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Genetic Divergence and Selection of Genitors Aiming to Generate Variability in Common Bean Lines

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Date Published: ABSTRACT

27 October 2022 The objective of this work was to evaluate the genetic divergence between common bean strains from the breeding program of the Agricultural Research Institute of Mozambique (IIAM)-Zonal Centre (CZC) and to identify potential

Keywords: genitors to generate segregating populations with high average and wide genetic variability. Five common bean lines were evaluated in a randomized block design with three repetitions. The quantitative characteristics evaluated were Leaf area index, days to 50% flowering, days to maturity, the number of pods per plant, 100-seed weight and yield, while the qualitative characteristics were Growth habit, Epicotyl colour, Leaf colour intensity, Grain colour and Grain size. The data obtained were submitted to variance analysis and grouping of means by the Scott-Knott test. The relative importance of characteristics was evaluated by the Singh. Clustering was performed by the Tocher optimization method and distance projection in the 2D plane, using the generalized Mahalanobis distance as a measure of dissimilarity. The degree of preservation of genetic distances in the 2D plane was assessed by the Cohenetic Correlation Coefficient, distortion, and stress. The LPA54 and Bonus lines were highlighted by direct selection based on the performance per se of their characteristics. Genetic variability was checked for days to 50%

Phaseolus vulgaris L.

Genetic Divergence

flowering pods per plant, and 100-seed weight and the strains were separated into two groups. The crosses between (G19833, LPA54, Manteiga, and Bonus) with the strain DOR364 there is the potential for obtaining hybrids with higher heterotic effect and segregating populations with high variability. The 100-seed weight, pods per plant, epicotyl colour, and grain size were the most important in the discrimination of the strains.

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INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is one of the agricultural products of major socio-economic importance in Mozambique, and with its remarkable source of protein, it is a staple food in the diet of the population and also a source of income for small and medium producers (Mercat et al, 2013). The bean improvement program in most African countries, and Mozambique in particular, is based on the selection of material introduced from other countries, with consolidated and stable programs, such as the Brazilian Agricultural Research Corporation (EMBRAPA), the Agronomic Institute of Campinas (IAC) in Brazil, and the International Centre for Tropical Agriculture (CIAT), Cali-Colombia. In addition, the low availability of resources and skilled labour makes it difficult to evaluate a set of genotypes jointly. This requires a greater technical knowledge of the genetic materials already introduced, to perform targeted crosses to

maximize the use of existing resources (FAO, 2019).

The genetic improvement programs for common beans are almost exclusively based on the hybridization of cultivars and obtaining superior lines from segregating populations (Tsutsumi et al., 2015). The choice of genitors to compose the hybridization block is a crucial step and should be carried out thoroughly and carefully since the success of the subsequent stages of the breeding program depends on it (Vale et al., 2015). Rinald et al. (2007) recommend inter-crossing between cultivars with superior and divergent performances to form the base population.

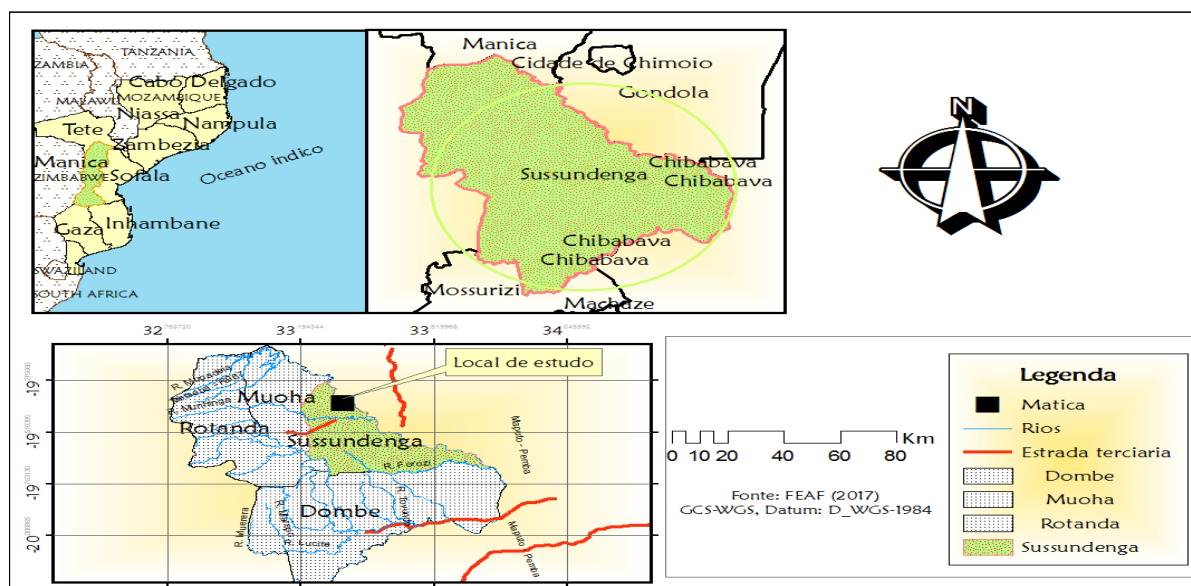
Predictive studies of genetic diversity aiming at the choice of genitors have been widely used by several researchers (Maziero et al., 2017; Sulzbacher et al., 2017; Vidyakar et al., 2017). This model does not require the prior obtaining of hybrid combinations, because it considers the agronomic, morphological, physiological, and/or molecular differences

