Genetic divergence among forage peanut genotypes based on agronomic and nutritional traits[1]

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ABSTRACT

Forage peanut is a perennial legume of high-quality forage production and persistence in mixed pastures that improves soil quality and becomes an efficient option for the recovery of degraded pastures. Therein context, the objective of this study was to cluster forage peanut genotypes based on their breeding values, considering structuring agronomic and nutritive traits to select parents for the hybridization program. Sixty-seven genotypes were evaluated in three separate cut trials in a randomized complete block design, with Belmonte and BRS Mandobi cultivars as a common control. Genotypic values were obtained for each trial by the mixed model methodology. Genetic divergence was analyzed by principal component analysis and hierarchical cluster analysis. There was variability for most traits, with variation among trials on genotypic, genotype x evaluation interaction, and permanent environment variances. There were similar structuring traits among trials and showed soil cover, total dry matter yield, and plant height as the most relevant traits. The hierarchical cluster analysis indicated genotype discrimination by dry matter and seed production. There is a possibility to select highly divergent superior genotypes as parental and breeding with a focus on forage and seed production.

Keywords: *Arachis pintoi* and *A. repens*; cluster; mixed models; multivariate analysis.

INTRODUCTION

Forage peanut (*Arachis pintoi* Krapov. & W. C. Greg. and *Arachis repens* Handro) is a perennial legume of geocarpic fruits and high-quality production of aerial biomass among tropical forage species (Carvalho *et al.*, 2009; Assis *et al.*, 2013). These characteristics are important because they provide more persistence, especially in mixed pastures, by the individuals' constant recruitment, and improved feed forage supply (Valentim *et al.*, 2003; Valentim & Andrade, 2003). Besides, because of biological nitrogen fixation, the introduction of forage peanut into pastures improves soil quality and becomes an efficient option for the recovery of degraded pastures (Assis *et al.*, 2013; Assis & Valentim, 2013). Sexual propagation for this forage represents an important trait since the currently used propagation by stolons is considered one of the limiting factors for its adoption in large areas (Shelton *et al.*, 2005; Assis *et al.*, 2013).

The cultivars already released in Brazil were obtained by mass selection and phenotypic evaluation of natural ecotypes (Assis *et al.*, 2013; Simeão *et al.*, 2017). Currently, the superior and divergent ecotypes, selected through network evaluation, constitute materials for intra and interspecific crosses (Assis & Valentim, 2013). The resulting hybrids are then evaluated in regions of interest for genetic materials selection with specific and more adapted characteristics (Simeão *et al.*, 2017).

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In that sense, genetic diversity studies are essential in the conservation, quantification, and direction of this variability to achieve superior genotypes with the interest traits (Resende, 2002; Cruz *et al.*, 2012). The characterization utilized in these studies makes it possible to maintain desirable traits of forage peanut genotypes in the new cultivars, such as rapid soil cover, high nutrient content, and resistance to pests and diseases (Assis *et al.*, 2008; Ferreira *et al.*, 2012; Menezes *et al.*, 2012).

In this context, the objective of this study was to cluster forage peanut genotypes based on their breeding values considering structuring agronomic and nutritive traits, previously selected, to identify parents for the hybridization program of this forage.

MATERIAL AND METHODS

The Embrapa Acre Active Germplasm Bank, located in Rio Branco, AC, Brazil (10°01'34"S, 67°42'13"W - Datum WGS 84, and 160 m altitude) was the source of 66 forage peanut genotypes evaluated (Table 1).

The region's climate is hot and humid equatorial, with average temperatures ranging from 21 °C to 31 °C, 80% relative humidity, and annual rainfall of 1900 mm. The rainy season spans from October to April, while the dry season lasts from June to September (Acre, 2010).

Three separated trials were conducted to evaluate the genotypes, beginning in December 2005 and concluding in April 2013. Based on soil analysis and pasture fertilization and liming recommendations to the Acre state, Brazil, fertilization was performed for the trials (Andrade *et al.*, 2014). Trial I was installed in Dystrophic Ultisol, while Trial II and III were in Dystrophic Oxisol (Embrapa, 2018).

Before planting in Trial I, which utilized conventional tillage, 500 kg ha^{-1} of dolomitic limestone was applied. Post-planting fertilization included 50 kg ha^{-1}triple superphosphate (P_2O_5), 30 kg ha^{-1} of potassium chloride (K_2O), and 40 kg ha^{-1} of micronutrients (FTE BR12). The trial was installed in December 2005, with the uniformization cut performed in October 2006. From December 2006 to November 2008, eight evaluations were conducted in 19 genotypes (seven during the rainy season and one during the dry season). Due to low leaf production in the dry season, no biomass cuts were performed.

Trial II was installed in December 2008, with post-planting application of 50 kg ha^{-1} of P_2O_5, 40 kg ha^{-1} of K_2O, and 40 kg ha^{-1} of FTE BR12. The uniformization cut was performed in April 2009, followed by side-dressing fertilization in February 2010 with 40 kg ha^{-1} of P_2O_5, 50 kg ha^{-1} of K_2O, and 40 kg ha^{-1} of FTE BR12, repeated one year later. From July 2009 to April 2011, 16 genotypes were evaluated across eight cuts (two in the dry season and six in the rainy season).

Trial III was installed in December 2010, with an application of 110 kg ha^{-1} of dolomitic limestone before planting. Post-planting fertilization in conventional tillage included 80 kg ha^{-1} of P_2O_5 and 40 kg ha^{-1} of K_2O, with side-dressing fertilization in March 2012 consisting of 15 kg ha^{-1} of P_2O_5, 15 kg ha^{-1} of K_2O, and 10 kg ha^{-1} of FTE BR12. The uniformization cut occurred in April 2011. From May 2011 to July 2013, 33 genotypes were evaluated in 12 evaluations and 11 cuts (eight evaluations with cuts in the rainy season and four evaluations with three cuts in the dry season). Systemic fungicides azoxystrobin and cyproconazole were applied at 0.3 mL L^{-1} in all plots in April and May 2012 for Rhizoctonia control.

