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Minimum Number of Measurements for Accurate Evaluation of Qualitative Traits in *Urochloa brizantha*

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ABSTRACT

This work aimed to identify the most effective method to estimate the coefficient of repeatability in genotypes of *U. brizantha* and predict the minimum number of measurements required for some qualitative traits. It were evaluated 9 genotypes in a randomized block design with two replications in the rainy season and drought in 2000. It were evaluated the following qualitative traits: volume of gas, in mL, packed in fast and slow fraction, crude protein; neutral detergent fiber, acid detergent fiber, cellulose, lignin sulfuric acid, silica and *in vitro* digestibility of organic matter. The repeatability coefficient (r) was estimated considering different strategies: Analysis of variance, principal component analysis based on the correlation matrix (CPCOR), principal components analysis based on the matrix of phenotypic variance and covariance and structural analysis based on the correlation matrix. The CPCOR method, provided more accurate estimates of r and the number of measurements required for the qualitative traits assessed due to the cyclical behavior of genotypes of *U. brizantha*. The traits neutral detergent fiber, cellulose and silica require two measurements, while the remaining characters require four measurements to predict the actual value of genotypes of *U. brizantha* with a minimum accuracy of 80%, by CPCOR method.

Key words: Coefficient of determination, experimental precision, repeatability

INTRODUCTION

Breeding program of *Urochloa brizantha* (Hochst. ex A. Rich) fodder grasses from Embrapa Gado de Corte works on developing of fodder cultivars in order to reduce the vulnerability of livestock production systems and which has high productivity, high animal performance and low seasonality between dry and wet season, minimizing the need for opening new areas of cultivation (Torres *et al*., 2015).

In these researches, for the correct discrimination of genotypes is important to assess he traits with precision (less dispersion) and accuracy (small bias between the estimate and the parameter). Thus, to achieve the desired accuracy is important to sized appropriately the number of measurements that should be performed to characterize the genotypes, because many years may be needed to quantify the expression of a trait which manifests itself over time (Cruz *et al*., 2004).

During the process of selection of genotypes with a view to launching cultivars or parental choice for recombination, it is important to make sure, the genetic superiority of individuals. To this end, are often carried out repeated measurements in the same individual (Negreiros *et al*., 2008). Therefore, determining the number of measurements to be performed by repeatability coefficient it is necessary to

perennial species because it provides an approximation of the maximum value that the heritability of a trait, in the broad sense, can achieve (Cruz *et al*., 2004). In addition, this information is valuable for breeding programs, since it allows estimating what the lowest possible selection cycle and allocating human and financial resources to the research.

The sizing of the number of measurements has been performed in several perennial crops, such as; acerola (Lopes *et al*., 2001), guava (Degenhardt *et al*., 2002), araçá and pitanga (Danner *et al*., 2010), peach (Bruna *et al*., 2012), sweet orange (Negreiros *et al*., 2014) and banana (Lessa *et al*., 2014). In general, the authors of these studies observed that the multivariate methods are more accurate, compared to univariate method, however, they recommend that researches are made with other crops before the generalizing these results. Thus, in order to generate information relevant to the genetic breeding of *U. brizantha*, the aim of the study was to identify the most effective method to estimate the coefficient of repeatability in nine genotypes of *U. brizantha* and to predict the minimum number of measurements required for some qualitative traits.

MATERIALS AND METHODS

The trial was conducted at Embrapa Gado de Corte, in Campo Grande-MS, localized at 20°28'S and 55°40'W, with average altitude of 520 m. The local climate, according to Köppen classification, is rainy tropical and savanna (AW), characterized by irregular rainfall distribution and with dry and rainy season well defined. The soil of the trial area was classified as Oxisol dystrophic. In the trial period the cumulative rainfall and average temperature during the rainy season were 640 mm and 29.4°C, respectively, and in the dry season reached 410 mm and 26.7°C.

Nine genotypes of *U. brizantha* (eight ecotypes-B1, B2, B3, B4, B5, B6 and B8 and cv. Marandu) were implemented in 1994 in pickets with 1000 m^2 each, in two replications, and with cultural practices performed as recommended by Hughes *et al.* (2000).

In the dry and wet seasons, when the fodder had accumulated growth of 42 days, ten blades were sampled in three transects lanes in each repetition, always collecting the second fully expanded blade. The 30 samples were taken to the laboratory where were evaluated according to the methodology recommended by Goering and van Soest (1970). It was evaluated nine qualitative traits; gas volume, in mL, accumulated at fast and slow fraction (A and D, respectively) Crude Protein (CP), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Cellulose (CEL), Lignin in Sulfuric Acid (LIG), Silica (SIL) and *in vitro* Digestibility of Organic Matter (DOM).

