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Univariate and multivariate analysis of genetic diversity in common bean[1]

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ABSTRACT

Genetic diversity is important for conservation and genetic improvement of common beans. This study aimed to estimate the genetic diversity among common bean genotypes from the Embrapa germplasm collection using univariate and multivariate analyses. The experiment was conducted in the region of Aquidauana-MS, at the State University of Mato Grosso do Sul, in a randomized block design with three replications and twenty-three genotypes, in 2021. The agronomic traits considered in the study were plant height, height to first pod, number of branches, number of pods per plant, number of grains per pod, hundred-grain mass, and grain yield. Descriptive analysis, univariate and multivariate variance analyses, mean clustering, phenotypic correlation network, UPGMA analysis (Unweighted Pair Group Method with Arithmetic Mean), and canonical variables were used to examine the data. The genotypes showed significant differences for plant height, height to first pod, number of branches per plant, number of pods per plant and yield, with potential for the selection of these traits. CNFC17278, CNFC17305, CNFC19133 and CNFC19198 showed superior yield potential compared to the other lines. The combined use of statistical methodologies can provide more information about the genotypes studied.

Keywords: UPGMA analysis; *Phaseolus vulgaris* L.; phenotypic correlation network; selection; canonical variables.

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INTRODUCTION

The characterization of genetic diversity among common bean genotypes is important for breeding programs of this species (Santos *et al.*, 2022) because knowing how this indicator is distributed, within and between groups, is fundamental for any system of conservation and genetic improvement of plants (Cruz *et al.*, 2014; Amabile *et al.*, 2018). Above all, statistical analyses help to identify and select promising individuals and agronomic variables of interest in these programs (Cruz *et al.*, 2020).

Clustering of means and correlations are auxiliary tools in the strategy for the selection of superior genotypes (Souza *et al.*, 2021). Phenotypic correlations are important for identifying favorable traits that can be used in the indirect selection of a desirable trait (Mendonça *et al.*, 2018), indicating the presence or absence of an association between two traits, whether positive or negative (Cruz & Nascimento, 2018; Cruz *et al*., 2014) and contributing to selection efficiency (Zuffo *et al.*, 2018).

Genetic diversity in germplasm banks can be predicted by multivariate analysis, using clustering methods, which are easy to interpret, but depend on previously estimated dissimilarity measures such as Euclidean distance or generalized Mahalanobis distance. In addition, scatter plot techniques, for example, using principal components and principal coordinates when the data have no repetitions and canonical variables when experiments have repetitions (Cruz *et al.*, 2020).

Studies that use univariate analysis have been conducted in economically important species to help in the selection of superior individuals with high grain yield in numerous regions (Silva *et al.*, 2017; Nobre *et al.*, 2018; Zuffo *et al.*, 2018; Gonçalves & Lima, 2021). Along with these studies, analyses of genetic divergence using agronomic traits in common bean, based on multivariate analysis, have also been performed by a number of researchers (Sulzbacher *et al.*, 2017; Tavares *et al.*, 2018; Leite *et al.*, 2019; Santos *et al.*, 2015; Santos *et al.* al., 2022).

Even though there are different statistical methods to assist in the selection of genotypes in the breeding of common bean, the relationship between these univariate and multivariate methodologies is a subject rarely addressed in research. Besides, there has been no information on this group of cultivars and advanced lines of common bean in the edaphoclimatic conditions of the Aquidauana-MS region, in the Cerrado-Pantanal ecotone. Furthermore, this study can contribute to the identification of genotypes that perform well in the region, in addition to promote the exploitation and cultivation of this crop in the municipality, mainly by family farming.

In the current study, the main objective was to estimate the genetic diversity among common bean genotypes from the Embrapa germplasm bank, cultivated in the region of Aquidauana-MS, using univariate and multivariate analyses to infer on the selection of superior genotypes and agronomic characteristics of interest, as well as to relate the statistical analyses used in the discrimination between materials.

MATERIAL AND METHODS

The experiment was conducted from April to July 2021, in the experimental field of the State University of Mato Grosso do Sul, at the University Unit of Aquidauana (UEMS/UUA), located at coordinates 20°27’5.30” South and 55°40’ 23.07” West, average altitude of 170 meters, in the Cerrado-Pantanal ecotone.

