MORPHO-AGRONOMIC GENETIC DIVERSITY IN COMMON BEAN LANDRACES BASED ON BLUP VALUES

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Abstract: The objective of the research was to evaluate morpho-agronomic of genetic diversity in common bean landraces based in predicted genotypic values, to identify divergent genotypes with agronomic potential in several traits for selection activities and crosses in the breeding program. Traits evaluated were plant height, branches per plant, days to flowering, days to maturity, pods per plant, seeds per pod, seeds mass and grain yield at Nampula Research Station, Mozambique, in an augmented design with four blocks, 27 genotypes (24 landraces and 3 checks). Data were subjected to analysis of deviance, variance components and genetic parameters. The genotypic values were estimated by REML/BLUP mixed model and were used in heat map clustering, pairwise distances and constellation plot. The results indicated genetic variability for all traits except branches per plant and showed six clusters of divergent genotypes. Days to flowering, pods per plant and plant height, had the highest contribution to genetic divergence. The morpho-agronomic analysis of genetic diversity in common bean landraces based in predicted genotypic values allowed to identify divergent genotypes with agronomic potential in several traits for selection activities and crosses in the breeding program.

Keywords: *Phaseolus vulgaris*, genetic variability, REML/BLUP.

Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important legume for human consumption worldwide and an important source of vegetable protein, minerals, antioxidants, and bioactive compounds (Karavidas et al., 2022). It is self-pollinated with low crossing frequency, diploid with 11 pairs of chromosomes ($2n = 2x = 22$) and genome size of 473 Mb (Schmutz et al., 2014). Belongs to the Fabaceae family, can fix atmospheric nitrogen in association with rhizobia bacteria, and provides a cheap source of protein for people in developing countries as Latin America and Africa (Schmutz et al., 2014; Jannat et

al., 2019), including in Mozambique, where fresh and dry grains represent a significant food for human nutrition and income generation for small farmers (Pedro et al., 2022).

Despite the notorious socio-economic importance, the yield (kg ha^{-1}) reached by farmers in Mozambique is around 533.8 (MADER, 2021), considered low compared to the average yield of some major producing countries such as China: 1744.1, United Republic of Tanzania: 1343.7, Myanmar: 911.6 (FAOSTAT, 2020) and Brazil: 1056 (CONAB, 2023). The low yield can be attributed to several factors, such as poor agricultural techniques, abiotic and biotic stresses and the use of varieties with low genetic potential (Pedro et al., 2022). However, the yield of cultivars can be improved through the evaluation and selection of parents with high genotypic value, or by crossing divergent parents, accessed from common bean landraces, cultivated for many years by small farmers and adapted locally, varying in different agronomic traits and many of them are at risk of extinction. According to Stoilova et al. (2013) and Abdollahi et al. (2016) landraces are a variety of plants domesticated from the wild through natural and artificial selection and helps farmers or agricultural programs to adapt to new challenges such as climate change.

The genetic diversity for morpho-agronomic traits in common bean landraces has been studied by some authors (Stoilova et al., 2013; Nadeem et al., 2020; Nogueira et al., 2021) through several multivariate methods, such as discriminant analysis, principal components and cluster analysis (Diniz Filho, 2000; Cruz et al., 2020). There are other studies, based on morphological and molecular information, that combine these methods with the heat map (Fiore et al., 2020; Androcioli et al., 2020; Laskar et al., 2021; Mecha et al., 2022), however, the tool is still little explored in the study of genetic divergence for morphological traits, and very little for the study of pairwise distances. In the context of mixed models, the evaluation of genotypes based on the REML/BLUP (Restricted Maximum Likelihood/Best Linear Unbiased Prediction) is an optimal procedure for more accurate estimates and predictions of genetic parameters and genotypic values that maximize selective precision, respectively (Resende, 2016). Thus, the use of multivariate analysis combined with heat map in the study of the genetic diversity using predicted genotypic values of the morphological data can be advantageous in the quick visual analysis of the agronomic potential of genotypes between and within the clusters and in the visualization of the magnitude of the distances between genotypes pairs, allowing to identify and select with higher efficiency and precision, divergent genotypes and with better performance in one or several traits to be used in the breeding program, as in crossing activities aiming at greater genetic variability and obtaining superior genotypes in segregating generations. Therefore, the objective of the research was to evaluate morpho-agronomic of genetic diversity in common bean landraces based in predicted genotypic values, to identify divergent genotypes with agronomic potential in several traits for selection activities and crosses in the breeding program.

