SOYBEAN CYST NEMATODE, *HETERODERA GLYCINES*, RESISTANCE GENES IN PI 89772 AND PI 209332 SOYBEAN

M. S. Assunção[1], N. Atibalentja[2], and G. R. Noel[3]

Soybean Breeder, EMPRAPA, Goainia, Goias, Brazil[1], Senior Research Specialist, Department of Crop Sciences, University of Illinois, Urbana, IL 61801, U.S.A.[2], and Research Plant Pathologist, USDA, ARS and Professor of Nematology, Department of Crop Sciences, University of Illinois, Urbana, IL 61801, U.S.A., E-mail: g-noel1@uiuc.edu.[3] A portion of a Ph.D. dissertation by the first author.

ABSTRACT

M. S. Assunção, N. Atibalentja and G. R. Noel. 2004. Soybean cyst nematode, *Heterodera glycines*, resistance genes in PI 89772 and PI 209332 soybean. Nematropica 34:165-181.

The number of resistance genes in PI 89772 and PI 209332 conferring resistance to *H. glycines* race 3 is not well defined. Crosses of PI 89772 × 'Lee 68', PI 88788 × PI 89772, and 'Lee 68' × PI 209332 were made in the field and greenhouse. Several F_1 and F_2 families from each cross, 98 F_3 families from cross PI 89772 × 'Lee 68', 74 F_3 families from cross PI 88788 × PI 89772, and 80 F_3 families from cross 'Lee 68' × PI 209332 were tested with an inbred line of *H. glycines* developed on PI 88788 to determine the level and inheritance of resistance. Approximately 8,000 individual plants growing in pots containing 200 cm^3 of sterilized sand were inoculated with 4,000 eggs and J2/pot. Thirty days after inoculation the number of females that developed on each plant was determined. Cluster analysis revealed sets of families with a low mean number of females and low variance, intermediate means and high variance, and high means with a low variance, indicating F_3 plants came from, respectively, homozygous resistant, heterozygous or segregating, and homozygous susceptible F_2 plants. Thus, resistance classes were considered as quantitative parameters having different levels of resistance as opposed to only two classes, either resistant or susceptible. Chi-square analysis of segregation of phenotypic data indicated two genes confer resistance to race 3 of *H. glycines*. The three *H. glycines*-resistant parents have at least two genes that express resistance to *H. glycines*. One gene acts as a major gene (Rhg_x) and the other a minor gene (Rhg_y) in conferring resistance of the parents PI 89772 ($Rhg_{x1?}Rhg_{x1?}Rhg_{y1?}Rhg_{y1?}$), PI 88788 ($Rhg_{x2?}Rhg_{x2?}Rhg_{y2?}Rhg_{y2?}$), and PI 209332 ($Rhg_{x3?}Rhg_{x3?}Rhg_{y3?}Rhg_{y3?}$) to *H. glycines* race 3. The same genes may occur in PI 209332 as in PI 89772, but support for this hypothesis must be obtained by studying the cross PI 209332 × PI 89772. The same major (Rhg_x) and minor (Rhg_y) genes occur in PI 89772 ($Rhg_{x1?}Rhg_{x1?}Rhg_{y1?}Rhg_{y1?}$) and PI 88788 ($Rhg_{x2?}Rhg_{x2?}Rhg_{y2?}Rhg_{y2?}$). The phenotypic ratios obtained in this research indicate that epistasis occurs between gene Rhg_x and gene Rhg_y. Results from this analysis indicated that the sensitivity of resistant genotypes to the environment is different from that of susceptible genotypes. In addition, a maternal effect was found for the inheritance of resistance of PI 88788 to *H. glycines* race 3, but not for PI 89772.

Key words: environmental effects, epistasis, genetics, *Glycine max*, *Heterodera glycines*, maternal inheritance, resistance, resistance genes, soybean, soybean cyst nematode.

RESUMO

Assunção, M. S., N. Atibalentja e G. R. Noel. 2004. Estudo de genes na PI 89772 e PI 209332 visando resistência ao nematóide de cisto da soja, *Heterodera glycines*. Nematropica 34:165-181.

O número de genes de resistência nas PI 89772 e PI 209332 que confere resistência á *H. glycines* raça 3 ainda não está bem definido. Foram feitos cruzamentos das PI 89772 × 'Lee 68', PI 88788 × PI 89772, e 'Lee 68' × PI 209332 em casa de vegetação e em campo. Várias famílias F_1 e F_2 de cada cruzamento, 98 famílias F_3 do cruzamento PI 89772 × 'Lee 68', 74 famílias F_3 do cruzamento PI 88788 × PI 89772 e 80 famílias F_3 do cruzamento 'Lee 68' × PI 209332 foram testadas com uma linhagem de *H. glycines* desenvolvida na PI 88788. Aproximadamente 8.000 plantas individuais desenvolvidas em

vasos de cerâmica contendo 200 cm^3 de areia esterilizada foram inoculados com 4.000 ovos e J2/vaso. Foi determinado o número de fêmeas que desenvolveram em cada planta aos 30 dias após a inoculação. Análise estatística de agrupamento (Cluster Analysis) revelou grupos de famílias com baixo número médio de fêmeas e com baixa variância, intermediário número de fêmeas e altas variâncias e alto número médio de fêmeas com baixa variância, indicando que plantas F_3 vieram, respectivamente, de plantas homozigotas resistentes, heterozigotas ou segregando e homozigotas susceptíveis. Portanto, as classes de resistência foram consideradas parâmetros quantitativos tendo diferentes níveis de resistência, opondo-se à tradicional denominação de duas classes resistente ou susceptível. Teste de Chi-quadrado dos dados da segregação fenotípica indicaram que dois genes conferem resistência à raça 3 de *H. glycines*. Os três parentais com resistência *H. glycines* têm pelo menos dois genes que expressam resistência a esta raça de *H. glycines*. Um gene age como gene principal (Rhg_x) e outro de menor efeito (Rhg_y) conferindo resistência a *H. glycines* raça 3 nos parentais PI 89772 ($Rhg_{x1?}Rhg_{x1?}Rhg_{y1?}Rhg_{y1?}$), PI 88788 ($Rhg_{x2?}Rhg_{x2?}Rhg_{y2?}Rhg_{y2?}$), e PI 209332 ($Rhg_{x3?}Rhg_{x3?}Rhg_{y3?}Rhg_{y3?}$). Pode ser que os mesmos genes que ocorrem na PI 209332 também ocorra na PI 89772, entretanto para confirmação desta hipótese, deve ser estudado o cruzamento PI 209332 × PI 89772. Os mesmos genes principais (Rhg_x) e os de menor efeito (Rhg_y) ocorrem nas PI 89772 ($Rhg_{x1?}Rhg_{x1?}Rhg_{y1?}Rhg_{y1?}$) e PI 88788 ($Rhg_{x2?}Rhg_{x2?}Rhg_{y2?}Rhg_{y2?}$). A taxa fenotípica obtida no presente trabalho indica que ocorre epistase entre os gene Rhg_x e o gene Rhg_y. Os resultados desta análise indicaram que a sensibilidade de genótipos resistentes ao ambiente é diferente aos genótipos susceptíveis. Adicionalmente, observouse que ocorreu um efeito materno para a herança da resistência na PI 88788 a *H. glycines* raça 3, mas não para a PI 89772.

Palavras chaves: efeito ambiental, epistase, gene de resistência, genética, *Glycine max*, herança maternal, *Heterodera glycines*, nematóide de cisto da soja, resistência, soja.

INTRODUCTION

Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is the most serious disease of soybean, *Glycine max* (L.) Merr., in the United States and also is a serious pest of soybean in Argentina and Brazil, two other major soybean producing countries. A study of soybean yield loss in the North Central United States from 1989 to 1991 showed *H. glycines* caused an annual loss of 1.33 million t (Doupnik, 1993). From 1996 to 2000 estimated crop loss in the United States caused by *H. glycines* ranged from 3.86 million to 7.59 million t (Wrather, 2002). If the price of soybean is $5.00 per bushel (60 lbs. Avdp.) this crop loss would be $700 million to $1.4 billion/year.