exposed by the genitors in determining genetic variability (Cruz et al., 2012). Thus, this study aimed to estimate the genetic divergence between strains of common bean and identify contrasting and superior genitors to produce segregating populations with high variability.

MATERIAL AND METHODS

The experiment was conducted in the 2021/2022 crop year at the Institute of Agrarian Research of Mozambique (IIAM)- Zonal Centre (ZC) at the Sussundenga Agricultural Station (EAS) in Manica Province-Mozambique. Geographically, the study site is located in the Revue River basin at latitude 19°32' South, longitude 33°35' east, and 635 m above mean sea level (*Figure 1*)

Figure 1: Geographical location of the experiment site



Five common bean lines were evaluated: Manteiga, DOR364, G19833, LPA54, and Bonus, from the grain legume program of (IIAM), Zonal Centre (ZC), at the Sussundenga Agricultural Station (EAS). The experimental design used was a randomized block design with five strains and three repetitions in which each plot was composed of four rows of plants four meters long. Sowing was performed at a spacing of 0.50 m between rows and 0.1 m between plants in the period from March to June 2022. The cultural treatments were performed as recommended by the bean agronomic management literature (Carneiro et al., 2015). The quantitative characteristics evaluated were: leaf area index (LAI), determined by the length-width method; days to 50% flowering (D50F); days to maturity (DM); the number of pods per plant (NPP); 100-seed weight (100S), and yield (Yield).

The quantitative variables were submitted to univariate analysis of variance to verify the

existence of variability among strains, at 5% probability, by the F test. The grouping of the means between the characteristics of the strains was performed using the Scott-Knott test at a 5% probability level. The dissimilarity between the lines was measured by the Mahalanobis distance.

The qualitative characteristics evaluated were: growth habit (GH), epicotyl colour (EC), leaf colour intensity (LCI), grain colour (GC), and grain size (GS). The variables were considered as binary multi-categorical using coding (1=presence, 0=absence) and the similarity between the strains was estimated by the simple coincidence method using the index: $= C / (C + D)$, where: C is the total category agreement for all variables considered and D is the total category disagreement for all variables considered.

In both data sets, clustering by Tocher's optimization method and graphical scatter was employed. The consistency of the graphical

dispersion clustering was checked using Cophenetic correlation, skewness, and stress.

The correlation between the distance measures of the genitors about the qualitative and quantitative characteristics was evaluated by the t-test and Mantel's (1967) test with 5000 simulations. Finally, the relative contribution of the characteristic to genetic divergence was quantified by Singh's (1981) criterion. All analyses were performed using the computational resources of the GENES software (Cruz, 2013).

RESULTS AND DISCUSSION

The lines showed a statistical difference of 1% for the characteristics, D50F, NPP, and 100-seed weight (*Table 1*), thus allowing the inference of the existence of variability among lines related to these characteristics. As for the characteristics LAI, DM, and YIELD were not significant, indicating that the means of the lineages for these characteristics were equal, which emphasizes the need for developing new cultivars. Similar results were reported by Gonçalves et al. (2016) who observed statistical differences in flowering, pods per plant, 100-seed weight, and non-significant grain yield.

