Revista Agrarian
ISSN: 1984-2538

Choice of parents of cowpea bean by means of multivariate analysis

Escolha de genitores de feijão caupi por meio de análise multivariada

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Recebido em: 28/03/2014 Aceito em: 22/05/2015

Abstract. The aim of this study was to quantify genetic divergence among cowpea bean genotypes by means of Integrated Multivariate Analysis, with the purpose of assisting selection of parents for development of new cultivars. A randomized block experimental design was used with 32 treatments and 4 replications. There is agreement among the multivariate techniques applied in clustering of the genotypes. The genotypes show genetic variability, exhibiting potential for use in breeding programs. The crosses carried out between the genotypes MNC02-675F-9-3 and MNC03-737F-5-10 with the genotypes MNC03-737F-5-11, MNC03-737F-5-1, MNC03-725F-3 and MNC03-737F-5-9 may lead to obtaining segregating progenies with high yield potential and an increase in the probability of select superior genotypes in the segregating generations.

Keywords: Hybridization, clustering techniques, selection, *Vigna unguiculata*

Resumo. O objetivo foi quantificar a divergência genética entre genótipos de feijão-caupi por meio de Análise Multivariada Integrada, com a finalidade de auxiliar na seleção de genitores para o desenvolvimento de novos cultivares. O delineamento experimental utilizado foi em blocos casualizados, com 32 tratamentos e 4 repetições. Existe uma concordância entre as técnicas multivariadas aplicadas na realização do agrupamento dos genótipos. Os genótipos apresentam variabilidade genética, possuindo potencial para uso em programas de melhoramento. O cruzamento dirigido entre os genótipos MNC02-675F-9-3 e MNC03-737F-5-10 com os genótipos MNC03-737F-5-11, MNC03-737F-5-1, MNC03-725F-3 e MNC03-737F-5-9, poderá propiciar a obtenção de progênies segregantes com elevado potencial produtivo e aumento na probabilidade de selecionar genótipos superiores nas gerações segregantes.

Palavras-chave: Hibridação, técnicas de agrupamento, seleção, *Vigna unguiculata*

Introduction

In Brazil, genetic breeding of cowpea bean has been developing gradually. According to Silva et al. (2011), the country has a history of low yields, from 500 to 700 kg ha^{-1}. Cowpea bean production is concentrated in the Northeast region (1.2 million ha) and North region (55.8 thousand ha) and has gained ground in the Center-West region. Although the mean bean yield is low, as a result of the low level of technology used in growing it, the states of Amazonas, Goiás, Mato Grosso do Sul, and Mato Grosso achieve yields greater than 1000 kg ha^{-1} (Silva, 2009).According to Bertini et al. (2010), genetic breeding is based on selection of parents, followed by hybridization for the purpose of forming a base population and advancement of the segregating generation, with simultaneous selection

For more than one trait. In identification of the parents for hybridization, genotype selection is recommended within the most divergent groups, with greater mean values in relation to the traits (Passos et al., 2007). According to Nagalakshmi et al. (2010), there are two reasons to identify genetic diversity among the most promising types, the first being related to genetic diversification of the parents for hybridization programs, subject to production of a high heterotic effect, and the second through expecting a broad range of variability in the

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ISSN: 1984-2538

segregation generation of crosses involving remotely related parents.

Thus, studies of genetic divergence of cowpea bean become important for identification of parent groups that show the best hybrid combination of greatest heterotic effect and greatest heterozygosis for traits related to growth habit and seed production and quality (Passos et al., 2007; Bertini et al., 2010).

Studies on genetic divergence with application of multivariate analysis can contribute to advances in cowpea bean breeding programs and in different crops as well. Among the studies on this topic, the following may be cited: Dias et al. (2009), Nagalakshmi et al. (2010), among others.

According to Sartorio (2008), multivariate statistics is a set of statistical methods that allows simultaneous analysis of diverse variables in each experimental unit, as well as an overall study of these variables, showing the connections, similarities or differences among them, seeking minimal loss of information of the original data.