Planting resistant cultivars to control *H. glycines* is used widely, and finding durable resistance remains a top research priority. Since the 1960s, researchers have studied cultivars and plant introductions (PI) resistant to *H. glycines* to determine the inheritance of resistance, which would provide a better understanding of how *H. glycines*-resistant soybean expresses different levels of resistance to various populations of *H. glycines*. The first of these studies reported resistance to *H. glycines* is controlled by three independent recessive genes known as rhg_1, rhg_2, and rhg_3 (Caldwell *et al.*, 1960). Another study determined the dominant gene Rhg_4, which also is linked to the *i* recessive allele that codes for black seed coat in Peking, provides a high level of resistance to races 1 and 3 of *H. glycines* (Matson and Williams, 1965). Other studies have demonstrated that certain lines have the same resistance genes to some populations of *H. glycines*. For example, PI 88788 and PI 416762 are resistant to races 3 and 14 (Anand, 1982), and

Peking and PI 438489B share the same genes for resistance to *H. glycines* race 3 (Rao-Arelli and Anand, 1988). Hancock *et al.* (1985) reported PI 88788 has a dominant gene controlling resistance to race 14. A single dominant (Rc_1) and two recessive (rc_1, rc_3) genes were reported to confer resistance to *H. glycines* race 14 (Myers and Anand, 1991). A study that evaluated crosses among PI 88788, PI 90763, Peking and the susceptible cv. Essex, indicated at least four genes, two dominant and two recessive, function in the resistance to *H. glycines* race 3 (Rao-Arelli *et al.*, 1992). The genetic pattern of resistance to *H. glycines* race 5 found in cv. Peking and PI 90763 is conditioned by two recessive genes rb_1 and rb_2 (Anand and Rao-Arelli, 1989). The *H. glycines*-resistant soybeans PI 424595, PI 438342, PI 90763, and PI 437654 have the same group of genes for resistance to race 5 (Young and Kileen, 1994). Results thus far indicate there are three dominant resistant genes (Rc_1, Rc_2, Rc_3) in PI 437654 (Myers and Anand, 1991).

After the discovery of variability in *H. glycines* populations, researchers realized resistance to *H. glycines* acted in a race specific manner. However, classification of the parasitic ability of populations (races and Hg types) has not proven completely satisfactory (Golden *et al.*, 1970; Niblack *et al.*, 2002; Riggs and Schmitt, 1988; Riggs *et al.*, 1988). The distinction between resistance and susceptibility is not always clear due to several factors including: a) different gene frequencies for parasitism in different *H. glycines* populations of the same race; b) different levels of inoculum used in experiments; c) different experimental conditions; d) genetic variability within the differential lines used by researchers; and e) the use of different criteria when evaluating resistance and susceptibility.

In most of the studies concerning *H. glycines* resistance, researchers worked with heterogeneous field populations. However, Faghihi *et al.* (1995) used an inbred *H. glycines* race 3 population to study resistance genes in cv. Hartwig (PI 437654 source of resistance). They reported that resistance in cv. Hartwig is controlled by one dominant and one recessive gene.

At present there are 700 cultivars marketed in the U.S. as being resistant to *H. glycines* (Shier, 2004). Most of the resistance for *H. glycines* in soybean cultivars available at present comes from a few resistant plant introductions (Diers *et al.*, 1997; Dong *et al.*, 1997). In the central United States, greater than 95% of the public and private varieties resistant to SCN derived their resistance from Fayette (PI 88788 source of resistance; Bernard *et al.*, 1988). A search for new sources of resistance genes and alleles is important to increase the gene pool of resistance to *H. glycines* in commercial cultivars. *Heterodera glycines* exists in heterogeneous field populations, and it is important to identify new sources of resistance to control *H. glycines* in fields with populations having different parasitic abilities. Efficacy of gene deployment as a management tactic requires knowledge of nematode parasitic ability and also the resistance genes available (Noel and Edwards, 1996). Thus, a clear understanding of the number of resistant gene(s) and their interaction(s) is highly desirable. Genetic variability of resistance in soybean to *H. glycines* also plays an important role in providing yield stability against the large spectrum of variability encountered in field populations of *H.* glycines. Since resistance to *H. glycines* race 3 in PI 89772 and PI 209332 is not well defined (Rao-Arelli, 1994), the objectives of this research were: (i) to develop a genetic model for the number of genes that confer resistance in PI 89772 and PI 209332; and (ii) to determine whether PI 89772 and PI 88788 have resistance genes in common.

MATERIALS AND METHODS

The research reported herein was accomplished by evaluating parents, F_1, F_2 and F_3 generations from three crosses, PI 89772 × 'Lee 68', PI 88788 × PI 89772, and 'Lee 68' × PI 209332, against an inbred population of *H. glycines* developed on PI 88788. Both PI 89772 and PI 88788 are considered resistant and PI 209332 moderately resistant to the *H. glycines* inbred population utilized in this research (Schmitt and Shannon, 1992). 'Lee 68' was used for all crosses, but sufficient seed could not be obtained to complete the research. 'Lee 74' was used as the susceptible standard for all evaluations of parasitism and did not differ in it's susceptibility to the *H. glycines* population used in this study (data not shown). Due to the destructive nature of the screening process, the F_2 plants evaluated could not generate F_3 families. Therefore, plants of $F_{2:3}$ families (F_2 derived F_3 families) evaluated against *H. glycines* were obtained from a random sample of F_2 seeds.

Development of Crosses, F_1, and Segregating Progenies

The crosses PI 89772 × 'Lee 68', PI 88788 × PI 89772, and 'Lee 68' × PI 209332 were made in May 1998 in the greenhouse. The reciprocal crosses 'Lee 68' × PI 89772 and PI 89772 × PI 88788, were made to determine possible maternal effects. Insufficient seed of the PI 209332 × 'Lee 68' were obtained. The same crosses also were made in the field during the 1998 growing season. Seeds from F_1 plants were planted in the greenhouse and advanced to F_2 in the winter of 1998/99.

The F_1 plants were threshed individually and approximately 400 F_2 seeds were harvested from each cross. A portion of the F_2 seeds was saved for evaluation against *H. glycines*, and the remaining planted and advanced to the F_3 in the spring of 1999. Each F_2 generation was derived from a single F_1 plant. A total of 150 F_2 plants were grown from the crosses PI 89772 × 'Lee 68', PI 88788 × PI 89772, and 'Lee 68' × PI 209332. Randomly selected plants were obtained from F_2 plots to produce $F_{2:3}$ families for each cross. The F_2 plants from the three crosses, PI 89772 × 'Lee 68', PI 88788 × PI 89772, and 'Lee 68' × PI 209332, were harvested and threshed individually resulting in 98, 74, and 80 F_3 families, respectively.

Verification of F_1 Hybrids

Flower color (when available) was used to verify that an F_1 plant came from a cross and not a selfed plant. 'Lee 68', PI 89772, and PI 209332 have purple flowers, and PI 88788 has white flowers. Therefore, flower color was utilized in PI 89772 × PI 88788 cross, which segregated for flower color in the F_2 and F_3 generations.

The simple sequence repeat (SSR) marker BARC-Satt 307 (Cregan *et al.*, 1999a) was used to confirm that F_1 plants were heterozygous plants instead of homozygous selfed plants. The hybrids analyzed were: PI 88788 × PI 89772; PI 89772 × PI 88788; 'Lee 68' × PI 209332; PI 89772 × 'Lee 68'; 'Lee 68' × PI 89772; PI 209332, PI 89772, PI 88788, and 'Lee 74'. Soybean DNA was extracted from small trifoliates following a modified CTAB method (Kisha *et al.*, 1997). The SSR marker analysis was done using the primer Satt 307, obtained from Research Genetics (Huntsville, Alabama). Amplification of DNA was done according to published procedures (Cregan and Quigley, 1997), and PCR products were separated in 3% metaphor agarose gel (FMC-Bio Products, Rockland, Maine).

Development of the Inbred Nematode Line and Inoculum

An inbred population of *H. glycines* was developed on PI 88788 by sib matings (P.

Esbenshade, pers. comm.). A cyst was crushed and eggs and J2 were inoculated onto a PI 88788 root system. This plant was transferred to hydroponic culture where males could be collected from the bottom of the container. Upon development of immature females on the roots, the roots were excised so that one female was on each root piece. The root piece with one female was transferred onto agar in a petri plate. A male collected from the bottom of the hydroponic solution was placed next to the female, and the nematodes were allowed to mate. The female was then added to a plant growing in fine sand. Infection by second-stage juveniles (J2) was allowed to occur for several days, and then plants were placed in the hydroponic culture. This procedure was repeated for approximately 12 generations, producing a highly inbred line of *H. glycines*.

The inbred *H. glycines* population was maintained on 'Lee 74' soybean. Females were extracted by gravity screening using nested 850-µm and 180-µm-pore sieves (Cobb, 1918) followed by a modified (50% sucrose) centrifugal flotation technique (Jenkins, 1964), crushed, and eggs and J2 were added to 'Lee 74' soybean. Approximately 30 days after inoculation, females were harvested as described above, and the procedure repeated as needed to ensure fresh inoculum of eggs and J2 during the evaluation phase.

Phenotype Evaluations and Genetic Study

The phenotypic evaluation for *H. glycines* was made on parents, F_1, F_2 and F_3 generations of the three crosses, PI 89772 × 'Lee 68', PI 88788 × PI 89772, and 'Lee 68' × PI 209332. The evaluation for *H. glycines* resistance was conducted in the greenhouse, from October 1999 to May 2000. Soil temperatures ranged from 24 to 28°C. The large number of plants evaluated (ca. 8,000), precluded planting of soybean and processing of *H. glycines* females at the same time. Therefore, the parents, F_1, F_2 and F_3 plants were divided into sets containing 300-550 plants depending on the generation and cross evaluated.

