Article

Integrated multivariate analysis to identify superior cowpea genotypes

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Abstract

Genetic divergence among 40 cowpea genotypes was quantified by means of integrated multivariate analysis aiming to assist the selection and development of new cultivars. The experiment was carried out in randomized blocks design with 40 treatments and 4 repetitions. The multivariate techniques applied were partially concordant, following the same trend for clustering of genotypes. Directed crosses between the genotypes belonging to group I with group IV and VI will be able to lead to the creation of segregating families with high yield potential and an increase in probability of recovering individuals. The variables of hundred grain weight, average pod length and number of seeds per pod are the main determinants in quantification of the genetic divergence among cultivars.

Keywords: Dissimilarity, selection, *Vigna unguiculata*

Análise multivariada integrada na identificação de genótipos superiores de feijão-caupi

Resumo

A divergência genética entre 40 genótipos de feijão-caupi, foi quantificada por meio da análise multivariada integrada, com a finalidade de auxiliar na seleção e no desenvolvimento de novos cultivares. O delineamento experimental foi em blocos casualizados, com 40 tratamentos e 4 repetições. As técnicas multivariadas aplicadas foram parcialmente concordantes entre si, seguindo a mesma tendência de agrupamento dos genótipos. O cruzamento dirigido entre os genótipos pertencentes ao grupo I com o grupo IV e VI poderá propiciar a criação de famílias segregantes com elevado potencial produtivo e aumento na probabilidade de recuperar indivíduos. As variáveis, massa de cem grãos, comprimento médio da vagem e número de grãos por vagem são os principais determinantes na quantificação da divergência genética entre as cultivares.

Palavras-chave: Dissimilaridade, seleção, *Vigna unguiculata*

Introduction

Breeding of cowpea is mainly based on parents selection, followed by hybridization, in order to form a base population base and generation advance, with simultaneous selection for more than one trait (Bertini et al., 2010). In identification of parents for hybridization, the selection of genotypes within the most divergent groups is recommended, with greater mean values for the characteristics that are the target of the breeding process (Passos et al., 2007).

Analysis of genetic divergence seeks to identify parents for creating populations with genetic variability and consequent genetic gain in successive selection cycles. Some methodologies have been used in the characterization of germplasm, such as the use of isoenzymes and molecular markers (Pinto et al. 2004 and Dutra Filho et al., 2013). Another choice that may be made is the multivariate analysis of morphological and agronomic data, which is a lower cost option, only requiring field evaluations and knowledge of statistical methods.

Multivariate statistics is a set of statistical methods used in situations in which different variables, continuous or not, are measured simultaneously in each experimental unit (Santos et al., 2010). Multivariate data analysis methods allow an overall study of these variables, placing the connections, similarities or differences among them in evidence, with minimal information losses. In forecasting genetic divergence, different multivariate methods may be applied. Among these methods, the most used are: principal component analysis, canonical variate analysis and the cluster analysis methods (Oliveira et al., 2003).

The most used cluster analysis methods in genetic breeding are the hierarchical cluster analysis and the cluster analysis optimization. In the hierarchical methods, the accessions are clustered by a process that is repeated in various levels until a dendrogram or graph is established, with no concern about the optimum number of groups, but rather with the topology of the dendrogram visually examined (Cruz et al., 2006 and Ferreira et al., 2007). For the optimization methods, location of accessions in subclusters is performed with a view toward the maximization or minimization of some pre-established measure. One of the most commonly used optimization methods in genetic breeding is Tocher's method.

The canonic variate analysis is an alternative when experimental data with replications is available and the weighting coefficient of the original variables may be estimated in each one of the canonical variates and their variances. Principal component analysis may be performed even when the researcher does not have experimental data with replications available, and this method evaluates if there is a small number of the first principal components which are responsible for explaining a high proportion of the total variation associated with the original set. Thus, it promotes elimination of traits that contribute little to the variation of the individuals evaluated and allows the clustering of similar individuals by means of graph dispersions (Gonçalves & Fritsche-Neto, 2012).