Thus, multivariate statistical analysis has diverse methods, among them the analysis of canonical variables, an alternative when experimental data with replication is available (Gonçalves & Fritsche-Neto, 2012); it may estimate the weighted coefficient of the original variables in each one of the canonical variables and their variances.

Another aspect of multivariate analysis which assists plant breeding by means of identification of contrasting genotypes used for obtaining segregating populations with greater variability refers to dissimilarity measures and clustering methods (Stähelin et al., 2011).

In this context, for analysis of genetic divergence, different multivariate methods may be applied. Among them, the most used are: principal component analysis (PCA), canonical variable analysis (CVA) and cluster analysis (CA), with the use of a dissimilarity measure (Oliveira et al., 2003). In addition, use of the Mahalanobis generalized distance (D^2) and the cluster technique by the Tocher method is recommended for quantification of divergence among the parents (Santos et al., 2012; Oliveria et al., 2003).

The choice of the most adequate method has been determined by the precision desired by the researcher, ease of analysis and the manner in which the data were obtained, whether by experimental means or by sampling (Bezerra Neto et al., 2010).

Thus, the purpose of this study was to quantify the genetic divergence among cowpea bean genotypes by means of Integrated Multivariate Analysis for the purpose of assisting in selection of parents for development of new cultivars.

Materials and Methods

The experiment was carried out with 32 cowpea bean genotypes, with 28 advanced lines and 4 commercial varieties, originating from the germplasm bank of Embrapa Meio Norte (Table 1). The study was conducted in the municipality of Chapadão do Sul, located in the state of Mato Grosso do Sul in the fall-winter period of 2011.

Harvest of the plots was performed manually and in each plot the plants were assessed in regard to the following traits: pod weight (PW), considering the average weight of the pods from five plants collected at random at physiological maturity, in grams (g); green pod length (GPL), average length in centimeters of the previously collected pods, in g; average weight of seeds per pod (WSP), average weight of seeds from five plants collected, in g; seed index (SI), refers to the green seed weight in green pods at physiological maturity; number of seeds per pod (NSP), performed through counting the seeds in the pods collected for the previous samples; average number of pods per plant (NPP), considering the average of the pods collected from five plants; hundred grain weight (HGW), weight of 100 seeds at 13% moisture, in g, and grain yield (YLD) estimated through the useful area collected in each plot, extrapolating the results to kg ha^{-1}, and adjusting the data to 13% moisture.

A randomized block experimental design was used with 32 treatments and 4 replications. The experimental unit consisted of four five-meter rows spaced at 0.50 meters, considering the two center rows as the useful area.

Initially, univariate analysis of variance was performed to obtain the estimate of the adjusted mean values and after that analysis of genetic divergence using the multivariate techniques of principal components, canonical variables and clustering. In application of the genotype clustering technique by the unweighted pair group method with arithmetic mean (UPGMA), the Mahalanobis generalized distance (D^2) was adopted as a measure of dissimilarity, taking into consideration the degree of dependence among the variables studied (Cruz & Regazzi, 2001).

In relation to the establishment of similar groups, the optimization based hierarchical agglomeration clustering method proposed by Tocher was applied, whose calculations were

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likewise based on the Mahalanobis generalized distance (D^2). Principal component analysis (PCA) was used, evaluating the relative contribution of each trait to the genetic divergence among them and graphic distribution was prepared as a function of the first three principal components. In addition, a distribution graph was drawn up using the canonical variables (Cruz & Regazzi, 2001). The GENES (Cruz, 2013) computing application was used to carry out statistical analyses.

