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CROP SCIENCE

REML/BLUP methodology for selection intraspecific hybrids of *Paspalum notatum* Flügge by multivariate analysis

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Abstract: The *Paspalum* genus has potential for further genetic improvement because of its adaptability to different ecosystems and production of high yields for grazing livestock. We estimate the genetic parameters of 195 intraspecific *P. notatum* hybrids using Restricted Maximum Likelihood (REML), followed by selection based on Best Linear Unbiased Prediction (BLUP) through multivariate analysis. The intraspecific hybrids studied showed considerable genetic variability in the evaluated forage traits, displaying their potential for progression in subsequent stages of the genetic improvement program. Notably, plant height emerged as an important trait for indirect selection to enhance forage production. The use of the REML/BLUP procedure proves to be a robust tool for data analysis, particularly for perennial species. Furthermore, multivariate analysis based on BLUPs should be used in the selection process within breeding programs. Based on the BLUP values, hybrids D3, D16, C17, C2 and B17 were identified as superior for forage production, and they hold promise for future breeding programs for future breeding initiatives aimed at direct selection to improve yield.

Key words: Bahiagrass, best linear unbiased prediction, genetic correlation, genetic parameters, heritability, restricted/residual maximum likelihood.

INTRODUCTION

In South America, the *Paspalum* genus includes many species with high potential forage production and nutritional quality (Sartor et al. 2011). This diversity stems from the existence of different modes of reproduction and ploidy levels within the genus (Ortiz et al. 2013). Within the southern region of Brazil, these species form an integral part of the natural grasslands in the Pampa biome, recognized for their exceptional foraging potential (Steiner et al. 2017). Moreover, these species exhibit substantial scope for genetic enhancement, as highlighted by previous studies (Motta et al. 2017), owing to their favorable forage traits suitable for animal production and an adaptability to different ecosystems (Novo et al. 2016). Additionally, the utilization of native forage species in pastoral agriculture systems not only contributes to the stability and conservation of natural resources but also reduces the costs and risks associated with livestock production, ultimately fostering long-term sustainability (Gasparetto et al. 2021).

Paspalum notatum Flügge, a native perennial grass species in South America, holds significant prominence within the genus (Chen et al. 2022). Its distribution primarily encompasses tropical and subtropical regions (Silveira et al. 2014), where is has greater economic importance (Fachinetto et al. 2021), particularly in terms of forage utilization and ground cover (Blount & Acuña 2009, Wawu et al. 2021). This grass species is renowned for its high forage yields (Steiner et al. 2017, Machado et al. 2019), making it a valuable resource. Recently, Motta et al. (2021) demonstrated that when intercropped with legumes, the dry matter production of *P. notatum* mixture was comparable to that of a monoculture fertilized with 240 kg N ha^{-1}, further emphasizing its potential for enhanced productivity.

Considering the substantial economic importance and remarkable forage potential of this species, the genetic improvement programs for forage species, including this particular one, they should include several critical steps. These steps encompass the selection of parental plants to generate genetic variability and the identification of desirable recombinants with specific traits (Resende et al. 2013, Asfaw et al. 2021). Therefore, it becomes essential to comprehend the genetic variability, heritability, and genetic correlation among the target traits, enabling the selection of superior genotypes (Majidi et al. 2009, Fogaça et al. 2012). In the context of pastoral forages, where economically important traits such as forage production are genetically complex with quantitative inheritance and influenced by genotype × environment interactions (Amini et al. 2013, Saeidnia et al. 2020), the genetic improvement of native forage species. Presents a sustainable alternative for optimizing livestock production (Silveira et al. 2022a).

The adoption of more efficient and robust statistical methodologies holds immense importance in guiding the process of genetic improvement, especially in perennial species (Capistrano et al. 2021). Accurate estimation of genetic parameters, which yield reliable predictions and information on genetic values, is crucial for the success of plant breeding programs (Resende 2016). Therefore, the combined use of Restricted Maximum Likelihood (REML) and Best Linear Unbiased Prediction (BLUP) emerges as the most effective approach for estimating genetic parameters and predicting genotypic values (Piepho et al. 2008, Faville et al. 2018). In the analysis of perennial plants, the REML/BLUP methodology is considered standard practice due to its accuracy (Silveira et al. 2022b), even in the context of unbalanced experimental designs (Piepho et al. 2008, Abu-Ellail et al. 2018). Recently, the REML/BLUP procedure was employed to determine genetic parameters and predict genotypic gain in forage traits of *P. notatum* (Marcón et al. 2021, Silveira et al. 2022b).

The objective of this study was to estimate the genetic parameters of a population consisting of intraspecific hybrids of *P. notatum* using REML and subsequently conduct selection based on BLUP through multivariate analysis.

MATERIALS AND METHODS

Experimental site

The experiment was conducted in the municipality of Eldorado do Sul, Rio Grande do Sul, Brazil (latitude 30°29'26'' S, longitud 51°06'42'' W, altitude 62 m above sea level). The local climate is classified as (Cfa) according to the Köppen classification (Moreno 1961), characterized as subtropical with no distinct dry season, and the average air temperature of the hottest month exceeds 22 °C. The long-term (1970-2009) average minimum and maximum annual air temperatures in the region

Table I. Comparison of average monthly minimum (min) and maximum (max) temperature (°C) and rainfall (mm) during the experimental period (December 2010- March 2012) with the 40-yr average (1970-2009).

Month	Temperature (°C, min-max)				Rainfall (mm)			
	2010	2011	2012	40-yr avg.	2010	2011	2012	40-yr avg.
Jan	-	20.7-30.8	17.6-30.4	19.3-30.2	-	130	43	106
Feb	-	19.7-29.0	19.5-31.6	19.0-29.4	-	259	137	106
Mar	-	17.2-27.4	14.8-28.9	18.0-28.2	-	101	112	102
Apr	-	13.3-25.3	-	14.5-25.2	-	172	-	110
May	-	9.4-20.9	-	11.2-21.8	-	30	-	108
Jun	-	7.0-18.0	-	8.7-18.6	-	116	-	154
Jul	-	6.8-17.0	-	8.5-18.7	-	246	-	144
Aug	-	8.1-17.3	-	9.7-20.2	-	221	-	164
Sep	-	9.1-22.1	-	11.2-21.4	-	85	-	142
Oct	-	12.9-24.5	-	13.8-24	-	133	-	129
Nov	-	13.4-27.5	-	15.4-26.6	-	40	-	109
Dec	16.5-28.8	15.8-28.6	-	17.5-29.0	99	56	-	111

were 14.0 °C and 24.2 °C, respectively (Table I), resulting in an average annual air temperature of 19.6 °C. The average annual rainfall in the area is 1400 mm. Detailed information on the average monthly minimum and maximum air temperature, as well as rainfall during the experimental period, is provided in Table I.

The soil at the experimental site was classified as an Ultisol according to the USDA Soil taxonomy (Santos et al. 2018b). Prior to establishment the experiment, soil samples were collected from a depth of 0-0.2 m. Test results revealed the following parameters: clay content of 15%, pH (H_2O) of 5.4, pH measured using the SMP method of 6.3, phosphorus(P) level of 15.6 mg dm^{-3}, potassium (K) level of 151.4 mg dm^{-3}, and organic matter content of 2.7%. Fertilizer requirements were determined based on the recommendations of the Soil Chemistry and Fertility Commission (CQFS 2004). Urea, containing 46% nitrogen (N), was applied at a rate equivalent to 160 kg N ha^{-1}.

