

Repeatability and minimum number of evaluations for morpho-agronomic characters of elephant-grass for energy purposes

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ABSTRACT

Due to the need to promote information relevant to elephant-grass breeding programs for energy purposes, this work aimed to estimate the coefficient of repeatability of the main morpho-agronomic characters and to predict the minimum number of cuts required for selection with greater efficiency. A total of 73 elephant-grass genotypes were evaluated in a randomized block design, in a subdivided plot scheme with two replicates. Nine cuts were performed at six-month intervals, from June 2012 to December 2016, in the municipality of Campos dos Goytacazes, RJ. We estimated the repeatability coefficients for dry matter yield (DMY), number of tillers (NT), plant height (PH), stem diameter (SD) and leaf blade width (LBW), using the methods of analysis of variance, principal components and structural analysis. At least nine, three, six, six and three cuts should be performed, considering the DMY, NT, PH, SD and LBW characteristics, respectively, to predict the true value of the genotypes with 80% reliability.

Key words: dry matter yield; Penissetum purpureum Schum.; selection efficiency

Repetibilidade e número mínimo de avaliações para caracteres morfoagronômicos de capim-elefante para fins energéticos

RESUMO

Devido à necessidade de promover informações relevantes para programas de melhoramento de capim-elefante para fins energéticos, este trabalho teve como objetivo estimar o coeficiente de repetibilidade dos principais caracteres morfoagronômicos e prever o número mínimo de cortes exigidos para seleção com maior eficiência. Avaliaram-se 73 genótipos de Capim-elefante em delineamento casualizado em blocos, no esquema de parcelas subdivididas, com duas repetições. Foram realizados nove cortes em intervalos de seis meses, no período de junho de 2012 a dezembro de 2016, no município de Campos dos Goytacazes, RJ.Estimou-se os coeficientes de repetibilidade para as características produtividade de matéria seca (PMS), número de perfilhos (NP), altura de plantas (ALT), diâmetro de colmo (DC) e largura de lâmina foliar (LL), utilizando-se os métodos de análise de variância, componentes principaise análise estrutural. Devem ser realizados, pelo menos, nove, três, seis, seis e três cortes, considerando as características PMS, NP, ALT, DC e LL, respectivamente, para predizer o valor real dos genótipos com 80% de confiabilidade.

Palavras-chave: produtividade de matéria seca; Penissetum purpureum Schum.; eficiência de seleção

Introduction

Energy production through the use of alternative forms, such as plant biomass, is a stimulus for researchers around the world, since the high emission of carbon dioxide (CO_2) from the use of fossil fuels and their derivatives has contributed to the greenhouse effect and consequently to the planet's climatic changes, with disastrous consequences (Morais et al., 2009). In this context, elephant-grass (*Pennisetum purpureum* Schum.) has emerged as a promising crop for use as an alternative source of energy because its several advantages for biomass production, such as: high productivity, less area required for production, smaller productive cycle allowing two cuts per year, high calorific power, better cash flow, possibility of mechanization and total and greater assimilation of carbon (Paterlini et al., 2013).

For all those reasons, the elephant-grass breeding program of the Northern Fluminense State University Darcy Ribeiro -UENF develops researches with the purpose of evaluating the viability of the use of elephant-grass biomass and to identify genotypes with high productive potential and quality of biomass suitable for use as a source of energy (Daher et al., 2014; Menezes et al., 2016; Sousa et al., 2016).

In these researches, for the efficient discrimination of genotypes, it is important to evaluate the characters with precision (less dispersion) and accuracy (small bias between the estimation and the parameter) (Torres et al., 2015). In order to achieve the desired accuracy, it is important to appropriately decide the number of measurements that must be performed to characterize the genotypes, since many years may be necessary to quantify the expression of a characteristic that is manifested over time (Cruz et al., 2012). During the selection process of genotypes, aiming at the launching new cultivars or the choice of parents for recombination, it is essential to be convinced of the genetic superiority of the individuals. For this, repeated measurements are often performed on the same individual (Neves et al., 2010).