PI 89772, PI 88788, and PI 209332, and the F_1 hybrids originating from crosses among these PIs as female parents, have a black seed coat. Some segregating progenies originated from the cross PI 89772 × 'Lee 68' also had black seed coats. All seeds with a black seed coat were scarified prior to germination. Seeds were germinated on paper towels for 48 hours at room temperature, and then one seedling was transplanted into a 7 cm-diameter clay pot containing 200 cm^3 of sterilized sand. Each of the three crosses, parents and F_1 hybrids were evaluated in the same set of plants, and the F_2 and F_3 progenies were evaluated sequentially in sets. Controls during each evaluation consisted of one set of seven replications of the four differential lines ('Peking', 'Pickett 71', PI 88788, PI90763, (Golden *et al.*, 1970)), the susceptible 'Lee 74', and resistant parents PI 89772 and PI 209332. These lines were evaluated to monitor the reproductive index of the nematode population and also to serve as the "anchor" for the cluster analysis. Approximately 10 days after transplanting, pots were infested with 4010 ± 68 eggs and J2/pot. The number of females on each plant was determined 30 days after inoculation.

Females were extracted using gravity sieving (Cobb, 1918). All females from each plant were counted using a stereoscopic microscope, and an index of parasitism (IP) was calculated where:

IP = (number of females from candidate plant/number of females from 'Lee 74') × 100.

Data Analysis

For the genetic study, attempts were made to classify F_3 families and parental genotypes into discrete groups of reaction to *H. glycines* based on the mean number of females per plant. Since discrete classification based on a quantitative variable such as the number of females per plant can be highly subjective, cluster analysis was done to provide an objective partitioning of the data (Johnson and Wichern, 1998). In the present study, segregation of F_2 populations was investigated using cluster analysis (SAS, Inc., Cary, NC) based on means and standard errors of the mean of F_2-derived F_3 families, rather than on single F_2 plants (Sebastian and Nickell, 1985). Therefore, to define the ratio of homozygous or heterozygous F_2 plants, 20 F_3 plants within each family of the three crosses (98 F_3 families from cross PI 89772 × 'Lee 68', 74 F_3 families from cross PI 88788 × PI 89772, and 80 F_3 families from cross 'Lee 68' × PI 209332) were planted. The means for number of females that developed for each F_3 family in each of the three crosses was partitioned into classes to determine the genotype of the F_2 plant produced by that family. Thus, it was possible to determine whether the F_3 families were derived either from heterozygous or homozygous F_2 plants.

The component of variance due to environment was estimated through the variance of the parents and F_1 hybrids. To estimate this variance, seven replications of the parents, and at least nine replications for each F_1 hybrid, were evaluated in each set of an experiment for a total of 10 sets for crosses PI 89772 × 'Lee 68', PI 88788 × PI 89772, and 'Lee 68' × PI 209332, and their progenies. Since there was only one replicate for each F_2 plant, it was not possible to distinguish with accuracy the homozygous and heterozygous genotypes. The assumptions made for the present research are based on F_3 families with low mean and low variance, intermediate mean and high variance, and high mean with low variance, indicating that the F_3 plants came from, respectively, homozygous resistant, heterozygous or segregating, and homozygous susceptible F_2 plants.

RESULTS AND DISCUSSION

The *H. glycines* inbred used in this study was identified as race 3 using the host differentials 'Peking', 'Pickett 71', PI 88788, and PI 90763, where the IPs were, respectively, 0 ± 0, 0 ± 0, 5.6 ± 0.9, and 0 ± 0 (Riggs and Schmitt, 1988). This population was able to develop on PI 209332 (IP = 17.5 ± 5.1), which was reported as resistant to race 3 (Diers *et al.* 1997), but did not develop on PI 89772 (IP = 0 ± 0). Thus, the population also was classified as HG Type 5 (Niblack *et al.*, 2002). Based on the scheme of Schmitt and Shannon (1992), PI 209332 was classified as moderately resistant to this inbred population of *H. glycines*. The population of *H. glycines* was highly virulent on 'Lee 74' as shown by the high level of development ($\bar{x}$ = 577 ± 20 females/plant) when inoculated with 4,010 eggs and J2.

The F_2 populations for the three crosses (PI 89772 × 'Lee 68', PI 88788 × PI 89772, and 'Lee 68' × PI 209332) studied in the present research exhibited a wide range of reactions to the inbred population of *H. glycines* race 3. A two-gene model of one major gene and one minor gene was hypothesized to explain the expression of resistance in PI 89772, PI 88788, and PI 209332 as illustrated in Table 1. Whether the loci and alleles proposed in this study are the same as reported previously is not known. Molecular markers have been developed to identify quantitative trait loci conferring resistance to some races of *H. glycines* (Concibido *et al.*, 1997, Webb *et al.*, 1995; Yue *et al.*, 2001). The *rhg1* locus, which confers resistance to *H. glycines* race 3,

Table 1. Model for F_2 segregation and F_3 family means for resistance to *Heterodera glycines* race 3.[z]

F_2 Genotype	Frequency	Number of females/F_2 plant	F_3 family mean number of females	Variance of F_3 family mean number of females
$Rhg_xRhg_xRhg_yRhg_y$	1/16	low	low	low
$Rhg_xRhg_xRhg_yrhg_y$	2/16	low	low	low
$Rhg_xRhg_xrhg_yrhg_y$	1/16	intermediate	intermediate	low
$Rhg_xrhg_xRhg_yRhg_y$	2/16	intermediate	intermediate	high
$Rhg_xrhg_xRhg_yrhg_y$	4/16	intermediate	intermediate	high
$Rhg_xrhg_xrhg_yrhg_y$	2/16	intermediate	intermediate	high
$rhg_xrhg_xRhg_yRhg_y$	1/16	intermediate	intermediate	low
$rhg_xrhg_xRhg_yrhg_y$	2/16	high	high	low
$rhg_xrhg_xrhg_yrhg_y$	1/16	high	high	low

[z]Adapted from Sebastian and Nickell (1985). The model assumes the genotypes are $Rhg_xRhg_xRhg_yRhg_y$ and $rhg_xrhg_xrhg_yrhg_y$, respectively, for the resistant and the susceptible parents.

on linkage group G has been confirmed in PI 88788 and PI 209332 used in this study, and the allele for partial resistance controlled 36% and 50% of the variation in PI 88788 and PI 209332, respectively (Caldwell *et al.*, 1960; Concibido *et al.*, 1997; Cregan *et al.*, 1999b). Studies also have identified other putative resistance loci on other linkage groups (Concibido *et al.*, 1994; Concibido *et al.*, 1996). However, data necessary to determine whether the gene in PI 89772 is *rhg1* was not conclusive (Yue *et al.*, 2001). Additionally, Yue *et al.* (2001) did not find the *Rhg4* locus on linkage group A2 in PI 89772 (Matthews *et al.*, 1998; Weisemann *et al.*, 1992), and concluded resistance at *Rhg4* was not required for race 3 or had a small, undetected effect. Therefore, symbols for *H. glycines* resistance genes are used but the genes are given the temporary labels $Rhg_{x?}$ or $Rhg_{y?}$ (Anon., 1997). The model assumes the genotypes of parents are $rhg_{x0?}rhg_{x0?}rhg_{y0?}rhg_{y0?}$, $Rhg_{x1?}Rhg_{x1?}Rhg_{y1?}Rhg_{y1?}$, $Rhg_{x2?}Rhg_{x2?}Rhg_{y2?}Rhg_{y2?}$, and $Rhg_{x3?}Rhg_{x3?}Rhg_{y3?}Rhg_{y3?}$, respectively, for 'Lee 68', PI 89772, PI 88788, and PI 209332, with $Rhg_{x1?}$ dominant over $rhg_{x0?}$ and $Rhg_{y1?}$ dominant over $rhg_{y0?}$, $Rhg_{x1?}$ dominant over $Rhg_{x2?}$ and $Rhg_{y2?}$ dominant over $Rhg_{y1?}$, $Rhg_{x3?}$ dominant over $rhg_{x0?}$, and $Rhg_{y3?}$ dominant over $rhg_{y0?}$.

Verification of F_1 Hybrid Plants

Segregation for flower color was observed in the F_2 progenies for the cross PI 89772 × PI 88788, thus, confirming this population resulted from a successful cross. However, as expected, segregation of flower color was not present in the crosses PI 89772 × 'Lee 68' and 'Lee 68' × PI 209332.

The electrophoretogram (data not shown) using the marker BARC-Satt 307 showed one band for the parents PI 209332, PI 89772, PI 88788, and 'Lee 68', and for the F_1 hybrid PI 88788 × PI 89772. In contrast, two bands indicating successful crosses were observed for F_1 hybrids 'Lee 68' × PI 209332 and PI 89772 × 'Lee 68'.