Plant material and experimental design

The plant material for this study consisted of three female tetraploid sexual genotypes, namely C44X (Quarin et al. 2001), Q4188 and Q4205 (Quarin et al. 2003), obtained from the Botanical Institute of Northeast Argentina (IBONE), Corrientes, Argentina. These genotypes were crossed with two male parent apomictic ecotypes, 'Bagual' and 'André da Rocha', which are elite tetraploid germplasm native to the state of Rio Grande do Sul (Table II). The crosses were performed using the methodology described by Burton (1948) and later adapted by Weiler et al. (2018) to produce hybrid progeny. The reproductive mode was determined following the approach of Weiler et al. (2017). A total of 195 genotypes of *P. notatum* were evaluated, which included 189 hybrids, the female parents (C44X, Q4188 and Q4205), male parents ('André da Rocha' and 'Bagual'), and the commercially available cultivar 'Pensacola', which served as a control.

Seeds were germinated on Germitest paper-lined Petri dishes in a germination chamber under controlled temperature and day length condition: 8 h of light at 30 °C and 16 h of darkness at 20 °C. Germinated seedlings were transplanted into honeycomb trays until they had five fully expanded

Table II. Female and male tetraploid parents and hybrids of *Paspalum notatum* evaluated.

Female parent	Male parent	Family	N° hybrids[1]	Hybrid ID
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
C4-4X	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
C4-4X	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

Note: [1]189 hybrids in total.

leaves. Seedlings were then transplanted into pots filled with Carolina Soil™, a commercial substrate composed of peat, vermiculite, organic residue and limestone. When the plants had four or more tillers, the tillers were separated into four different pots to obtain four clones, which served as replicates in the field.

The field experiment followed a randomized complete block design with four replicates and was established at the UFRGS (Universidade Federal do Rio Grande do Sul) Experiment Station. The clones were transplanted into the field with a spacing of 1.0 m within and between rows on 11/26/2010. Sprinkler irrigation was applied after sowing to facilitate seedling establishment.

Procedures and traits

Throughout the 2-year evaluation period, a total of five cuts were performed on the following dates: 1st cut on 02/22/2011, 2nd cut on 04/06/2011, 3rd cut on 11/17/2011, 4th cut on 01/09/2012, and 5th cut on 03/16/2012. Various traits were quantified, including plant height (PH, cm), tiller population density (TPD, tiller plant^{-1}), leaves dry mass (LDM, g plant^{-1}), stem dry mass (SDM, g plant^{-1}), inflorescence dry mass (IDM, g plant^{-1}), total dry mass (TDM, g plant^{-1}), and growth habit (GH). Non-destructive observations were made before each cutting event.

Plant height was measured from the soil surface to the curvature of the leaves, while TPD was determined by counting all tillers with expanded leaves. Growth habit (GH) was classified on a scale of 1 to 5, where 1 represented a prostrate habit and 5 represented an erect habit. Plants were cut when they reached an average height of 20 cm, leaving a residual height of 5 cm. After cutting, the harvested material was sorted into morphological components: leaves (leaf blades), stems (including stems and sheaths), and inflorescences. The samples were then dried in an oven at 60 °C until constant weight was achieved. The leaf-to-stem ratio (LSR) was subsequently calculated based on the LDM and SDM values.

Statistical analysis

The estimation of variance components and prediction of breeding values were performed using the Restricted Maximum Likelihood (REML) and Best Unbiased Linear Prediction (BLUP) methodology. Furthermore, the genetic correlation (r) between forage characters was estimated by utilizing genotypic values (hybrid means estimated by BLUP). The correlation matrix was generated using the ''corrplot'' statistical package (Wei et al. 2017) within the R environment (R Core Team 2019).

The statistical analysis was performed using a complete randomized block model, which considered data from an individual location, multiples harvests, and one observation per plot. The model used in this study can be represented as:

$$y = X_r + Z_g + W_p + T_i + e$$

Where: *y* is the data vector; *r* is the vector of the effects of the measurement-repetition combinations (assumed to be fixed) added to the overall mean; *g* is the vector of the genotypic effects (assumed to be random); *p* is the vector of permanent environment effects (plots in this case) (random); *i* is the vector of the effects of the genotypes x measurements interaction, and e is the vector of errors or residuals (random). The capital letters represent the incidence matrices for the aforementioned effects.

The mixed model equations are equivalent to:

$$\begin{bmatrix} X'X & X'Z & X'W & X'T \\ Z'X & Z'Z + I^{-1}\lambda 1 & Z'W & Z'T \\ W'X & W'Z & W'W + I^{-1}\lambda 2 & W'T \\ T'X & T'Z & T'W & T'T + I^{-1}\lambda 3 \end{bmatrix} \begin{bmatrix} \tilde{m} \\ \tilde{g} \\ \tilde{p} \\ \tilde{i} \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \\ W'y \\ T'y \end{bmatrix}$$

In which:

$\lambda_1 = \frac{1-\rho}{h^2} = \frac{\hat{\sigma}^2_e}{\hat{\sigma}^2_g}$; $\lambda_2 = \frac{1-\rho}{c^2} = \frac{\hat{\sigma}^2_e}{\hat{\sigma}^2_c}$; $\lambda_3 = \frac{1-\rho}{p^2} = \frac{\hat{\sigma}^2_e}{\hat{\sigma}^2_p}$;

Individual heritability in the broad sense within the block is given by $h^2 = \frac{\hat{\sigma}^2_g}{\hat{\sigma}^2_g + \hat{\sigma}^2_c + \hat{\sigma}^2_p + \hat{\sigma}^2_e}$

The individual repeatability in the block is given by $\rho = \frac{\hat{\sigma}^2_g + \hat{\sigma}^2_c + \hat{\sigma}^2_p}{\hat{\sigma}^2_g + \hat{\sigma}^2_c + \hat{\sigma}^2_p + \hat{\sigma}^2_e}$

The coefficient of determination of the permanent effects of the plot is given by $P^2 = \frac{\hat{\sigma}^2_p}{\hat{\sigma}^2_g + \hat{\sigma}^2_c + \hat{\sigma}^2_p + \hat{\sigma}^2_e}$

The common environmental correlation between plots is given by $c^2 = \frac{\hat{\sigma}^2_c}{\hat{\sigma}^2_g + \hat{\sigma}^2_c + \hat{\sigma}^2_p + \hat{\sigma}^2_e}$

The iterative estimators of the variance components in REML were obtained using the Expectation-Maximization (EM) algorithm (Dempster et al. 1977).

$$\hat{\sigma}^2_e = \left[y'y - \widehat{m}'X'y - \hat{g}'Z'y - \hat{p}'W'y - \hat{i}'T'y\right]/[N - r(X)]$$

$$\hat{\sigma}^2_g = \left[\hat{g}'^{-1}\hat{g} + \hat{\sigma}^2_e \, tr(I^{-1}C^{22})\right]/q$$

$$\hat{\sigma}^2_p = \left[\hat{p}'p + \hat{\sigma}^2_e \, tr \, C^{33}\right]/s$$

$$\hat{\sigma}^2_i = \left[\hat{i}'i + \hat{\sigma}^2_e \, tr \, C^{44}\right]/q$$

In which C^{22}, C^{33} and C^{44} comes from:

$$C^{-1}=\begin{bmatrix} C_{11} & C_{12} & C_{13} & C_{14} \\ C_{21} & C_{22} & C_{23} & C_{24} \\ C_{31} & C_{32} & C_{33} & C_{34} \\ C_{41} & C_{42} & C_{43} & C_{44} \end{bmatrix}^{-1} = \begin{bmatrix} C^{11} & C^{12} & C^{13} & C^{14} \\ C^{21} & C^{22} & C^{23} & C^{24} \\ C^{31} & C^{32} & C^{33} & C^{34} \\ C^{41} & C^{42} & C^{43} & C^{44} \end{bmatrix}$$

where: C = matrix of coefficients of the mixed model equations; tr = matrix trace operator; r(X) = rank of matrix X; N = total number of data; q = number of individuals; s = number of genotype x harvests. The variance components associated with the model effects correspond to: $\widehat{h}^2_g = \frac{\hat{\sigma}^2_g}{\hat{\sigma}^2_f}$ = heritability of individual plots in the broad sense, that is, of total genotypic effects; C^2_p = determination coefficient of plot effects.