Table 1. Description of the genotypes used in the experiment

NIG	Genotypes	Commercial subclass	Variety/Line
1	MNC02-675F-4-9	Mulatto	Line
2	MNC02-675F-4-2	Mulatto	Line
3	MNC02-675F-9-2	Mulatto	Line
4	MNC02-675F-9-3	Mulatto	Line
5	MNC02-676F-3	Mulatto	Line
6	MNC02-682F-2-6	White	Line
7	MNC02-683F-1	White	Line
8	MNC02-684F-5-6	White	Line
9	MNC03-725F-3	White	Line
10	MNC03-736F-7	White	Line
11	MNC03-737F-5-1	White	Line
12	MNC03-737F-5-4	White	Line
13	MNC03-737F-5-9	White	Line
14	MNC03-737F-5-10	White	Line
15	MNC03-737F-5-11	White	Line
16	MNC03-737F-11	White	Line
17	BRS-Tumucumaque	White	Variety
18	BRS-Cauame	White	Variety
19	BRS-Itaim	Fradinho	Variety
20	BRS-Guariba	White	Variety
21	MNCO1-649F-1-3	Mulatto	Line
22	MNCO1-649F-2-1	White	Line
23	MNCO1-649F-2-11	White	Line
24	MNCO2-675F-4-9	Ever Green	Line
25	MNCO2-675F-9-5	Ever Green	Line
26	MNCO2-676F-1	Striped	Line
27	MNCO2-677F-2	Mulatto	Line
28	MNCO2-677F-5	Ever Green	Line
29	MNCO2-680F-1-2	Mulatto	Line
30	MNCO2-689F-2-8	Striped	Line
31	MNCO2-701F-2	Striped	Line
32	MNCO3-736F-2	Ever Green	Line

NIG: Genotype identification number

Results and Discussion

The traits that most contributed to genetic diversity were the NVP (30.93%), the NGV (17.74%) and the IDG (15.72%) (Table 2), indicating the existence of greater genetic variability for these traits in the germplasm studied. Elias et al. (2007) and Cabral et al. (2011), studying genetic divergence in common bean by means of

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multivariate analysis, indicated hundred grain weight as the variable that most contributed to genetic divergence.

Table 2. Relative contribution of each trait to genetic dissimilarity (S.j') in 32 cowpea bean genotypes.

Variable	S.j'	%	% accumulated
PW[1/]	310.2552	6.6956	6.695
GPL	468.2365	10.105	16.801
WSP	531.5836	11.4721	28.273
SI	728.4428	15.7205	43.993
NSP	822.2709	17.7454	61.739
NPP	1433.4816	30.936	92.675
HGW	22.1959	0.479	93.154
YLD	317.2375	6.8463	100.000

[1/]PW: pod weight (g); GLP: green pod length (cm); WSP: weight of seeds per pod (g); SI: seed index (%); NSP: number of seeds per pod; NPP: number of pods per plant; HGW: hundred grain weight; YLD: grain yield (kg ha^{-1}).

For cowpea bean, studies on genetic divergence have shown differing results in regard to the contribution of each component to diversity. Dias et al. (2009) observed that the variables that most contributed to divergence in cowpea bean were the maturation cycle, number of nodes on the main stem and the beginning of flowering. In their study, Nagalakshmi et al. (2010), described grain yield and 100 grain weight as the traits that most contributed to divergence. In this study, the number of pods per plant, number of seeds per pod and seed index were the traits that most contributed.

It may furthermore be observed in relation to the relative contribution of each trait, that the traits HGW, YLD and PW, had low power of divergence among the genotypes, even showing productive heterogeneity among the genotypes, forming two distinct groups by the Scott-Knott clustering test (Table 3).

Table 3. Mean values of the eight traits assessed in 32 cowpea bean genotypes.

