



RESEARCH ARTICLE

MULTIVARIATE ANALYSIS APPLIED TO GENOTYPE DISTINCTION IN COMMON BEAN

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ABSTRACT

This study aimed to determine the dissimilarity among landrace common bean genotypes through multivariate techniques aiming the selection of individuals to compose the crossing blocks and determine among the evaluated genotypes, which ones have proximity to an agronomic ideotype. The experiment was carried out during the 2014/2015 agricultural year in Tenente Portela- RS, southern Brazil. The augmented blocks design was composed by the control treatments: BRS Estilo, IPR Uirapuru, IPR Tangara, BRSMG Realce, IAC Formoso, IPR Imperado, and IPRTiziu, and the regular treatments were constituted by the genotypes: LMGPP01, LMGPP2, LMGPP3, LMGPP4, LMGPP5, LMGPP6, LMGPP7, LMGPP8, LMGPP9, LMGPP10, LMGPP11, PORTELA16, PORTELA37, PORTELA38, PORTELA39, PORTELA64, and PORTELA65. The genotypes LMGPP09 and PORTELA16 are the most dissimilar, while LMGPP01 and LMGPP02 are the most similar ones. The BRSMG REALCE and PORTELA16 genotypes can be used as genitors in combination with other genotypes in the formation of segregating populations with wide genetic variability. The hierarchical UPGMA clustering method, the Tocher optimizing method, and the principal component analysis are consistent and complementary, enabling a reliable response in the data interpretation on the genetic dissimilarity in common bean.

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INTRODUCTION

The grains of common bean (*Phaseolus vulgaris*) are utilized as a food source of protein and energy in different regions of the world, having special importance in the human diet (GOMES JUNIOR et al. 2005). The common bean yield of Brazil is far below the cultivars potential, being the average grain yield of 1000 kg ha⁻¹ (CONAB 2015). There are several factors that influence the crop grain yield, such as the cropping environment factors, genotype traits, technologies employed, cropping systems (MINGOTTE et al. 2014), nutritional management mainly of nitrogen (DEMARI et al. 2015), and water supply (CARVALHO et al. 2013). The search for genotypes with higher yield and adapted to the most varied cropping conditions presents as one of the main interests of the genetic breeding.

Thus, plant breeders seek to understand the genetic variability available and better determine which genotypes are agronomically adequate and responsive to environmental effects (RAMALHO et al. 2012). Phenotypic differentiation are important to orientate available genotypes, which meet the agronomical requirements and lead to crop breeding gains. Thus, the phenotype is defined as a collection of biological information, resulting from the expression of genetic traits and by the environmental action (RAMALHO et al. 2012). While the breeder has genetic variability available in common bean breeding program, it is necessary to understand the genetic variation and discriminate the genetic distance among the genotypes without studies or information. Thus, with the purpose of support in choosing which genotypes better represent the agronomic ideotype, techniques of multivariate analysis are used in the genotype discrimination.

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Among these techniques, the Standardized Euclidean Distance is emphasized because it indicates the genetic dissimilarity by the group formation, pointing the closest and distantly related genotypes (CRUZ *et al.* 2012). Cluster analysis techniques aim assemble the genotypes into several groups by some criteria in a way that homogeneity occurs within the group and heterogeneity occurs among groups. These methods are used efficiently by directing the selection of genitors to compose the crossing blocks (CRUZ *et al.* 2012). Multivariate methods that summarize the set of original variables in a few components involving the majority of the found variability are also very useful in the analysis of genetic dissimilarity, highlighting the principal components analysis (CRUZ & REGAZZI 1997). Therefore, this study aimed to determine the dissimilarity among landrace common bean genotypes through multivariate techniques aiming the selection of individuals to compose the crossing blocks and determine among the evaluated genotypes, which ones have proximity to an agronomic ideotype.