$C^2_{gm} = \frac{\hat{\sigma}^2_{int}}{\hat{\sigma}^2_f}$ = coefficient for determining the effects of the genotypes x measurements interaction;

$r_{gmed} = \frac{\hat{\sigma}^2_g}{(\hat{\sigma}^2_g + \hat{\sigma}^2_{int})}$ genotypic correlation through measurements

$h^2_{mg} = \frac{\hat{\sigma}^2_g}{\hat{\sigma}^2_g + \left(\frac{\hat{\sigma}^2_i}{b}\right) + \left(\frac{\hat{\sigma}^2_r}{nb}\right)}$ genotype mean heritability; n: number of plots; b: number of blocks

$\hat{r}_{gg} = \sqrt{h^2_{mg}}$ = accuracy in genotype selection

$CV_g\ (\%) = \frac{\sqrt{\hat{\sigma}^2_g}}{\mu} * 100$ = coefficient of genotypic variation

$CV_e\ (\%) = \frac{\sqrt{\hat{\sigma}^2_e}}{\mu} * 100$ = coefficient of environmental variation

$CV_r = \frac{CVg}{CVe}$ = relative variation coefficient

The gain calculated via selection between genotypes was given by

$$\text{Gain } (\%) = 100 \text{ x } \left(\frac{GAs - OGm}{OGm}\right)$$

where GAs is the genotypic mean of the selected and OGm is the general genotypic mean.

The genetic divergence among the cultivars was estimated using the genetic distances matrix of Mahalanobis (Resende 2007). Predicted values were obtained from the variance and covariance matrix of these genetic values, calculated as follows: $D^2_{ii'}$=δ'Gδ, where $D^2_{ii'}$ represents the Mahalanobis distance between genotypes *i* and *i'*, G is the matrix of genotypic variance and covariance, δ is the vector $[d_1, d_2, \ldots d_j]$, with $d_j = Y_{ij} - Y_{i'j}$, and Y_{ij} represents the mean of the *i*-th genotype in relation to the *j*-th variable.

Grouping of genotypes was performed using the hierarchical method unweighted pair group method with arithmetic mean (UPGMA) and Tocher's Optimization method (Rao 1952). The importance of forage characteristics was evaluated using the methodology of Singh (1981), which assesses the total observed dissimilarity for each characteristic, estimated through the participation of the components of the generalized Mahalanobis distance (D^2).

Principal component analysis (PCA) was conducted to eliminate characteristics with less importance based on the criterion of Jolliffe's criterion (1972, 1973). This method identifies variables with greater weight in the last components of lesser importance. The criteria for discarding the main components was set at 80%. These methodologies were employed to assess similarities between the variables with lower participation according to Singh's method (1981) and the variables discarded by the PCA analysis.

All analyzes were conducted using the SELEGEN-REML/BLUP genetic-statistical computational application developed by Resende (2016) and the GENES software package (Cruz 2016) for obtaining multivariate analyzes.

RESULTS

Deviation analysis indicated that all traits exhibited a significant genotypic effect, as determined by the likelihood ratio test (LRT) at a 1% probability level (Table III). This finding confirmed the existence of genetic variability among the hybrids evaluated. The genotype x environment (GxE) interactions were also found to be significant (p<0.01) for all traits studied, except for LSR. This suggests that hybrid selection strategies can be employed across both years to achieve genetic gains.

The experimental variation coefficient (CV_e) ranged from 16.9% for PH to 295% for LSR, indicating substantial variation in trait measurements within the experimental setup. The coefficients of genetic variation (CV_g) ranged from 13.2% for GH to 78.8% for LSR. Notably, only the PH trait displayed a higher genetic variation coefficient (CV_g; 22.1%) compared to the experimental variation coefficient (CV_e; 16.9%), indicating a dominant role of genetic effects in determining this trait. Consequently, the

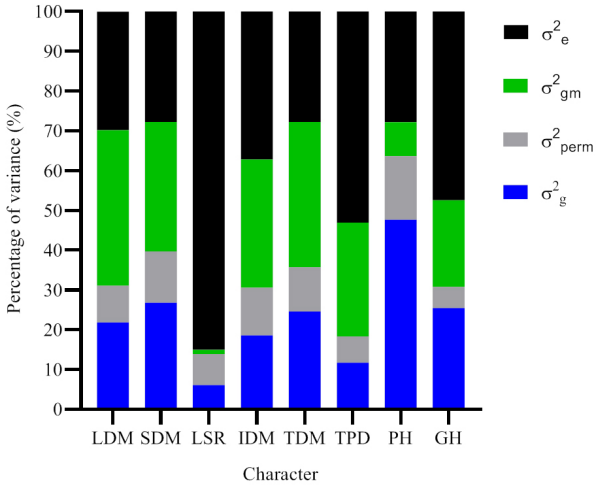

Figure 1. Decomposition of variance components for mixed model of forage characters. σ^2_e: environmental variance; σ^2_{gm}: variance of genotypes x measurements interaction; σ^2_{perm}: permanent ambient variance; σ^2_g: genotypic variance. LDM: Leaves dry mass; SDM: stem dry mass; LSR: leaf: stem ratio; IDM: inflorescence dry mass; TDM: total dry mass; TPD: tiller population density; PH: plant height; GH: Growth habit.

Table III. Principle components (PC), estimates of variances (eigenvalue λj), percentage of variance explained by components (importance %) and accumulated variance (% accumulated) of accessions of *Paspalum notatum*.

Importance (%)	% Accumulated	Highlights	Recomendation*
60.67	60.67	TDM	
17.15	77.82	GH	
11.69	89.51	LSR	
6.41	95.92	IDM	Discard
2.10	98.03	PH	Discard
1.45	99.47	TDM	Discard
0.52	99.99	SDM	Discard
0.00	100.00	TDM	Discard

***According to the criterion of Jolliffe (1972). SDM: stem dry mass; LSR: leaf: stem ratio; IDM: inflorescence dry mass; TDM: total dry mass; PH: plant height; GH: growth habit.**

PH characteristic exhibited a favorable scenario for generating genetic gains among the evaluated hybrids.

The relative expressions of environmental (σ^2_e), genotype x harvest variance (σ^2_{gm}), permanent environment variance (σ^2_{perm}), and genotypic (σ^2_g) variance are depicted in Figure 1. The estimates of genotypic variances, compared to those of environmental, genotype x measurements, and permanent environment variances, provided evidence of genetic variability for the PH trait.

Regarding genotype x harvest variance (σ^2_{gm}), the traits LDM (39.1%), TDM (36.6%), SDM (32.6%), and IDM (32.3%) exhibited the highest percentage variance. This indicates that these traits were significantly influenced by interaction with the environment. For permanent environment variance (σ^2_{gm}), the traits PH (15.9%), SDM (12.9%), IDM (12.0%), and TDM (11.1%) showed the highest values, indicating substantial variation attributable to permanent environmental factors. Finally, the genetic variance for PH was estimated at 47.7%, making it the trait with the highest genetic variance. This was followed by SDM (26.8%), GH (25.4%), TDM (24.6%), and LDM (21.8%). These results highlight the contribution of genetic factors to the variation observed in these traits.

