Qualitative and quantitative descriptors for quantifying the genetic diversity of bean seeds

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Abstract: Beans are one of the main vegetable protein sources for human consumption. Breeders always seek a diversity of bean genotypes to establish and select the best genotypes that promote the greatest genetic gain for the crop. This study aimed to determine the genetic diversity of bean genotypes based on the characterization of qualitative and quantitative descriptors. Samples of 17 bean cultivars were acquired from the local seed market in Cassilândia, MS, Brazil. Four quantitative and eight qualitative descriptors were used. The data were subjected to analysis of variance using a completely randomized design with three replications. Quantitative data means were grouped using the Scott-Knott test at a 5% probability level. Principal component analysis (PCA) was used for the qualitative and quantitative data. Genetic diversity was reported for all quantitative and qualitative traits of bean seeds. The weight of 1,000 seeds (169 to 629 g), seed width (4.8 to 8.0 mm), seed length (8 to 17 mm), and seed thickness (3.7 to 7.4 mm) showed highly significant differences ($P<0.001$) between the genotypes. Seed color (2 classes), primary color (3 classes) and secondary color (3 classes), seed shape (5 classes), degree of seed flattening (3 classes), seed brightness (3 classes), halo (2 classes) and halo color (2 classes) were the qualitative descriptors measured. The qualitative and quantitative descriptors used in this study proved important for describing the germplasm of the bean crop. The principal component analysis showed that the first two components retain 58% of the data variability, forming five divergent groups. The characterization of the bean genotypes showed genetic diversity in their seeds, and the qualitative and quantitative descriptors used contributed to the adequate separation of the genotypes.

Keywords: *Phaseolus vulgaris* L.; *Vigna unguiculata* L. Walp.; multivariate analysis; selection; seed conservation.

1. Introduction

Common beans (*Phaseolus vulgaris* L.) and cowpeas (*Vigna unguiculata* L. Walp.) are important components of the diet of the human population in several countries around the world, especially due to their high nutritional value and protein content (Fao, 2023). In Brazil, beans are part of the daily diet, especially for the population with lower per capita income (Tavares et al., 2018; Oliveira et al., 2023). Brazil is one of the world's largest producers of beans, with production of around 3 million tons for the 2022 harvest (Conab, 2022), and family farming represents 80% of this national production.

The conservation and selection of genotypes is one of the first actions to learn about the diversity present in any crop (Aguilera et al., 2011; Aguilera et al., 2019; Elsayed et al., 2023;

Morales-Aranibar et al., 2023; Sampaio et al., 2023), and the characterization of these genotypes is fundamental for the creation and maintenance of germplasm banks (Silva et al., 2015). In addition, the conservation and selection of genetic resources represent important sources for guaranteeing food security for future generations (Guerra et al., 2015).

Numerous studies involving beans have guided breeders in the appropriate choice of genitors and at the same time have shown the genetic dissimilarity that the crop presents (Coelho et al., 2007; Cargnelutti Filho et al., 2009; Coelho et al., 2010; Cabral et al., 2011; Correa & Gonçalves, 2012; Gonçalves et al., 2014; Tavares et al., 2018; Bisneto et al., 2022; Oliveira et al., 2023). The genetic variability in beans can be observed in the morphological characteristics of seeds and seedlings, as well as in the growth development characteristics of the crop (Tavares et al., 2018; Oliveira et al., 2023). Studies related to genetic diversity between genotypes collected in different regions result in information of interest in selecting potential genitors for breeding programs (Correa & Gonçalves, 2012). Bean breeding programs are mainly based on hybridizing cultivars and obtaining lines from segregating populations (Tsutsumi et al., 2015). Therefore, the choice of genitors for hybridization is a crucial step since the success of subsequent stages of the breeding program depends on it (Vale et al., 2015).

The germplasm characterization from seeds provides information regarding the genetic variability of each genotype, thus identifying possible duplicates and potential parents. As they are less costly assessments, they are always the first assessments carried out in the pre-improvement process of numerous crops (Aguilera et al., 2011). This study aimed to evaluate the genetic divergence in common bean and cowpea accessions using qualitative and quantitative descriptors in seeds collected in the municipality of Cassilândia, MS, Brazil.