Another indication that F_2 progenies came from a hybrid F_1 instead of a selfed plant was the wide segregation of reaction to the *H. glycines* race 3 population obtained in the F_2 generations in the three

crosses (Figs. 2A, 4A, and 6A). Since the F_2 progenies represented a wide range of variability to *H. glycines*, the F_1 plants resulted from a successful crossing.

Evaluation of Cross PI 89772 × 'Lee 68'

The mean number of females for the F_1 hybrid PI 89772 × 'Lee 68' was 318.0 ± 58.7 (IP = 55.1 ± 10.8), and for the reciprocal hybrid, 'Lee 68' × PI 89772, the number of females was 240.2 ± 34.6 (IP = 41.6 ± 6.0). These two means did not differ (t = 1.10; P = 0.29), indicating no maternal effect in the inheritance of resistance in PI 89772 to *H. glycines* race 3. Therefore, an overall mean number of females, 281.4 ± 35.4 (IP = 48.7 ± 6.4), was calculated from all the 17 F_1 plants involved in the cross PI 89772 × 'Lee 68'. This number of females was intermediate to both parents (Fig. 1).

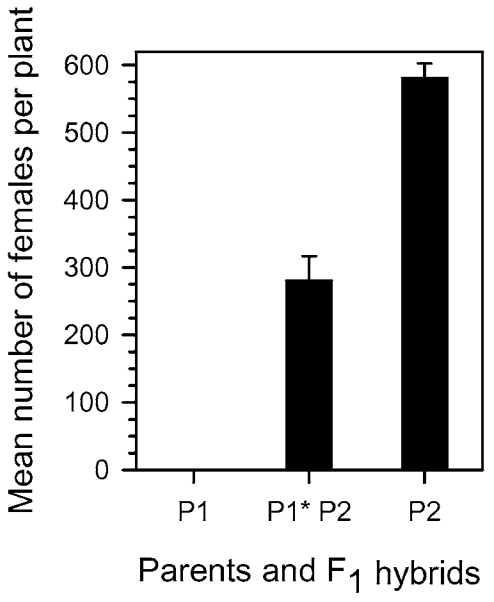

Fig. 1. Mean number of females per plant for parents PI 89772 (P1) and Lee 68 (P2), respectively, resistant and susceptible to *Heterodera glycines* race 3, and for the F_1 hybrid PI 89772 × Lee 68. Bars indicate standard error of the mean.

The number of females that developed on the 222 F_2 progenies ranged from 0 to 678, with a frequency distribution (Fig. 2A) not normally distributed as expected (P = 0.0001). The frequency distribution of 98 F_3 families, and the mean number of females and IPs for these families are presented in Figure 2B and Table 2, respectively. In both generations, a wide range of nematode development was observed. The initial cluster analysis revealed that the 98 F_3 families could be partitioned into two classes. The first class included 89 F_3 families with IPs ranging from 0 to 60, and the second class was comprised of nine F_3 families with IPs greater than 60. This partition fits a 15:1 ratio based on F_3 family means and not individual F_3 plants (χ^2 = 1.44; P = 0.23; R^2 = 0.32), which supported a two-gene model. The 15:1 ratio was also supported (χ^2 = 0.64; P = 0.42; R^2 = 0.75) by cluster analysis of the 222 F_2 plants, which formed one group of 211 plants with IPs ranging from 0 to 90, and another group of 11 plants with IPs greater than 90. However, cluster analysis was used to further partition the 89 F_3 families of the "15 class" into three classes as follows: 7 F_3 families with 0 ≤ IP ≤ 10, 14 F_3 families with 10 ≤ IP < 30 and 68 F_3 families with 30 ≤ IP ≤ 60. Consequently the 98 F_3 families from the cross PI 89772 × 'Lee 68' were grouped into four classes of reaction to *H. glycines* race 3. These four classes as shown in Table 2 are referred herein as resistant (R): 0 ≤ IP < 10; moderately resistant (MR): 10 ≤ IP < 30; moderately susceptible (MS): 30 < IP ≤ 60; and susceptible (S): IP > 60) (Schmitt and Shannon, 1992). The later partition, which accounted for most of the variability in F_3 families (R^2 = 0.88), fits a 12:2:1:1 segregation ratio (Table 5) and also suggests a model of two resistance genes ($Rhg_{x1?}$ and $Rhg_{y1?}$) from PI 89772. The IPs resulting from the cross PI 89772 × 'Lee 68' supported the proposal for ranking resistance

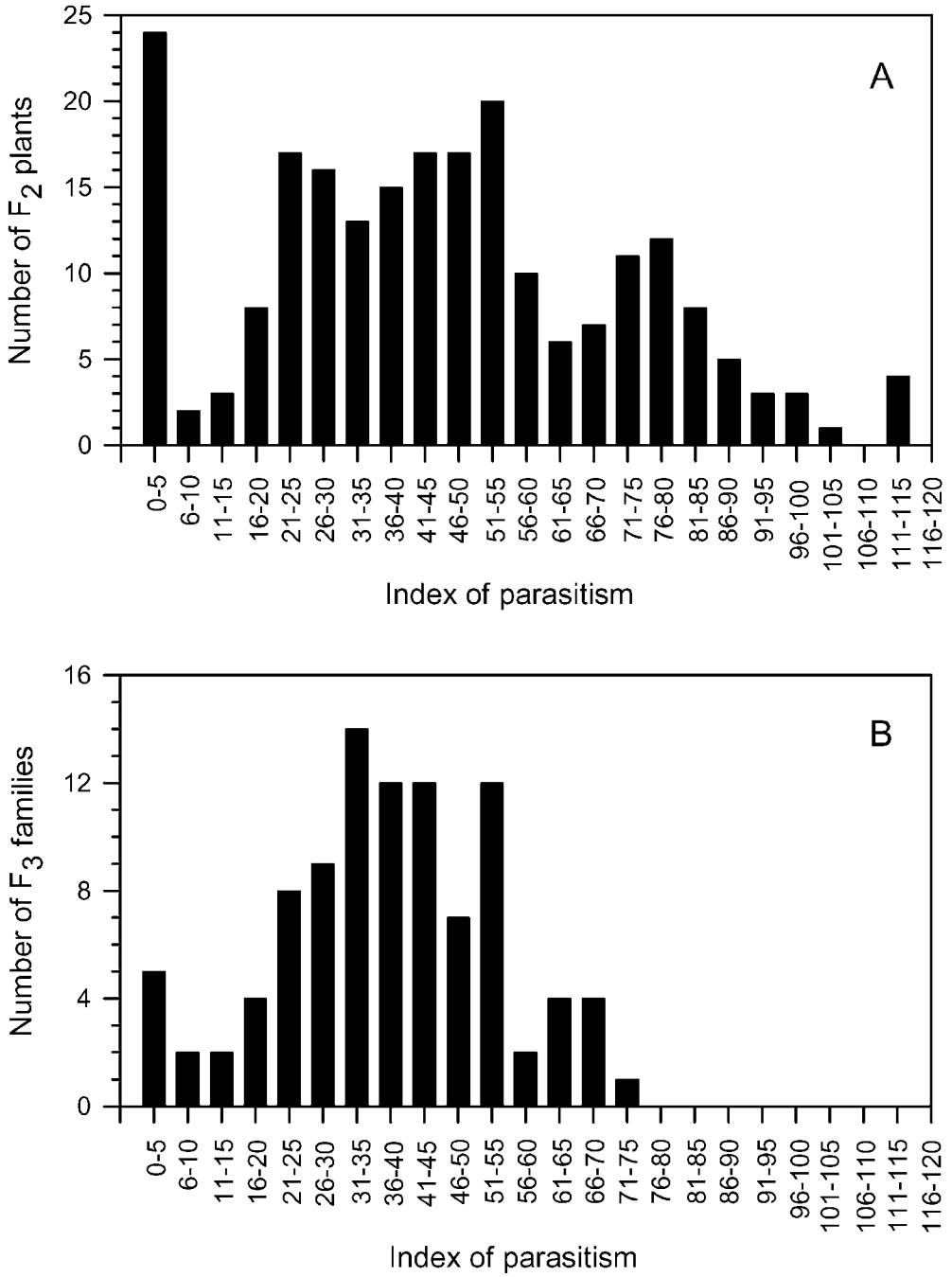

Fig. 2. (A) Frequency distribution of the index of parasitism (IP) of 222 F_2 soybean plants from the cross PI 89772 and Lee 68, resistant and susceptible, respectively, to *Heterodera glycines* race 3, where IP = [(number of females developed on the candidate plant)/(mean number of females developed on Lee 74) × 100]. (B) Frequency distribution of 98 F_3 families from the same cross.