Material and methods

The trial was carried out at Nampula Research Station (15°09'01.8"S 39°18'47.0"W) of the Mozambique Agricultural Research Institute. Nampula is a district from Nampula province, located in the Northern Region of Mozambique, Eastern Africa continent. Nampula city has a tropical climate considered to be Aw according to the Köppen-Geiger climate classification. The summers are much rainier than the winters. The average temperature is 23.9 °C and the annual rainfall is 959 mm.

Twenty-seven common bean genotypes, being 24 landraces collected in the Northern Region of Mozambique and 3 checks (Figure 1) were used to evaluate plant height (PH), branches per plant (BP), days to flowering (DF), days to maturity (DM), seeds per pod (SPO), pods per plant (POP), seeds mass (SM) and grain yield (GY) according to the methodology of IPGRI (2001), in an experiment with augmented design (Federer, 1956) with four blocks.

Figure 1. 27 common bean (*Phaseolus vulgaris*) genotypes (24 landraces and 3 checks). CZnd = Centro Zonal Nordeste. C (1, 2, 3) = checks. In parentheses is the genotype number.

Sowing was carried out on 02/25/2021, with a density of 10 seeds per linear meter in plots of two meters in length and two row, spaced 0.5 meters apart. The crop management consisted of two weedings, carried out 15 and 30 days after plant emergence. Two foliar fertilization with codafol NPK liquid fertilizer (7-21-7) and two applications of the insecticide Cypermethrin (Hitcel 44EC) for pest control were carried out at phenological stages V3 and V6. In each plot, the operations of harvesting, drying, threshing and selection of seeds were carried out, followed by weighing the 100 seeds mass (SM) in grams and the seeds mass per plot converted to grain yield (GY) in kilograms per hectare.

Data analysis were carried out by the Restricted Maximum Likelihood (REML) and Best Linear Unbiased Predictor (BLUP) methods. The analysis of deviance (ANADEV) was performed considering the following statistical model (Resende, 2016):

$$y = Xf + Zg + Wb + \varepsilon,$$

where y is the vector of observations, f is the vector of fixed effects (overall mean), g is the vector of the genetic effects of the common bean landraces and checks (assumed as random), b is the vector of the effects of the blocks (assumed as random), and ε is the vector of errors (random). X, Z and W represent the incidence matrices for f, g and b respectively. The significance of all random effects from the ANADEV was verified by the Likelihood Ratio Test (LRT) at 1% of probability. Were estimated the variance components: genotypic variance ($\hat{\sigma}_g^2$), environmental variance between blocks ($\hat{\sigma}_b^2$), error variance ($\hat{\sigma}_e^2$), and phenotypic variance ($\hat{\sigma}_f^2$). The estimated genetic parameters were broad

sense heritability: $h^2 = \dfrac{\hat{\sigma}_g^2}{\hat{\sigma}_g^2 + \hat{\sigma}_e^2}$, and genetic

selection accuracy: $Ac = \sqrt{1 - \dfrac{PEV}{\hat{\sigma}_g^2}}$, where

PEV is the prediction error variance of the genotypic values. Genotypic value (u+g) was predicted, with: u is the overall mean, and g is the predicted genotypic effects. Later they were plotted in the boxplots to verify the genotypic variation.

For cluster analysis, Euclidean distances between pairwise genotypes were estimated based on the predicted genotypic values of the traits. These measurements were used in a heat map pairwise to identify the most dissimilar and similar, and in the hierarchical heat map clustering based on Ward's method, whose consistency was verified by the cophenetic correlation between the values of the original and graphic Euclidean distances. The heat map clustering is advantageous in the quick visual analysis of the agronomic potential of the genotypes between and within the clusters and the pairwise, it will allow visualizing the magnitude of the distances of the pairs of genotypes through the color of the coordinates, allowing to identify and select with greater efficiency and accuracy, divergent genotypes and better performance on one or several traits. The formed clusters were represented in the constellation plot to verify their proximity and distance. The relative contribution of traits to genetic divergence was estimated according Singh (1981). Statistical analysis of the data were performed using Genes (Cruz, 2016), Selegen (Resende, 2016) R version 4.0.0 (R Core Team, 2021) and JMP Version 16 (SAS Institute, 2022) softwares.