Once the variance components were obtained (Figure 1), several parameters were estimated. The heritability in the broad sense (H^2) was calculated, and it ranged from 0.06 (LSR) to 0.48 (PH) (Figure 2). These values supported the findings from the variance components analysis, indicating that environmental variance had a greater influence on the hybrids compared to genetic variance

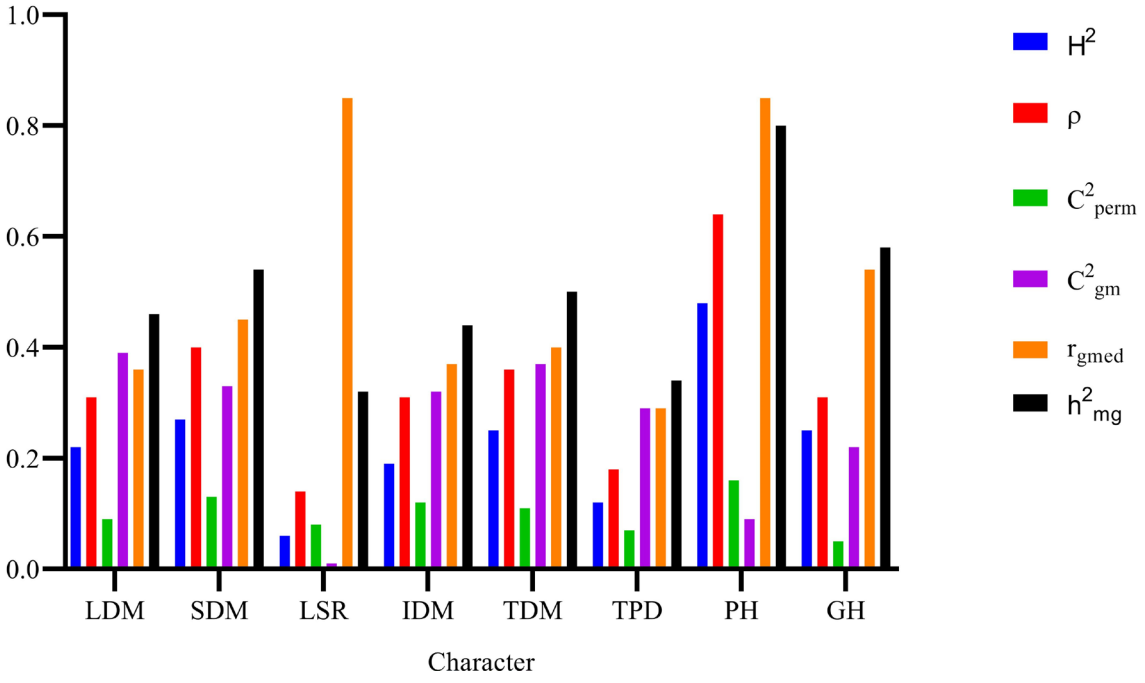

Figure 2. Estimation of variance components and genetic parameters for forage traits in intraspecific hybrids of *Paspalum notatum*. H^2: individual plot heritability in the broad sense, of total genotypic effects; ρ: repeatability at the plot level; C^2_{perm} = coefficient of determination of plot effects; C^2_{gm} = coefficient for determining the effects of the genotype x measurement interaction; r_{gmed} = genotypic correlation through measurements; h^2_{mg} = genotype mean heritability. LDM: Leaves dry mass; SDM: stem dry mass; LSR: leaf: stem ratio; IDM: inflorescence dry mass; TDM: total dry mass; TPD: tiller population density; PH: plant height; GH: Growth habit.

(Figure 1). The repeatability at the plot level (ρ) varied from 0.14 (LSR) to 0.64 (PH) (Figure 2). The coefficient of determination of plot effects (C^2_{perm}) ranged from 0.05 (GH) to 0.16 (PH) (Figure 2), providing insights into the contribution of permanent environmental effects to the overall variation. The coefficient for determining the effects of the genotype x measurement interaction (C^2_{gm}) ranged from 0.01 (LSR) to 0.39 (LDM), indicating the extent to which the interaction between genotypes and measurements influenced the trait variation. The genotypic correlation through measurements (r_{gmed}) ranged from 0.29 (TPD) to 0.85 (LSR), reflecting the level of consistency in the performance of genotypes across different measurements. Finally, the mean genotype heritability (h^2_{mg}) ranged from 0.32 (LSR) to 0.80 (PH) (Figure 1). This parameter represents the proportion of phenotypic variation attributed to the genetic effects of individual genotypes, indicating their potential for transmitting desirable traits to the next generation.

The genetic correlation coefficients between forage traits are presented in Figure 3. Total dry matter production (TDM) exhibited strong positive correlations with LDM (r = 0.95, p<0.01), TPD (r = 0.91, p<0.01), SDM (r = 0.87, p<0.01), and IDM (r = 0.80, p<0.01). It also showed a moderate correlation with PH (r = 0.67, p<0.05). The correlation between TDM and LSR was negative (r = -0.24), but it was not statistically significant (Figure 3). Leaf dry matter (LDM) exhibited strong positive correlations with the TPD trait (r = 0.86, p<0.01) and moderate correlations with SDM (r = 0.68, p<0.05), PH (r = 0.68, p<0.05), and IDM (r = 0.60, p<0.05). Plant height (PH), which showed significant genetic control (Figure 1) and high heritability (Figure 2), had moderate correlations with LDM (r = 0.68, p<0.05), TDM (r = 0.67, p<0.05), and TPD (r=0.54, p<0.05) (Figure 3).

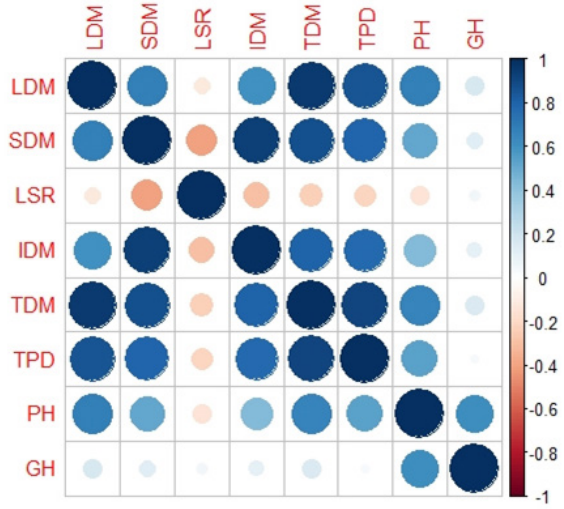

Figure 3. Genotypic correlation between eight forages traits of 195 *Paspalum notatum* intraspecific hybrids. LDM: Leaves dry mass; SDM: stem dry mass; LSR: leaf: steam ratio; IDM: inflorescence dry mass; TDM: total dry mass; TPD: tiller population density; PH: plant height; GH: Growth habit.

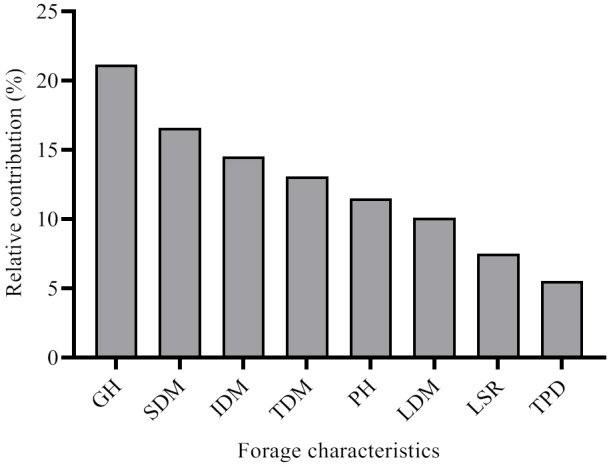

Figure 4. Relative contribution of forage traits to the genetic diversity in 195 *Paspalum notatum* intraspecific hybrids, based on the Mahalanobis (D^2) genetic distance. LDM: Leaves dry mass; SDM: stem dry mass; LSR: leaf: steam ratio; IDM: inflorescence dry mass; TDM: total dry mass; TPD: tiller population density; PH: plant height; GH: Growth habit.