Table 2. Partition of 98 F_3 families from the cross PI 89772 (resistant) × 'Lee 68' (susceptible) into four classes of reaction to *Heterodera glycines* race 3.[y]

F_3 Families	Index of parasitism ($\bar{x} \pm S_{\bar{x}}$)		
	Resistant[z]		
1…7	0.2 ± 0.0	…	5.6 ± 2.5
	Moderately resistant		
8…21	16.0 ± 1.8	…	29.9 ± 4.9
	Moderately susceptible		
22…89	31.4 ± 4.1	…	61.1 ± 5.4
	Susceptible		
90…98	67.2 ± 6.6	…	79.1 ± 5.3

[y]Cluster analysis was based on F_3 family means and standard error of the mean. The index of parasitism (IP) = (number of females developed on a candidate plant)/(mean number of females developed on 'Lee 74') × 100, and the mean number of females for the parents were 0.2 ± 0.1 (IP = 0 ± 0) and 506.3 ± 9.9 (IP = 100.0 ± 3.9), respectively, for PI 89772 and 'Lee 68'.
[z]Classification of levels of resistance are defined as: resistant (R): $0 \leq IP < 10$; moderately resistant (MR): $10 \leq IP < 30$; moderately susceptible (MS): $30 < IP \leq 60$; and susceptible (S): $IP > 60$ (Schmitt and Shannon, 1992).

of soybean genotypes based on reproduction of *H. glycines* on host differentials (Schmitt and Shannon, 1992). The categorization of IPs of soybean cultivars as resistant, moderately resistant, moderately susceptible and susceptible to *H. glycines* are nearly identical to the cluster analysis resulting from crossing the immune PI 89772 with the susceptible 'Lee 68'.

Evaluation of Cross PI 88788 × PI 89772

The mean number of females for the F_1 hybrid (PI 88788 × PI 89772) was 22.3 ± 2.6 (IP = 3.9 ± 0.5), and this result was different (t = 3.7; P =0.001) from that of the reciprocal hybrid, 38.0 ± 3.5 (IP = 6.6 ± 0.6). Since the environmental variances data for these reciprocal crosses were low, the difference for these two hybrids indicates a maternal effect in the inheritance of resistance of PI 88788 to *H. glycines* race 3 (Fig. 3). This result contrasts with the cross PI 89772 × 'Lee 68' in which no maternal effect was observed. The mean number of females for the F_1 hybrid from the cross PI 88788 × PI 89772 (22.3 ± 2.6) was closer to the PI 88788 parent (32.1 ± 8.0; Fig. 3).

In the F_2 generation for the cross PI 88788 × PI 89772, the number of females varied from 0 to 100, with a skewed distribution (P = 0.0001) toward the most resistant parent PI 89772 (Fig. 4A). The partition of the 74 F_3 families using cluster analysis resulted in two classes: the first included 69 F_3 families with IPs ranging from 0 to 17, and the second class grouped five F_3 families with IPs ranging from 20 to 30 (Table 3). This partition fits a 15:1 ratio (χ^2 = 0.03;

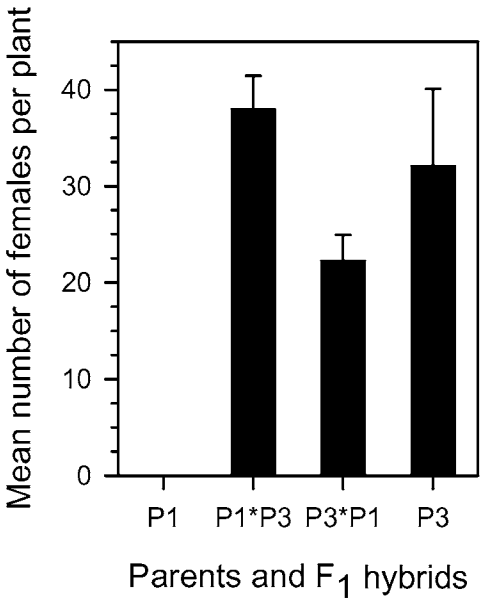

Fig. 3. Mean number of females per plant for parents PI 89772 (P1), and PI 88788 (P3), two lines resistant to *Heterodera glycines* race 3, and F_1 hybrids PI 89772 × PI 88788 (P1 × P3), and PI 88788 × PI 89772 (P3 × P1). Bars indicate standard error of the mean.

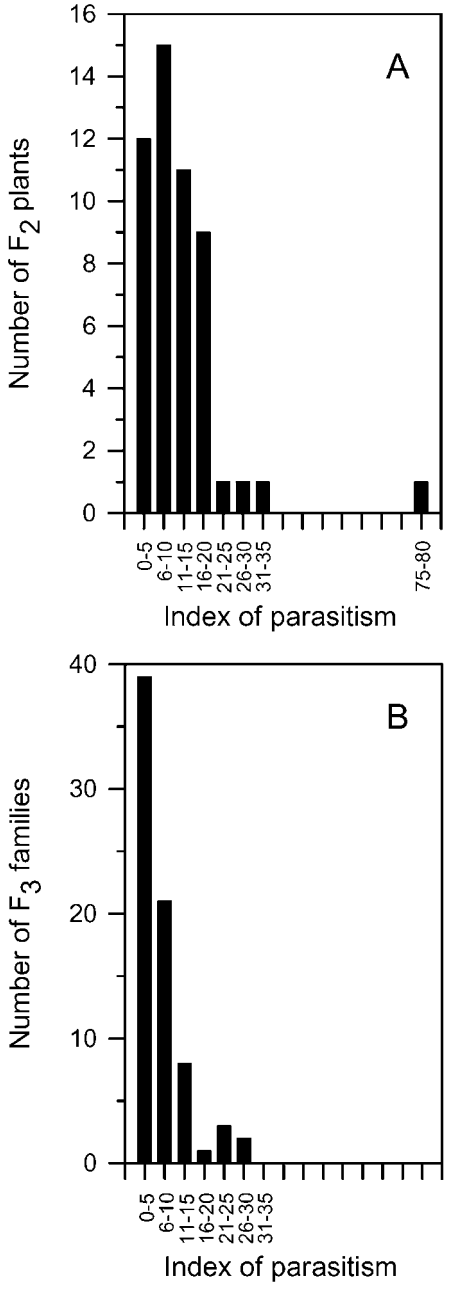

Fig. 4. (A) Frequency distribution of the index of parasitism (IP) of 164 F_2 soybean plants from the cross PI 89772 and PI 88788, two lines resistant to *Heterodera glycines* race 3, where IP = [(number of females developed on the candidate plant)/(mean number of females developed on 'Lee 74') × 100]. (B) Frequency distribution of 74 F_3 families from the same cross.

$P = 0.86$; $R^2 = 0.87$; Table 5) based on F_3 family means and not individual F_3 plants. Results from the analysis of F_3 families also were supported by the analysis of 164 F_2 plants, although to a lesser extent (χ^2 = 5.47; $P = 0.02$; $R^2 = 0.92$). Since both parents (PI 89772 and PI 88788) had different levels of resistance, the phenotypic classes formed from the cross PI 88788 × PI 89772 are not as diverse as the ones formed in the cross PI 89772 × 'Lee 68', due to the narrow range of IPs for *H. glycines* (Fig. 4B). The cluster analysis resulted in two groups (resistant and moderately resistant) and the coefficient of variation (R^2) explained 87% of the variation in the F_3 families (Table 5). This model suggests both parents share the same two loci (Rhg_x and Rhg_y) for resistance to *H. glycines*, but have different alleles. Results reported herein are not in complete agreement with previous reports indicating PI 88788 has two dominant genes

Table 3. Partition of 74 F_3 families from the cross PI 88788 × PI 89772, both resistant to *Heterodera glycines* race 3, into two classes of resistance.[y]

F_3 Families	Index of parasitism ($\bar{x} \pm S_x$)		
	Resistant to lower moderately resistant[z]		
1…69	0.0 ± 0.0	…	16.8 ± 5.9
	Upper moderately resistant		
70…74	20.8 ± 2.1	…	30.2 ± 3.5

[y]Cluster analysis was based on F_3 family means and standard error of the mean. The index of parasitism (IP) = (number of females developed on the candidate plant)/(mean number of females developed on 'Lee 74') × 100, and the mean number of females for the parents were 0.4 ± 0.2 (IP = 0.1 ± and 18.2 ± 2.5 (IP = 5.9 ± 0.8), respectively, for PI 89772 and PI 88788.

[z]Classification of levels of resistance are defined as: resistant (R): 0 ≤ IP < 10; moderately resistant (MR): 10 ≤ IP < 30; moderately susceptible (MS): 30 < IP ≤ 60; and susceptible (S): IP > 60 (Schmitt and Shannon, 1992).

Table 4. Partition of 80 F_3 families from the cross 'Lee 68' (susceptible) × PI 209332 (moderately resistant), into four classes of reaction to *Heterodera glycines* race 3.[y]

F_3 Families	Index of parasitism ($\bar{x} \pm S_{\bar{x}}$)
	Upper resistant to lower moderately resistant[z]
1…4	8.0 ± 0.9 … 17.7 ± 1.7
	Upper moderately resistant to mid moderately susceptible
5…57	21.6 ± 3.0 … 47.8 ± 3.4
	Upper moderately susceptible to mid susceptible
58…77	50.7 ± 6.2 … 76.1 ± 5.1
	Upper susceptible
78…80	83.5 ± 4.7 … 88.0 ± 6.2

[y]Cluster analysis was based on F_3 family means and standard error of the mean. The index of parasitism (IP) = (number of females developed on a candidate plant)/(mean number of females developed on 'Lee 74') × 100, and the mean number of females for the parents were 418.7 ± 30.5 (IP = 100.0 ± 7.3) and 68.2 ± 9.9 (IP = 16.3 ± 2.4), respectively, for 'Lee 68' and PI 209332.

