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Genetic Relationships for Resistance to *Heterodera glycines* Races 3 and 5 in Soybean[1]

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Abstract: *Heterodera glycines* is the most damaging root pathogen of soybean in the United States. Plant introduction (PI) 90.763 is resistant to both races 3 and 5, whereas PI 424.595 is resistant only to race 5. Genetic relationships in these PI lines for resistance to *H. glycines* are not known. Crosses between PI 90.763 and PI 424.595 and susceptible cv. Essex were studied in the F_1, F_2, and F_3 generations to compare the genes involved in resistance to race 3 with those for resistance to race 5. The F_2 plants also were studied separately for reaction to the two races by dividing roots into separate pots. Plants were screened using conventional techniques and classified as either resistant or susceptible based on an index of parasitism. Data were analyzed using χ^2 to determine goodness of fit between observed and expected genetic ratios. The cross PI 90.763 × Essex segregated 3 resistant: 13 susceptible plants in the F_2 generation, which indicated one dominant and one recessive gene, conditioned resistance to race 3. All progenies of the cross PI 424.595 × Essex, involving both susceptibles parents, were susceptible, indicating no genetic interaction. The cross of PI 90.763 × PI 424.595 showed monogenic inheritance with a dominant gene in PI 90.763. The divided root study of the cross PI 90.763 × Essex indicated that all race 5 resistant F_2 plants also were resistant to race 3, whereas, in the cross PI 90.763 × PI 424.595, some of the race 5 resistant plants were susceptible to race 3. Similarly, all plants susceptible to race 3 also were susceptible to race 5 in cross PI 90.763 × Essex, but not for PI 90.763 × PI 424.595. The results indicated that the dominant resistance gene and one of the recessive resistance genes in PI 90.763 which conditioned resistance to race 5 also imparted resistance to race 3. The additional recessive gene that controlled resistance to race 5 in PI 424.595 was ineffective against race 3.

Key words: genetics, *Glycine max, Heterodera glycines,* host-plant resistance, resistance, soybean, soybean cyst nematode

Soybean cyst nematode (SCN) *Heterodera glycine* Ichinohe is the most serious root pathogen of soybean (*Glycine max* L. Merr.) in the United States (7,13). Since its discovery in the United States (14), efforts were made to identify resistant germplasm to develop resistant cultivars (12). Subsequent research identified a large number of plant introductions (PI) resistant to races 3, 4, or 5 (3,4,8). Among these, PI 90.763 was resistant to both SCN races 3 and 5 (4). Three additional lines were identified that were resistant to race 5 but susceptible to race 3 (15).

Genetics of resistance to SCN indicated the presence of three recessive genes conditioning resistance in Peking to a North Carolina field population (6). An additional dominant gene was subsequently added to this list (10). Different genes were reported to condition resistance to SCN race 5 in certain PIs (5). One dominant and two recessive genes in PI 437.654 conferred resistance to both races 3 and 5 (11). One dominant and two recessive genes in PI 90.763 and three recessive genes in PI 424.595 conditioned resistance to race 5 (1). No serious attempt has been made to study relationships for SCN resistance against different races. The objective of this study was to determine genetic relationships among PI 90.763 and PI 424.595 for resistance to races 3 and 5, as both PIs are resistant to race 5 but have different reactions to race 3.

Materials and Methods

Crosses PI 90.763 × Essex, PI 424.595 × Essex, and PI 90.763 × PI 424.595 were made to generate F_1 seeds at the University of Missouri, Delta Center at Portageville, Missouri. PI 90.763 and PI 424.595 are resistant to race 5 (4,15), whereas PI 90.763 also is resistant to race 3

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(3). Both PI 424.595 and Essex are susceptible to race 3 (15). A part of the F_1 seeds were used to develop an F_2 population, and a random sample of F_2 was grown to generate F_3 progenies. The study of each cross included 10 plants of each of the parents, and F_1s, 350 to 498 F_2 plants, and 25 plants from each of the 50 randomly selected F_3 progenies. The four SCN race differentials, cv. Pickett, cv. Peking, PI 88.788, and PI 90.763, also were included (9).

