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Assessment of genetic divergence in runner peanut genotypes grown in the Brazilian Northeast environments

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Runner cultivars are widely demanded by peanut farmers because of their high oil and grain productions. As those are late cycles, the management is limited in environments with water restriction. For improvement of runner genotypes to these environments is necessary to identify genotypes adapted and the knowledge of genetic divergence is quite relevant to assist the breeding procedures. In this work, ten morphological and agronomic traits were measured on 13 runner peanut genotypes grown in sandy loam textured soils during the rainy season in three environments on Brazilian Northeast region. These measurements included harvest index (%), main stem height (cm), 100 seed weight, 100 pod weight, pod length (mm), number of pod/plant, blooming (days after emergence, dae), number of seed/plant, physiological maturation of pods (dae) and oil content in seeds (%). The genetic divergence of genotypes was estimated by multivariate methods. Data set was analyzed by canonical variable (CV) in combination with UPGMA-clustering analyses. The statistical analysis was performed using the GENES program. We found that the first two CV were significant and accounted for 82.13% of the total variation. Three groups were clearly formed, separated by earliness and pod production. This arrangement was further attested by the dendrogram generated by UPGMA. The CV indicated that physiological maturation of pods, main stem height, number of pods/plant and 100 seed weight were the most differentiating traits among the genotypes. These two last allowed high magnitude associations and were contributive to selection procedures in improvement works. We provide indications of the best genotypes with potential to generate robust progenies, in a peanut breeding program.

Key words: *Arachis hypogea* L., canonical variables, traits associations, breeding.

INTRODUCTION

Peanut (*Arachis hypogea* L.) is an oilseed crop grown in many countries and is stands out commercially due to high value of grains, indicated to both food and oil markets. The species has two subspecies: The fastigiata,

Table 1. Geographical coordinates, soil and climate characteristics of environments.

Environment	Coordinates	Climate	Soil	T (°C)/RH (%)[1]	Rainfall (mm)[2]
Barbalha, CE	07º18'S; 39º18'W; 415 m	Semiarid	Vertisol	32/60	520
Campina Grande, PB	07º13'S; 35º53'W; 552 m	Tropical	Vertisol	28/68	730
Abreu e Lima, PE	07º54'S; 34º54'W; 19 m	Tropical	Entisol	29/66	820

[1]Means of temperature (T) and relative humidity (RH) fair during experimental period; [2]Total volume during experiment.

which includes Valencia and Spanish types, both upright and short cycle; and hypogea, that include Virginia and Runner types, both high yield and late cycles (Gibbons et al., 1977). The accessions from hypogaea usually have large seed and high oil yield, attracting to both food and oil market segments, but have limited adaptation to environments with water restriction (Isleib et al., 2004).

The Brazilian Company of Agricultural Research (EMBRAPA) coordinates a robust peanut breeding program targeted to oil and food markets, focusing on the development of Valencia and Runner cultivars adapted to environments with water restriction (Santos et al., 2013). Annually EMBRAPA has invested a large sum in generation of populations derived from interspecific genotypes in order to combine yield and early maturity, concentrating efforts in environments with semiarid climates (Luz et al., 2014; Vasconcelos et al., 2015; Pereira et al., 2015).

Analysis of genetic relationships is an important component of crop improvement programs because it provides information about genetic diversity, and is a platform for stratified sampling of breeding populations (Mohammadi and Prasanna, 2003). Such analysis can facilitate reliable classification of genotypes with possible utility for specific breeding purposes.

The univariate methods are widely adopted by breeders in several statistical models, in which each trait is analyzed separately allowing for substantial overlapping of results to occur (Yeater et al., 2004). Moreover, these methods do not explain how genotypes differ when all measured traits are considered jointly. In multivariate methods, all traits are considered simultaneously in the differentiation of populations, resulting in a more reliable comparison of germplasm. These methods are more suitable for assessing genetic divergence because it allow a more holistic interpretation of data, once consider the potential of the random trait sets, setting them at the same level of importance (Cruz and Regazzi, 2014). Among them, clustering and graphic dispersion techniques have been widely adopted by breeders (Pitta et al., 2010; Cruz and Regazzi, 2014).