[z]Classification of levels of resistance are defined as: resistant (R): 0 ≤ IP < 10; moderately resistant (MR): 10 ≤ IP < 30; moderately susceptible (MS): 30 < IP ≤ 60; and susceptible (S): IP > 60 (Schmitt and Shannon, 1992).

(*Rhg*$_4$ and *Rhg*$_5$) and one recessive (*rhg*) that confer resistance to race 3 (Rao-Arelli and Anand, 1988; Myers and Anand, 1991; Rao-Arelli *et al.*, 1992,). Those reports relied on the "10 per cent rule" (Golden *et al.*, 1970) for resistance vs. susceptibility and used selected but not inbred nematode lines. Analysis of the PI 88788 × 'Lee 68' cross using the inbred *H. glycines* race 3 would provide additional insight into the total number of genes and gene action that controls resistance in this widely used source of resistance to *H. glycines*.

Evaluation of Cross 'Lee 68'× PI 209332

The mean number of females for the F_1 hybrid was 170.7 ± 27.6 (IP = 31.1 ± 5.0), which is closer to the PI 209332 parent (Fig. 5). A total of 176 F_2 plants was evaluated from this cross. The range in number of females developed per plant was large, varying from 6 to 692. The frequency distribution of the F_2 plants was skewed (P = 0.0001) toward the moderately resistant parent PI 209332 (Fig. 6A). The frequency distribution of the 80 F_3 families for this cross (Fig. 6B) was almost normal (P = 0.035). The partition of the 80 F_3 families derived from cluster analysis is presented in Table 4. Initially, partitioning the 80 F_3 families using cluster analysis formed two different classes: one class with four F_3 families with IPs ranging from 0 to 20, and the second class with 76 F_3 families with IPs ranging from 21 to 88. This partition fits a 15:1 ratio (χ^2 = 0.21; P = 0.64; R^2 = 0.75) based on F_3 family means but not individual F_3 plants and supported a two-gene model. The F_2 analysis utilizing the 15:1 ratio supported the F_3 partition (χ^2 = 3.49; P = 0.061; R^2 = 0.78). Additional analysis further partitioned the 76 F_3 families into groups of 54, 19 and 3 F_3 families. The 80 F_3 families were partitioned into four classes of reaction to *H. glycines* race 3, classified herein as: a) upper-R to lower-MR, where IP = 8-20; b) upper-MR to Mid-MS, where IP = 21-50; c) upper-MS to mid-S, where IP = 51-80; and d) upper-S, where IP > 80 (Table 4). The entire group of 80 F_3 families fit the ratio 9:5:1:1 and explained the most variability (R^2 = 0.93) for the F_3 families in this cross (Table 5 and Fig. 6) and also supported a two-gene model.

Since the crosses PI 209332 × PI 89772 and PI 209332 × PI 88788 were not analyzed in this research, it was not possible to determine if PI 209332 may have gene(s) in common with both PI 89772 and PI

Table 5. Models for inheritance of soybean resistance to *Heterodera glycines* race 3, based on the reaction of 98, 74, and 80 F_3 families, respectively, from crosses PI 89772 (resistant) × 'Lee 68' (susceptible), PI 88788 (resistant) × PI 89772, and 'Lee 68' × PI 209332 (moderately resistant), as determined by cluster analysis.[u]

Cross	Genetic model[v]	Observed/expected ratio	χ^2	*P* value	R^2[w]
PI 89772 × 'Lee 68'	12:2:1:1[x]	68:14:7:9/74:12:6:6	2.14	0.54	0.88
PI 88788 × PI 89772	15:1[y]	69:5/69.4:4.6	0.03	0.86	0.87
'Lee 68' × PI 209332	9:5:1:1[z]	54:19:3:4/45:25:5:5	4.24	0.24	0.93

[u]The analysis was based on F_3 family means and standard error of the mean, although the reaction classes are described here in terms of index of parasitism (IP), where IP = (number of females developed on a candidate plant)/(mean number of females developed on 'Lee 74') × 100.

[v]The models assume the genotype of parents are $rhg_{x0}rhg_{x0}rhg_{y0}rhg_{y0}$, $Rhg_{x1}Rhg_{x1}Rhg_{y1}Rhg_{y1}$, $Rhg_{x2}Rhg_{x2}Rhg_{y2}Rhg_{y2}$, and $Rhg_{x3}Rhg_{x3}Rhg_{y3}Rhg_{y3}$, respectively, for Lee 68, PI 89772, PI 88788, and PI 209332, with Rhg_{x1} dominant over Rhg_{x2} and Rhg_{y2} dominant over Rhg_{y1}.

[w]R^2 = proportion of variation explained by the suggested partition.

[x]Rhg_{x1} Rhg_{x1} rhg_{y0} rhg_{y0} + Rhg_{x1} rhg_{x0} _ _ + rhg_{x0} rhg_{x0} Rhg_{y1} _ = 12/16 [moderately susceptible (MS): 30 ≤ IP ≤ 60]; $Rhg_{x1}Rhg_{x1}Rhg_{y1}rhg_{x0}$ = 2/16 [moderately resistant (MR): 10 ≤ IP < 30]; $Rhg_{x1}Rhg_{x1}Rhg_{y1}Rhg_{y1}$ = 1/16 [resistant (R): 0 ≤ IP < 10]; and $rhg_{x0}rhg_{x0}rhg_{y0}rhg_{y0}$ = 1/16 [susceptible (S): IP > 60] (Schmitt and Shannon, 1992).

[y]Rhg_{x1} _ _ _ + Rhg_{x2} Rhg_{x2} _Rhg_{y2} = 15/16 [R to lower-MR (10 ≤ IP < 20, i.e., PI 209332-type of reaction)]; and $Rhg_{x2}Rhg_{x2}Rhg_{y1}Rhg_{y1}$ = 1/16 [upper-MR (20 ≤ IP < 30)] (Schmitt and Shannon, 1992).

[z]Rhg_{x3} Rhg_{x3} _rhg_{y0} + $Rhg_{x3}rhg_{x0}$ Rhg_{y3} _ = 9/16 [upper-MR to mid-MS (40 ≤ IP ≤ 50)]; $Rhg_{x3}rhg_{x0}rhg_{y0}rhg_{y0}$ + rhg_{x0} rhg_{x0} Rhg_{y3} _ = 5/16 [upper-MS (50 < IP ≤ 60) to mid-S]; rhg_{x0} rhg_{x0} rhg_{y0} rhg_{y0} = 1/16 [upper-S (IP > 80)]; and $Rhg_{x3}Rhg_{x3}Rhg_{y3}Rhg_{y3}$ = 1/16 [upper-R (8 ≤ IP < 10, i.e., PI 88788-type of reaction) to lower-MR] (Schmitt and Shannon, 1992).

88788. However, the simplest model would explain that the same loci identified in the crosses PI 89772 × 'Lee 68' and PI 88788 × PI 89772 confer resistance in PI 209332 parent. Therefore, the major gene Rhg_x and minor gene Rhg_y from parents PI 89772 and PI 88788, were considered as different alleles ($Rhg_{x3}Rhg_{y3}$), which confer a moderate level of resistance to PI 209332.

Bartlett's test for homogeneity of variance (Gomez and Gomez, 1984) was used to estimate the environmental standard error of the mean number of females per plant, based on variance of parents and F_1 hybrids. The various estimates were as follows: 27.4 (χ^2 = 7.92; *P* = 0.0190), 2.5 (χ^2 = 5.54; *P* = 0.0628) and 15.1 (χ^2 = 0.33; *P* = 0.8463), respectively, for PI 89772 × 'Lee 68', PI 88788 × PI 89772, and 'Lee 68' × PI 209332. Since these three estimates were not homogeneous (χ^2 = 87.29; *P* < 0.0001) no attempt was made to combine them into a pooled estimate of the component of variance due to the environment. Results from this analysis clearly indicated that the sensitivity of resistant genotypes to the environment is different from that of susceptible genotypes (Figs. 1, 3, and 5). Differing sensitivity to environment questions the common practice of estimating the component of variance due to the environment from the variances of parents and F_1 hybrids, a practice that assumes that all genotypes are equally sensitive to the environment, which is not true (Falconer, 1996).

