Animal Science/ Original Article

Repeatability and divergence among genotypes of *Desmanthus* sp. in a semiarid region

Abstract – The objective of this work was to evaluate the divergence among genotypes of *Desmanthus* sp. regarding morphological, productive, and qualitative characteristics, as well as to estimate the number of observations necessary to predict the real value of these characteristics. The experiment was conducted in the semiarid of the state of Pernambuco, Brazil, using 26 genotypes of *Desmanthus* sp. from different locations in the region. Seven evaluations were carried out from July 2013 to July 2014. Tocher's grouping method and standardized average Euclidean distance were used. The coefficients of repeatability (r) and determination (R^2) were estimated using two models of the analysis of variance, principal components based on the correlation or covariance matrix, and structural analysis based on the correlation matrix. The variables with the greatest weights were stem diameter, leaf area index, and forage yield, with frequencies of 22.05, 17.57, and 14.58%, respectively. Morphological, productive, and qualitative variability was observed among the genotypes of *Desmanthus* sp. All characteristics presented r and R^2 of high magnitude in the methods of analysis. Up to four evaluation cycles are required to predict (R^2=95%) the real value of stem diameter, peduncle length, plant height, leaf length and width, number of leaves, and pod length.

Index terms: forage, native legume, plant breeding.

Repetibilidade e divergência entre genótipos de *Desmanthus* sp. em região semiárida

Resumo – O objetivo deste trabalho foi avaliar a divergência entre genótipos de *Desmanthus* sp. quanto a características morfológicas, produtiva e qualitativa, bem como estimar o número de observações necessárias para predizer o valor real destas características. O experimento foi conduzido no semiárido do estado de Pernambuco, Brasil, com uso de 26 genótipos de *Desmanthus* sp. de diferentes localidades nessa região. Foram realizadas sete avaliações de julho de 2013 a julho de 2014. Utilizou-se o método de agrupamento de Tocher e a distância euclidiana média padronizada. Os coeficientes de repetibilidade (r) e determinação (R^2) foram estimados por dois modelos de análise de variância, componentes principais baseados na matriz de correlação ou covariância, e análise estrutural baseada na matriz de correlação. As variáveis com maiores pesos foram diâmetro do caule (DC), índice de área foliar e produção de forragem, com frequências de 22,05, 17,57 e 14,58%, respectivamente. Observou-se variabilidade morfológica, produtiva e qualitativa entre os genótipos de *Desmanthus* sp. Todas as características apresentaram r e R^2 de alta magnitude nos métodos de análise. Até quatro ciclos de avaliação são necessários para prever (R^2=95%) o valor real de diâmetro do caule, comprimento do pedúnculo, altura da planta, comprimento e largura da folha, número de folhas e comprimento da vagem.

Termos para indexação: forrageira, leguminosa nativa, melhoramento de plantas.

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Introduction

The *Desmanthus* genus is composed of 24 legume species naturally distributed in tropical and subtropical regions of the Americas (Muir et al., 2014). However, the genus also occurs naturally in other edaphoclimatic conditions around the world (Sonawane et al., 2019; Patil et al., 2020), such as in the Brazilian semiarid (Costa et al., 2017; Muir et al., 2019). Legumes of the genus have shown potential to optimize animal production (Santos et al., 2019) and stand out as some of the few to adapt and persist under heavy grazing on clay soils in seasonally dry regions (Gardiner et al., 2013).

It should be noted that genotypes of *Desmanthus* may show variability regarding morphological, productive, and qualitative characteristics, as is the case of those collected in the Brazilian semiarid region (Calado et al., 2016; Diniz, 2016). Costa et al. (2017) found that the number of seeds was the morphological descriptor that contributed the most to characterize the genetic divergence among 26 *Desmanthus* genotypes.

Therefore, it is essential to expand knowledge on the variability of naturally occurring genotypes as the basis for future forage breeding programs, especially with native plants that still require additional information to improve the efficiency of their use. This is important since the genetic breeding of tropical forages is still a very recent activity compared with that of temperate forages (Jank et al., 2014). In addition, the gradual degradation process of Brazilian native biomes highlights the need to obtain more information on available plant genetic resources, an aspect of great interest for germplasm collection and assessment activities.