The choice of the most adequate method has been determined by the precision desired by the researcher, by the ease of analysis and by the manner that the data were obtained (Bezerra Neto et al., 2010). Multivariate analysis may furthermore be applied in an integrated manner, where a set of multivariate techniques is used simultaneously in such a way as to provide for better interpretation of the results.

Therefore, the goal of this study was to quantify the genetic divergence of 40 cowpea genotypes by means of Integrated Multivariate Analysis for the purpose of assisting the parents choice and for new cultivars development..

Materials and Methods

The experiment was performed with 40 cowpea genotypes, 24 advanced lines and 16 commercial varieties originating from the germplasm bank of Embrapa Meio Norte. The genotypes from 1 to 20 are of upright and semi-upright growth habit and from 21 to 40 of prostate and semi-prostate growth habit (Table 1). The studies were conducted in two municipalities of Mato Grosso do Sul State (Dourados and Chapadão do Sul), Brazil, during the fall-winter period of 2009.

Table 1. Description of the genotypes used in the experiment.

I.G	Genotypes	Parents/origin
1	MNC99-537F-1	TE96-282-22G x IT87D-611-3
2	BRS Tumucumaque	TE96-282-22G x IT87D-611-3
3	MNC99-541F-5	TE93-210-13F x TE96-282-22G
4	MNC99-541F-8	TE93-210-13F x TE96-282-22G
5	BRS Potengi	TE96-282-22G x TE93-210-13F
6	BRS Novaera	TE97-404-1F x TE97-404-3F
7	MNC00-553D-8-1-2-3	TE97-404-1F x TE97-404-3F
8	MNC99-557F-2	(TE96-282-22G x IT87D-611-3) x TE97-411-1F
9	MNC01-627F-14-2	TE99-496-1F x TE97-411-15F-2-1
10	MNC01-627F-14-5	TE99-496-1F x TE97-411-15F-2-1
11	MNC03-720C-20	MNC01-625D-10-2 x TE99-499-1F-2-1
12	MNC03-720C-31	MNC01-625D-10-2 x TE99-499-1F-2-1
13	MNC03-731C-21	TE99-499-1F-2-3 x MNC01-627D-5-1
14	MNC03-732C-5	MNC01-627D-5-1 x TE99-499-1F-2-3
15	TVx-5058-09C	IITA, Nigéria
16	MNC05-784b-38-2	TE99-499-1F-2-3 x MNC99-544D-10-1-2-2
17	MNC05-832b-234-5	[(MNC00-553D-8-1-2-3 x MNC99-544D-10-1-2-2)] x Urubuquara -113
18	Vaina Blanca	Iguitos, Peru
19	californiablackeye-27	Univ. da California, Riverside
20	BRS Guariba	IT85-2687 x TE87-98-8G
21	MNC99-510F-16-1	Paulista x TE90-180-88F
22	MNC99-510F-16-3	Paulista x TE90-180-88F
23	MNC99-537F-14-2	TE96-282-22G x IT87D-611-3
24	MNC01-611F-11	TE97-340-4E x TE93-222-11F
25	MNC01-614F-15	TE97-404-1F-15 x TE93-242-10E-6-1-1
26	MNC01-631F-11	CNCx 409-11F-P2-195 x TE97-341-1E-1-1
27	MNC01-631F-15	CNCx 409-11F-P2-195 x TE97-341-1E-1-1
28	MNC01-631F-20-5	CNCx 409-11F-P2-195 x TE97-341-1E-1-1
29	MNC01-649E-2	TE97-309G-24 x MNC-01-608D-2-5
30	Canapuzinho	São Raimundo Nonato, PI
31	Canapuzinho-2	São Raimundo Nonato, PI
32	Inhuma	Inhuma, PI
33	Pingo-de-ouro-1-2	Iguatu, CE
34	Pingo-de-ouro-2	Iguatu, CE
35	paulistinha	Juazeiro, CE
36	Patativa	CNC1735 x (CNCx 926-5F x Paulista)
37	BRS Paraguassu	BR10-Piauí x Aparecido Moita
38	BRS Milênio	Tracuateua, PA
39	BR 17-Gurguéia	BR 10-Piauí x CE-315
40	BRS Marataoã	Seridó x TVx1836-013J

I.G: Identification of the genotypes.