Results and discussion

The deviance revealed significant differences among the morpho-agronomic traits of the 27 genotypes ($P \leq 0.01$) except for BP, and non-significant for the blocks ($P \geq 0.05$), which indicates blocks homogeneity (Table 1).

The genotypic variances ($\hat{\sigma}_g^2$) were higher than the environmental variances between blocks ($\hat{\sigma}_b^2$) and error variances ($\hat{\sigma}_e^2$), indicating that the high proportion of phenotypic variance ($\hat{\sigma}_f^2$) is explained by genetic effects. The high heritability (h^2) observed in the traits, expresses high genetic variability and correlation between phenotype and genotype. According to Resende and Duarte (2007) criteria, the selection accuracy (Ac) was very high (≥ 0.90) for PH, DM, POP, SM and GY and high (≥ 0.70) for SPO and DF,

Table 1. Analysis of deviance, Likelihood Ratio Test, variance components and genetic parameters. Plant height (PH: cm), branches per plant (BP), days to flowering (DF), days to maturity (DM), pods per plant(POP), seeds per pod(SPO), seeds mass (SM: g) and grain yield (GY: Kg ha^{-1}).

| Effects	Deviance[+]							
	PH	BP	DF	DM	POP	SPO	SM	GY
Genotype	300.90	-7.77	102.02	151.48	133.43	-5.03	183.18	487.35
Block	279.26	-9.25	99.19	124.24	121.52	-8.61	166.96	474.40
Model	278.95	-10.74	95.58	124.23	120.92	-12.06	166.94	473.63
Likelihood Ratio Test: LRT(X^2)								
Genotype	21.95**	2.97	6.44**	27.25**	12.51**	7.03**	16.24**	13.72**
Block	0.31	1.49	3.61	0.01	0.60	3.45	0.02	0.77
Variance components and genetic parameters								
$\hat{\sigma}_g^2$	1636.31	0.20	5.98	23.69	17.2	0.29	70.38	419.87
$\hat{\sigma}_b^2$	21.61	0.03	0.85	0.01	0.31	0.03	0.12	8.64
$\hat{\sigma}_e^2$	95.74	0.08	0.87	0.85	1.12	0.04	3.93	24.23
$\hat{\sigma}_f^2$	1753.66	0.32	7.70	24.55	18.63	0.35	74.43	452.73
h^2	0.93±0.46	0.64±0.38	0.78±0.42	0.96±05	0.92±0.45	0.81±0.42	0.95±0.46	0.93±0.15
Ac	0.97	0.80	0.88	0.98	0.96	0.90	0.97	0.96
μ	62.91	3.97	27.80	72.52	10.14	3.90	45.19	1453.74

[+]: Model deviation adjusted without corresponding effects, X^2: Chi-square, **: Significant by chi-square test at 0.01 probability. $\hat{\sigma}_g^2$: genotypic variance, $\hat{\sigma}_b^2$: environmental variance between blocks, $\hat{\sigma}_e^2$: error variance, $\hat{\sigma}_f^2$: individual phenotypic variance, h^2): heritability in the broad sense, Ac: genetic selection accuracy and μ = Overall mean.

indicating high experimental precision and correlation between predicted and true genotypic values of genotypes. These parameters indicate a high possibility of precise selection of common bean genotypes with agronomic potential to be used in the breeding program. Similar results were observed by Androcioli et al. (2020), Basavaraja et al. (2021) and Nogueira et al. (2021).

The common bean genotypes present wide genotypic variation in the trait (Figure 2).