The climate of the region according to the Köppen-Geiger classification is type Aw (tropical sub-humid), with a rainy season in summer and a dry season in winter, average annual rainfall of 1,200 mm and average annual temperature of 24ºC. The maximum, minimum and average temperatures during the experiment were recorded at the Meteorological Data Collection Platform of the Laboratory for Management and Conservation of Soil and Water in Aquidauana-MS. The maximum temperature recorded was 35.3ºC in May and the minimum was 3.3ºC in July. The rainfall reached 92.4 mm during the cycle.

The soil in the area was classified by Schiavo *et al.* (2010), according to Embrapa (2006) criteria, as dystrophic Red Argisol with sandy texture, moderately deep, well drained and good fertility. Soil chemical properties were analyzed in the 0-20 cm layer, according to the Manual of Chemical Analysis of Soils, Plants and Fertilizers (Silva, 2009) and showed base saturation (V%) of 64% and total acidity or potential (H+Al) of 1.80 cmol dm^3 of soil. Potassium (K), Calcium (Ca), and Magnesium (Mg) contents were 0.27, 2.40 and 0.60 cmol dm^3 of soil, respectively. The soil cationic exchange capacity (CEC) was measured at 5.07, pH at 5 (CaCl), and Phosphorus (P) at 18 mg dm^3.

Soil was prepared in the conventional tillage, with plowing and leveling harrowing. Planting furrows were manually opened to a depth of 5 centimeters. Sowing fertilization was applied in the furrows at the rate of 150 kg ha^{-1} of the formula 04-20-20, following the routinely recommended fertilization for common bean of applying part of the nitrogen in the furrow, with phosphorus and potassium at sowing, and part in top dressing (Barbosa Filho & Silva, 1994; Barbosa Filho & Silva, 2000; Barbosa Filho, 2004; Barbosa Filho *et al*., 2005). After the fertilizer incorporation, manual sowing was carried out on 04/17/2021, and the first emerged seedlings appeared from 04/21/2021.

Due to the expected low rainfall in the winter, sprinklers were installed in the experimental area and periodic irrigation was carried out to complement the water demand of 250 mm required by the crop, as described by Oliveira *et al.* (2018).

Hand weeding was performed from the phenological stage V2 to R5. Topdressing nitrogen fertilization was carried out when the crop entered the V4 stage. Urea at the rate 133 kg ha^{-1} of the commercial fertilizer was distributed along the row in thin strips. Afterward, irrigation was applied for incorporation of the fertilizer.

The plots consisted of four rows of plants 4 m long, spaced 0.5 m apart, with density of 12 plants m^{-1}. The experiment was arranged in a randomized block design, with three replications. The treatments consisted of 23 bean genotypes from the Embrapa Rice and Beans germplasm bank, among 19 advanced lines and 4 cultivars of the commercial group Carioca, as described below: CNFC17182, CNFC17259, CNFC17260, CNFC17264, CNFC17270, CNFC17273, CNFC17275, CNFC17278, CNFC17302, CNFC17303, CNFC17304, CNFC17305, CNFC17310, CNFC17328, CNFC17589, CNFC19133, CNFC19193, CNFC19198, CNFC19205, BRS Estilo, BRSFC402, BRSFC414 and Pérola.

At the end of the cycle, 83 days after emergence (DAE), with the plants at the phenological stage R9 (maturation), 10 plants were randomly selected per plot, harvested and evaluated for the following agronomic characteristics:

a) Plant height (ALP): height from the root collar to the apex of the main stem using a flexible tape measure (cm);

b) Height to first pod (AIV): height from soil at the base of plant to the first pod using a tape measure (cm);

c) Number of secondary branches per plant (NRP): count of the number of existing secondary branches;

d) Number of pods per plant (NVP): count of the average number of pods;

e) Number of grains per pod (NGV): count of the average number of grains per pod, evaluating 3 pods of each of the 10 plants sampled for the trait NVP;

After the harvest, the following traits were also evaluated:

f) Hundred-grain dry mass (MCG): weight measured on a semi-analytical scale, a sample of 100 grains from each plot, then converting moisture to 13% (g);

g) Dry grain yield (PROD): weight of all the grains in the plot, and adjusted moisture content to 13% and transformed into kg ha^{-1}.