MATERIALS AND METHODS

The experiment was carried out during 2014/2015 agricultural year in Tenente Portela – RS, southern Brazil. The corresponding coordinates are latitude of 27°22'10.20"S and longitude of 53°45'23.00"O, with an altitude of 420 meters. The soil is classified as a "Latossolo Vermelho Férreo típico". According to Köppen climate classification, the climate is classified as humid subtropical Cfa. The experimental design utilized was augmented blocks, where the common treatments were composed by seven control treatments: BRS Estilo, IPR Uirapuru, IPR Tangara, BRSMG Realce, IAC Formoso, IPR Imperado, and IPR Tiziu, and the regular treatments were constituted by 17 genotypes: LMGP01, LMGP02, LMGP03, LMGP04, LMGP05, LMGP06, LMGP07, LMGP08, LMGP09, LMGP10, LMGP11, PORTELA16, PORTELA37, PORTELA38, PORTELA39, PORTELA64, and PORTELA65, as proposed by Ramalho *et al.* (2012).

The experimental units were composed by two rows of one meter and spacing of 0.45 meters. The employed population density was ten seeds per linear meter ($s\ m^{-1}$), based on the no-till seeding system. Basic fertilization of 300 kg ha^{-1} of N-P-K with the formulation 10-20-20 was performed. Posteriorly, topdressing fertilization of 90 kg ha^{-1} of nitrogen in the amide form was performed during V4 crop vegetative stage. Phytosanitary management to control weeds, insect pests, and diseases were carried out preventively in order to minimize interference on experimental results. The following traits were measured in ten plants per treatment: days to flowering (DTF), plant height at flowering (PHF), days to maturity (DTM), plant height at maturity (PHM), insertion height of the first pod (IFP), number of branches per plant (NBP), number of pods per plant (NPP), number of grains per pod (NGP), grain weight per pod (GWP), number of grains per plant (NGP), grain weight per plant (GWP), length of grains (LGG), and width of grains (WDG), based on methodologies proposed by Silva (2005) and Demari *et al.* (2015). The obtained data were submitted to descriptive analysis through phenotypic frequencies distributions. Subsequently, the analysis of genetic dissimilarity was performed through Standardized Euclidean Distance and the dendrogram was based on Unweighted Pair

Group Method Arithmetic Average (UPGMA) grouping. The Tocher group analysis and the relative contribution of traits proposed by Singh (1981) were performed. Moreover, the principal components analysis was carried out, where scores of each genotype were plotted in a three-dimensional distribution. All analyzes were performed as Cruz *et al.* (2012), utilizing the Genes statistical software (CRUZ 2013).

RESULTS AND DISCUSSION

Descriptive analyzes by the frequency distributions showed the formation of different phenotypic classes for the measured traits. For the trait days to flowering (DTF), the formation of five phenotypic classes with range from 25 to 55 days (Figure 1A) was observed. Classes of 40 and 45 DTF comprised 74.5% of genotypes. The plant height at flowering (Figure 1B) showed values from 18 cm to 66 cm, with the majority of the present genotypes among 18 cm and 30 cm. In this regard, the great range of variation observed in PHF can enable obtaining segregating populations with high genetic variation for this trait. For the trait days to flowering (Figure 1C), early genotypes with up to 75 days to maturation and late genotypes with up to 115 days for maturation were observed. The data indicated that there is the possibility of selecting contrasting superior genotypes in terms of cycle, allowing their insertion in specific niches of market, which require early genotypes to enable the production system in rotation with other crops.

The plant height at maturity (Figure 1D) appeared as one of the traits with high variation among genotypes, ranging from 15 cm to 75 cm of PHM, evidencing the difference among the studied genotypes. The same can be observed for the trait insertion height of the first pod (Figure 1E) with genotypes distributed among measures of two cm to 22 cm. Concerning the number of branches per plant (Figure 1H), most genotypes revealed magnitudes of 0.75 branches per plant.