The experimental design was randomized blocks with 40 treatments and 4 replications. The experimental unit consisted of four 5-meter rows spaced at 0.50 meters, considering the two center rows as the useful area.

Harvesting of the plots was performed manually, and in each plot the plants were evaluated for the following traits: green pod length (GPL), average length in cm of the pods of five plants harvested at random at physiological maturity; pod weight (PW), considering the average weight of the previously harvested pods; number of seeds per pod (NSP), obtained through counting of the seeds in the pods harvested for the previous sampling; seed index (SI) refers to the green seed weight in green pods at physiological maturity; average number of pods per plant (NPP), considering the average of the pods harvested from five plants; hundred grain weight (HSW), weight of 100 seeds with 13% of moisture; and grain yield (YLD) estimated through the useful area harvested in each plot, converting the results to kg ha^{-1} and adjusting the data to 13% of moisture.

Initially, analysis of variance was performed for each environment in an isolated

manner, checking the uniformity of residual variance. Afterwards, the variance analysis was performed, considering the two environments.

Analysis of genetic divergence was performed using multivariate techniques. In application of the genotype cluster analysis technique, the Mahalanobis generalized distance (D²) was adopted as a measure of dissimilarity, taking into consideration the degree of dependence among the studied variables .

In relation to the establishment of similar groups, the agglomerative hierarchical clustering method of optimization proposed by Tocher was applied, whose calculations were equally based on Mahalanobis generalized distance (D²). Principal component analysis was used, evaluating the relative contribution of each trait to the genetic divergence among them and graph dispersion was developed as a function of the first three principal components. All statistical analyses was carried out using the GENES software (Cruz, 2013).

Results and Discussion

It may be observed that the mean squares presented a significant difference for all studied characteristics in the GxE interaction by the F test (Table 2). This result indicates the existence of phenotypic variability, i.e., the genotypes behave in a differentiated manner in the environments, which benefits their characterization process. The significance of the interaction of all the agronomic traits suggests its contribution for identification of the genetic divergence among them.

Table 2. Summary of joint analysis of variance for the seven traits evaluated on 40 cowpea genotypes.

S.V	D.F	GPL	PW	NSP	SI
Genotypes (G)	39	39.2400**	21.4631*	16.4242*	48,3620*
Environment (E)	1	174.8671**	1536.6228**	1.6531^{ns}	330,9954**
G. x E.	39	2.9943*	17.5782**	8.7364**	30,6857**
Residue	240	1.9231	2.6744	0.8760	7,2837
Mean		18.26	14.32	8.89	74,0
CV (%)	-	7.59	11.41	10.52	5,61
S.V		NPP	HSW	YLD	
Genotypes (G)	39	24.3714**	46.9176**	90562.5278**	
Environment (E)	1	5.7432*	55.1556**	10026122.5377**	
G. x E.	39	29.1317**	32.5606**	60218.8948**	
Residue	240	2.6480	1.1332	8972.1239	
Mean		9.07	18.76	642.81	
CV (%)		17.92	5.67	14.73	

S.V: source of variation; D.F: degree of freedom: GPL: pod length (cm); PW: pod weight (grams); NSP: number of seeds per pod: SI: seed index; NPP: number of pods per plant; HSW: hundred grain weight (grams); YLD: grain yield (kg ha⁻¹).

The coefficients of heritability based on the mean of families ($h_{\bar{x}}^2$) showed magnitudes from moderate to high for the characteristics of pod length (GPL), pod weight (PW), number of seeds per pod (NSP), seed index (IS), number of pods per plant (NPP), hundred grain weight (HSW) and grain yield (YLD), confirming that most of the phenotype is attributed to genetic causes (Table 3). The low value of heritability observed for grain yield can be explained by the polygenic origin of the character, coming from the sum of the environmental effect of each gene.