Descriptive analysis, univariate and multivariate variance analyses, grouping of means, phenotypic correlation network, UPGMA analysis (Unweighted Pair Group Method with Arithmetic Mean), and canonical variables were used to examine the data.

The data were analyzed by analysis of variance (ANOVA) at 5% significance, with contrast of the controls (cultivars), and correlations, in the GENES software (Cruz, 2013; Falconer, 1987). Phenotypic correlation network, Multivariate Analysis of Variance (MANOVA), and canonical variables were performed using the Rbio software, with all residual assumptions met for each test (Cruz, 2013). In addition, descriptive analysis and UPGMA (unweighted pair group method average) cluster analysis were performed with the RStudio program, in the respective packages, ExpDes.pt version 1.2.2 and MultivariateAnalyses version 0.4.4.

Multivariate statistical analyzes were carried out to investigate the relative importance of the traits and the relationship between the methodologies, the genetic diversity was evaluated by UPGMA clustering and canonical variables. Dissimilarity between genotypes was measured using the generalized Mahalanobis distance, and the subsamples were grouped by UPGMA using the dissimilarity matrix, as in Cruz & Regazzi (2020). The analysis of canonical variables showed the genetic divergence through the dispersion of scores in a graph, with the axes represented by the first two variables, as described by Cruz & Regazzi (2020).

RESULTS AND DISCUSSION

The analysis of variance showed the existence of genetic variation between the genotypes, with significant differences for the agronomic traits plant height, height to first pod, number of branches per plant, number of pods per plant and yield (Table 1). The results indicate that this group, in theory, is promising for the selection of these components, despite the fact that the contrast between the lines and the cultivars (controls) showed no significant difference for plant architecture traits. These traits are primary components of production and grain yield, indicating that the lines in the study are as good as the cultivars already available in the market.

The existence of genetic variability in the germplasm is fundamental for crop improvement, as it allows for the selection of superior genotypes and the combination of desirable traits, thereby increasing the efficiency of the breeding process. Based on the study results, the next steps include field validation of the selected genotypes and continued evaluation of their agronomic performance to ensure the development of new varieties with higher productivity and resistance to biotic and abiotic stresses.

The existence of genetic variation in the group is one of the determining factors for improvement programs (Cruz *et al.*, 2014). Variation originated mainly from heritable mutations (Ramalho *et al.*, 2012) is one of the principles of life, and without this condition, the plant kingdom and all other kingdoms would not have evolved (Borém *et al.*, 2021).

The findings of this study indicate that there is significant genetic variability among the evaluated genotypes, which is essential for the breeding process. The lines identified as superior can be used in crossing programs to develop new varieties with better agronomic performance and greater resistance to environmental stresses. For future studies, we propose validating these lines under different field conditions and evaluating other agronomic and quality traits to produce even more adapted and productive cultivars.

The univariate and multivariate analysis of genetic diversity in common bean enables the understanding of genetic variability in beans, revealing patterns and relationships among genetic characteristics. The results benefit plant breeding, especially in selecting bean genotypes that are more adapted to different environments, with greater disease resistance, productivity, and grain quality, contributing to sustainability and food security in agriculture (Cruz *et al*., 2021).

The coefficients of variation for plant height, 100-grain mass, number of grains per pod and yield (Table 1) were less than 20%, which were good, according to the classification of Gomes (2009); however, the other traits had coefficients of variation moderately good, between 20% and 40%. This parameter is frequently used by researchers as an indicator of experimental quality and accuracy. These results are in the range similar to those reported by authors who evaluated agronomic traits in common bean genotypes (Silva *et al.*, 2017; Hiolanda *et al.*, 2018; Terra *et al.*, 2019; Santos *et al.*, 2022).

The clustering of means (Table 2) confirmed the diversity among individuals for the traits plant height, height to first pod, number of branches per plant, number of pods per plant, and yield. Table 2 shows that the genotypes that presented component means followed by the letter a were superior to the others within the group.