The trait number of pods per plant (Figure 1G) also showed contrast, being observed genotypes with three to 27 pods, wherein 75% of the genotypes evidenced among three to nine pods per plant. For the number of grains per pod (Figure 1H), we observe similar distributions, ranging from 1.3 to six grains per pod. The results showed the potential for selection of superior genotypes, since the number of pods per plant (KUREK *et al.* 2012) and the number of grains per pod (CABRAL *et al.* 2011) are the traits with great potential aiming indirect selection for increased grain yield. The grain weight per pod (Figure 2A), the distribution showed that most genotypes revealed magnitudes of 0.6 g to 1.2 g per pod. The number of grains per plant (Figure 2B) also presented great contrast, ranging from one to 75, which represents the existing large variation among the assessed genotypes. For the grain weight per plant (Figure 2C), the majority of genotypes concentrated in the magnitude from 2.5 g to 7.5 g per plant. For the trait length of grains (Figure 2D), genotypes ranging from nine to 15 mm were found. Distributions ranging from 5.7 mm to 8.1 mm were also presented for the width of the grains (Figure 2E). Multivariate techniques with the aim of identifying the existing dissimilarity among genotypes and cultivars revealed that there is dissimilarity among the studied genotypes.

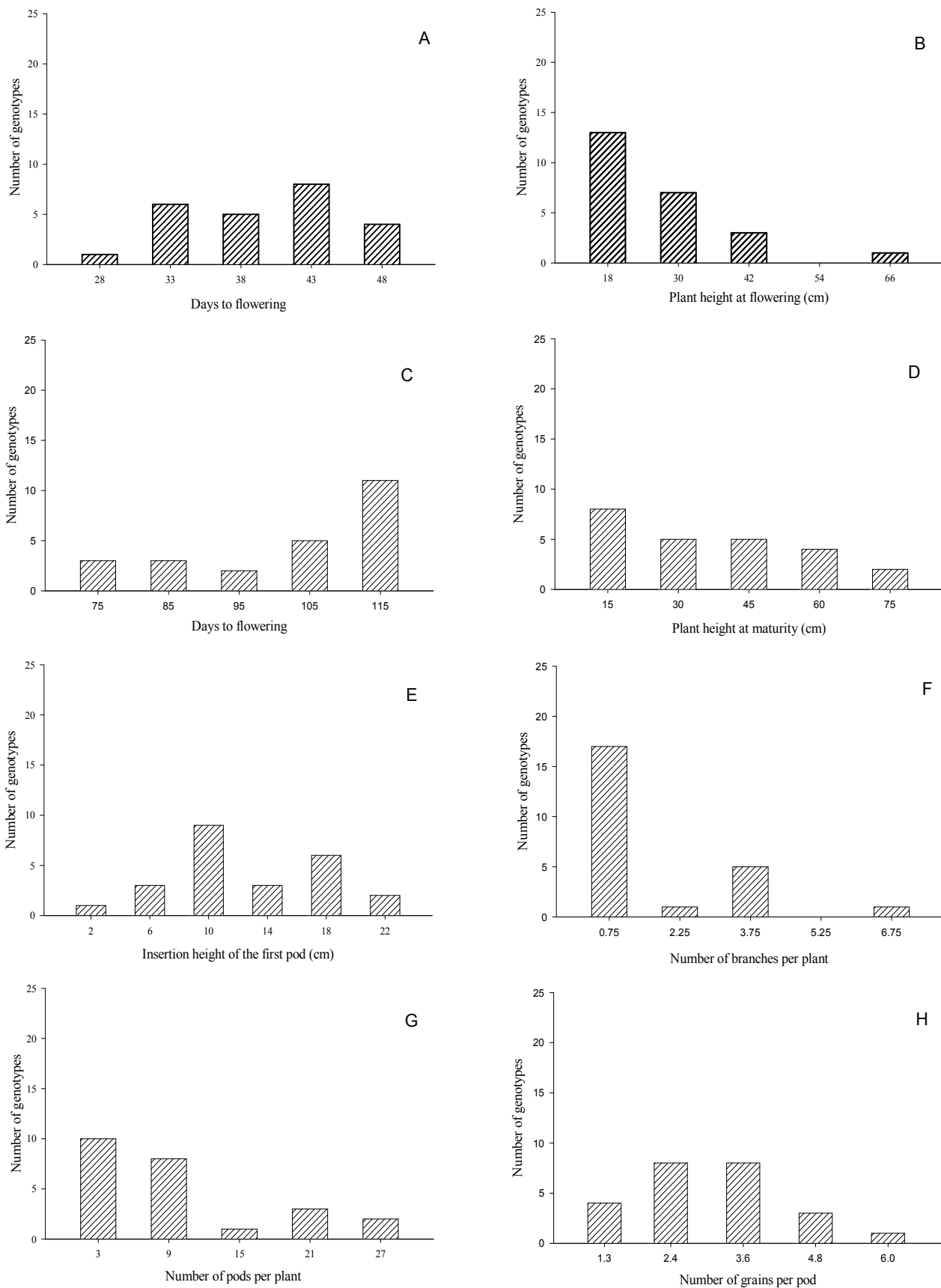


Figure 1. Frequency distribution of phenotypic classes for: graph (A) days to flowering (DTF); graph (B) plant height at flowering (PHF); graph (C) days to flowering (DTM); graph (D) plant height at maturity (PHM); graph (E) insertion height of the first pod (IFP); graph (F) number of branches per plant (NBP); graph (G) number of pods per plant (NLP); and graph (H) number of grains per pod (NGP) measured in the 2014/2015 agricultural year

