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Genetic diversity in soybean genotypes with resistance to *Heterodera glycines*

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ABSTRACT – *The purpose of this study was to analyze the genetic diversity among soybean genotypes inoculated with* Heterodera glycines *race 3. The experiments were conducted in a greenhouse. In two performance tests of morphological characteristics and resistance to the pathogen, 27 soybean genotypes were assessed. The coefficient of genotypic determination was estimated by the method of analysis of variance and the genetic diversity analyzed based on dendrograms and optimization method. The estimated coefficients of determination indicated a predominantly genetic origin of the genotypic differences in the traits. The genetic variability was maintained in the superior genotypes, which can be used in breeding programs for resistance to soybean cyst nematode.*

Key words: Glycine max, *soybean cyst nematode, soybean breeding, resistant cultivars.*

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is one of the most important crops of the global agribusiness, and Brazil is the second largest producer, with an expectation of surpassing the United States within the next 10 years (Sediyama et al. 2009). With the expansion of the crop and of continuous cultivation systems, the number of pathogenic microorganisms of soybean has increased and so, some absent or economically unimportant diseases have become limiting factors for soybean production (Dhingra et al. 2009).

The soybean cyst nematode (*Heterodera glycines*), causal agent of the disease "soybean yellow dwarf", is considered one of the major nematodes of the crop, causing yield losses ranging from 15 % to 100 % (Dhingra et al. 2009, Embrapa 2010). Since its identification in Brazil in the growing season of 1991/1992 (Lima et al. 1992, Lordello et al. 1992, Monteiro and Morais 1992), the infested area has grown rapidly to over 2.0 million hectares in 10 Brazilian states (Embrapa 2010). The control is based primarily on crop rotation with non-host species and the use of resistant cultivars (Dias et al. 2009a, Embrapa 2010).

The successful development of resistant cultivars has been one of the greatest contributions of plant breeding (Sediyama et al. 2009), owing to the fact that genetic diversity between the parents is available for breeding. The reason is that a number of authors reported that a maximum

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heterosis expressed in hybrids is desirable to increase the possibility of superior segregants in advanced generations and to help broaden the genetic base (Carpentieri-Pipolo et al. 2000).

In studies of genetic diversity allelic differences in a population can be identified, which is considered essential in the choice of genotypes to be used as parents (Falconer 1981). Thus, an analysis of the base population and establishment of well-defined groups based on a number of traits (agronomic, morphological, molecular, etc.) are important steps in the planning of crosses (Cruz and Carneiro 2006, Setotaw et al. 2010). Thus, the availability and use of cyst-nematode-resistant germplasm requires the development of strategies of plant breeding for the introgression of resistance genes into commercial cultivars. According to Embrapa (2010), 14.1 % of the commercial cultivars of Brazil are resistant to *H. glycines*. However, the pedigree information of the genotypes is generally not available (Zhang et al. 1999). In this situation, analyses of genetic diversity can identify promising genotypes for breeding programs and eventually to the rural entrepreneurs.

For soybean, analyses of genetic diversity have been widely applied. The presence of genetic variability among genotypes was observed by Miranda et al. (2001), Miranda et al. (2007), Matsuo et al. (2009), Vieira et al. (2009) and Almeida et al. (2011). Also focusing on the soybean cyst nematode, Ma et al. (2006) studied the genetic diversity of soybean and created a core collection based on this resistance. For other crops, studies of genetic divergence have been conducted, e.g., in snapbean (Krause et al. 2009), cowpea (Dias et al. 2009b), passion fruit (Cerqueira-Silva et al. 2009), in wine grape accessions (Borges et al 2010), barley (Setotaw et al. 2010) and turmeric (Sigrist et al. 2011).

The selection, identification and genetic studies of soybean cultivars with *H. glycines*-resistance have been conducted mainly in inoculated greenhouses (Silva et al. 2000, Moura et al. 2008). However, according to Dias et al. (2009a), the populations developed by Embrapa Soja, from the F_4 generation, whenever possible, are grown in areas infested with *H. glycines*, where the best plants are selected (in height, lodging resistance, cycle, disease resistance and yield potential). Additionally, they reported that to increase the frequency of cyst-nematode-resistant soybean lines, where possible, the lines can be evaluated (scored according to the number of females in the roots), in the field or in polyethylene bags filled with infested soil.

