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Repeatability and genotypic stability in intraspecific hybrids of *Paspalum notatum* Flügge

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ABSTRACT - The objective of this study was to verify the repeatability of the expression of forage characters in intraspecific hybrids of *Paspalum notatum* Flügge to aid early selection. Across five harvests, plant height, tiller population density, leaf dry matter, stem dry matter, inflorescence dry matter, total dry matter, and growth habit were quantified for five parents, 189 hybrids, and a commercially available cultivar as a control (n = 195). Analysis of variance, principal components analysis, and structural analysis methods were used to determine the repeatability coefficients. The repeatability coefficients ($\hat{\rho}$) for all evaluated characteristics generated by the different methods were between 0.05 (ANOVA II) and 0.95 (PCACov). For most of the characteristics studied, repeatability coefficients and determination coefficients were considered high. The repeatability coefficients estimates obtained for the eight characteristics evaluated with the ANOVA I and II methods were almost always lower than those obtained by PCA and structural analysis methods. Based on the covariance matrix, the principal component method generated higher estimates than those produced by ANOVA or structural analysis. Assuming a minimum 80% reliability to verify the relative superiority of the hybrids across all assessed traits, the five harvests proved adequate for selecting the optimal plant materials to advance to the next phase of the breeding program. However, reliable early selection for leaf dry matter, leaf:stem ratio, and total dry matter required a minimum of two harvests. The genetic parameters (h^2 and CVg) showed a favorable scenario for direct selection to increase forage production.

Keywords: analysis of variance, breeding, early selection, heritability, principal components, structural analysis

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1. Introduction

Grasslands provide critical ecosystem services for humanity (Sollenberger et al., 2019). In Brazil, pastures occupy 156 million hectares across six previously defined biomes (Projeto MapBiomas, 2021). These pastures support a national herd of ~224 million cattle (IBGE, 2019) and places Brazil as the world's second-largest beef producer, after the United States (Jank et al., 2014). Pastoral farming systems in Brazil are heavily reliant on native forage species for productive stability and conservation

of natural resources. Their use can reduce costs and risks associated with livestock production, which increases long-term system sustainability (Gasparetto et al., 2021).

The genus *Paspalum* comprises several native species with important forage characteristics for animal production. They exhibit adaptability to the range of different ecosystems where they are present as a pasture component (Novo et al., 2016), which means there is high potential for genetic improvement (Motta et al., 2017). The center of genetic diversity is located in the tropical region of South America (Chase, 1929; Valls, 1987). The genus is native to the southern grasslands, is most abundant in Brazil (Rio Grande do Sul), Uruguay, and Argentina, and has previously been recognized for its high yield and forage quality (Steiner et al., 2022). In Argentina, the region with the greatest diversity of species is Mesopotamia (Morrone et al., 2012).

A major objective of forage plant breeding programs in southern Brazil is to obtain hybrids adapted to diverse edaphoclimatic conditions, which have superior biomass production compared with cultivars already on the market (Saraiva et al., 2021). There is considerable commercial and academic interest in improvement of different *Paspalum* species to increase the productivity of native pastures and extend their use as improved cultivated pastures (Steiner et al., 2022).

The significant time and resource requirements involved in obtaining consistent data within breeding programs, considering the extensive number of genotypes and characters studied (Jank et al., 2014), requires the determination of the minimum number of measurements for the selection of superior genotypes (Rodrigues et al., 2020). This is a major issue faced by forage breeding programs, which need to determine the number of measurements necessary to accurately estimate the differences between genotypes (Toebe et al., 2020). This is generally determined through repeatability analysis, which aims to predict the genotypic value of a genotype over time with predefined determination coefficients (Chaves et al., 2018). The repeatability analysis reduces time, costs, and labor within the experimental period to optimize the process of launching new cultivars into the market (Torres et al., 2015; Rodrigues et al., 2020).

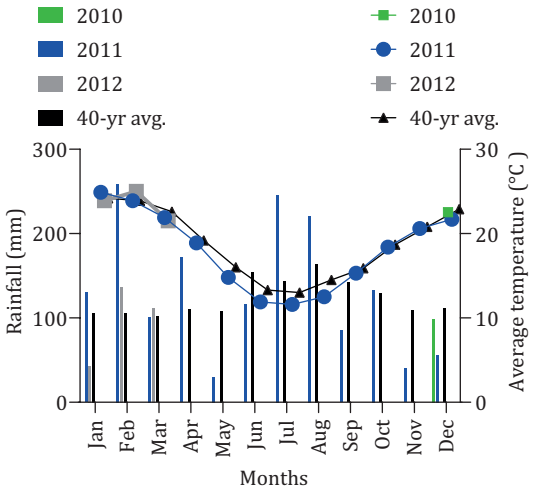
The objective was to estimate the repeatability coefficients of forage characteristics in intraspecific hybrids of *P. notatum* Flügge using different methods.

2. Material and Methods

2.1. Experimental site

The experimental site is located in Eldorado do Sul, Rio Grande do Sul, Brazil (at 30°29'26" S latitude, 51°06'42" W longitude, and 62 m asl altitude). The local climate is classified as Cfa according to the Köppen classification (Moreno, 1961): subtropical with no defined dry season, and the average air temperature of the hottest month (February) exceeds 22 °C. The 40-year (1970-2010) average minimum and maximum annual air temperatures in the region are 14.0 and 24.2 °C, respectively, and the annual average air temperature is 19.6 °C. The 40-year average annual rainfall is 1398 mm. Total monthly rainfall (mm) and mean air temperature (°C) during the experimental period are reported in Figure 1.

The soil is classified as an Ultisol (USDA Soil taxonomy; Santos et al., 2006). Prior to establishment of the experiment, soil samples (0-0.2 m) were collected. The soil analysis showed: clay = 15%; pH (H_2O) = 5.4; SMP pH = 6.3; P (mg dm^{-3}) = 15.6; K (mg dm^{-3}) = 151.4; and organic matter = 2.7%. The protocol for basal and maintenance fertilization for perennial grasses followed the recommendation of the “Comissão de Química e Fertilidade do Solo RS/SC” (CQFS RS/SC, 2004). A total of 160 kg N/ha, in the form of urea (46% N), was distributed across five split applications of 32 kg N/ha at the beginning of each regrowth period.



Black lines and bars are the 40-yr average (1970-2010).

Figure 1 - Total monthly rainfall (mm; bars) and mean monthly air temperature (°C; lines) during the experimental period.

2.2. Plant material and experimental design

Three tetraploid female sexual genotypes C44X (Quarin et al., 2001), Q4188, Q4205 (Quarin et al., 2003) were sourced from the Instituto de Botánica del Nordeste (IBONE), Corrientes, Argentina. They were crossed with two elite male tetraploid germplasm lines (ecotypes Bagual and André da Rocha) native to the state of Rio Grande do Sul (Table 1), Brazil. The crosses were performed using the methodology described by Burton (1948) and later adapted by Weiler et al. (2018) to create hybrid progeny. The reproduction mode was determined based on Weiler et al. (2017). A total of 195 *P. notatum* Flügge genotypes were evaluated, including 189 hybrids, three female (C44X, Q4188, and Q4205) and two male (André da Rocha and Bagual) cultivars, and the commercially available cultivar 'Pensacola'.