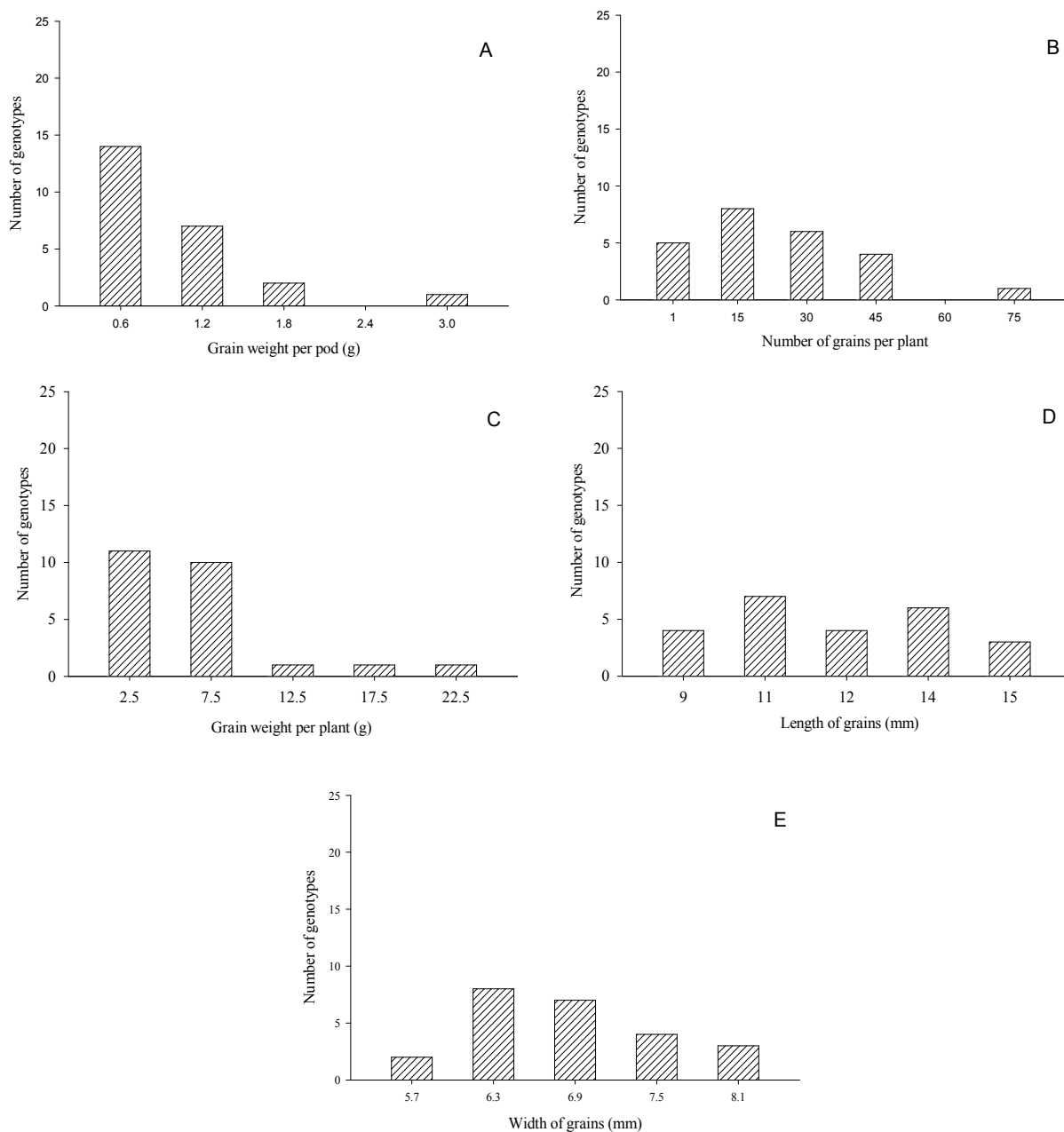


Figure 2. Frequency distribution of phenotypic classes for: graph (A) grain weight per pod (GWPD); graph (B) number of grains per plant (NGP); graph (C) grain weight per plant (GWP); graph (D) length of grains (LGG); and graph (E) width of grains (WDG) measured in the 2014/2015 agricultural year

According to Table 1, which presents the dissimilarity matrix among the 24 common bean genotypes based on the Standardized Euclidean Distance, the greatest distance was 2.25 and it was observed between the pair of genotypes 19 (PORTELA16) and 16 (LMGPP09). The highlighted pairs of genotypes represented the largest found distances, being indicated as potential genitors to compose the crossing blocks. While the genotypes 8 and 9 were the most similar ones, showing the smallest distance (0.27). Although revealing the genetic distance among each genotype combination, complementary analyzes are necessary in order to design the crossing blocks, aiming to identify promising genotypes.

The formation of three distinct groups with the greatest distance between groups of 1.86 ($D_{I,III}$) was observed by grouping the genotypes by the Tocher optimization method (Table 2). Also, the largest group was formed by group I, composed by 22 genotypes. Groups II and III were composed by one genotype in each group. When comparing the results of Tocher grouping with genetic dissimilarity matrix (Table 1), the most distant genotypes by the dissimilarity matrix were reliably separated in both multivariate methods. Comparing the group formation, the cultivars remained in similar groups, such as IPR UIRAPURU, IPR TANGARÁ, IPR IMPERADOR, IPR TIZIU cultivars, being the group I.

Table 1. Genetic dissimilarity matrix among 24 common bean accessions (listed from 1 to 24), estimated from 13 morphological traits in Tenente Portela – RS in the 2014/2015 agricultural year

