

Genetic divergence in cowpea genotypes with upright growth and early cycle

Francisco Tiago Cunha Dias¹, Ana Paula Moura da Silva¹, and Cândida Hermínia Campos de Magalhães Bertini^{1*}

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ABSTRACT – The objective of this study was to group cowpea genotypes with upright growth and early maturity using multivariate techniques to estimate the relative contribution of the response variables to genetic divergence studied. Twenty-eight lines of the Genbank of the Universidade Federal do Ceará and Embrapa Meio Norte were used. For the analysis of genetic divergence the canonical variables, Mahalanobis' distance and the Tocher cluster method were used. Most of the maximum distances were observed when combined with CE-46 genotypes. The crosses between the groups VIII and XI and groups VII and VIII may result in new gene combinations. The variables beginning of flowering and crop cycle contributed most to the genetic divergence among the genotypes.

Key words: *Vigna unguiculata*, dissimilarity, multivariate techniques.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is one of the most important and strategic food sources in tropical and subtropical regions of the world (Freire-Filho et al. 2005) and is one of the most adapted, versatile and nutritive Fabaceas among the cultivated species.

The species is well-adapted to the climate and soil conditions in the North and Northeast of Brazil. Unlike common bean (*Phaseolus vulgaris* L.) and other Fabaceas, cowpea can be grown in Brazil in both the dry climate of the Northeast as well as the humid climate in the North, from latitudes 5° N to 18 S. The temperature range for an adequate development is between 20 °C and 35 °C (Araújo 1988).

In recent years the yield of this crop has gradually declined in the state of Ceará, to yields as low as 75 to

473 kg ha⁻¹ (CONAB 2008), which does not represent the full genetic potential of the cultivars used in the state. A number of factors are responsible for the low yields, including: use of cultivars with low genetic potential and extensive intercrop cultivation, in rotation with other crops, mainly maize, cassava and cotton (Teófilo et al. 2003).

The genetic variability of cowpea is high for almost all important agronomic traits, but in comparison with other crops little breeding work has been done, focused mainly on the improvement of yield and increased disease resistance (Hall et al. 2003).

The genetic improvement of cowpea begins with the selection of parents and formation of the base population generating segregating populations (Freire-Filho et al. 2005) in which superior lines are selected,

¹ Universidade Federal do Ceará (UFC), Departamento de Fitotecnia, Bloco 805, 2977, 60356-001, Fortaleza, CE, Brazil. *E-mail: candida@ufc.br

making knowledge on the dissimilarity between the parents particularly important. Studies on genetic diversity are therefore of great importance in breeding programs, since they permit the identification of appropriate parents of hybrids with greater heterotic effect and with greater segregation in recombinations, allowing the appearance of transgressive genotypes (Cruz and Carneiro 2003).

Several multivariate techniques can be applied to predict genetic divergence, such as clustering methods, analysis by principal components and/or canonical variables. The most appropriate method should be chosen according to the desired precision, ease of analysis and the way the data were collected (Rangel et al. 1991).

Cluster analysis designates a group of multivariate techniques whose primary purpose is to group plants based on their traits, so the internal homogeneity and the external heterogeneity of the resulting groups should be high (Hair et al. 2005). Among the dissimilarity methods, Mahalanobis' generalized distance (D^2) has been widely used in clustering analysis, where the most common methods are hierarchical (Ferreira 1993). This method does not only apply a process of data standardization, establishing a scale in terms of standard deviations, but also summarizes the accumulated variance-covariance within groups, which adjusts the intercorrelations between variables. The Mahalanobis' distance (D^2) therefore computes a distance measure between comparable plants by the coefficient of determination in regression (Hair et al. 2005).

By the method of canonical variables and the main components however, the objective is to evaluate the similarity between genotypes based on graphical dispersion, generally on two Cartesian axes (Cruz and Regazzi 1997). The different multivariate techniques have been successfully applied for diverse crops such as cassava (Nick et al. 2008), assai palm (Oliveira et al. 2007), coffee (Fonseca et al. 2006) and grass (Shimoya et al. 2002).