This process involves many steps, evaluation of many characters and a great expenditure of costs and workmanship. Because of this, it is necessary to determine the number of measurements to be made through the repeatability quotient because this is an approximation of the maximum value that the inheritance of a characteristic can reach, in broadest sense (Cruz et al., 2012). This information is valuable for breeding programs, since it allows estimating the least selection cycle possible and also allows allocating human and financial resources to the research (Torres et al., 2015)

With regard to elephant-grass, it has been found that most of the scientific studies (Daher et al., 2004; Oliveira et al., 2011; Cavalcante et al., 2012) show results oriented to the use of this grass for animal production; no literature, to our knowledge, has used the abovementioned statistical tool for this grass with the purpose of studying it with bio-energetic purposes.

Thus, in order to promote information relevant to the genetic improvement of elephant-grass for energy purposes, the objective of this study was to estimate the repeatability coefficient of the main morpho-agronomic characters and predict the minimum number of cuts required for selection with greater efficiency.

Material and Methods

The experiment was conducted at the experimental station of PESAGRO-RJ in Campos dos Goytacazes, in the northern region of the state of Rio de Janeiro, located at 21°44'47"S and 41°18'24"W with altitude between 20 and 30 m. According to Köppen classification system (1948), the climate of the northern region of Rio de Janeiro is of Aw, tropical wet and dry with dry season in the winter and rainy season in the summer, with annual rainfall around 1023 mm (Mendonça et al., 2007). The rainfall during the period of the research was obtained from the Evapotranspirometric Station - Irrigation and Agrometeorology Sector of the UENF/PESAGRO (Table 1).

The soil of the experimental area was classified as dystrophic argisol (Embrapa, 2006) and presented the following characteristics in the 0-20 cm depth layer: pH = 5.5; $P = 18mg - dm^{-3}$; $K = 83mg - dm^{-3}$; Ca = 4.6cmolc $- dm^{-3}$; Mg = 3.0cmol $- dm^{-3}$; Al = 0.1cmolc $- dm^{-3}$; H + Al = 4.5cmolc dm^{-3} and C = 1.6%.

The planting of the experiment was carried out in February 2011 using whole stems arranged in tipped foot form, distributed in 10 cm deep furrows. After distribution of the stems, they were cut into pieces containing two or three buds. For planting fertilization, 60 g of simple superphosphate were distributed in each line, and 50 days after planting, the same quantity was used as cover fertilization using 70 g of urea and 40 g of KCl (potassium chloride) per line, corresponding to 28.6 kg of N (nitrogen) and 24 kg of K₂O (potassium oxide) per hectare. After the establishment phase, on December 15, 2011, all genotypes were cut close to the soil (cut of uniformity) and it a reforestation was made to minimize emergence failures in the planting lines.

A total of 73 elephant-grass genotypes (Table 2) were obtained from the elephant-grass Germplasm Active Bank (BAG-CE) from Embrapa, located in Coronel Pacheco, Minas Gerais, MG. The experiment was carried out in a completely randomized block design with two replicates. Each experimental unit consisted of a genotype planted in the row of 5.5 m spaced in 2 m, totaling 11 m². The useful area comprised $2m^2$ to the center of the plot. Nine cuts were performed at sixmonth intervals from one evaluation to another, from June 2012 to December 2016.

The evaluated variables are: number of tillers (NT), count of the number of tillers in the useful area; plant height (PH),

Table 1. Monthl	y rainfall (mm)	recorded	during the	e experimenta	period.

Month	2012	2013	2014	2016
Jan	216.50	125.70	26.50	131.10
Feb	11.70	44.30	4.00	64.90
Mar	73.60	230.20	143.30	19.20
Apr	14.20	103.20	103.10	14.00
May	147.20	41.60	25.70	14.50
Jun	74.00	8.70	30.80	37.70
Jul	5.90	67.10	139.60	12.20
Aug	59.80	57.00	14.20	22.80
Sep	21.60	45.20	8.10	6.4
Oct	12.50	26.40	17.70	73.3
Nov	133.70	158.40	69.10	161
Dec	10.40	289.40	32.00	148.1
Total	781.10	1197.20	614.10	705.20

Source: Evapotranspirometric Station - Irrigation and Agrometeorology sector of UENF/PESAGRO.