G*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	0																							
2	1,51	0																						
3	1,69	0,48	0																					
4	1,35	1,85	2,15	0																				
5	1,17	0,94	0,97	1,62	0																			
6	1,21	1,07	1,35	1,39	1,04	0																		
7	1,25	1,20	1,59	1,30	1,21	0,97	0																	
8	1,62	0,98	0,74	2,05	0,86	1,51	1,79	0																
9	1,61	0,95	0,69	2,10	0,76	1,48	1,77	0,27	0															
10	1,32	1,04	1,28	1,62	0,87	0,79	0,92	1,46	1,36	0														
11	1,05	1,08	1,31	1,47	1,12	1,01	1,03	1,37	1,39	0,89	0													
12	1,19	1,05	1,07	1,83	0,73	1,21	1,29	1,12	1,08	0,82	0,89	0												
13	0,81	1,35	1,59	1,42	1,25	1,15	0,91	1,69	1,69	1,10	0,62	1,08	0											
14	1,09	1,29	1,59	1,63	1,54	1,42	1,03	1,88	1,88	1,41	1,08	1,38	0,85	0										
15	0,96	1,56	1,75	1,67	1,34	1,46	1,23	1,79	1,79	1,48	1,34	1,33	1,14	1,02	0									
16	1,54	0,81	0,76	2,12	0,88	1,31	1,55	0,89	0,71	0,99	1,19	0,97	1,53	1,64	1,71	0								
17	1,28	1,38	1,52	1,73	1,07	1,15	1,10	1,57	1,52	0,98	0,95	1,14	0,88	1,41	1,34	1,38	0							
18	1,37	1,50	1,60	1,84	1,35	1,29	1,34	1,73	1,74	1,30	1,12	1,20	1,02	1,34	1,23	1,68	0,79	0						
19	1,68	2,01	2,18	1,51	1,84	1,89	1,77	1,96	2,09	2,00	1,50	1,84	1,59	1,80	1,62	2,25	1,77	1,77	0					
20	1,32	1,16	1,49	1,42	1,29	0,93	0,83	1,70	1,71	0,82	0,72	1,00	0,92	1,15	1,44	1,49	1,28	1,30	1,64	0				
21	1,50	1,43	1,70	1,75	1,61	1,13	1,11	1,92	1,97	1,24	1,03	1,32	1,11	1,27	1,40	1,79	1,36	1,06	1,83	0,77	0			
22	1,31	0,82	1,03	1,77	1,14	0,94	1,11	1,37	1,34	0,84	0,63	0,89	0,90	1,09	1,49	1,06	1,00	1,01	1,91	0,83	0,97	0		
23	1,65	0,61	0,62	2,08	1,00	1,46	1,49	0,79	0,74	1,24	1,26	1,13	1,58	1,56	1,72	0,58	1,53	1,77	2,15	1,51	1,78	1,14	0	
24	1,31	1,11	1,40	1,77	1,23	0,91	0,88	1,72	1,64	0,79	0,92	1,21	0,94	1,07	1,29	1,26	0,81	1,09	1,93	1,01	1,18	0,78	1,43	0