A race 3 population was obtained from the Ames Plantation, Tennessee, and maintained on cv. Essex in a greenhouse. A homogenous SCN race 5 was developed as outlined previously (5). Briefly, white females were collected from the roots of PI 88.788 soybean grown at the Rhodes Farm, University of Missouri, near Clarkton, Missouri, in a field primarily infested with SCN race 5. Inoculum consisting of eggs and second-stage juveniles (J2) from the white females was added to PI 88.788 seedlings growing in sterilized soil in the greenhouse, and white females were collected 30 to 35 days after inoculation. The cycle of greenhouse selection was repeated 10 times to obtain a homogenous population of SCN race 5, which was maintained on PI 88.788 in a greenhouse. Following inoculation with 1,000 eggs and J2, the typical race 3 population produced an average of 180 white females on cv. Lee 74 and cv. Essex, 8 females on cv. Pickett, 5 on Peking, and 1 to 3 on PI 88.788 and PI 90.763. The race 5 population produced 165 white females on Lee 74 and Essex, 150 on PI 88.788, 130 on Pickett, 8 on Peking, and 1 to 2 on PI 90.763.

Seeds were germinated in vermiculite, and one seedling with a 2.4- to 3.5-cm-long radicle was transplanted into 20 × 2.5-cm plastic tube filled with steam-sterilized Brosely fine sandy loam (loamy, mixed, thermic Arenic Hapludalf) soil. Soil pH was adjusted to 6.5 by adding $CaCO_2$. White females collected from 30- to 35-day-old seedlings were crushed to release eggs and J2 just before inoculation. Each seedling was inoculated with a 5-ml water suspension containing approximately 1,000 eggs and J2 5 days after transplanting. Fifty tubes were placed in a 50-cm-diam. pot and kept at 27 °C in a growth chamber.

One hundred plants from each F_2 population also were screened separately for reaction to both races 3 and 5. Two, 7.5 cm-diam. styrofoam cups were tied together with masking tape and filled with steam-sterilized soil. Seedlings were grown in germination pouches for 10 days in the growth chamber at 27 °C. The main tap root of each seedling was excised, leaving several lateral roots. The remaining roots were divided in two parts planted in each of the adjoining styrofoam cups. One cup was infested with 1,000 eggs and J2 of race 3, and the adjoining cup was infested with 1,000 eggs and J2 of race 5. Ten checks also were provided in which both cups either were infested with the same race or one of the cups was not infested. These cups were placed in the growth chamber and maintained as described above. Seedlings were pruned at the V_2 stage to the unifoliate leaves to reduce top growth (2).

Thirty days after inoculation, roots were soaked in water, gently removed from soil, and washed with a high-pressure water stream to dislodge white females. In case of divided roots, each part was washed separately. White females were counted using a gridded glass petri plate under a dissecting microscope. The index of parasitism (IP) for each plant was calculated as (number of white females on a given plant per mean number of white females on susceptible-Essex) ×100. Plants with an IP $\geqslant 10\%$ or more were classified as susceptible, whereas plants with an IP < 10 were considered resistant (9). Based on the reaction of 25 plants in a family, each F_3 family was categorized as resistant, segregating, or susceptible. Segregating and susceptible families were grouped together for analysis. Chi-square analyses were used to test goodness of fit between observed and expected ratios based on segregation of genes in a given cross.

RESULTS

The reaction of the parental lines PI 90.763, PI 424.595, Essex, and F_1, F_2, and F_3 families of the cross PI 90.763 × Essex, PI 424.595 × Essex, and PI 90.763 × PI 424.595 to race 3 are presented in Table 1. In cross PI 90.763 × Essex, all 10 F_1 plants were susceptible to race 3. In the F_2, 92 plants were resistant and 358 plants were susceptible. These results closely fitted a ratio of 3 resistant:13 susceptible (P = 0.25–0.50), indicating that resistance in this cross was conditioned by one dominant and one recessive gene. The F_3 data confirmed the results of F_2 studies (χ^2 = 0.05, P = 0.75–0.90).

The F_1, F_2, and all F_3 progenies of cross PI 424.595 × Essex were susceptible, indicating no genetic interaction for resistance. In cross PI 90.763 × PI 424.595, the F_1 plants were resistant to race 3 and the F_2 population segregated 380 resistant:118 susceptible plants. This was in close agreement with the expected ratio of 3:1 (P = 0.50–0.75), which suggested a single dominant gene in PI 90.763 conditioning resistance to race 3. The F_3 data with 9 resistant and 41 susceptible or segregating progenies supported the expected ratio of 1:3.

The results of the divided-roots study in the F_2 population of the cross PI 90.763 × Essex indicated that (i) all six plants resistant to race 5 also were resistant to race 3, (ii) all 94 plants susceptible to race 5 were not all susceptible to race 3, and (iii) all plants susceptible to race 3 were also susceptible to race 5.