Table 2. Identification of the 73 genotypes of the UENF Elephant Grass Germplasm Active Bank, used in the repeatability study.

Id.	Genotype	Origin	Id.	Genotype	Origin	Id.	Genotype	Origin
1	Elefante Colombia	Colombia	26	Mole de Volta Grande	Brazil	51	Cameroon	Brazil
2	BAGCE 2	Brazil	27	Porto Rico	Brazil	52	BAGCE 69	Brazil
3	Três Rios	Brazil	28	Napier	Brazil	53	Guaçu	Brazil
4	Napier Volta Grande	Brazil	29	Mercker Comum	Brazil	54	Napierzinho	Brazil
5	Mercker Santa Rita	Brazil	30	Teresopólis	Brazil	55	IJ 7125 cv EMPASC 308	Brazil
6	Pusa Napier Nº 2	India	31	Taiwan A -46	Brazil	56	IJ 7136 cv EMPASC 307	Brazil
7	Gigante de Pinda	Brazil	32	Duro de Volta Grande	Brazil	57	IJ 7139	Brazil
8	Napier Goiano	Brazil	33	Mercker Comum Pinda	Brazil	58	Goiano	Brazil
9	Mercker S. E. A	Brazil	34	Turrialba	Brazil	59	CAC 262	Brazil
10	Taiwan A -148	Brazil	35	Taiwan A -146	Brazil	60	Ibitinema	Brazil
11	Porto Rico 534-B	Brazil	36	Taiwan A -121	Brazil	61	Australiano	Brazil
12	Taiwan A -25	Brazil	37	Vrukwona	Brazil	62	13 AD	Brazil
13	Albano	Colombia	38	P 241 Piracicaba	Brazil	63	10 AD IRI	Brazil
14	Pusa Gigante Napier	Colombia	39	BAGCE 51	Brazil	64	07 AD IRI	Brazil
15	Elefante Híbrido 534-A	India	40	Elefante Cach.Itapemirim	Brazil	65	Pasto Panamá	Panama
16	Costa Rica	Costa Rica	41	Capim Cana D'África	Brazil	66	BAGCE 92	Brazil
17	Cubano Pinda	Brazil	42	Gramafante	Brazil	67	05 AD IRI	Brazil
18	Mercker Pinda	Brazil	43	Roxo	Brazil	68	13 AD IRI	Brazil
19	Mercker Pinda México	Brazil	44	Guaçu/I.Z.2	Brazil	69	03 AD IRI	Brazil
20	Mercker 86 México	Colombia	45	Cuba-115	Cuba	70	02 AD IRI	Brazil
21	Napier S.E.A.	Brazil	46	Cuba-116	Cuba	71	08 AD IRI	Brazil
22	Taiwan A -143	Brazil	47	King Grass	Cuba	72	BAG 86	Brazil
23	Pusa Napier № 1	India	48	Roxo Botucatu	Brazil	73	BAG 87	Brazil
24	Elefante de Pinda	Colombia	49	Mineirão IPEACO	Brazil			
25	Mineiro	Brazil	50	Vruckwona Africano	Brazil			

expressed in m, obtained with a tape measure, measured from the soil to the point of inflection of the last fully expanded leaf; stem diameter (SD), expressed in mm, obtained with a digital caliper to measure the stem diameter at 10 cm from the ground level of three plants randomly chosen within each plot; leaf blade width (LBW), expressed in mm, obtained with a graded ruler randomly taking three plants from each plot; and dry matter yield (DMY), in t - ha⁻¹ – cutting, plants in the useful area of the plot were weighed, to which a sample was taken and packed in a paper bag, weighed and placed in an oven at 65°C for 72 hours, then the samples were weighed again to obtain the air dried sample (ADS). This material was crushed in a 1 mm sieve and kept in an oven at 105°C for 12 h for determination of the oven dried sample (ODS). The values of ADS and ODS were obtained by the percentage of dry matter and estimated DMY.