After the establishment period, cuts were made: 10 months after planting for Trial I, and 4 months for Trials II and III. Bromatological analyses were conducted approximately 70 days after regrowth in both rainy and dry seasons.

The three trials were implanted using vegetative propagation, with two stolons planted per pit, spaced 0.5 m apart both between pits and between rows. To ensure uniformity, each stolon measured about 25 cm in length with five internodes, three of which were buried in soil. In Trial II, cv. BRS Mandobi was also implanted by seeds, maintaining the same spacing of 0.5 m between pits and rows, with two seeds per pit. The trials used the cultivars BRS Mandobi and Belmonte (now known as cv. Belomonte [Mapa, 2020]) as control, propagated vegetatively. The experimental design was a randomized complete block, with four replications for Trial I and III and five replications for Trial II. Each trial had a plot area of 1 m^2 for practical use.

The evaluations involved measuring agronomic traits, seed production, and conducting bromatological analysis of the harvested aerial biomass. Pests and diseases occurrences, plant vigor, and flowering were assessed visually using a grading scale based on the increasing intensity observed for each trait, following the methodology described by Menezes *et al.* (2012).

Soil cover (SC) was visually estimated as a percentage using a subdivided 1 m x 1 m square. The stand height was measured in centimeters, taking an average of three measurements per plot, as described by Menezes *et al.* (2012). Total dry matter yield (TDMY) and leaf dry matter yield (LDMY), were quantified after each evaluation by separating leaf blades and cutting the aerial biomass at 2 cm above the ground. The biomass was dried using forced air at 55 ºC for

Table 1: Forage peanut genotypes used in three trials at Rio Branco, AC, Brazil

Code	BRA	Specie	Code	BRA	Specie
	Trial I			**Trial III**	
1	014931	*A. pintoi*	16	032409	*A. pintoi*
2	033260	*A. repens*	35	030082	*A. repens*
3	039799	*A. pintoi*	36	035122	*A. pintoi*
4	035068	*A. p* x *A. r*[1]	37	032387	*A. repens*
5	035041	*A. p* x *A. p*[2]	38	032280	*A. repens*
6	035033	*A. p* x *A. p*	39	031909	*A. pintoi*
7	040894	*A. pintoi*	40	040223	*A. pintoi*
8	030333	*A. pintoi*	41	039195	*A. pintoi*
9	039187	*A. pintoi*	42	030635	*A. pintoi*
10	015083	*A. pintoi*	43	031275	*A. pintoi*
11	014991	*A. pintoi*	44	031461	*A. pintoi*
12	035114	*A. pintoi*	45	031526	*A. pintoi*
13	032352	*A. repens*	47	031984	*A. pintoi*
14	034436	*A. repens*	48	012114	*A. repens*
15	032379	*A. repens*	49	040193	*A. pintoi*
16	032409	*A. pintoi*	50	015121	*A. pintoi*
17	034142	*A. pintoi*	51	016683	*A. pintoi*
18	037036	*A. pintoi*	52	032280	*A. repens*
19	52*	*A. pintoi*	53	040088	*A. repens*
68	031828	*A. pintoi*	54	016357	*A. pintoi*
69	040550	*A. pintoi*	55	037443	*A. repens*
	Trial II		56	014788	*A. repens*
20	039985	*A. pintoi*	57	032361	*A. repens*
21	029220	*A. repens*	58	022683	*A. pintoi*
22	012122	*A. pintoi*	59	040185	*A. repens*
23	014982	*A. pintoi*	60	036544	*A. pintoi*
24	030325	*A. pintoi*	61	034363	*A. repens*
25	030601	*A. pintoi*	62	034355	*A. pintoi*
26	039772	*A. pintoi*	63	032433	*A. pintoi*
27	040045	*A. pintoi*	64	032492	*A. repens*
28	012106	*A. repens*	65	030872	*A. pintoi*
29	029190	*A. repens*	66	030899	*A. pintoi*
30	029203	*A. repens*	67	030929	*A. pintoi*
31	035076	*A. p* x *A. r*	68	031828	*A. pintoi*
32	038857	*A. p* x *A. r*	69	040550	*A. pintoi*
33	030384	*A. pintoi*	-	-	-
34	013251	*A. pintoi*	-	-	-
68	031828	*A. pintoi*	-	-	-
69	040550	*A. pintoi*	-	-	-
70	040550[3]	*A. pintoi*	-	-	-

*Local identification (without BRA). [1] Interespecific *A.pintoi* x *A. repens* hybrid; [2] Intraspecific *A. pintoi* x *A. pintoi* hybrid; [3]propagation by seeds.

72 hours and the yield was estimated in kg ha^{-1}. Nutritional traits, assessed after weighing the sampled total dry matter, included neutral detergent fiber (NDF) and acid detergent fiber (ADF), following the method by Goering & Van Soest (1970), and crude protein content (CP), determined by the modified Kjeldahl method (Silva & Queiroz, 2001), all expressed in kg ha^{-1} of dry matter. Seed production was evaluated at the end of each trial (November 2008, August 2011, and August 2013), through manual harvesting of the top 10 cm of soil, and was estimated in kg ha^{-1}.

Data for each trial were analyzed using the SAS program (SAS, 2010). The Restricted Maximum Likelihood (REML) method (Patterson & Thompson, 1971) was employed to estimate variance components, and the Best Linear Unbiased Prediction (BLUP) method (Henderson, 1975) was used to predict genotypic values. The models applied were based on those proposed by Resende (2002) for the analysis of unrelated perennial plants and one plant per plot.

