

GENETIC VARIABILITY AMONG SOYBEAN BIPARENTAL CROSSES EVALUATED BY MULTIVARIATE ANALYSIS

VARIABILIDADE GENÉTICA ENTRE CRUZAMENTOS BIPARENTAIS EM SOJA AVALIADA POR ANÁLISES MULTIVARIADA

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ABSTRACT: This work aims to study the genetic variability of 22 biparental crosses of soybean through multivariate techniques. The experiment was carried out in a randomized complete block design with three replications, consisting of 110 genotypes from 22 biparental crosses and cultivars UFUS Riqueza, UFUS Impacta, UFUS Xavante UFUS Millionária and MSoy 8211 which were used as control. The characters evaluated were number of days until flowering and until maturity, plant height at flowering and at maturity, number of pods with one, two or three seeds, total number of pods, weight of plant and first pod yield. The population evaluated showed genetic variability for most traits. Plant height at maturity, pods with one seed and grain yield were the traits that contributed the most to genetic diversity among the soybean crosses studied. The three clustering methods used in this study were effective in representing the genetic distance in soybean. Hybridizations between lines derived from crosses CR13 and CR14 with cultivar UFUS Impact or hybridizations between lines derived from crosses CR5 and CR10 with lines derived from crosses CR21 and CR12 show promise for obtaining segregating soybean populations.

KEYWORDS: *Glycine max.* L. Genetic distance. Breeding.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is one of the most important crops worldwide and especially in Brazil (LI et al., 2008), which is the second largest producer, due to a productive and technological chain developed around this oleaginous species.

The advancement of soybean production in Brazil is due in large part to the results achieved by soybean breeding programs. With new market demands, a portion of these results is subject to the progress of research to improve important traits such as protein and oil content in grains, which coupled with adequate yield levels can increase the possibility of Brazil becoming the world's leading soybean producer in the coming years.

Agronomic traits of greatest interest in breeding programs are of complex inheritance, as is the case with grain yield. These traits have continuous distribution, are highly influenced by the environment, and are controlled by a large number of genes. (FALCONER; MACKAY, 1996; LYNCH; WALSH, 1998).

Studies of genetic divergence in soybean are of great relevance during the process of developing

new genotypes. Studies noted that the Brazilian soybean germplasm has a narrow genetic base having its origin in few ancestral lines (HIROMOTO; VELLO, 1986; PRIOLLI et al., 2002).

According to Iqbal et al. (2010) genetic divergence is a key component of any agricultural production system. Studies on degrees of relationship and divergence between genotypes have proven that most of the cultivars on the market show high similarity (KISHA et al., 1997; PRIOLLI et al., 2002; BONATO et al., 2006; HYTEN et al., 2006).

Multivariate techniques, used to estimate genetic divergence, permit the simultaneous evaluation of several traits (WANG et al., 2006; FU et al., 2007; LEE et al., 2010; MIN et al., 2010; SHI et al., 2010). These techniques can generate important information for genetic resource maintenance, germplasm bank simplification, and core collection generation as well as assist in choosing descriptors that best represent the population diversity.

Evaluation of agronomic traits, pedigrees, geographic origins, isozymes, and DNA markers have been used to assess soybean genetic diversity

(PERRY; MCINTOSH, 1991; GRIFFIN; PALMER, 1995; GIZLICE et al., 1996; DONG et al., 2004).

This study aimed to assess the genetic variability of 22 biparental crosses of soybean and five soybean cultivars used as a control group using multivariate techniques.

MATERIAL AND METHODS

The experiment was carried out in an experimental area of 0.26 hectares in Uberlândia, Minas Gerais state (18° 55' 08" S, 48° 16' 37" W, 805 m a.s.l.).

The area where the experiment was carried out is located on a dystrophic Dark Red Latosol. The seeding was performed on Feb. 22, 2010. The experimental design was a randomized complete block with three replications of 110 progenies (F5 generation) that were assessed through 22 biparental crosses (five progenies from each cross) and five cultivars used as controls which were UFUS Riqueza, UFUS Impacta, UFUS Xavante, UFUS Milionaria and MSoy 8211, that were selected based on the indication of the area and high performance. The plot consists of four rows of soybean plants with four meters in length, 0.5 m apart from each other. The density used was ten plants per linear meter, spaced 0.10 m between plants and 0.5 m between rows, ignoring rows of edging. The plot's size was 1.5 m².