One of the complexities of forage breeding is related to the determination of the number of evaluations necessary to estimate, with accuracy, differences among the studied genotypes (Toebe et al., 2020). Using repeatability analysis, it is possible to determine the number of assessments required to predict the genotypic value of an individual along sequenced measurements throughout time (Chaves et al., 2018), obtaining predefined coefficients of determination (Cruz et al., 2014). These coefficients allow the reduction of the trial period, costs, and labor, optimizing the process for launching new cultivars (Torres et al., 2015; Rodrigues et al., 2020).

The objective of this work was to evaluate the divergence among genotypes of *Desmanthus* sp. regarding morphological, productive, and qualitative characteristics, as well as to estimate the number of observations necessary to predict the real value of these characteristics.

Materials and Methods

The research was carried out in Serra Talhada, a semiarid locality in the state of Pernambuco, Northeastern Brazil (07º59'31"S, 38º17'54"W). The average rainfall from planting in November 2011 until the last cut in July 2014 was 34.36 mm per month (Figure 1). The soil of the experimental area was classified as a Latossolo Vermelho-Amarelo (IUSS Working Group WRB, 2015), with the following physical-chemical characteristics in the 0 to 20 cm layer: 102.6 g kg^{-1} clay, 54.8 g kg^{-1} silt, 842.6 g kg^{-1} sand, pH 7.9 (water – 1.0:2.5), 348 mg dm^{-3} P, 0.39 cmol$_c$ dm^{-3} K, 0.48 cmol$_c$ dm^{-3} Na, 3.2 cmol$_c$ dm^{-3} Ca, 0.1 cmol$_c$ dm^{-3} Mg, 0.0 cmol$_c$ dm^{-3} Al, 2.01 cmol$_c$ dm^{-3} H + Al, 19 g kg^{-1} organic carbon, and 33 g kg^{-1} organic matter.

The evaluated genotypes of *Desmanthus* sp. and their respective collection sites in the state of Pernambuco were: 25A in Serra Talhada; 92C, 100C, and 235C in the municipality of Sertânia; 65F, 89F, 94F, and 100F in the municipality of Bom Jardim; 15L and 45L in the municipality of Jataúba; and 11SA, 12SA, 13SA, 15SA, 16SA, 17SA, 19SA, 20SA, 21SA, 22SA, 23SA, 24SA, 27SA, 28SA, 29SA, and 39SA in the semiarid region. The active germplasm bank was formed in Serra Talhada by plants collected from April to August 2011 in different municipalities in the semiarid region of the state (Queiroz, 2012), and was maintained with sprinkler irrigation in the dry period, under 1.0 m spacing between rows and 0.5 m within rows, with the application of 0.5 kg organic fertilizer at the base of each pit at the time of planting.

In May 2013, the plants were staged at 40 cm in relation to the ground. From July 2013 to July 2014, evaluations were carried out at an interval of 60 days (seven evaluations), by measuring the following variables: plant height, stem diameter, leaf length and width, number of leaves, and peduncle (PEDL) and pod (PODL) lengths. Plant height, leaf length and width, PEDL, and PODL were evaluated with a measuring tape, whereas stem diameter was obtained with a caliper.

In July 2014, the leaf area index (LAI) was measured using the LAI-2000 Plant Canopy Analyzer (LI-COR Inc., Lincoln, NE, USA). Forage yield (FY) was obtained by cutting the plants at 40 cm in relation to the ground. From the collected plant material, leaves and stems ($\varnothing \leq 5$ mm) were weighed and taken to a forced-air circulation oven, at 55 ± 5ºC, until they reached a constant weight. The material was then weighed to determine dry weight and estimate the dry matter and crude protein (CP) content of the forage according to the procedure described by Association of Official Agricultural Chemists International (Latimer Jr., 2016).