Thus, the estimated genetic variability in soybean genotypes with resistance to cyst nematode can be of great importance in breeding programs for resistance to *H. glycines*. The reason is that the identification of genetically distinct genotypes can be considered promising for use in crossing blocks. The objective of this study was to analyze the genetic diversity among soybean genotypes inoculated with *H. glycines*, race 3.

MATERIAL AND METHODS

The experiment was arranged and conducted in a greenhouse as part of the soybean breeding program and evaluated in a laboratory of the Federal University of Viçosa (UFV), Minas Gerais. During the experiment, the mean maximum and minimum temperatures were 19.8 ºC and 35.6 ºC, respectively.

The plant material consisted of promising genotypes (Table 2) with desirable traits in the second year of assessment of the value of cultivation and use (VCU). A completely randomized design with six replications was used, in which each experimental unit was represented by one plant.

The soil infested with *H. glycines*, previously identified as race 3 by Silva et al. (1999), was taken from the nematode bank of the soybean breeding program of the UFV (BNPMGS-UFV). Each of the 10 nematode populations from BNPMGS-UFV were multiplied from 3 dm^3 soil, which was homogenized and distributed in pots to plant soybean cultivar Quartzo as host. To obtain the inoculum, the plants were removed from the pots and the root system was placed on a double sieve (mesh 20 and 100) and washed under a strong jet of water. Females retained in the 100 mesh sieve were transferred to a double sieve (mesh 100 and 500) on which they were crushed. The eggs retained on the 500 mesh were transferred to a beaker for subsequent microscopic quantification. The egg concentration was determined using a 1-ml Peters counting slide, standardizing the suspension to 1,000 eggs per ml. The genotypes were assessed in two trials; for experiment 1 the test inoculum was obtained from eggs of females and for experiment 2 from soil infested with cysts. The seeds of the genotypes were pre-germinated in sand and in the VE stage, according to Fehr and Caviness (1977), the plants were standardized (size and vigor) and transplanted, one per pot.

In experiment 1, each pot contained 0.8 dm^3 of sand mixed with clay soil (1:1) (v/v). Around each plant, 10 days after planting, 4,000 eggs were distributed in four holes drilled in the ground with a glass rod (depth of 2 cm), 2 cm

away from the main stem. Thirty-four days after inoculation, the root system of each plant was removed and washed under a strong jet of water on a double sieve (mesh 20 and 160). Females retained in the 160 mesh sieves were collected with water in a beaker. These females were counted under a stereoscopic microscope (20x) on a plastic score board with a checkerboard pattern. The eggs were obtained from the females as described above. The number of females, number of eggs, number of eggs per female, plant height, number of nodes, fresh and dry matter weight of shoots.

In experiment 2, pre-germinated plants were transplanted into pots containing soil infested with cysts of *H. glycines* race 3 from BNPMGS-UFV. The inoculum level was 29,356 eggs per 100 dm^3 soil. On the 35^{th} day after inoculation (transplantation), the number of females, the root vigor and vigor of the plant shoots were visually scored, comparing test genotypes with cultivar Lee 74 (susceptible control). The number of females was also evaluated with a magnifying glass (power 6x) on a black polyethylene film. The counting was done by three raters, trained to identify females on the root system (NF-1: rater 1, NF-2: rater 2, and NF-3: rater 3). The plant height, number of nodes, fresh and dry matter weight of shoots were also analyzed.

Firstly, tests for normality and homogeneity of variance were performed. The data of number of females and of eggs, plant height and fresh matter weight, in phase 1, and the number of females obtained by the raters A, B and C, average of the raters, visual score of the number of females, and plant vigor, in experiment 2 were transformed by $\sqrt{(x+1)}$. Data regarding plant height, fresh and dry matter weight of experiment 2, were transformed by *log (x + 1)*.

The coefficient of genotypic determination was estimated by the method of analysis of variance, where:

$$H^2 = \frac{\hat{\Phi}_g}{\hat{\sigma}_f^2}, \text{ where:}$$

H^2 = genotype determination coefficient based on the genotype mean.

$$\hat{\Phi}_g = \frac{QMG - QMR}{r}, \text{ where}$$

$\hat{\Phi}_g$ = square component of the genotypic variability

QMG = mean square of the genotype

QMR = mean square of the error

r = number of experimental replications

$$\hat{\sigma}_f^2 = \frac{QMG}{r}, \text{ where:}$$

$\hat{\sigma}_f^2$ = mean phenotyc variance.

The genetic diversity in promising soybean genotypes was studied and patterns of resistance and susceptibility in each test, based on all traits and those related to resistance (number of females, eggs and eggs per female). The dissimilarity matrices were estimated using the mean phenotypic values by Mahalanobis´ distance. Based on these values, the genetic diversity was studied based on the dendrogram using the Unweighted Pair-Group Method using Arithmetic Averages (UPGMA) and the genotypes were clustered by the Tocher optimization method. The statistical analyses were performed using software Genes (Cruz 2008).

RESULTS AND DISCUSSION

In experiment 1, the coefficients of genotypic determination for the traits number of females, number of eggs and plant height ranged from 88.42 % to 93.63 %. But for the trait number of females obtained by the raters A, B and C and in the average of the raters, for the visually scored number of females, and plant and root vigor, the genotypic correlation coefficients estimated in experiment 2 were ≥ 80 % (Table 1). High values indicate the existence of large genetic variation for the traits studied, i.e., a greater chance of successful breeding with simple methods and significant selection gains (Aragão et al. 2001, Nascimento Filho et al. 1994). Falconer (1981) reported that these coefficients express the validity of a phenotypic value as representative of the genotypic value of a population or of a set of genotypes.

The result of genetic divergence among the genotypes based on all traits in Experiment 1 indicated 'Lee 74' as the most dissimilar, followed by 'BCR945G110' and by genotype BCR1070G229 and UFVS2010 (Figure 1-A). Considering only the resistance-related traits, it was found that genotype BCR945G110 was most distant from the other genotypes. The dissimilarity of the genotypes BCR945G114 and BCR1070G229 to 'UFVS2010' was 4 and 8 %, respectively. The dissimilarity of these last three was 60 % in relation to genotype Lee 74 (Figure 1-B).

When analyzing the results obtained in experiment 2, considering all variables, a dissimilarity value of 30 % was found among the genotypes BCR132390, BCR945G114, BCR945G110, BCR1070G229, BCR1057G163, and UFVS2010 and 100 % compared to the others. Among

Table 1. Coefficient of genotypic determination (H^2), based on different traits of soybean genotypes, artificially inoculated with *H. glycines*, race 3, in a greenhouse

Experiment 1 – inoculated with eggs of *Heterodera glycines*	
Traits	H^2
Number of females	93.63
Number of eggs	91.38
Number of eggs per female	51.94
Plant height	88.42
Experiment 2 – inoculated with cysts of *Heterodera glycines*	
Traits	H^2
Number of females (Rater A)	88.36
Number of females (Rater B)	87.18
Number of females (Rater C)	90.31
Number of females (Mean of raters)	91.18
Visual score of number of females	94.72
Visual score of root vigor	80.00
Visual score of plant vigor	53.37
Plant height	66.06