Soil preparation was done conventionally, i.e. with two plows and a harrow. Before seeding, the area was fertilized according to soil analysis. Regarding cultural practices used in weed control, herbicides were used in pre-and post-emergence, supplemented with hoeing when necessary. Diseases and pest insects were controlled in accordance with appropriate technical recommendations for the culture under suitable temperature, humidity and plant cycle conditions (EMBRAPA, 2013).

The agronomic traits evaluated were the most relevant in analyzing soybean cultivars, this procedure being performed by means of visual observation and accurate measurements according to the crop's developmental stages as proposed by Fehr and Caviness (1977). The traits evaluated were: plant height at flowering (PHF), plant height at maturity (PHM) Number of days until flowering (NDF) Number of days until maturity (NDM), height of first pod (HFP), grain number per pod with 1, 2 or 3 grains and total number of pods (TP) Weight of plant (WP) and grain yield (GY).

Initially variance analyses were performed followed by multivariate analyses to study the genetic divergence, which was determined by the

Mahalanobis distance (D^2) between all pairs of crosses and controls. Based on the genetic distance matrix a dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) and clustering by the Tocher optimization method. In order to estimate the adjustment between the dissimilarity matrix and the dendrogram generated the cophenetic correlation coefficient was calculated. In order to determine the relative importance of the evaluated traits as to the genetic dissimilarity observed among the crosses, the methodology proposed by Morais et al. (1998) was used, as well as through the participation of the components of the Mahalanobis distance (D^2). The statistical analyses were performed with the aid of software in Genetics and Statistics - GENES software (CRUZ, 2013).

In order to assist in the recommendation of superior combinations based on the magnitude of the genetic distance, one index with the highest yielding crosses and another index regarding content over the ideotype (ideal genotype proposed by the breeder) based on the best behavior observed in each evaluated trait were employed. Next, the Mahalanobis distances (D^2) between the studied genotypes (22 crosses and five controls) and the ideotype were estimated. Thus, the crosses and controls were ranked according to the distance that they presented in relation to both the most productive cross and the ideotype, the crosses which presented the smallest distances being considered the best crosses.

RESULTS AND DISCUSSION

There were significant differences ($p \leq 0.05$) in the F test in almost all traits, except for the traits number of pods with three seeds (P3), weight per plant (PP) and height of first pod insertion (HFPI), which indicates the existence of sufficient genetic variability. Assessing the genetic divergence among the crosses, by means of multivariate analysis, the relative contribution of each trait is highlighted. This analysis is based on the data for eleven quantitative traits, which contributed variably to the evaluation of the degree of divergence of the crossings. The relative contribution of each trait can be seen in Figure 1, with emphasis on plant height at maturity (PHM), which contributed over 31 % of divergence followed by the number of pods with one grain (P1), grain yield (PROD) and total number of pods (TP), with 19.6; 13.2 and 13.0 % respectively. However, because for this group of genotypes the trait P1 was not relevant in the selection of promising materials, it can be discarded

as a selection criterion. The other variables presented estimates of relative contribution of low magnitude, indicating that these traits did not

materially influence the dissimilarity among the genotypes.