Estimates of the relative contribution of traits to genetic divergence ranged from 5.51 (TPD) to 21.13 (GD) (Figure 4). The traits GH (21.1%), SDM (16.6%), IDM (14.5%), and TDM (13.1%) exhibited the highest discrimination power among the genotypes evaluated (Figure 4). These four traits together contributed to 65.4% of the total genetic diversity, indicating that they are sufficient to quantify the genetic variability among *P. notatum* hybrids. On the other hand, PH, LDM, LSR, and TPD made smaller contributions, accounting for 34.6% of the total genetic diversity (Figure 4).

The first three principal components (PC1-PC3) explained 89.5% of the total variation across all evaluated traits (Table IV). The remaining principal components (PC4-PC8) had eigenvalues (λj) <0.7 (Table IV), indicating that variables with greater weight in these components of lesser importance can be discarded. Based on this analysis, it is recommended to exclude IDM, PH, TPD, SDM, and TDM be discarded from future genetic diversity studies, as they contribute little to discrimination between the studied hybrids (Table IV).

For the genetic distance matrix based on the BLUP values, two clustering methods were employed: the Tocher optimization method (Table V) and the hierarchical UPGMA method (Figure 5). The Tocher optimization method resulted in the identification of five groups (Table V), while the UPGMA hierarchical clustering method identified six groups (Figure 5). However, genotypes D3 (Group V) and

Table IV. Principle components (PC), estimates of variances (eigenvalue λj), percentage of variance explained by components (importance %) and accumulated variance (% accumulated) of accessions of *Paspalum notatum*.

PC	λj	Importance (%)	% accumulated	Highlights	Recomendation*
PC1	4.85	60.67	60.67	TDM	
PC2	1.37	17.15	77.82	GH	
PC3	0.94	11.69	89.51	LSR	
PC4	0.51	6.41	95.92	IDM	Discard
PC5	0.17	2.10	98.03	PH	Discard
PC6	0.12	1.45	99.47	TPD	Discard
PC7	0.04	0.52	99.99	SDM	Discard
PC8	0.00	0.00	100.00	TDM	Discard

***According to the criterion of Jolliffe (1972). SDM: stem dry mass; LSR: leaf: stem ratio; IDM: inflorescence dry mass; TDM: total dry mass; TPD: tiller population density; PH: plant height; GH: growth habit.**

Table V. Group composition based on Mahalanobis genetic (D^2) distance matrix using original Tocher optimization methods in *Paspalum notatum*.

Group	Hybrid IDs
I	E11; F8; E16; F14; F11; E4; B9; E1; E24; E12; B36; C44X; E6; F13; E19; E5; C30; E17; E15; B3; B41; A23; B21; F10; Pensacola; F23; F7; E14; E10; F3; E3; F26; B23; E22; A35; F9; F31; F33; F20; E7; E8; E13; B5; E20; D21; D27; B44; B34; F16; A26; A27; A38; A17; B1; C21; F1; A12; C36; A2; F6; A28; E18; A7; B31; A36; D14; B25; B18; E2; B42; A15; A32; B12; B20; E9; C29; A10; C7; D26; C13; A14; C10; C16; A8; D13; F5; C31; F27; Q4188; B10; F32; B33; B7; F18; B8; B27; D12; B30; D4; B40; C3; C34; B52; C26; D10; B38; F17; D19; B16; E21; F21; A33; C27; B32; B22; D9; D2; C20; A29; B14; D24; D8; C1; D5; F22; A18; D18; C19; C4; F25; A25; F2; B4; C25; B19; C14; C11; Q4205; C5; A16; C28; B2; D11; A11; B13; A21; D22; A31; C12; B11; D20; C35; A37; F30; D6; D7; A20; F28
II	Bagual; F15; F24; F29; B43; AR; F12; B35; F4; A13; C6; C32; B37; B17; C9; C2; C18; D16; C23; C8; C24; D25; C15; C17; D17; C22; B26; D1; B28; B29
III	B15; B39; A22; A24; B6
IV	D23
V	D3

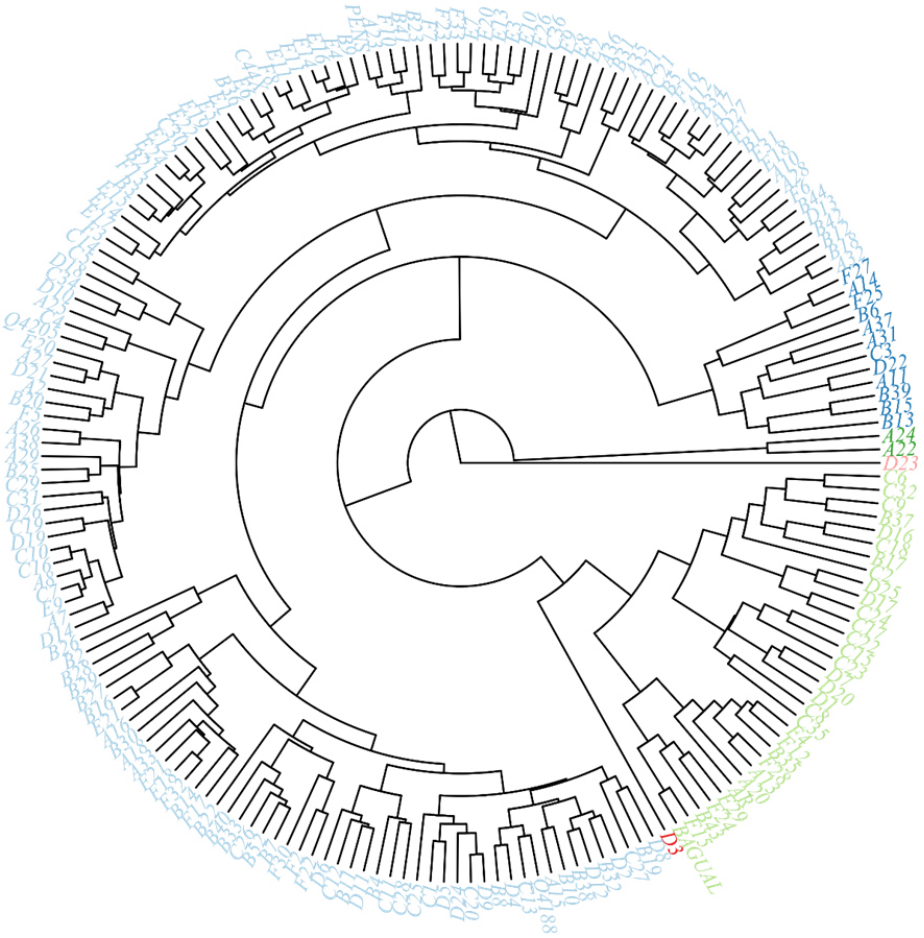

Figure 5. Dendrogram of genetic dissimilarity among hybrids of *P. notatum*, obtained by the UPGMA method, based on the Mahalanobis (D^2) genetic distance matrix.

D23 (Group IV) remained in separate groups regardless of the clustering method used. These two genotypes exhibited the highest and third-highest average TDM among all 195 hybrids evaluated. Group II, formed by Tocher's optimization method (Table V), had the second-highest average TDM. Groups V, II and IV, as identified by the Tocher optimization method, demonstrated the highest values for the commercially and agronomically important forage traits: TDM, LDM and PH (Table V). The cophenetic correlation coefficient, which measures the representativeness of the data within the dendrogram dissimilarity matrix, was 0.77. This coefficient indicates a satisfactory fit in the graphical representation of the dendrogram (Figure 5). Furthermore, the distortion and stress were calculated as 7.47% and 27.3%, respectively.