The amplitude of the means indicates that the PH ranged from 18.5 to 163.3 cm, with 37% of the genotypes above and 63% below the overall mean (62.91). The BP ranged from 3.29 to 5.17, with 37% of the genotypes above and 67% below the overall mean (3.97). The DF ranged from 24.81 to 32.78, with 29.63% of the genotypes above and 70.37% below the overall mean (27.80). The DM ranged from 62.38 to 83.61, with 48.15% of the genotypes above and 51.85% below the overall mean (72.52). According to Araujo et al. (1996) the genotypes are early maturity, when it occurs between 60 and 70 days and medium, between 85 and 90 days after emergence, while (Ribeiro et al., 2004)

consider 70 to 73 days for medium maturity, indicating that 59.26% of the genotypes are earlier (62-70 days) and 40.74% are of medium maturity (73.50 to 84.42). The use of early genotypes reduces losses and production costs, because the time of exposure of the crop in the field to insects, diseases, weeds and drought is short, in addition to allowing early harvest. The POP ranged from 2.92 to 17.81, with 40.74% of the genotypes above and 59.26% below the overall mean (10.14). The SPO ranged from 2.95 to 5.30, with 33.33% of the genotypes above 66.66% below the overall mean (3.90). The SM ranged from 22.43 to 54.89 g, with 7.41% from the Mesoamerican gene pool (22.43 to 25 g) and 92.59% from the Andean gene pool (>25 to 54.89 g), according to Singh et al. (1991) who classify, based on the 100 seeds mass, genotypes with a medium size (25-40 g) and large (>40 g) as Andean and with small size (<25 g) as Mesoamerican. Seeds from both gene pools are commercially important, but Andean grains are generally more preferred in eastern Africa. Yield (kg ha-1) ranged from 699 to 3.412, with 37% above and 63% below the overall mean (1453.74).
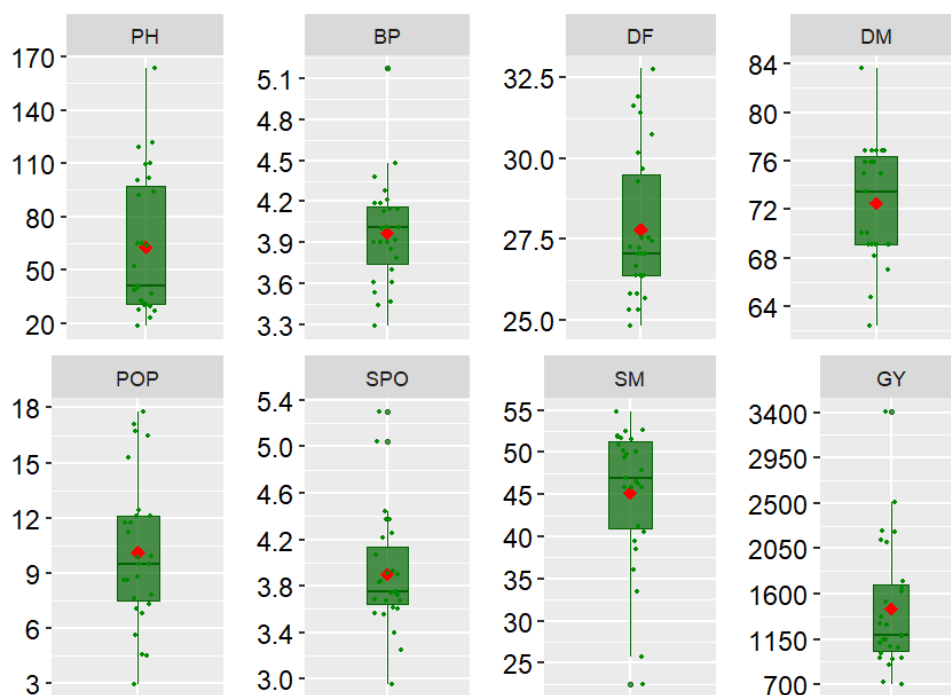

Figure 2. Genotypic variation of morpho-agronomic traits of 27 common bean (*Phaseolus vulgaris*) genotypes. The darkgreen risk is the median, the redpoint shape is the mean. Plant height (PH: cm), branches per plant (BP), days to flowering (DF), days to maturity (DM), pods per plant (POP), seeds per pod (SPO), seeds mass (SM: g) and grain yield (GY: Kg ha-1).