For each trait within each trial, the repeatability model was applied: , where y is the data vector, u represents the vector of fixed-repetition combinations plus the general mean, g is the vector of random genotypic effects, p is the vector of random permanent environment effect (plots), m is the vector of the genotype x evaluations interaction effects, and e is the vector of random errors or residuals. The capital letters represent the incidence matrices for these effects. For traits with only one evaluation (such as nutritional traits in Trial I and seed production in each trial), the model used was: , where y is the data vector, r is the vector of fixed repetition effects plus the general mean, g is the vector of random genotypic effects, and e is the vector of random errors or residuals. The capital letters represent the incidence matrices for these effects.

Were tested and selected several residual structures for the repeatability model based on the likelihood ratio test (LRT) and the Akaike (AIC) and Bayesian Information (BIC) criteria. This was necessary because of the presence of the effect of serial correlation, inherent in repeated measurement data. The selection process was conducted for each matrix in the models where convergence was achieved (Littel *et al.*, 2000). Specifically, were selected the variance components matrix, unstructured matrix (first-order), and analytical factor matrix (first-order). The components of variance obtained by the REML method and the genotypic, permanent plot, and genotype x evaluations interaction variability were verified by the deviance analysis, also based on the LRT test, following the methodology described by Resende (2007). This identical procedure was employed in the selection of the residual structure matrices.

For the application of multivariate analyses, the genotypic values of the traits with variability obtained by the BLUP method were standardized as follows: $Z_i = \frac{g_i - \overline{g}}{s}$, where Z_i is the standardized genotypic, g_i is the genotypic value of the genotype i, and $\bar{g}$ is the genotypic value mean of each trial and s is the standard deviation of genotypic values in each trial.

Principal component analysis (PCA) was applied to verify the influence and the possible structuring of traits (variables) on genotype discrimination. The choice of the number of components followed the criteria recommended by Khattree & Naik (1999), which indicates fixing the percentage of variance to be explained and selecting the minimum component number that satisfies such value. The cumulative variance used was 80%, as suggested by Cruz *et al.* (2012).

Multicollinearity was diagnosed by PCA, variable by variable within each trial, according to Cruz *et al.* (2014), with only the variables with weak multicollinearity remaining. The multicollinearity problem (high correlation) must be diagnosed and solved, since according to Cruz *et al.* (2014), it can compromise the matrix estimators and, consequently, the interpretation of the results. Structural consistency and genotype distinction were made by hierarchical cluster analysis based on Euclidean distance from genotypic values. The genotypes were grouped by the Ward method, which considers the variation between the analyzed values (Ward, 1963). The criterion for the formation of the clusters was the visual one, considering the ACP's previous information.

Analytical procedures via mixed models (REML/BLUP) were performed using PROC MIXED from the SAS program (SAS, 2010) and multivariate analysis by the GENES program (Cruz, 2016).

RESULTS AND DISCUTION

Genotypes variability

The genotypic, genotype x evaluation interaction, and permanent plot variances varied among trials for all traits (Table 2).

The observed genotypic variation indicates that, besides the environmental influence present in each trial, the evaluated genotypes suggested a broad genetic basis, which has also been observed in studies showing high

Table 2: Genotypic (σ^2_g), genotype x evaluations interaction (σ^2_m), permanent plot (σ^2_p), and residual (σ^2_e) variances and means of evaluation trial of forage peanut access at Rio Branco, AC, Brazil

	Traits	σ^2_g	σ^2_m	σ^2_p	σ^2_e	Mean
Trial I	Pests	0.0036**	0.0035	0.0001	0.0792	2.72
	Disease	0.0161**	0.0353**	0.0001	0.2383	3.03
	Vigor	0.8542**	0.2748**	0.2035**	0.7979	6.56
	Flower	0.7573**	0.4944**	0.0207*	1.0543	2.49
	SC	341.3000**	236.3800**	64.9096**	147.1954	80.58
	Height	4.8674**	2.8336**	3.0912**	4.1055	6.63
	CP[1]	3.6576*	-	-	3.0184	206.25
	ADF[1]	1.4841	-	-	6.3771	337.03
	NDF[1]	2.3323	-	-	11.7290	427.43
	TDMY	566237**	457251**	280591**	599601	2326.05
	LDMY	189798**	79500**	74927**	280060	1363.21
	Seed[1]	4064**	-	-	3403	47.88
Trial II	Pests	0.1249**	0.2420**	0.0120	0.7477	2.09
	Disease	0.1672**	0.4129**	0.0192	0.6047	2.06
	Vigor	0.1417**	0.1491**	0.0278**	0.3537	7.03
	Flower	0.6760**	0.9469**	0.0493*	0.7417	1.78
	SC	20.9472**	25.1139**	3.0112**	68.2724	93.31
	Height	1.1009**	0.6386**	0.2678**	0.9193	5.50
	CP	1.0475**	0.4304**	0.2157	1.4015	212.26
	ADF	1.4190**	0.7446**	0.2581	3.4728	336.38
	NDF	0.7958	1.4552*	0.0001	10.2332	591.05
	TDMY	248972**	125387**	95603**	155229	2327.36
	LDMY	83224**	35011**	31363**	59330	1373.78
	Seed[1]	506993**	-	-	150244	676.36
Trial III	Pests	0.0607**	0.0651**	0.0018	0.4923	2.35
	Disease	0.1456**	0.2731**	0.1329**	0.9797	2.14
	Vigor	0.1696**	0.1246**	0.0302**	0.6288	7.36
	Flower	0.6782**	0.7393**	0.0267**	0.4268	1.17
	SC	8.2556**	5.6870**	2.6547**	69.7336	94.07
	Height	1.5757**	0.8845**	0.3860**	0.9952	5.29
	CP	0.2224**	0.4250**	0.1003	2.0319	233.12
	ADF	0.3348**	0.2571	0.1876	5.1841	302.61
	NDF	1.1415**	0.1628	0.1070	8.6268	535.42
	TDMY	98139**	42867**	33300**	51328	1695.02
	LDMY	23699**	17974**	12641**	38822	844.17
	Seed[1]	91637**	-	-	78263	258.46

