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Selection of earliness peanut accessions for genetic improvement in a water-restricted environment¹

Seleção de acessos de amendoim precoce para melhoramento genético em ambiente com restrição hídrica

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HIGHLIGHTS:

Peanut accessions were differentiated by multivariate statistical methods.

Differentiated access groups were indicated to generate variability.

Productive peanut accessions are recommended as parental.

ABSTRACT: Peanut production is growing annually in Brazil, but with frequent droughts throughout the country, it is necessary to identify an earliness germplasm for the improved environmental adaptation of peanuts. Here, a mini-core collection of peanuts was studied to select germplasms for further use in breeding, with a focus on dry environments. Twelve agronomic traits were adopted and later used in multivariate methods based on hierarchical models (UPGMA) and graphical dispersion (canonical variables), which aimed to identify earliness accession clusters. Statistically significant differences were found for most traits, indicating that there was genetic variability among accessions. Multivariate analyses subsequently revealed coherence in the formation of three clusters, one of which (G3: PI-268-689 and AHK-85-3) combined the most promising accessions for pod production and reproductive efficiency, previously identified through analysis of variance. Therefore, both germplasms are recommended here for use in future breeding studies focused on water-restricted environments.

Key words: *Arachis hypogaea*, germoplasm clustering, genetic diversity

RESUMO: A produção de amendoim tem crescido anualmente no Brasil. Com as secas que ocorrem frequentemente em todas as regiões brasileiras, é necessário identificar germoplasma precoce para ser utilizado em trabalhos de melhoramento visando à adaptação ambiental. Aqui, uma mini coleção de amendoim foi estudada com o objetivo de selecionar germoplasma para uso posterior em procedimentos de melhoramento, com foco em ambiente com restrição hídrica. Doze características agrônomicas foram adotadas e posteriormente utilizadas em métodos multivariados, baseados em modelos hierárquicos (UPGMA) e dispersão gráfica (variáveis canônicas), visando identificar agrupamentos de acessos precoces. Diferenças estatísticas significativas foram encontradas para a maioria das variáveis, indicando variabilidade genética nos acessos estudados. Com base nas análises multivariadas, verificou-se coerência na formação de três agrupamentos, um deles (G3: PI-268-689 e AHK-85-3) aglomerou os acessos mais promissores para produção de vagens e eficiência reprodutiva, previamente identificados na análise de variância. Portanto, ambos germoplasma são recomendados para uso em trabalhos de melhoramento voltados para o ambiente com restrição hídrica.

Palavras-chave: *Arachis hypogaea*, agrupamento de germoplasma, diversidade genética

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INTRODUCTION

Germoplasm conservation is a global concern, considering that it aims to maintain genetic resources that will retain sources of food and medication for future generations. Knowledge of the genetic variability within a population is essential for the development and adoption of strategies in improvement programs. Conventional methods can classify and estimate superior materials with the potential to define commercial cultivars (Silva et al., 2016). Additionally, multivariate models have been very useful in this process, especially when dealing with autogamous-hermaphrodite plants, in which genetic homogeneity can hinder selection when a small number of variables are adopted (Ramos et al., 2015).

The peanut (*Arachis hypogaea* L.) fits this example, as it is a leguminous plant with a high rate of autogamy, benefitted by hermaphrodite and cleistogamic flowers. Brazilian germplasm banks contain more than 3,000 accessions, some of which are responsible for commercial cultivars (RNC, 2021). The identification of earliness and high yield accessions, alongside broad nutritional value, is widely sought by peanut researchers (Santos et al., 2013; Massarioli et al., 2022). Additionally, the aggregation of traits such as tolerance to biotic and abiotic stresses can greatly enhance the genetic value of germplasms for further use in breeding programmes (Santos et al., 2013; Silva et al., 2016). Multivariate statistical methods have contributed to the identification of the most important germplasms so far in terms of improvements in environmental adaptation (Luz et al., 2014; Ramos et al., 2015). Ramos et al. (2015) previously used three multivariate methods to estimate genetic divergence in 77 peanut accessions, whilst aiming to identify an earliness germplasm for future use in crossing. Subsequently, it was found that all methods showed consistent results and provided interesting combinations of high-yield and earliness accessions, which could be adopted in further improvement strategies directed towards water-restricted environments.