*1: BRS ESTILO; 2: IPR UIRAPURU; 3: IPR TANGARA; 4: BRSMG REALCE; 5: IAC FORMOSO; 6: IPR IMPERADOR; 7: IPR TIZIU; 8: LMGPP01; 9: LMGPP02; 10: LMGPP03; 11: LMGPP04; 12: LMGPP05; 13: LMGPP06; 14: LMGPP07; 15: LMGPP08; 16: LMGPP09; 17: LMGPP10; 18: LMGPP11; 19: PORTELA16; 20: PORTELA37; 21: PORTELA38; 22: PORTELA39; 23: PORTELA64; 24: PORTELA65.

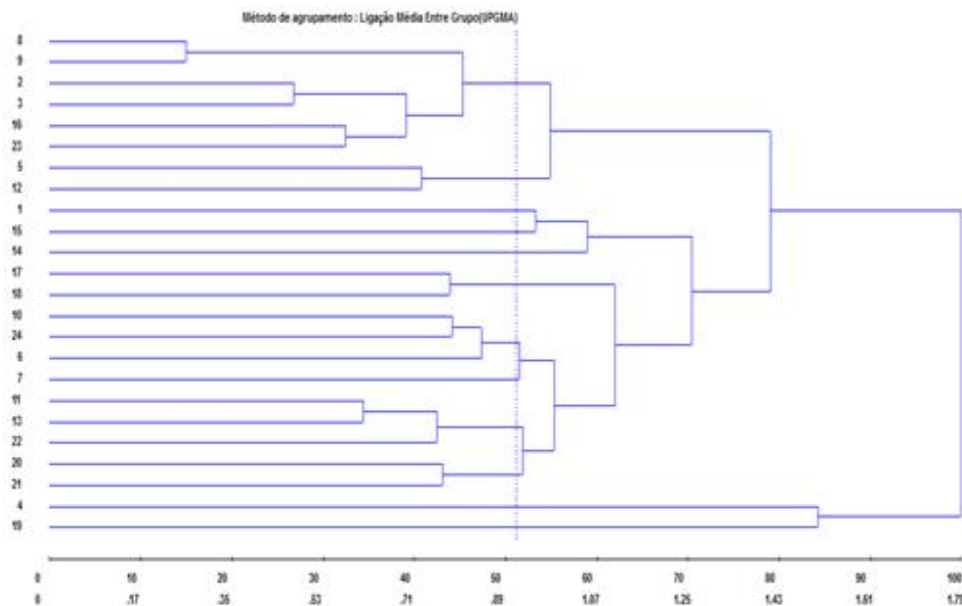


Figure 3. Representative dendrogram of genetic dissimilarity among 24 common bean genotypes, obtained by the method of average links between groups UPGMA and using the Euclidean Distance as dissimilarity measure. The values found in each cluster represents the percentage of times that the genotypes were grouped together in 2000 simulations of bootstrap resampling. The cophenetic correlation coefficient (r) was 0.82, with estimates of 2.42% distorting and 15.58% stress