Table 1: Summary of variance analysis of Leaf area index (LAI), days to 50% flowering (D50F), days to maturity (DM), the number of pods per plant (NPP), 100-seed weight (100-seed) and yield (YIELD) in common bean lines

SV	GL	SM ¹					
		LAI	D50F	DM	NPP	100 SEEDS	YIELD
Blocks	2	3.80	1.07	16.47	27.80	24.11	40333.45
Treatments	4	3.90	29.57**	106.43	28.83**	132.54**	304319.67
Residua	8	3.30	3.07	29.38	2.13	8.06	182495.82
Mean		7.40	39.73	79.47	12.00	55.93	2067.06
CV (%) ²		24.55	4.41	6.82	12.17	5.08	20.67

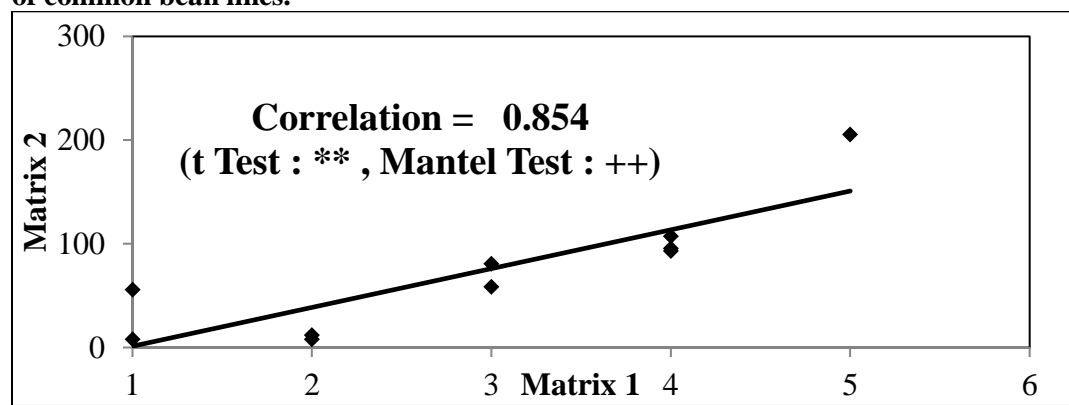
SM¹- Mean Square; CV (%)²:- Coefficient of Variation; **³-significant by the F test at 1% probability.

In *Table 2*, the distances obtained in quantitative characteristics through Mahalanobis dissimilarity, below the diagonal, show that strains 2 and 3 were the most genetically distant, followed by pairs of strains 2 x 4, 1 x 2 2 x 5, and 4 x 3 respectively. Whereas, line pairs 1 x 4, and for the distances obtained in qualitative characteristics through the measure of simple coincidence dissimilarity, above the diagonal, highlight the pairs of the lines 2 x 3, 2 x 4, 2 x 5, 1 x 2 as the most distant, followed by pairs of the lines 1 x 3, 3 x 4, while, pairs 1 x 4, 3 x 5, 1 x 5, and 4 x 5 were the most similar. The pairs 2 x 3, 2 x 4, 2 x 5, 1 x 2, and 3 x 4 were found to be the most distant, and the pairs 1 x 4, 1 x 5, and 3 x 5 were found to be the most similar. The similarity of the combinations verified in the two different dissimilarity measures is justified by analysing the

magnitude of the correlation between the distances of the quantitative and qualitative matrix presented in *Figure 1*, which was high. The most distant combinations indicate a higher possibility of success in the hybridization work, while the most similar ones indicate a lower possibility of success with hybridizations, which offers a higher probability of loss of time and resources in the breeding program

Table 2: Mahalanobis distances (below the diagonal) and simple coincidence (above the diagonal) for quantitative and qualitative characteristics among five common bean strains, respectively, in the 2016/2017 crop, in Manica Province, Mozambique

Linhagens	Mantega (1)	DOR 364 (2)	G 19833 (3)	LPA 54 (4)	Bonus (5).
Mantega (1)	0	4	3	1	2
DOR 364 (2)	95.463	0	5	4	4
G 19833 (3)	58.404	205.465	0	3	1
LPA 54 (4)	7.756	107.034	80.796	0	2
Bonus (5).	7.817	93.045	55.709	11.809	0

Figure 2: Correlation of simple coincidence distances (matrix1) and Mahalanobis distances (matrix2) of common bean lines.