Based on estimates of genetic gains predicted via BLUP, a selection process was conducted to classify the best twenty genotypes, representing approximately 10% of the total genotypes evaluated (Table VI). For the LDM trait, the genetic gain (Gain; Table VI) ranged from 37.6 (C15) to 77.2 g plant^{-1} (D3). The D3 hybrid exhibited a 124% increase in the new average (ISG; Table VI). The top 20 hybrids, on average, showed a 60.4% increase in LDM compared to the average of the total studied population of 195 genotypes. Regarding the LSR trait, the genetic gain ranged from 11.4 (C4) to 26.8 g plant^{-1} (A24), with the A24 hybrid more than tripling the new average (ISG; Table VI). The TDM trait ranged from 59.7 (C15) to 105.3 g plant^{-1} (D3), and the D3 hybrid more than doubled the new average (ISG 119.7%; Table VI). Genetic gain for the TPD trait ranged from 32.6 (B26) to 102.2 tillers plant^{-1} (D23), with the D23

Table VI. Estimates predicted genetic gain (BLUP) for forage traits in *P. notatum* hybrids based on average performance of years of experiment.

	LDM (g plant^{-1})							SDM (g plant^{-1})						
Order	**Hybrids**	**g**	**u+g**	**Gain**	**$\bar{X}_{new}$**	**u+g+gem**	**ISG**	**Hybrids**	**g**	**u+g**	**Gain**	**$\bar{X}_{new}$**	**u+g+gem**	**ISG**
1	D3	77.21	139.37	77.21	139.37	208.63	124.19	Bagual	31.16	50.09	31.16	50.09	69.02	164.54
2	C17	49.56	111.73	63.38	125.55	156.18	79.72	F15	29.40	48.34	30.28	49.22	66.20	155.26
3	B26	47.12	109.29	57.96	120.13	151.56	75.80	C2	25.61	44.55	28.72	47.66	60.11	135.25
4	D16	45.89	108.06	54.95	117.11	149.22	73.82	F29	25.39	44.33	27.89	46.83	59.75	134.08
5	B29	44.72	106.89	52.90	115.07	147.00	71.93	C32	22.21	41.15	26.75	45.69	54.64	117.30
6	C24	41.95	104.11	51.07	113.24	141.74	67.47	B43	21.90	40.84	25.95	44.88	54.14	115.67
7	C18	40.44	102.61	49.56	111.72	138.88	65.05	F24	20.78	39.71	25.21	44.14	52.34	109.73
8	B17	40.21	102.38	48.39	110.55	138.45	64.68	F28	19.86	38.79	24.54	43.47	50.85	104.86
9	D17	38.02	100.19	47.24	109.40	134.30	61.17	F12	19.64	38.58	23.99	42.93	50.51	103.72
10	B28	35.10	97.26	46.02	108.19	128.74	56.45	D3	19.15	38.09	23.51	42.45	49.72	101.13
11	D25	34.02	96.19	44.93	107.10	126.70	54.72	C6	19.06	38.00	23.11	42.04	49.58	100.67
12	C9	32.91	95.08	43.93	106.10	124.60	52.94	AR	18.37	37.31	22.71	41.65	48.46	97.01
13	B2	32.75	94.92	43.07	105.24	124.29	52.68	D17	18.23	37.17	22.37	41.30	48.24	96.29
14	C2	31.54	93.70	42.25	104.41	121.99	50.73	A13	17.92	36.86	22.05	40.99	47.75	94.66
15	B43	31.51	93.68	41.53	103.70	121.95	50.69	C15	17.92	36.86	21.77	40.71	47.75	94.66
16	B37	31.35	93.52	40.89	103.06	121.64	50.43	D25	17.22	36.15	21.49	40.43	46.61	90.93
17	A18	25.55	87.72	39.99	102.16	110.64	41.10	D16	16.88	35.82	21.22	40.15	46.07	89.15
18	A16	24.63	86.79	39.14	101.30	108.88	39.61	B17	16.67	35.61	20.97	39.90	45.74	88.05
19	B6	23.84	86.01	38.33	100.50	107.39	38.35	F4	16.45	35.39	20.73	39.66	45.38	86.88
20	C15	22.63	84.80	37.55	99.71	105.10	36.41	C23	15.61	34.54	20.47	39.41	44.02	82.42

	LSR							IDM (g plant^{-1})						
Order	**Hybrids**	**g**	**u+g**	**Gain**	**$\bar{X}_{new}$**	**u+g+gem**	**ISG**	**Hybrids**	**g**	**u+g**	**Gain**	**$\bar{X}_{new}$**	**u+g+gem**	**ISG**
1	A24	26.79	37.32	26.79	37.32	39.76	254.57	Bagual	11.18	18.88	11.18	18.88	28.60	145.33
2	A22	24.72	35.24	25.75	36.28	37.50	234.85	F15	10.51	18.20	10.85	18.54	27.33	136.55
3	B39	17.71	28.23	23.07	33.60	29.85	168.25	F24	9.04	16.73	10.24	17.94	24.58	117.42
4	B15	16.30	26.82	21.38	31.90	28.31	154.83	B43	8.97	16.66	9.92	17.62	24.45	116.53
5	A37	13.87	24.40	19.88	30.40	25.66	131.82	AR	8.05	15.74	9.55	17.24	22.73	104.58
6	D22	13.41	23.94	18.80	29.32	25.16	127.45	C2	7.92	15.61	9.28	16.97	22.50	102.92
7	A11	13.11	23.63	17.99	28.51	24.83	124.54	F29	7.23	14.92	8.98	16.68	21.20	93.91
8	B6	13.08	23.61	17.37	27.90	24.80	124.31	D23	7.18	14.88	8.76	16.45	21.12	93.37
9	A31	12.31	22.83	16.81	27.34	23.96	116.95	C8	7.16	14.86	8.58	16.28	21.08	93.10
10	B13	10.51	21.04	16.18	26.71	22.00	99.89	B35	6.50	14.20	8.37	16.07	19.85	84.51
11	F25	10.38	20.90	15.65	26.18	21.85	98.63	F12	6.43	14.13	8.20	15.89	19.71	83.58
12	A25	8.14	18.66	15.03	25.55	19.40	77.30	F4	6.05	13.74	8.02	15.71	19.00	78.58
13	F27	6.93	17.46	14.40	24.93	18.09	65.89	F30	5.67	13.37	7.84	15.53	18.29	73.70
14	E2	6.91	17.43	13.87	24.39	18.06	65.63	B17	5.43	13.12	7.67	15.36	17.84	70.51
15	C3	6.25	16.78	13.36	23.89	17.35	59.39	C32	5.31	13.00	7.51	15.20	17.62	69.00
16	A14	6.16	16.69	12.91	23.44	17.25	58.54	D25	5.30	12.99	7.37	15.07	17.59	68.83
17	A36	6.06	16.58	12.51	23.03	17.13	57.55	C12	4.81	12.50	7.22	14.91	16.68	62.49
18	Q4205	5.65	16.18	12.13	22.65	16.69	53.73	F22	4.48	12.17	7.07	14.76	16.07	58.20
19	E8	4.48	15.01	11.72	22.25	15.42	42.59	C6	4.47	12.17	6.93	14.63	16.05	58.11
20	C4	4.36	14.88	11.36	21.88	15.28	41.41	A13	4.43	12.13	6.81	14.50	15.98	57.58