Genotypes	PV[1/]	CVV	PGV	IDG	NGV	NVP	P100G	PROD
MNC02-675F-4-9	14.9 a*	19.2 a	11.1 a	74.8 a	11.6 a	8.8 b	19.2 a	1585.0 a
MNC02-675F-4-2	14.9 a	18.6 a	10.6 a	71.5 b	10.6 b	7.6 c	20.2 a	1331.1 a
MNC02-675F-9-2	12.6 b	17.1 b	9.3 b	73.5 a	9.2 b	8.4 b	20.2 a	1295.1 a
MNC02-675F-9-3	14.6 a	18.7 a	11.1 a	75.8 a	10.9 b	8.6 b	20.3 a	1541.3 a
MNC02-676F-3	11.2 b	15.7 b	8.1 b	64.0 c	9.8 b	11.6 a	14.6 b	1313.9 a
MNC02-682F-2-6	13.9 a	20.5 a	9.9 b	71.2 b	10.4 b	6.9 c	19.1 a	1095.6 b
MNC02-683F-1	14.5 a	18.8 a	10.9 a	75.1 a	10.7 b	6.7 c	20.3 a	1189.2 b
MNC02-684F-5-6	12.1 b	18.1 b	9.0 b	75.1 a	11.1 b	9.8 b	16.7 b	1449.6 a
MNC03-725F-3	14.7 a	18.6 a	10.9 a	74.5 a	11.9 a	10.3 b	18.5 a	1817.8 a
MNC03-736F-7	15.0 a	19.7 a	11.0 a	73.2 a	11.0 b	7.5 c	20.1 a	1328.5 a
MNC03-737F-5-1	13.7 a	18.8 a	10.7 a	78.7 a	11.9 a	11.0 a	18.1 a	1909.7 a
MNC03-737F-5-4	13.4 b	18.7 a	10.3 b	76.8 a	11.1 b	9.8 b	18.4 a	1564.7 a
MNC03-737F-5-9	12.3 b	17.9 b	9.3 b	75.6 a	10.35 b	11.9 a	18.1 a	1758.5 a
MNC03-737F-5-10	14.6 a	18.7 a	11.0 a	75.4 a	11.0 b	7.9 c	20.0 a	1392.2 a
MNC03-737F-5-11	12.2 b	17.2 b	9.6 b	78.7 a	10.4 b	13.4 a	19.3 a	2090.0 a
MNC03-737F-11	14.1 a	19.5 a	10.8 a	76.8 a	11.7 a	7.8 c	18.4 a	1357.8 a
BRS-Tumucumaque	13.8 a	19.0 a	10.2 b	73.9 a	11.3 b	9.9 b	18.2 a	1623.6 a

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BRS-Cauame	14.0 a	19.2 a	10.7 a	76.8 a	10.8 b	6.4 c	19.8 a	1127.4 b
BRS-Itaim	12.5 b	17.9 b	9.8 b	78.7 a	9.1 b	9.2 b	21.8 a	1451.5 a
BRS-Guariba	14.9 a	18.9 a	11.4 a	76.5 a	11.7 a	8.6 b	19.5 a	1604.2 a
MNCO1-649F-1-3	16.5 a	19.8 a	12.1 a	73.4 a	12.1 a	4.6 c	19.9 a	869.3 b
MNCO1-649F-2-1	14.0 a	19.9 a	10.0 b	71.8 b	11.7 a	6.6 c	17.1 b	1066.1 b
MNCO1-649F-2-11	15.0 a	19.4 a	11.1 a	74.3 a	12.7 a	6.3 c	17.6 b	1128.5 b
MNCO2-675-4-9	15.1 a	19.5 a	10.9 a	71.9 b	14.8 a	5.8 c	14.9 b	997.4 b
MNCO2-675F-9-5	14.3 a	19.1 a	10.2 b	71.5 b	11.9 a	6.0 c	17.3 b	1005.1 b
MNCO2-676F-1	14.7 a	19.5 a	10.9 a	74.2 a	11.8 a	6.0 c	18.6 a	1036.2 b
MNCO2-677F-2	16.1 a	20.5 a	11.8 a	73.4 a	12.05 a	4.2 c	19.6 a	789.8 b
MNCO2-677F-5	15.2 a	18.4 a	11.5 a	75.9 a	12.0 a	4.5 c	19.3 a	836.1 b
MNCO2-680F-1-2	14.6 a	19.0 a	10.5 a	71.3 b	10.85 b	4.9 c	19.3 a	798.3 b
MNCO2-689F-2-8	14.2 a	18.9 a	9.9 b	69.0 b	9.75 b	4.8 c	20.3 a	738.6 b
MNCO2-701F-2	16.8 a	20.0 a	13.0 a	77.2 a	13.5 a	4.5 c	19.1 a	948.8 b
MNCO3-736F-2	15.6 a	18.3 b	12.0 a	76.9 a	13.1 a	4.0 c	18.7 a	744.8 b

*Mean values followed by the same letter belong to the same group according to the Scott-Knott test, (P<0.05). PW: pod weight (g); GLP: green pod length (cm); WSP: weight of seeds per pod (g); SI: seed index (%); NSP: number of seeds per pod; NPP: number of pods per plant; HGW: hundred grain weight; YLD: grain yield (kg ha^{-1}).