At first, an individual variance analysis was performed applying the F test for each cut. After verifying the homogeneity of the residual variances, by the ratio between the largest and the smallest mean square of the residue (MSR), the analysis of variance of the nine cuts altogether was performed, according to Ramalho et al. (2005):

$$Y_{IJK} = m + G_i + B_i + \varepsilon_a + C_k + \varepsilon_b + GC_{ik} + \varepsilon_c$$

where:

 $Y_{_{\rm IJK}}\,$ - value observed in the i-nth genotype in j-nth block, in k-nth cutting;

m - test constant;

G_i - effect of the i-nth genotype;

B_j - effect of the j-nth block;

 $\varepsilon_{a}^{'}$ - error a associated to the i-nth genotype in the j-nth block;

 C_{μ} - effect of the k-nth cutting;

 $\boldsymbol{\epsilon}_{b}$ - error b associated to the j-nth block in the k-nth cutting;

 GC_{ik} - effect of the interaction of the i-nth genotype with the k-nth cutting; and,

 $\epsilon_{\rm c}~$ - error c associated to the i-nth genotype in the j-nth block, in the k-nth cutting.

It is worth noting that as the cuts were performed in the same experimental plot, it is necessary to include the cuts x block interaction in the model. This is because there was no randomization of cuts in the different blocks; consequently, the measurements made over time may not be independent. Thus, in this analysis, three experimental errors were considered: the error *a* related to the genotype x block interaction, the error *b* to the cuts x block interaction and the error *c* to the genotype x cuts interaction (Ramalho et al., 2005).

Repeatability analyses (estimates) were performed from the mean of the replicates. The repeatability coefficient was estimated through four different statistical procedures: analysis of variance, principal component method (based on covariance and correlation matrix) and structural analysis (based on the correlation matrix).

For the estimation of the repeatability coefficient through analysis of variance (Anova), we used the statistical model with two variation factors:

$$Y_{ij} = m + G_i + C_j + \epsilon_{ij}$$

where:

Y_{ii} - mean of the i-nth genotype in the j-nth cutting;

m - general constant;

G_i - random effect of the i-nth genotype under permanent environmental influences (cutting);

C_i - fixed effect of temporary in the j-nth cutting; and,

 ϵ_{ij} - experimental error established by temporary effects in the j-nth random cutting that involves other causes of variation, which are not included in the model.

The repeatability coefficient was obtained by:

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

where:

 $\sigma_{g}^{\ 2}~$ - esstimation of the genotypic variance component associated with permanent environmental effects; and,

 σ^2 - estimation of the environmental variance component.

The principal components methodology used by Abeywardena (1972) allows estimating the repeatability coefficient in two ways, through the correlation matrix and through the genotype covariance matrix obtained in each pair of measurements. When based on the correlation matrix, the eigenvalues (λ) and the normalized eigenvectors (α) of R are determined. The eigenvector whose elements have the same sign and magnitudes is the one that expresses the tendency of the genotypes to maintain their relative positions in the various intervals of cuts. The repeatability coefficient (Rutledge, 1974) is estimated based on this eigenvalue:

$$\mathbf{r} = \frac{\hat{\lambda}_1 - 1}{\eta - 1}$$

where:

 λ_1 - eigenvalue obtained in the correlation matrix associated with the eigenvector, whose elements have the same sign and similar magnitudes; and,

 η - number of cuts.

When based on the phenotypic variance and covariance matrix, the repeatability coefficient was estimated by:

$$r = \frac{\hat{\lambda}_1 - \hat{\sigma}_Y^2}{\hat{\sigma}_Y^2 (\eta - 1)}$$

where:

 λ_1 - eigenvalue of phenotypic variance and covariance matrix associated with the eigenvector whose elements have the same sign and similar magnitudes;

 $\sigma_{\rm Y}^{2}$ - variance of the variable Y; and,

 η - number of cuts.

The method of structural analysis to obtain the repeatability coefficient proposed by Mansour et al. (1981) was based only on the correlation matrix. Thus the estimator of the repeatability coefficient was determined by:

$$r = \frac{\alpha'\hat{R}\hat{\alpha} - 1}{\eta - 1}$$

where:

 α' - is eigenvector with parametric elements associated to the greater eigenvalue of the uniform correlation matrix, given by $\alpha' = [(1/\sqrt{n})...(1/\sqrt{n})]$; and

 η - number of cuts.