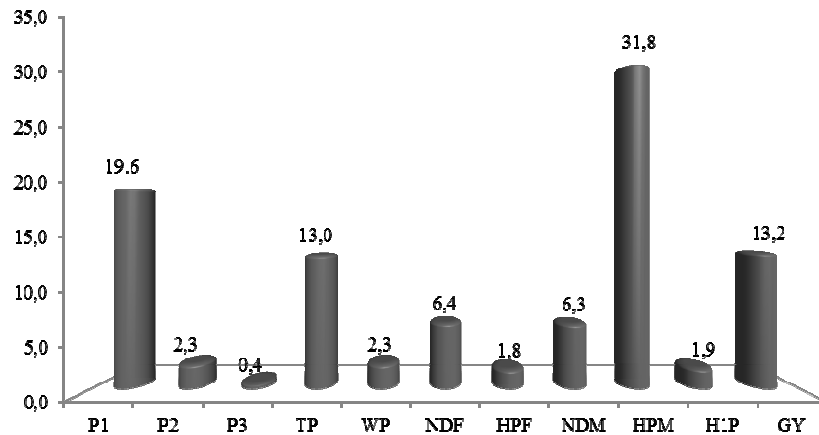


Figure 1. Relative contribution of 11 soybean agronomic traits to the genetic divergence: P1: number of pods with one grain, P2: number of pods with two grains, P3: number of pods with three grains, TP: total number of pods, WP: weight per plant (grams), NDF: number of days until flowering, HPF: plant height at flowering (cm), NDM: number of days until maturity, HPM: plant height at maturity (cm), H1P: height of insertion of first pod (cm) and GY: grain yield (kg ha^{-1}) to quantify the genetic dissimilarity between 22 biparental crosses of soybean and five control cultivars. Uberlândia, Minas Gerais, 2010.

Bharadwaj et al. (2009) in work done in India, assessing the genetic divergence among 85 soybean genotypes and two control cultivars, concluded that the character that most contributed to the divergence between genotypes was the total number of pods with 29.7 % of importance, followed by plant height at maturity with 17.5 %. The trait grain yield came in at eighth place with 3.4 %. Similar results were found by Guerra et al. (1999), working with 104 soybean genotypes, which concluded that the trait that most contributed to genetic divergence was plant height at maturity, with 23.3 % of contribution, followed by plant height at flowering with 19.2 %.

The measures of genetic divergence estimated by the Mahalanobis generalized distance (D^2), based on the eleven evaluated characters, presented distances with a minimum range of 3.2 between the crosses of CR6 and CR16 are the maximum value of 155.5 between cultivar UFUS Impact and CR21. Thus, from a genetic point of view, UFUS Impact and CR21 are the most

divergent and crosses CR6, CR16 are the most similar.

The dendrogram generated by the UPGMA method is shown in Figure 2. This method has been widely used in studies of genetic diversity of several species, including soybeans. Using this method, the characterization of genetic diversity by extreme values among genotypes is avoided (CRUZ et al., 2011). The delimitation of the groups was made by visual analysis of the dendrogram, observing there the occurrence of high-level change. When performing a cut in 43 % of genetic distance, a division of the crosses into eight groups was found: 1) CR6, CR16, CR18, CR19, CR8, CR4, CR15, CR17, CR7 and UFUS Milionaria (26); 2) CR9 and CR11; 3) CR14; 4) CR1, CR20, CR2, CR3, CR12, CR21 and CR22; 5) CR13; 6) UFUS Riqueza (23), UFUS Xavante (25) and M-SOY 8211 (27); 7) Test2 (24) and 8) CR5 and CR10. Based on the averages of the traits it was noticed that the crosses within the same group showed similar behavior in relation to the evaluated agronomic traits.