The use of the Tocher optimization method, based on dissimilarity, expressed by the Mahalanobis distance (D^2), allowed the formation of three distinct groups, with most of the genotypes belonging to group I, with 93.7% of the genotypes. Groups II and III were made up of individual genotypes (Table 4).

Table 4. Grouping and sub-grouping of 32 cowpea bean genotypes by the Tocher optimization method.

Groups	Genotypes											
I	4	14	7	20	1	10	17	16	26	18	23	29
	25	2	22	12	9	11	8	3	30	21	28	27
	6	31	13	32	19	15						
II	24											
III	5											

Maximum values for D^2 were obtained between groups I and III (35.1014), corresponding to greater divergences among groups and probably indicating the best combinations for crosses (Table 5). Identification of superior genotypes based on genetic divergence is the most adequate strategy for a breeding program. In this case, it is more effective to perform crosses between the genotypes with high yield potential from group I with those of group III, which are highly divergent.

Table 5. Mean distances within the groups on the main diagonal and among groups outside of the main diagonal corresponding to the three groups formed by the 32 cowpea bean genotypes.

Groups	I	II	III
I	7.3941	20.8692	25.203
II		-	35.1014
III			-

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Based on the clustering cut in the dendrogram, at 50% of dissimilarity, four groups were formed. The least dissimilarity was between genotypes 4 and 14 (MNC02-675F-9-3 and MNC03-737F-5-10) (Figure 1).