This study aimed to contribute to the ongoing research into the identification of promising accessions, by selecting peanut accessions for their genetic adaptation to dry environments.

MATERIAL AND METHODS

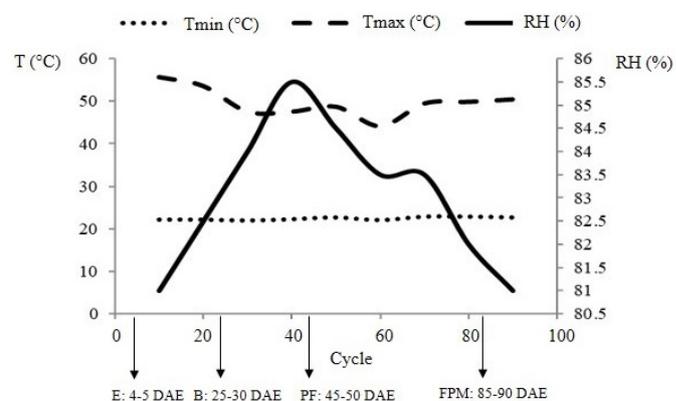
Eleven accessions of *A. hypogaea* subsp. *fastigiata* (upright growth habit) (Table 1) were sown in a greenhouse (Campina Grande, PB, Brazil, 7° 13' 50" S, 35° 52' 52" W, 551 m) in 30 L vases containing basaplant substrate. This consisted of a

balanced mixture of the following components: Pine bark, peat, coal, and vermiculite, all supplemented with NPK and micronutrients. The humidity, electrical conductivity, water-holding capacity, and pH of the substrate were: 50%, 2.5 mS cm⁻¹, 150% and 5.8, respectively. Furthermore, 100 g of bovine manure was added to each pot at the time of planting.

To analyze biological traits, a completely randomized design using five replicates was implemented here. The experimental unit comprised two plants per plot, which were manually watered daily to maintain the soil humidity close to the pot capacity (Duarte et al., 2013). The maximum (Tmax) and minimum (Tmin) air temperatures as well as relative air humidity (RH), in the greenhouse were all recorded daily during the plant cycle (Figure 1). Their respective mean values throughout the phenological cycle were 49.4, 22.3, and 83.5%.

Harvest began 75 days after emergence (DAE) for earliness accessions and continued until 90 DAE, corresponding with the full pod maturation period described by Santos et al. (2013). The following biological traits were recorded for the accessions: Blooming (B), which refers to the beginning of the flowering process, when half of plants have emitted their first flower (Silva et al., 2016; Ramos et al., 2020), main stem height (MSH), number of mature pods per plant (NPP), weight of dry pods per plant (WPP), 100-pod weight (100PW), 100-seed weight (100SW), and full pod maturation (FPM), which occurred when 70% of the plant's pods were fully mature (Santos et al., 2013).

The WPP, 100PW and 100SW data were all measured when seed moisture content was 4-10%. Additionally, to estimate the



E - Emergence; B - Blooming; PF - Peak of flowering; FPM - Full pod maturation; DAE - Days after emergence

Figure 1. Maximum (Tmax) and minimum (Tmin) air temperatures, and the relative air humidity (RH) recorded in the greenhouse during the phenological cycle of peanut accessions

Table 1. Passport data for the peanut accessions of this study

Accession	Origin	Botanic type	Seed color	Genetic basis
GDM	India	Valencia	Red	Cultivar
GDM (M1)	Brazil	Valencia	Red	Mutation from GDM
Georgia	USA	Spanish	Tan	Cultivar
Dixie-Spanish	USA	Spanish	Tan	Cultivar
PI 268-689	USA	Spanish	Tan	Introduction
Dwarf	Brazil	Valencia	Red	Mutation from BR 1
AHK-85-3	Senegal	Spanish	Tan	Top line from Senegal
Feuri-11	Senegal	Spanish	Tan	Top line from Senegal
55114	India	Spanish	Tan	Cultivar
SR-1-4	Senegal	Spanish	Tan	Top line from Senegal
R-31-1	India	Spanish	Tan	Cultivar