The minimum number of measurements required to predict the real value of the individuals, based on a pre-established coefficient of determination (R^2) (0.80, 0.85, 0.90, 0.95 and 0.99). The prediction of this value was calculated as proposed by Cruz et al. (2012):

$$\eta_0 = \frac{R^2 \left(1 - r\right)}{\left(1 - R^2\right)r}$$

where:

 η_0 - number of measurements to predict the real value;

 R^2 - coefficient of determination; and,

r - repeatability coefficient obtained according to one of the different methodologies used.

For each characteristic, based on the mean of the η cuts and in the estimation of the repeatability coefficients obtained by the methods used, the coefficient of determination (R²) was calculated by means of the following expression:

$$R^2 = \frac{\eta r}{1 + r(\eta - 1)}$$

All statistical analyses were performed using the computational resources of the Genes program (Cruz, 2013).

Results and Discussion

We observed that the effects of genotypes (G), cuts (C) and the genotypes x cuts (G x C) interaction were significant (P < 0.01) for all traits studied (Table 3). Thus, the significance found for the source of variation genotypes gives evidence of the existence of genetic variability, enabling the identification of promising individuals to be used in breeding programs. On the other hand, the significance of the genotypes x cuts (G x C) interaction indicates that some genotypes were influenced by the cut, that is, the response of these genotypes to the evaluated characters may vary according to the evaluation cut, which is associated with environmental conditions, especially rainfall (Table 1).

For this type of statistical analysis, there are three types of errors, each error associated with a source of variation, that is, the CV (%) of the error a is related to the genotypes, the error b is related to the cuts, and the error c is related to the interaction G x C. However, in this study only the coefficients of variation of error a were taken into account, and the values ranged from 11.69% to 42.69% for PH and DMY, respectively (Table 3).

The repeatability coefficients (r) observed for elephantgrass genotypes ranged from 0.234 to 0.647 (Table 4). The highest estimates were recorded for blade width (principal components method: covariance), while the lowest value was found for dry matter yield (variance analysis method). In addition, the values of the coefficient of determination for all the characteristics, in all the methodologies tested were

Table 3. Analysis of variance of cuts altogether and significance for the mean squares for the six evaluated traits, means and coefficients of variation of the	
experiment in nine semester cuts, in 73 elephant-grass genotypes for energy purposes.	

	DE	DMY	NT	PH	SD	LBW
FV	DF	(t ha ⁻¹ cut)	IN I	(m)	(mm)	
Blocks	1	263.45	6.44	1.40	54.75	46.31
Genotypes (G)	72	183.03**	2197.12**	0.47**	30.36**	387.00**
Error A	72	56.19	184.39	0.09	2.75	28.01
Cuts (C)	8	3868.06**	4669.41**	48.22**	374.98**	5863.32**
Error B	8	77.15	622.25	0.79	17.02	318.43
GxC	576	48.74**	184.65**	0.09**	4.33**	23.57**
Error C	576	34.67	136.94	0.05	2.79	14.27
Mean	-	17.56	42.76	2.55	13.79	33.43
CV(%) error a	-	42.69	31.76	11.69	12.02	15.83
CV (%) error b	-	50.02	58.33	34.84	29.92	53.38
CV (%) error c	-	33.53	27.37	8.66	12.12	11.30

** Significant at 1% probability, according to the F test. DMY: dry matter yield; NT: number of tillers; PH: plant height; SD: stem diameter; LBW: leaf blade width.

superior to 75%, except for DMY by Anova, and this value is considered reliable by Oliveira & Moura (2010).

Considering the DMY, a highly relevant feature from the point of view of selection, smaller estimates of repeatability were observed, regardless of the method of estimation adopted (Table 4). There was no regularity in the repetition of the performance of the genotypes along the successive cuts. In a way, this result was already expected, since the dry matter yield is a character governed by several genes, presenting low heritability. This character is strongly influenced by climatic variations throughout the year, particularly the irregularity of the precipitation (Table 1). It is also worth emphasizing the random errors peculiar to the quantification of this characteristic, since for the measurement of the DMY, this comprises the relationship between three variables: green mass, air dried mass in a forced air circulation oven at $55 \,^{\circ}$ C, and dry mass in an oven at 105° C.