Seeds were initially germinated in Germitest paper lined in petri dishes, under controlled temperature and day length (8 h of light at 30 °C and 16 h of darkness at 20 °C) in a germination chamber. Seedlings were transplanted into seedling trays until they had five fully expanded leaves. Seedlings were then transplanted into pots filled with Carolina Soil™, a commercial substrate composed of peat, vermiculite,

Table 1 - Female (♀ 4x; n = 3) and male (♂ 4x; n = 2) parents and hybrids (n = 189) of *Paspalum notatum* Flügge evaluated

♂ 4x	♀ 4x	Family	No. hybrids	Hybrid
Q4188	André da Rocha	A	29	A10, A11, A12, A13, A14, A15, A16, A17, A18, A2, A20, A21, A22, A23, A24, A25, A26, A27, A28, A29, A31, A32, A33, A35, A36, A37, A38, A7, A8
Q4188	Bagual	B	44	B1, B10, B11, B12, B13, B14, B15, B16, B17, B18, B19, B2, B20, B21, B22, B23, B25, B26, B27, B28, B29, B3, B30, B31, B32, B33, B34, B35, B36, B37, B38, B39, B4, B40, B41, B42, B43, B44, B5, B52, B6, B7, B8, B9
Q4205	André da Rocha	C	35	C1, C10, C11, C12, C13, C14, C15, C16, C17, C18, C19, C2, C20, C21, C22, C23, C24, C25, C26, C27, C28, C29, C3, C30, C31, C32, C34, C35, C36, C4, C5, C6, C7, C8, C9
Q4205	Bagual	D	26	D1, D10, D11, D12, D13, D14, D16, D17, D18, D19
				D2, D20, D21, D22, D23, D24, D25, D26, D27, D3, D4, D5, D6, D7, D8, D9
C44X	André da Rocha	E	23	E1, E10, E11, E12, E13, E14, E15, E16, E17, E18, E19, E2, E20, E21, E22, E24, E3, E4, E5, E6, E7, E8, E9
C44X	Bagual	F	32	F1, F10, F11, F12, F13, F14, F15, F16, F17, F18, F2, F20, F21, F22, F23, F24, F25, F26, F27, F28, F29, F3, F30, F31, F32, F33, F4, F5, F6, F7, F8, F9

The commercially available cultivar Pensacola was included as a control. Total lines evaluated n = 195.

organic residue, and limestone. When the plants had four or more tillers, the tillers were separated and re-potted into four different pots to obtain four clones to be used as replicates in the field experiment.

The field experiment followed a randomized complete block design with four replicates and was established at the experiment station of the Universidade Federal do Rio Grande do Sul on 12/26/2010. Clones were transplanted into the field spacing of 1.0 m within and between rows. Immediately after transplanting, the plants were watered by sprinkler irrigation to facilitate seedling establishment.

2.3. Procedures and traits

The plants were cut to a residual height of 5 cm when they reached an average height of 20 cm. Five harvests were made between sowing and 2012 (1st harvest on 02/22/2011, 2nd harvest on 04/06/2011, 3rd harvest on 11/17/2011, 4th harvest on 01/09/2012, and 5th harvest on 03/16/2012). Measurements included plant height (PH, cm), tiller population density (TPD, tiller plant^{-1}), leaf dry matter (LDM, g plant^{-1}), stem dry matter (SDM, g plant^{-1}), inflorescence dry matter (IDM, g plant^{-1}), total dry matter (TDM, g plant^{-1}), and growth habit (GH).

Non-destructive observations were made before cutting on each date. Plant height was measured from the soil surface to the curvature of the leaves; then, the TPD was quantified by counting all tillers that had expanded leaves. Growth habit was determined by visual observation scale, in which 1 = prostrate and 5 = erect habit.

Samples were separated into morphological components: leaves (leaf blades), stems (stems and sheaths), and inflorescences, then dried in an oven at 60 °C until constant weight. Subsequently, the leaf:stem ratio (LSR) was calculated from LDM and SDM.

2.4. Statistical analysis

Data were subjected to analysis of variance according to the following model:

$$Y_{ijk} = \mu + G_i + B_k + A_j + GA_{ij} + E_{ijk},$$

in which Y_{ijk} is the observed value of the i-th genotype in the k-th block and within the j-th environment, μ is the mean for the characteristic, G_i is the fixed effect of the i-th genotype (i = 1, 2, 3, ... 195), B_k is the fixed effect (k = 1, 2, 3 and 4) of the k-th block (replicate), A_j is the random effect of the j-th environment, GA_{ij} is the random effect of the interaction of the i-th genotype with the j-th environment, and E_{ijk} is the experimental error. Therefore, $G_i \sim (0, \hat{\sigma}^2_g)$; $A_j \sim N(0, \hat{\sigma}^2_a)$; $B_k \sim N(0, \hat{\sigma}^2_k)$; $GA_{ij} \sim N(0, \hat{\sigma}^2_{ga})$; and $E_{ijk} \sim N(0, \hat{\sigma}^2_e)$.

Heritability (h^2) was calculated from:

$$h^2 = \frac{\hat{\sigma}^2_g}{\hat{\sigma}^2_f}$$

in which $\hat{\sigma}^2_g$ is the genotypic variance and $\hat{\sigma}^2_f$ is the phenotypic variance.

Three analysis methods were applied to the data to quantify the consistency of the estimates and obtain more precise conclusions about the observed forage characteristics. Firstly, two analysis of variance (ANOVA) models (ANOVA I and ANOVA II) were used to estimate the repeatability coefficients. Then, Principal component analysis (PCA) quantified the matrix of variance and covariance (PCACov) and the intraclass correlation matrix (PCACor). Finally, structural analysis (SA) quantified the variance and covariance matrix (SACov) and correlation matrix (SACor). The ANOVA was obtained through two models:

ANOVA I

$$Y_{ij} = \mu + g_i + \varepsilon_{ij},$$

in which Y_{ij} is the observation referring to the i-th genotype in the j-th harvest, μ is the overall average, g_i is the random effect of the i-th genotype under the influence of the permanent environment

(i = 1, 2, ..., p = 195 genotypes), 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 repeatability coefficient (r) was obtained by:

$$\frac{C\hat{o}v\,(Y_{ik}, Y_{ik'})}{\sqrt{\hat{V}\,(Y_{ik})\,\hat{V}\,(Y_{ik'})}} = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_y^2} = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_e^2 + \hat{\sigma}_g^2}$$

ANOVA II

$$Y_{ij} = \mu + g_i + a_j + \varepsilon_{ij},$$

in which Y_{ij} is the observation referring to the i-th genotype in the j-th harvest, μ is the overall average, g_i is the random effect of the i-th genotype under the influence of the environment (i = 1, 2, ..., ρ), a_j quantifies the fixed effect of the temporary environment on the j-th measurement (j = 1, 2, ..., η_i), and ε_{ij} quantifies experimental error established by temporary effects of the environment on the j-th measurement of the i-th genotype. The repeatability coefficient is calculated by the same equation described above for ANOVA I.

Two PCA models were then applied. The first evaluated the matrix of phenotypic variances and covariances (PCACov) by:

$$r = (\hat{\rho}) = \frac{\lambda_1 - \hat{\sigma}_Y^2}{\hat{\sigma}_Y^2\,(\eta - 1)}$$

in which λ_1 is the largest eigenvalue, associated with the eigenvector, whose elements have the same sign and close magnitude; $\hat{\sigma}_Y^2 = \hat{\sigma}_g^2 + \hat{\sigma}_e^2$ and η are the number of harvests. The PCACor, consisted of obtaining a correlation matrix between the genotypes in each pair of harvests. In this matrix, the eigenvalues were determined (λ) and the eigenvectors (α) normalized. The eigenvector whose elements have the same sign and close magnitudes quantified the tendency of the genotypes to maintain their relative positions over time. Therefore, the estimator of the repeatability coefficient is the proportion of the eigenvalue associated with this eigenvector, expressed by:

$$r = \frac{\lambda_k}{\sum_j \lambda_k}$$

Being j = 1, 2, ..., η in which η is the number of harvests and λ_k is the eigenvalue associated with the eigenvector, whose elements have the same sign and similar magnitude; and λ_k is influenced by the number of genotype measurements (Rutledge, 1974). Therefore, r becomes more suitable for calculating the repeatability coefficient, which is obtained by:

$$r = \frac{\lambda_1 - 1}{\eta - 1}$$

in which $\lambda_1 = 1 + (\lambda - 1)\rho$ in which λ_1 is the eigenvalue of r associated with the eigenvector whose elements have the same sign and magnitude; η = number of harvests; ρ = genotypes (195). Both methods (PCACov and PCACor) were pioneered by Abeywardena (1972).