The traits that most contributed to genetic diversity were hundred grain weight (HSW) (36.96%) pod length (GPL) (16.29%) and the number of seeds per pod (NSP) (15.54%), indicating the existence of greater genetic variability among these traits in the germplasm studied. What can be seen also by higher heritability values and relation CV_g/CV_e obtained by these characters. In common bean genotypes, the hundred grain weight, which is a high heritability trait, is the variable which most contributes to genetic divergence (Elias et al., 2007 and Cabral et al., 2011).

In regard to cowpea, studies on genetic divergence have shown discordant results in relation to the contribution of each component to the diversity. In their study, Nagalakshmi et al. (2010) describe that grain yield and hundred

grain weight were the traits that most contributed to divergence. Nevertheless, it should be highlighted that seed production is a complex trait, generally of quantitative inheritance and with high environment influence, which may be the reason for this greater contribution.

Table 3. Genetic parameters and relative contribution of each trait to genetic dissimilarity (S.j') in 40 cowpea genotypes.

Variable	$h_{\bar{x}}^2$	CV_g	CV_g/CV_e	S.j'	%	accumulated %
GPL	95.18	11.82	1.57	3472.1196	16.2929	16.293
PW	87.97	10.72	0.95	1026.8763	4.8186	21.112
NSP	94.55	15.67	1.47	3312.4336	15.5436	36.655
SI	64.10	2.66	0.47	643.0035	3.0173	39.672
NPP	63.40	18.56	1.31	2895.2734	13.586	53.258
HSW	83.78	12.75	2.27	7875.6307	36.9563	90.215
YLD	61.38	9.58	0.65	2085.3185	9.7853	100.000

GPL: pod length (cm); PW: pod weight (grams); NSP: number of seeds per pod; SI: seed index; NPP: number of pods per plant; HSW: hundred grain weight (grams); YLD: grain yield (kg ha^{-1}).

Dias et al. (2009) verified that the variables that most contributed to divergence in cowpea were the maturity cycle, number of nodes on the main stem and the beginning of flowering.

It may furthermore be observed, regarding the relative contribution of each trait that grain yield had low discriminating power among the accessions, even showing productive heterogeneity among the genotypes (Table 3).

The maximum value of genetic divergence ($D^2 = 219.49$) was obtained for the pairs MNCO3-731C-21 and California Blackeye-27 from different geographic origin, Brazil and USA, respectively, which may have contributed to the greater dissimilarity between them. Nevertheless, the lower values of D^2 shown by the genotypes TVx-5058-09C with MNC03-732C-21 (1.60) and the genotype Viana Blanca with BRS Potengi 13.62), also from different geographic origins, showing little effect of geographic origin on the dissimilarity between these pairs of genotypes. The lowest values of D^2 were observed for the pairs MNC01-614F-15 with Paulistinha (0.72) and MNC99-537F-14-2 with MNC01-649E-2 (0.95), meaning greater similarity among the considered traits (Table 4).

Moreover in relation to Table 4, it should be noted that the maximum values of D^2, regardless the genotypes, were obtained whit a combination of MNCO3-731C-21 and California Blackeye-27, indicating these as the most divergent among the evaluated germplasm group. Maximum values of D^2 of high magnitude were obtained between the genotype MNCO3-731C-21 when combined with MNC05-832b-234-5, MNC01-649E-2, Paulistinha, Canapuzinho, BRS-Paraguassu and BRS-Milênio and between the genotype California Blackeye-27 with the genotype Viana-Blanca.