The methods of graphic dispersion more often used are Principal Components (PC) and Canonical Variables (CV) analyses, that are linear combinations of the original quantitative measurements that contain the highest possible multiple correlation with each group and that best summarize among-class variation (Pitta et al., 2010; Cruz and Regazzi, 2014). To plant breeder, CV are more contributive because it allows the interpretation of the data with repetition, taking into account the residual covariance between the means of genotypes (Oliveira et al., 2003). As to clustering techniques, Hierarchical-UPGMA (Unweighted Pair Group Method with Arithmetic Mean) is widely adopted, which classifies similar individuals by clustering, based on similarity index (Bassab et al., 1990; Cruz and Regazzi, 2014). This method is based on genetic distances and has been considered an efficient estimator of phylogenetic linkages (Nei et al., 1983).

The combination of these techniques allow to clearly estimate the genetic interrelations in bred lines or core collections, providing valuable information to contribute to progress of allogamous or autogamous plant improvements. In this work we used UPGMA and CV analyses in order to assessing the genetic divergence in runner peanut genotypes grown in the Brazilian Northeast environments.

MATERIALS AND METHODS

The experiment were carried out in 2014 in three environments located in Brazilian Northeast region (Table 1), in sandy loam texture soils, previously limed and fertilized according to the needs of culture, revealed in soil analysis. The assays were performed in dry station of each places, at February, April and May in Barbalha (CE), Campina Grande (PB) and Abreu and Lima (PE), respectively.

Thirteen runner genotypes were evaluated, whose agronomic traits are shown in Table 2. The plot consisted of three 5 m-rows, spaced between other in 0.7 m. Plants (2/hole) were spaced in 0.3 m. Data were collected from central row. A randomized block design with three replications was adopted.

The crop management was performed according peanut crop recommendations described in Santos et al. (2010). The harvest took place between 115 to 130 days to earliness and late cycle earlier genotypes, respectively. These traits were collected: 100pod-weight (100P), 100 seeds-weight (100S), pod length (PL), height of main stem (HMS), number of pods/plant (PPI), number of

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Table 2. Origin, genealogy and some agronomical traits of runner genotypes.

Genotype	Origin	Genealogy	Seed			SP	PMP (dae)	Oil (%)	MSH (cm)
			Size	Color					
BRA 02179201	DF, Brazil	Accession	M	T	P	2	118-120	46	23
BRS Pérola Branca	PB, Brazil	Cultivar	L	W	P	3-4	110-115	50	25
Florunner	EUA	Cultivar	L	T	D	2-3	125-130	52	12
M407.F(B)	PB, Brazil	Top line	M	T	P	2	115-120	48	27
L50	Senegal	Top line	M	T	P	2	113-115	48	22
F.M424(B)	PB, Brazil	Top line	L	T	P	2-3	115-120	48	26
M424.F(B)	PB, Brazil	Top line	L	T	P	2	115-118	49	25
IAC Caiapó	SP, Brazil	Cultivar	L	T	D	2	125-130	51	13
Porto Alegre	PR, Brazil	Accession	M	T	P	2	118-120	48	24
Cavalo	RB, Brazil	Accession	EL	T	D	2	130-135	52	12
LGoPE-06	PE, Brazil	Top line	EL	T	D	2	125-130	52	13
BR1xAnonV	PE, Brazil	Top line	G	R	P	2-3	110-115	50	24
LViPE-06	PE, Brazil	Top line	EL	T	D	2-3	125-130	52	12

Seed size: M - mid, L - large, EL - extra large; Seed color: T - tan, W - white, R - red, C - Canopy: P - prominent main stem and side branches up to 1 m; D - decumbent, with no-prominent main stem and side branches up to 1 m; NSP - number of seeds/pod; PMP - physiological maturation of pods; MSH - Main stem height.

seeds/pod (S/P), harvest index (HI), Blooming (B), physiological maturation of pods (PMP), and oil content in the seeds (O). The height and production data were taken after harvest of genotypes. Blooming data was estimated as 50% of plants started the flowering, in central rows. The harvest index was estimated by dry weight of pod yield/ dry weight of total plant ratio (Duarte et al., 2013). The oil content (H1 OXFORD MQA 7005) estimated by Nuclear Magnetic Resonance spectrometer H1 OXFORD MQA 7005, using from 20 g of seeds of each genotype (AOCS, 2004).