reproductive efficiency and harvest indexes of the accessions, the following traits were added: Total number of pegs (NP), total number of flowers (NF), period of flowering (PF), dry weight of plant (DWP), and fresh weight of plant (FWP), according to Luz et al. (2014). The flowering duration was estimated using the period from the beginning of flowering until the day of harvest for each accession. Flower viability (FV%) was estimated using Eq. 1:

$$FV\% = \frac{NP}{NF} \times 100 \quad (1)$$

where:

- FV - Flower viability;
- NP - Number of pegs; and,
- NF - Number of flowers.

The harvest index was estimated using Eq. 2, in accordance with Coffelt et al. (1989):

$$HI\% = \frac{WPP}{DWP} \times 100 \quad (2)$$

where:

- HI - Harvest index;
- WPP - Weight of dry pods per plant; and,
- DWP - Dry weight of plant.

Reproductive efficiency was estimated by the production capacity of flowers (Eq. 3) according to Coffelt et al. (1989), and pegs using Eq. 4, based on Santos et al. (2013), considering that not all flowers can form a gynophore and, later, a pod.

$$REF\% = \frac{NPP}{NF} \times 100 \quad (3)$$

where:

- REF - Reproductive efficiency based on production of flowers;
- NPP - Number of mature pods per plant; and,
- NF - Total number of flowers.

$$REP\% = \frac{NPP}{NP} \times 100 \quad (4)$$

where:

- REP - Reproductive efficiency based on production of pods;
- NPP - Number of mature pods per plant; and,
- NP - Total number of pegs.

Statistical analyses were performed using GENES software, version 1990.2018.71 (Cruz, 2013). Prior to this, the data were subjected to the Lilliefors test to verify normality. For univariate analyses, the F-test and Scott-Knott test ($p \leq 0.5$) were used for ANOVA and comparison of means, respectively. For multivariate analyses, hierarchical (Unweighted Pair Group Method using arithmetic averages, UPGMA) and non-hierarchical (canonical variables, CV) models were adopted to verify the coherence of clustering. Mahalanobis distance (D^2) was also used to estimate genetic similarity between accessions.

RESULTS AND DISCUSSION

Statistically significant differences were found for the majority of peanut traits assessed in this study ($p \leq 0.01$), except for blooming (B), period of flowering (PF), and full pod maturation (FPM) (Table 2), indicating that although the accessions exhibited variations in productive aspects, they were uniform in earliness for both blooming and pod maturation. These results are relatively unusual in the germoplasm of the subsp. *fastigiata* (Santos et al., 2000; Ramos et al., 2015). Early flowering and pod maturation is highly desirable, particularly in water-restricted environments or in crop rotation management involving off-season production (Santos et al., 2013; Ramos et al., 2022). The variability of these traits has often been found in accessions derived from crosses between *hypogaea* and *fastigiata*, whose combinations are highly desired by peanut breeders because, in addition to earliness, their progenies also carried an increased yield (Patil et al., 2020; Ramos et al., 2020).

The average traits recorded for each accession are shown in Table 3. PI-268-689 combined the most relevant agronomic traits for breeding work, such as number of mature pods per plant (NPP), weight of dry pods per plant (WPP), reproductive efficiency based on number of pegs (REP), and harvest index (HI), with respective means of 36.0, 39.0, 58.9, and 51.7%, which overcame the overall means of the accessions by 80.0, 50.0, 26.0, and 31.0%, respectively. Additionally, focusing on REP, the accessions Dwarf, AHK-85-3, and Feuri-11 all

Table 2. Summary of ANOVA for MSH - Main stem height; B - Blooming; PF - Period of flowering; NPP - Number of mature pods per plant; WPP - Weight of dry pods per plant, 100PW- 100-pod weight, 100SW-100-seed weight; FV- Flower viability; REP - Reproductive efficiency based on number of pegs; REF - Reproductive efficiency based on number of flowers; HI - Harvest index; FPM - Full pod maturation for the peanut accessions