These genotypes are therefore the most indicated forthe hybrid combination in the initial stages of a breeding program, with the expectation that, due to genetic divergence, hybrid production with greater heterotic effect will be obtained, raising the chances of favorable gene combinations that allow the selection of superior genotypes. The large amplitude of D^2 and the high values estimated for most of the pairs of cultivars reveal the great genetic variability existing in this group of genotypes, which makes identification of parents, possible by the formation of a population with a broad genetic base, increasing the probability of obtaining superior genotypes in the segregating generations.

Considering Tocher´s grouping, minimum values for D^2 were obtained between groups I and V (29.4135), group I and III (34.8662) and group I and II (36.9961); these low values mean that crossing the genotypes of these respective pairs of groups may not be greatly indicated for obtaining superior genotypes in the segregating generations, due to their proximity (Table 5).

Table 4. Least and greatest Mahalanobis distances (D²) among 40 cowpea genotypes.

| | Genotypes | D² Distance between genotypes | | | |
		Shortest		Greatest	
1	MNC99-537F-1	3.87	20	86.38	13
2	BRS Tumucumaque	4.91	20	102.39	13
3	MNC99-541F-5	3.95	10	91.72	13
4	MNC99-541F-8	2.34	5	69.10	13
5	BRS Potengi	1.05	7	57.54	19
6	BRS Novaera	1.07	12	76.56	19
7	MNC00-553D-8-1-2-3	1.05	5	59.11	19
8	MNC99-557F-2	13.34	7	97.85	19
9	MNC01-627F-14-2	2.37	16	112.86	13
10	MNC01-627F-14-5	2.50	11	71.65	13
11	MNC03-720C-20	1.68	5	65.75	13
12	MNC03-720C-31	1.07	6	67.20	19
13	MNC03-731C-21	27.76	18	219.49	19
14	MNC03-732C-5	1.60	15	75.06	19
15	TVx-5058-09C	1.60	14	85.94	19
16	MNC05-784b-38-2	2.37	9	104.02	13
17	MNC05-832b-234-5	3.18	9	133.78	13
18	Vaina Blanca	13.62	5	108.26	19
19	californiablackeye-27	23.19	17	219.49	13
20	BRS Guariba	3.87	1	97.93	13
21	MNC99-510F-16-1	2.28	23	72.13	13
22	MNC99-510F-16-3	2.56	5	59.38	19
23	MNC99-537F-14-2	0.95	25	66.27	13
24	MNC01-611F-11	1.75	32	111.57	13
25	MNC01-614F-15	0.95	23	66.72	13
26	MNC01-631F-11	5.78	29	111.48	13
27	MNC01-631F-15	7.53	32	74.78	13
28	MNC01-631F-20-5	8.33	30	98.88	13
29	MNC01-649E-2	0.72	35	131.98	13
30	Canapuzinho	6.04	31	128.04	13
31	Canapuzinho-2	4.63	24	112.22	13
32	Inhuma	1.75	24	97.75	13
33	Pingo-de-ouro-1-2	2.43	34	112.26	13
34	Pingo-de-ouro-2	2.43	33	113.64	13
35	paulistinha	0.72	29	131.64	13
36	Patativa	4.56	21	66.69	19
37	BRS Paraguassu	7.20	24	128.15	13
38	BRS Milênio	3.46	24	125.41	13
39	BR 17-Gurguéia	3.57	40	98.24	13
40	BRS Marataoã	2.47	23	88.9	13

The greatest average distances were obtained between groups IV and V (219.4953), groups II and VI (103.0585) and groups III and IV (102.3998), corresponding to the greatest divergences among groups and probably, indicating the best combinations for crosses. Identification of superior genotypes based on genetic divergence is an adequate strategy for a breeding program. However, the choice of genotypes must also be made considering their behaviors *per se*; that is, crosses are recommended between divergent genotypes, but which also show superior performance in relation to the main agronomic characteristics.

The use of the Tocher optimization method based on dissimilarity expressed by the Mahalanobis distances (D²), allowed the formation of six distinct groups, with 85% of the genotypes belonging to group I. Groups III, IV, V and VI were formed by individual genotypes (Table 6).