	TDM (g plant^{-1})							TPD (tillers plant^{-1})						
Order	**Hybrids**	**g**	**u+g**	**Gain**	**$\bar{X}_{new}$**	**u+g+gem**	**ISG**	**Hybrids**	**g**	**u+g**	**Gain**	**$\bar{X}_{new}$**	**u+g+gem**	**ISG**
1	D3	105.34	193.37	105.34	193.37	271.82	119.67	D23	102.22	187.50	102.22	187.50	312.17	119.85
2	D16	70.10	158.12	87.72	175.75	210.33	79.63	D3	41.47	126.76	71.84	157.13	177.33	48.62
3	C17	69.57	157.60	81.67	169.70	209.40	79.03	B17	40.40	125.69	61.36	146.65	174.96	47.37
4	C2	67.53	155.56	78.13	166.16	205.84	76.71	Bagual	33.10	118.38	54.30	139.58	158.75	38.81
5	B17	65.82	153.84	75.67	163.70	202.86	74.77	F15	31.90	117.19	49.82	135.10	156.09	37.40
6	D17	62.56	150.59	73.49	161.51	197.18	71.07	F29	31.43	116.72	46.75	132.04	155.05	36.85
7	B43	62.21	150.24	71.88	159.90	196.57	70.67	D16	30.62	115.90	44.45	129.73	153.25	35.90
8	Bagual	61.23	149.25	70.54	158.57	194.85	69.55	F24	29.59	114.88	42.59	127.88	150.97	34.70
9	C24	60.62	148.65	69.44	157.47	193.80	68.87	B43	29.59	114.88	41.15	126.43	150.97	34.70
10	D25	59.44	147.47	68.44	156.47	191.73	67.52	D17	28.99	114.28	39.93	125.22	149.64	34.00
11	C18	59.03	147.06	67.59	155.61	191.02	67.06	C18	27.11	112.40	38.77	124.05	145.47	31.79
12	F15	57.33	145.36	66.73	154.76	188.06	65.13	D25	26.86	112.15	37.77	123.06	144.90	31.49
13	F29	53.95	141.98	65.75	153.78	182.15	61.28	B37	26.39	111.68	36.90	122.18	143.86	30.94
14	B26	52.90	140.93	64.83	152.86	180.33	60.10	C24	26.13	111.42	36.13	121.42	143.29	30.64
15	B37	50.23	138.26	63.86	151.89	175.66	57.06	AR	26.00	111.29	35.45	120.74	143.01	30.49
16	F24	48.91	136.93	62.92	150.95	173.35	55.56	C6	25.79	111.08	34.85	120.14	142.53	30.24
17	C32	47.51	135.54	62.02	150.04	170.93	53.98	C32	24.38	109.67	34.23	119.52	139.40	28.59
18	C9	46.78	134.81	61.17	149.20	169.64	53.14	C2	24.12	109.41	33.67	118.96	138.83	28.29
19	C6	46.59	134.62	60.40	148.43	169.31	52.93	C17	24.08	109.37	33.17	118.45	138.74	28.24
20	C15	45.98	134.01	59.68	147.71	168.25	52.24	B26	21.56	106.85	32.59	117.87	133.14	25.28

	PH							GH (1 to 5)						
Order	(cm)	g	u+g	Gain	$\bar{X}_{new}$	u+g+gem	ISG	Hybrids	g	u+g	Gain	$\bar{X}_{new}$	u+g+gem	ISG
1	D17	8.04	23.07	8.04	23.07	23.80	53.50	C22	0.60	3.20	0.60	3.20	3.45	22.97
2	C24	7.88	22.91	7.96	22.99	23.62	52.39	D7	0.60	3.20	0.60	3.20	3.45	22.97
3	C22	7.81	22.85	7.91	22.94	23.55	51.98	C19	0.52	3.12	0.57	3.17	3.35	20.16
4	C15	7.53	22.56	7.81	22.85	23.24	50.08	C14	0.52	3.12	0.56	3.16	3.35	20.16
5	C17	7.29	22.33	7.71	22.74	22.98	48.52	D18	0.52	3.12	0.55	3.15	3.35	20.16
6	D3	7.18	22.21	7.62	22.65	22.86	47.75	D25	0.52	3.12	0.55	3.15	3.35	20.16
7	D25	7.11	22.14	7.55	22.58	22.78	47.31	C34	0.45	3.05	0.53	3.14	3.25	17.37
8	D1	7.03	22.06	7.48	22.52	22.69	46.75	D1	0.45	3.05	0.52	3.12	3.25	17.37
9	C5	5.56	20.59	7.27	22.30	21.09	36.97	D10	0.45	3.05	0.52	3.12	3.25	17.37
10	D16	5.46	20.49	7.09	22.12	20.98	36.30	D17	0.45	3.05	0.51	3.11	3.25	17.37
11	C18	5.42	20.45	6.94	21.97	20.94	36.07	D20	0.45	3.05	0.50	3.11	3.25	17.37
12	C35	5.27	20.31	6.80	21.83	20.78	35.08	B28	0.45	3.05	0.50	3.10	3.25	17.37
13	D20	5.06	20.09	6.66	21.70	20.54	33.63	C5	0.45	3.05	0.50	3.10	3.25	17.37
14	D6	4.82	19.85	6.53	21.56	20.29	32.08	D19	0.45	3.05	0.49	3.09	3.25	17.37
15	B28	4.69	19.72	6.41	21.44	20.14	31.18	C15	0.45	3.05	0.49	3.09	3.25	17.37
16	B29	4.59	19.62	6.30	21.33	20.03	30.52	C8	0.45	3.05	0.49	3.09	3.25	17.37
17	D7	4.49	19.52	6.19	21.22	19.92	29.85	C4	0.45	3.05	0.49	3.09	3.25	17.37
18	C8	4.42	19.45	6.09	21.12	19.85	29.41	A29	0.45	3.05	0.48	3.08	3.25	17.37
19	C14	4.39	19.43	6.00	21.03	19.82	29.23	A25	0.38	2.98	0.48	3.08	3.14	14.56
20	C23	4.22	19.25	5.91	20.95	19.63	28.09	B13	0.38	2.98	0.47	3.07	3.14	14.56

Notes: g: genotypic effects; u + g: predicted genotypic values, free of all interaction with years; G: gain; $\bar{X}_{new}$: new average; u + g + gem: average genotypic value in the years studied and capitalizes on an average interaction with all evaluated environments; ISG (%): individual gain of selection in relation to the general average of the genotypes in percentage. LDM: Leaves dry mass; SDM: stem dry mass; LSR: leaf: stem ratio; IDM: inflorescence dry mass; TDM: total dry mass; TPD: tiller population density; PH: plant height; GH: growth habit

hybrid raising the new mean by 119.9% (ISG; Table VI). Finally, the genetic gain for plant height (PH) ranged from 5.91 (C23) to 8.04 cm plant^{-1} (D17), with the hybrid D17 increasing the average by 53.5% (ISG; Table VI). The D3 hybrid ranked first for the LDM and TDM traits, second for TPD and sixth for PH (Table VI). The remaining characters were comparatively less important, and the results are provided for completeness in Table VI.

DISCUSSION

All variables, except for the leaf/stem ratio (LSR) trait, showed significant genetic effects and genotype-by-environment (GxE) interaction, indicating the presence of genetic variability among the hybrids (Table III). Notably, the plant height (PH) trait displayed a greater genetic influence compared to other traits (Figure 1), indicating its potential for inclusion in forage breeding programs. This underscores the genetic potential of the *P. notatum* population studied.

The coefficient of experimental variation (CV_e) is commonly used to assess experimental precision (Albuquerque et al. 2022). In this study, the estimated CVe values for the evaluated traits (Table II) exceeded the observed range in previous studies with *P. notatum* (Machado et al. 2021, Silveira et al. 2022b). Literature suggested that an increase in CV_e indicates greater phenotypic variation (Paw et al. 2020, Wang et al. 2022). Since expected gain are directly correlated with the existence and magnitude of genetic variation (Bush et al. 2013), the evaluated hybrids demonstrated significant variability (Table III). When selecting genotypes for breeding purposes, it is crucial to maximize genetic gain without reducing genetic variability (Santos et al. 2022). Here, the quantification of genetic (CV_g) and relative (CV_r) coefficients of variation can aid in designing future strategies and ensuring a successful selection within a breeding program (Paw et al. 2020, Riva et al. 2020).