In cross PI 90.763 × PI 424.595, (i) all 85 plants resistant to race 5 were not all resistant to race 3, (ii) all 15 plants susceptible to race 5 were also susceptible to race 3, and (iii) all plants susceptible to race 3 were not all susceptible to race 5.

DISCUSSION

A previous study on the genetics of resistance to race 5 in PI 90.763 indicated that resistance was governed by two recessive genes and one dominant resistance gene, aa bb CC (1). In the present investigation, resistance to race 3 was conditioned by one dominant and one recessive gene. Since PI 90.763 is resistant to both race 3 and 5, it is postulated that the dominant gene CC and one of the recessive genes, aa or bb, confer resistance to race 3 as well. In the cross PI 90.763 × Essex, all F_2 plants that were resistant to race 5 would have the dominant and both the recessive resistance genes, a and b. Since only one of the re-

TABLE 1. Reaction of parents, F_1, F_2 plants, and F_3 families from three soybean crosses to *Heterodera glycines* race 3.

	Number of parents,[a] F_2 plants or F_3 families				
PI line or cross	Resistant	Segregating or susceptible	Expected ratio	Chi-square value	Probability range
Essex	0	50			
PI 90.763	50	0			
PI 424.595	0	50			
PI 90.763 × Essex (F_1)	0	10			
PI 90.763 × Essex (F_2)	92	358	3:13	0.85	0.25–0.50
PI 90.763 × Essex (F_3)	3	47	1:15	0.05	0.75–0.90
PI 424.595 × Essex (F_1)	0	10			
PI 424.595 × Essex (F_2)	0	350			
PI 424.595 × Essex (F_3)	0	50			
PI 90.763 × PI 424.595 (F_1)	10	0			
PI 90.763 × PI 424.595 (F_2)	380	118	3:1	0.45	0.50–0.75
PI 90.763 × PI 424.595 (F_3)	9	41	1:3	1.31	0.25–0.50

[a] Based on index of parasitism (number of white females on a given plant per mean number of white females on cv. Essex) × 100. Plants with IP < 10 were classified as resistant, and plants with IP ≥ 10 were classified as susceptible.

TABLE 2. Genotypic and phenotypic reaction of F_2 populations of two soybean crosses to *Heterodera glycines* races 3 and 5.

Cross-genotype	Genotype	Phenotype	
		Race 3	Race 5
PI 90.763 (aa bb CC DD)	aa B_ C_	Resistant	Susceptible
× Essex (AA BB cc DD)	aa bb C_	Resistant	Resistant
	All other combinations	Susceptible	Susceptible
PI 90.763 (aa bb CC DD)	aa bb cc D_	Susceptible	Susceptible
× PI 424.595 (aa bb cc dd)	aa bb cc dd	Susceptible	Resistant
	All other combinations	Resistant	Resistant

cessive genes is required for resistance to race 3, all race 5 resistant plants would be resistant to race 3 (Table 2). Similarly, all plants susceptible to race 3 also would be susceptible to race 5, whereas some of the plants susceptible to race 5 which are lacking one of the recessive resistance genes will be resistant to race 3. Thus, the results of the divided-root study confirmed that the dominant resistance gene and one of the recessive genes in PI 90.763 which control resistance to race 5 also were responsible for resistance to race 3.

The genes in cross PI 90.763 × PI 424.595 were described as aa bb CC DD and aa bb cc dd, respectively, for race 5 reaction (1). The dominant gene C in PI 90.763 provided resistance to both race 3 and 5, whereas the recessive gene dd in PI 424.595 conditioned resistance to race 5 only. The cross PI 90.763 × PI 424.595 segregated for a single dominant gene C for race 3 reaction as locus "D" is not functional. The divided-root study showed that all plants susceptible to race 5 (cc) also were susceptible to race 3, whereas some of the plants susceptible to race 3 (cc dd) were resistant to race 5. Similarly, all plants resistant to race 5 were not all resistant to race 3. These results explain the genetic makeup for resistance in PI 90.763 and PI 424.595, and describe genetic relationships for reaction to SCN races 3 and 5.

These findings are unique, because they describe for the first time in resistance to *H. glycines* a genetic system controlling resistance to more than one race. The C locus along with aa or bb gives resistance to race 3 only and to both race 3 and 5 when coupled with aa and bb loci in PI 90.763. The dd locus along with aa and bb condition resistance to race 5 only in PI 424.595. These results elucidate some of the complexities in understanding the inheritance of resistance to *H. glycines* in soybean.

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