The data were subjected to the principal component analysis (Cruz, 2013). The standardized average Euclidean distance was calculated as a measure of dissimilarity, and genotype groups were formed using Tocher's grouping method. Repeatability coefficients were estimated based on five different methods described by Cruz et al. (2014): two models of the analysis of variance (ANOVA 1 and 2), principal component analysis (PCA) based on the correlation (PCACOR) or covariance (PCACOV) matrix, and structural analysis based on the correlation matrix (SACOR).

Using the ANOVA method, the repeatability coefficient was estimated with two models. The first was: $Y_{ij} = \mu + g_i + \varepsilon_{ij}$, where Y_{ij} is the observation regarding the i-th environment (evaluation), μ is the overall mean, g_i is the random effect of the i-th genotype under the influence of the permanent environment (i = 1, 2, ..., p), and ε_{ij} is the effect of the temporary environment associated with the j-th measurement on the i-th genotype (j = 1, 2, ..., η_i). The second was: $Y_{ij} = \mu + g_i + a_j + \varepsilon_{ij}$, where Y_{ij} is the mean of the i-th genotype in the j-th cycle, μ is the overall mean of the experiment, g_i is the effect of the i-th genotype under the influence of the permanent environment, a_j is the a-th cycle effect, and ε_{ij} is the random error involving other causes of variation not included in the model. In these two models, the repeatability coefficient (r) for each characteristic was obtained by:

$$ r = \frac{\widehat{Cov}\left(Y_{ij}, Y_{ij'}\right)}{\sqrt{\widehat{V}\left(Y_{ij}\right)\widehat{V}\left(Y_{ij'}\right)}} = \frac{\widehat{\sigma}_g^2}{\widehat{\sigma}_Y^2} = \frac{\widehat{\sigma}_g^2}{\widehat{\sigma}^2 + \widehat{\sigma}_g^2} $$

In the PCACOR method, the repeatability estimate was calculated using the R correlation matrix. The repeatability coefficient (r) was determined by: $r = \left(\widehat{\lambda}_1 - \widehat{\sigma}_Y^2\right)/\left[\widehat{\sigma}_Y^2(\eta-1)\right]$, where $\widehat{\lambda}_1$ is the largest eigenvalue obtained from 'Γ', associated with the
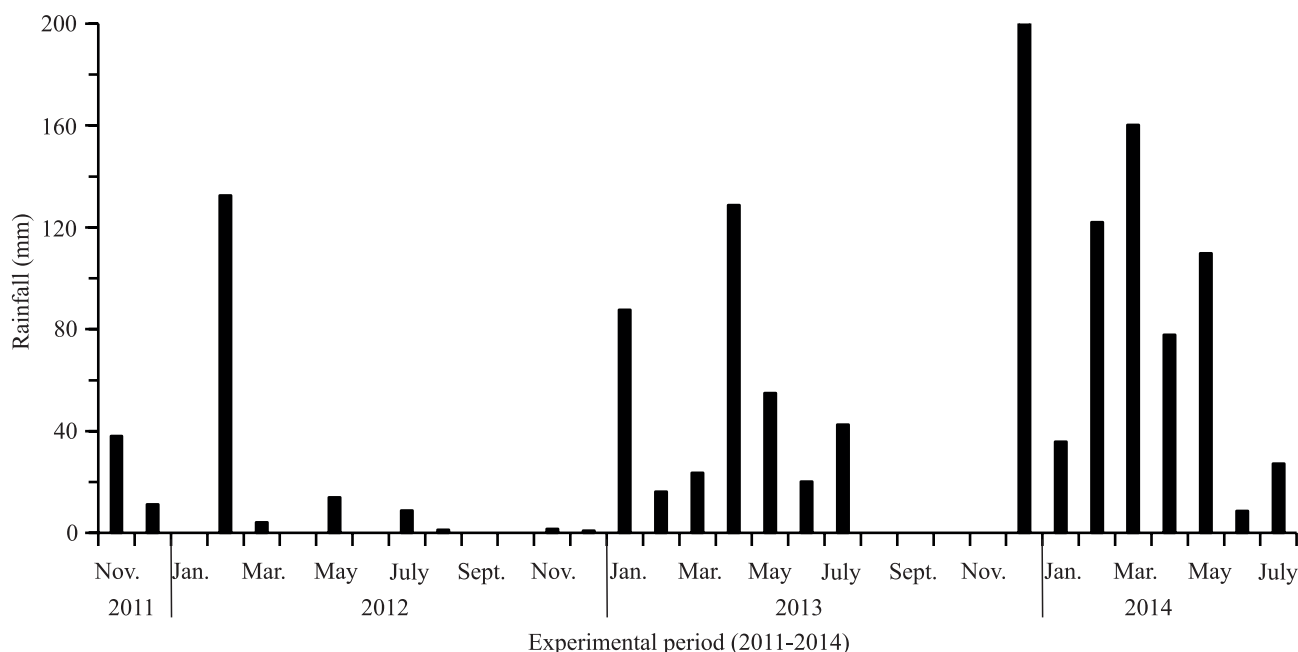

