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Genetic Diversity for Resistance to *Heterodera glycines* Race 5 in Soybean.[1]

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Abstract: *Heterodera glycines* is a serious pest of soybean in the United States. Plant introductions 90763 and 424595 are reported to be resistant to *H. glycines* race 5; however their genetic relationship for resistance is unknown. Crosses between these two lines and the susceptible cultivar Essex were studied in the F_1, F_2, and F_3 generations to determine the number of genes involved in inheritance of resistance. The plants were screened using conventional techniques based on the index of parasitism. The data were subjected to analyses using chi-square test to determine goodness of fit between observed and expected genetic ratios. The cross PI 424595 × Essex segregated 1 resistant:63 susceptible in the F_2 generation, which indicated the presence of three recessive genes controlling resistance to race 5. In the cross PI 90763 × Essex, resistance was conditioned by one dominant and two recessive genes. The cross between PI 424595 and PI 90763 segregated into 13 resistant:3 susceptible. The data fit a four-gene model with two recessive and two dominant genes with epistasis. PI 90763 has a dominant gene, whereas PI 424595 has a recessive gene; both share two additional recessive genes for resistance to race 5. This information is important to geneticists and soybean breeders for the development of cultivars resistant to *H. glycines*.

Key words: breeding, genetic diversity, *Glycine max*, *Heterodera glycines*, host-plant resistance, nematode, *soybean cyst nematode, soybean.*

Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is a serious pest of soybean, *Glycine max* L. Merr., in the United States. Soon after its discovery in 1954 (14), a search was made to find sources of resistance. This resulted in the identification of resistance in plant introduction lines 90763, 84751, 209332, and cvs. Ilsoy and Peking to field population of SCN in North Carolina (12). After the recognition of physiological races in SCN in 1970 (8), eight lines with a high level of resistance to SCN race 4 were reported (7). Anand and Gallo (3) and Anand et al. (4) screened approximately 10,000 plant introductions (PI) lines from the U.S. Soybean World Collection against homogeneous SCN populations and found 45 lines resistant or moderately resistant to race 3. Twelve lines had moderate or high levels of resistance to race 4, whereas eight were resistant to race 5. Recently, Young (15) screened soybean lines added to the collection since the earlier work by Anand and Gallo (3) and reported three more lines resistant to race 5.

Inheritance studies by Caldwell et al. (6) indicated the presence of three recessive genes in Peking that conditioned resistance to the North Carolina field population of SCN (6). An additional dominant gene for resistance was subsequently reported (10). Thomas et al. (13) reported that one dominant and two recessive genes controlled resistance to race 4 in certain crosses. Anand and Rao-Arelli (5) reported that resistance to race 5 in some PI lines was conditioned by alleles at the same loci, whereas in other PI lines resistance alleles were located at different loci. Myers and Anand (11) reported segregation ratios of two dominant and two recessive genes for race 5 resistance in the cross PI 437654 × Essex.

The objective of this research was to study the genetics and degree of similarity for resistance to *H. glycines* race 5 in PI 90763 and PI 424595.

MATERIALS AND METHODS

Crosses PI 90763 × Essex, PI 424595 × Essex, and PI 90763 × PI 424595 were made to generate F_1 seed at the Delta Center, University of Missouri, Portageville. PI

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90763 and PI 424595 are resistant to race 5 and Essex is susceptible (4,11,15). Some F_1 seeds were planted in Puerto Rico to develop an F_2 population. A random sample of the F_2 seed was grown at Portageville to produce F_3 progenies. Each cross included 10 plants of each of the parents and F_1's, 446 to 476 F_2 plants, and 25 plants from each of the 50 randomly selected families. In addition, the four SCN race differentials (8), 'Pickett,' Peking, PI 88788, and PI 90763, were included.

A homogeneous SCN race 5 was developed as outlined by Anand and Rao-Arelli (5). The steps for developing and maintaining this population included (i) collecting white females from the roots of PI 88788 soybean grown at the Rhodes Farm, University of Missouri, near Clarkton, MO, in a field primarily infested with SCN race 5, (ii) obtaining inoculum (eggs and juveniles) from crushed white females, (iii) growing PI 88788 seedlings in sterilized soil in the greenhouse and inoculating the roots, and (iv) collecting white females from the roots of inoculated PI 88788 plants after a period of 30 to 35 days. The cycle was repeated 10 times to obtain a homogeneous population of SCN race 5, which was maintained on the roots of PI 88788 under isolation in the greenhouse. The typical race 5 population produced an average of 200 white females on 'Lee 74,' 'Essex,' and PI 88788, 150 females on Pickett, 12 on Peking, and 1 to 3 on PI 90763.

Seedlings to be screened for resistance were germinated in vermiculite, and single seedlings with 2.4–3.5 cm radicles were transplanted to 20 × 2.5 cm plastic tubes filled with steam-sterilized Brosely fine sandy soil (loamy, mixed, thermic Arenic Hapludalf). Soil pH was adjusted to 6.5 by adding $CaCO_2$. White females collected from 30 to 35-day-old seedlings of PI 88788 were crushed to release eggs and juveniles just before inoculation. Each seedling was inoculated with a 5-ml water suspension containing approximately 1,000 eggs and juveniles 5 days after transplanting. Fifty tubes were placed in a 50-cm-d pot and kept at 27 C in a growth chamber. Seedlings were pruned at the V_2 stage to the unifoliates to reduce top growth (2). Thirty days after inoculation, plant roots were washed with a high-pressure water stream to dislodge white females (hereafter called cysts). Cysts 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 cysts on a given plant per mean number of cysts on susceptible cv. Essex × 100. Plants with an IP of 10% or more were classified as susceptible, whereas plants with an IP of less than 10 were considered resistant (8). Based on the reaction of 25 plants in a family, each F_3 family was categorized as resistant, segregating, or susceptible. For analysis, segregating and susceptible families were grouped together. Chi-square (χ^2) analyses was 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 424595, PI 90763, Essex, and of F_1, F_2, and F_3 populations of the crosses PI 424595 × Essex, PI 90763 × Essex, and PI 90763 × PI 424595 are given in Table 1. The cross PI 424595 × Essex segregated six resistant and 470 susceptible plants in the F_2 population, which closely fitted a ratio of one resistant:63 susceptible (P = 0.5–0.75). This indicated that resistance to race 5 in this cross was conditioned by three recessive genes. The F_3 results confirmed the results of F_2 studies, with the presence of 1 resistant and 49 susceptible or segregating families, which was in good agreement with the expected 1:63 (χ^2 = 0.063, P = 0.75–0.90).