Data were submitted to variance analysis by F test (p < 0.05) and the means were submitted to the canonical variables (CV) analysis. The genetic distance between accessions was estimated by Mahalanobis distance (D^2), expressed by:

$$D^2_{i'i} = \delta\psi^{-1\delta}$$

Where: $D^2_{i'l}$ = Mahalanobis distance between l and l' genotypes; ψ: variances and covariance matrix residuals; δ'= (d1 d2 ... dv), where dj = Y; ij - Yi'j and Yij: mean of the ith genotype in relation to j[th] trait (Cruz and Regazzi, 2014).

The UPGMA method was adopted to clustering analysis. In order to eliminate the non-hierarchical effects, the cophenetic correlation coefficient was estimated (Sneath and Sokal, 1973), which indicates the distortion produced by clustering in the original genetic distances. This coefficient is a matrix correlation between original genetic distances and a new distance matrix (the cophenetic matrix), derived directly from the UPGMA dendrogram. Additionally, linear associations between traits were estimated adopting Pearson correlation method. Statistical analyzes were performed using the GENES software (Cruz, 2006).

RESULTS AND DISCUSSION

A summary of variance analysis for all eight traits is shown in Table 3. Statistical differences were found to genotypes (G). To environments (E) and G x E interaction, statistical differences were found for most traits, excepting 100P, PL, S/P, PMP and O, indicating that the environments did not influence their expressions.

In Table 4 are shown the eigenvalues, individual variation (%) and accumulated variation associated to CV, based on ten traits from runner genotypes. It was found that the first two CV explained approximately 83.11% of the total variance (CV1 = 68.91%; CV2 = 14.19%), indicating that the most of variability is summarized in these two component. Then, the classification of genotypes by plotting of values in the two-dimensional level is feasible.

The graphical dispersion of genotypes is found in Figure 1. Three groups were formed with heavy contribution of traits associated with earliness and pod production.

Group 1 - clustered earliness and mid-cycle genotypes, with physiological maturation of pods ranging from 110 to 120 days, and oil content ranging from 47 to 50%. They are F.M424B, L50, M424.F (B), F.M407 (B), BR 1xAnonV, BRS Pérola Branca and Porto Alegre. These materials have prominent main stem, side branches up to 1 m and main axis up to 20 cm (Table 2). The highlight of this group is BRS Pérola Branca, the most earliness genotype, with maturation cycle starting from 110 days and mild tolerance to indian summers (Pereira et al., 2012). BRS Pérola Branca was developed by EMBRAPA to semiarid environment and inherited robust earliness and yield traits from their parents: BR 1, an earliness Valencia type and LViPE-06, a Runner-high yield material (Pereira et al., 2012). The isolines F.M407 (B), M424.F (B) and F.M424 (B), all generated by crossings between Argentina (Manfredi) and North American (Florunner) cultivars also offer interesting contributions to runner

Table 3. Summary of variance analysis for runner peanut traits.

		Mean square									
SV	FD	HI	MSH	100S	100P	PL	NSP	PPI	B	PMP	O
B/E	6	7.68	0.92	6.62	382.01	0.25	0.06	6.81	1.10	7.53	9.16
B	2	17.72	1.19	1.43	888.49	0.03	0.19	6.72	2.67	6.33	10.80
B x E	4	2.67	0.78	9.22	128.78	0.36	0.01	6.85	0.31	8.14	8.34
G	12	226.19**	408.87**	859.63**	9422.26*	157.09*	1.20**	842.90*	58.97*	658.38*	41.99*
E	2	670.49**	12.88**	48.26*	35.35	3.30**	0.02	719.44**	6.72*	19.92	9.80
G x E	24	27.73**	8.32	16.78*	86.28	3.71**	0.01	29.93**	3.17**	5.03	8.13
Error	72	2.85	4.18	9.72	83.39	1.51	0.03	8.91	1.17	7.32	7.86
CV (%)	-	3.77	9.42	5.28	6.26	4.10	8.82	9.16	3.11	2.22	5.67
Mean	-	44.66	21.69	59.05	145.68	29.94	2.12	32.55	34.85	121.66	49.40