In view of the importance of cowpea in the North and Northeast of Brazil, the purpose of this study was to group cowpea genotypes with upright growth and early maturity according to the genetic dissimilarity, for the formation of cross blocks and indicate the relative contribution of the studied response variables to the genetic divergence, to increase the efficiency of the selection process.

MATERIAL AND METHODS

The experiment was conducted on the Fazenda Lavoura Seca, of the Center for Agrarian Sciences of the Universidade Federal do Ceará (CCA / UFC), in Quixadá, in the central Sertão region of the state of Ceará (lat 4 59 ' S; long 39 01 ' W; 190 m asl), with an Aw' climate (Köppen 1918), in the 2008 growing season.

The experiment was evaluated in a randomized block design with four replications. The experimental unit consisted of four 5.0-m-rows, rows spaced 0.5 m and plants 0.2 m apart. The two central rows were used for data collection, reduced by 0.5 m at either end of each row, for a total area of 8 m² per plot.

The treatments consisted of 27 cowpea lines with upright plant type and early maturity (cycle <70 days) and one cultivar with a semi-upright plant type and early maturity (BR9-Longá) whose seeds came from the Genbank of the UFC and from the cowpea breeding program of Embrapa Meio Norte. The following variables were measured: beginning of flowering, insertion angle of lateral branches (IALB), number of nodes on the main branch (NMB), number of pods per plant (NPP), number of seeds per pod (NSP), pod length (Pod Leng.), 100-seed weight (W100), rate of grain (GI), yield (kg ha⁻¹) and crop cycle.

A univariate analysis of variance was performed first, to evaluate the variation between the tested lines, and later the cluster analysis using the average-linkage (UPGMA) method and Mahalanobis' generalized distance (D^2) as genetic dissimilarity measure. To estimate Mahalanobis' generalized distance, the means of all variables for each cultivar were initially computed, and then the residual covariance matrix, the data transformation matrix, the variance of the transformed variables, the means of the non-correlated variables and, finally, the technique of pivotal condensation to solve the dispersion matrix were established.

To establish similar groups, the method of hierarchical cluster optimization was applied, as proposed by Tocher (Rao 1952), whose calculations were based on Mahalanobis' generalized distance (D^2), assuming that the mean intragroup should be smaller than the intergroup distances (Cruz and Regazzi 2001).

In addition to the clustering study the following steps were performed: investigation of the relative contribution of each trait to the genetic divergence among them, estimation of the greatest distance in the cluster of shortest distances between each line, and

estimation of the mean intergroup distances corresponding to the groups formed. Then the graphical dispersion was outlined, based on results of the cluster analysis of intra and intergroups of the lines, also obtained based on Mahalanobis' D^2 statistic. The analysis of canonical variables was also used, to evaluate the relative contribution of each trait to the divergence. All tests were performed using software Genes (Cruz 2001).

RESULTS AND DISCUSSION

The shortest and longest D^2 distances between the studied genotypes are shown in Table 1. It was observed that the D^2 distances of 89.2% of the genotypes were highest when combined with genotype CE-46 (1); the highest genetic divergence was observed between the genotypes CE-46 (1) and

MNC 03-720C-11 (22) ($D^2=438.585704$), indicating genotype CE-46 as the most divergent of all.

The highest D^2 distance values were observed in the combination of line CE-46 with MNC 03-720C-11 and of CE-46 with CE -76 (3) (Table 1). These combinations deserve special attention in cowpea breeding programs, since they are the most suitable genotypes for hybrid combinations, due to the high genetic diversity and higher probability of finding favorable gene combinations for the selection of superior genotypes. According to Oliveira et al. (2003), the wide range of D^2 and the high estimated values for pairs of lines show the great genetic variability in this group of genotypes, which makes the identification of parents for the formation of a population with a broad genetic base possible, thereby increasing the probability of obtaining superior genotypes in segregating generations.