Assuming the genotypes of the three resistant parents, PI 89772, PI 88788 and PI 209332, carry the homozygous genes $Rhg_{x1}Rhg_{x1}Rhg_{y1}Rhg_{y1}$, $Rhg_{x2}Rhg_{x2}Rhg_{y2}Rhg_{y2}$, and $Rhg_{x3}Rhg_{x3}Rhg_{y3}Rhg_{y3}$, respectively, and also applying the two gene model for these parents as outlined in Table 1, several geno-

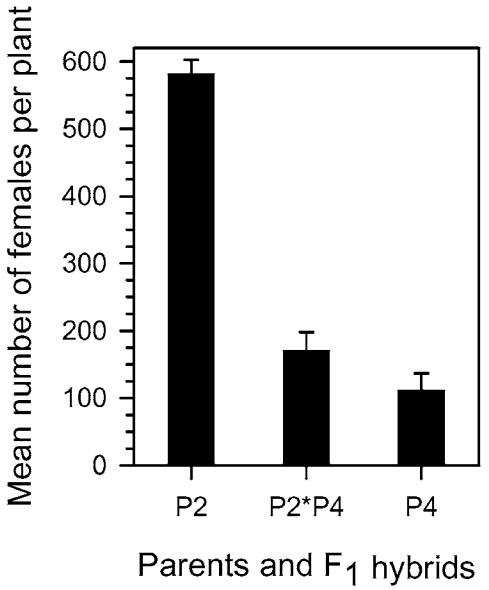

Fig. 5. Mean number of females per plant for parents Lee 68 (P2), and PI 209332 (P4), respectively, susceptible and moderately resistant to *Heterodera glycines* race 3, and for the F_1 hybrid Lee 68 × PI 209332 (P2 × P4). Bars indicate standard error of the mean.

types for the offspring from each cross studied in this research are presented in Table 5. To understand how the *H. glycines* resistant gene model functions in the proposed models for the three crosses, a major gene (Rhg_x) and minor (Rhg_y) were assigned to the parents and the genotypes for the crosses PI 89772 × 'Lee 68', PI 88788 × PI 89772, and 'Lee 68' × PI 209332 are as shown on Table 5. The model of two resistant genes for PI 89772 and PI 209332 proposed in this research indicates that a major and minor gene not a dominant and recessive (*Rhg, rhg*) confer resistance (Rao-Arelli, 1994). However, the F_2 results suggesting the dominant and recessive model were not supported by F_3 family partitioning obtained within the same cross. Cluster analysis reported herein resulted in sets within F_3 families, in which the homozygous resistant families have low means of females and low variance; heterozygous or segregating families have intermediate means of females and high variance, and the homozygous susceptible families have high means of females and low variance. The array of phenotypes obtained from crosses between these genotypes may have intermediate levels of either resistance or susceptibility, depending on how one gene interacts in the presence or absence of the other. Categorizing soybean response to *H. glycines* based on single F_2 plants is not as precise as using data obtained from F_3 families. The results reported herein used means and standard error of the mean in the F_3 families arrayed according to cluster analysis, and grouped genotypes as $Rhg_{x1?}rhg_{x0?}Rhg_{y1?}rhg_{y0?}$ and $Rhg_{x1?}rhg_{x0?}Rhg_{y1?}Rhg_{y1?}$ or $Rhg_{x3?}rhg_{x0?}Rhg_{y3?}rhg_{y0?}$ and $Rhg_{x3?}Rhg_{x3?}Rhg_{y3?}Rhg_{y3?}$. Cluster analysis was important in avoiding grouping of discrete levels of resistance into qualitative phenotypes based on arbitrary definitions of resistance and susceptibility. Since the three crosses produced a non-significant Chi-square ratio of 15:1, the data herein suggest a duplicate dominant epistatic gene effect for the resistant genes for the PIs between the two loci as opposed to dominant and recessive epistasis, ratio 13:3, reported previously (Rao-Arelli, 1994).

The two-gene model provided the best explanation of the data obtained in the research reported herein. The three parents studied in this research have at least two genes that confer resistance to *H. glycines*. One gene acts as a major gene (Rhg_x) and the other acts as a minor gene (Rhg_y) in conferring resistance of the parents PI 89772 ($Rhg_{x1?}Rhg_{x1?}Rhg_{y1?}Rhg_{y1?}$), and PI 209332 ($Rhg_{x3?}Rhg_{x3?}Rhg_{y3?}Rhg_{y3?}$) to *H. glycines* race 3. The same genes may occur in PI 209332 as in PI 89772, but support for this hypothesis must be obtained by studying the cross PI 209332 × PI 89772. The same major ($Rhg_{x1?}Rhg_{x1?}$) and minor ($Rhg_{y1?}Rhg_{y1?}$) genes occur in PI 89772

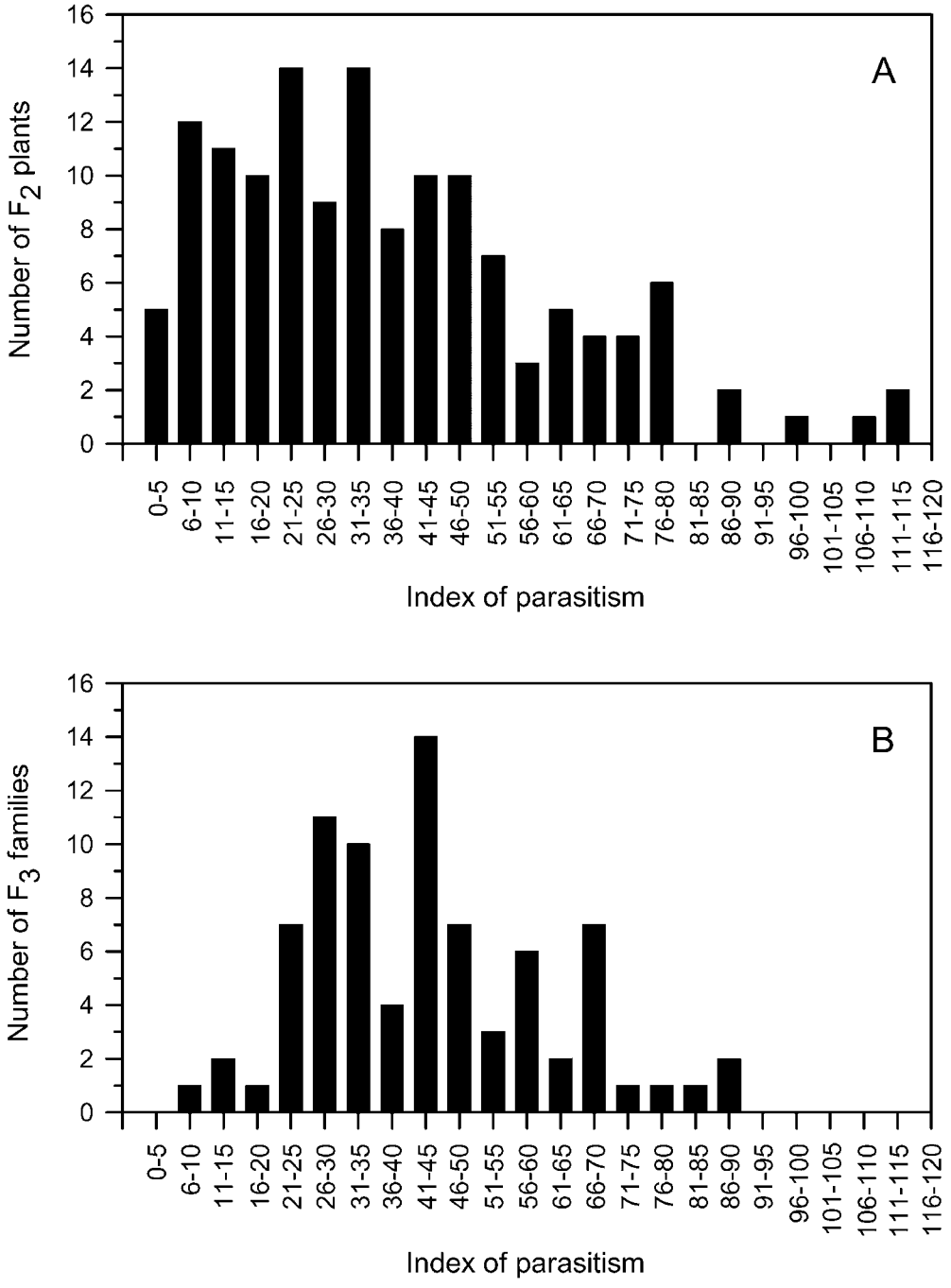

Fig. 6. (A) Frequency distribution of the index of parasitism (IP) of 176 F_2 soybean plants from the cross PI 209332 × Lee 68, moderately resistant and susceptible, respectively, to *Heterodera glycines* race 3, where IP = [(number of females developed on the candidate plant)/(mean number of females developed on Lee 74) × 100]. (B) Frequency distribution of 80 F_3 families from the same cross.

($Rhg_{x1?}Rhg_{x1?}Rhg_{y1?}Rhg_{y1?}$) and PI 88788 ($Rhg_{x2?}Rhg_{x2?}Rhg_{y2?}Rhg_{y2?}$). Also, the phenotypic ratios obtained in this research indicate epistasis occurs between gene Rhg_x and gene Rhg_y.