SV	DF	Mean square						
		MSH	B	PF	NPP	WPP	100PW	
Accession	10	217.3**	5.13 ^{ns}	42.68 ^{ns}	196.21**	98.45**	769.88**	
Error	22	30.83	4	35.66	6.87	9.93	22.78	
CV (%)		17.05	7.79	12.49	11.29	12.51	4.08	
		100SW	FV	REP	REF	HI	FPM	
Accession	10	47.29**	823.83**	275.79**	212.44**	122.61**	1.47 ^{ns}	
Error	22	4.43	31.39	18.54	6.18	13.68	0.78	
CV (%)		4.31	9.77	9.38	9.33	9.21	0.98	

** - Significant (F-test, $p \leq 0.01$); ^{ns} - Non-significant; SV - Source of variation; DF - Degrees of freedom; CV - Coefficient of variation

Table 3. Mean and percentage of the amplitude of the MSH - Main stem height; NPP - Number of mature pods per plant; WPP - Weight of dry pods per plant; 100PW - 100-pod weight, 100SW - 100-seed weight; FV - Flower viability; REP - Reproductive efficiency based on number of pegs; REF - Reproductive efficiency based on number of flowers; HI - Harvest index in the peanut accessions

Accession	MSH (g)	NPP	WPP	100PW (g)	100SW (g)	FV	REP (%)	REF (%)	HI (%)
G.D.M	43.00 a	16.00 c	22.00 c	133.00 a	51.00 a	48.00 d	42.47 b	21.55 c	39.59 c
G.D.M-MUT-1	13.00 e	14.00 c	17.00 d	94.00 d	42.00 c	63.00 c	46.76 b	32.57 b	38.76 c
Georgia	22.00 d	20.00 b	20.00 c	109.00 c	47.00 b	43.00 d	42.81 b	19.38 c	38.93 c
Dixie-Spanish	38.00 b	22.00 b	22.00 c	122.00 b	50.00 a	44.00 d	39.51 b	17.69 c	31.69 d
PI-268-689	31.00 c	36.00 a	39.00 a	113.00 c	48.00 b	37.00 d	58.92 a	32.94 b	51.71 a
Dwarf	37.00 b	20.00 b	26.00 b	124.00 b	51.00 a	70.00 b	52.50 a	39.27 a	45.30 b
AHK-85-3	38.00 b	40.00 a	27.00 b	87.00 d	41.00 c	59.00 c	52.30 a	32.85 b	45.13 b
Feuri-11	30.00 c	24.00 b	26.00 b	128.00 b	51.00 a	74.00 b	51.64 a	38.37 a	41.92 c
55114	35.00 b	24.00 b	22.00 c	131.00 a	52.00 a	39.00 d	43.47 b	16.94 c	42.25 c
SR-1-4	36.00 b	17.00 c	26.00 c	104.00 c	46.00 b	59.00 c	51.44 a	31.74 b	37.86 c
R-31-1	30.00 c	19.00 c	27.00 b	134.00 a	53.00 a	90.00 a	22.82 c	20.60 c	28.50 d
AM (%)	324	275	226	153	129	240	258	231	181
Mean	36	20	26	122	50	59	46.76	31.74	39.59

Means with the same letter did not differ statistically (Scott-Knott test, $p \leq 0.05$); AM - Amplitude of the mean, based on the variation from the lowest to the highest mean of each trait

showed promising results with a mean of 52%. The amplitude values of each trait revealed a large variability to be explored in selection procedures, based on high variations ranging from 129 (100SW) to 324% (MSH).

The reproductive efficiency (RE) of the accessions, a particularly relevant trait in the estimation of pod-yield capacity, was considered satisfactory, although they were from the subsp. *fastigiata*, which typically exhibits an RE between 30 and 40%. Despite the ability of plants to produce a large number of flowers, RE is a trait dependent on main stem height as well as lateral branches. Considering that peanut pods develop below the soil surface, the higher the flowers, the lower the viability of the gynophores (pegs), which carry the pods in their extremities (Santos et al., 2000; Luz et al., 2010). RE was estimated here based on the total number of flowers (REF) and pegs (REP) produced by the accessions. According to the literature, plants with REF below 30% indicated that their flowers were inefficient in pod production pods, either because of the height of the flower in the main stem or nutritional aspects. In the case of REP, values below 40% are usually related to greater plant height, which typically prevents the survival of fertilized eggs, although this is dependent on the distance from the peg to the soil surface (Coffelt et al., 1989; Luz et al., 2010; Santos et al., 2013).