Similar to the PCA method, SA can be obtained by covariance matrix (SACov), in which r is calculated, using the eigenvector (α) and the covariance matrix:

$$r = \frac{\alpha' \hat{I} \alpha - \hat{\sigma}_Y^2}{\hat{\sigma}_Y^2\,(\eta - 1)}$$

The structural analysis based on the correlation matrix (SACor) is determined by:

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

in which $\alpha' = \left[\frac{1}{\sqrt{\eta}} \ldots 1/\sqrt{\eta}\right]$ represents the eigenvector with parametric elements associated with the highest eigenvalue of *r*. The repeatability estimator is the arithmetic mean of the phenotypic correlations between genotypes, considering each part of evaluations and expressed by:

$$r = \frac{2}{\eta(\eta - 1)} \sum_j \sum_{<j'} rjj'$$

The estimator is equivalent to that obtained by the analysis of variance. The genotypic stabilization of forage characters was evaluated by ANOVA II and PCA methods based on the intraclass correlation matrix for successive measures until all evaluations were performed. Therefore, $\eta-1$ analyzes were performed on two consecutive measurements, and $\eta-2$ analyzes were performed in three consecutive evaluations until all five measurement dates had been evaluated. All data were analyzed with GENES (Cruz, 2016) statistical software.

3. Results

The analysis of variance of the forage variables was statistically significant for all effects tested (genotype, harvest, and their interaction), except PH which showed significant differences for main effects (genotype and harvest) only (Table 2). Experimental (CV_e; 38.3 (GH) to 128.4% (TPD)) and genetic (CV_g; 18.1 (GH) to 77.9% (SDM)) coefficients of variation were high for all characters evaluated (Table 2). Heritability estimates ranged from 0.33 (LSR) to 0.95 (PH). Characters of interest within the

Table 2 - Summary of analysis of variance for forage traits in 195 genotypes of *Paspalum notatum* Flügge for leaf dry matter (LDM), stem dry matter (SDM), leaf:stem ratio (LSR), inflorescence dry matter (IDM), total dry matter (TDM), tiller population density per plant (TPD), plant height (PH), and growth habit (GH)

Source of variation	GL	Medium square			
		LDM	SDM	LSR	IDM
Blocks	3	11580.56	1158.24	1150.13	201.93
Genotype	194	5066.38**	951.85**	1431.43**	159.59**
Harvest	4	214768.30**	49129.64**	59181.89**	10134.09**
Interaction	776	739.84**	328.80**	953.36**	73.07**
Error	2922	347.33	75.54	522.90	19.57
Maximum	-	228.70	151.20	520.00	82.90
Minimum	-	0.10	0.03	0.10	0.02
Mean	-	25.46	10.57	13.04	5.13
CVg (%)	-	61.86	77.91	40.22	74.76
CVe (%)	-	78.38	121.32	188.14	159.01
h^2	-	0.85	0.65	0.33	0.54
Source of variation	GL	Medium square			
		TDM	TPD	PH	GH
Blocks	3	24253.66	122018.51	208.98	17.10
Genotype	194	11155.69**	67292.50**	329.70**	5.07**
Harvest	4	578112.61**	6038487.70**	15160.67**	36.82**
Interaction	776	2126.06*	30099.07*	16.75ns	1.31**
Error	2922	744.79	13467.87	22.83	0.84
Maximum	-	440.10	6223.00	56.00	5
Minimum	-	0.10	1.00	3.00	1
Mean	-	36.15	96.82	14.66	3
CVg (%)	-	62.93	47.71	28.92	18.13
CVe (%)	-	80.83	128.40	34.93	38.31
h^2	-	0.81	0.55	0.95	0.74

CVg - genetic coefficient of variation; CVe - experimental coefficient of variation; h^2 - heritability.
* P<0.01; ** 0.05; ns = not significant.

forage plant genetic improvement program, associated with forage quality and production, such as LDM (0.85) and TDM (0.81), also had high heritability values.

The presence of significant genotype × harvest interactions for all forage characters, except PH, reinforced the need to estimate the repeatability coefficients for the characters because responses vary over time (Table 3). For TDM, $\hat{\rho}$ ranged from 0.19 (ANOVA II) to 0.88 (PCACov). With the exception of the ANOVA II method (54%), the coefficient of determination (R^2) exceeded 80%, which indicated reliability in identifying *P. notatum* genotypes with superior TDM (Table 3). The pattern of the PCACov and ANOVA II, which produced the highest and lowest $\hat{\rho}$, respectively, was repeated for the other characteristics evaluated in the study. The $\hat{\rho}$ values obtained for TDM and LDM traits were closely correlated, which indicated that the genotypes evaluated in this experiment performed well for important agronomic traits required for future genetic improvement. The LSR $\hat{\rho}$ were poor and ranged from 0.03 (ANOVA II) to 0.49 (PCACov), with associated R^2 from 12 to 83%, respectively. Analysis showed that PCACov, PCACor, and SACov methods almost consistently gave the highest $\hat{\rho}$ for all characteristics evaluated, while ANOVA I, ANOVA II, and SACor had the lowest $\hat{\rho}$ values.

The estimated minimum number of harvests needed to select the best hybrids identified by the five analysis methods for TDM, with a precision of 0.85, ranged from 1 (PCACov) to 24 (ANOVA II; Table 4). When the precision level was increased to 0.95, minimum harvest numbers for TDM increased for all methods and ranged from 3 (PCACov) to 79 (ANOVA II). For LDM, an agronomically important trait that provides an indirect measure of forage quality, the estimated number of harvests required for a precision of 0.85 ranged from 1 (PCACov) to 16 (ANOVA II). Increasing the required precision level to 0.95 meant the minimum number of harvests increased to 3 and 54, respectively. Across all traits evaluated, the estimated minimum number of harvests identified by all five analysis methods was the highest for LSR and varied substantially across methods within a specific precision level. For example, the minimum number of harvests ranged from 6 (PCACov) to 198 (ANOVA II) with precision of 0.85 and from 20 (PCACov) to 663 (ANOVA II) with a precision of 0.95.

Estimated minimum harvest numbers were higher for ANOVA II and I methods when compared with PCACov, PCACor, and SA. The principal components method generated the lowest estimates, especially when based on the variance and covariance matrix (PCACov). Structural analysis (SA) produced values very close to PCACor for most of the characteristics studied.