Table 2. Genotype groups established by the Tocher method based on dissimilarity expressed by the Euclidean Distance, estimated from 13 morphological traits

Group	Common bean accessions	Distance	
		Intragroup	Intergroup
I	8 9 3 23 16 2 5 12 22 10 11 6 24 20 17 13 7 18 1 14 21 15	1,22	$D_{I,II} = 1,72$
II	4		$D_{I,III} = 1,86$
III	19		$D_{II,III} = 1,51$

1: BRS ESTILO; 2: IPR UIRAPURU; 3: IPR TANGARA; 4: BRSMG REALCE; 5: IAC FORMOSO; 6: IPR IMPERADOR; 7: IPR TIZIU; 8: LMGPP01; 9: LMGPP02; 10: LMGPP03; 11: LMGPP04; 12: LMGPP05; 13: LMGPP06; 14: LMGPP07; 15: LMGPP08; 16: LMGPP09; 17: LMGPP10; 18: LMGPP11; 19: PORTELA16; 20: PORTELA37; 21: PORTELA38; 22: PORTELA39; 23: PORTELA64; 24: PORTELA65

Table 3 – Results of relative contribution of each morphological-agronomic trait to the total variation found among 24 common bean genotypes estimated by the method of Singh (1981)

Traits	S _j	Relative Contribution (%)
Daystoflowering	15116,00	2,14
Plantheightatflowering	85973,75	12,15
Daystomaturity	116864,00	16,52
Plantheightatmaturity	224315,00	31,71
Insertion height of the first pod	14099,00	1,99
Number of branches per plant	1808,00	0,26
Number of pods per plant	28911,00	4,09
Number of grains per pod	862,51	0,12
Grainweight per pod	180,49	0,03
Number of grains per plant	201399,00	28,47
Grainweight per plant	15519,95	2,19
Lengthofgrains	2091,94	0,30
Width of grains	243,97	0,03

Table 4. Estimate of the principal components (PC), eigenvalues (λ_i), percentage of variance explained by the component (%VEC) and cumulative variance by the traits (%CVT) obtained from 13 traits evaluated in 24 common bean genotypes

Principal Components	λ_i	% VEC	% CVT
PC ₁	4,689	36,071	36,071
PC ₂	2,354	18,108	54,179
PC ₃	1,480	11,388	65,568
PC ₄	1,156	8,896	74,464
PC ₅	1,004	7,725	82,188
PC ₆	0,593	4,560	86,748
PC ₇	0,576	4,433	91,182
PC ₈	0,397	3,053	94,235
PC ₉	0,323	2,485	96,720
PC ₁₀	0,219	1,687	98,407
PC ₁₁	0,160	1,231	99,638
PC ₁₂	0,038	0,294	99,932
PC ₁₃	0,009	0,068	100,000

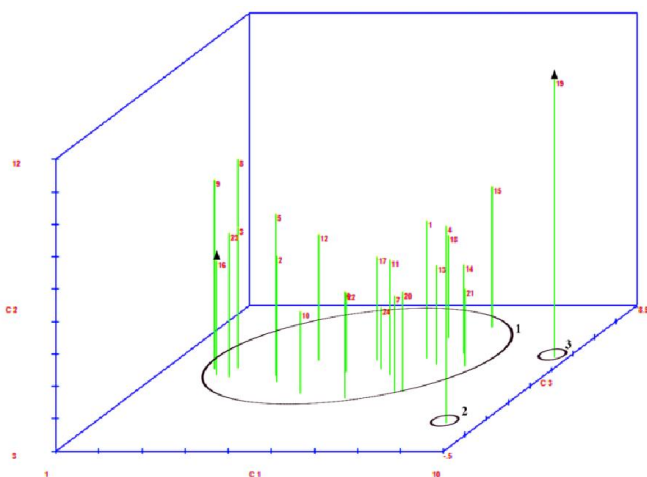


Figure 4. Three-dimensional graphic dispersion of scores obtained in the first three principal components, estimated by the weighting coefficients and the standard mean of each trait measured in 24 common bean genotypes