[1]Only one evaluation. * and ** significant at 5% and 1%, respectively, by deviance analysis based on LRT test. (-) with no available data. Occurrence of pests and disease: visual scale of 0 to 10; Vigor: visual scale of 0 to 9; Flower: visual scale of 0 to 10; SC: soil cover %; Height: plant height, cm; CP: crude protein content in the aerial biomass in kg ha^{-1}; ADF and NDF: acid and neutral detergent fiber content, in kg ha^{-1}; TDMY: total dry matter yield per cut, in kg ha^{-1}; LDMY: leaf dry matter yield per cut, in kg ha^{-1}; Seed: seed production, in kg ha^{-1}.

phenotypic, genotypic, and molecular variability of forage peanut (Palmieri *et al.*, 2010; Carvalho & Quesenberry, 2012; Simeão *et al.*, 2017). On the other hand, bromatological traits have revealed less variability in forage peanut (Ferreira *et al.*, 2012; Menezes *et al.*, 2012), which was confirmed by the results (Table 2). Lascano (1994) points out forage peanuts as high-quality legumes and low nutritional variation, which confers better stability in forage production. According to Ferreira *et al.* (2012), this minor variation is predominantly because of local edaphoclimatic differences, which may affect the development and, consequently, its maturation, and variation in the chemical plant composition.

The genotype x evaluation interaction was equally significant, except for the occurrence of pests in Trial I and ADF and NDF in Trial III, which suggests the tendency of variation in genotype behavior between evaluations for most traits. However, its magnitude was lower than genotypic variances, especially for forage yield traits such as vigor, SC, height, and TDMY. These traits, despite being influenced by seasonality, that is, climatic variation throughout the year, presented greater genetic control than pest and disease occurrences and flowering, with a marked influence of the seasons (Carvalho *et al.*, 2009; Dávila *et al.*, 2011; Menezes *et al.*, 2012).

Permanent plot variances, which reflect plot microclimate throughout the evaluations (Braz *et al.*, 2013), despite the low magnitude, were also significant for most traits, especially for forage production. This permanent nature variance is confused with the genetic effects on genotype development and, through repeatability, expresses the maximum genotypic potential achieved at each experimental site (Resende, 2002). That way, this variance contributes to genotypic variation in the selection of highly adapted superior materials.

Genotypic means for pests and disease occurrences were low (Table 2). This corroborates the few reports of insect, mite, and microorganism damage to forage peanuts (Assis *et al.*, 2011; Menezes *et al.*, 2012). However, these occurrences must be permanently monitored. Recently the first report of a population outbreak of the *Tetranychus ogmophallos* mite in the Western Amazon, Brazil, was published by Santos (2016), which should be followed and considered in future actions of the breeding program. In these cases, the possibility of phytopathological studies with pathogen inoculation and introduction of pests of critical economic impact should be considered, since the current evaluations regard only their natural occurrence, to allow future control and minimization of production losses.

The mean values of forage yield (vigor, soil cover [SC], height, and total dry matter yield [TDMY]) were high, indicating high culture yield potential. As already observed in these study conditions (Valentim *et al.*, 2003; Assis *et al.*, 2008) and indicate the elevated forage potential of the culture. Despite the low variation, bromatological traits presented adequate content and were balanced among trials (Ferreira *et al.*, 2012; Paulino *et al.*, 2012).

Seed production was variable among trials, with a lower yield in Trial I (despite higher flower production) and a higher yield in Trial II. This reflects its marked variation among edaphoclimatic cultivation conditions (Carvalho *et al.*, 2009).

Structuring of agronomic and bromatological traits

Despite the wide variation among trials, the traits presented constant structuring in the genotypes discrimination by principal component analysis (PCA) (Table 3).

The same variables among the trials were diagnosed with severe multicollinearity and were removed from the analysis, namely pest and disease incidences, ADF, NDF, vigor, and leaf dry matter yield (LDMY). Vigor and LDMY were highly coincident with SC and TDMY, respectively. The other traits, pest and disease occurrences, ADF and NDF, presented low variability.

The first three principal components of the trials separately analyzed had values close to or above 80% of the original data cumulative variance (Table 3). Overall, the statistics related to the eigenvalues of Trial III were lower, possibly because of the larger number of genotypes analyzed.

The first two principal components (PC) of the three trials discriminated against genotypes mainly based on forage yield traits, which had the highest load in the weighting of components. In Trial I and II, the highest eigenvectors in the first PC were SC and TDMY and in the second PC, it was height. In Trial III, height was the highest eigenvector trait in the first PC, and SC and TDMY were higher in the second component. Flowering trait in Trial I and III and crude protein content (CP) in Trial II were the ones with the highest weight for genotype discrimination in the third PC.

The consistent structuring of agronomic and bromatological traits among trials suggests that, even with the inversion in the participation of the first and second components of each trial, the relationship of traits in genotype discrimina-

Table 3: Eigenvalues, eigenvectors, and accumulated variances of principal components (PC) of forage peanut trials

	PC	Eigenvalue	Accumulated Variance%	Flower	SC	Height	CP	TDMY	Seed
				Eigenvectors					
Trial I	1	2.951	49.178	0.331	-0.511	-0.223	-0.365	-0.534	0.401
	2	1.274	70.410	0.331	0.182	0.728	-0.373	0.248	0.355
	3	0.746	82.846	0.802	0.151	-0.052	0.567	-0.070	-0.076
	4	0.511	91.366	0.238	-0.202	0.187	-0.390	-0.101	-0.840
	5	0.443	98.746	-0.242	-0.524	0.592	0.503	-0.252	-0.015
	6	0.075	100.000	0.150	-0.606	-0.179	0.033	0.758	0.042
				Eigenvectors					
Trial II	1	2.543	42.389	0.457	-0.558	-0.166	-0.121	-0.558	0.356
	2	1.646	69.825	0.185	0.120	0.708	-0.225	0.242	0.583
	3	1.012	86.689	0.370	0.154	0.019	0.901	0.047	0.156
	4	0.489	94.837	0.729	0.331	-0.330	-0.338	0.330	-0.167
	5	0.240	98.838	0.222	-0.633	0.385	0.081	0.351	-0.522
	6	0.070	100.000	-0.200	-0.373	-0.462	0.044	0.630	0.457
				Eigenvectors					
Trial III	1	2.244	37.402	-0.360	0.359	-0.567	0.337	-0.302	-0.464
	2	1.533	62.952	0.138	0.539	0.262	0.270	0.692	-0.265
	3	0.825	76.696	0.883	-0.160	-0.252	0.217	-0.139	-0.254
	4	0.776	89.622	-0.127	-0.274	0.220	0.875	-0.070	0.300
	5	0.533	98.508	-0.206	-0.569	0.299	-0.025	0.100	-0.730
	6	0.090	100.000	0.112	0.396	0.640	-0.015	-0.629	-0.163