Table 1. Greatest and shortest Mahalanobis' distance D^2 between 28 cowpea genotypes

Genotypes	D^2 Distance between genotypes			
	Shortest		Greatest	
1-CE-46	126.24	2	438.59	22
2-CE-73	37.79	4	299.51	16
3-CE-76	25.52	22	432.25	1
4-CE-77	37.79	2	314.38	16
5-CE-103	31.00	6	302.20	1
6-CE-104	31.00	5	339.14	1
7-CE-113	46.90	4	357.80	1
8-CE-796	23.75	27	378.39	1
9-CE-542	20.18	17	352.63	1
10-CE-790	18.59	27	286.24	1
11-CE-798	62.92	12	317.05	1
12-CE-866	25.95	15	201.67	1
13-BR9-Longá	16.86	24	341.05	1
14-CB-3	9.12	15	280.54	1
15-CB-27	9.12	14	280.08	1
16-IT 870-611-3	9.93	21	386.95	1
17-IT82D-60	19.11	27	339.37	1
18-AU94-418-07-01	35.97	15	188.80	1
19-UCR-95-701	18.27	15	319.59	1
20-MNC03-731C-21	17.16	15	365.56	1
21-IT87D-611-3	9.93	16	356.53	1
22-MNC03-720C-11	25.52	3	438.59	1
23-MNC-00-5190-1-1-5	19.39	13	284.97	1
24-MNC-00-553-D-81-2-3	16.86	13	366.41	1
25-MNC01-627D-65-1	19.17	20	300.95	1
26-IT91K-118-2	28.77	12	315.29	1
27-IT93-10-93	18.59	10	278.66	1
28-MNC03-720-11	21.27	23	395.96	1
Of the set of shortest distances	9.12	14 and 15	188.80	1 and 15
Of the set of greatest distances	126.24	1 and 2	438.59	1 and 22

The lowest D^2 value was found for the pair CB-3 and CB-27, indicating high similarity of these two genotypes for the traits studied. The smallest genetic distance of 17.8% of the genotypes was lowest when combined with genotype CB-27, indicating this genotype as the most similar among the evaluated (Table 1).

Using Tocher's optimization procedure based on dissimilarity, expressed by Mahalanobis' distances (D^2), the studied genotypes could be separated into 11 different groups (Table 2). There is a wide distribution of genotypes in different groups, indicating a wide diversity among the genotypes. Oliveira et al. (2003) studied the genetic diversity among 16 cowpea cultivars by the Tocher optimization method, based on dissimilarity expressed by Mahalanobis' distance (D^2), grouping them in six groups. On the other hand, Passos et al. (2007) quantified the genetic diversity among 22 genotypes with prostrate and 20 with upright growth and grouped them by the Tocher method in nine groups for the prostrate group and in eight groups for the upright growth genotypes.

The greatest distances were observed between the groups VIII and XI (343.2) and VII and VIII (323.2) (Table 3), indicating the importance of group VIII as a source of parentals. Rahman et al. (2002) claimed that the identification of superior genotypes based on genetic divergence is the most appropriate strategy to start a breeding program. It is important to emphasize that crosses between highly divergent genotypes with a good yield potential are most effective.

The lowest D^2 distance values were observed in the intercross between the groups VII and XI (22.5) and

VI and IX (36.4) indicating that the intercross of the genotypes that make up these respective pair groups may not produce superior genotypes in segregating generations. However, Nick et al. (2008) emphasized the importance of knowledge of similarity between pairs of plants or groups; this information can be used in programs involving backcrosses, where the use of similar parentals, which differ basically in terms of the allele one wants to transfer, facilitates the recovery of the recurrent parental.

The variables that contributed most to genetic divergence were cycle (20.12%), number of nodes on the main branch (19.54%) and beginning of flowering (17.56%), indicating the existence of genetic variability for these response variables in the studied lines (Table 4). The variables with lowest contribution to the genetic divergence were grain yield (1.72%), number of pods per plant (5.26%) and grain index (5.54%).