Figure 1. Rainfall during the experimental period in the region of Serra Talhada, in the state of Pernambuco, Brazil. Source: APAC (2019).

eigenvector whose elements have the same sign and similar magnitudes;

$$\hat{\sigma}_Y^2 = \frac{1}{\eta} \sum_j \hat{\sigma}_j^2 = \hat{\sigma}^2 + \hat{\sigma}_g^2 \; ;$$

and η is the number of evaluated cycles. In the SACOR method, the repeatability estimate was calculated using the R correlation matrix, as already shown, for each pair of measurements carried out in the different genotypes. The repeatability coefficient (r) was given by: $r = (\alpha'\hat{R}\alpha - 1)/(\eta - 1)$ where $\hat{R}$ is the estimator of R and $\alpha' = \left[1/\sqrt{\eta} \ldots 1/\sqrt{\eta}\right]$ is the auto vector with parametric elements associated with the largest eigenvalue of R.

The estimate of the minimum number of measurements required to obtain the real value (η_0) of the evaluated characteristics, based on pre-established coefficients of determination (0.80, 0.85, 0.90, and 0.95), was obtained through the expression: $\eta_0 = \left[R^2(1-r)\right]/\left[(1-R^2)r\right]$, where R^2 is the coefficient of determination. Based on the mean of η cycles ($\eta = 7$) and on the estimates of the repeatability coefficient according to the different methodologies used, the coefficient of determination (R^2) was calculated by the expression: $R^2 = \eta r/\left[1 + r(\eta - 1)\right]$, where η is the number of measurements required and r is the repeatability coefficient. Principal component and repeatability analyses were performed using the GENES software (Cruz, 2013).

Results and Discussion

The accumulated variance in the principal components totaled 88.82% for the evaluated plants of the genus *Desmanthus* (Table 1). According to Cruz et al. (2014), the number of principal components to be

considered should explain at least 80% of the observed variation. This means that the first six principal components satisfactorily explained the variation among genotypes. The first two principal components were used, as they explained most (39.62%) of the variation found in the original data.

When evaluated using the principal component analysis, the following variables carried the greatest weights: stem diameter, LAI, and forage yield, with frequencies of 22.05, 17.57, and 14.58%, respectively. Stem diameter becomes important because it affects the transport of water and nutrients in the plant, whereas the LAI is a variable directly related to canopy photosynthesis and, consequently, to forage biomass (Edvan et al., 2016), which can affect animal performance.

Queiroz (2012) studied the environmental characteristics of different genotypes of *Desmanthus* sp. in the semiarid region of Pernambuco, but only observed the formation of three groups when using Tocher's grouping method. In the present study, nine distinct groups were formed with the 26 assessed genotypes of *Desmanthus* sp.: group 1, 11SA, 12SA, 15SA, 16SA, 22SA, 27SA, 39SA, 235C, and 100F; group 2, 17SA, 19SA, 20SA, 21SA, 28SA, 65F, and 25A; group 3, 13SA and 92C; group 4, 29SA and 94F; group 5, 23SA and 15L; group 6, 24SA; group 7, 89F; group 8, 45L; and group 9, 100C. It is worth mentioning that the first two principal components explained only 39.62% of the data variation, a value lower than the 80% recommended by Cruz et al. (2014), which may influence the association of variables with groups.