** significant at 1% probability by t-t-test; ++ significant at 1% probability by Mantel test with 5000 simulations.

The grouping by the Tocher method allowed the distribution of the five common bean strains into two groups and the Scott Knott test, allowed the evaluation of the groups of higher means in a univariate way (Table 3). Group I, formed by the strains Butter, G19833, LPA54, and Bonus was characterized by having higher 100-seeds (g) (54.60 to 60.43), lower NPP (9.67 to 11.67), lower D50F (36.67 to 42), green epicotyl, light green leaves, medium-sized grains, cream, and cream streaked to dark brown grain colour. Whereas, group II, formed by genotype DOR364 was characterized by having lower 100-seed (g) (44.83), higher NPP (17.33), and higher D50F (43.67), purple epicotyl, dark green leaves, black grain colour, and small grain size. Within-a group I, access G19833 is distinguished by

its indeterminate growth habit and higher D50F, and access LPA54 by brown grain colour.

The characteristic D50F ranged from 36.67 to 43.67, and NPP ranged from 9.67 to 17.33 among the strains, being similar to the averages observed by Gonçalves et al. (2016). For 100-seeds (g), the average among the evaluated strains ranged from 44.83 to 60.43 g. As pointed out by Gepts and Bliss (1986) and Coelho et al. (2007), 100-seed weight less than 25 g indicates beans belonging to the Mesoamerican centre and, when higher than 33 g, to the Andean centre.

Table 3: Grouping of the strains by the Tocher method and the means of the common bean quantitative characteristics and respective qualitative characteristics, evaluated in the 2016/2017 harvest, in Manica Province, Mozambique.

GROUPS	I				II
Strains:	Mantega (1)	G19833 (3)	LPA54 (4)	Bonus (5)	DOR364 (2)
Characteristics					
LAI	9.00 ^a	6.67 ^a	7.67 ^a	7.67 ^a	6.00 ^a
D50F	36.67 ^b	42.00 ^a	36.67 ^b	39.67 ^b	43.67 ^a
DM	77.33 ^a	78.00 ^a	71.33 ^a	85.00 ^a	85.67 ^a
NPP	9.67 ^b	10.00 ^b	11.67 ^b	11.33 ^b	17.33 ^a
100-Seed	59.83 ^a	54.60 ^a	60.43 ^a	59.97 ^a	44.83 ^b
Yield	1887.7 ^a	2061.23 ^a	2524.5 ^a	2183.17 ^a	1678.7 ^a
GH	Determined	Undetermined	Determined	Determined	Determined
EC	Green	Green	Green	Green	Purple
LCT	Light Green	Light green	Light Green	Light Green	Dark green
GC	Cream	Cream- streak	Dark brown	Cream- streak	Black
GS	Large	medium	Large	Medium	Small

Averages followed by the same letter do not differ significantly at 5% probability by the Scott-Knott test. Leaf area index (LAI), days to 50% flowering (D50F), days to maturity (DM), the number of pods per plant (NPP), 100-seed weight (100-seed) and yield (YIELD). Growth habit (GH), Epicotyl colour (EC), Leaf colour intensity (LCI), Grain colour (GC) Grain size (GS).

The estimates of eigenvalues for data of qualitative and quantitative nature in Andean bean strains were presented, as a premise for representing the distances using graphic dispersion (*Table 4*). The first two cumulative values were sufficient to explain the variation in the data with the percentage values of 83.02% and 95.5% for the quantitative and qualitative characteristics, respectively, indicating that they are favourable for studying diversity through scatter plotting. Similar results were observed by Correia and Gonçalves (2012).

Table 4: Estimated eigenvalues of qualitative and quantitative characteristics evaluated in common bean lines, in the 2016/2017 crop, in Manica Province, Mozambique.

	ROOT	ROOT (%)	%ACCUMULATED
Quantitative characteristics	6.84208	52.63138	52.63138
	3.951021	30.39247	83.02385
	1.25	9.615385	92.63923
	0.9569	7.360766	100
Qualitative characteristics	26.2746	72.65231	72.65231
	8.264469	22.85221	95.50452
	1.033645	2.858148	98.36267
	0.592137	1.637328	100

In *Table 5*, the estimates of the fit between the dissimilarity matrix and the 2D graphical dispersion are presented. The cophenetic correlation coefficient (r) was greater than 0.97 for both groups of characteristics, and the distortion and stress of the