ACKNOWLEDGMENTS

The authors thank T. Cary and S. Bauer of the Department of Crop Sciences, University of Illinois, and M. Zimmerman USDA, ARS for technical assistance.

LITERATURE CITED

ANAND, S. C. 1982. New soybean strain resistant to soybean cyst nematode: PI416.762. Plant Disease 66:933-934.

ANAND, S. C., and A. P. RAO-ARELLI. 1989. Genetic analysis of soybean genotypes resistant to soybean cyst nematode Race 5. Crop Science 29:1181-1184.

ANONYMOUS. 1997. Rules for genetic symbols. Soybean Genetics Newsletter 24:19-20.

BERNARD, R. L., G. R. NOEL, S. C. ANAND, and J. G. SHANNON. 1988. Registration of 'Fayette' soybean. Crop Science 28:1028-1029.

CALDWELL, B. E., C. A. BRIM, and J. P ROSS. 1960. Inheritance of resistance of soybeans to the cyst nematode (*Heterodera glycines*). Agronomy Journal 52:635-639.

COBB, N. A. 1918. Estimating the nema populations of soil. U.S. Department of Agriculture Technology Circular 1. Washington, DC: U.S. Government Printing Office.

CONCIBIDO, V. C., R. L. DENNY, S. R. BOUTIN, R. HAUTEA, J. H. ORF, and N. D. YOUNG. 1994. DNA marker analysis of loci underlying resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe). Crop Science 34:240-246.

CONCIBIDO, V. C., N. D. YOUNG, D. A. LANGE, R. L. DENNY, D. DANESH, and J. H. ORF. 1996. Targeted comparative genome analysis and qualitative mapping of a major partial-resistance gene to the soybean cyst nematode. Theoretical and Applied Genetics 93:234-241.

CONCIBIDO, V. C., D. A. LANGE, R. L. DENNY, J. H. ORF, and N. D. YOUNG. 1997. Genome mapping of soybean cyst nematode resistance genes in 'Peking', PI 90763, and PI 88788 using DNA markers. Crop Science 37:258-264.

CREGAN, P. B., and C. V. QUIGLEY. 1997. Simple sequence repeat DNA marker analysis. Pp. 173-185 *in* G. Caetano-Anolles and P. M. Gresshoff, eds. DNA markers: Protocols, applications and overviews. John Wiley and Sons, New York. 364 pp.

CREGAN, P. B., T. JARVIK, A. L. BUSH, R. C. SHOEMAKER, K. G. LARK, A. L. KAHLER, N. KAYA, T. T. VANTOAI, D. G. LOHNES, J. CHUNG, and J. E. SPECHT. 1999a. An integrated genetic linkage map of the soybean genome. Crop Science 39:1464-1490.

CREGAN, P. B., J. MUDGE, E. W. FICKUS, D. DANESH, R. DENNY, and N. D. YOUNG. 1999b. Two simple sequence repeat markers to select for soybean cyst nematode resistance conditioned by the rhg1 locus. Theoretical and Applied Genetics 99:811-818.

DIERS, B. W., H. T. SKORUPSKA, A. P. RAO-ARELLI, and S. R. CIANZIO. 1997. Genetic relationships among soybean plant introductions with resistance to soybean cyst nematodes. Crop Science 37:1966-1972.

DONG, K., K. R. BARKER, and C. H. OPPERMAN. 1997. Genetics of soybean *Heterodera glycines* interactions. Journal of Nematology 29:509-522.

DOUPNIK, B., JR. 1993. Soybean production and disease loss estimates for north central United States from 1989-1991. Plant Disease 77:1170-1171.

FAGHIHI, J., R. A. VIERLING, J. M. HALBRENDT, V. R. FERRIS, and J. M. FERRIS. 1995. Resistance genes in a 'Williams 82' × 'Hartwig' soybean cross to an inbred line of *Heterodera glycines*. Journal of Nematology 27:418-421.

FALCONER, D. S. and T. F. C. MacKAY. 1996. Introduction to quantitative genetics. Longman, New York. 464 pp.

GOLDEN, A. M., J. EPPS, R. D. RIGGS, L. A. DUCLOS, J. A. FOX, and R. L. BERNARD. 1970. Terminology and identity of infraspecific forms of the soybean cyst nematode (*Heterodera glycines*). Plant Disease Reporter 54:544-546.

GOMEZ, A. A. 1984. Statistical procedures for agricultural research. 2nd ed. John Wiley & Sons, New York. 680 pp.

HANCOCK, F. G., C. E. CAVINESS, and R. D. RIGGS. 1985. Heritability and inheritance of resistance to Race 4 and 'Bedford' biotype of soybean cyst nematode. Agronomy Abstracts. P. 56. American Society of Agronomy, Madison, WI.

JENKINS, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

JOHNSON, R. A., and D. W. WICHERN. 1998. Applied multivariate statistical analysis. 4th ed. Prentice Hall, Upper Saddle River, NJ. 767 pp.

KISHA, T. J., C. H. SNELLER, and B. W. DIERS. 1997. Relationship between genetic distance among

parents and genetic variance in populations of soybean. Crop Science 37:1317-1325.

MATTHEWS, B. F., M. H. MacDONALD, J. S. GEBHARDT, and T. E. DEVINE. 1998. Molecular markers residing close to the *Rhg4* locus conferring resistance to soybean cyst nematode race 3 on linkage group A of soybean. Theoretical and Applied Genetics 97:1047-1052.

MATSON, A. L., and L. F. WILLIAMS. 1965. Evidence of a fourth gene for resistance to the soybean cyst nematode. Crop Science 5:477.

MYERS, G. O., and S. C. ANAND. 1991. Inheritance of resistance and genetic relationships among soybean plant introductions to races of soybean cyst nematode. Euphytica 55:197-201.

NIBLACK, T. L., P. R. ARELLI, G. R. NOEL, C. H. OPPERMAN, J. H. ORF, D. P. SCHMITT, J. G. SHANNON, and G. L. TYLKA. 2002. A revised classification scheme for genetically diverse populations of *Heterodera glycines*. Journal of Nematology 34:279-288.

NOEL, G. R., and D. I. EDWARDS. 1996. Population development of *Heterodera glycines* and soybean yield in soybean-maize rotations following introduction into a noninfested field. Journal of Nematology 28:335-342.

RAO-ARELLI, A. P. 1994. Inheritance of resistance to *Heterodera glycines* race 3 in soybean accessions. Plant Disease 78:898-900.

RAO-ARELLI, A. P., and S. C. ANAND. 1988. Genetic relationships among soybean plant introductions for resistance to race 3 of soybean cyst nematode. Crop Science 28:650-652.

RAO-ARELLI, A. P., S. C. ANAND, and J. A. WRATHER. 1992. Soybean resistance to soybean cyst nematode race 3 is conditioned by an additional dominant gene. Crop Science 32:862-864.

RIGGS, R. D., and D. P. SCHMITT. 1988. Complete characterization of the race scheme for *Heterodera glycines*. Journal of Nematology 20:392-395.

RIGGS, R. D., D. P. SCHMITT, and G. R. NOEL. 1988. Variability in race tests with *Heterodera glycines* Ichinohe. Journal of Nematology 20:565-572.

SCHMITT, D. P., and G. SHANNON. 1992. Differentiating soybean response to *Heterodera glycines* races. Crop Science 32:275-277.

SEBASTIAN, S. A., and C. D. NICKELL. 1985. Inheritance of brown stem rot resistance in soybeans. Journal of Heredity 76:194-198.

SHIER, M. 2004. Soybean varieties with soybean cyst nematode resistance: Soybean variety listing (Available on line at http://www.ag.uiuc.edu/~wardt/cover.htm).

WEBB, D. M., B. M. BALTAZAR, A. P. RAO-ARELLI, J. SCHUPP, K. CLAYTON, P. KEIM, and W. D. BEAVIS. 1995. Genetic mapping of soybean cyst nematode Race-3 resistance loci in the soybean PI 437654. Theoretical and Applied Genetics 91:574-581.

WEISEMANN, J. M., B. F. MATTHEWS, and T. E. DEVINE. 1992. Molecular markers located proximal to the soybean cyst nematode resistance gene, *Rhg4*. Theoretical and Applied Genetics 85:136-138.

WRATHER, J. A. 2002. Soybean disease loss Estimates for the United States, 1996-2002 (Available on line at http://aes.missouri.edu/delta/research/soyloss.stm).

YOUNG, L. D., and T. C. KILEEN. 1994. Genetic relationships among plant introductions for resistance to soybean cyst nematode race 5. Crop Science 34:936-939.

YUE, P., D. A. SLEEPER, and P. R. ARELLI. 2001. Mapping resistance to multiple races of *Heterodera glycines* in soybean PI 89772. Crop Science 41:1589-1595.

Received: *30.III.2004*

Recibido:

Accepted for publication: *14.I.2005*

Aceptado para publicación: