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Research Article

Multivariate analysis of combining ability for soybean resistance to *Cercospora sojina* Hara

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Abstract

Seven soybean cultivars (Bossier, Cristalina, Davis, Kent, Lincoln, Paraná and Uberaba), with different levels of resistance to *Cercospora sojina*, race 04, were crossed according to a diallel design, with no reciprocals, to determine the general and the specific combining abilities for the resistance. The evaluations of the reaction to the disease were performed 20 days after the inoculation of the fungus on the most infected leaflet of the plant, in the parents and in the F1 hybrids. To quantify the resistance, the following characteristics were evaluated: infection degree (ID); number of lesions per leaflet (NLL); lesion mean diameter (LMD); lesioned leaf area (LLA); percentage of lesioned leaf area (PLLA); number of lesions per square centimeter (NLC) and disease index (DI). The relative importance of each characteristic was evaluated by the canonical variables analysis and the LLA and NLL characteristics were eliminated from the multivariate function. With the remaining five characteristics, a multivariate index was created using the first canonical vector, which was submitted to the diallel analysis, according to Griffings fixed model, method 2. The most important characters to discriminate resistant from susceptible soybean plants to *C. sojina* were: ID, LMD, NLC, DI and PLLA. Cristalina, Davis and Uberaba cultivars are the best ones among those tested that can be recommended as parents in soybean breeding programs seeking resistance to *Cercospora sojina*. The additive, dominant and epistatic genetic effects were important for the expression of the resistance, although the additive genetic effect was the most important component.

Key words: *Glycine max*, diallel, canonical variables, frogeye leaf spot.

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Introduction

The world production of soybean grains (*Glycine max* (L.) Merrill), in 2001/2002 growing season, was estimated to be 181 million tons, resulting in trade of more than 30 billion dollars worldwide. Brazil, the world's second largest producer, participated with approximately 22.85% of the world production, just behind the United States (Toledo, 2002). The average Brazilian productivity of soybean grain is still low; according to IBGE (2004) it was about 2798 kg/ha in 2003. The productivity potential of this crop is more than 4000 kg/ha and the State of Mato Grosso has been producing around 3000 kg/ha in the last years, showing the highest performance of the country. Soybeans are responsible for approximately 42% of Brazilian agricultural products for export, making it the most important crop for income generation to Brazil.

The cultivated area of soybean in Brazil has increased in the last years - in 2002 the planted area was 16,345,223 ha and 2003 was 18,293,128 ha, with an increase of 11.92% in relation to 2002 (IBGE, 2004). The consequence of this large cultivated area has been the increasing incidence of diseases, which are responsible for annual losses of about two billion dollars. One of the most important diseases is the cercospora leaf spot also known as frogeye leaf spot, caused by the *Cercospora sojina* Hara fungus (Yorinori, 1997). It is one of the main foliar diseases of soybean, but the fungus can also attack the stem, the pods and the seeds (Sinclair and Backman, 1989). The incidence of the disease is favored by hot and humid environment and its influence in the reduction of the productivity is derived from the reduction of the photosynthetic area, premature defoliation and damage to the seeds (Akem and Dashiell, 1994).

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In Brazil, this disease currently is under control through genetic resistance and its occurrence is sporadic, and it is limited to the south of Maranhão (Balsas) and north of the state of Goiás (Niquelândia). More than 23 races have already been identified in Brazil and new epidemics are likely to occur (Yorinori, 1997).

The use of resistant cultivars and the incorporation of resistance genes into susceptible commercial cultivars are the most economical and efficient means of control of frogeye leaf spot, because the chemical control with fungicides, in addition to being expensive, has not been shown to be viable in controlling the disease.

In the initial stages of a breeding program seeking resistance to this disease, it is important to understand the genetic mechanisms of the inheritance and the genetic parameters underlying the resistance to increase the efficiency in the selection of the parents to be used in the crossings and in the choice of selection methods to be used. Therefore it is important to obtain information on the genetic potential of the cultivars as well as on their combining abilities that would result in hybrids producing promising segregating populations (Griffing 1956).

The general combining ability (GCA) refers to the average behavior of an inbred parent in a series of hybrid combinations, and it is associated with the additive action of the genes. The specific combining ability (SCA) refers to those instances in which certain hybrid combinations are either better or poorer than would be expected on the average performance of the inbred parents considered, and it is associated to non-additive genetic effects (Rojas and Sprague, 1952, Miranda *et al.* 1988). The diallel analysis permits the evaluation and identification of more promising crossings for the development of superior segregating lines or for use in generating hybrid populations.

Most of the time, a great number of variables are evaluated in the populations involved in a breeding program. One of the problems that appears when several characteristics are considered is the understanding of the relative importance of each of them on treatment discrimination, to allow the elimination of those characteristics of lesser importance due to their non-variance, redundancy, and/or correlation with other characteristics present in the analysis.

The multivariate analysis, through the canonical variables, enables the determination of the relative importance of those characters, allowing the simplification of data, summarizing the information originally contained in a small number of variables, which present the property of retaining the maximum of the variation originally available and independence from each other. This analysis was first proposed by Rao (1952). It can be used on studies of genetic divergence between individuals or parents and in the determination of the relative importance of the characteristics (Cruz, 2001).

According to Mardia *et al.* (1979), the elimination of characters is accomplished by choosing those variables associated with the largest elements of the last canonical variable. After the elimination of characters, the first canonical variable may still involve most of the estimated variance. The coefficients associated with the first canonical variable of the analysis involving only the remnant characteristics may be used to generate a function that serves as a multivariate index to represent the group of the evaluated characters in the experiment (Martins Filho *et al.*, 1992).

The objective of this work was to study the resistance of soybean to *C. sojina* through the estimation of the general and specific combining abilities and of the genetic parameters involved in the determination of the resistance by means of Griffings diallel analysis based on a multivariate index.

Materials and Methods

Cultivars and crosses

Seven soybean cultivars (Bossier, Cristalina, Davis, Kent, Lincoln, Paraná and Uberaba) were artificially crossed in a diallel, to produce 21 hybrid combinations. The parents and the F_1s were evaluated on the basis of seven characteristics associated with the disease, in a completely randomized design with 12 replications, in a greenhouse. Kent and Lincoln cultivars were supplied by Embrapa - The National Center for Soybean Research, Londrina, PR; the other five cultivars were supplied by the Germoplasm collection of the Departamento de Fitotecnia of Universidade Federal de Viçosa, Viçosa, MG. These cultivars were chosen because they present different levels of resistance to *Cercospora sojina* Hara. The hypocotyl, flower and pubescence colors, and days to flower and to maturity data were used as phenotypic markers to eliminate the selfings. For those crosses where it was not possible to use these phenotypic markers, DNA markers were used to distinguish the hybrid from the selfing. The DNA extraction was made according to the methodology proposed by Doyle and Doyle (1990), and the RAPD technique was used for the identification of hybrid plants (Alzate-Marin *et al.*, 1996). The F_1 hybrids resulting from the cross Uberaba x Bossier were identified using the OPB-03 or OPAG-10 RAPD primers, and the F_1 hybrids from Kent x Lincoln with the AB-4 RAPD primer or microssatellite AN1.

Inoculation of pathogen

The race 04 isolate of *Cercospora sojina* Hara was supplied by the Embrapa - The National Center for Soybean Research, Londrina, PR. The fungus was multiplied in Petri dishes containing agar-tomato juice (Superbom) without spices culture medium, prepared similarly to the V8-agar medium, just substituting the V8 for tomato juice.

The inoculation of the pathogen was made when the soybean seedlings presented a completely developed third trifoliate leaf, spraying about 10 mL per plant of a 40,000 conidia per milliliter suspension. Immediately after the inoculation, the pots were taken to a mist chamber where they remained for three days under a relative humidity of 100%.

Evaluation of the disease

Twenty days after the inoculation, when the disease symptoms were considered well-established, the following characteristics were evaluated: a) infection degree (ID) - visually evaluating the symptoms and using a grading scale, where 1.0 indicates no apparent infection, and 5.0 -indicates the maximum degree of infection; b) number of lesions per leaflet (NLL) - on the most infected leaflet; c) lesion mean diameter (LMD) - average of the 10 largest lesions of the most infected leaflet, in millimeters; d) number of lesions per square centimeter (NLC) - by dividing the number of lesions per leaflet by the leaflet area; e) lesioned leaf area (LLA); f) percentage of lesioned leaf area (PLLA) - by dividing the lesioned leaflet area by the area of the leaflet and multiplying by one hundred; and g) disease index (DI) - by multiplying the number of lesions per leaflet by the square of lesion mean diameter, $DI = NLL \times LMD^2$. All variables were evaluated in accordance to the methods used by Veiga (1973), Cordeiro (1992) and Martins Filho (1992 and 1999).

Statistical-genetic analysis

The canonical variables multivariate analysis was used to determine the relative importance of the characters and to simplify the data, summarizing the originally contained information in lesser variables. The analysis was performed using the GENES software (Cruz, 2001). The relative importance of the characters evaluated in the studied genotypes was evaluated by means of the coefficients of the eigenvectors associated with the last canonical variable. The variables associated with the largest coefficients of this last canonical variable were eliminated from the study and canonical variables were recalculated with the remaining variables. The new first canonical variable coefficients were used to multiply the original values of the characters to obtain a multivariate index (composed character). This index was submitted to the analysis of variance to verify the existence of significant genetic variability among the entries (parents and F_1's) and was analyzed according to the diallel analysis fixed model method-2, with no-reciprocals, by Griffing (1956). The treatment effect was considered fixed and decomposed into general and specific combining abilities. The means were compared by the Scott-Knotts test at 5% probability level.

Results and Discussion

In the first run of the canonical variable analysis, it was observed that the first two canonical variables involved 86.88% of the total variance, which is an indication that the appraised compound can be simplified, and that there are non-variant or redundant characters, which are suitable for elimination. In his work, the relative importance of the characters was evaluated by the coefficient obtained by the multiplication of the residual standard deviation of the character by the respective coefficient weight according to Singh (1981).

The eliminated characters were those that presented the largest coefficients of the last eigenvector. The eliminated characters were NLL and LLA, which contributed less to the evaluation of the disease symptoms. After the elimination of these two variables, new canonical variables were created with the remaining variables, so that the first canonical variable involved more than 75% of the estimated variance and its coefficients were used to multiply the original variables to compose the following multivariate index (compound character): $Y_i = 1{,}1344ID + 0{,}6155LMD + 0{,}1265NLC - 0{,}0261PLLA - 0{,}0548DI$, where Y_i represents the index values used in the subsequent analyses of the experiment. The analysis of variance of the index values, in a completely randomized design, showed significant effect of treatments (parents and their F_1 hybrid combinations), implying that the entries showed significant differences in the manifestation of the resistance to the disease when the symptoms were pooled in the multivariate function. It is worthwhile to note that the included characteristics are inversely related to the resistance; in other words, the smaller the value the higher is the resistance.

Table 1 shows, in the diagonal, the average values of the multivariate index of the parents and on the off-

Table 1 - Estimated means for the multivariate index evaluated in the assay of soybean reaction to *C. sojina* involving seven cultivars and their 21 hybrid combinations, in a diallel cross[1/].

Cultivars	Bossier	Cristalina	Davis	Kent	Lincoln	Paraná	Uberaba
Bossier	7.89a	1.13f	1.71f	6.46	5.72c	6.12c	2.42e
Cristalina		1.36f	1.13f	1.13f	1.40f	1.13f	1.20f
Davis			1.22f	2.41e	1.90f	2.29e	1.13f
Kent				3.94d	6.18c	5.97c	3.05e
Lincoln					6.18c	6.90b	3.75d
Paraná						2.99e	1.92f
Uberaba							1.87f

[1/] Means followed by the same letter represent the same group by the Scott-Knott test to 5% probability.

diagonal the averages for the F_1s. The parents were discriminated into five groups, according to the Scott-Knotts test. Bossier and Lincoln cultivars presented reactions of high susceptibility to *C. sojina*; Kent and Paraná showed intermediate reactions and Cristalina, Davis and Uberaba were resistant. The F_1 hybrids derived from different crosses were highly different from each other in relation to their reaction to *C. sojina*. Crosses where at least one of the parents was resistant yielded plants that presented few or no disease symptoms, indicating dominance of the resistance. Hybrids from crosses between two susceptible cultivars or between cultivars with intermediate resistance presented more disease symptoms.

Cristalina, Davis and Uberaba cultivars carry alleles for resistance to frogeye leaf spot. In these cultivars, practically no infection symptoms were observed. Possibly, these cultivars possess resistance alleles in highly favorable combinations that optimize the expression of the resistance. In Cristalina, the combination of resistance genes is so favorable that no other entry showed higher resistance expression.

The mean squares for general (GCA) and specific combining abilities (SCA) were highly significant for the multivariate index, indicating that the crosses were heterogeneous. The significance of GCA and SCA indicates the existence of additive and non-additive genetic variability among the evaluated genotypes. According to Ramalho *et al.* (1993), GCA is of great importance to the breeders that work with autogamous species because it depends on the additive genetic variance. In breeding programs, selections are performed on segregating generations and in advanced generations several pure lines with additive gene effect are obtained.

In the present work, although neither GCA nor SCA were significant for the multivariate index, there is indication of the superiority of GCA due to the great magnitude of the mean squares for GCA in relation to the mean square for SCA (MS of CGA = 303.39 against MS of SCA = 17.77). Thus, there is indication of efficiency for genetic progress in the advanced segregating generations, due to the larger additive effect of the genes in these populations. Oliveira Júnior *et al.* (1999) concluded that the GCA effect is a good predictor for the performance of segregating field beans F_3 populations. The same authors base this fact on the principle that the phenotypic value of F_1 is strongly determined by the heterotic effects conditioned by the dominance deviations, which are not transferred to posterior generations. As the heritable genetic factors are of additive nature, it is acceptable that the prediction is more efficient when the information of the hybrid is based solely on the effect of the general combining ability.

Parents with higher GCA estimates (gi) are indicated for the constitution of new populations, aiming at the attainment of higher genetic progress in breeding programs. Therefore, for the case of disease resistance evaluation, the interest concentrates on genotypes with lower severity of the disease, or either, genotypes that contribute to diminish the expression of the character and, consequently, show negative estimates of gi (Cruz and Regazzi, 2001).

In the present study, the estimates of the gi of each cultivar are shown in Table 2. Negative values indicate the contribution for the resistance to *C. sojina* in soybean, which were observed for resistant parents (Cristalina, Davis and Uberaba). Positive estimates were observed on susceptible and intermediately resistant parents (Bossier, Lincoln, Kent and Paraná).

Bossier cultivar presented the most unfavorable general combining abilities estimate, and is, therefore, the genitor that presents the lowest capacity to contribute with resistance alleles to the genetic pool under study.

According to Cruz and Regazzi (2001), the SCA effect is the deviation of the hybrid in relation to that would be expected based on the GCA effects of its parents. Thus, the low absolute values for SCA indicate that the F_1 hybrid derived from crosses between the studied parents behaved as expected, while high absolute values of $\hat{s}_{ij}$ demonstrate that the behavior of a given cross is relatively better or worse than the expected value on the basis of the GCA of the parents. The estimates of $\hat{s}_{ij}$ show the importance of the genes that show non-additive effects.

In the present study, hybrids of lower SCA estimates are distinguished by having Cristalina, Davis or Uberaba (Table 3) as one of the parents. The magnitude of the values of the SCA of each parent ($\hat{s}_{ii}$) may also be interpreted as an indication of the genetic divergence of the cultivar in relation to the average of the other parents tested in the diallel. The greater the absolute value of $\hat{s}_{ii}$ the greater is the effect of heterosis inherent to the cultivar, which has been manifested in all F_1 populations from the cross (Cruz and Regazzi, 2001).

Cristalina, Davis and Uberaba presented the highest values of $\hat{s}_{ii}$ (absolute values), evidencing their genetic divergence in relation to the average of the other parents involved in the diallel, as well as the higher effect of the heterosis reveled in the F_1 of the crosses between them. This indicates the existence of unidirectional dominance deviations (Silva *et al.* 2000) and the negative manifestation of heterosis in the hybrid combinations, involving divergent ancestors (Table 3, main diagonal). Negative values of $\hat{s}_{ii}$ were observed for the parents Bossier, Kent and Paraná, revealing positive heterosis in their hybrids, which in this case is disadvantageous, because it leads to increased disease symptoms.

Table 2 - Estimates of general combining ability effect (gi) of seven soybean cultivars, for the evaluated index.

Effect	g_1	g_2	g_3	g_4	g_5	g_6	g_7
Estimate	1.52	-1.80	-1.42	0.80	1.37	0.50	-0.96

Table 3 - Estimates of the specific combining abilities ($\hat{s}_{ii}$) for the characters evaluated in the assay of soybean reaction to *C. sojina*, involving seven cultivars and their 21 hybrid combinations, in a diallel cross.

Cultivars	Bossier	Cristalina	Davis	Kent	Lincoln	Paraná	Uberaba
Bossier	-1.61	-1.59	-1.62	0.90	-0.40	0.87	-1.38
Cristalina		1.51	1.13	-1.10	-1.40	-0.79	0.74
Davis			0.84	-0.19	-1.28	-0.01	0.29
Kent				-0.90	0.77	1.44	-0.02
Lincoln					0.20	1.80	0.11
Paraná						-1.23	-0.85
Uberaba							0.56

In this present work, it was concluded that the most important characters to discriminate resistant from susceptible plants of soybean to *C. sojina* are ID, LMD, NLC, DI and PLLA. Cristalina, Davis and Uberaba cultivars may be recommended as parents in breeding programs of soybean seeking resistance to *C. sojina*. The additive and non-additive genetic effects are important for the expression of the resistance, although the additive genetic effect is the most important component.

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