Lines CNFC17278, CNFC17305, CNFC19133 and CNFC19198 expressed the highest yields, with 1,117.88 kg ha^{-1}; 1,158.12 kg ha^{-1}; 1,197.90 kg h^{-1}; and 1,104.86 kg ha^{-1}, respectively, which were higher than the Brazilian average yield for the 2021/2022 harvest of 1,102 kg ha^{-1} (Conab, 2022). Among these four lines, CNFC19133 and CNFC17278 were also superior in plant height (71.63 cm and 80.59 cm, respectively), height to first pod (12.88 cm and 10.55 cm, respectively), number of branches per plant

Table 1: Analysis of variance of plant architecture traits, primary components of production and grain yield, among 23 common bean genotypes cultivated in Aquidauana-MS, in 2021

Source of Variation	DF	ALP	AIV	MCG	NGV	NRP	NVP	PROD
Blocks	2	530.20	5.30	16.62	1.27	10.24	17.10	75375.57
Genotypes	22	332.61**	10.65**	8.16^{ns}	0.52^{ns}	2.02*	16.69*	162858.98**
Lines	18	276.84*	11.52**	7.89^{ns}	0.58^{ns}	2.29**	19.32*	186729.70**
Cultivars (controls)	3	769.26**	5.26^{ns}	12.09^{ns}	0.21^{ns}	0.18^{ns}	6.25^{ns}	73311.73*
Lines vs Cultivars	1	26.36^{ns}	11.19^{ns}	1.23^{ns}	0.36^{ns}	2.66^{ns}	0.59^{ns}	1827.82^{ns}
Residue	44	145.66	4.63	4.72	0.44	0.96	8.58	19195.7
CV (%)		18.07	22.65	8.28	14.82	36.95	30.02	16.30
Contrast between Lines vs Cultivars								
Lines		67.07a	9.69a	26.28a	4.46a	2.74a	9.80a	1001.50a
Cultivars		65.44a	8.63a	25.93a	4.65a	2.22a	9.56a	911.38a
Overall Mean		66.79	9.50	26.21	4.49	2.65	9.75	849.99

ALP = plant height (cm); AIV = height to first pod (cm); MCG = 100-grain mass (gram); NGV = number of grains per pod; NRP = number of secondary branches per plant; NVP = number of pods per plant; PROD = grain yield (kg ha^{-1}); **; *; ns = significant and non-significant at 1% and 5% by F-test. respectively; DF = Degree of freedom; CV = coefficient of variation (%); Means followed by different letters differ by the contrast estimated by the analysis of variance.

(5.00 and 2.29, respectively), and number of pods per plant (11.38 and 10.59, respectively) along with the other genotypes followed by the same letter. Height to first pod of these lines were 12.88 cm and 10.55 cm, respectively, and are considered promising, as they allow for a good mechanized harvest, minimizing losses (Moura *et al.*, 2013).

The Scott-Knott test is used to infer the selection of promising materials within the group such as the lines CNFC17278, CNFC17305, CNFC19133, and CNFC19198 that showed superior yields among the cultivars studied. The superiority of these lines meets one of the objectives of plant breeding programs, which is to provide genotypes with superior agronomic performance than those already available on the market, due to gains in the selection of individuals or selection of agronomic traits of interest (Machado, 2014; Cruz *et al.*, 2014; Borém & Miranda, 2013; Borém *et al.*, 2021).

Figure 1 illustrates the phenotypic correlation network between the seven agronomic traits evaluated in the 23 studied genotypes. The number of branches per plant and the number of pods per plant showed the highest correlation, followed by the number of pods per plant and yield, and then, plant height and height to first pod. The network shows that the highest correlations with yield were number of pods per plant, number of grains per pod and plant height. Pointing up that the highest plants had the largest numbers of pods and grains per pod, and consequently highest grain yields. Once the selection is applied to one of these correlated traits, it may cause significant changes in yield (Ramalho *et al.*, 2012).

The distance between traits is proportional to the value of the existing correlation between them. The intensity of these correlations is represented by the edge thickness and the vector indicates the direction of the influence between the components. The green color represents a positive correlation, while the red color represents a negative correlation.