In general, the genotypes can be summarized as being of short stature, early maturity, low pod and yield and Andean gene pool, offering possibilities for the exploitation of genetic material for various purposes of genetic improvement. Similar results were observed for DF, DM, POP, SPO, SM and GY (Mambrin et al., 2015), PH, DM, POP, SPO and SM (AlBallat and Al-Araby, 2019) POP, SM and GY (Nadeem et al., 2020), SPO and SM (Basavaraja et al., 2021) for different common bean genotypes and environmental conditions in the study of genetic divergence.

The hierarchical heat map of genotypic values showed six genetically divergent clusters of the 27 genotypes and the agronomic potential of each genotype (Figure 3A).
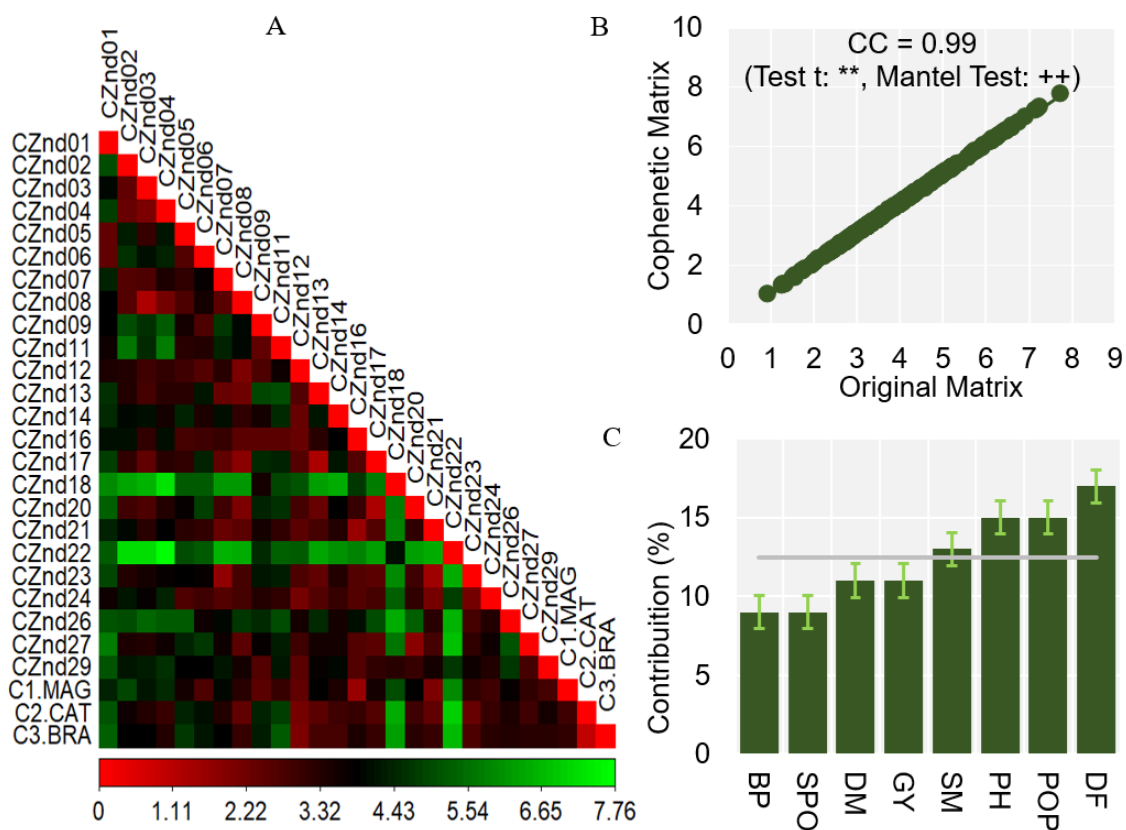
The means clusters of common bean genotypes allow characterizing clusters with the traits' highest and lowest agronomic potential (Figure 3B). The cluster VI, formed with nine genotypes were characterized by the lowest DF, POP and SPO. This