** and * - significant by F test F ($p<0.01$ and $p<0.05$, respectively); B - block; E - Environment; G - Genotype; CV - Coefficient of variation; SV - source of variation; FD - freedom degree; HI - harvest index (%); MSH - main stem height (cm); 100S - 100 seed weight (g); 100P - 100 pod weight (g); PL - pod length (mm); NSP - number of seed/plant; PPI - number of pod/plant; B - blooming (days after emergence, dae), PMP - physiological maturation of pods (dae), O - oil content in seeds (%).

Table 4. Estimate of variance (eigenvalues and accumulated variation) of the canonical variables for ten peanut traits.

Canonical variable	Eigenvalue	%	Acumulated variation (%)
VC1	65.35	68.91	68.91
VC2	13.46	14.19	83.11
VC3	6.56	6.92	90.04
VC4	4.31	4.54	94.59
VC5	2.43	2.56	97.15
VC6	1.34	1.41	98.57
VC7	0.96	1.01	99.59
VC8	0.28	0.29	99.89
VC9	0.07	0.08	99.97
VC10	0.02	0.02	100.00

peanut breeding due to their better productivity, in tropical climate (Santos et al., 2010).

Group 2: Clustered late materials, with maturation cycle from 120 days. They are: IAC Caiapó, Florunner, Cavalo, LviPE-06 and LgoPE-06, all with decumbent canopy and no-prominent main stem. Side branches reach up to 1.5 m and oil content are up to 50% (Table 2). These genotypes have high oil and grain yield, are sensitive to Indian summers and broad tolerant to leaf diseases (Isleib et al., 2004; Santos et al., 2010; Duarte et al., 2013). They are, therefore, excellent genetic resources for breeding works, aiming to broadening the genetic basis of Valencia or Spanish materials.

The Group 3 contained only one genotype (BRA02179201), newly incorporated in EMBRAPA *A. hypogaea* collection, and involved in pre-breeding procedures. This genotype is a mid-cycle material, and shows no-typical traits of Runner types, such as low harvest index and oil content below 46%. In field, the plants showed a flow of pods concentrated next to main stem. This ideotype can later be interesting to generate runner cultivars indicated to no-mechanized management.

Based on the group compositions formed by the CV analysis, we recommended adopting genotypes from Groups 1 and 2 in breeding program in order to obtain promising progenies for further use in selection procedures focusing on environment adaptation to Brazilian Northeast region.

The dendrogram generated by UPGMA is found in Figure 2. The comparison of the distance matrices and clustering were based on the estimative of cophenetic correlation (0.74). Two groups were formed and similar as found in Figure 1, excepting that BRA02179201 was clustered in the same group of earliness and mid cycle genotypes (Group 1).