The importance degree of the variables calculated in this study increased the divergence of results in terms of the importance of each response variable for genetic divergence in cowpea, since the results in the literature are contradictory, e.g., Oliveira et al. (2003) studied the importance of several traits for divergence in cowpea and indicated pod length (36.87%), 100-seed weight (19.21%) and number of seeds per pod (9.62%) as the most important. On the other hand, in the study of Passos et al. (2007) the traits of greatest magnitude for divergence in cowpea were pod yield (43.91%) for genotypes of prostrate growth and pod yield (35.92%), pod length (28.56 %) and weight of grains per pod (28.14%) for genotypes of semi-upright growth.

Table 2. Grouping of 28 cowpea genotypes evaluated by the method of Tocher optimization, based on the dissimilarity expressed by Mahalanobis' distance (D^2)

Group	Genotypes	Mean distances
I	IT82D-60. MNC-00-553-D-81-2-3. MNC 03-720C-11. AU94-418-07-01	56.99
II	CE-104. CE-866. CE-103. CE-790	92.38
III	UCR-95-701. MNC-00-5190-1-1-5. CB-27. CE-798. MNC01-627D-65-1	79.32
IV	CE-113. CE-542. CE-76	161.17
V	CE-796. IT93-10-93	23.74
VI	CB-3. IT91K-118-2	65.73
VII	IT87D-611-3. MNC03-720-11	53.13
VIII	CE-46. CE-73	126.24
IX	BR9-Longa. MNC03-731C-21	30.58
X	CE-77	-
XI	IT 870-611-3	-

Table 3. Mean distances among and within 11 groups formed by 28 cowpea genotypes

Groups	I	II	III	IV	V	VI	VII	VIII	IX	XI	XI
I	56.9	87.1	84.7	101.3	41.6	78.8	62.9	263.9	51.8	208.7	67.8
II		92.3	95.1	110.3	96.8	71.6	122.9	199.7	72.8	124.8	134.7
III			79.3	136.1	99.1	54.2	81.7	238.1	50.1	199.6	97.2
IV				161.1	103.6	125.7	144.6	273.9	103.6	144.2	157.1
V					23.7	94.4	52.8	265.4	64.7	218.0	49.0
VI						65.7	75.8	227.9	36.4	174.1	86.9
VII							53.1	323.2	282.0	22.5	126.2
VIII								126.2	271.5	159.3	343.2
IX									30.5	185.2	58.7
X										-	314.3
XI											-

Table 4. Relative contribution of the response variables to the genetic divergence in 28 cowpea lines by the method of Singh (1981)

Response variable	Relative contribution of the response variables to the divergence (%)
Flowering (days)	17.16
Insertion angle of lateral branches	6.46
Number of nodes on main branch	19.54
Number of pods per plant	5.26
Number of seeds per pod	7.92
Pod length (cm)	5.70
100-seed weight (g)	10.53
Grain index	5.54
Yield (kg ha ⁻¹)	1.72
Cycle (days)	20.12

Based on the magnitude of the response variables to the genetic divergence evaluated by the canonical

variable method, the correlation coefficients between the principal canonical variables and the 10 response variables used in the study of genetic divergence in cowpea genotypes were determined (Table 5). Significant correlations indicate a partial contribution of each response variable to total variability; this shows that the variables that were significant for more than one canonical variable were beginning of flowering, insertion angle of lateral branches, number of seeds per pod, pod length, 100-seed weight and the crop cycle. In a comparison of this result with the study of relative contribution of these variable responses to genetic divergence by the methodology of Singh (1981) agreement was observed between the variables beginning of flowering and crop cycle, since these variables contributed most to genetic divergence and the variables grain yield, grain index and number of pods per plant as those with lowest contribution to the divergence by the two methodologies.

