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ORIGINAL ARTICLE

Agronomic performance and estimated genetic diversity among soybean inbred lines based on quantitative traits¹

Desempenho agronômico e estimativa da diversidade genética entre linhagens de soja baseada em caracteres quantitativos

Guilherme S. Medeiros², Pablo D. S. Cabral²*, Fernando H. L. e Silva², Jôsie C. de O. Freitas³, Luís H. R. de Campos² & Arthur M. M. F. Carrijo²

¹ Research developed at Instituto Federal de Ciência e Tecnologia Goiano, Rio Verde, GO, Brazil

² Instituto Federal de Ciência e Tecnologia Goiano/Campus Rio Verde, Rio Verde, GO, Brazil

³ Universidade Estadual de Goiás/Campus Oeste/Unidade de Palmeiras de Goiás, Palmeiras de Goiás, GO, Brazil

HIGHLIGHTS:

Soybean accessions were differentiated by multivariate statistical methods. Soybean accessions are recommended as parent plants. Differentiated accession groups were indicated to generate genetic diversity.

ABSTRACT: Although soybean is one of the most important agricultural commodities in the world, it has been losing genetic diversity in recent decades, due to the intense breeding process and reproduction method. Thus, the objective of this study was to evaluate the agronomic performance and estimate the genetic diversity among inbred lines, and identify promising crossings. A total of 66 soybean genotypes were evaluated, 61 of which were pre-commercial lines, and five commercial cultivars (AS3797, DESAFIO, M7110, M7739, M8210) as controls. The experimental design consisted of randomized blocks with three replicates. The distance matrix between the inbred lines was calculated by Mahalanobis distance and grouping by the unweighted pair group method with arithmetic mean (UPGMA). The maximum distance was 1,776.26 between cultivar M7110 and the G23 inbred line, and the minimum 1.50 between inbred lines G13 and G36, with an average distance of 364.40, demonstrating genetic diversity. The traits that contributed most to genetic divergence were number of days to flowering (66.7%) and physiological maturation (28.55%). Inbred lines G60 (higher grain yield), G51 and G33 (lower physiological maturation) and cultivar M7110 (greater divergence) stood out. Four groups were formed, with G1 having the highest grain yield and fewest days to flowering and physiological maturation. The indicated crossings are between G60, G57, CD2728, M7110, G33 and G32 (group G1) and G47 (group G4). ABSTRACT: Although soybean is one of the most important agricultural commodities in the world, it has been

Key words: Glycine max (L.) Merril, genetic variability, Mahalanobis distance, UPGMA, phenotypic correlation

RESUMO: A soja é uma das mais importantes comodities agrícolas mundiais, no entanto, devido ao intenso **RESUMO:** A soja é uma das mais importantes comodities agrícolas mundiais, no entanto, devido ao intenso processo de melhoramento e sua forma de reprodução, vem perdendo diversidade genética ao longo das últimas décadas. Assim, o objetivo deste estudo foi avaliar o desempenho agronômico, estimar a diversidade genética entre as linhagens e identificar cruzamentos promissores. Para isto, foram avaliados 66 genótipos de soja, sendo 61 linhagens pré-comerciais e cinco cultivares comerciais (AS3797, DESAFIO, M7110, M7739, M8210) como controles. O delineamento experimental foi o de blocos ao acaso com três repetições. A matriz de distância entre as linhagens foi calculada pela distância de Mahalanobis e o agrupamento pelo método da ligação média entre grupos (UPGMA). A distância máxima foi de 1.776,26 entre as linhagens M7110 e G23, e a mínima de 1,50 entre as linhagens G13 e G36, sendo a distância média de 364,40, demonstrando a existência de diversidade genética. Os caracteres que mais contribuíram para a divergência genética foram número de dias para o florescimento (66,7%) e maturação fisiológica (28,55%). As linhagens G60 (maior produtividade de grãos), G51 e G33 (menor maturação fisiológica) e a cultivar de grãos e menor número de dias para o florescimento sendo o grupo G1 com maior produtividade de grãos e menor número de dias para o forescimento estre fueros indicados são entre de grãos e menor número de dias para o florescimento e maturação fisiológica. Os cruzamentos indicados são entre G60, G57, CD2728, M7110, G33 e G32 (grupo G1) com a G47 (grupo G4).

Palavras-chave: Glycine max (L.) Merril, variabilidade genética, distância de Mahalanobis, UPGMA, correlação fenotípica

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INTRODUCTION

In recent years, soybean (*Glycine max* (L.) Merrill) has established itself as the main crop in Brazilian agribusiness, playing an important role in the national economy (Campos et al., 2023). In the 2021/22 harvest, the planted area was 41.452 million hectares, with a production of 125,552.3 million tonnes of grain, 77,199.2 million tons of which were exported, making Brazil the world's largest producer and exporter of this grain (CONAB, 2022).

Much of the success of soybean in Brazil is due to the breeding process, as evidenced by the increase in average grain yield over the last 50 years, from 1,748 to 3,517 kg ha⁻¹ (Todeschini et al., 2019). On the other hand, Swarup et al. (2021) and Valliyodan et al. (2021) reported that the modern breeding process, which includes the selection of high-yielding lines in populations with a low effective size and high degree of relatedness, may have reduced the genetic diversity of the crop.

According to Vello et al. (1988), in the 1980s, 15% of the area cultivated with soybeans in Brazil involved cultivars that were introduced from the Southern United States and the remaining 85% came from hybridization of North American varieties, generating low genetic diversity and high phenotypic similarity among Brazilian cultivars, corroborating Rodrigues et al. (2017), Bruce et al. (2019), La et al. (2019) and Bayer et al. (2022).

Information on genetic diversity among genotypes and agronomic performance are essential to breeding programs (Shilpashree et al., 2021), since they make it possible to identify hybrid combinations that can provide greater genetic variability in segregating generations (Rodrigues et al., 2017; Silva et al., 2017; Kachare et al., 2020).

In view of the above, the objective of this study was to evaluate the agronomic performance and estimate the genetic diversity among advanced soybean inbred lines based on agronomic traits, and identify promising crosses.

MATERIAL AND METHODS

The experiment was carried out between October 2017 and January 2018 (2017/18 growing season) at the Cooperativa Central de Pesquisa Agrícola (COODETEC) research station, in the municipality of Rio Verde, Goiás state, Brazil (17° 45' 57" S, 51° 01' 44" W, and average altitude of 844 m). The city of Rio Verde is located in soybean macro-region 3 and micro-region 301, according to the third approach to agricultural zoning proposed by Santos et al. (2018). The soil was classified as dystrophic Red Latosol (Oxisol) and the physicochemical characteristics of the soil in the experimental area are: Texture: clay = 420 g kg⁻¹, silt = 50 g kg⁻¹ and sand = 520 g kg⁻¹; Chemistry: pH = 5.5, CTC = 6.4, base saturation = 57.3%, K = 60 mg dm⁻³, P = 11.5 mg dm⁻³, Ca = 2.5 cmol dm⁻³, Mg = 1.0 cmol dm⁻³, Al = 0.1 cmol dm⁻³, Organic matter = 22.7 g dm⁻³.

The climate in the region is tropical Aw (Köppen-Geiger), with rainfall concentrated between October and April and a well-defined dry season in winter, with average annual rainfall between 1,200 and 1,500 mm (Alvares et al., 2013). The average annual minimum and maximum temperature is 18.8 and 28.4 °C, and average relative air humidity 65.25%.

This study examined 61 pre-commercial soybean inbred lines from a breeding program in the Brazilian Midwest and five cultivars (AS3797 (IPRO), DESAFIO (RR), M7110 (IPRO), M7739 (IPRO), M8210 (IPRO)) as controls. Between July and October 2017, all the cultivars were multiplied to standardize vigor and germination for the experiments. Sowing took place on 17/10/2017 at the COODETEC research station.

A randomized block design with three replicates was used. Plots consisted of 4-m rows spaced 0.50 m apart, with a final stand of 300,000 plants. The two central rows were considered the study area for the evaluations. All cultivation treatments were carried out in accordance with EMBRAPA (2013).

The following traits were assessed: grain yield (GY), obtained by weighing the grains of each plot, adjusting the result for 13% moisture and extrapolating it to kg ha⁻¹; 100-seed weight (100SW, in g), by averaging a random collection of 100 grains three times in each plot and weighing on a digital scale; number of pods per plant (NPP), by counting and averaging the number of pods from six plants randomly sampled in each plot; number of seeds per pod (NSP), by dividing the number of seeds by the number of pods; number of days to flowering (NDF), number of days from seedling emergence to the day when 50% of the plot was in the flowering stage; physiological maturation (PM), number of days from seedling emergence to the day when 50% of the plot was at the R8 stage (physiological maturation/harvest point); and plant height (PH, in cm), determined by measuring the main stem of six random plants in each plot at the physiological maturity stage.

Data were submitted to analysis of variance (ANOVA), $p \le 0.05$, using the F test, to verify the existence of variability between accessions and obtain the means and matrix of residual variance and covariance. A simple linear correlation analysis was performed, with significance determined by the t-test.

The dissimilarity matrix between accessions was obtained from the residual variance and covariance matrix, using the generalized Mahalanobis distance (D2). The relative contribution of traits was estimated by the Singh method (S.j). The unweighted pair group method with arithmetic mean (UPGMA) was used to group the accessions and the cophenetic correction coefficient to determine grouping accuracy in relation to the original distance. Genes and R software (Cluster and Qgraph packages) were applied for statistical analysis.

RESULTS AND DISCUSSION

A significant difference was observed by the F test at $p \le 0.01$ for all evaluated traits and considerable variation between the highest (Max) and lowest average (Min) (Table 1). These results demonstrate the existence of genetic diversity among the soybean inbred lines under study. Torres et al. (2015) observed genetic diversity among soybean cultivars in Aquidauana, Mato Grosso do Sul state, Brazil. Shilpahree et al. (2021) found genetic variation in morphological traits among 28 soybean genotypes.

The coefficients of variation (CV) were 1.11 and 28.58% for physiological maturation (MF) and number of seeds per plant (NSP), respectively. Since they are quantitative traits,

Table 1. Analysis of variance and descriptive statistics for the traits grain yield - GY, 100-seed weight - 100SW, number of pods
per plant - NPP, number of seeds per plant - NSP, number of days to flowering - NDF, physiological maturation - PM, and plant
height – PH, evaluated in 66 soybean inbred lines

SV	Root mean square						
0V	GY	100SW	NPP	NSP	NDF	PM	PH
IL	85,982,212.0**	18.2**	579.7**	3,056.8**	199.8**	314.9**	669.7**
Block	1,257,723.0	19.5	640.6	5,686.6	4.91	0.02	4.9
Error	376,070.0	4.2	254.7	1,326.4	0.58	2.14	39.1
Max.	6,680.0	24.6	122.5	237.7	61.0	145.0	150.1
Mean	4,281.0	15.2	61.1	127.4	47.0	131.0	95.6
Min.	2,254.2	8.38	20.5	48.8	32.0	107.0	50.0
CV (%)	14.32	13.45	26.11	28.58	1.61	1.11	6.53

SV - Source of variation; IL - Inbred Lines; CV - Coefficient of variation; ** - Significant at p \leq 0.01 according to the F test; Max. - Highest average; Min. - Lowest average

these values are within acceptable limits (Leite et al., 2016). Similar CV results were observed by Ferreira Júnior et al. (2015), Torres et al. (2015), Oliveira et al. (2017), and Campos et al. (2023).

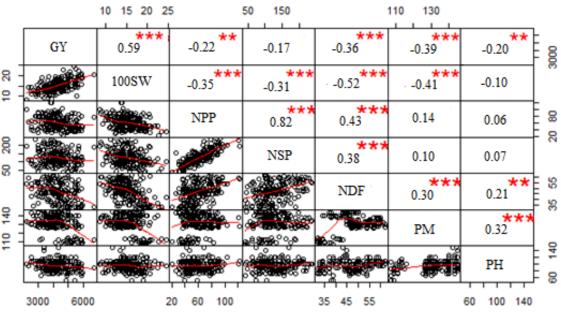
Grain yield (GY) showed a positive and significant correlation with 100-seed weight (100SW) (0.59, $p \le 0.01$) and a negative correlation with number of pods per plant (NPP) (-0.22, $p \le 0.05$), number of days to flowering (NDF) (-0.36, $p \le 0.01$), physiological maturation (PM) (-0.39, $p \le 0.01$) and plant height (PH) (-0.20, $p \le 0.05$) (Figure 1). This indicates that gains in GY are directly related to the increase in 100SW. Rigon et al. (2012) assessed the correlation between the quantitative traits of soybean, observing a positive and high magnitude of correlation between GY and 100SW, as did Campos et al. (2023).

La et al. (2019) studied wild soybean accessions, and found a positive correlation between PM and 100SW, different from that observed in the present study with commercial cultivars and lines originating from breeding programs aimed at reducing PM and increasing yield.

However, Malek et al. (2014) found a positive correlation between GY and NDF (0.62), PM (0.62) and PH (0.66), contrasting with the present study. It is important to note that the inbred lines in this study come from a breeding program aimed at launching cultivars in the Brazilian Midwest, where there is a second crop (off-season) (February to June). Thus, short-cycle cultivars and high grain yields are desired. The strategy of prioritizing the selection of cultivars with semidetermined and indeterminate growth habits adopted by soybean breeding programs in Brazil, makes it possible to obtain early and highly productive cultivars (Gesteira et al., 2018). This probably explains the negative correlations between GY and the aforementioned traits.

The number of days to flowering (NDF) exhibited the highest relative contribution to the separation of inbred lines (66.7%), followed by PM, with 28.55% (Figure 2). These results indicate that there is high variability between strains for these two traits and lower for the others. Similar results were obtained by Almeida et al. (2011) and Ferreira Júnior et al. (2015).

Soybeans are very sensitive to photoperiod and temperature. In Rio Verde, Brazil, the historical average rainfall for the period during which the experiment was conducted (October to February) is 214 mm, minimum and maximum temperature 20.4 and 30.9 °C, respectively (INMET, 2023) and photoperiod less than 13 hours (Sentelhas et al., 2017). Thus, NDF and PM are important traits in cultivar selection, since selection based on these



***, ** - Significant at $p \leq 0.001$ and $p \leq 0.01$ by t-test

Figure 1. Simple linear correlation between grain yield - GY; 100-seed weight - 100SW; number of pods per plant - NPP; number of seeds per plant - NSP; number of days to flowering - NDF; physiological maturation - PM, and plant height - PH

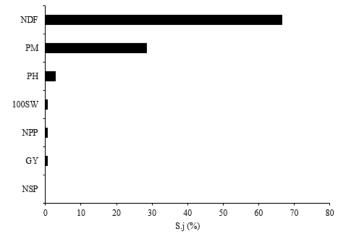


Figure 2. Relative percentage contribution (S.j%) of traits in descending order for the genetic dissimilarity of 66 soybean inbred lines, using the method proposed by Singh for grain yield - GY; 100-seed weight - 100SW; number of pods per plant - NPP; number of seeds per plant - NSP; number of days to flowering - NDF; physiological maturation - PM, and plant height - PH

traits makes it possible to stagger planting and harvesting, in order to prevent water stress during reproductive stages, and excess water during harvest (Almeida et al., 2011). Shorter-cycle cultivars (lower physiological maturity) are desired, given the off-season crop in Rio Verde (February to June) in succession to soybeans. As such, the inbred lines evaluated show variability for these traits of interest in soybean breeding.

Villela et al. (2014) studied the phenotypic and molecular diversity among Brazilian soybean cultivars carrying the RR gene, reporting that NDF accounted for 36% of genetic diversity, followed by GY (21.19%), results similar to those of Rigon et al. (2012) and the present study.

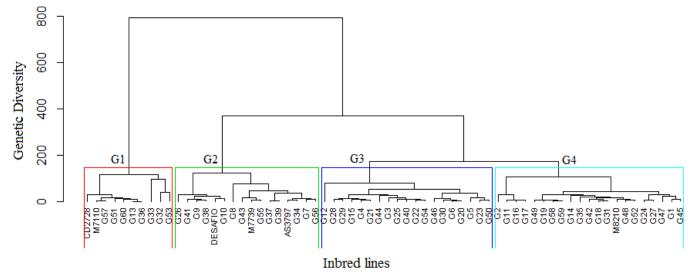
The 66 inbred lines were assessed using the generalized Mahalanobis distance and UPGMA grouping (Figure 3), divided into four groups, with Group 1 (G1) comprising: CD2728, M7110, G57, G51, G60, G13, G36, G33, G32 and G53

(15.2% of the inbred lines); Group 2 (G2): G26, G41, G9, G38, DESAFIO, G10, G8, G43, M7739, G55, G37, G39, AS3797, G34, G7 and G56 (24.2% of the inbred lines); Group 3 (G3): G12, G28, G29, G15, G4, G21, G24, G3, G25, G40, G22, G54, G46, G30, G6, G20, G5, G23 and G50 (28.8% of the inbred lines); and Group 4 (G4): G2, G11, G16, G17, G49, G19, G58, G59, G14, G35, G42, G31, M8210, G48, G52, G24, G27, G47, G1 and G45 (31.8% of the inbred lines).

The maximum distance found was 1,776.26 between cultivar M7110 and the G23 inbred line, while the minimum distance was 1.50 between the G13 and G36 inbred lines. Cultivar M7110 had the highest average difference in relation to the other inbred lines (792.4), and the average distance between all inbred lines was 364.40, demonstrating genetic variability between the inbred lines under study. Almeida et al. (2011) studied the genetic difference between soybean cultivars under irrigated floodplain conditions in southern Tocantins state, Brazil, using the generalized Mahalanobis distance, observing genetic variability between cultivars, with the genetic distance ranging from 2.65 to 374.06, while Rodrigues et al. (2017) observed a genetic distance between soybean cultivars ranging from 0.11 to 251.2.

Valliyodan et al. (2021) studied genetic diversity using SNP molecular markers between elite cultivars, wild species and local or landrace soybean, observing the formation of three distinct groups, and variation between commercial cultivars. Shipahree et al. (2021) conducted the morphological characterization of 28 soybean genotypes, reporting the formation of eight different groups. Bayer et al. (2022) conducted a pangenomic study of 1000 soybean accessions from the USDA germplasm collection, including wild and cultivated lines, reporting that with the advancement of soybean domestication through plant breeding, numerous genes were lost, and the genetic base was narrowing. They also observed that the commercial cultivars formed a larger group, divided into subgroups, demonstrating low genetic dissimilarity.

The cophenetic correlation coefficient (CCC) was 0.8802, considered satisfactory, since it represents the group's efficiency



G1 - group 1; G2 - group 2, G3 - group 3 and G4 - group 4

Figure 3. Genetic diversity dendrogram of 66 soybean inbred lines in relation to seven agronomic traits, based on the generalized Mahalanobis distance and UPGMA clustering

in capturing the information contained in the distance matrix. Similar results were reported by Rodrigues et al. (2017).

According to the four groups shown in Figure 3, group G1 obtained the highest mean GY (5,135 kg ha⁻¹), showing variability between the inbred lines with a lower (LI) and upper limit (LS) of 2,254 and 6,680 kg ha⁻¹, respectively (Figure 4A). The inbred lines with the highest averages in this group were G60, G47 and G57 with 5,830, 5,513 and 5,406 kg ha⁻¹, respectively. The average GY of groups G4, G2 and G3 were similar (4,215, 4,163 and 4,003 kg ha⁻¹, respectively). Similar behavior was observed for 100SW (Figure 4B), where the mean of the strains contained in group G1 was the highest (19.04 g), followed by G2 (15.63 g), G4 (14.61 g) and G3 (13.75 g), while inbred lines G36, G60 and G13 displayed the highest means. The grain yields found in this study are higher than the national average, which was 3,029 kg ha⁻¹ in the 2021/2022 growing season (CONAB, 2022).

For NPP, group G3 showed a wide variability between inbred lines (LI= 36.33 and LS= 122.5) and the highest average, with 70.69 pods, followed by groups G4 (63.9), G2 (54, 8) and G1 (46.9), while the inbred lines with the highest means were G22, G6 and G5. Similar behavior was observed for NSP, where group G3 obtained a wide variability (LI= 61.7 and LS= 237.7) and the highest average (148.8 seeds), followed by G4, G2 and G1, while G29, G6 and G30 obtained averages of 207, 204 and 192 seeds, respectively.

Thus, the inbred lines with the highest GY are associated with the highest 100SW and not the highest NPP and NSP, as shown in Figures 4A, B, C and D. These results corroborate Figure 1, where GY obtained a positive correlation with 100SW and negative with NPP.

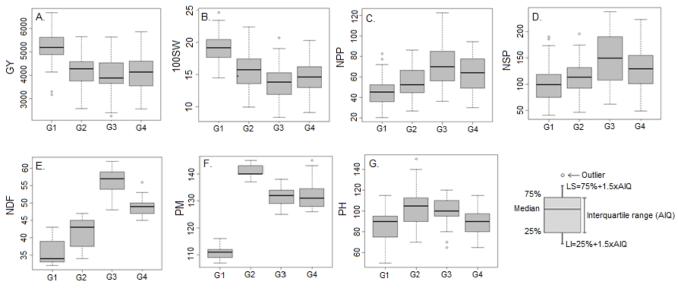
The results obtained in the present study allow inferring that short-cycle cultivars (lower NDF and PM) with a lower PH, and a smaller number of pods (NPP) obtained the highest grain yield. This is probably due to a shorter vegetative growth period and greater photoassimilate redistribution for grain yield, which may be associated with greater selection pressure in the crop breeding program for short-cycle cultivars with high grain yield.

In soybean breeding programs for the Brazilian Midwest, short-cycle cultivars are desirable, given the second crop in this region (off-season). Thus, the inbred lines of group G1 had lower mean NDF and PM, with 35.6 and 110.7 days, respectively (Figures 4E and F). Inbred lines M7110 (32 days), G57 (32.6 days) and G60 (33 days) for NDF and inbred lines G51, G33 and CD2728 for PM, with 107, 107 and 109 days, respectively, obtained the lowest means for these traits. Figure 4F shows a large discrepancy between the averages of the inbred lines of group G1 and the other groups (G2, G3 and G4), demonstrating the significant contribution of this trait to the separation of the inbred lines in the groups, as shown in Figure 2.

Average PH was 86, 89, 101 and 103 cm for groups G1, G4, G3 and G2, respectively. According to Silva et al. (2017), tall plants (above 90 cm) generally do not show much resistance to lodging, which may cause grain losses during mechanized harvesting.

The choice of parents to form the base population is one of the main steps in a breeding program. According to Silva et al. (2017), the relative performance of the agronomic traits of interest and genetic diversity must be considered to identify hybrid combinations with a greater heterotic effect. Thus, the existence of phenotypic variation due to genetic variation in the available germplasm contributes to improving the trait in question (Swarup et al., 2021).

On the other hand, it was observed that the inbred lines with the best agronomic performance (highest GY and lowest PM) were from group G1 (Figures 4A and F). As such, the recommended crosses are between G60, G57, CD2728, G33, M7110 and G32 from group G1 and the G47 inbred line of group G4, which had a high GY (5513 kg ha⁻¹) and medium PM (130 days), requiring high selection pressure to reduce PM, NDF and PH in the segregating population.



LS - Upper limit and LI- Lower limit

Figure 4. Boxplot of descriptors: grain yield - GY (A); 100-seed weight - 100SW (B); number of pods per plant - NPP (C); number of seeds per plant - NSP (D); number of days to flowering - NDF (E); physiological maturation - PM (F); plant height - PH (G) of the four groups formed (G1, G2, G3 and G4) using Mahalanobis dissimilarity and UPGMA clustering

CONCLUSIONS

1. There is genetic diversity among the soybean inbred lines under study.

2. Four groups were formed, with group G1 exhibiting the highest grain yield and lowest number of days to flowering and physiological maturation.

3. The traits that most contributed to genetic divergence were number of days to flowering (66.7%) and physiological maturation (28.55%).

4. Inbred lines G60 (higher grain yield), G51 and G33 (lower physiological maturation) and cultivar M7110 (greater divergence) stood out.

5. The indicated crossings are between pre-commercial inbred lines G60, G57, G33 and G32 and commercial cultivars M7110 and CD2728 of group G1 and the G47 pre-commercial inbred line of group G4 to obtain new recombinants, higher grain yield and shorter cycles.

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