The different groups formed characterize the genetic divergence among the genotypes of *Desmanthus* sp. However, in the present study, the majority of the genotypes (62%) were found in the first two groups, implying that the genetic divergence among the genotypes was not high. Despite this, groups formed by isolated individuals (such as groups 6, 7, 8, and 9) presented the greatest divergence within the evaluated set; different genotypes for future recombination schemes can, therefore, be identified, facilitating the projection of breeding programs (Resende et al., 2014).

Group one, made up of the largest number of individuals, had an average forage yield of 81.25 g per plant, while group seven, with only the 89F genotype, even with a lower plant height, had a forage yield of 222.70 g per plant (Table 2). However, group six

Table 1. Individual (Vi) and accumulated (Va) variances of the principal components on the ten evaluated descriptors in 26 genotypes of *Desmanthus* sp.

Principal components	Vi (%)	Va (%)
C1	22.05	22.05
C2	17.57	39.62
C3	14.58	54.20
C4	11.56	65.76
C5	9.79	75.55
C6	7.72	83.27
C7	5.55	88.82

(genotype 24SA) presented a LAI of 0.3 and a forage yield of 14.4 g per plant, lower than those of the other groups. In contrast, group nine (genotype 100C) had the greatest stem diameter of 3.0 cm. The group that had the greatest forage yield (group 7) had the second highest crude protein (CP) content of 201 g kg⁻¹, only below that of 212 g kg⁻¹ of the genotypes in group 5. The 201 g kg⁻¹ value represents an increase of 17, 26, 41, 101, 41, 18, and 15% in CP over groups 1, 2, 3, 4, 6, 8, and 9, respectively. These results highlight that the genetic divergence found among genotypes allows forage breeders to produce several alternative crossbreeds, while seeking to complement characteristics of interest in offspring, such as forage yield and CP content. The CP values reported here ranged from 100 to 212 g kg⁻¹ dry matter (Table 2), being similar to that of 195 g kg⁻¹ obtained by Patil et al. (2020). The higher content of CP observed in the present trial shows the great nutritional value of the plant and its potential for use in animal feed in tropical regions. It is worth mentioning that this legume persists and is selected by browsing animals, despite large forage mass fluctuations between rainy and dry seasons and browser preference (Santos et al., 2019).

According to Resende (2015), in the selection of a group of individuals, R^2 values are considered adequate when they are above 80%, whereas repeatability values are classified as low when below 0.30, medium when between 0.30 and 0.60, and elevated when above 0.60. In general, it was found that, in the different methods used, all evaluated characteristics showed a high magnitude of repeatability and R^2 (Table 3), which indicates that the mathematical models adopted were satisfactorily fitted to the data set. Furthermore, the high magnitude of these coefficients shows reliability in estimating the number of observations required to

Table 2. Means for stem diameter (SD), leaf area index (LAI), forage yield (FY), peduncle length (PEDL), crude protein (CP) content, plant height, leaf length (LL), leaf width (LW), number of leaves (NL), and pod length (PODL) of genotypes of *Desmanthus* sp. for the groups obtained by Tocher's grouping method.

Characteristic	Group								
	1	2	3	4	5	6	7	8	9
Stem diameter (SD, cm)	1.1	2.1	0.7	1.9	1.4	1.5	1.9	1.2	3.0
LAI	1.2	0.9	1.6	3.2	1.4	0.3	1.0	0.3	1.4
Forage yield (g per plant)	81.2	98.2	160.3	214.7	115.2	14.4	222.7	31.1	172.3
PEDL (cm)	2.4	1.4	2.2	1.6	2.6	1.2	1.1	2.5	1.9
Crude protein (CP, g kg⁻¹)	171	160	143	100	212	143	201	170	174
Plant height (cm)	53.5	53.7	46.0	55.5	60.5	64.0	40.1	46.0	65.4
Leaf length (LL, cm)	5.0	5.6	5.5	5.0	5.5	4.5	7.1	9.2	9.1
Leaf width (LW, cm)	5.9	4.6	3.6	6.4	5.0	3.8	4.4	6.3	5.5
Number of leaves (NL)	9.0	5.8	6.5	6.0	10.1	13.0	7.0	11	6.0
Pod length (PODL, cm)	7.6	4.4	7.5	7.0	4.5	6.0	6.0	6.3	6.5