To estimate with greater consistency the coefficient of repeatability (r) were used four procedures: Analysis of variance (ANOVA), Principal component analysis based on the correlation matrix (PCCOR), principal component analysis based on the matrix of phenotypic variance and covariance (PCCOV) and structural analysis based on correlation matrix (r-average SACOR).

To ANOVA method, it were obtained estimates of the mean square of measurements (MS_M), mean square of genotype (MS_G) and mean square of residue (MS_R). After, it was estimated the coefficient of repeatability, given by:

$$r = \frac{MS_G - MS_R}{MS_G + (\eta - 1)MS_R}$$

where in MS_G is the mean square of genotype, MS_R is the mean square of residue and η is the number of measurements conducted.

The PCCOR method consists of obtaining a correlation matrix among the genotypes at each pair of measurement. We determined in the matrix, the eigenvalues and its associated normalized eigenvectors. The eigenvector, whose elements have the same sign and magnitudes nearby, is one that expresses the tendency of genotypes to maintain, over the measurements, their relative position in relation to others (Abeywardena, 1972; Cruz *et al*., 2004). Its estimator is given by:

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

where in $\hat{\lambda}_1$ is the eigenvalues of the covariance matrix ($\hat{\Gamma}$) or of the correlation matrix ($\hat{R}$).

For the principal components method PCCOV, the estimator of repeatability is given by:

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

where in $\hat{\sigma}^2_Y$ is the phenotypic variance (Cruz *et al*., 2004). Obtaining the coefficient of repeatability by the method SACOR shows only conceptual differences in relation to methods based on principal components. Mansour *et al.* (1981), authors of this method, consider R the correlation parametric matrix between treatments in each pair of assessments and $\hat{R}$ its estimator. Obtaining the coefficient of repeatability based on this method it is made by the use of the equation:

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

being $\hat{\alpha}$ the eigenvector associated with the greater eigenvalue $\hat{R}$.

We determined for each trait the minimum number of measurements required to predict the actual value of individuals (η), based on a coefficient of determination (R^2) pre-set (80 and 85%), according to Cruz *et al*. (2004), given by:

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

wherein r is the coefficient of repeatability, obtained according to one of the different methods used.

The coefficient of genotypic determination (R^2), which is the certainty percentage of predict of the real value of the genotypes based on measurements η it was obtained by the expression:

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

Statistical analyzes of the data were performed using the softwares Genes (Cruz, 2013) and Microsoft Excel®.

RESULTS AND DISCUSSION

It is possible verify in Table 1 that estimates of the coefficient repeatability (r) by ANOVA method were lower than the other methods, regardless of the trait in question. The estimates of r by principal components (PCCOR and PCCOV) were higher in comparison with other methods, resulting in fewer measurements required to identify superior genotypes of *U. brizantha* (Table 2). This possibly was due to the cyclical behavior observed from one measurement to another, physiological changes, regular, irregular or systematic (Cruz *et al*., 2004).

Thus, how this effect may vary in different way and intensity between *U. brizantha* genotypes, ANOVA method, usually used to estimate r, does not allow isolate the factor measurements which, when it occurs, is included in the experimental error, raising its value and causing the underestimation of repeatability and overestimation of the number of measurements required (Cruz *et al*., 2004). In this case, principal components method, which takes into account the cyclical behavior of the trait, is the most recommended to estimate r with greater accuracy. This occurs because in this methodology the eigenvector, whose elements exhibit the same sign and magnitudes nearby, is one that expresses the tendency of genotypes to maintain its relative positions in the measurement periods (Abeywardena, 1972).

Table 1: Estimates of different parameters evaluated in *Urochloa brizantha* genotypes in two measurements

Trait	ANOVA	PCCOR	PCCOV	SACOR
CP				
r	0.53	0.63	0.56	0.56
R^2	0.69	0.77	0.72	0.72
NDF				
r	0.49	0.49	0.49	0.49
R^2	0.65	0.65	0.65	0.65
ADF				
r	0.63	0.75	0.69	0.69
R^2	0.77	0.85	0.82	0.82
CEL				
r	0.81	0.81	0.81	0.81
R^2	0.90	0.90	0.90	0.90
LIG				
r	0.40	0.76	0.53	0.53
R^2	0.57	0.87	0.69	0.69
SIL				
r	0.73	0.91	0.87	0.87
R^2	0.84	0.95	0.93	0.93
DOM				
r	0.50	0.55	0.51	0.51
R^2	0.66	0.71	0.67	0.67
A				
r	0.34	0.49	0.36	0.36
R^2	0.51	0.65	0.53	0.53
D				
r	0.50	0.78	0.62	0.62
R^2	0.66	0.87	0.77	0.77