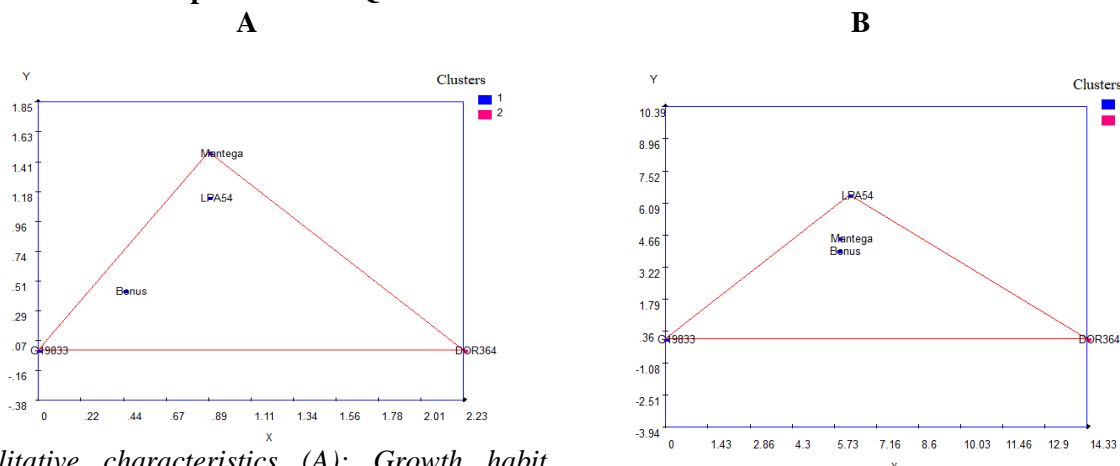
estimates were below 20%, indicating reliability between the dissimilarity matrix and the graphical representation obtained (Sokal & Rohlf, 1962; Rohlf & Fisher, 1968).

Table 5: Efficiency calculation of the projection of original distances between common bean lines based on qualitative and quantitative characteristics in two-dimensional space

Efficiency Calculation	Qualitative Characteristics	Quantitative Characteristics
Distortion (%)	15.5730	4.7718
Correlation (r)	0.9740	0.9950
Stress (%)	19.9718	9.7151

Thus, in *Figure 2*, it was verified the formation of two groups of genetically distinct strains in their qualitative and quantitative characteristics. The first group was represented by the strains Butter, G19833, DOR364, and Bonus, and the second by only one genotype DOR364, similar to what was verified in the clustering presented by Tocher's method. According to Rotili et al. (2012); Vidyakar et al. (2017), the formation of distinct groups confirms the existence of genetic divergence, which comes to be a positive aspect from the point of view of the use of these strains in artificial hybridizations to expand the genetic variability. Analysing the distances within the group, it can be seen that in *Figure 3A*, the strains Manteiga and G19833 are

more divergent and are the most distant points about the genotype DOR364 of the second group. In *Figure 3B*, the strains G19833 and LPA54, are the most divergent and stand out as the most distant points about the genotype DOR364 of the second group. These points of greater diversity indicate strains that have a greater possibility of success in hybridization work. In general, the dispersion of the strains in space showed a similar clustering pattern in *Figures 3A* and *3B*, although in *Figure 3B*, strains G19833 and LPA54 were more distant in the first group and formed points of higher diversity with strain DOR364 in the second group. However, the Butter and LPA54 strains are closer in both figures.

Figure 3: 2D scatter plot based in Qualitative traits

Qualitative characteristics (A): Growth habit (GH), Epicotyl colour (EC), Leaf colour intensity (LCI), Grain colour (GC) Grain size (GS).

Quantitative characteristics (B): Leaf area index (LAI), days to 50% flowering (D50F), days to maturity (DM), the number of pods per plant (NPP), 100-seed weight (100-seed) and yield (YIELD) evaluated in common bean lines: Manteiga (1), DOR 364 (2), G 19833 (3), LPA 54 (4), Bonus (5), using the methodology of SINGH (1981) in the 2021/2022 crop in Manica Province, Mozambique. Canonical variables (CV).