Table 5. Mean distances within the groups in the main diagonal and among groups outside of the main diagonal corresponding to the six groups formed by 40 cowpea genotypes by the Tocher optimization method.

Groups	I	II	III	IV	V	VI
I	19.3785	36.9961	34.8662	88.8202	29.4135	47.9283
II		19.69	37.7893	38.8558	48.0672	103.0585
III			-	102.3998	76.2151	47.4862
IV				-	98.8877	219.4953
V					-	68.9792
VI						-

Table 6. Clustering and sub-clustering of 40 cowpea genotypes by Tocher´s optimization method

Groups					Access							
	14	15	7	5	12	11	22	25	4	10	23	29
I	35	38	24	26	32	31	39	40	33	30	9	17
	16	3	21	36	1	20	6	34	37	27		
II	8	18										
III	2											
IV	13											
V	28											
VI	19											

Regarding the cut in the dendrogram, in approximately 50% of dissimilarity, the formation of three groups was observed (Figure 1). The least dissimilarity was between genotypes 29 and 35 (MNC01-649E-2 and Paulistinha). These results indicates that hybridizations between MNC01-649E-2 and Paulistinha may result in the generation of very similar progenies with a very narrow genetic base.On the other hand, depending on the strategy of the breeding program, this type of cross, considered as convergent, may facilitate the work of breeders in the selection of superior lines in less time because both cultivars have superior performance in important agronomic characteristics, such as productive potential.

The Cophenetic Correlation Coefficient (CCC) obtained was 0.892 and significant (p < 0.1) by the t test. Only cophenetic correlation values greater than 0.80 indicates a good fit between the original matrices of distance and the graph distances (Sokal and Rohlf, 1962), with cophenetic correlation being adequate, showing that the representativeness of the real distance between the genotypes by means of graphic representation of the dendrogram is trustworthy (Figure 1).

It may be observed that in both cluster analysis techniques, there was the formation of groups with prostrate growth habit genotypes (genotypes from 21 to 40) and upright growth habit genotypes (genotypes from 1 to 20). Passos et al. (2007), evaluating genetic divergence in cowpea, divided the genotypes by upright and prostrate growth habit; however, it may be suggested that the growth habit of the plant does not have an effect on the variables used for cluster analysis in this study.

The first three principal components (CP1, CP2 and CP3) explained 83% of the total variation. Thus, the study of genetic dissimilarity in the three-dimensional space was able to be performed (Figure 2A). With the evaluation of the groups formed by means of this clustering technique, the establishment of six groups may be observed, as the individualization of the genotypes MNC03-731C-21 and California Blackeye-27 (13 and 19).