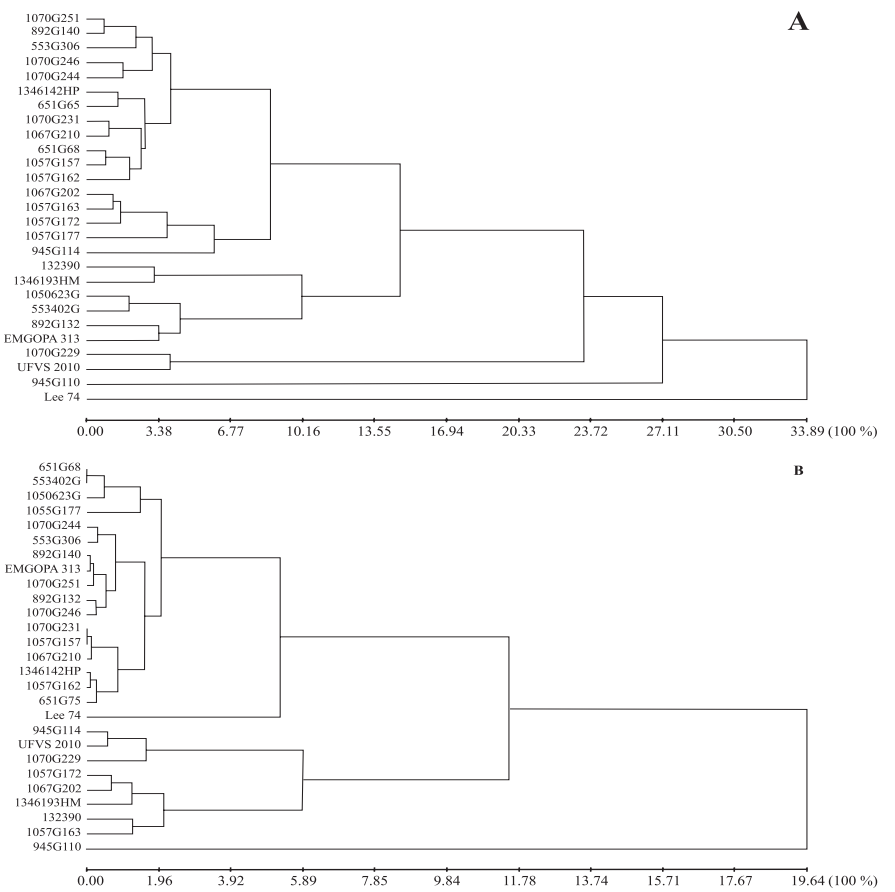

Figure 1. Dendrogram of the dissimilarity in 27 soybean genotypes in experiment 1, obtained by UPGMA algorithm, based on Mahalanobis' distance, including traits number of females, of eggs, and of eggs per female, plant height, number of nodes, fresh and dry matter weight (A) and resistance-related traits number of females, of eggs and of eggs per female (B).

these, the least dissimilar pair of genotypes was BCR1070 G229 and UFVS2010 (Figure 2-A). The dendrogram of the analysis of resistance-related traits (Figure 2-B) showed similar results to Figure 2-A, for the dissimilarity of the genotypes in relation to UFVS2010, although the values were lower.

Genotype UFVS2010 was the resistance pattern to cyst nematode. In all dendrograms it was observed that the dissimilarity of 'BCR1070G229' was less than 15 % in relation to UFVS2010, and in all cluster analyses, the two genotypes were included in the same group. This allowed the conclusion that the phenotypic performance of

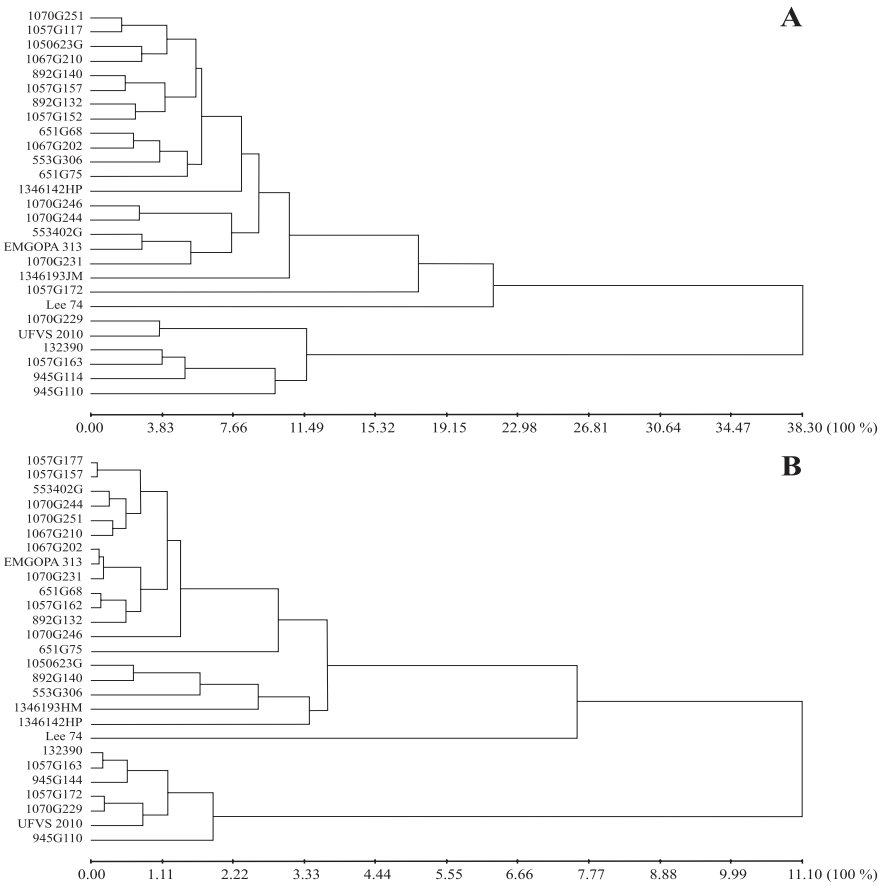

Figure 2. Dendrogram resulting from the dissimilarity in 27 soybean genotypes in experiment 2, obtained by UPGMA algorithm, based on Mahalanobis´ distance, based on traits number of females as scored by the rater A, B and C and mean of the raters, visual score of number of females, of root vigor, plant vigor, plant height, number of nodes, and fresh and dry matter weight (A) and resistance-related traits number of females, according to rater A, B and C and mean of raters (B).

The grouping results showed that the genotypes in experiment 1 were allocated into four groups. Considering all traits, the genotypes BCR132390, BCR945G114, BCR1070G229 and UFVS2010 were classified in Group II. However, when considering only the resistance-related traits, the group additionally included genotype BCR1057 G163. In the results of both tests, it was found that genotype BCR945G110 was allocated in a group of its own (Table 2).

In experiment 2, considering all traits, the genotypes formed mutually exclusive groups. Group II was composed of the genotypes BCR132390, BCR945G114, BCR1057G163, BCR1070G229, BCR945G110, and UFVS2010. For the resistance-related traits only, the formation of five groups was observed, three consisting of one genotype each. Group II consisted of the genotypes BCR132390, BCR945G114, BCR1057G163, BCR1070G229, BCR945G110, UFVS2010, and BCR1057G172 (Table 2).

Table 2. Genotypes clustered by Tocher´s optimization method based on Mahalanobis´ generalized distance estimated in experiment 1, from all traits (number of females, of eggs and of eggs per female, plant height, number of nodes, fresh and dry matter weight) and of resistance-related traits (number of females, of eggs, and of eggs per female) and in experiment 2, based on the traits (number of females counted by rater A, B and C and mean of raters, visual score of number of female, root vigor, plant vigor, plant height, number of nodes, fresh and dry matter weight) and of resistance-related traits (number of females according to rater A, B and C and mean of raters)

Experiment 1 – inoculated with eggs of *Heterodera glycines*	
Groups	**Genotype grouping based on all traits**
I	BCR1070G251, BCR892G140, BCR1067G210, BCR1057G157, BCR1070G244, BCR553G306, BCR1057G162, BCR1346142HP , BCR1070G231, BCR651G68, BCR651G75, BCR1057G177, BCR1070G246, BCR1057G172, BCR1067G202, BCR1057G163, EMGOPA313, BCR1346193HM, BCR892G132 and BCR553402G
II	BCR1070G229, UFVS2010, BCR945G114 and BCR132390
III	BCR1050623G and Lee74
IV	BCR945G110
Groups	**Genotype grouping based on resistance-related traits**
I	BCR651G68, BCR553402G, BCR1057G157, BCR1070G231, BCR1067G210, BCR1070G244, BCR1346142HP, BCR1057G162, BCR651G75, EMGOPA313, BCR1070G251, BCR553G306, BCR892G140, BCR1070G246, BCR892G132, BCR1057G177, BCR1050623G , BCR1057G172, BCR1067G202 and BCR1346193HM
II	BCR945G114, UFVS2010, BCR1070G229, BCR1057G163 and BCR132390
III	Lee74
IV	BCR945G110
Experiment 2 – inoculated with cysts of *Heterodera glycines*	
Groups	**Genotype grouping based on all traits**
I	BCR1070G251, BCR1057G177, BCR1067G210, BCR1050623G, BCR651G68, BCR1067G202, BCR892G140, BCR1057G157, BCR1057G162, BCR892G132, BCR553G306, BCR651G75, BCR1070G244, BCR1070G246, EMGOPA313, BCR553402G, BCR1070G231, BCR1346193HM and BCR1346142HP
II	BCR1070G229, UFVS2010, BCR1057G163, BCR132390, BCR945G114 and BCR945G110
III	BCR1057G172
IV	Lee74
Groups	**Genotype grouping based on resistance-related traits**
I	BCR1057G177, BCR1057G157, BCR1070G251, BCR1070G244, BCR553402G, BCR1067G210, EMGOPA313, BCR1067G202, BCR892G132, BCR1070G231, BCR1057G162, BCR651G68, BCR1070G246, BCR1050623G, BCR892G140, BCR553G306 and BCR651G75
II	BCR132390, BCR1057G163, BCR1057G172, BCR1070G229, UFVS2010, BCR945G114 and BCR945G110
III	BCR1346193HM
IV	BCR1346142HP
V	Lee 74

'BCR1070G229' was quite similar to UFVS2010 and may be desirable for breeding programs for resistance to *H. glycines* race 3. However, based on the results it is emphasized that the genetic dissimilarity of these genotypes is low. To exemplify this, genotype BCR945G110 with resistance to cyst nematode, race 3, in phase 1 can be used, which was found to have a genetic similarity of at least 80 % from all genotypes and was separately classified into one group. Consequently, this genotype may be promising for breeding programs for resistance to *H. glycines* race 3.