Table 3. Estimates of the coefficients of repeatability (r) and determination (R^2, %) for stem diameter (SD), peduncle length (PEDL), plant height, leaf length (LL), leaf width (LW), number of leaves (NL), and pod length (PODL) of 26 genotypes of *Desmanthus* sp. using five different methods.

Method[1]	SD		PEDL		Height		LL		LW		NL		PODL	
	r	R^2	R	R^2	r	R^2	r	R^2	r	R^2	r	R^2	r	R^2
ANOVA 1	0.91	98.71	0.97	99.56	0.89	98.26	0.97	99.58	0.99	99.94	0.89	98.00	0.87	98.08
ANOVA 2	0.91	98.73	0.97	99.56	0.85	97.62	0.97	99.59	0.99	99.94	0.89	98.33	0.88	98.10
PCACOR	0.92	98.87	0.97	99.62	0.89	98.37	0.97	99.60	0.99	99.94	0.89	98.38	0.88	98.11
PCACOV	0.92	98.88	0.97	99.60	0.91	98.63	0.97	99.61	0.99	99.94	0.89	98.31	0.88	98.12
SACOR	0.91	98.76	0.97	99.62	0.88	98.15	0.97	99.60	0.99	99.94	0.89	98.35	0.88	98.09
Mean	0.91	98.79	0.97	99.59	0.88	98.21	0.97	99.60	0.99	99.94	0.89	98.27	0.88	98.10

[1]ANOVA 1 and ANOVA 2, analysis of variance based on models 1 and 2, respectively; PCACOR and PCACOV, principal component analysis based on the correlation and covariance matrices, respectively; and SACOR, structural analysis based on the correlation matrix.

predict the real value of the assessed characteristics (Rodrigues et al., 2020).

The expression of all variables showed high regularity between evaluations, and the values found in the present study indicate the proportion of the real value of each characteristic that was repeated throughout the assessments (Table 3). By increasing the number of measurements performed for a given variable, the temporary variance caused by the environment is reduced and, consequently, phenotypic variance is also reduced, optimizing the accuracy of the repeatability coefficient. However, when the temporary environmental variance is low and repeatability is high, the increase in the number of measurements carried out will add little to the inference of the individual genotypic value (Martuscello et al., 2015).

The estimates of the repeatability coefficient and R^2 for each variable were almost always lower when obtained by the ANOVA methods than by the other methods used (Table 3). However, when determined by PCACOR and PCACOV, the estimates were always greater than or equal to the general average. This result is in alignment with those of Cruz et al. (2014), who suggested that the repeatability analysis can be more efficiently estimated by the principal component method and also concluded that the ANOVA method can, in some cases, lead to undersized estimates of the repeatability coefficient.

High repeatability coefficients are desired, because as the repeatability coefficient is increased, the number of evaluations necessary to predict the real value of an individual is reduced (Rodrigues et al., 2020). In the five methods evaluated, one assessment cycle would be

Table 4. Estimates of the number of measurements (η) for stem diameter (SD), peduncle length (PEDL), plant height, leaf length (LL), leaf width (LW), number of leaves (NL), and pod length (PODL) of 26 genotypes of *Desmanthus* sp. using five different methods.