Flower: flowering; SC: soil cover %; Height: plant height; CP: crude protein content; TDMY: total dry matter yield per cut; Seed: seed production.

tion remained constant, with SC, TDMY, and height being the most influential. However, this structure may be different in other experimental conditions, since the agronomic traits are quantitative in nature and are highly influenced by the environment and the plant developmental stage.

The trait height is an example, listed as one of the most influential in the dry and rainy periods by Menezes *et al.* (2012) and, however, discarded by Valentim *et al.* (2003) as a variable and considered of minor interest in the forage peanut establishment phase. This is because, during that period, plants tend to invest more in horizontal expansion. Menezes *et al.* (2012) also observed the association of flowering and CP with height and SC in the discrimination of genotypes after establishment throughout the year and Valentim *et al.* (2003) point to CP as the main discriminating factor of genotypes in the establishment period.

The flowering and CP traits were the most weighted in the third PC for the genotype discrimination (Table 3). In general, the CP content tends to be more stable in the species (Lascano, 1994; Paulino *et al.*, 2012). In contrast, flowering varies significantly among genotypes, also depending on climatic conditions and management (Carvalho *et al.*, 2009; Dávila *et al.*, 2011). This suggests a potential for field access differentiation considering the flowering trait.

Therein context, the structuring of traits assists in the genotype selection process, as it can indirectly facilitate the increase of a target trait that is difficult to obtain or possesses low heritability, especially if the responses between the traits are highly correlated (Resende, 2002). This response is important in multi-trait selection because it determines the traits to be used (Basso *et al.*, 2009).

In addition to the possibility of indirect selection, the structuring of traits is also important in genotype discrimination, as it helps in reducing the number of traits used because of information redundancy and the use of the most influential and informative variables in each trial (Menezes *et al*., 2012).

Clustering and discrimination of genotypes

Considering the traits used in the PCA, the hierarchical clustering by the Ward method based on Euclidean distance indicated the formation of three genotype clusters in Trial I and II and five clusters in Trial III (Figure 1).

The clusters were divided by seed and forage production which is possibly the result of the physiological pattern observed in plants with high seed production. According to Martiniello (1998;1999), these plants tend to reallocate reserves to favor sexual propagation, without completely restoring photoassimilates for vegetative growth, which negatively affects dry matter production throughout their development.

In Trial I, the first cluster aggregated genotypes that increased the means of flowering and seed production about the mean of the trial, with lower values of SC, CP, and TDMY (Table 4). The intraspecific hybrids of *A. pintoi* BRA 035033 (6) and 035041 (5) (Table 1), are also included in this cluster. The formation of this cluster probably was favored by the inclusion of these hybrids, which were obtained by selected parents crossing based on flower morphological traits (Oliveira & Valls, 2002).

Cluster 3 gathered the genotypes with higher TDMY, SC, and height, with the Belmonte cultivar (68) and the genotypes BRA 039799 (3) and 039187 (9), both with high performance for forage production. This behavior is already observed in the Brazilian Cerrado region for most of the genotypes of this cluster (Simeão *et al.,* 2017). Such highly contrasting clusters can generate hybrids that favor seed production concomitantly with forage production, which would be of great commercial interest to the culture (Assis & Valentim, 2013).

The BRS Mandobi cultivar (69) was segregated from Belmonte cv and is allocated to cluster 2, with low seed production because of its inferior performance for this trait in Trial I. Besides, the means of this cluster were intermediate for all traits. On the other hand, the poor performance for cv. BRS Mandobi and the population evaluated as a whole may be a result of the time and harvest conditions, which occurred during the rainy season and shortly after the last trial biomass cut. Probably the harvest in November may have favored the recruitment of new individuals and reduced the number of seeds to be harvested. The seed bank may also have been affected by the longer period of establishment of Trial I than the other trials, so the seeds were harvested 35 months after planting. Harvesting is recommended between 18 and 21 months after planting, a period of higher pod production and fruit accumulation (Pizarro *et al.*, 1998; Valentim *et al.*, 2009). Cluster 2 also added an interspecific hybrid of *A. repens* and *A. pintoi* and *A. repens* genotypes (BRA 035068 [4] and three *A. repens*