The classification method of germplasm using UPGMA offers various advantages to the plant breeder, such as the sharp perception of similarity level among genotypes. Sometimes this proximity may not be noticeable on the
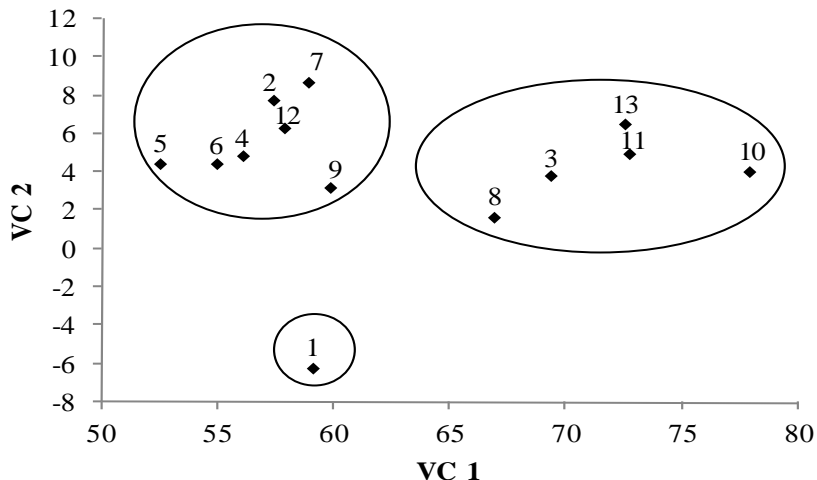
Figure 1. Graphical dispersion of scores in relation to first two canonical variables, based on ten agronomic traits obtained in the 13 runner peanut genotypes. 1 - BRA02179201, 2 - BRS Pérola Branca, 3 - Florunner, 4 - F.M424 (B), 5 - L50, 6 - M424.F (B), 7 - M407.F (B), 8 - IAC Caiapó, 9 - Porto Alegre; 10 - Cavalo, 11 - LGoPE-06, 12 - BR1xAnon, 13 - LViPE-06.
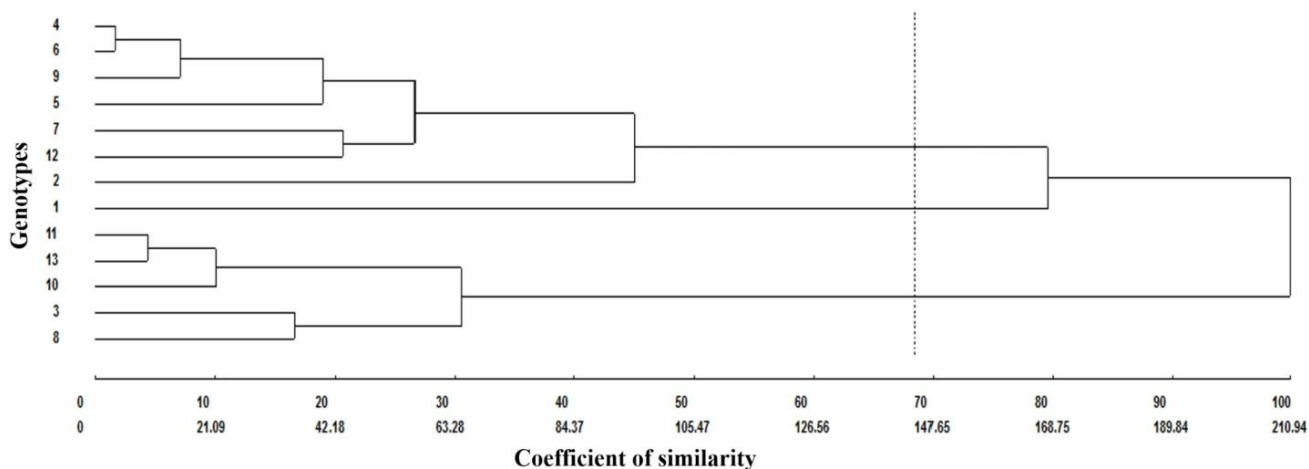

Figure 2. Dendrogram generated by NTSYS-pc software 2:10 using UPGMA clustering method, based on ten traits obtained from runner peanut genotypes. 1 - BRA02179201, 2 - BRS Pérola Branca, 3 - Florunner, 4 - F.M424 (B), 5 - L50, 6- M424.F (B), 7 - M407.F (B), 8 - IAC Caiapó, 9 - Porto Alegre; 10 - Cavalo, 11 - LGoPE-06, 12 - BR1xAnon, 13 - LViPE-06. Cophenetic correlation coefficient: 0.74.

graphic plan. In Figure 2 we confirmed the high relationship between M424.F (B) (4) and F.M424 (B) (6), and also between LVIPE-06 (11) and LGoPE-06 (13), that have closely related to each other (Duarte et al., 2013). LVIPE-06 and LgoPE-06 are late cycle genotypes widely used by EMBRAPA breeders due to high capacity to pod production and resistance do leaf diseases (Santos et al., 2013). Luz et al. (2014) estimated the genetic gain of progenies derived from these genotypes and cv. BR 1 (earliness, Valencia type) and found gains ranging from 6 to 15% to number of peg, 4 to 17% to number of pod/plant and 3 to 17% to 100 pod weight.