Method[1]	R^2	SD	PEDL	Height	LL	LW	NL	PODL
	0.80	0.34	0.12	0.49	0.11	0.02	0.48	0.54
	0.85	0.49	0.17	0.69	0.16	0.02	0.68	0.77
ANOVA 1	0.90	0.78	0.27	1.10	0.26	0.03	1.08	1.22
	0.95	1.65	0.58	2.34	0.54	0.08	2.29	2.59
	0.99	8.61	3.04	12.20	2.86	0.41	11.96	13.51
	0.80	0.35	0.12	0.68	0.11	0.02	0.47	0.54
	0.85	0.50	0.17	0.96	0.16	0.02	0.67	0.76
ANOVA 2	0.90	0.80	0.27	1.53	0.25	0.03	1.06	1.21
	0.95	1.69	0.58	3.23	0.54	0.08	2.25	2.56
	0.99	8.85	3.02	16.85	2.83	0.41	11.72	13.38
	0.80	0.31	0.10	0.46	0.11	0.02	0.45	0.53
	0.85	0.45	0.15	0.65	0.15	0.02	0.64	0.76
PCACOV	0.90	0.71	0.23	1.04	0.24	0.04	1.03	1.21
	0.95	1.51	0.50	2.20	0.52	0.07	2.17	2.56
	0.99	7.90	2.62	11.47	2.72	0.38	11.34	13.34
	0.80	0.31	0.11	0.38	0.10	0.02	0.47	0.53
	0.85	0.44	0.15	0.54	0.15	0.02	0.67	0.75
PCACOR	0.90	0.70	0.24	0.86	0.24	0.03	1.07	1.20
	0.95	1.49	0.52	1.83	0.50	0.07	2.27	2.54
	0.99	7.79	2.72	9.56	2.65	0.36	11.84	13.25
	0.80	0.35	0.10	0.52	0.11	0.02	0.46	0.54
	0.85	0.49	0.15	0.74	0.15	0.02	0.66	0.77
SACOR	0.90	0.78	0.24	1.18	0.24	0.04	1.05	1.22
	0.95	1.66	0.50	2.49	0.52	0.07	2.21	2.58
	0.99	8.67	2.63	13.01	2.72	0.38	11.55	13.44

[1]ANOVA 1 and ANOVA 2, analysis of variance based on models 1 and 2, respectively; PCACOR and PCACOV, principal component analysis based on the correlation and covariance matrices, respectively; and SACOR, structural analysis based on the correlation matrix.

needed to predict the real value of *Desmanthus* genotypes sp. for PEDL, leaf length and width; two cycles for stem diameter; and three cycles for number of leaves and PODL, if $R^2 = 95\%$ is considered (Table 4). To predict the actual height of the genotypes of *Desmanthus* sp. ($R^2 = 95\%$), two evaluation cycles would be necessary with the PCACOR method and four cycles with the ANOVA 2 method, whereas three evaluations would be necessary with the other methods.

It was found that the increase in precision from 95 to 99% in relation to the prediction of the real value of the evaluations (Table 4), in all used methods, implied a considerable increase in the number of measurements for stem diameter, plant height, number of leaves, and PODL, making its application unfeasible. This information becomes important, especially at the beginning of a breeding program, in which a large number of genotypes are evaluated simultaneously and the selection of superior individuals must be made with greater accuracy (Torres et al., 2015), with the possibility of reducing the number of evaluations, costs, and the time required for the selection of promising genotypes.

In general, morphological, productive, and qualitative variability was observed among the genotypes of *Desmanthus* sp.; however, most of it was found in the first two groups formed, which implies that the genetic divergence among them was not high. For the studied characteristics, up to four evaluation cycles would be necessary for the selection process to have a R^2 equal to 95% and for the superior or inferior behavior of the genotypes to be maintained. Moreover, the obtained estimates of the repeatability coefficient were of high magnitude, showing the reliability of the results.

Conclusions

1. There is a morphological, productive, and qualitative variability among the evaluated genotypes of *Desmanthus* sp., and forage yield, stem diameter, and leaf area index are the characteristics that contribute the most to the divergence among genotypes.

2. The estimates of the coefficients of repeatability and determination are of high magnitude for all variables by the different methods used.

3. Up to four evaluation cycles are required to predict the real value of *Desmanthus* sp. genotypes

for stem diameter, peduncle length, plant height, leaf length and width, number of leaves, and pod length, considering a coefficient of determination of 95%.

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