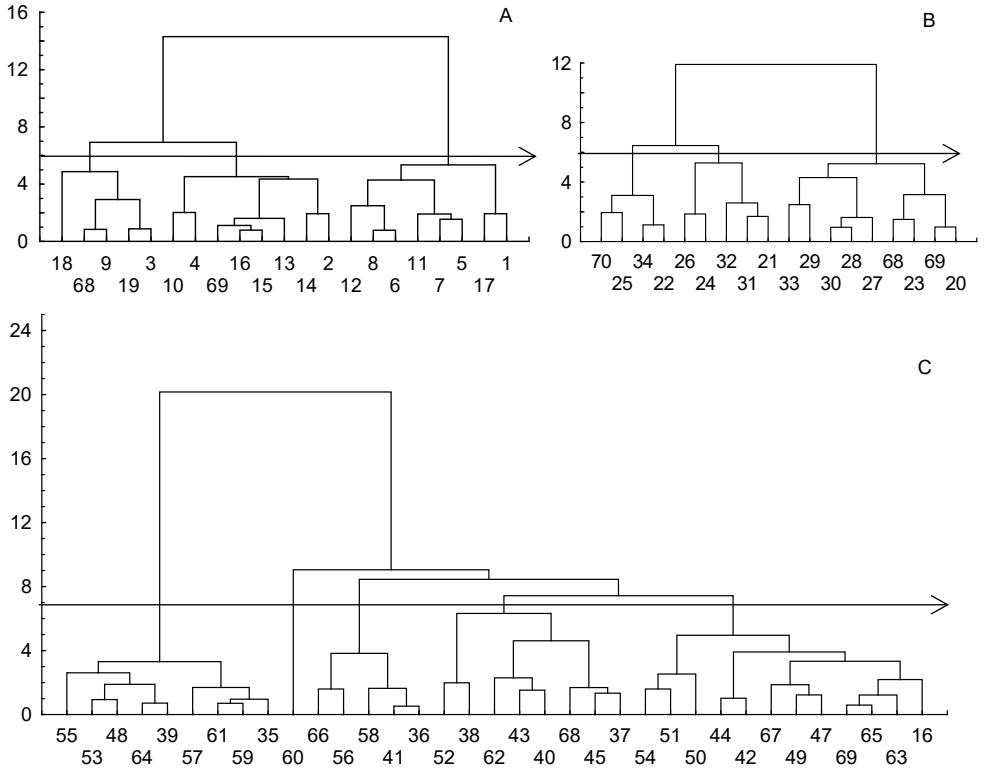


Figure 1: Hierarchical clustering of forage peanut genotypes by Ward method based on Euclidian distance of genotypic values, according to the flowering, soil cover, plant height, crude protein content, total dry matter yeld and seed production traits of the three trials. A): Trial I; B): Trial II; and C): Trial III. Identification of genotypes: see Table 1.

Table 4: Genotypic averages and standard deviations of clustered genotypes of forage peanut evaluated in three trials, according to hierarchical clustering

Cluster	Genotypes	Flower[1]	SC	Height	CP	TDMY	Seed
				Trial I			
1	1 5 6 7 8 11 12 17	3.18 ±0.58	65.13 ±16.16	6.13 ±0.68	197.02 ±1.17	1790.11 ±301.43	94.35 ±72.06
2	2 4 10 13 14 15 16 69	2.17 ±0.68	86.55 ±10.34	5.65 ±0.97	210.54 ±2.05	2361.04 ±371.54	13.83 ±8.15
3	3 9 18 19 68	1.88 ±0.58	95.73 ±2.86	9.02 ±2.71	214.15 ±1.60	3127.54 ±242.87	28.02 ±21.72
				Trial II			
1	20 23 27 28 29 30 33 68 69	1.40 ±0.80	96.44 ±0.73	5.45 ±0.96	215.36 ±0.69	2665.19 ±330.90	246.94 ±340.07
2	21 31 32 24 26	2.09 ±0.59	88.08 ±3.00	4.79 ±0.64	208.07 ±1.35	1857.34 ±284.40	678.68 ±424.85
3	22 25 34 70	2.25 ±0.32	92.79 ±2.21	6.50 ±0.54	210.53 ±0.60	2154.77 ±266.05	1639.65 ±610.50
				Trial III			
1	35 39 48 53 55 57 59 61 64	0.39 ±0.34	95.24 ±0.77	3.84 ±0.65	235.36 ±0.27	1396.11 ±129.16	84.60 ±77.28
2	60	0.70	82.30	7.43	230.07	1344.67	971.28
3	36 41 56 58 66	2.48 ±0.32	92.29 ±1.87	5.35 ±0.42	233.20 ±0.22	1743.24 ±273.33	72.87 ±32.25
4	37 38 40 43 45 52 62 68	0.75 ±0.47	94.94 ±1.48	6.05 ±1.24	232.57 ±0.44	1961.41 ±268.90	122.40 ±118.17
5	16 42 44 47 49 50 51 54 63 65 67 69	1.51 ±0.25	94.34 ±1.35	5.67 ±0.52	232.03 ±0.36	1750.70 ±192.05	497.48 ±239.66

[1]Flower: flowering in visual scale of 0 to 10; SC: soil cover %; Height: plant height, in cm; CP: crude protein content of aerial biomass, in kg ha^{-1}; TDMY: total dry matter yield, in kg ha^{-1}; Seed: seed production, in kg ha^{-1}. Identification of genotypes: see Table 1.

genotypes), generally considered to have lower forage production and SC (Assis *et al.*, 2008; Simeão *et al.*, 2017), reducing the means for these traits.

In Trial III, the largest number of genotypes generated the formation of more clusters, aggregating most *A. repens* genotypes, discriminating clusters with high forage production and clusters with high seed production, and segregating the contrasting behavior. In Trial II, the highest TDMY and SC mean were from cluster 1, composed of the cultivars vegetatively propagated BRS Mandobi and Belmonte, reaching 2660 kg ha^{-1} of TDMY and 96% of CS. Cluster 3 presented high flowering and seed production, with a mean above 1600 kg ha^{-1}, and was composed of cultivars BRS Mandobi propagated by seeds (70) and Amarillo (34). Cluster 2, composed of the interspecific hybrids BRA 035076 (31) and 038857 (32) and one genotype of *A. repens*, presented the lowest mean of TDMY and SC, but high seed production, above 670 kg ha^{-1}.