Table 3 - Estimates of the repeatability coefficient ($\hat{\rho}$) and their respective determination coefficients (R^2) derived from different analysis methods for leaf dry matter (LDM), stem dry matter (SDM), leaf:stem ratio (LSR), inflorescence dry matter (IDM), total dry matter (TDM), tiller population density per plant (TPD), plant height (PH), and growth habit (GH) measured in 195 genotypes of *Paspalum notatum* Flügge

Method	LDM		SDM		LSR		IDM	
	$\hat{\rho}$	R^2	$\hat{\rho}$	R^2	$\hat{\rho}$	R^2	$\hat{\rho}$	R^2
ANOVA I	0.54	85.40	0.27	65.46	0.09	33.40	0.19	54.22
ANOVA II	0.26	63.73	0.11	39.16	0.03	12.54	0.05	21.89
PCACov	0.85	96.71	0.90	97.78	0.49	82.97	0.93	98.58
PCACor	0.76	93.94	0.53	84.87	0.23	59.67	0.44	79.75
SACov	0.75	93.90	0.51	84.08	0.15	47.80	0.40	77.22
SACor	0.54	85.40	0.27	65.46	0.09	33.40	0.19	54.22
Method	TDM		TPD		PH		GH	
	$\hat{\rho}$	R^2	$\hat{\rho}$	R^2	$\hat{\rho}$	R^2	$\hat{\rho}$	R^2
ANOVA I	0.46	80.94	0.20	55.27	0.79	94.92	0.37	74.23
ANOVA II	0.19	54.46	0.02	9.48	0.33	71.37	0.32	70.64
PCACov	0.88	97.30	0.95	98.87	0.81	95.58	0.46	81.01
PCACor	0.74	93.56	0.67	90.94	0.82	95.85	0.43	79.16
SACov	0.74	93.54	0.66	90.82	0.82	95.81	0.36	73.39
SACor	0.46	80.94	0.20	55.27	0.79	94.92	0.37	74.23

ANOVA - analysis of variance; PCACov - principal components analysis based on the residual variance and covariance matrix; PCACor - principal components analysis based on the correlation matrix; SACov - structural analysis based on the covariance matrix; SACor - structural analysis based on the correlation matrix.

Table 4 - Minimum number of measurements required by different analysis methods to identify superior hybrids for leaf dry matter (LDM), stem dry matter (SDM), leaf:stem ratio (LSR), inflorescence dry matter (IDM), total dry matter (TDM), tiller population density per plant (TPD), plant height (PH), and growth habit (GH) in 195 hybrids of *Paspalum notatum* Flügge

R^2	LDM					R^2	SDM				
	ANOVA I	ANOVA II	PCACov	PCACor	SA		ANOVA I	ANOVA II	PCACov	PCACor	SA
0.80	3	11	1	1	1	0.80	11	31	0	4	4
0.85	5	16	1	2	2	0.85	15	44	1	5	5
0.90	8	26	2	3	3	0.90	24	70	1	8	9
0.95	16	54	3	6	6	0.95	50	148	2	17	18
0.99	85	282	17	32	32	0.99	261	769	11	88	94
R^2	LSR					R^2	IDM				
	ANOVA I	ANOVA II	PCACov	PCACor	SA		ANOVA I	ANOVA II	PCACov	PCACor	SA
0.80	40	140	4	14	22	0.80	17	71	0	5	6
0.85	57	198	6	19	31	0.85	24	101	0	7	8
0.90	90	314	9	30	49	0.90	38	161	1	11	13
0.95	189	663	20	64	104	0.95	80	339	1	24	28
0.99	987	3453	102	335	541	0.99	418	1766	7	126	146
R^2	TDM					R^2	TPD				
	ANOVA I	ANOVA II	PCACov	PCACor	SA		ANOVA I	ANOVA II	PCACov	PCACor	SA
0.80	5	17	1	1	1	0.80	16	191	0	2	2
0.85	7	24	1	2	2	0.85	23	270	0	3	3
0.90	11	38	1	3	3	0.90	36	430	1	4	5
0.95	22	79	3	7	7	0.95	77	907	1	9	10
0.99	117	414	14	34	34	0.99	401	4725	6	49	50
R^2	PH					R^2	GH				
	ANOVA I	ANOVA II	PCACov	PCACor	SA		ANOVA I	ANOVA II	PCACov	PCACor	SA
0.80	1	8	1	1	1	0.80	7	8	5	5	7
0.85	2	11	1	1	1	0.85	10	12	7	7	10
0.90	2	18	2	2	2	0.90	16	19	11	12	16
0.95	5	38	4	4	4	0.95	33	39	22	25	34
0.99	26	199	23	21	22	0.99	172	206	116	130	179

R^2- determination coefficient; ANOVA - analysis of variance; PCACov - principal components analysis based on the residual variance and covariance matrix; PCACor - principal components analysis based on the correlation matrix; SA - structural analysis.

In general, forage characteristics evaluated by PCACor had higher genotypic stability values than those produced by the ANOVA II model (Table 5). For LDM, SDM, LSR, IDM, and TDM variables, the highest $\hat{\rho}$ were obtained when harvests 1 and 2 were correlated, regardless of the method used. The highest $\hat{\rho}$ for TPD ($\hat{\rho}$ = 0.84; R^2 = 91.5%) and GH ($\hat{\rho}$ = 0.67; R^2 = 80.2%) traits were observed for the correlation of harvests 3 and 4, and for PH using harvests 4 and 5 ($\hat{\rho}$ = 0.94; R^2 = 96.7%). High $\hat{\rho}$ were observed for most of the characters studied. The exception was the LSR trait, which indicated that it was not stable among the genotypes evaluated.

4. Discussion

The basic premise for selection is the presence and knowledge of genetic variability within the population (Nielsen et al., 2014; Figueiredo et al., 2019; Sant'Anna et al., 2021; Steiner et al., 2022). The findings of the present study indicated that genetic variability existed among the hybrids evaluated, which allows genetic gain via direct selection in all traits quantified (Table 2).

The experimental (CV_e) and genetic (CV_g) variation coefficients were higher than those found by other authors who have previously evaluated forage characteristics (Machado et al., 2021; Silveira et al., 2022) or seed production traits (Lopes et al., 2017; Lopes et al., 2019) of *P. notatum* hybrids. The current experiment found that CV_e was always greater than CV_g (Table 2), which showed that the environmental

Table 5 - Repeatability ($\hat{\rho}$) and determination (R^2) coefficients for comparisons of harvest times generated by ANOVA II and PCACor analysis methods to evaluate genotypic stability of leaf dry matter (LDM), stem dry matter (SDM), leaf:stem ratio (LSR), inflorescence dry matter (IDM), total dry matter (TDM), tiller population density per plant (TPD), plant height (PH), and growth habit (GH) in 195 hybrids of *Paspalum notatum* Flügge