Table 2: Grouping of means by the Scott-Knott test of plant architecture traits, primary components of production and grain yield, among 23 genotypes of common bean cultivated in Aquidauana-MS in 2021

Genotype	ALP	AIV	NRP	NVP	PROD
CNFC17182	58.96 b	11.17 a	2.59 a	10.92 a	733.37 c
CNFC17259	90.56 a	10.75 a	4.00 a	13.19 a	720.72 c
CNFC17260	74.33 a	12.59 a	1.38 b	6.42 a	836.00 b
CNFC17264	76.63 a	12.04 a	2.67 a	9.92 a	903.20 b
CNFC17270	61.88 b	11.38 a	2.56 a	9.44 a	768.61 c
CNFC17273	72.84 a	9.38 b	3.46 a	13.04 a	989.06 b
CNFC17275	60.42 b	9.34 b	3.38 a	10.29 a	621.57 c
CNFC17278	80.59 a	10.55 a	2.29 a	10.59 a	1.117.88 a
CNFC17302	70.04 a	11.13 a	3.34 a	4.88 b	239.04 d
CNFC17303	61.54 b	9.55 b	2.29 a	8.30 a	704.10 c
CNFC17304	58.00 b	7.75 b	1.56 b	6.88 a	893.99 b
CNFC17305	63.09 b	7.50 b	2.79 a	10.09 a	1.158.12 a
CNFC17310	64.75 b	7.13 b	2.75 a	13.38 a	989.43 b
CNFC17328	74.38 a	8.42 b	1.92 b	7.88 a	912.10 b
CNFC17589	65.42 b	6.21 b	3.04 a	14.29 a	982.28 b
CNFC19133	71.63 a	12.88 a	5.00 a	11.38 a	1.197.90 a
CNFC19193	59.13 b	10.25 a	2.75 a	7.96 a	933.13 b
CNFC19198	59.67 b	8.84 b	2.62 a	8.71 a	1.104.86 a
CNFC19205	50.50 b	7.25 b	1.63 b	8.63 a	389.44 d
BRSESTILO	45.09 b	9.38 b	1.92 b	8.13 a	620.99 c
BRSFC402	78.63 a	8.67 b	2.13 a	11.50 a	923.26 b
BRSFC414	60.21 b	6.75 b	2.38 a	8.92 a	835.23 b
PÉROLA	77.84 a	9.71 b	2.46 a	9.67 a	975.64 b

Notes: Means followed by the same letters in the same column belong to the same group by the Scott Knott test; at 95% probability ALP = plant height (cm); AIV = height to first pod (cm); NRP = number of secondary branches per plant; NVP = number of pods per plant. PROD = grain yield (kg ha^{-1}).

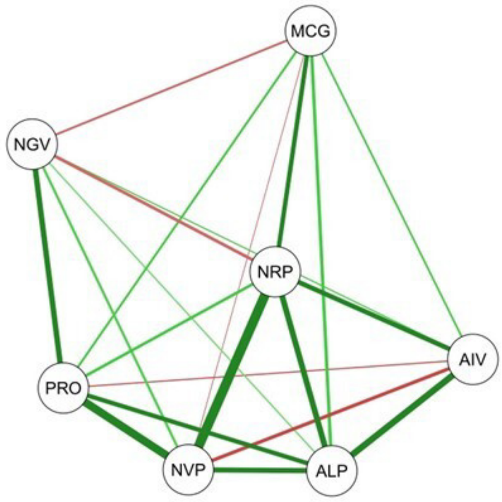

Figure 1: Phenotypic correlation network of common bean (*Phaseolus vulgaris* L) traits of the 23 genotypes.

The correlation network has been used in the characterization of complex systems in several areas of knowledge, but more recently in the genetic improvement of plants (Formagio *et al.*, 2021; Cogo *et al.*, 2022). The use of this technique facilitates the visualization of correlations between the traits studied, requiring less time for evaluation and fewer resources for use in genetic improvement programs (Mendes *et al.*, 2019).

Multivariate analysis using the UPGMA clustering, based on the Mahalanobis generalized distance to measure dissimilarity, clustered the genotypes into three groups (Figure 2). Similarly, Tavares *et al.* (2018), in a study with nineteen common bean cultivars, reported a not so large discrepancy between the genotypes.

Group I comprised the lines CNFC17302 and CNFC19205. Group II separated 8 genotypes with genetic similarity, including 6 lines and the 2 cultivars BRSESTILO and BRSFC414. Group III included 13 genotypes, 11 lines and the 2 cultivars Pérola and BRSFC402. It is worth noting that the 4 lines that had superior yield by the Scott-Knott test, CNFC17278, CNFC17305, CNFC19133 and CNFC19198, were clustered into group III, along with the other similar genotypes.