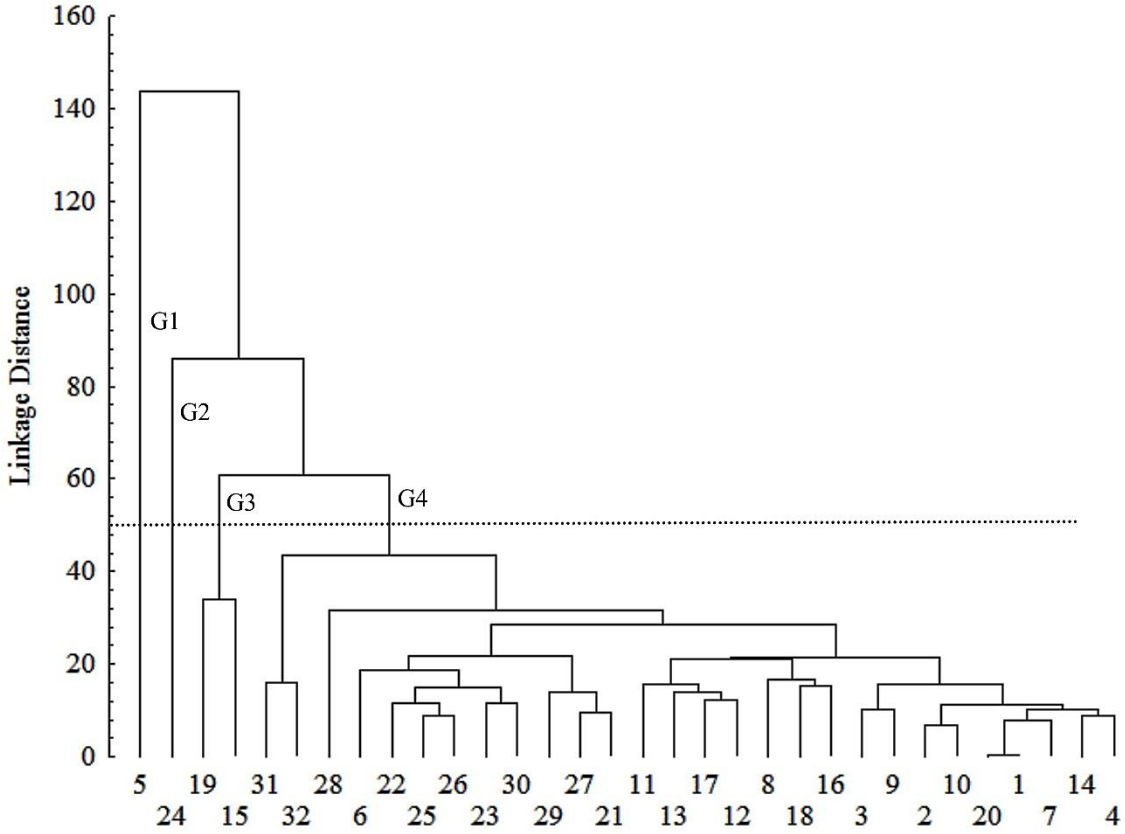


Figure 1. Dendrogram representing genetic dissimilarity among the 32 genotypes studied obtained by the unweighted pair group method with arithmetic mean (UPGMA), using the Mahalanobis generalized distance as a measure of dissimilarity. Cophenetic correlation (0.855**). (G1, G2, G3 and G4 indicate groups 1, 2, 3, and 4. respectfully).

This result is an indication that the hybridizations among these genotypes may result in the generation of very similar progenies with a very narrow genetic base so as to make the gains to be obtained by selection unviable. Nevertheless, depending on the strategy and goals of the breeding program, this type of cross, considered to be convergent, may be used, to facilitate the work of breeders in selection of superior lines in a shorter time because both cultivars have better performance in important agronomic characteristics, such as yield potential.

It may be observed that group one (G1) and group two (G2), were made up of only one genotype and group three (G3) by two, with the rest of the genotypes belonging to group one (G1). This result allows one to infer that hybridization between groups G1, G2 and G3 with G4 is the most advisable strategy to begin a breeding program, or otherwise a diallel among groups G1, G2 and G3.

The Cophenetic Correlation Coefficient (CCC) obtained was 0.855 and significant ($p < 0.1$) by the t test, which represents a good fit between the cophenetic matrix and the dissimilarity matrix constructed as based on the Mahalanobis generalized distance. Such a coefficient, according to Sokal e Rohlf (1962), indicates good reliability of the clusters established.

The first two canonical variables explained 89% of the total variation (Figure 2), which allows the study of genetic dissimilarity in the two-dimensional space, according to the criterion adopted by Cruz & Regazzi (2001). In regard to the principal components, the first three components alone (CP1, CP2 and CP3) explained more than 80% of the total variation (92.20%).