Harvest	N	LDM				SDM			
		ANOVA II		PCACor		ANOVA II		PCACor	
		$\hat{\rho}$	R^2	$\hat{\rho}$	R^2	$\hat{\rho}$	R^2	$\hat{\rho}$	R^2
1-2	2	0.92	95.86	0.92	95.87	0.83	90.76	0.84	91.57
2-3	2	0.57	72.58	0.77	87.32	0.24	38.13	0.39	56.20
3-4	2	0.83	90.91	0.89	94.13	0.17	29.37	0.30	46.72
4-5	2	0.61	75.75	0.87	93.15	0.35	51.96	0.77	86.73
1-3	3	0.60	81.59	0.81	92.56	0.50	75.30	0.55	78.45
2-4	3	0.70	87.71	0.80	92.24	0.31	57.35	0.38	64.42
3-5	3	0.69	87.10	0.86	94.79	0.21	44.21	0.51	75.72
1-4	4	0.67	88.94	0.78	93.59	0.41	73.24	0.46	76.99
2-5	4	0.59	85.19	0.78	93.53	0.25	57.31	0.52	81.46
1-5	5	0.54	85.40	0.76	93.94	0.27	65.46	0.53	84.87
Harvest	N	LSR				IDM			
		ANOVA II		PCACor		ANOVA II		PCACor	
		$\hat{\rho}$	R^2	$\hat{\rho}$	R^2	$\hat{\rho}$	R^2	$\hat{\rho}$	R^2
1-2	2	0.37	53.96	0.39	55.61	0.65	78.67	0.76	86.10
2-3	2	0.00	0.79	0.01	1.50	0.12	21.43	0.20	33.02
3-4	2	0.28	43.68	0.30	45.99	0.11	19.01	0.16	28.22
4-5	2	0.03	5.77	0.03	6.32	0.25	39.48	0.63	77.33
1-3	3	0.00	0.13	0.20	42.89	0.37	63.84	0.42	68.72
2-4	3	0.14	32.73	0.15	34.90	0.23	47.22	0.25	49.61
3-5	3	0.13	30.88	0.15	34.67	0.14	32.62	0.36	62.99
1-4	4	0.09	27.34	0.14	39.73	0.30	63.16	0.35	67.86
2-5	4	0.12	34.73	0.22	52.45	0.20	50.05	0.42	74.46
1-5	5	0.09	33.40	0.23	59.67	0.19	54.22	0.44	79.75
Harvest	N	TDM				TPD			
		ANOVA II		PCACor		ANOVA II		PCACor	
		$\hat{\rho}$	R^2	$\hat{\rho}$	R^2	$\hat{\rho}$	R^2	$\hat{\rho}$	R^2
1-2	2	0.93	96.24	0.93	96.28	0.77	86.85	0.88	93.65
2-3	2	0.69	81.92	0.75	85.52	0.72	84.00	0.76	86.56
3-4	2	0.82	90.12	0.83	90.66	0.84	91.48	0.85	91.67
4-5	2	0.52	68.16	0.92	95.68	0.26	40.84	0.71	82.96
1-3	3	0.74	89.31	0.79	91.96	0.64	84.37	0.77	90.74
2-4	3	0.74	89.44	0.76	90.36	0.72	88.71	0.74	89.76
3-5	3	0.53	77.16	0.84	93.92	0.26	51.60	0.75	90.04
1-4	4	0.73	91.44	0.76	92.52	0.63	87.29	0.71	90.86
2-5	4	0.48	78.64	0.77	92.99	0.23	54.63	0.70	90.16
1-5	5	0.46	80.94	0.74	93.56	0.20	55.27	0.67	90.94
Harvest	N	PH				GH			
		ANOVA II		PCACor		ANOVA II		PCACor	
		$\hat{\rho}$	R^2	$\hat{\rho}$	R^2	$\hat{\rho}$	R^2	$\hat{\rho}$	R^2
1-2	2	0.80	89.04	0.87	92.91	0.60	74.67	0.61	75.86
2-3	2	0.77	86.92	0.77	87.08	0.63	77.10	0.63	77.17
3-4	2	0.88	93.68	0.88	93.68	0.67	80.26	0.67	80.29
4-5	2	0.94	96.66	0.94	96.83	0.02	3.18	0.02	3.20
1-3	3	0.75	90.09	0.79	91.85	0.59	81.27	0.60	81.95
2-4	3	0.79	91.85	0.79	91.87	0.61	82.64	0.61	82.70
3-5	3	0.92	97.19	0.92	97.30	0.25	49.52	0.34	60.24
1-4	4	0.75	92.35	0.78	93.41	0.56	83.76	0.57	84.40
2-5	4	0.85	95.74	0.85	95.83	0.33	65.94	0.41	73.53
1-5	5	0.79	94.92	0.82	95.85	0.37	74.23	0.43	79.16

ANOVA - analysis of variance; PCACor - principal components analysis based on the correlation matrix.

effect dominated results rather than genetic effects. This result is contradictory to previous studies because with greater experimental precision the greatest expressions of genotypic variability were not expressed (Shimoya et al., 2002). The tendency of a greater CV_e compared with CV_g was not observed by Lopes et al. (2019). Precision (CV_e) and repeatability ($\hat{\rho}$) are influenced by management decisions, experimental design, and/or biotic and abiotic factors (Johnson and Frey, 1967; Vela-Cardenas and Frey, 1972; Neyhart et al., 2022). Furthermore, early selection in perennial plants can be affected by numerous factors, such as slow establishment, which would lead to high coefficients of experimental variation. Additionally, the seedlings were very young when transplanted, which may have led to an increase in the coefficients of variation. Conversely, high CV_e values can mean greater phenotypic variation (Wang et al., 2022). At the same time, CV_g provides information about the magnitude of the variability present within the population. This allows comparison of the levels of genetic variability present among different genotypes, environments, and traits (Ferrão et al., 2008). The current experiment confirmed that genetic variability studies within breeding programs must quantify both CV_e and CV_g during analysis (Cortes et al., 2019). Populations that exhibit high CV_g indicate potential for improvement via genetic gains in the breeding program (Zanata et al., 2010). The main advantage of utilizing the coefficient of variation in trait analysis is to enable the quantification and weighting of the proportion of variation within a population, which exists due to genetic and environmental factors (Kampa et al., 2020). This parameter, along with the h^2, provides the basis for decision-making to increase forage production within the current *P. notatum* Flügge breeding program.

Heritability values quantify how much of the total observed variation was caused by genotypic factors (Ferreira et al., 2020). Resende (2015) classified h^2 into three classes; low ($h^2 < 0.15$), moderate ($0.15 < h^2 < 0.50$), and high ($h^2 > 0.50$). Accordingly, the h^2 parameter generated in the analysis of this experiment (Table 2) was high for most forage traits evaluated. The exception was LSR, which is greatly influenced by environmental conditions over time. Higher h^2 values allow the identification of superior hybrids within the population studied. Selection of these hybrids in the next stage of the breeding program could improve forage gains obtained in subsequently selected progeny (Majidi et al., 2009).

The high number of genotypes and characters studied within breeding programs (Jank et al., 2014), requires significant time and resources to obtain consistent data and needs an estimate of the minimum number of measurements for selection of superior genotypes (Rodrigues et al., 2020). Therefore, several studies have been conducted to estimate the repeatability ($\hat{\rho}$) of characteristics of interest in forage plants including *Urochloa spp.* (Basso et al., 2009; Souza Sobrinho et al., 2010; Teixeira et al., 2011; Martuscello et al., 2013; Matias et al., 2016; Coêlho et al., 2018; Figueiredo et al., 2019), *Megathyrsus maximus* (Martuscello et al., 2007; Braz et al., 2015; Martuscello et al., 2015; Coêlho et al., 2018; Ferreira et al., 2019), *Medicago sativa* L. (Botrel et al., 2000; Ferreira et al., 2010), *Pennisetum purpureum* Schum. (Shimoya et al., 2002; Rodrigues et al., 2020), and *Lolium multiflorum* Lamarck (Rios et al., 2019). Until now this information has not been available for the *Paspalum* species. The $\hat{\rho}$ value indicates a consistent ranking of genotypic performance in a specific location over time (Neyhart et al., 2022). In our study, $\hat{\rho}$ values were medium-to-high for most characteristics (Table 3), based on the criteria of Resende (2002). In most cases, the highest estimates were obtained through the PCACov analysis method, followed by PCACor. These results are supported by previously published work, which also showed that $\hat{\rho}$ estimates obtained by ANOVA are generally lower than the estimates obtained by other methods (Cargnelutti Filho et al., 2004; Martuscello et al., 2007; Martuscello et al., 2015). Our work showed that the PCACov method estimates of $\hat{\rho}$ tended to be higher than those obtained by the other methods, which is supported by the results of Martuscello et al. (2015). The difference among $\hat{\rho}$ estimates from the analysis methods indicated that evaluation by more than one analysis method is required to obtain a reliable parameter. Evaluation of the analysis procedure as a whole means the real value can probably be found within the range of estimates calculated (Martuscello et al., 2007). In this context, identification of the most appropriate analysis method should be used as a strategy to improve parameter estimates for future decision making (Martuscello et al., 2015).