Considering the structure of correlation existing between the evaluated agronomic traits, the genetic divergence was analyzed by the canonical variables based on the multivariate analysis of variance (MANOVA) (Table 3). The Fischer F test approximation indicates that there is difference between the genotypes, taking all the characteristics evaluated simultaneously. Ledo *et al.* (2003), Vieira *et al.* (2007), and Taveira *et al.* (2020) also used MANOVA to analyze the data in order to verify the presence of genetic diversity among the individuals studied.

Figure 3 shows the genotype scatter plot of the first two canonical variables, which explain 66.7% of the total variation. The dispersion of the scores is formed by discriminating between the genotypes, to study the main traits that accounted for the difference between them (Cruz & Regazzi, 2020). The strain CNFC17278 stood out for yield, while CNFC19133 for plant height. The lines CNFC 19198 and CNFC17305 were important for the number of grains per pod.

The clusters obtained by the scatter plot in the two canonical variables were in agreement with those obtained by the UPGMA method and by the Scott-Knott test, mainly for the lines CNFC17278, CNFC17305, CNFC19133 and CNFC19198, which showed the highest yields and the other agronomic traits.

Table 3: Multivariate analysis of variance (MANOVA) of genotypes considering all traits evaluated at the same time

Source of Variation	**DF**	**Pillai Test**	**Approximation F**
Genotypes	22	0.00239	2.1964^{***}
Blocks	2	0.318877	3.8309^{***}
Residue	44		

***: significant by F-test at 0.1%.

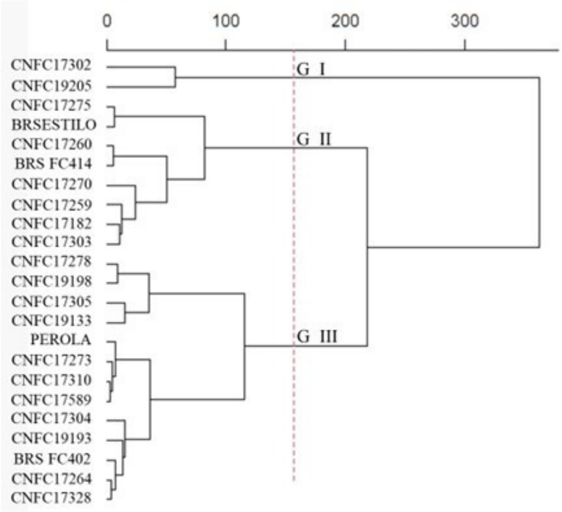

Figure 2: Dendrogram of genetic dissimilarity between 23 genotypes by the UPGMA clustering method using the generalized Mahalanobis distance.

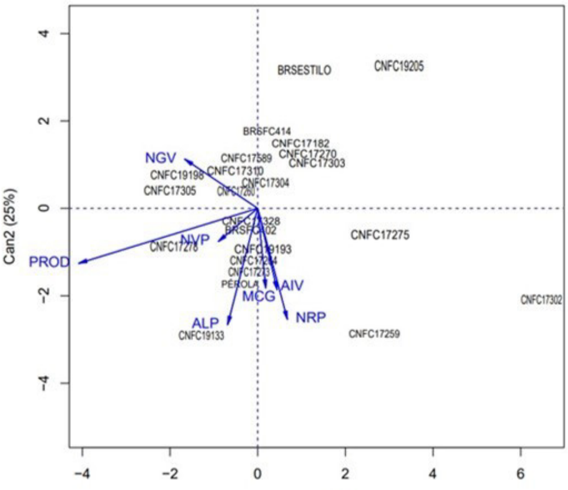

Figure 3: Scatter plot of scores of the first two canonical variables (Can1. Can2) from seven agronomic traits evaluated in 23 common bean genotypes.

CONCLUSIONS

In this study, we reveal considerable diversity among common bean genotypes from the Embrapa germplasm collection.

Lines CNFC17278, CNFC17305, CNFC19133 and CNFC19198 showed yield performances over the other genotypes, allowing the inference of selection of these materials.

Multivariate analyses grouped genotypes into different groups, confirming the existence of genetic diversity among individuals.

The combined use of statistical methodologies provides more information about the studied group, allowing the selection of superior individuals and agronomic traits of interest for the region of Aquidauana-MS.

These findings are of great importance for the bean breeding process, as they identify genetically distinct and agronomically superior materials that can be used in crossing programs to develop new varieties with better performance and greater resistance to environmental stresses.

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