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ISSN: 1984-2538

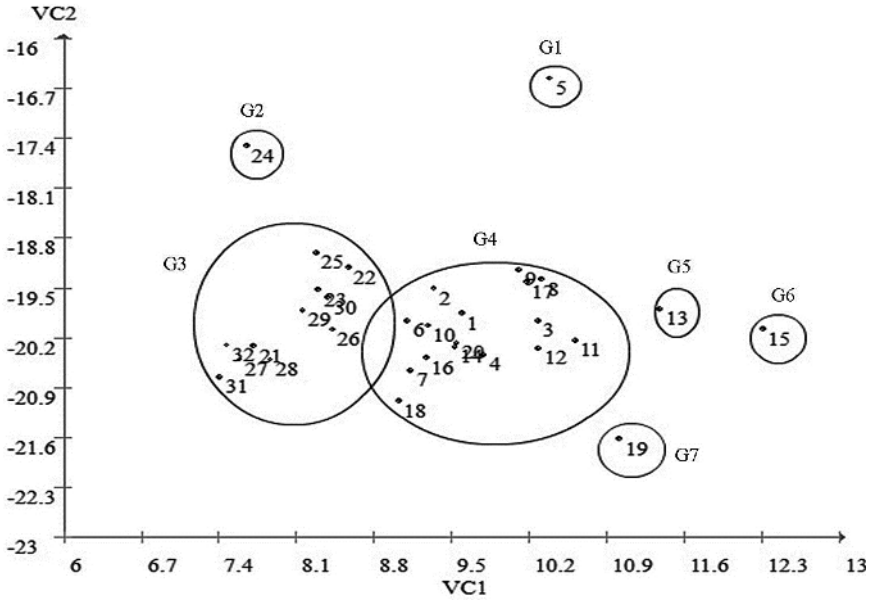


Figure 2 – Score distribution of 32 cowpea bean genotypes in relation to two canonical variables (VC1 and VC2), as based on assessment of morphological and agronomic characteristics (G1, G2, G3, G4, G5, G6 and G7 indicate groups 1, 2, 3, 4, 5, 6, and 7 respectively).

That way, the study of genetic dissimilarity was able to be performed in the three dimensional space (Figure 3). With the assessment of the groups formed by both techniques, the formation of seven groups may be observed, and the individualization of genotypes 5 (MNC02-675F-9-3) and 24 (MNC03-737F-5-10) occurred in both.

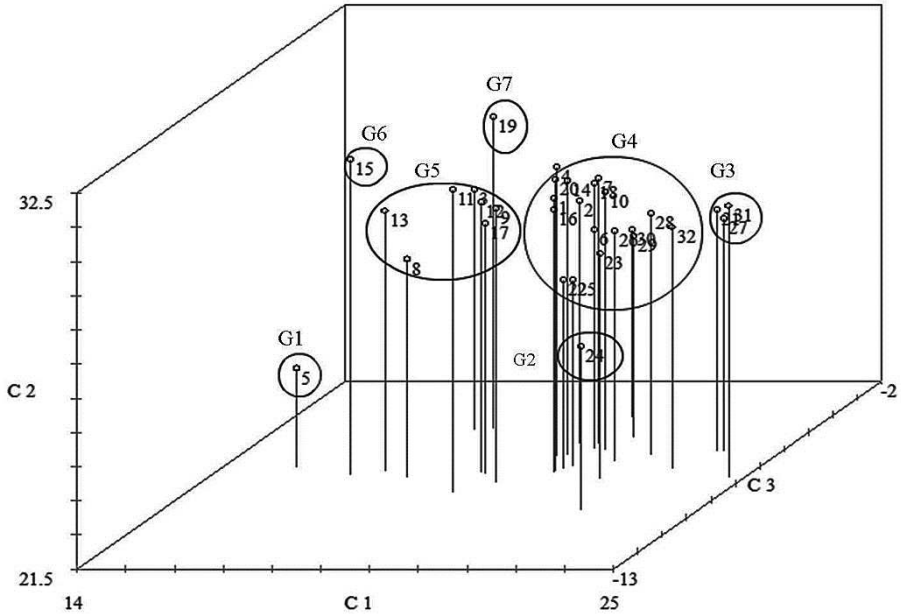


Figure 3. Graphic distribution obtained by means of the principal components assessed based on eight morphological and agronomic traits in 32 cowpea bean genotypes. (G1, G2, G3, G4, G5, G6 and G7 indicate groups 1, 2, 3, 4, 5, 6, and 7 respectively).

These are therefore the most suitable genotypes in hybrid combination in the initial stages of a breeding program, with the expectation that due to genetic divergence, there will be production of hybrids of greater heterotic effect, so as to increase the chances of favorable gene combinations which allow the selection of superior genotypes.

Even when the clustering methods used are different, a certain similarity may be observed in the order of formation of the groups, where the greatest similarities in the formation of the groups were between the Tocher method with the dendrogram, and the canonical variables with the principal components. In this context, both methods were efficient in placing genotypes 5 and 24 in individual groups.

Conclusion

There is agreement among the multivariate techniques applied in the trend toward clustering of genotypes.

The genotypes show genetic variability and have potential for use in breeding programs.

The crosses carried out between the genotypes MNC02-675F-9-3 and MNC03-737F-5-10 with the genotypes MNC03-737F-5-11, MNC03-737F-5-1, MNC03-725F-3 and MNC03-737F-5-9 may lead to the creation of segregating families with high yield potential and an increase in the probability of recovering superior genotypes in the segregating generations.

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