Cluster 1 was divergent from Cluster 3 about flowering and seed production, composed of cultivars highly productive for this trait (Jones *et al.*, 1993; Assis *et al.*, 2013). Because of the high seed production of some cultivars, vegetatively propagated cultivars were kept in the same cluster. However, the Belmonte and BRS Mandobi cultivars have had different productive performances, since Belmonte cv. hardly produces seeds (Valentim & Andrade, 2003). Besides, this cultivar has a higher TDMY than BRS Mandobi cv., reaching 11300 kg ha^{-1} in the Cerrado region, while BRS Mandobi reaches 6800 kg ha^{-1} (Fernandes *et al.*, 2017). Another important factor to be considered in genotype analysis is the propagation form, which tends to influence seed production, TDMY, and SC, as highlighted by Ferguson (1994) and Valentim *et al.* (2009). Sexual propagation for BRS Mandobi cv. increases seed production, but tends to reduce TDMY (Balzon *et al.*, 2005), which may have caused dissimilarity within the cultivar, propagated by both seeds and vegetatively in this trial. In fact, there is variability for some traits within the cultivar, which may have contributed to this distinction (Assis *et al.*, 2018).

In Trial III, Cluster 1, which aggregated most *A. repens* genotypes and only one *A. pintoi* genotype, had high SC and lower means for TDMY, height, and low seed production. Seed production was also low in cluster 3, despite the high flowering. Cluster 2 was formed only by *A. pintoi* genotype BRA 036544 (60), with high seed production and height. Cluster 4 presented the highest TDMY mean, aggregating the Belmonte cv. Cluster 5, with BRS Mandobi cv., presented an intermediate TDMY mean and the second-largest seed production. Despite the higher seed production, the inclusion of this discrepant genotype in the selection of parents should be cautious. The high seed deposition of the BRS 036544 (60) genotype has been possibly a survival strategy, since its low performance in the field, with smaller TDMY, SC, and low flowering also led to high discrimination against other genotypes, and is not, therefore, suitable for cross-breeding.

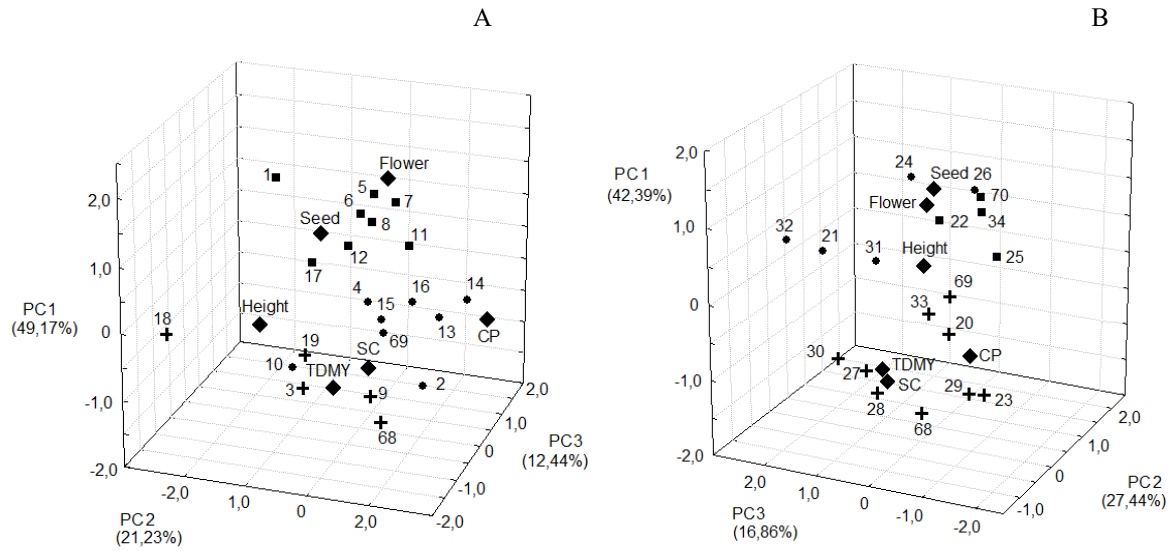
Considering the classification by hierarchical clustering of genotypes (Table 4), there was high discrimination in Trial I (Figure 2A) of BRA 014931 (1), 035041 (5), 035033 (6) and 030333 (8) genotypes, all with high flower and seed production compared to BRA 039799 (3), 039187 (9) genotypes and Belmonte (68) cv., with elevated forage production.

Similarly, in Trial II (Figure 2B), BRS Mandobi propagated by seeds (70) and Amarillo (34) cultivars and BRA 030601 (25) genotype, good forage producers with high seed production, were highly divergent from BRA 014982 (23), 029190 (29), 030384 (33) genotypes and Belmonte (68) cv., high TDMY and SC.

The genotypes that integrated cluster 5 from the cluster analysis, BRA 015121 (50), 016357 (54), 016683 (51) and 030929 (67), 030635 (42), 031461 (44), 032433 (63) and BRS Mandobi cv., all with high seed production, high SC and intermediate levels of TDMY, were contrasting to Belmonte (68) cv and BRA 040223 (40), 031275 (43), 031526 (45) and 032387 (37) genotypes, allocated to cluster 4 and presenting high TDMY and SC and lower seed production (Figure 3).

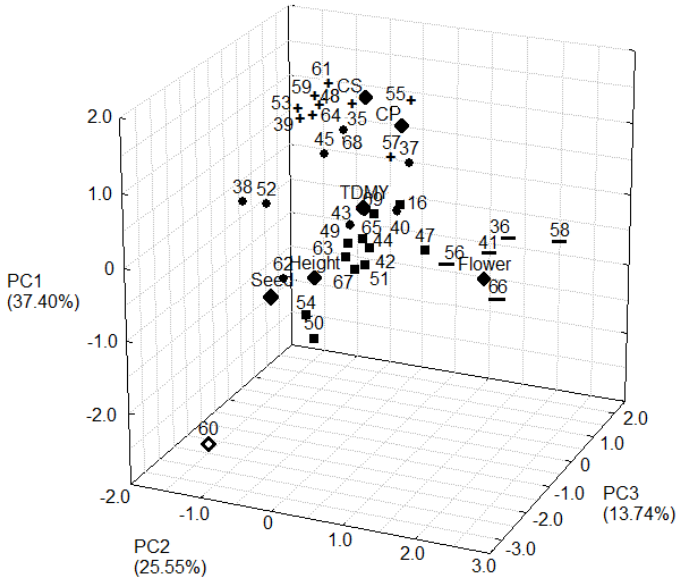
The high flower production associated with intermediate TDMY of the cluster 3 genotypes, BRA 035122 (36), 039195 (41), and 022683 (58), also suggests divergence from the cited genotypes of cluster 5. This could lead to combinations of interest to obtain highly productive forage and seed-propagated genotypes.