As presented in Table 3, the respective means of REP and REF were 46.76 and 31.74%, while PI-268-689, Dwarf, AHK-85-3, Feuri-11, and SR-1-4 all exhibited means above 51 and 31%, respectively. The lowering of the maximum temperature from the flowering peak (Figure 1) favored the germplasm in the emission of flowers, and these five accessions benefited the most, and as a result, were the most important germplasm for breeding studies aimed at pod production and earliness. The adoption of top parents in hybridization is a promising strategy employed by plant breeders which can add value to these traits.

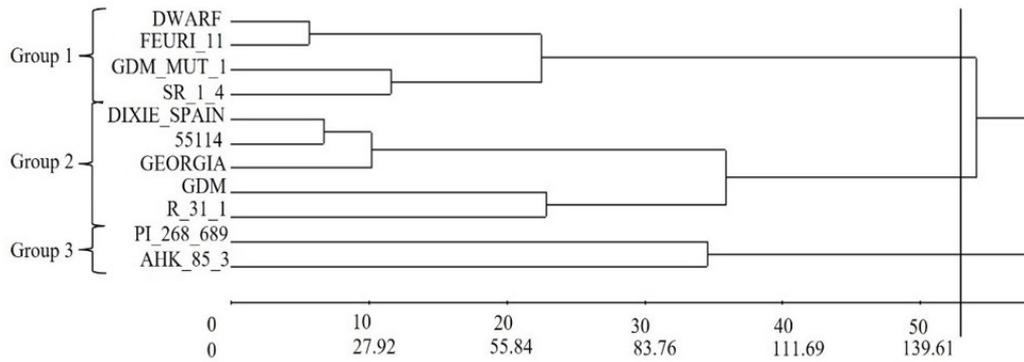
Luz et al. (2010) crossed the early peanut cultivar BR 1 with runner lines from Argentina, aiming to improve pod yield and tolerance to leaf diseases. In the F_2 generation, the authors estimated the REP of progenies and found broad variability in these traits (mean of 34%), thereby overcoming the mean of the parentals by 19%. Furthermore, Sousa et al. (2020) estimated the REP of seven upright and semi-upright peanut genotypes and subsequently obtained a mean of 21%. This was considered low, possibly because of the narrow genetic base of the germplasm,

considering that they were all collected from the same geographic region in Ceará state, Brazil. Despite difficulties in estimating this trait, it is highly valuable for peanut genetic improvement, and although plant height was negatively correlated with pod yield in upright species, this trend may change when the genetic base of the plant contains an interspecific germoplasm (Santos et al., 2000; Silva et al., 2016; Ramos et al., 2020).

The genetic diversity of peanut accessions, estimated using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), is shown in Figure 2. A dendrogram was generated using 5,000 bootstraps, with a cophenetic correlation coefficient of 0.79. As a result, three groups (G) were formed with the following compositions: G1- Dwarf, Feuri-11, G.D.M-MUT-1, and SR-1-4; G2, Dixie-Spanish, 55114; Georgia, G.D.M, and R-31-1; and G3- PI-268-689 and AHK-85-3, the latter containing the two most robust accessions based on pod production and reproductive efficiency, in addition to earliness. No accessions differed significantly in terms of blooming and full pod maturation. This same grouping trend was found in the non-hierarchical model, represented by canonical variables (Figure 3), whose accumulated variance in the first two CVs explained 79.93% of the total variance (Table 4), thereby justifying the representation of accessions in a two-dimensional plane.