cluster also showed high SM, moderate DM and GY and low means for PH, but the genotypes CZnd21 showed moderate PH, CZnd07 high DF, CZnd07, CZnd21, CZnd23, CZnd26, and CZnd24 high DM. The CZnd12, CZnd14, C2.CAT, C3.CAT showed low DM, CZnd12 and CZnd14 showed high POP, CZnd07, CZnd12 and CZnd24 moderate SPO, CZnd14 moderate SM, CZnd14 and CZnd24 high GY, CZnd12 and CZnd26 moderate GY. Cluster V with five genotypes was characterized by lowest DF and GY. Showed high SPO, moderate
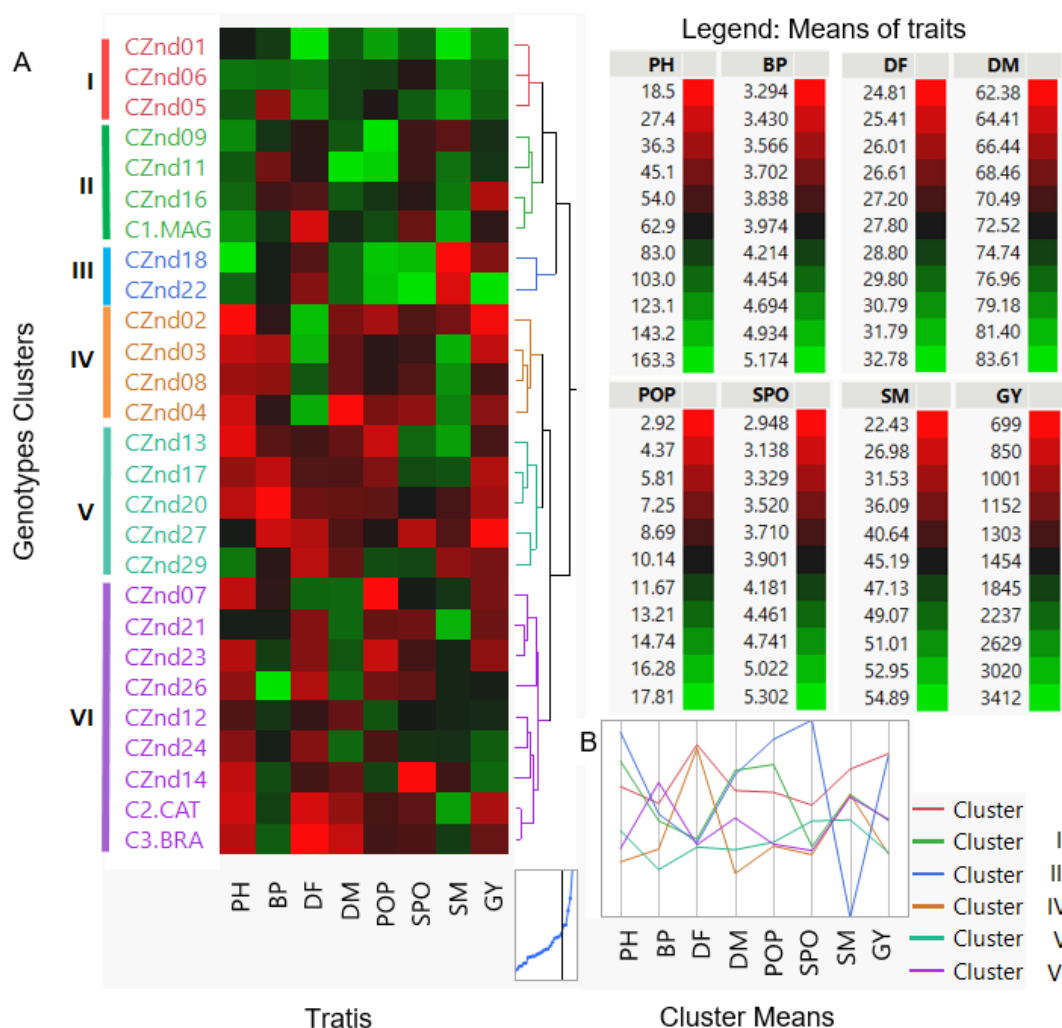
Figure 3. Ward's hierarchical heat map clustering (A) for morpho-agronomic traits of 27 common bean (*Phaseolus vulgaris*) genotypes. Analysis was performed using standardized Euclidean distance. Cluster means (B). The green to red color gradients indicate high to low means, respectively. Plant height (PH: cm), branches per plant (BP), days to flowering (DF), days to maturity (DM), pods per plant (POP), seeds per pod (SPO), seeds mass (SM: g) and grain yield (GY: Kg ha^{-1}).