The estimates of the eigenvalues corresponding to the first three canonical variables explained 82% of the total variation, with the study of genetic dissimilarity also having been performed in the three-dimensional space (Figure 2B). The formation of four groups can be observed, with agreement with the results obtained by UPGMA methods. Santos et al. (2011) also verified that the cluster methods by the average connection among groups and the canonical variables were previously in agreement among themselves.
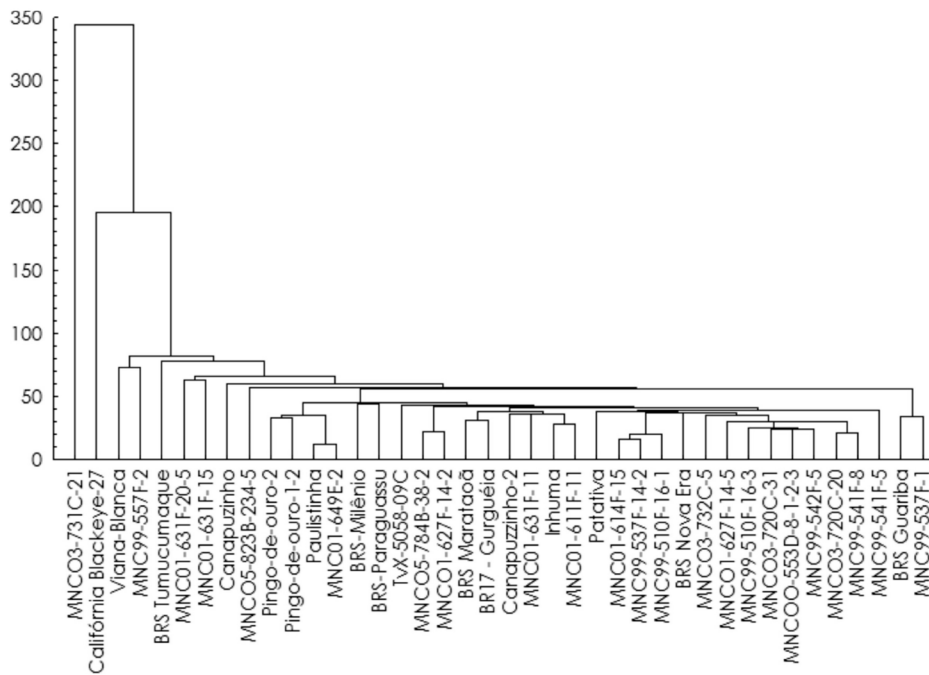
Figure 1. Dendrogram representing genetic dissimilarity among the 40 genotypes studied, obtained by the mean connection among groups (UPGMA), using the Mahalanobis generalized distance as a dissimilarity measure. Cophenetic correlation (0.892). (G1, G2, and G3 indicate groups 1, 2 and 3, respectively).
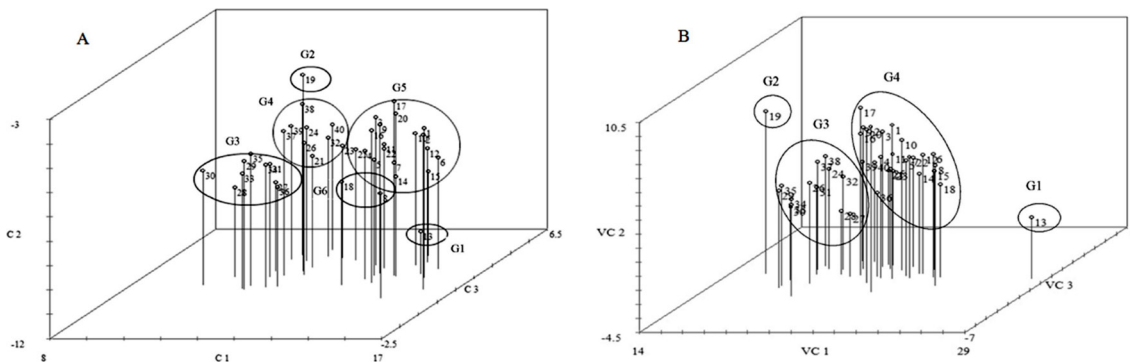
Figure 2. Graph dispersion obtained by means of the principal components evaluated as of seven agronomic traits in 40 cowpea genotypes. (G1, G2, G3, G4, G5 and G6 indicate groups 1, 2, 3, 4, 5 and 6, respectively) (A). Score dispersion of 40 cowpea genotypes in relation to three canonical variables (VC1, VC2 and VC3), based on evaluation of morphoagronomic characteristics (G1, G2, G3 and G4 indicate groups 1, 2, 3 and 4, respectively) (B).

Even though the cluster methods used are different, there was a certain similarity in the order of group formation, concludingthat the methods were efficient in clustering the genotypes MNC03-731C-21 and California Blackeye-27 (13 and 19, respectively) in individual groups.

The multivariate techniques applied were partially in agreement among themselves, following the same tendency in clustering the genotypes. Directed crosses between the genotypes belonging to group I with group IV and VI will be able to lead to the creation of segregating families with high productive potential and an increase in the probability of recovering superior genotypes. The variables of hundred grain weight, average pod length and number of seeds per pod are the main determinants in quantification of the genetic divergence among the cultivars.

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