Table 5. Coefficient of correlation between 10 response variables and 4 main canonical variables that explained more than 80% of the total variation

Response variables	Canonical variables			
	1	2	3	4
Flowering (days)	-0.17	0.85**	0.50	-0.45*
Insertion angle of lateral branches	-0.46*	-0.27	-0.59**	-0.05
Number of nodes on main branch	0.87**	-0.17	0.26	-0.26
Number of pods per plant	-0.66**	-0.14	-0.32	-0.18
Number of seeds per pod	0.63**	0.07	0.49**	0.10
Pod length (cm)	0.37*	0.44*	-0.10	0.56**
100-seed weight (g)	0.15	0.60**	-0.21	0.40*
Grain index	-0.26	0.67	-0.05	-0.41*
Yield (kg ha ⁻¹)	0.49**	0.27	0.22	0.18
Cycle (days)	0.66**	0.12	-0.70**	-0.14

* and ** Significant by the t test at 5% and 1% probability, respectively

The dendrogram (Figure 1) was a result of intra- and intergroup cluster analysis of the 28 cowpea lines, based on dissimilarity measures of Mahalanobis' generalized distance (D^2), grouped into 11 different groups, based on the method of Tocher optimization.

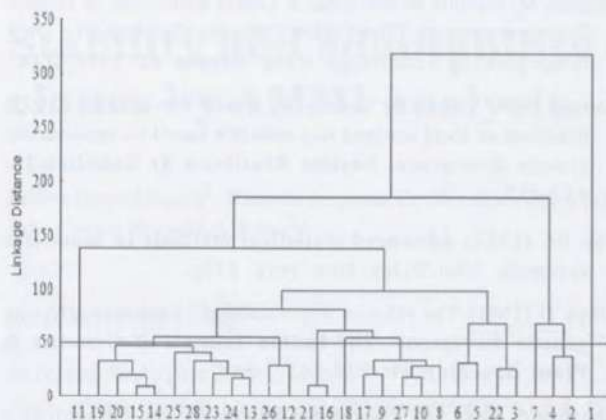


Figure 1. Dendrogram representing the genetic dissimilarity in 28 cowpea genotypes, obtained by the Unweighted Pair-Group Method with the Arithmetic average (UPGMA), using Mahalanobis' generalized distance (D^2) as dissimilarity measure

Genotype CE-46 (1) was confirmed as the most divergent of all, considering that it is in a group separated clearly from other groups.

The 28 genotypes were separated into 11 groups, when a horizontal cut was made at a linkage distance of around 48 in the hierarchical grouping of genotypes based on the measured response variables. This cutoff value allows the visualization of 11 groups formed by the Tocher method and separation of the genotype of the genotypes CE-46 from CE-76 and MNC 03-720C-11 in different groups and to join CB-3 and CB-27 in a same group, confirming these genotypes as the most similar among the evaluated.

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Divergência genética de genótipos de feijão-caupi de porte ereto e ciclo precoce

RESUMO – *Objetivou-se com esse trabalho realizar o agrupamento de genótipos de feijão-caupi de porte ereto e ciclo precoce utilizando técnicas multivariadas e estimar a contribuição relativa das variáveis respostas estudadas para a divergência genética. Utilizou-se 28 linhagens do Banco de Germoplasma da Universidade Federal do Ceará e Embrapa Meio Norte. Para a análise da divergência genética utilizou-se as variáveis canônicas, distância de Mahalanobis e o método aglomerativo de Tocher. A maioria das distâncias máximas observadas foi obtida quando combinadas com os genótipos CE-46. Os cruzamentos entre os grupos VIII e XI e grupos VII e VIII podem resultar na produção de novas combinações gênicas. As variáveis respostas floração inicial e ciclo da cultura contribuem em maior magnitude para a quantificação da divergência genética entre os genótipos avaliados.*

Palavras-chave: *Vigna unguiculata*, dissimilaridade, técnicas multivariadas.

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