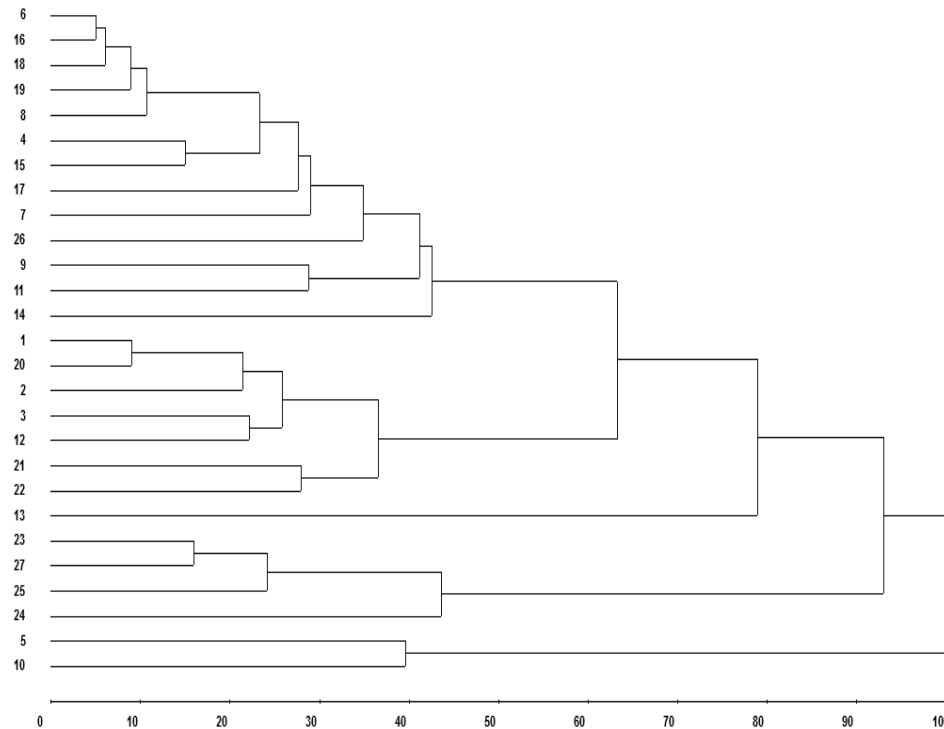


Figure 2. Dendrogram generated by the Unweighted Pair Group Method with Arithmetic mean (UPGMA) from the matrix of the Mahalanobis generalized distance between crosses 22 (1-22) and five soybean control cultivars. The value of cophenetic correlation coefficient (r) was 0.70, significant at 5% probability by the t test. Uberlandia, Minas Gerais, 2010.

According to Li and Nelson (2001) the formation of groups by the UPGMA method is useful for the selection of the progenitors, since the new hybrid combinations to be established must be based on the magnitude of their dissimilarities and potential of the parents per se. The cultivars grouped into more distant groups are more likely to be dissimilar. They may be considered promising in artificial crosses. However, even being dissimilar, it is necessary that the parents combine an elevated average and variability for the traits to be improved.

The coefficient of cophenetic correlation of the dendrogram obtained in this study was 0.70. According to Sokal and Rohlf (1962) higher values of the coefficient of cophenetic correlation indicate a good adjustment between the graphical representation of the distances and the original matrix. Priolli et al. (2010) evaluating the genetic diversity among 168 soybean cultivars through molecular markers, identified seven distinct groups. A similar result was found in this study assessing the phenotypic diversity, as well as in Mannan et al. (2010), Bharadwaj et al. (2009) and Guerra et al. (1999).

Regarding the Tocher grouping method (RAO, 1952), based on the dissimilarity matrix expressed by the Mahalanobis distances (D^2), the distribution of crosses occurred in nine groups; one

more than the grouping through UPGMA, as illustrated in Table 1.

The first group was designated as the principal group as it encompasses 52 % of the assessed genotypes, in group 1 alone 63 % of the crosses are represented. Group 2 is formed only by controls.; Groups 3, 4 and 5 each represent 9% and groups 6, 7 and 8, each with only one component, each representing 4.5 %, a result that resembled that found by Iqbal et al. (2008) and Matsuo et al. (2011).

Cruz et al. (2011) do not suggest the involvement of individuals of the same standard of dissimilarity in the crosses so that genetic variability is not restricted thus preventing negative effects on the gains to be obtained by selection. As reported by Abreu et al. (1999) and Li et al (2008) the best hybrid combinations to be tested in a breeding program must involve parents that are divergent and have high average performance.

Analyzing the average values of each crossing considering the eleven traits evaluated, it was noted that in group 1 (CR6, CR16, CR18, CR19, CR8, CR4, CR12, CR17, CR15, CR7, CR1, CR2, CR3 and CR20) the later crossings were assembled, with the group average number of days to maturity (NDM) of 125 days and more pods per plant (TP). Group 2 consists of the three controls,

UFUS Riqueza, UFUS Xavante and MSoy 8211, which were of earlier maturity than group 1 with average DTM of 117 days. Group 3 gathered crosses, CR21 and CR22, with the greatest heights at flowering and greatest number of days to

maturity, with an average of 45 cm and 130 days respectively. Group 4 (C29 and CR11) contains two crosses that showed great similarity to group 1, and the lowest yield.

