ N/A, not available.

§ Actual mean number of females (10 replications) occurring on Hutcheson soybean, not Index of parasitism.

the number of females developing on each accession in each replication (Golden et al., 1970).

Data for three tests for each race were combined for analysis of variance of parasitism indices by the statistical analysis system software (SAS, 1988), and means were separated with Fisher's LSD based on a significant F test. Date of testing was considered a random effect, and the interaction term between soybean accession and testing date was used as the error term to test differences among accessions. Ratings of resistant (IP = 0–9%), moderately resistant (IP = 10–30%), moderately susceptible (IP = 31–60%), and susceptible (IP = >60%) were used to classify the accessions (Schmitt and Shannon, 1992).

RESULTS AND DISCUSSION

All accessions bioassayed for *H. glycines* Races 1 and 2 were from PI54591 through PI567660B, which included 86 lines (Table 1). These bioassays identified 52 accessions with various levels of resistance to *H. glycines* Race 1 (Table 1). These included 18 resistant, 12 moderately resistant, and 22 moderately susceptible soybean accessions (Table 1). For Race 2, 24 soybean accessions had some resistance, and these included six resistant, nine moderately resistant, and nine moderately susceptible (Table 1).

Young (1990) reported that Race 3 was capable of parasitizing three plant introductions (PI24595, PI399061, and PI438342) that were resistant to Race 5. This was the first reported evidence contrary to the hypothesis that Race 3 did not have any genes to parasitize resistant cultivars (Triantaphyllou, 1975). In our study, PI438342, which was susceptible to Race 3, was also moderately susceptible to Race isolates 1 and 2. Additionally, PI567415A and PI567445, both susceptible to Race 3 (Nelson et al., 1994), were also found with various levels of resistance in this study.

Soybean PI lines 89772, 90763, 404198A, 437654, and 438489B were resistant to both Races 1 and 2. PI437679 was moderately resistant to both Races. The PI lines which were resistant to Race 1 and were moderately resistant to Race 2 included 404166, 437690, 438497, 567491A, 438498, and 567516C. PI line 567432 was resistant to Race 2 and moderately resistant to Race 1. These are potential sources of resistance to *H. glycines* Races 1 and 2 for use in developing resistant soybean cultivars in the U.S.A. Soybean PI567516C was the only line with resistance to Races 1 and 2 that is yellow seeded. The rest are all black seeded. PI437654 still is the only known source with comprehensive resistance to Races 1, 2, 3, 5, and 14.

The value of the soybean germplasm collection as a reservoir of genes for genetic improvement of soybean continues to be demonstrated. The soybean germplasm collection is also an invaluable gene pool resource for pest resistance and genetic studies. DNA fingerprinting studies in collaboration with Southern Illinois University, Carbondale, are currently underway to estimate the genetic relationship among several of these PIs to determine the diversity of *H. glycines* resistance genes for use in developing soybean cultivars.

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