The study of genotypic stabilization contributes to increasing the reliability of the selection process for a trait by identifying groups of repeated measurements with a higher level of association. This is based on the assumption that gene expression can be influenced not only by the stage of development but also by various climatic conditions and management changes that plants experience throughout the year (Braz et al., 2015). For most traits studied here, genotypic stabilization between two harvests showed the highest correlation (Table 5). The evaluation between successive harvests may not represent genotypic stabilization, but the occurrence of two very similar harvests (Ferreira et al., 2019). This probably reflects they have been exposed to similar environmental conditions, rather than non-adjacent harvests, which likely experience less similar environmental conditions due to seasonal changes. It is necessary to include longer evaluation periods with more harvests to determine genotypic stabilization. Thus, the evaluation of stabilized genotypes to obtain $\hat{\rho}$ estimates is extremely important for selecting material within breeding programs (Martuscello et al., 2015).

Finally, our study reports, for the first time, estimates of the repeatability coefficient for forage production traits in hybrids of *P. notatum* Flügge. Our results showed values differed among the methodologies applied (Table 3). In general, the repeatability coefficient estimates were of medium to high magnitude for most traits evaluated and indicated reliability in identifying superior hybrids of *P. notatum* Flügge. For all methods evaluated, increasing the accuracy from 0.85 to 0.95 would require a large increase in the number of harvests, but this adds little in terms of precision, especially when there are many hybrids to be evaluated within the improvement program. In addition, the h^2 and CV_g genetic parameters indicated a favorable situation for genetic gains with selection within the studied population.

5. Conclusions

For reliable early selection based on LDM, LSR, and TDM traits of *P. notatum* Flügge, two to four harvests are recommended. Broad heritability for most of the characters studied suggests this would provide a favorable situation for direct selection for increased forage production. The principal component analysis, based on the covariance matrix, has the highest repeatability estimates compared with the other methods. For early selection, this method is recommended for identification of superior hybrids of *P. notatum* Flügge.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: R.L. Weiler, A.P. Brunes, C. Simioni and M. Dall'Agnol. Data curation: D.C. Silveira. Formal analysis: D.C. Silveira. Funding acquisition: R.L. Weiler and M. Dall'Agnol. Investigation: R.L. Weiler, A.P. Brunes, C. Simioni, J. Longhi, M.V.S. Corrêa, C. Nauderer, A. Valentini, W.M. Santos and M. Dall'Agnol. Methodology: R.L. Weiler, C. Simioni and M. Dall'Agnol. Project administration: R.L. Weiler, C. Simioni and M. Dall'Agnol. Resources: R.L. Weiler and M. Dall'Agnol. Supervision: R.L. Weiler and M. Dall'Agnol. Validation: R.L. Weiler. Visualization: R.L. Weiler and M. Dall'Agnol. Writing – original draft: R.L. Weiler, D.C. Silveira and A. Mills. Writing – review & editing: R.L. Weiler, D.C. Silveira and A. Mills.

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References

Abeywardena, V. 1972. An application of principal component analysis in genetics. Journal of Genetics 61:27-51. https://doi.org/10.1007/BF02984099

Basso, K. C.; Resende, R. M. S.; Valle, C. B.; Gonçalves, M. C. and Lempp, B. 2009. Avaliação de acessos de *Brachiaria brizantha* Stapf e estimativas de parâmetros genéticos para caracteres agronômicos. Acta Scientiarum. Agronomy 31:17-22. https://doi.org/10.4025/actasciagron.v31i1.6605

Botrel, M. A.; Ferreira, R. P.; Cruz, C. D.; Pereira, A. V.; Viana, M. C. M.; Rocha, R. and Miranda, M. 2000. Estimativas de coeficientes de repetibilidade para produção de matéria seca em cultivares de alfafa, sob diferentes ambientes. Revista Ceres 47:651-663.

Braz, T. G. S.; Fonseca, D. M.; Jank, L.; Cruz, C. D. and Martuscello, J. A. 2015. Repeatability of agronomic traits in *Panicum maximum* (Jacq.) hybrids. Genetics and Molecular Research 14:19282-19294. https://doi.org/10.4238/2015.december.29.38

Burton, G. W. 1948. Artificial fog facilitates Paspalum emasculation. Agronomy Journal 40:281-282. https://doi.org/10.2134/agronj1948.00021962004000030010x

Cargnelutti Filho, A.; Castilhos, Z. M. S.; Storck, L. and Savian, J. F. 2004. Análise de repetibilidade de caracteres forrageiros de genótipos de *Panicum maximum*, avaliados com e sem restrição solar. Ciência Rural 34:723-729. https://doi.org/10.1590/S0103-84782004000300011

Chase, A. 1929. The North American species of Paspalum. Contributions from the United States National Herbarium 28:1-310.

Chaves, G. G.; Cargnelutti Filho, A.; Carini, F.; Kleinpaul, J. A.; Neu, I. M. M. and Procedi, A. 2018. Tamanho de parcela e número de repetições para avaliação de caracteres vegetativos em centeio. Revista Brasileira de Ciências Agrárias 13:1-11.

Coêlho, J. J.; Mello, A. C. L.; Santos, M. V. F.; Dubeux Junior, J. C. B.; Cunha, M. V. and Lira, M. A. 2018. Prediction of the nutritional value of grass species in the semiarid region by repeatability analysis. Pesquisa Agropecuária Brasileira 53:378-385. https://doi.org/10.1590/S0100-204X2018000300013

Cortes, D. F. M.; Santa-Catarina, R.; Vettorazzi, J. C. F.; Ramos, H. C. C.; Viana, A. P. and Pereira, M. G. 2019. Development of superior lines of papaya from the Formosa group using the pedigree method and REML/Blup procedure. Bragantia 78:350-360. https://doi.org/10.1590/1678-4499.20180253

CQFS RS/SC - Comissão de Química e Fertilidade do Solo RS/SC. 2004. Manual de adubação e calagem para os estados do Rio Grande do Sul e Santa Catarina. 10.ed. Sociedade Brasileira de Ciência do Solo - Núcleo Regional Sul, Porto Alegre.

Cruz, C. D. 2016. Genes Software-extended and integrated with the R, Matlab and Selegen. Acta Scientiarum. Agronomy 38:547-552. https://doi.org/10.4025/actasciagron.v38i3.32629

Ferrão, R. G.; Cruz, C. D.; Ferreira, A.; Cecon, P. R.; Ferrão, M. A. G.; Fonseca, A. F. A.; Carneiro, P. C. S. and Silva, M. F. 2008. Parâmetros genéticos em café Conilon. Pesquisa Agropecuária Brasileira 43:61-69. https://doi.org/10.1590/S0100-204X2008000100009

Ferreira, F. M.; Carvalho Rocha, J. R. A. S.; Alves, R. S.; Elizeu, A. M.; Benites, F. R. G.; de Resende, M. D. V.; Souza Sobrinho, F. and Bhering, L. L. 2020. Estimates of repeatability coefficients and optimum number of measures for genetic selection of *Cynodon* spp. Euphytica 216:70. https://doi.org/10.1007/s10681-020-02605-x

Ferreira, M. R.; Martuscello, J. A.; Braz, T. G. S.; Nascimento, A. A.; Jank, L.; Assis, J. A.; Almeida, O. G.; Reis, G. A.; Santos, M. V. and Santos, M. F. 2019. Repeatability and genotypic stability of agronomic characteristics in *Panicum maximum* Jacq. Chilean Journal of Agricultural Research 79:547-556. https://doi.org/10.4067/S0718-58392019000400547

Ferreira, R. P.; Vasconcelos, E. D.; Cruz, C. D.; Barioni Júnior, W.; Rassini, J. B.; Freitas, A. R.; Vilela, D. and Moreira, A. 2010. Determinação do coeficiente de repetibilidade e estabilização genotípica das características agronômicas avaliadas em genótipos de alfafa no ano de estabelecimento. Revista Ceres 57:642-647. https://doi.org/10.1590/S0034-737X2010000500012

Figueiredo, U. J.; Berchembrock, Y. V.; Valle, C. B.; Barrios, S. C. L.; Quesenberry, K. H.; Muñoz, P. R. and Nunes, J. A. R. 2019. Evaluating early selection in perennial tropical forages. Crop Breeding and Applied Biotechnology 19:291-299. https://doi.org/10.1590/1984-70332019v19n3a41

Gasparetto, B. F.; Radunz, L. L.; Lopes, R. R.; Franke, L. B. and Martinelli, J. A. 2021. Fungi associated with *Paspalum guenoarum* seeds: their impact on physiology and control. Ciência Rural 51:e20200497. https://doi.org/10.1590/0103-8478cr20200497

IBGE - Instituto Brasileiro de Geografia e Estatística. 2019. Censo agropecuário 2017: Resultados definitivos. IBGE, Rio de Janeiro.