Table 1. Grouping of 22 biparental crosses and five controls cultivars of soybean by the method of Tocher. Uberlandia, Minas Gerais, 2010.

Group	Number of genotypes	Crosses and controls
1	14	CR6, CR16, CR18, CR19, CR8, CR4, CR12, CR17, CR15, CR7, CR1, CR2, CR3, CR20
2	3	UFUS Riqueza, UFUS Xavante, MSoy 8211
3	2	CR21, CR22
4	2	CR9, CR11
5	2	CR5, CR10
6	1	UFUS Milionaria
7	1	CR14
8	1	CR13
9	1	UFUS Impacta

Group 5 (CR5, CR10) deserves more emphasis, because it contains higher yielding crosses, averaging 2812 kg ha⁻¹, approximately 32% higher than the yield of the best control (MSoy 8211). Groups 6 (UFUS Milionaria), 7 (CR14), 8 (CR13) and 9 (UFUS Impacta) presented average behavior for the eleven evaluated traits.

The clustering by the UPGMA method is similar to the Tocher method regarding the formation of groups among the most divergent genotypes. The agreement of these two techniques is confirmed by the fact that the crossings belonging to

groups 2, 5, 7, and 9 in Tocher's were the ones with the greatest distances from each other by UPGMA's method. This result is similar to recent studies (MULATO et al., 2010).

The graphic dispersion using traits that contributed most to the genetic divergence, plant height at maturity and grain yield is presented in Figure 3. There was agreement between the dispersion of crossings and/or cultivars and the UPGMA method and Tocher, because the crosses which were the same groups were by the farthest by graphic dispersion.

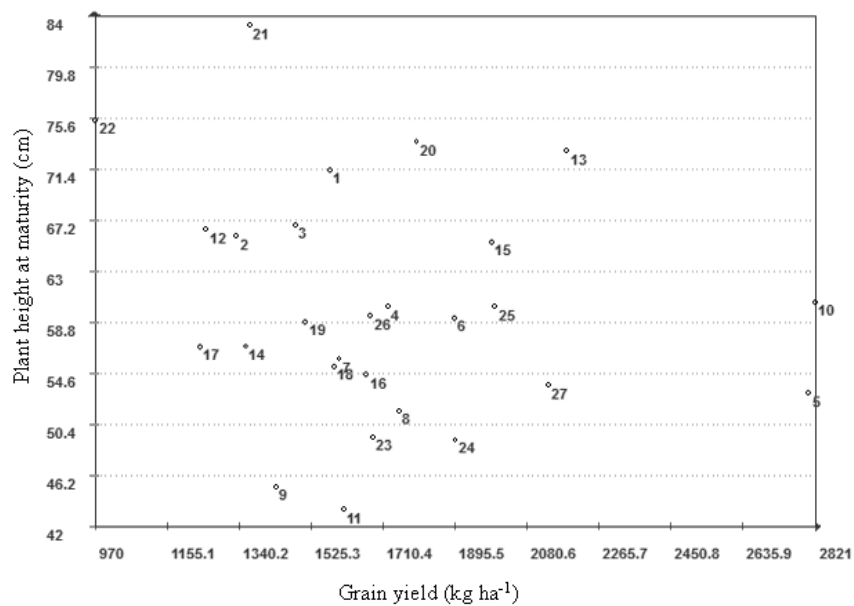


Figure 3. Graphical Dispersion of 22 crosses and five control cultivars, based on two characters: Plant height at maturity (APM) and grain yield (PROD). The genotypes 23, 24, 25, 26 and 27 refer to the controls. Uberlandia, MG, 2010.

Considering that the efficiency of a grouping method (the ability to graphically represent the contrasts between genotypes) depends on the distribution of genetic divergence among the tested genotypes (BERTAN et al., 2006) we can conclude that the Tocher technique, UPGMA and graphic dispersion were efficient.

Regarding the selection index based on distance from the most productive crossing (CR10

in Table 2) it is evident that most genotypes with intermediate genetic distances from each other (distances similar in the three methods of evaluation), were those that were closest to CR10 (CR5, CR6, CR18, CR16 and CR8). On the ranking scale, the descendants of the crosses of lesser distance from the most productive intersection can be used as sources of high grain yield in soybean breeding programs that perform hybridizations.

Table 2. Distance and ranking of the 22 of soybean crosses and five controls evaluated in the study in relation to the best intersection (CR10) and ideotype. Uberlândia, 2010.

Crosses	CR10	Crosses	Ideotype	Ranking
CR10	0	CR21	96,68	1
CR5	25,21	CR12	98,40	2
CR6	31,18	CR15	107,06	3
CR18	36,70	CR20	109,69	4
CR16	39,18	CR22	113,38	5
CR8	39,85	UFUS Milionária	115,93	6
CR15	41,22	CR1	117,85	7
UFUS Xavante	42,50	CR14	121,86	8
CR7	42,60	CR4	122,64	9
CR13	42,70	CR3	127,05	10
CR4	44,51	CR19	135,97	11
CR19	53,53	CR6	137,51	12
CR17	53,76	CR8	139,99	13
CR11	56,78	CR16	139,83	14
CR2	57,79	CR7	147,77	15
CR1	58,81	UFUS Xavante	152,69	16
UFUS Milionária	59,03	CR5	155,51	17
MSoy 8211	61,36	CR2	160,51	18
CR9	64,33	CR18	162,46	19
CR14	65,03	CR13	167,46	20
CR20	66,70	CR9	169,61	21
CR12	73,22	CR11	172,73	22
UFUS Riqueza	79,45	CR17	180,91	23
CR3	80,19	UFUS Riqueza	181,18	24
UFUS Impacta	101,91	MSoy 8211	189,83	25
CR21	107,16	CR10	201,82	26
CR22	125,55	UFUS Impacta	259,14	27

The high agreement among the employed techniques provides support for safer inferences about the distance between the crosses evaluated. Thus, it is possible to infer that the crosses CR13, CR14 and UFUS Impacta are divergent from each other and that the crossings CR5, CR10, CR21 and CR12 are recommended as they stood out when combined with the more distant ones in the studies of genetic divergence in this work. However, besides showing divergence, it is expected that the crosses involving high performance for these traits, allow the selection of superior individuals, mainly for grain yield.

CONCLUSIONS

The Tocher, UPGMA and graphic dispersion grouping methods were efficient and consistent in representing the genetic diversity between the crosses and commercial soybean cultivars.

The characters plant height at maturity, number of pods with one grain and grain yield were those that contributed most to the genetic divergence among soybean cultivars and crosses.

Hybridizations between lines derived from crosses CR13, CR14 with cultivar UFUS Impact or hybridizations of lines derived from crosses CR5

and CR10 with crossovers CR21 and CR12 are promising for obtaining segregating soybean populations.

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RESUMO: O objetivo deste trabalho foi estudar a variabilidade genética de 22 cruzamentos biparentais de soja por meio de técnicas multivariadas. O experimento foi conduzido em delineamento blocos completos casualizados com 3 repetições, constituídos por 110 genótipos provenientes de 22 cruzamentos biparentais e cinco testemunhas: UFUS Riqueza, UFUS Impacta, UFUS Xavante, UFUS Milionária e MSoy 8211. Avaliaram-se os caracteres número de dias para floração e maturidade, altura da planta na floração e maturidade, número de vagens com 1, 2 e 3 grãos na vagem, número total de vagens, peso da planta, altura da inserção da primeira vagem e a produtividade de grãos. A população exibiu variabilidade genética para a maioria dos caracteres estudados. A altura de planta na maturidade, vagem de um grão e produtividade de grãos foram os que mais contribuíram para a diversidade genética entre os cruzamentos estudados. Os três métodos de agrupamento empregados nesse trabalho foram eficientes em representar a distância genética em soja. As hibridações entre linhagens provenientes dos cruzamentos CR13, CR14 com a cultivar UFUS Impacta ou hibridações de linhagens provenientes dos cruzamentos CR5, CR10 com linhagens dos cruzamentos CR21, CR12 são promissoras para obtenção de populações segregantes de soja.

PALAVRAS-CHAVE: *Glycine max*. L. Cruzamentos. Distância genética.

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