Vasconcelos et al. (2015) estimated the stability and adaptation of runner genotypes to semiarid environment during three years, including LVIPE-06, LGoPE-06, BRS Pérola Branca, Florunner and IAC Caiapó. The authors found best results to BRS Pérola Branca and LVIPE-06, while LGoPE-06 showed excellent results to pod production, but was highly dependent upon favorable environments.

The relative contribution of traits to genetic divergence among genotypes, based on D2, is found in Table 5. The following traits were more contributive: physiological maturation of pods, main stem height, number of

Table 5. Relative contribution of traits to genetic divergence of runner peanut genotypes.

Trait	PMP	MSH	PPI	100S	HI	100P	B	PL	NSP	O
%	16.18	15.97	14.69	14.09	12.59	11.95	5.76	4.75	3.82	0.15

PMP - physiological maturation of pods (dae); MSH - main stem height (cm); PPI - number of pod/plant; 100S - 100 seed weight (g); HI - harvest index (%); 100P - 100 pod weight (g); B - blooming (days after emergence. dae); PL - pod length (mm); NSP - number of seed/plant; O - oil content in seeds (%).

Table 6. Linear associations between traits collected in 13 runner peanut genotypes.

	HI	100P	PL	NSP	100S	MSH	PPI	B	PMP	O
HI	1	0.66*	0.60*	-0.45	0.85**	-0.62*	0.65*	0.91**	0.70**	0.64*
100P		1	0.89**	-0.00	0.87**	-0.35	0.73**	0.78**	0.69**	0.59*
PL			1	0.13	0.87**	-0.33	0.73**	0.74**	0.60*	0.75**
NSP				1	-0.07	0.11	0.08	-0.37	-0.21	0.20
100S					1	-0.56*	0.77**	0.85**	0.65*	0.77**
MSH						1	-0.33	-0.65*	-0.64*	-0.52
PPI							1	0.61*	0.42	0.72**
B								1	0.82**	0.67*
PMP									1	0.59*
O										1

HI - harvest index (%); 100P - 100 pod weight (g); PL - pod length (mm); NSP - number of seed/plant; 100S - 100 seed weight (g); MSH - main stem height (cm); PPI - number of pod/plant; B - blooming (days after emergence - dae); PMP - physiological maturation of pods (dae); O - oil content in seeds (%).

pods/plant and 100 seed weight. These results were also found by Santos et al. (2000) and Ajay et al. (2012) using principal component analysis, indicating that all of them are responsive to discrimination of runner genotypes. The linear associations generated from means of traits are shown in Table 6. Several significant associations were verified; some of them had high magnitude, indicating possible relation with linked genes or pleiotropic effects. They are B x 100S, 100P x PL, 100P x 100S, 100P x PPI, 100P x B, 100S x PL, PL x PPI, PL x B, 100S x PPI and 100S x B. These results are widely useful to seletion procedures in peanut breeding program. The length of the main stem, for example, negatively correlated with blooming (-0.65) and physiological maturation of pods (-0.64), are a clear demonstration of robustness of these traits to selection of runner genotypes, serving even as a reference for selection in progenies derived from cross between fastigiata x hypogeae. It is quite interesing to realizes that the decrease the main stem height does not contribute to the reduction of seed production and therefore, main stem height is a good demonstration of the practical use of correlation estimates in breeding programs because it is a trait that can be measured at the beginning of the cycle, anticipating the selection to productive genotypes.

Negative associations were found only to HI x MSH (r = -0.62), MSH x 100S (r = 0.57), MSH x PMP (-0.64) and MSH x B (r = -0.65), all of which involving plant height, indicating that lower plants tend to be late and more productive. These results are consistent with pattern of runner plants that often are decumbent, fostering a better exploitation of pegs to facilitate the formation of pods (Gomes and Lopes, 2005).

Conclusions

1. The runner genotypes showed variability based on traits selected, however, they showed G x E interactions to traits associated with earliness and production, such Blooming, 100 seed weight, harvest index, pod length and number of pod/plant.
2. The high magnitude-associations here identified were important for setting selection strategies in runner peanut breeding, focused on Northeast region.

Conflict of Interests

The authors have not declared any conflict of interests.

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