Jank, L.; Barrios, S. C.; Valle, C. B.; Simeão, R. M. and Alves, G. F. 2014. The value of improved pastures to Brazilian beef production. Crop & Pasture Science 65:1132-1137.

Johnson, G. R and Frey, K. J. 1967. Heritabilities of quantitative attributes of oats (*Avena* sp.) at varying levels of environmental stress. Crop Science 7:43-46. https://doi.org/10.2135/cropsci1967.0011183X000700010016x

Kampa, M. B.; Homczinski, I.; Roque, R. H.; Figueiredo Filho, A.; Peres, F. S. B. and Tambarussi, E. V. 2020. Variabilidade genética em progênies de *Campomanesia xanthocarpa* Mart. ex O. Berg em viveiro. Scientia Forestalis 48:e2935. https://doi.org/10.18671/scifor.v48n125.10

Lopes, R. R.; Franke, L. B.; Souza, C. H. L.; Bertoncelli, P.; Graminho, L. A.; Ávila, M. R.; Pereira, E. A. and Motta, E. A. M. 2019. Genetic assessment of seed yield-related traits in superior hybrids of *Paspalum plicatulum* × *Paspalum guenoarum.* Revista Brasileira de Zootecnia 48:e20190075. https://doi.org/10.1590/rbz4820190075

Lopes, R. R.; Souza, C. H. L.; Pereira, E. A.; Gasparetto, B. F.; Dall'Agnol, M. and Franke, L. B. 2017. Genetic variability of the components of seed yield in interspecific hybrids of *Paspalum*. Revista Brasileira de Zootecnia 46:296-302. https://doi.org/10.1590/S1806-92902017000400004

Machado, J. M.; Motta, E. A. M.; Barbosa, M. R.; Weiler, R. L.; Simioni, C.; Silveira, D. C.; Mills, A.; Pereira, E. A. and Dall'Agnol, M. 2021. Multivariate analysis reveals genetic diversity in *Paspalum notatum* Flügge. Revista Brasileira de Zootecnia 50:e20200252. https://doi.org/10.37496/rbz5020200252

Majidi, M. M.; Mirlohi, A. and Amini, F. 2009. Genetic variation, heritability and correlations of agro-morphological traits in tall fescue (*Festuca arundinacea* Schreb.). Euphytica 167:323-331. https://doi.org/10.1007/s10681-009-9887-6

Martuscello, J. A.; Braz, T. G. S.; Jank, L.; Cunha, D. N. F. V.; Souza, M. W. M.; Brito, G. F. and Oliveira, L. P. 2013. Repeatability of agronomic characters in *Brachiaria brizantha* cultivars. Revista Brasileira de Zootecnia 42:30-35. https://doi.org/10.1590/S1516-35982013000100005

Martuscello, J. A.; Braz, T. G. S.; Jank, L.; Cunha, D. N. F. V.; Lima, B. P. S. and Oliveira, L. P. 2015. Repeatability and phenotypic stabilization of *Panicum maximum* accessions. Acta Scientiarum. Animal Sciences 37:15-21. https://doi.org/10.4025/actascianimsci.v37i1.23206

Martuscello, J. A.; Jank, L.; Fonseca, D. M.; Cruz, C. D. and Cunha, D. N. F. V. 2007. Repetibilidade de caracteres agronômicos em *Panicum maximum* Jacq. Revista Brasileira de Zootecnia 36:1975-1981. https://doi.org/10.1590/S1516-35982007000900005

Matias, F. I.; Barrios, S. C. L.; Valle, C. B.; Mateus, R. G.; Martins, L. B. and Moro, G. V. 2016. Estimate of genetic parameters in *Brachiaria decumbens* hybrids. Crop Breeding and Applied Biotechnology 16:115-122. https://doi.org/10.1590/1984-70332016v16n2a18

Moreno, J. A. 1961. Clima do Rio Grande do Sul. Secretaria da Agricultura, Porto Alegre.

Morrone, O.; Aagesen, L.; Scataglini, M. A.; Salariato, D. L.; Denham, S. S.; Chemisquy, M. A.; Sede, S. M.; Giussani, L. M.; Kellogg, E. A. and Zuloaga, F. O. 2012. Phylogeny of the *Paniceae* (Poaceae: Panicoideae): integrating plastid DNA sequences and morphology into a new classification. Cladistics 28:333-356. https://doi.org/10.1111/j.1096-0031.2011.00384.x

Motta, E. A. M.; Dall'Agnol, M.; Pereira, E. A.; Machado, J. M. and Simioni, C. 2017. Valor forrageiro de híbridos interespecíficos superiores de *Paspalum*. Revista Ciência Agronômica 48:191-198.

Neyhart, J. L.; Gutierrez, L. and Smith, K. P. 2022. Optimizing the choice of test locations for multitrait genotypic evaluation. Crop Science 62:192-202. https://doi.org/10.1002/csc2.20657

Nielsen, H. B.; Almeida, M.; Juncker, A. S.; Rasmussen, S.; Li, J.; Sunagawa, S.; Plichta, D. R.; Gautier, L.; Pedersen, A. G.; Le Chatelier, E.; Pelletier, E.; Bonde, I.; Nielsen, T.; Manichanh, C.; Arumugam, M.; Batto, J.-M.; dos Santos, M. B. Q.; Blom, N.; Borruel, N.; Burgdorf, K. S.; Boumezbeur, F.; Casellas, F.; Doré, J.; Dworzynski, P.; Guarner, F.; Hansen, T.; Hildebrand, F.; Kaas, R. S.; Kennedy, S.; Kristiansen, K.; Kultima, J. R.; Léonard, P.; Levenez, F.; Lund, O.; Moumen, B.; Le Paslier, D.; Pons, N.; Pedersen, O.; Prifti, E.; Qin, J.; Raes, J.; Sørensen, S.; Tap, J.; Tims, S.; Ussery, D. W.; Yamada, T.; Renault, P.; Sicheritz-Ponten, T.; Bork, P.; Wang, J.; Brunak, S. and Ehrlich, S. D. 2014. Identification and assembly of genomes and genetic elements in complex metagenomic samples without using reference genomes. Nature Biotechnology 32:822-828. https://doi.org/10.1038/nbt.2939

Novo, P. E.; Valls, J. F. M.; Galdeano, F.; Honfi, A. I.; Espinoza, F. and Quarin, C. L. 2016. Interspecific hybrids between *Paspalum plicatulum* and *P. oteroi*: a key tool for forage breeding. Scientia Agricola 73:356-362. https://doi.org/10.1590/0103-9016-2015-0218

Projeto MapBiomas. 2021. Mapeamento anual de cobertura e uso da terra do Brasil - Coleção 6. Available at: <https://mapbiomas-br-site.s3.amazonaws.com/Fact_Sheet_PASTAGEM_13.10.2021_ok_ALTA.pdf.>. Accessed on: Dec. 09, 2022.