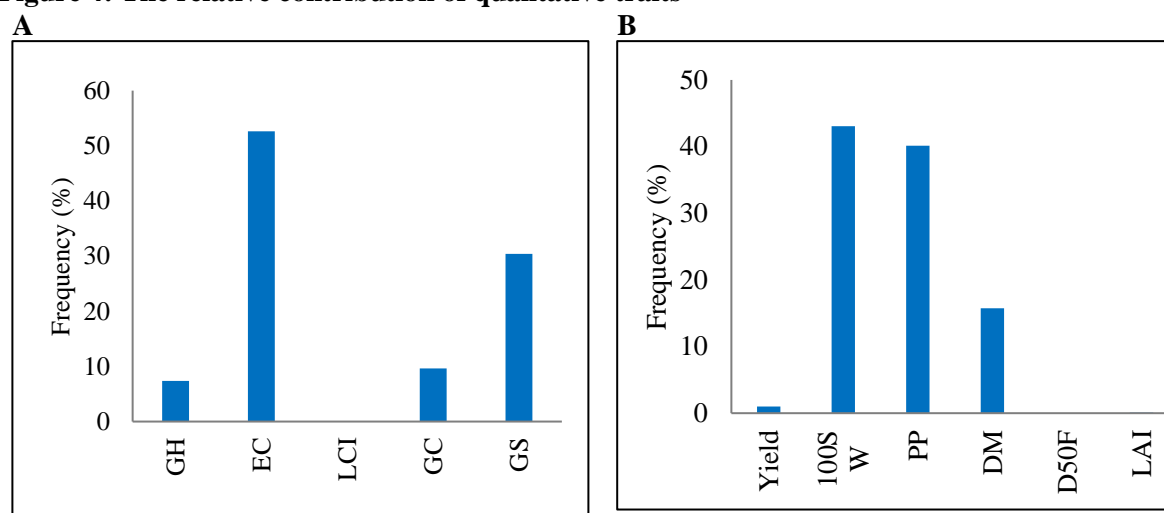
However, for the choice of genitors, not only the genetic contrast between the lines should be considered, but also the agronomic performance of the desired quantitative characteristics presented in table 3. Thus, depending on the objective of improvement, it is recommended to inter-cross the lines (G19833, LPA54, Manteiga, and Bonus) with line DOR364 for the development of segregating populations with high genetic variability. If the selection criterion is based on performance per se, the strains LPA54 and Bonus are recommended as they showed better performance in 100-seed weight, numerically high productivity, shorter days to flowering, and large and medium seed size respectively. As for the selection criteria for qualitative characteristics, it is recommended that they be made based on market preferences.

When verifying the relative contribution of each variable, it is possible to observe that the quantitative characteristics of 100-Seed and NPP were responsible for 83.16% of the total divergence (Fig. 3B) and the characteristics EC and GS were responsible for 81 % of the total divergence (Fig.

3A), indicating these, to be more important in the discrimination of the evaluated lineages (Singh, 1981). Similar results were observed by Correia and Gonçalves (2012), who observed the weight of 100 seeds as a characteristic of greater importance, and Coimbra et al (1998) who observed greater importance for the characteristic pods per plant. The other quantitative and qualitative characteristics evaluated were of less importance in the discrimination of strains.

This fact demonstrates that such variables are not the most relevant for the study of genetic divergence of the material evaluated, although they are important in providing information about the morpho-agronomic potential of the strains. With this, these characteristics that are not very representative can be discarded thus reducing the labour, time, and costs allocated to experimentation (Cruz et al., 2012) and can be maintained, depending on the objective of the breeding program.

Figure 4: The relative contribution of qualitative traits



(A): Growth habit (GH), Epicotyl colour (EC), Leaf colour intensity (LCI), Grain colour (GC) Grain size (GS)

(B): Leaf area index (LAI), days to 50% flowering (D50F), days to maturity (DM), the number of pods per plant (NPP); 100-seed weight (100-seed) and yield (YIELD), evaluated in common bean strains, through the methodology of SINGH (1981), crop, in Manica Province, Mozambique.

CONCLUSIONS

The evaluated lines present genetic dissimilarity regarding the agronomic and morphological characteristics. To obtain hybrids with greater heterotic effect and high variability in segregating populations crosses between (G19833, LPA54, Manteiga, and Bonus) with the line DOR364 may be recommended. For selection by performance per se, the lines LPA54 and Bonus are recommended. The characteristics of 100 seed weight, pods per plant, epicotyl colour, and grain size contributed the most to the study of the genetic dissimilarity of the material studied.

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CONFLICTS OF INTEREST

For this research entitled a Divergence and selection of genitors aiming to generate variability in common bean lines, we declare no disagreements between the authors.

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