The results obtained using the two multivariate methods indicated that 75% of the traits examined in this study were responsive to the clustering of peanut accessions. The relatively low contributions of B, PF, and FPM were due to the genetic nature of the germoplasm, which belonged to the upright-subsp. *fastigiata*, between the Spanish and Valencia botanical types. Since they accumulated many similarities between morphological and agronomic traits, the use of two multivariate methods to cluster genotypes of *fastigiata* has often been adopted to verify the coherence between the groups. Thus, the clusters found here provide reliability for plant breeders when choosing parents that will contribute to improvement work (Dutra et al., 2018; Ramos et al., 2020).

Although most of these accessions were introduced to Brazil during the 1980's, some studies initiated by the Federal University of Ceará have involved mutations in the upright germplasm. However, this research was uncertain due to a lack of biological improvements and knowledge, as was the case with



The vertical line represents the cut-out for selection based on a dissimilarity index greater than 55%

Figure 2. Dendrogram obtained by the UPGMA hierarchical clustering method from the dissimilarity matrix of 11 peanut accessions and nine traits

Table 4. Estimates of variance (Eigenvalues and accumulated variance) of the canonical variables (CV) for nine peanut traits

Canonical variable	Eigenvalue	%	Accumulated variance
CV1	48.83	58.49	58.49
CV2	17.89	21.43	79.93
CV3	8.79	10.53	90.47
CV4	3.35	4.02	94.49
CV5	2.51	3.0	97.50
CV6	1.39	1.67	99.17
CV7	0.62	0.75	99.93
CV8	0.05	0.06	99.99
CV9	0.006	0.007	100.00

with BR 1 (Valencia), which would add earliness, pod yield, and adaptation to drought, to the progenies, thus increasing the competitiveness of peanut agribusiness in semiarid regions.

CONCLUSIONS

1. The peanut accessions PI-268-689 and AHK-85-3 were valuable germplasms for peanut improvement in a semiarid region.
2. Peanut accessions PI-268-689, Dwarf, AHK-85-3, Feuri-11, and SR-1-4 had high reproductive efficiency, and therefore are promising for pod yield and earliness.

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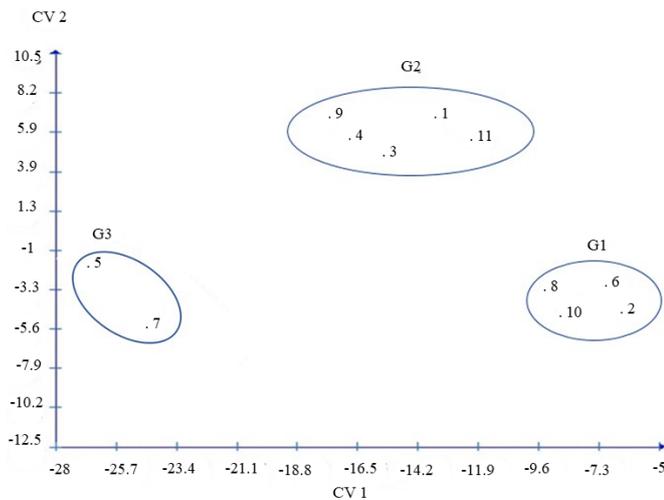
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Peanut accessions: 1- G.D.M, 2- G.D.M-MUT-1; 3- Georgia; 4- Dixie-Spanish; 5- PI 268-689; 6- Dwarf; 7- AHK-85-3; 8- Feuri-11; 9- 55114; 10- SR-1-4; 11- R-31-1; G1, G2, G3 - Peanut accession groups; CV 1 and CV 2-canonical variables in X and Y axes, respectively

Figure 3. Graphical dispersion of scores in relation to the first two canonical variables based on nine agronomical traits obtained from the 11 peanut accessions

the mutant Dwarf, which was described as a mutation from BR 1, an early upright cultivar widely adapted to the Northeast semiarid region (Dutra et al., 2018; Santos et al., 2013). There is currently no published record of the mutation technique used to generate Dwarf; although, it is identical to BR 1, with no additional biological traits that make up for commercial investment. However, since the peanut market in the Brazilian northeast is dominated by the Valencia type (red seeds), an interesting strategy for an improvement program could involve crossings between earliness accessions of group 3 (Spanish)

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