For all the methods used, the repeatability coefficients for plant height (PH) and stem diameter (SD) were considered to be of medium magnitude (0.3 < r < 0.60) according to Resende's classification (2002). Besides these estimates, coefficients of determination above 80% were observed. These results show a moderate regularity in the repetition of performance of the genotypes from one evaluation to another for these characters. The evaluation of these two characters is of major relevance for forage breeding programs, because they correlate positively with DMY (Menezes et al., 2014).

The analysis of the number of tillers (NT) per linear meter showed low amplitude in the repeatability coefficients obtained by the different methods, with 0.548 being the lowest r value,

Table 4. Estimates of repeatability coefficients (r) and respective coefficients of determination (R^2 , in brackets) for the five characteristics evaluated in the 73 elephant-grass genotypes for energetic purposes obtained through different estimation methods.

Mathadalasias	Characters						
Methodologies	DMY	NT	PH	SD	LBW		
Analysis of Variance	0.234	0.548	0.312	0.400	0.631		
(Anova)	(73.37)	(91.60)	(80.28)	(85.73)	(93.91)		
Principal components	0.305	0.592	0.437	0.437	0.647		
(covariance) - (PCcov)	(79.81)	(92.88)	(87.48)	(87.50)	(94.29)		
Princpal components	0.281	0.550	0.349	0.425	0.635		
(correlations) - (PCcor)	(77.88)	(91.68)	(82.88)	(86.96)	(94.02)		
Structural analysis	0.261	0.554	0.346	0.410	0.634		
(correlations) – (SAcor)	(76.08)	(91.47)	(80.63)	(85.73)	(93.98)		

DMY - dry matter yield; NT - number of tillers; PH - plant height; SD - stem diameter; LBW - leaf blade width. estimated by the Anova method. Daher et al. (2004) estimated genetic parameters and repeatability coefficients in elephantgrass in the edaphoclimatic conditions of the north of Rio de Janeiro and found estimates of r for NT ranging from 0.5864 to 0.6714 by Anova and PCcov, respectively.

Regarding the character leaf blade width (LBW), highmagnitude repeatability coefficient estimates (r> 0.60) were obtained, with coefficients of determination above 90%, regardless of the methodology used, indicating that the genotypes were highly regular in the repetition of responses from one evaluation to another. This accredits LBW as a highly repetitive character over time. These results agree with those found by Cavalcante et al. (2012), who worked with interspecific hybrids of *Pennisetum purpureum* and obtained estimates of repeatability of high magnitude (r > 0.80) for the same variable, regardless of the estimation method.

Estimates of repeatability coefficients obtained through the different methodologies showed good agreement, with consistency and reliability of the observed results (Table 4). In general, the estimated repeatability coefficients (r) obtained through the Analysis variance (Anova) were lower than those obtained through the other methodologies, regardless of the evaluated character. On the other hand, the highest estimates of repeatability were found through the principal components method, especially the one based on the phenotypic variance and covariance matrix (PCcov), which results in a lower number of evaluations to detect the superior elephant-grass genotypes for energy purposes (Table 5). This result was probably due to the seasonal behavior of the genotypes in relation to the characters studied (Cruz et al., 2012).

The inferiority of the repeatability estimates observed by the Anova method is explained because this methodology does not allow isolating the seasonality factor from the studied characteristics. By incorporating it into the estimation, the analysis increases the value of the experimental error, thus underestimating the repeatability and, consequently, overestimating the number of measurements required (Negreiros et al., 2008). In this sense, the principal component method, considering the seasonal behavior, is more recommended to estimate the repeatability with greater accuracy.