The cross Pi 90763 × Essex gave 17 resistant and 439 susceptible plants in the F_2 generation. The data fitted a ratio of three resistant:61 susceptible (P = 0.25–0.50), indicating that resistance to race 5 in this cross was controlled by one dominant and two recessive genes. In F_3, all 50 families were susceptible or segregating, which was close to the expected ratio of 1:6 (P = 0.25–0.50).

TABLE 1. Reaction of parents F_1, F_2 plants, and F_3 families from crosses to *Heterodera glycines* race 5.

	Number of parents, F_2† plants or F_3 families				
	Resistant	Segregating or susceptible	Expected ratio	Chi-square value	Probability range
Essex		50			
PI 424595	50	0			
PI 90763	50	0			
PI 424595 × Essex (F_1)	0	10			
PI 424595 × Essex (F_2)	6	470	1:63	0.28	0.50–0.75
PI 424595 × Essex (F_3)	1	49	1:63	0.06	0.75–0.90
PI 90763 × Essex (F_1)	0	10			
PI 90763 × Essex (F_2)	17	439	3:61	0.94	0.25–0.50
PI 90763 × Essex (F_3)	0	50	1:63	0.79	0.25–0.50
PI 90763 × PI 424595 (F_1)	10	0			
PI 90763 × PI 424595 (F_2)	358	88	13:3	0.28	0.50–0.75
PI 90763 × PI 424595 (F_3)	18	32	7:9	1.22	0.25–0.50

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

The cross between PI 90763 and PI 424595, both resistant, segregated into 358 resistant and 98 susceptible plants in the F_2 generation. This was a close fit to the expected ratio of 13 resistant:3 susceptible (P = 0.5–0.75), indicating the presence of one dominant and one recessive gene. The F_3 data of 17 resistant and 33 segregating or susceptible families were consistent with the F_2 results.

DISCUSSION

Genetics of resistance to *H. glycines* in soybean is complex and is controlled by multiple genes (6,13). Both dominant and recessive genes are reported to condition resistance to race 5 (11), and in some PI lines resistance alleles are located at different loci (5). In this study, crosses between PI 90763 and PI 424595 with Essex had a different segregation pattern. This indicated that resistance to SCN in these two lines was conditioned by different genes. Symbolically the genotype of the parental lines in the cross PI 90763 × Essex with two recessive and one dominant genes could be described as r_ar_a r_br_b R_cR_c for PI 90763, and R_aR_a R_bR_b r_cr_c for Essex. Both recessive r_ar_a and r_br_b, along with R_c–, are required for resistance. In the cross PI 424595 × Essex, which segregated for three recessive genes, the genotype of PI 424595 could be designated as r_ar_a r_br_b r_dr_d, whereas Essex genotype would be R_aR_a R_bR_b R_dR_d. All three recessive genes, r_ar_a, r_br_b, and r_dr_d, are required for resistance. The d locus in PI 90763 would be R_dR_d, whereas the c locus in PI 424595 would be r_cr_c. The requirement for resistance would be either the dominant R_c locus as in PI 90763, or the recessive r_dr_d as in PI 424595. Thus, the cross of PI 90763 with a genotype of r_ar_a r_br_b R_cR_c R_dR_d and PI 424595 with a genotype of r_ar_a r_br_b r_cr_a r_dr_d will segregate 13 resistant:3 susceptible in the F_2 generation. The susceptible plants would occur with a genotypic frequency of 1/16 r_ar_a r_br_b r_cr_c R_dR_d and 2/16 r_ar_a r_br_b r_cr_c R_dr_d. The genotypes of the three soybean lines and their crossess could be summarized as follows:

Cross/genotype	Gene action
PI 90763 × Essex r_ar_a r_br_b R_cR_c R_dR_d × R_aR_a R_bR_b r_cr_c R_dR_d	R_c- along with r_ar_a and r_br_b are resistant.
PI 424595 × Essex/ r_ar_a r_br_b r_cr_c r_dr_d × R_aR_a R_bR_b r_cr_c R_dR_d	r_dr_d along with r_ar_a and r_br_b are resistant.
PI 90763 × PI 424595/r_ar_a r_br_b R_cR_c R_dR_d × r_ar_a r_br_b r_cr_c r_dr_d	r_cr_c R_d- are susceptible; all other combinations are resistant.

In the present study involving three crosses, the data fitted a four-gene model with two recessive and two dominant genes in epistasis. In an earlier study we observed two dominant genes, one each in PI 90763 and Peking, controlling resistance to SCN race 5 (5). On the other hand, PI 437654 was found to have two dominant and two recessive genes (11). Our present study indicates that although PI 424595 and PI 90763 share several genes for SCN resistance, there is an additional recessive gene for race 5 resistance present in PI 424595. Thus, PI 424595 could provide greater genetic diversity for resistance, which would be useful in resistant cultivar development.

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