In all three trials, Belmonte cv. tended to form clusters with high TDMY genotypes and BRS Mandobi cv., with intermediate TDMY genotypes associated with high seed production. However, the forage peanut production traits both forage and seed are influenced by environmental management and edaphoclimatic conditions. Successive cuts tend to modify the structure of the canopy surface



PC: principal component; Flower: flowering; CP: crude protein content; TDMY: total dry matter yield; SC: soil cover; Height: plant height; Seed: seed production. Genotypes classification based on clusters formed by clustering analysis (Table 4): Trial I (A): (♦) traits; (■) cluster 1; (●) cluster 2; (+) cluster 3.Trial II (B): (♦) traits; (+) cluster 1; (●) cluster 2; (■) cluster 3. Identification of genotypes: see Table 1.

Figure 2: Tridimensional dispersion of forage peanut genotypes in the Trial I (A), performed between 2006 and 2008, and Trial II (B), performed between 2009 and 2011, according its scores in the first three principal components.



PC: principal component; Flower: flowering; CP: crude protein content; TDMY: total dry matter yield; SC: soil cover; Height: plant height; Seed: seed production. Genotypes classification based on clusters formed by clustering analysis (Table 4): (♦) traits; (+) cluster 1; (◊) cluster 2; (−) cluster 3; (●) cluster 4; (■) cluster 5. Identification of genotypes: see Table 1.

Figure 3: Tridimensional dispersion of forage peanut genotypes in the Trial III, performed between 2011 and 2013, according its scores in the first three principal components.

cover and increase the number of leaves produced because of regrowth induction (Dávila *et al.*, 2011; Ferreira *et al.*, 2013) and seed production may also be favored by cutting height and soil type (Carvalho *et al.*, 2009; Dávila *et al.*, 2011). Besides, the form of planting also influences forage and seed productions, which can cluster genotypes with variable performance (Balzon *et al.*, 2005; Valentim *et al.*, 2009).

According to Cruz *et al.* (2012), the genotype divergence analysis should be performed based on the potential of the genotypes themselves and the magnitude of their dissimilarities as a function of the graphic distances presented among them. Consequently, with hierarchical clustering information, genetic divergence among specific genotypes can be facilitated by the graphic dispersion of PCA.

Thus, the classification by hierarchical grouping (Figures 2 and 3) indicated combinations that can generate highly productive hybrids, both for seeds and forage, further increasing genetic variability and exploiting heterosis in the F1 generation, as highlighted by Assis & Valentim (2009). Among genotypes with low seed yield, hybrid vigor can be easily exploited by stolon propagation (Assis *et al.*, 2008).

The structuring of the variables, previously selected based on their multicollinearity, proved to be useful in genotype discrimination. In this case, the indication of genetic materials of interest is now satisfied by multicriteria of greater influence and weight in the selection. Indirect selection can equally benefit from such structuring, especially if the traits involved are highly correlated.

Overall, both methods presented agreement on genotype discrimination, especially for those with more divergent behavior, with analysis by graphic dispersion of PCA facilitating individual selection associated with predicted genotypic values. The general trend in both methods was the segregation of genotypes based on forage and seed production, as observed by the constant discrimination of Belmonte and BRS Mandobi cultivars. Also, the association of satisfactory TDMY and high seed production was observed in the clusters where BRS Mandobi cv. was allocated. In this sense, studies indicate variability in the relationship between forage and seed production and highlight the marked environmental influence on these traits, which reinforces the complex association between forage and seed production and the need for further investigations (Valentim & Andrade, 2003; Carvalho & Quesenberry, 2012).

Considering the possibility of parental selection for cross-breeding as a source of variability, the genotypes cited as the most divergent of each trial, according to the traits analyzed, may be indicated for the next step of the forage peanut breeding program, involving hybridization

to obtain genotypes with high forage yield and seed propagation primarily (Assis & Valentim, 2013).

However, from the favorable combinations obtained in the F1 generation, the superior genotypes, but with low seed production, can be considered for a future release of vegetatively propagated cultivars, reinforcing the importance of the traits related to forage and seed production as the most influential in discriminating genotypes.

CONCLUSIONS

The forage peanut genotypes analyzed are divergent for most of the variables analyzed and were discriminated mainly by traits aimed at forage production, such as dry matter production, soil cover, height, and seed production.

The highly contrasting genotypes indicated as possible parents are: Trial I - BRA 014931 (1), 035041 (5), 035033 (6) and 030333 (8) (high flower and seed production) divergent from BRA 039799 (3), 039187 (9), and Belmonte (68) cv. (elevated forage production); Trial II - BRS Mandobi by seeds (70) and Amarillo (34) cv. and BRA 030601 (25) (good forage and high seed producers) divergent from BRA 014982 (23), 029190 (29), 030384 (33), and Belmonte (68) cv. (high TDMY and SC); Trial III - BRA 015121 (50), 016357 (54), 016683 (51), 030929 (67), 030635 (42), 031461 (44), 032433 (63), and BRS Mandobi cv. (high seed production, high SC and intermediate TDMY), contrasting to Belmonte (68) cv. and BRA 040223 (40), 031275 (43), 031526 (45), and 032387 (37) (high TDMY and SC and lower seed production), both groups contrasting to BRA 035122 (36), 039195 (41), and 022683 (58) (high flower production and intermediate TDMY).

The selection of genotypes through multivariate analyses based on genotypic values allows the efficient identification and selection of the most divergent genotypes in forage peanuts which are capable of selecting highly contrasting genotypes for crossing, aimed mainly at high forage production with seed propagation.

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