The number of cuts needed to characterize the DMY with 80% probability of its real value was at least nine according to the principal components method based on the covariance matrix and at most 14 according to the Anova method (Table

5). Considering 90% efficiency to discriminate the real value of the genotypes, it was observed that at least 21 measurements would be necessary according to the PCcov method, which would mean an increase of 12 cuts in relation to 80% of certainty. Considering that evaluations in elephant-grass experiments for energetic purposes require a longer interval (6-8 months) to increase fiber (lignin, cellulose), according to Queiroz Filho et al. (2000), the selection based on this character would need about 10 years and a half of evaluations performed every semester, which is unfeasible in practice, since it would be a costly and time-consuming process.

It was observed that none of the methodologies used presented a viable number of measurements to values above 90% certainty, which makes the characterization work extremely difficult and even unfeasible. Therefore, the definition of the ideal R^2 should prioritize, besides the minimum expected reliability of the data, the availability of resources and workmanship to carry out the characterization, considering data with 80% reliability as reliable in this study. Resende (2002) emphasizes that coefficients of determination of 80% can be considered adequate.

For all the analysis methods, it was verified that the number of tillers (NT) and leaf blade width (LBW) demanded a smaller number of measurements than the other characteristics, and the number of cuts needed to predict the true value of the individuals with 80% reliability were a maximum of four and three for NT and LBW, respectively, obtained in common through the Anova, PCcor and SAcor methods and only three evaluations for both variables according to the principal components method (Table 5). Therefore, we can state that these characteristics can be selected earlier with a good reliability in genetic improvement programs for elephantgrass for energy purposes, resulting in saving time, financial resources and manpower.

With respect to the prediction of the genetic value reached with the total number of nine cuts effectively performed in the

Table 5. Estimates of the number of measurements needed (η), for five characteristics in 73 elephant-grass genotypes for energy purposes under different confidence levels (R^2), according to different estimation methods.

Mathadalagiag	\mathbb{R}^2	Characteristics					
Methodologies	(%)	DMY	NT	PH	SD	LBW	
	80	14	4	9	6	3	
Analysia of Variance	85	19	5	13	9	4	
Analysis of Variance	90	30	8	20	14	6	
(Anova)	95	62	16	42	29	12	
	99	324	82	219	149	58	
	80	9	3	6	6	3	
	85	13	4	8	8	4	
Principal components (covariance) (PCcov)	90	21	7	12	12	5	
	95	44	14	25	25	11	
	99	226	69	128	128	54	
	80	11	4	8	6	3	
	85	15	5	11	8	4	
Principal components	90	23	8	17	13	6	
(correlation) (PCcor)	95	49	16	36	26	11	
	99	395	81	184	134	57	
	80	12	4	9	6	3	
0	85	16	5	13	9	4	
Structural analysis	90	26	8	20	13	6	
(correlation) (SAcor)	95	54	16	41	28	11	
	99	439	83	214	142	58	

DMY - dry matter yield; NT - number of tillers; PH - plant height; SD - stem diameter; LBW - leaf blade width.

conduction of this experiment, we can affirm that none of the characteristics reached values of $R^2 \ge 95\%$. Only the NT and LBW characters reached R^2 estimates of 90% for all methods used. Meanwhile, PH and SD achieved 85% reliability only through the principal components method based on the covariance matrix. In turn, DMY reached only 80% through the same method. In general, the nine cuts were able to predict the true value of the genotypes with 80% of reliability through the principal components method.

We consider that for the beginning of a genetic improvement program aiming at the use of elephant-grass for energy purposes, the selection based on these evaluated characteristics can be considered adequate, since they present positive genotype correlation with dry matter yield, being this the main characteristic to be selected (Menezes et al., 2014). Therefore, higher investments may be employed in more advanced stages of the breeding process when the number of genotypes is reduced, such as more detailed evaluations of biomass quality, or increased experimental accuracy with a greater the number of repetitions.

Conclusions

The characteristics number of tillers, plant height, stem diameter and leaf blade width presented repeatability estimates of medium to high magnitude, while those of dry matter yield had low magnitude.

At least nine cuts are necessary so that the selection of elephant-grass for energy purposes can be practiced with 80% accuracy of identification of superior genotypes, based on productivity, number of tillers, plant height, stem diameter and leaf blade width, through the principal components method using the covariance matrix.

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