The results demonstrated the existence of genetic variability in the analyzed plant material when the resistance data were analyzed separately or together with morphological traits. This may be desirable, since it was found that the genetic variability of the populations was maintained and that the genotypes can be used in crossing blocks in order to avoid that the developed lines would have a narrow genetic base and pathogen resistance. The success of a breeding program depends on the existence of variability in the work population, so the use of tools such as the

study of genetic diversity of the parents has been recommended by breeders with the purpose to form a base population with a view to intercrossing between superior and divergent cultivars (Cruz and Carneiro 2006).

The results of experiments 1 and 2 indicated that the magnitude of dissimilarity between genotypes was greater when all traits were used, than in the analysis considering only the resistance traits. This demonstrated the benefit of analyzing the genotypes based on two criteria, since in the absence of morphological characteristics undesirable parents might be selected, ie, without information on the genetic diversity of genotypes based on the plant shoot as well. Thus, the importance of evaluating all traits is emphasized, because this ensures that aspects of resistance to pathogens and agricultural traits of soybean are taken into consideration.

In this context, the scientific gain of the study was the possibility of analyzing the genetic diversity of cyst-nematode-resistant soybean cultivars and contributes to scientific knowledge, with a view to the development of resistant lines, since it was possible to identify promising genotypes for hybridizations, with resistance to *H. glycines*, race 3, and genetic divergence from the other analyzed genotypes. The most promising crosses are BCR1057G172 x UFVS2010 and BCR1057G172 x BCR1070G229, because the diversity values were below 30 % considering the resistance-related traits and above 68 % when considering all traits analyzed. Therefore, parents can be chosen with cyst-nematode-resistance and adequate genetic diversity based on the analysis of resistance traits together with morphological aspects. Besides these, crosses between genotypes with a resistance level similar to the standard (BCR132390, BCR945G110, BCR1070G229, BCR945G114, and UFVS2010) may be suggested with genotypes not grouped with UFVS2010 and with wide genetic diversity with regard to the traits analyzed (BCR1057G157, BCR1057G162, BCR1057G177, BCR1067G210, BCR1070G231, BCR1070G244, BCR1070G246, BCR1070G251, BCR1346142HP, BCR553G306, BCR651G68, BCR651G75, and BCR892G140). In the crosses that involved only one pathogen-resistant parent, the probability of identifying a cyst-nematode-resistant line in the segregating population or in the advanced lines is lower due to the crosses of resistant parents. Therefore, efforts are needed to identify soybean genotypes with resistance to the pathogen as well as genetically divergent and necessary, since the recombination of these genotypes would be more desirable.

CONCLUSIONS

The estimated coefficients of determination indicated a predominantly genetic origin of the genotypic differences in the traits. The genetic variability was maintained in the superior genotypes, which can be used in breeding programs for resistance to soybean cyst nematode, race 3.

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Diversidade genética em genótipos de soja com resistência ao *Heterodera glycines*

RESUMO – *Este trabalho teve como objetivo analisar a diversidade genética entre genótipos de soja inoculados com* Heterodera glycines*, raça 3. Os experimentos foram conduzidos sob condições de casa de vegetação. Foram avaliados 27 genótipos de soja sob dois ensaios de análise do comportamento quanto às características morfoagronomicas e de resistência ao patógeno. Estimou-se o coeficiente de determinação genotípica, a partir do método da análise de variância, e análise de diversidade genética por meio de dendrogramas e método de otimização. Verificou-se que as estimativas dos coeficientes de determinação genotípica demonstraram que as diferenças observadas nos caracteres avaliados foram de natureza, predominantemente, genética; e os genótipos superiores mantiveram a variabilidade genética, podendo ser utilizados em programas de melhoramento para resistência a nematoide de cistos.*

Palavras-chave: Glycine max*, nematoide de cistos da soja, melhoramento de soja, cultivares resistentes.*

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