Quarin, C. L.; Espinoza, F.; Martinez, E. J.; Pessino, S. C. and Bovo, O. A. 2001. A rise of ploidy level induces the expression of apomixis in *Paspalum notatum*. Sexual Plant Reproduction 13:243-249. https://doi.org/10.1007/s004970100070

Quarin, C. L.; Urbani, M. H.; Blount, A. R.; Martinez, E. J.; Hack, C. M.; Burton, G. W. and Quesenberry, K. H. 2003. Registration of Q4188 and Q4205, sexual tetraploid germplasm lines of bahiagrass. Crop Science 43:745-746. https://doi.org/10.2135/cropsci2003.7450

Resende, M. D. V. 2015. Genética quantitativa e de populações. Suprema, Viçosa, MG. 463p.

Resende, M. D. V. 2002. Genética biométrica e estatística no melhoramento de plantas perenes. Embrapa Informação Tecnológica, Brasília.

Rios, E. F.; Kenworthy, K. E.; Gezan, S. A. and Munoz, P. R. 2019. Genetic parameters for phenotypic traits in annual ryegrass. Crop Science 59:2128-2140. https://doi.org/10.2135/cropsci2019.02.0126

Rodrigues, E. V.; Daher, R. F.; Gravina, G. A.; Viana, A. P.; Araújo, M. S. B.; Oliveira, M. L. F.; Vivas, M.; Menezes, B. R. S. and Pereira, A. V. 2020. Repeatability estimates and minimum number of evaluations for selection of elephant-grass genotypes for herbage production. Bioscience Journal 36:30-41.

Rutledge, J. J. 1974. A scaling which removes bias of Abeywardena's estimator of repeatability. Journal of Genetics 61:247-250.

Sant'Anna, I. C.; Gouvêa, L. R. L.; Martins, M. A.; Scaloppi Junior, E. J.; de Freitas, R. S. and Goncalves, P. S. 2021. Genetic diversity associated with natural rubber quality in elite genotypes of the rubber tree. Scientific Reports 11:1081. https://doi.org/10.1038/s41598-020-80110-w

Santos, H. G.; Jacomine, P. K. T.; Anjos, L. H. C.; Oliveira, V. A.; Oliveira, J. B.; Coelho, M. R.; Lumbreras, J. F. and Cunha, T. J. F. (eds.). 2006. Sistema brasileiro de classificação de solos. 2.ed. Embrapa Solos, Rio de Janeiro.

Saraiva, K. M.; Dall'Agnol, M.; Da Motta, E. A. M.; Pereira, E. A.; De Souza, C. H. L.; Simioni, C.; Weiler, R. L.; Kopp, M. M.; Schneider-Canny, R. and Barbosa, M. R. 2021. Hybrids of *Paspalum plicatulum* × *P. guenoarum*: selection for forage yield and cold tolerance in a subtropical environment. Tropical Grasslands-Forrajes Tropicales 9:138-143. https://doi.org/10.17138/tgft(9)138-143

Shimoya, A.; Pereira, A. V.; Ferreira, R. P.; Cruz, C. D. and Carneiro, P. C. S. 2002. Repetibilidade de características forrageiras do capim-elefante. Scientia Agricola 59:227-234. https://doi.org/10.1590/S0103-90162002000200004

Silveira, D. C.; Machado, J. M.; Motta, E. A. M.; Barbosa, M. R.; Simioni, C.; Weiler, R. L.; Mills, A.; Sampaio, R.; Brunes, A. P. and Dall'Agnol, M. 2022. Genetic parameters, prediction of gains and intraspecific hybrid selection of *Paspalum notatum* Flügge for forage using REML/BLUP. Agronomy 12:1654. https://doi.org/10.3390/agronomy12071654

Sollenberger, L. E.; Kohmann, M. M.; Dubeux Jr, J. C. B. and Silveira, M. L. 2019. Grassland management affects delivery of regulating and supporting ecosystem services. Crop Science 59:441-459. https://doi.org/10.2135/cropsci2018.09.0594

Souza Sobrinho, F.; Borges, V.; Lédo, F. J. S. and Kopp, M. M. 2010. Repetibilidade de características agronômicas e número de cortes necessários para seleção de *Urochloa ruziziensis*. Pesquisa Agropecuária Brasileira 45:579-584. https://doi.org/10.1590/S0100-204X2010000600007

Steiner, M. G.; Weiler, R. L.; Brunes, A. P.; Mills, A.; Dall'Agnol, M.; Nabinger, C., Motta, E. A. M.; Silveira, D. C.; Sampaio, R. and Tessis, G. 2022. Characterization and genetic diversity in *Paspalum notatum* Flügge accessions: Morphological and geographical distance. Revista Brasileira de Zootecnia 51:e20220015. https://doi.org/10.37496/rbz5120220015

Teixeira, V. I.; Dubeux Jr, J. C. B.; Mello, A. C. L.; Lira Jr, M. A.; Lira, M. A. and Saraiva, F. M. 2011. Repetibilidade de variáveis produtivas e qualitativas da forragem e da excreta bovina em pastagem de braquiária. Pesquisa Agropecuária Brasileira 46:655-662. https://doi.org/10.1590/S0100-204X2011000600012

Toebe, M.; Cargnelutti Filho, A.; Mello, A. C.; Souza, R. R.; Soares, F. S.; Silva, L. S. and Segatto, A. 2020. Plot size and replications number for triticale experiments. Ciência Rural 50:e20200222. https://doi.org/10.1590/0103-8478cr20200222

Torres, F. E.; do Valle, C. B.; Lempp, B.; Teodoro, P. E.; Santos, A. and da Silva Junior, C. A. 2015. Minimum number of measurements for accurate evaluation of qualitative traits in *Urochloa brizantha*. Journal of Agronomy 14:180-184. https://doi.org/10.3923/ja.2015.180.184

Valls, J. F. M. 1987. Recursos genéticos de espécies de *Paspalum* no Brasil. p.3-13. In: Anais do Encontro Internacional sobre Melhoramento Genético de *Paspalum*. Instituto de Zootecnia, Nova Odessa.

Vela-Cardenas, M. and Frey, K. J. 1972. Optimum environment for maximizing heritability and genetic gain from selection. Iowa State Journal of Science 46:381-394.

Wang, X.; Yan, M.; Wang, X.; Wu, Z.; Zhou, J.; Wang, C.; Chen, R.; Qin, X.; Yang, H.; Wei, H. and Gu, W. 2022. The phenotypic diversity of *Schisandra sphenanthera* fruit and SVR model for phenotype forecasting. Industrial Crops and Products 186:115162. https://doi.org/10.1016/j.indcrop.2022.115162

Weiler, R. L.; Dall'Agnol, M.; Simioni, C.; Krycki, K. C.; Dahmer, N. and Guerra, D. 2017. Determination of the mode of reproduction of bahiagrass hybrids using cytoembryological analysis and molecular markers. Revista Brasileira de Zootecnia 46:185-191. https://doi.org/10.1590/S1806-92902017000300002

Weiler, R. L.; Dall'Agnol, M.; Simioni, C.; Krycki, K. C.; Pereira, E. A.; Machado, J. M.; Motta, E. A. M. 2018. Intraspecific tetraploid hybrids of *Paspalum notatum*: agronomic evaluation of segregating progeny. Scientia Agricola 75:36-42. https://doi.org/10.1590/1678-992X-2016-0354

Zanata, M.; Freitas, M. L. M.; Silva, M. T.; Morais, E.; Zanatto, A. C. S. and Sebbenn, A. M. 2010. Parâmetros genéticos e ganhos na seleção em teste de progênies de polinização aberta de *Eucalyptus pellita*, Batatais–SP. Revista do Instituto Florestal 22:233-242.