SM and low PH, DM, POP, but the genotype CZnd29 showed high PH and POP, CZnd27 moderate PH, POP and low SPO, CZnd20 moderate SPO, CZnd13 and CZnd17 high SM, CZnd20, CZnd27 and CZnd29 low SM. Cluster IV with four genotypes was characterized by highest DF and lowest PH, DM, POP, SPO and GY. Showed high SM, low BP and DF, but the genotypes CZnd03 and CZnd08 Showed moderate DM. Cluster II with four genotypes was characterized by the highest DM and lowest SPO. Showed high PH, SM, moderate GY and low DF, but the genotype C1.MAG Showed the lowest DF and moderate DM, and CZnd16 low GY. Cluster I with three genotypes was characterized by the highest DF, SM, GY. Showed high PH, DM, POP, SPO and SM. Cluster III with two genotypes was characterized by the highest PH, DM, POP, SPO, GY and lowest SM. Showed low DF, but the genotypes CZnd22 showed the highest GY and CZnd18 low GY.

The results of the heat map cluster together with the characterization of each genotype show the possibility of obtaining superior genotypes by performance per se, furthermore, we can predict a high potential for crosses between genotypes from clusters I, II and III, and between superior genotypes belonging to more distant clusters. Taking yield as the main trait in the breeding program, the selection for highest GY can be in the genotypes CZnd22 and CZnd09. For higher GY and lower PH in genotypes CZnd01, CZnd04 and CZnd24. For higher GY and SM in genotypes CZnd01, CZnd05 and CZnd06. For higher GY, lower PH and DM in genotypes CZnd12 and CZnd14.

The genotypes clusters IV, V and VI are similar and genetically more distant from the similar clusters I, II and III (Figure 4). The selection of genotypes for intercrossing, the agronomic potential of the traits associated with higher pairwise genetic distance should be considered (Figure 5A). Based in this criterion, the genotypes pairs CZnd22 x CZnd12, CZnd22 x CZnd14, CZnd22 x CZnd24, CZnd01 x CZnd14, CZnd01 x CZnd22,

Figure 4. Constellation plot of 27 common bean (*Phaseolus vulgaris*) genotypes.

Figure 5. Heat map pairwise of Euclidean distances (A) among 27 common bean (*Phaseolus vulgaris*) genotypes. The green to red color gradients indicate high to loweuclidiandistances.Cophenetic correlation (CC) (B) and contribution (C). Plant height (PH: cm), branches per plant (BP), days to flowering (DF), days to maturity (DM), pods per plant (POP), seeds per pod (SPO), seeds mass (SM: g) and grain yield (GY: Kg ha^{-1}).

CZnd05 x CZnd22, CZnd06 x CZnd22, CZnd11 x CZnd22, CZnd01 x CZnd26, CZnd05 x CZnd14, CZnd05 x CZnd26 and CZnd11 x CZnd14, are recommended for crosses, aiming at higher variability and breeding for plant height, early maturity, seed mass and yield. According to Ghaderi et al. (1984) and Cruz et al. (2020), so that they can present complementary genes that provide, in F1, higher heterosis and, in segregating generations, transgressive individuals.

The hierarchical heat map clustering was significantly consistent (Figure 5B), indicating perfect reproduction of the projection of Euclidean distances on heat map pairwise, which allows higher precision in the selection of genotypes with desired agronomic potential associated with higher genetic distance for crosses. The PH, DF and POP had a higher contribution (47%) to the genetic divergence of 27 common bean genotypes (Figure 5C). Similar results were reported by Stoilova et al. (2013) and Nadeem et al.

(2020) who observed PH and POP as traits with the highest contribution to genetic divergence in common bean genotypes.

Conclusions

The morpho-agronomic analysis of genetic diversity in common bean landraces based in pre-dicted genotypic values allowed to identify divergent genotypes with agronomic potential in several traits for selection activities and crosses in the breeding program.

Acknowledgment

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