The presence of genotypes derived from the same breeding program in the same group shows the similarity among them (FALCONER 1987). Thereby, possibly only one of genotypes would be incorporated to the crossing block due to its close relation with the other genotypes. Moreover, the genetic distance (1.34) among the cultivars originated from the same program that integrated different groups in the distance matrix

indicated that genetic constitutions with greater amplitude of genetic variation can be obtained from the same breeding program, as the example of BRS ESTILO (group I) and BRSMG REALCE (group II) cultivars. The formation of groups II (BRSMG REALCE) and III (PORTELA16), composed of only one genotype each, evidenced the great genetic distance of these with the other genotypes. The distance evidenced between groups II and III was 1.51, which corresponds to the distance between 4 and 19 genotypes (Table 1). The dendrogram (Figure 3) presented significant cophenetic correlation (r) of 0.82. The higher this coefficient, the smaller is the distortion between the original dissimilarity matrix and the graphic grouping (BUSSAB *et al.* 1990). In this regard, Sokal & Rohlf (1962) reported that ratios above 0.80 are considered satisfactory, providing reliability to the original matrix. The calculated values of distortion (2.42%) and stress (15.58%) also indicate the reliability of graphical representation of the original matrix.

As in Tocher optimization method, the same genotypes were observed composing groups. The first group was composed of 22 genotypes with approximately 92% of all genotypes. Moreover, the second group was composed by the BRSMG REALCE genotype and the third group was composed by PORTELA16 genotype (Figure 3). These results indicated being possible the combination of these with the genotypes present in group I. The most favorable combinations utilizing the check varieties in the crossing blocks are with genotype 4 (BRMSG REALCE): G4 x G8; G4 x G9; G4 x G16, and G4 x G23. While the most promising combinations using genotype 19 (PORTELA16) are: G19 x G2; G19 x G3; G19 x G9; G19 x G10; G19 x G16, and G19 x G25 (Table 1). The use of more than one grouping method allowed the complementarities between them, generating a reliable information data and avoiding erroneous inferences that may occur depending on the various criteria that each method uses. When assessing the relative trait contribution (Table 3), the traits that contributed more to the dissimilarity among the analyzed genotypes were the plant height at maturity (31.71%), the number of grains per plant (28.47%), days to maturity (16.52%) and plant height at flowering (12.15%), which accounted for approximately 90% of the total variation among genotypes. The traits number of branches per plant, number of grains per pod, grain weight per pod, length of the grain, and width of the grain did not come to explain 1% of the total variation found among genotypes.

Regarding the analysis of the first principal components (Table 4), the feasibility of using principal components depends of the possibility of the original set, composed by variables, being summed in a two-dimensional or three-dimensional space without loss of information. In this way, the first three main components were utilized, which accounted for approximately 65.56% of the total variation. The three-dimensional projection of the genotypes in function of estimated scores for the first three principal components (Figure 4), formed groups similar to Tocher optimization method and hierarchical clustering UPGMA into three groups. The method enabled to identify the distance among the genotypes 19 and 16 (represented by a triangle), which were also contrasting according to the original dissimilarity matrix (Table 1).

Although the first three principal components had just explained 65.56% of the total variation found (Table 4), this method was consistent with other dissimilarity analysis methods among genotypes, being complementary with the results found in other methods. In order to exploit the maximum genetic variability in crossings and obtain segregating populations, with the possibility of obtaining superior genotypes, the genetic basis of common bean is promising to be expanded. Whereas the multivariate analysis revealed that there are indications of dissimilarity among genotypes. In this sense, the multivariate methods are promising and useful to plant breeders in order to facilitate the interpretation of data showing the dissimilarity of the genotypes and optimizing the working time as well as human and financial resources.

Conclusion

The LMGPP09 and PORTELA16 genotypes are the most dissimilar, while LMGPP01 and LMGPP02 are the most similar. The BRSMG REALCE and PORTELA16 cultivars can be used as genitors in combination with other genotypes in the formation of segregating populations with wide genetic variability. The hierarchical UPGMA clustering method, the Tocher optimizing method, and the principal component analysis are consistent and complementary, enabling a reliable response in data interpretation on the genetic dissimilarity in common bean.

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