ANOVA: Analysis of variance method, PCCOR: Principal component analysis method based on the correlation matrix, PCCOV: Principal component analysis method based on the phenotypic variances and covariances, SACOR: Structural analysis method based on the correlation matrix (r average) r: Coefficients of repeatability, R^2: Coefficients of determination for the traits CP: Crude protein, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, CEL: Cellulose, LIG: Lignin in sulfuric acid, SIL: Silica, *in vitro* DOM: Digestibility of Organic Matter and gas volume accumulated at fast and slow fraction (A and D, respectively)

Table 2: Number of measurements estimates associated with different parameters evaluated in nine *Urochloa brizantha* genotypes in two measurements

Trait	ANOVA	PCCOR	PCCOV	SACOR
CP				
0.80	4	2	3	3
0.85	5	3	4	4
NDF				
0.80	4	4	4	4
0.85	6	6	6	6
ADF				
0.80	2	1	2	2
0.85	3	2	3	3
CEL				
0.80	1	1	1	1
0.85	2	2	2	2
LIG				
0.80	6	1	4	4
0.85	9	2	5	5
SIL				
0.80	1	1	1	1
0.85	2	1	1	1
DOM				
0.80	4	4	4	4
0.85	6	5	5	5
A				
0.80	8	4	7	7
0.85	11	7	10	10
D				
0.80	4	1	2	2
0.85	6	2	3	3

ANOVA: Analysis of variance method, PCCOR: Principal component analysis method based on the correlation matrix, PCCOV: Principal component analysis method based on the phenotypic variances and covariances, SACOR: Structural analysis method based on the correlation matrix (r average) R^2: Coefficients of determination for the traits CP: Crude protein, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, CEL: Cellulose, LIG: Lignin in sulfuric acid, SIL: Silica, *in vitro* DOM: Digestibility of Organic Matter and gas volume accumulated at fast and slow fraction (A and D, respectively)

Similar results were obtained in several researches with cultures that has been expressed cyclical behavior, like acerola (Lopes *et al*., 2001), guava (Degenhardt *et al*., 2002), araçá and pitanga (Danner *et al*., 2010), peach (Bruna *et al*., 2012), sweet orange (Negreiros *et al*., 2014) and banana (Lessa *et al*., 2014), in which the ANOVA method overestimated the r estimates and overestimated the number of measurements required compared to method based on principal component (PCCOR and PCCOV). Thus, in view of the results obtained and its consistency with published researches with perennial species, we recommended for future studies with perennial species the use of methods based on the principal components (PCCOR and PCCOV) to estimate r, R^2 and number of measurements for selection of genotypes with greater prediction of real value.

For the traits ADF, CEL and SIL, values of r and R^2 exceeded the limits ($r \geq 0.40$; $R^2 \geq 0.75$) considered reliable by Bergo *et al.* (2013) and De Oliveira and Moura (2010), respectively. In addition, the r values denotes good ability of the genotypes to repeat the expression of a trait throughout the measurements, while the estimates R^2 express safety in the genetic superiority of selected *U. brizantha* genotypes.

For other traits, where has been found smaller estimates for R^2, increasing the number of measurements cannot be the solution to the problem. In some cases, absence of measurements in initial phases, which there is no full genetic potential expression of the studied material can increase the r estimated more accurately predict the genetic value of *U. brizantha* genotypes (Laviola *et al*., 2013).

In all analysis methods was found that traits AFD, CEL and SIL require fewer measurements when compared to other traits (Table 2), demonstrating the greater tendency of individuals regularity of a measurement to another (Cruz *et al*., 2004). This indicates that these traits can be selected earlier in breeding programs of *U. brizantha*, providing savings in time and labor. On the other hand, in view of a minimum coefficient of determination of 80% among the traits evaluated the LIG and A required greater the number of measurements, indicating the greater the effect of the environment on these characters, i.e., the environmental variance is greater as compared with the genetic variance among genotypes. Moreover, this indicates that these characters are determined by a number of different genes that can be expressed in greater or lesser quantity, according to the season in which the cut is made in the *Urochloa*, inflating the minimum number of measurements to identify genotypes for these characters (Cruz *et al*., 2004).

CONCLUSIONS

CPCOR method, provided more accurate estimates of r and the number of measurements required for the qualitative traits assessed due to the cyclical behavior of genotypes of *U. brizantha*.

The traits neutral detergent fiber, cellulose and silica require two measurements, while the remaining characters require four measurements to predict the actual value of genotypes of *U. brizantha*, with a minimum accuracy of 80%, by CPCOR method.

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