2. Material and Methods

The work was conducted at the State University of Mato Grosso do Sul (UEMS) in Cassilândia, MS, Brazil. A total of 17 bean genotypes, 12 common bean genotypes, and 5 cowpea genotypes were purchased from the local seed market in the municipality of Cassilândia, MS, Brazil. Seed collection is part of a practical activity for 4th-grade Agronomy students. Common bean seeds were collected in different commercial stores located in the city of Cassilândia (MS). The seeds of cowpea genotypes are part of the UEMS/Cassilândia seed bank. The detailed description of the 17 bean genotypes used in this study is shown in Table 1.

Table 1. Description of the species and collection origin of the 17 bean genotypes used in this study.

Genotypes	Name described	Species	Origin
G1	Paquito	*Phaseolus vulgaris* L.	Cassilândia-MS market
G2	Rajado 1	*Phaseolus vulgaris* L.	UEMS/Cassilândia seed bank
G3	Caupi Sempre Verde	*Vigna unguiculata* (L.) Walp.	UEMS/Cassilândia seed bank
G4	Caupi Nova Era	*Vigna unguiculata* (L.) Walp.	UEMS/Cassilândia seed bank
G5	Caupi BRS Guariba	*Vigna unguiculata* (L.) Walp.	UEMS/Cassilândia seed bank
G6	Caupi BRS Itaim	*Vigna unguiculata* (L.) Walp.	UEMS/Cassilândia seed bank
G7	Caupi BRS Tamucumaqui	*Vigna unguiculata* (L.) Walp.	UEMS/Cassilândia seed bank
G8	Vô Cid	*Phaseolus vulgaris* L.	Cassilândia-MS market
G9	Rajado 2	*Phaseolus vulgaris* L.	Cassilândia-MS market
G10	Vermelho Dark	*Phaseolus vulgaris* L.	Cassilândia-MS market
G11	Vermelho	*Phaseolus vulgaris* L.	Cassilândia-MS market
G12	Bolhinha	*Phaseolus vulgaris* L.	Cassilândia-MS market
G13	Bem Te-vi	*Phaseolus vulgaris* L.	Cassilândia-MS market
G14	Branco	*Phaseolus vulgaris* L.	Cassilândia-MS market
G15	Branco Dorama	*Phaseolus vulgaris* L.	Cassilândia-MS market
G16	Feijão Preto	*Phaseolus vulgaris* L.	Cassilândia-MS market
G17	TAA-Marhe	*Phaseolus vulgaris* L.	Cassilândia-MS market

2.1 Evaluation of quantitative descriptors

To assess the genetic divergence of the 17 genotypes, seeds with a moisture content of 12-14% were used (Brasil, 2009). Three repetitions of 25 seeds were used to determine the weight of 1000 seeds (1,000-W, in g), seed width (SW, in mm), seed length (SL, in mm), and seed thickness (ST, in mm). The weight of 1,000 seeds was determined on an analytical balance with an accuracy of 0.0001 g. The seeds' width, length, and thickness were determined using a digital caliper with an accuracy of 0.01 mm.

2.2 Evaluation of qualitative descriptors

To assess genetic divergence in terms of qualitative descriptors, Embrapa's recommendation was used (Silva, 2005), which lists the minimum descriptors indicated for characterizing common bean cultivars/varieties (*Phaseolus vulgaris* L.). A sample of 10 seeds was used, and the qualitative characteristics were checked.

Seed color (SC) was assessed by considering the uniformity of the color in the seeds, assigning a score of 1-Uniform or 2-Uniform.

Primary color (PCo, in %) and secondary color (SCo, in %) by evaluating the percentage of color occurrence in the seed.

The seed shape (SS) is based on the coefficient J (mm) = SL/SW, according to Puerta-Romero (1961) where the following shapes are obtained: Spherical (1.16 to 1.42), Elliptical (1.43 to 1.65), Oblong/Short Reniform (1.66 to 1.85), Oblong/Medium Reniform (1.86 to 2.00) and Oblong/Long Reniform (> 2.00).

The degree of seed flattening (DF) is based on the coefficient H (mm) = ST/SW, according to Puerta Romero (1961): where the following shapes are obtained: Flattened (< 0.69), Semi-filled (0.70 to 0.79), and Filled (> 0.80).

Seed brightness (SB) was assessed by considering the shade of color in the seeds and assigning a score of 1-Opaque, 3-Intermediate, and 5-Bright.

The seed halo (SH) was evaluated by considering the presence of the seed halo and assigning the score 1- Absent and 2- Present.

The color of the seed halo (C-SH) was evaluated by considering the color of the seed halo and assigning a score of 1- Same color as the seed and 2- Different color from the seed.

2.3 Statistical analysis

The data from the quantitative descriptors was subjected to analysis of variance using a completely randomized design, with the F test applied at the 5% probability level. The means of the bean genotypes were compared using the Scott-Knott test at the 5% probability level. The data from the qualitative descriptors was used in descriptive analyses. The principal component analysis (PCA) was used for the quantitative and qualitative data. The statistical analyses used RBio software version 166 for Windows (Bhering, 2017). The graphs were drawn using the SigmaPlot program version 11.0 (Systat Software, Inc., San Jose, CA, USA).

3. Results

3.1 Quantitative descriptors

The results of the analysis of variance for the four quantitative descriptors involving the 17 genotypes are shown in Table 2. High experimental precision was found, measured by the coefficient of variation (CV) with values below 10%. It was, therefore, possible to detect highly

significant differences (*P<0.001*) for all the descriptors tested using the ANOVA F-test (Table 2).

Table 2. Summary of variance analysis of four quantitative descriptors evaluated in seeds of 17 bean genotypes. Cassilândia-MS, 2023.

Features	Mean squares			CV (%)	Average	Minimum	Maximum	H²
	Genotypes	Blocks	Error					
1,000-W (g)	49621 ***	733	481	6.84	320.76	169.2	629.6	99.03
SW (mm)	1.9997 ***	0.0258	0.0638	3.88	6.51	4.78	7.96	96.81
SL (mm)	18.047 ***	0.279	0.198	3.98	11.16	8.11	17.00	98.91
ST (mm)	1.9845 ***	0.0537	0.0382	3.87	5.05	3.68	7.42	98.07

CV: coefficient of variation, H²: broad sense heritability. 1,000-W: weight of 1000 seeds, SW: seed width, SL: seed length, ST, seed thickness. *** represents highly significant differences by the F test at 0.1% probability.

Heritability values for all the descriptors were high (>95%), which suggests that it is possible to select for these characteristics and gain from selection if they are used in a breeding program (Table 2). For all the descriptors, great variability was obtained if we consider the variations shown by the data for the four quantitative descriptors (1,000-W from 169.2 g to 629.6 g, SW from 4.78 mm to 7.96 mm, SL from 8.11 mm to 17.00 mm and ST from 3.68 mm to 7.42 mm, described in Table 2).

Table 3. Mean data for four quantitative descriptors evaluated in seeds of 17 bean genotypes. Cassilândia-MS, 2023.

Genotypes	1,000-W (g)	SW (mm)	SL (mm)	ST (mm)
G1	275.33 e	6.95 b	11.48 e	3.81 f
G2	344.67 d	5.32 e	11.39 e	5.32 c
G3	200.00 g	5.25 e	8.35 h	4.70 d
G4	240.13 f	7.08 b	9.45 g	4.79 d
G5	175.33 g	6.25 d	8.20 h	4.57 d
G6	210.67 g	4.84 f	9.64 g	4.19 e
G7	213.73 g	6.66 c	9.09 g	4.50 d
G8	256.53 f	6.49 c	9.33 g	4.72 d
G9	443.33 b	6.96 b	13.85 c	5.75 b
G10	447.47 b	7.75 a	13.55 c	5.67 b
G11	565.73 a	7.80 a	16.52 a	5.35 c
G12	369.73 c	6.16 d	10.42 f	7.32 a
G13	228.00 f	6.69 c	9.81 g	4.58 d
G14	581.07 a	7.21 b	15.43 b	6.01 b
G15	418.67 b	6.51 c	12.89 d	5.08 c
G16	242.00 f	6.11 d	9.97 g	4.79 d
G17	240.53 f	6.69 c	9.83 g	4.77 d
Groups formed	7	6	8	6

1,000-W: weight of 1,000 seeds, SW: seed width, SL: seed length, ST, seed thickness. Different lowercase letters in the columns represent statistical differences at 5% by the Scott-Knott test.

The results of the post hoc, using the Scott-Knott test for the four quantitative descriptors involving the 17 genotypes are shown in Table 3. It was found that more than six groups were formed for all the descriptors, considering that seven different groups were formed for 1000-W, six groups were formed for SW, 8 groups were formed for SL, and six groups were formed for ST.

For the 1000-W descriptor (Table 3), genotypes G14 and G11, with 581 g and 566 g, were the ones with the highest seed weight, respectively, but four of the five cowpea genotypes had the lowest seed weight (G3, G5, G6 and G7 with 200 g, 175 g, 210 g, and 214 g, respectively). For the SW descriptor, genotypes G11 and G10 had the widest seeds, with 7.80 mm and 7.85 mm, respectively. However, the one with the smallest seed width was genotype G6, with 4.84 mm (Table 3).

For the SL descriptor (Table 3), genotype G11, with 16.52 mm, had the longest seeds, while the genotypes G3 and G5, with 8.35 mm and 8.20 mm, respectively, had the shortest seeds. For the

ST descriptor, genotype G12 (7.32 mm) had the thickest seeds, while genotype G1 (3.81 mm) had the thinnest seeds (Table 3).

3.2 Qualitative descriptors

When considering the qualitative descriptors evaluated in 17 bean genotypes, the variability of the characteristics is shown in Table 4. The qualitative characteristics evaluated show genetic variability between the genotypes evaluated, as evidenced by the classifications of eight descriptors (Table 4).

Table 4. Qualitative descriptors assessed in seeds of 17 bean genotypes. Cassilândia-MS, 2023.

Genotypes	SC[1]	PCo	SCo	SS	DF	SB	SH	C-SH[a]
G1	Uneven	95	5	Elliptical	Flattened	Intermediate	Present	1
G2	Uneven	90	10	Oblong/Reniform	Flattened	Intermediate	Present	2
G3	Uniform	100	0	Elliptical	Filled	Opaque	Present	2
G4	Uniform	100	0	Spherical	Flattened	Opaque	Present	2
G5	Uniform	100	0	Spherical	Semi-filled	Opaque	Present	2
G6	Uniform	100	0	Oblong/Reniform medium	Filled	Opaque	Present	2
G7	Uniform	100	0	Spherical	Flattened	Opaque	Present	2
G8	Uneven	90	10	Elliptical	Semi-filled	Intermediate	Present	1
G9	Uneven	90	10	Oblong/Reniform medium	Filled	Brilliant	Present	2
G10	Uniform	100	0	Oblong/Reniform short	Filled	Intermediate	Present	2
G11	Uniform	100	0	Oblong/Reniform	Flattened	Brilliant	Present	2
G12	Uniform	100	0	Oblong/Reniform short	Filled	Brilliant	Present	2
G13	Uneven	95	5	Elliptical	Flattened	Opaque	Present	2
G14	Uniform	100	0	Oblong/Reniform	Filled	Opaque	Present	1
G15	Uniform	100	0	Oblong/Reniform medium	Semi-filled	Opaque	Absent	1
G16	Uniform	100	0	Elliptical	Semi-filled	Intermediate	Present	2
G17	Uneven	95	5	Elliptical	Semi-filled	Opaque	Present	1

[1] seed color (SC), primary color (PCo), secondary color (SCo), seed shape (SS), degree of seed flattening (DF), seed brightness (SB), seed halo (SH), and color of the seed halo (C-SH). [a] 1-same color as the seed, 2-different color from the seed.

Among the descriptors, the smallest variations, with only two classes, were obtained in SC, SH, and C-SH. The SS had 35% elliptical seeds, 47% oblong seeds, and 18% spherical seeds. The DF had 35% flattened seeds, 35% filled seeds, and 30% semi-filled seeds. SB showed variations in brightness with 29% of seeds having an intermediate brightness, 53% of seeds having an opaque brightness and 18% of bright seeds (Table 4).

3.2 Principal component analysis

The qualitative and quantitative data obtained from the evaluation of 12 descriptors was used in a principal component analysis and is shown in Figure 2. The analysis shows that the first two components account for 58% of the total variability in the data. Among the descriptors, the PCA shows that the variables with the greatest contribution are 1,000-W, SC, PCo, and SCo, in contrast to SH, C-SH, and DF, which have the least contribution to the total variability of the data (Figure 2A).

When combined in the representation of the genotypes and descriptors (Figure 2B), the genotypes are distributed in four groups, with G9 individually separated from the rest of the genotypes. The second group was formed by the proximity of genotypes G2, G8, G1, G17 and G13 and close to the descriptors SC and SCo, which determines their grouping (Figure 2B). A third group comprises cowpea genotypes (G3 to G7) and a common black bean (G16). A fourth group comprises genotypes G11, G14, G10, G12, and G15, which are closely associated

according to the representation of the descriptors with 1,000-W, ST, and DF characteristics,
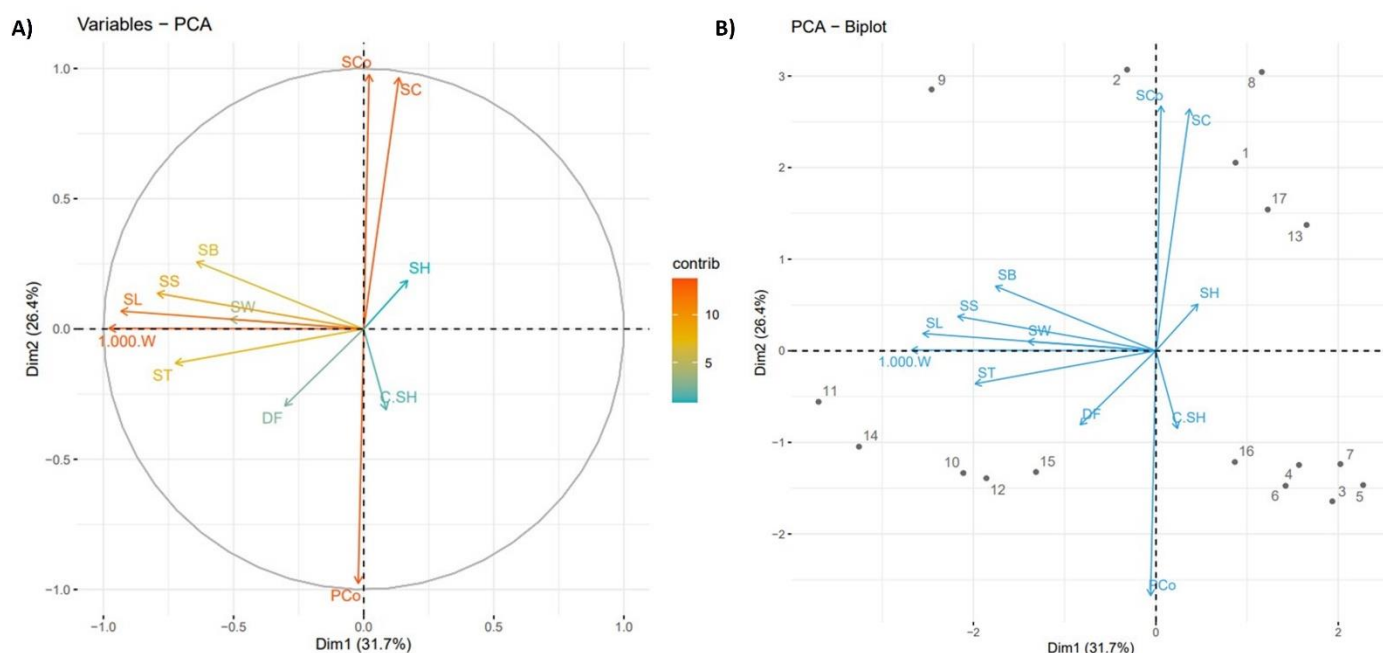which determine this grouping (Figure 2B).

Figure 2. The principal component analysis result obtained from four quantitative and eight qualitative descriptors when characterizing 17
bean genotypes collected in Cassilândia-MS, 2023. The contribution of the descriptors (A) and the representation of the genotypes and
descriptors (B). 1,000-W: weight of 1000 seeds, SW: seed width, SL: seed length, ST: seed thickness, SC: seed color, PCo: primary color, SCo:
secondary color, SS: seed shape, DF: degree of seed flattening, SB: seed brightness, SH: seed halo and C-SH: color of the seed halo. The
numbers in (B) represent the genotypes described in Table 1.

4. Discussion

The morphological characterization of germplasm collections forms the basis of breeding
programs for most crops (Cruz & Regazzi, 2001; Bisneto et al., 2022; Oliveira et al., 2023).
Germplasm collections need to be characterized to identify the best genotypes in terms of
agronomic performance, resistance to pests and diseases, among other attributes (Silva, 2005).
The maintenance and use of the diversity present in germplasm collections is only employed if
it is characterized, so the possibility of making selections and recommending crosses is always
guaranteed (Aguilera et al., 2019).

The characterization of bean seeds using qualitative and quantitative descriptors is efficient in
determining contrasting genotypes of interest for plant breeding (Coelho et al. 2007;
Cargnelutti-Filho et al. 2009; Coelho et al. 2010; Cabral et al. 2011; Correa & Gonçalves, 2012;
Gonçalves et al. 2014; Tavares et al.., 2018; Bisneto et al., 2022; Oliveira et al., 2023). Given the
wide diversity of bean crops, the main objective of this study was to characterize the seeds of
17 genotypes from the collection of bean genotypes in the municipality of Cassilândia, MS,
Brazil. Characterization using four quantitative descriptors (Table 2 and Table 3) and eight
qualitative descriptors (Table 4) was carried out, and with the combination of these two sets of
descriptors a PCA was made (Figure 2).

For the seeds evaluated, it was observed that there is high genetic variability for the two types
of descriptors used, which shows the great variation existing in beans germplasm. The variation
showed that the genotypes had a 1000-seed weight of 169 to 629 g, seed width of 4.8 to 8.0 mm,
seed length of 8 to 17 mm and seed thickness of 3.7 to 7.4 mm, with highly significant
differences ($P<0.001$) between the genotypes (Table 2 and Table 3). Nadeem et al. (2020) when
characterizing a total of 183 common bean accessions, including three commercial varieties
collected from 19 Turkish provinces, showed the occurrence of genotypic variations that can
be used to develop candidate varieties that respond to the preferences of the breeder, the farmer,
and the consumer.

Within the characterized material there are five cowpea genotypes, and the rest are common beans (12 genotypes). The quantitative descriptors made it possible to highlight the differences between these two groups of bean species. Andrade et al. (2010) when characterizing cowpeas observed that the characters associated with production (which include characteristics associated with seeds) exhibited a high genetic component in the phenotypic expression of all the characters evaluated, with a high probability of genetic gains in additional cycles of selection based on phenotype. The same behavior was obtained in this study if we consider that the H^2 obtained was higher than 95% for all the quantitative descriptors tested, regardless of the bean species (Table 2).

When considering the qualitative descriptors, the genotypes showed seed color (2 classes), primary color (3 classes) and secondary color (3 classes), shape (5 classes), degree of flattening (3 classes), gloss (3 classes), halo (2 classes) and halo color (2 classes), and these classes identified in each of the descriptors show the genetic variability that this bean material has (Table 4). The traits evaluated do not interact with the environment and are therefore of interest when classifying bean genotypes according to Embrapa's recommendations (Silva, 2005). From the point of view of consumption, these characteristics associated with the seed (shape, color, and size) are important for the consumer when selecting which bean to buy, and this is also taken into account by crop improvement (Carvalho et al., 2023), which is why it is important to measure these attributes.

The combination of qualitative and quantitative characters has been a powerful strategy employed by numerous research groups in the characterization of tomato germplasm (Aguilera et al., 2019), chili peppers (Sampaio et al., 2023), cotton (Morales-Aranibar et al., 2023) and beans (Cabral et al., 2011; Correa & Gonçalves, 2012; Bisneto et al., 2022; Carvalho et al., 2023, Oliveira et al., 2023). According to Bertini et al. (2010), to identify superior genotypes, it is necessary to consider a combination of several characteristics, which is successfully obtained through multivariate analysis. Multivariate techniques have helped to combine different data (quantitative and qualitative) and have promoted the better selection of parameters or characteristics of greater importance, as well as superior genotypes (Cruz & Regazzi, 2001; Aguilera et al., 2019; Leite et al., 2019).

When we combine the data obtained (four quantitative descriptors and eight qualitative descriptors) in a PCA, we see that 58% of the variation in the data was retained in the first two components (Figure 2). The PCA shows that the variables with the greatest contribution were W1000s, SCo, PCo and SCo, in contrast to SH, SLH and DF, which have the least contribution to the variability of the data obtained (Figure 2A).

Depending on the focus of the breeding program, we could be proposing direct selection on these traits and thus have a greater chance of selecting superior and contrasting genotypes, thereby giving us a greater genetic gain (Cruz & Regazzi, 2001). Using the ACP technique, the genotypes were separated into five groups (Figure 2B). The 12 characteristics made it possible to differentiate the cowpea genotypes from the rest of the genotypes, highlighting the main differences between them and the rest of the common bean genotypes and the power of the PCA to discriminate between them. Among the common bean genotypes, G9 stood out because it differed from the rest of the genotypes, being allocated to an individual group according to the PCA result (Figure 2B), thus showing that the characteristics it possesses are different from the rest of the genotypes.

The characterization of the beans collected showed diversity at seed level. The qualitative and quantitative descriptors contributed to the separation of the genotypes and the data generated confirms the possibility of using this type of work as a strategy in divergence studies to identify duplicates in working collections, allowing for the planning of conservation management and use in breeding programs.

5. References

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6. Additional information

6.1 Acknowledgements

6.2 Conflicts of interest

The authors declare that there is no conflict of interest.