Original Research Article

Morphological and agronomic variability between seven local cowpea genotypes in acre, Brazil

ABSTRACT

Local varieties of cowpea are promising for breeding programs, as they harbor an important source of genetic variability. The objective of this study was to estimate the genetic variability among cowpea genotypes based on morphological and agronomic descriptors. The experiment was carried out in a randomized complete block design with three replications in the experimental field at Universidade Federal do Acre, in Rio Branco, Acre, Brazil. The experimental unit consisted of six lines of 5.0 m, with a spacing of 0.80 m x 0.50 m, with the four central lines used in the evaluations of 21 characters. The treatments consisted of seven local cowpea genotypes. The Mahalanobis generalized distances (D^2) were calculated and used in the Tocher and UPGMA clusters. Principal component analysis, relative contribution of traits and analysis of variance were also performed. Manteiguinha and Mudubim de Rama were the most dissimilar genotypes, while Ceará Mercado and Baiano were the most similar. The main component of greater explanation illustrated the separation of genotypes into two groups, with the union between Manteiguinha and Manteigão forming one group and the other genotypes forming the other group. The traits grain yield, number of grains per pod and grain mass of fifty pods were the main determinants in the quantification of genetic divergence. The combination of the Manteiguinha with Mudubim de Rama genotypes is the most suitable for obtaining promising segregating populations.

Keywords: Vigna unguiculata; genetic distance; *legume breeding*; *cluster analysis*.

1. INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is a dicotyledonous angiosperm of great importance among cultivated leguminous species as it is a versatile crop, which increases soil fertility through biological nitrogen fixation [1]. In addition, it has phenotypic plasticity and high genetic variability, which gives it a high adaptive capacity to different edaphoclimatic conditions [2].

Brazil produces 3.3 million tons in an area of 3.7 million ha^{-1} of cultivated beans annually, with cowpea being responsible for 15.5% of the total production, occupying 40% of the planted area. The North region concentrates 8.8% of the total produced nationally, representing the second position among the largest producing regions [3]. Thus, the cowpea stands out socioeconomically in the agricultural scenario of the Brazilian Amazon, assuming great importance in human nutrition. Furthermore, as it is cultivated by small and medium-sized producers, who practice agriculture characterized as extensive and subsistence, it contributes to the generation of employment and income for local populations [4].

As a result of the agricultural model mentioned above, there is the conservation of little studied genotypes, which could be worked on in genetic improvement programs for the selection or improvement of morphophysiological characteristics, citing grain quality, plant architecture and resistance to biotic stress and abiotic [5]. One of the necessary prerequisites in breeding programs is the knowledge of the available genetic variability, which allows evaluating the parents as to the similarity or divergence between them. This contributes to determining the hybrid combinations with greater heterotic effect and high heterozygosity and, consequently, greater potential to generate promising segregating populations for obtaining superior cultivars [6]. This knowledge can be obtained by applying clustering methods such as Tocher and UPGMA, based on dissimilarity measures such as the Mahalanobis distance [6].

Genetic divergence among cowpea genotypes has been previously reported [7, 8, 9]. However, many local varieties of cowpea, such as those grown in the State of Acre, remain unknown in terms of genetic variability related to morphoagronomic aspects. Therefore, in order to deepen the knowledge of the species, genetic variability studies are still necessary, considering that Acre is one of the granaries of diversity of this culture [3].

Given this scenario, the aim of this study was to verify the genetic divergence between seven traditional varieties of cowpea from Acre through the use of morphoagronomic traits.

2. MATERIAL AND METHODS

The experiment was conducted in the experimental area of the research group on genetic improvement at the Federal University of Acre (GPMG/UFAC), in Rio Branco (lat 09°58'29"S; long 67°48'36"W, 164 m asl). According to Koppen's classification, the climate in the region is equatorial, hot and humid, with temperature, humidity and annual precipitation ranging from 24.5 ºC to 32 ºC, 80% to 90% and 1,700 mm to 2,400 mm, respectively [10]. The soil in the area is classified as a plinthic elliptic red-yellow argisol [11].

A randomized complete block design (RCBD) with seven treatments and three replications was used. The experimental unit consisted of six rows of 5.0 m, with a spacing of 0.80 m x 0.50 m, considering only the four central rows, where the evaluations were carried out, as a useful portion. Sowing was carried out on May 5, 2016 and studies were carried out from May to August 2016. Planting was carried out in furrows. The seeds used came from collections carried out in the municipalities of Sena Madureira, Rodrigues Alves and Cruzeiro do Sul, in the biennium 2012/2013. For legal purposes, the collections were carried out with authorization required from the Biodiversity Information and Authorization System with registration 34945-1.

The treatments consisted of seven cowpea genotypes: Baiano, Ceará Caeté, Ceará Mercado, Costa de Vaca, Manteigão, Manteiguinha and Mudubim de Rama. In the central lines of each plot, evaluations of 21 morphoagronomic descriptors were carried out, which are described in Table 1. Soil preparation consists of plowing and harrowing in the 0-20 cm layer, 30 days before the installation of the experiment. To correct soil acidity, 300 kg ha^{-1} of dolomitic lime were applied, as recommended by Uchôa et al [12] for the soil of the experimental area used. The planting furrows were opened manually with the use of hoes, where 6 liters of cured earthworm humus was deposited, the result of mixing grass clippings, leaves and plant branches.

Table 1. Descriptors of 21 traits of the seven cowpea varieties.

Initials	Traits	Description
HP	Height of plants	Measurement of the plant's neck to the apical gem
NMB	Number of Main Branches	Average main branches observed in the 8th week
ALW	Apical Leaflet Width	Distance from extreme to the other of the apical leaflet
BF	Beginning of Flowering	Number of days until the first flowers appear
NDFM	Number of days for maturation of the pods	Number of days from sowing to maturity of 50% of the pods
AF	Average Flowering	Number of days between emergence, up to 50% of plants showing open flower
EF	End of Flowering	Number of days from sowing to completion of flowering.
HL	Hypocotyl Length in mm	Hypocotyl measuring from the collar to the node of the cotyledons
AM	Average Maturation	Number of days from sowing up to 50% of the plants have mature pods
L25PL	Twenty-five Pod Length	Length of 25 ripe pods, selected at random
NLP1	Number of Locules per Pod	Number of locules per pod of the first harvest
NLP2	Number of Locules per Pod	Number of locules per pod of the second harvest
SL	Seed Length	Average length of 50 seeds in mm
SW	Seed Width in mm	Average width of hilo to keel of 50 seeds
ST	Seed Thickness in mm	Average of 50 mature seeds.

HY	Harvest Yield	Estimate based on production per useful area of th experimental plot.
NPB	Number of pod beans	Number of pod grains from the first harvest.
MB50P	Mass of Pod Beans	Grain mass of 50 pods.
M100G	Mass of 100 Grains	Average mass of 100 grains.
PGH	Plant Growth Habit	Evaluated in the 6th week after sowing.
PV	Plant Vigor	Plant height and width, evaluated 3-4 weeks after sowing.

Genetic divergence analyzes were performed using the Mahalanobis generalized distance (D2) as a measure of dissimilarity between all pairs of genotypes. With the D^2 in hand, the grouping analyzes were performed using the Tocher method and the UPGMA (Unweighted Pair Group Method Arithmetic Average). The analyzes were performed with the help of the Genes software [13].

Another multivariate method approached with a view to grouping through variances was the principal components method. The components were calculated using the data of repetitions and centroid of the 21 variables. Principal component analysis (PCA) was performed using commands from the factoextra package [14] in the R programming environment, 4.1.0 (https://cran.r-project.org/).

In addition to the grouping study, an investigation was carried out to verify the relative importance of each trait for the genetic divergence between varieties, using the method of [15]. Additionally, only for the traits that presented considerable (above 10%) relative importance, an analysis of variance was performed, with application of the F test. For comparisons between the means of the varieties, the Scott-Knott test was used, at the level of 5% probability.

3. RESULTS AND DISCUSSION

The generalized Mahalanobis distances (D^2) among the seven cowpea varieties are shown in Table 2. The lowest genetic similarity was observed between the Ceará Mercado and Baiano genotypes (0.92), showing that these genotypes are the most similar for the studied traits and, therefore, the least indicated for the selection of superior genotypes. Genetic variability is essential in any breeding program, so that similar genotypes are not recommended for use in hybridization, as it makes selection gains impossible [6].

Table 2. Measures of genetic dissimilarity among seven cowpea genotypes in relation to 21 traits, based on generalized Mahalanobis distance (D^2).

Genotypes	Genotypes					
	2	**3**	**4**	**5**	**6**	**7**
1	11.701	11.349	4.209	8.306	8.027	9.267
2		4.009	6.646	1.701	1.834	2.552
3			9.713	1.619	2.865	0.922
4				6.101	5.441	7.201
5					1.129	0.993
6						1.370

Genotypes: 1 = Manteiguinha; 2 = Mudubim de Rama; 3 = Ceará Mercado; 4 = Manteigão; 5 = Costela de Vaca; 6 = Ceará Caeté; 7 = Baiano.

At first, it is recommended to cross between pairs of more divergent genotypes, aiming to maximize heterosis in the progenies and increase the possibility of occurrence of segregants in advanced generations [6]. In this study, the most divergent combinations were formed by the genotypes Manteiguinha and Mudubim de Rama (D2 = 11.70) followed by Manteiguinha and Ceará Mercado (11.35). Therefore, due to the high genetic distance, such arrangements deserve special attention in cowpea breeding programs, as they can provide favorable genetic combinations for the selection of superior genotypes.

The Tocher grouping method allowed the formation of two groups (Table 3). Group I gathered 71% of the studied genotypes, demonstrating that they are little variable among themselves, showing inter-genotypic homogeneity. The genotypes grouped in group I come from the municipalities of Sena Madureira and Cruzeiro do Sul. In general, it was observed that the groupings between genotypes indicated little relationship with the place of occurrence. The separation of genotypes into distinct groups by the Tocher method is a common feature among studies with cowpea [7, 16].

Table 3. Grouping of seven cowpea genotypes by the Tocher optimization method, based on Mahalanobis Generalized Distance (D^2).

Groups	Genotypes	Average distance
I	Ceará Mercado, Baiano, Costela de Vaca, Ceará Caeté, Mudubim de Rama	1,9
II	Manteiguinha, Manteigão	4,21
I x II	-	8,38

Group II gathered only two genotypes (29%), from the municipalities of Rodrigues Alves and Sena Madureira (Manteiguinha and Manteigão), demonstrating a close relationship between them. The combination between these two genotypes, as well as between the genotypes that form group I, are less suitable for use in future breeding programs for the species in question, considering that within the group there is homogeneity between the genotypes, whereas between the groups heterogeneity predominates. Therefore, combinations capable of generating promising hybrid populations are more easily achieved when crossing genotypes from different groups [7].

Differently from the grouping by the Tocher optimization method, where two different groups were formed, in the dendrogram obtained by the UPGMA method, the genotypes were arranged in three groups from a cut at approximately 35% of the maximum distance (Figure 1). Although there were differences in the number of groups formed by the two methods used, group I of both methods had exactly the same components (Ceará Mercado, Baiano, Costela de Vaca, Ceará Caeté and Mudubim de Rama), confirming that the genotypes of this group have a greater source of kinship.

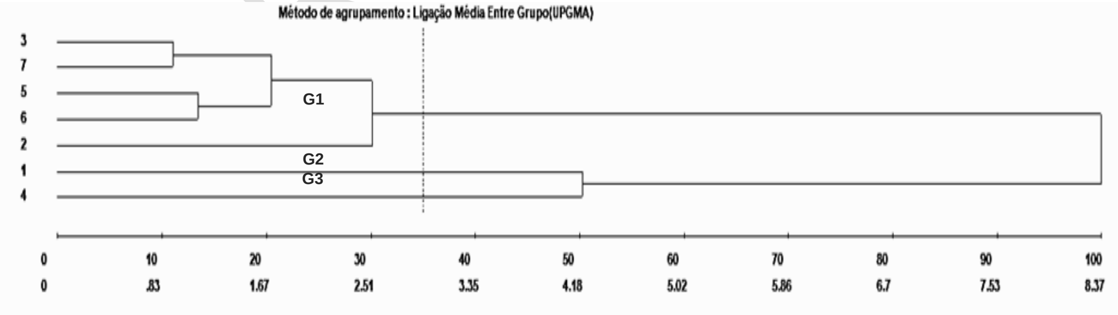

Figure 1. Dendrogram generated by the UPGMA method, representing the genetic dissimilarity among seven cowpea genotypes, using the Generalized Mahalanobis Distance (D^2) as a dissimilarity measure. Genotypes: 1 = Manteiguinha; 2 = Mudubim de Rama; 3 = Ceará Mercado; 4 = Manteigão; 5 = Costela de Vaca; 6 = Ceará Caeté; 7 = Baiano.

Groups II and III generated by the UPGMA method were unitarily formed by the Manteiguinha and Manteigão genotypes, respectively, components of group II of the Tocher optimization method. This shows that despite using the same genetic distance values (D2), the results do not fully corroborate. This difference is due to the peculiarity with which each method carries out its conglomerates. However, even with small discrepancies, both clustering methods are considered efficient in discriminating genotypes by genetic dissimilarity [17]. However, the UPGMA allows for better genotypic characterization, allowing the identification of individuals with great genetic distance and using them as parents, in order to increase the probability of reaching populations with great genetic variability [18].

It is noteworthy that, as in the Tocher method, the UPGMA method also did not group the genotypes by place of collection, showing the existence of wide divergence between genotypes of the same origin. Similar observations showed that common bean genotypes did not group by progeny or place of collection, demonstrating that geographic origin does not necessarily reflect genetic diversity, which is a measure of variability related to the genes they possess [19].

The genetic distance relationship between the seven genotypes was also verified by the principal components technique. Figure 2 shows the graphical dispersion of the seven genotypes based on the combination of the first two main components in relation to the 21 traits studied. These components explained 73.30% of the total variation (PC1 = 59.3% and PC2 = 19%). A useful interpretation of principal component analysis is that the total percent change in the data is explained by principal components (CPs), with each CP being a linear combination of the original variable [20]. However, all components are not commonly used, as most data variation patterns are limited to PC1 and PC2, which can be summarized in scatter plots [21]. The component with greater explanation (CP1) clearly illustrated the separation of genotypes into two groups, with the union between Manteiguinha and Manteigão forming one group and the other genotypes forming the other group.

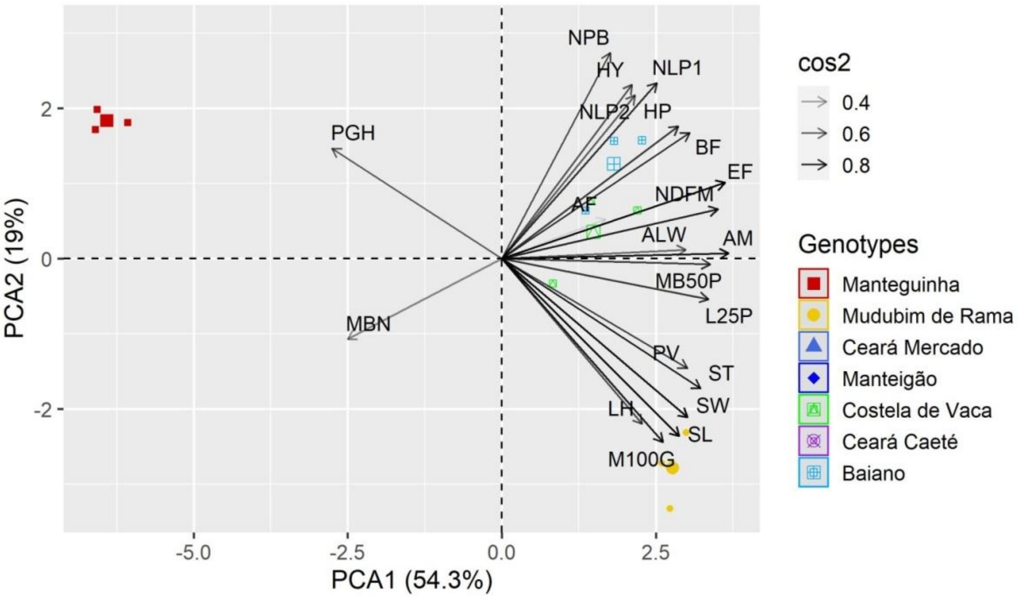

Figure 2. Graphic dispersion of seven traditional cowpea genotypes and 21 morpho-agronomic traits obtained through the first and second main components. The traits are: Height of plants (HP), Main Branch Numbers (MBN), Apical leaflet Width (ALW), Beginning of flowering (BF), Number days until first pod maturation (NDFM), Average flowering (AF), end of flowering (EF), hypocotyl length in mm (HL), Average maturation (AM), Twenty-five pod length (L25P), Number of locules per pod (NLP1), Number of locules per pod (NLP2), Seed length (SL), Harvest yield (HY), Number of grains per pod (NPB), Grain weight of 50 pods (MB50P), Seed width in mm (SW), Seed thickness in mm (ST), Mass of 100 grains (M100G), Plant growth habit (PGH), Plant vigor (PV).

The traits number of main branches (NRP) and growth habit (HC) were the ones with the greatest discriminatory power in the segregation of the Manteiguinha and Manteigão genotypes. The relationship of similarity between these two genotypes and dissimilarity between the others is consistent with Tocher's analysis. The group formed by the genotypes Ceará Mercado, Baiano, Costela de Vaca, Ceará Caeté and

Mudubim de Rama had the traits average maturity, end of flowering and number of days until first maturity as the main influencers on the separation and formation of the group, corroborating with the Tocher and UPGMA methods.

The relative contribution of traits to the genetic divergence of the seven cowpea genotypes is shown in Figure 3. This contribution is of great importance, as it allows the identification of traits with greater influence in the determination of dissimilarity measures, and likewise, help in the discard of those that are not very expressive, and that contribute minimally to the discrimination of genotypes. Therefore, it is essential to know the influence of the different traits studied on the genetic divergence between the different accessions, as these will allow directing the improvement work, increasing the efficiency in the synthesis of promising genotypes [17].

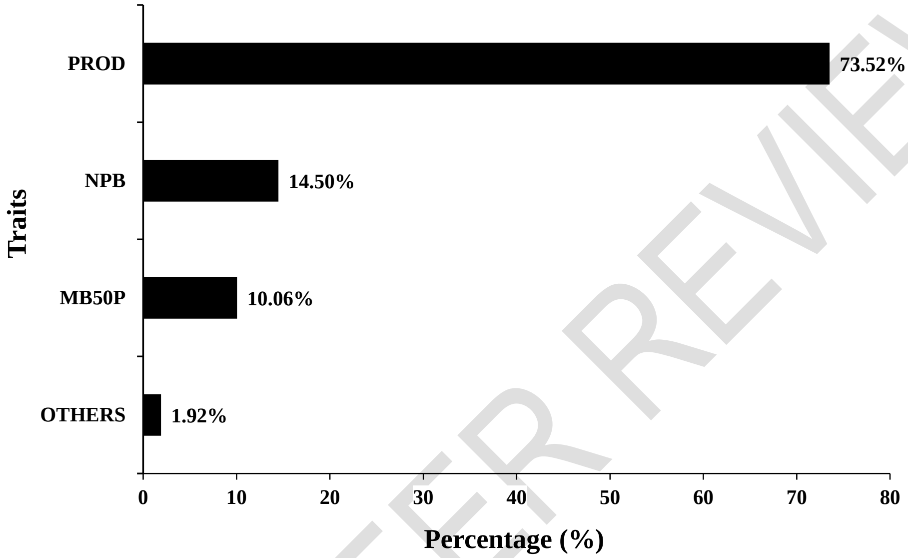

Figure 3. Relative contribution of the traits Harvest yield (HY), Number of grains per pod (NPB), Grain weight of 50 pods (MB50P) and OTHERS: Height of plants (HP), Main Branch Numbers (MBN), Apical leaflet Width (ALW), beginning of flowering (BF), number days until first pod maturation (NDFM), Average flowering (AF), end of flowering (EF), hypocotyl length in mm (HL), Average maturation (AM), Twenty-five pod length (L25P), Number of locules per pod (NLP1), Number of locules per pod (NLP2), Seed length (SL), Seed width in mm (SW), Seed thickness in mm (ST), Mass of 100 grains (M100G), Plant growth habit (PGH), Plant vigor (PV), for genetic dissimilarity of seven cowpea cultivars, determined by the Singh method (1981).

Grain yield contributed considerably to the dissimilarity between the evaluated genotypes, 74% of the total observed. This trait has been the attribute with the greatest influence on genetic divergence in studies with cowpea genotypes (99.8% [7]; 24.3% [8]). This fact suggests that grain yield should be prioritized in studies of genetic divergence in cowpea, as it is the most economically important trait. In addition to yield, the number of grains per pod and the grain mass of 50 pods were the traits that most contributed to the dissimilarity between the genotypes, 14% and 10.06%, respectively. Such traits are extremely important, as they are directly related to productivity [22]. For Santos et al [23] One hundred grain mass is the trait that most contributed to the separation of common bean accessions and should be prioritized in genetic improvement programs.

The other traits contributed in an inexpressive way for the determination of the dissimilarity measures between the studied genotypes (1.92%), indicating less influence in the gain of genetic variability and little relevance for the identification of genetic divergence. Low contribution traits are not relevant in studies of genetic divergence or duplication of genotypes in germplasm banks, although they are important to be evaluated for the knowledge of the productive potential of the genotypes [24]. Furthermore, low expressive traits make the selection of parents in breeding programs unfeasible [8]. In contrast, high-contribution traits make it possible to reduce labor, time and experimental costs [25].

Finally, for the characteristics that presented relative importance above 10% (grain yield, number of grains of pods and weight of grains of 50 pods) an analysis of variance was performed. There was a significant difference (p<0.01) between the genotypes for the three traits analyzed, confirming the existence of genetic variability between the genotypes (Figure 4A; Figure 4B; Figure 4C). The variability based on these traits, mainly grain yield, corroborates previous studies in cowpea [7, 8]. This aspect is relevant, as it allows maximizing the possibility of success in using genotypes in breeding programs.

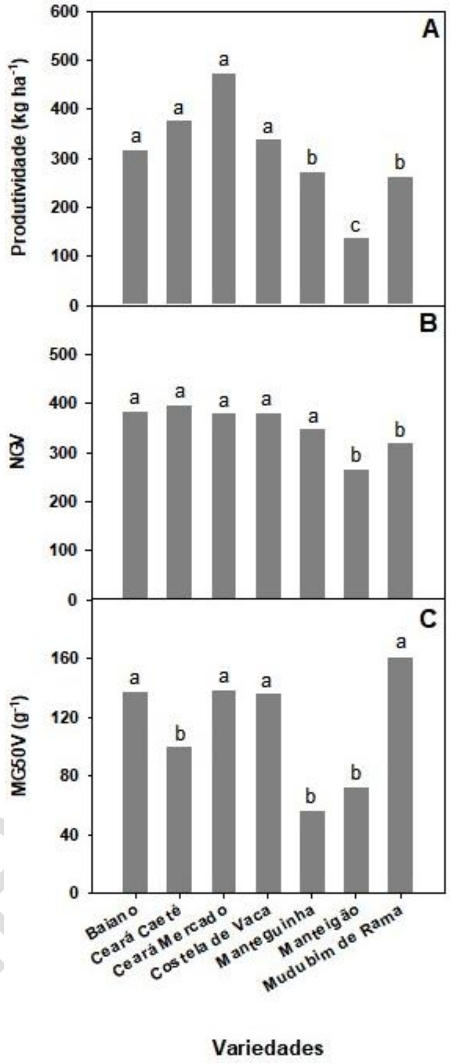

Figure 4. Means of grain yield (A), number of grains per pod (B) and grain weight of 50 pods (C) of seven traditional varieties of cowpea from Acre. Means followed by the same lowercase letter belong to the same statistical group by the Scott-Knott test at the 5% probability level.

The number of grains per pod and the grain mass of 50 pods are traits that contribute to productivity. Even with this relationship, it was found that the variation between genotypes in these three traits coincided only partially. This is the case of Baiano, Ceará Mercado and Costela de Vaca, which together had the highest averages in the three traits (Figure 4A; Figure 4B; Figure 4C). Ceará Caeté, on the other hand, also had the highest average yield and number of grains per pod, but was not among the varieties with the highest average grain mass of 50 pods. Manteigão has always been at the lower end, with low averages in the three variables analyzed. On the other hand, o Manteiguinha e o Mundubim de Rama showed statistically superior means only for number of grains per pod and grain mass of 50 pods, respectively. This shows that traits have different weights between genotypes to maintain productivity.

Comparatively, the yield results verified in this study were lower than the national average (712.6 kg ha^{-1}) in the 2019/2020 harvest [26]. However, the traditionally native varieties with a vast evolutionary history and adaptation to stressful environments are ideal genetic resources to explore new genetic variations that overcome the challenges of agricultural production, such as: resistance to diseases, pests and abiotic imbalances [27]. In addition, seeds from traditional varieties are cheaper in local markets and can be reproduced and stored for use in subsequent crops, which reduces the cost of production [28].

4. CONCLUSION

The combinations between the genotypes Manteiguinha with Mudubim de Rama and Manteiguinha with Ceará Mercado are the most suitable for obtaining promising segregating populations.

Grain yield, number of grains per pod and grain weight of 50 pods are the traits that most contribute to genetic dissimilarity and should be 6prioritized in the selection of cowpea genotypes.

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