The presence of CV_g values exceeding CV_e values indicates promising genetic gains for the PH trait, with a CV_r >1, and for the TDM trait, with a CV_r close to 1 (Table III). A CV_r values above 1 signifies greater certainty in the selection process (Silveira et al. 2022a). The population's variability consists of both hereditary characteristics represented by CV_g and non-hereditary characteristics represented by CV_e (Hamidou et al. 2018). These CV_g findings, expressed as a percentage of the overall mean for each trait, are crucial for understanding the genetic structure of the population, as they demonstrated the amount of variability present and allows for estimates of genetic gains.

The results of this study revealed a high level of genetic control, as indicated by the by genotypic variance $\left(\sigma_g^2\right)$ in Figure 1 and the mean genotype heritability $\left(h_{mg}^2\right)$ in Figure 2, in the *P. notatum* hybrids. This suggests the potential for achieving genetic gains through selection, particularly for the plant height (PH) and total dry matter (TDM) traits. Comparing the obtained results with the heritability scale established by Resende (2015), it can be expected that the hybrids would exhibit good genetic gains, given the substantial genetic control observed for the PH trait. However, for the other traits, there was a strong a strong influence of environmental factors, as depicted in Figure 1 and Figure 2, indicating that in addition to genetic factors, environmental conditions strongly influenced the performance of the hybrids (Santos et al. 2018a, Santos et al. 2022). The repeatability parameter (ρ) exceeded 40% only for the PH trait (Figure 2). According to Almeida et al. (2019), a repeatability value >40% suggest the possibility of identifying superior genotypes, considering the significant variance among treatments based on the average genotypic value.

In the context of forage production, the complexity arises from its dependence on multiple factors and their interactions. Therefore, understanding these interactions becomes crucial for the genetic improvement of any species (Bonilla et al. 2022). In this regard, it is essential to comprehend the traits closely associated with forage production for the selection of superior genotypes. Correlation coefficients play a significant role in indicating the relationship and nature of the association between the traits of interest for the breeding program (Thondaiman & Rajamani 2014). The results obtained for CV_r (Table II), σ_g^2 (Figure 1) and h_{mg}^2 (Figure 2) revealed a strong genetic control for the PH trait, suggesting the possibility of indirect selection to enhance forage production. Comparing the results obtained with the correlation scale established by Silveira et al. (2021), genetic correlations indicated moderate to strong positive associations between the PH trait and leaf dry matter (LDM), total dry matter (TDM), and tillers per plant (TPD) forage characteristics (Figure 3). As expected, direct selection based on TDM exhibited very strong associations with the LDM and TPD (Figure 3). The genetic correlation results (Figure 3) were highly consistent with those reported by Machado et al. (2021). It is worth noting that high positive genetic correlations can arise due to pleiotropy or genetic linkage, causing transient correlations, particularly in populations resulting from crosses between divergent parents (Falconer & Mackay 1996). These findings demonstrated the potential for indirect selection through the PH trait to increase TDM, given its strong genetic control within the studied population compared to the other traits (Figure 1).

Two methods were employed to assess the relative contribution of observed traits to genetic divergence. Singh's method (1981) identified four traits (GH, SDM, IDM and TDM; Figure 4) that made a significant contribution to discrimination among the evaluated hybrids. Subsequent principal component analysis (PCA) indicated that three traits (TDM, GH and LSR; Table III) would suffice to capture the greatest genetic dissimilarity among the hybrids. The disparity between the two methodologies underscores the importance of employing both approaches in studies focusing on characterization and genetic diversity (Steiner et al. 2022). Singh's method (1981) quantifies the "weight" of a variable in the composition of the Mahalanobis generalized distance matrix. Accordingly, this method considers highly variable traits as crucial and permits the exclusion of traits that contribute minimally to dissimilarity. This reduces the workload, time, and additional costs associated with data collection (Valadares et al. 2017). Singh's (1981) method has been previously used in *P. notatum* evaluations (Machado et al. 2021, Steiner et al. 2022) to identify forage production and morphological traits responsible for greater discrimination among the studied genotypes. Conversely, PCA analysis eliminates variables that carry greater "weight" in the less important components (Jolliffe 1972, 1973). Jolliffe's pioneering work (1972, 1973) focused on character discards. The author examined four discard methods using simulated (Jolliffe 1972) and real (Jolliffe 1973) data and concluded that the procedure was satisfactory when the number of discarded traits equaled the number of principal components with eigenvalues <0.7. Based on this criterion, components PC3-PC8 (Table III) were discarded in this study.

The utilization of phenotypic traits to assess genetic variability is the oldest, direct, and most practical method employed in breeding programs (Wang et al. 2022). When combined with multivariate analyzes, these traits have become routine approaches in genetic improvement programs, particularly for the selection of divergent parents (Leite et al. 2018). Principal component analysis and cluster analysis are considered the primary multivariate statistical tools utilized to

evaluate genetic dissimilarity based on phenotypic traits (Denwar et al. 2019, Boutsika et al. 2021). In order to quantify the dissimilarity among the studied hybrids, a cluster analysis was conducted, as the formation of groups is crucial for parent identification, especially in recommending superior genotypes. Parent selection can rely on the magnitude of dissimilarity among hybrids for the traits of interest. In this evaluation, two types of grouping were performed. Cluster analysis using the Tocher optimization method (Table IV) revealed a high concentration of hybrids in Group I, encompassing 81.0% of the genotypes studied. Group II contained 15.4%, Group III 2.56%; while Groups IV and V contained a single genotype each, representing 0.51% of the total number of genotypes evaluated. The UPGMA hierarchical grouping method exhibited high concentration in Group I (75.4% of the genotypes), followed by Group V (16.4%), Group II (6.15%), Group III (1.02%), and Groups IV and VI with 0.51% (Figure 5). Interestingly, a greater number of groups was expected given the large number of genotypes evaluated. The data from the Mahalanobis genetic matrix (D2) demonstrated a satisfactory fit in the dendrogram (Figure 5). Silveira et al. (2022b) suggested that a cophenetic correlation index above 0.70 indicates satisfactory results. The high concentrations of genotypes assigned to the same group indicates a high level of similarity among those genotypes (Silveira et al. 2021, Steiner et al. 2022). The concurrent use of different grouping methods should be considered standard practice to enhance genotype discrimination (Sant'Anna et al. 2021, Silveira et al. 2022b). By employing different multivariate methods, the accuracy of the results is improved (Azevedo et al. 2015), which is advantageous within a breeding program.

The identification of the best parents for future crosses is crucial for the success of a breeding program (Marostega et al. 2021). For selection of new genotypes, Best Linear Unbiased Prediction (BLUP) is a method known for shrinking estimators towards the mean, reducing their variance while increasing their predictive accuracy (Robinson 1991). The genotypes values obtained using the u + g + gem criterion are higher due to the incorporation of the average interaction (Capistriano et al. 2021), which is why we chose to use this criterion. Resende & Barbosa (2006) described the genotypic value, which combines the genotypic effect and the general mean, as the best parameter to explain the superiority of a particular cross. In our study, the top twenty most productive genotypes were selected for the eight forage traits under investigation (Table IV). To enhance forage production, we recommend selecting hybrids D3, C17, B26, D16, and B29 for increase LDM; A24, A22, B39, B15, and A37 for improvements in LSR; D3, D16, C17, C2, and B17 for enhanced forage yield (TDM). Hybrids D23, D3, B17, Bagual, and F15 offer opportunities for increased TPD, while hybrids D17, C24, C22, C15, and C17 could contribute to an improved PH. These genotypes will be prioritized for future stages of the breeding program, as they rank among the top ten for the most important forage traits (Table V). Among these superior hybrids, the D3 hybrid shows the most promise as it performed well across multiple key forage traits.

The presence of genetic variability in forage production indicates a high potential for genetic improvement of important forage traits by selecting from ranked hybrids in future crosses. The average genotype heritability was found to be higher for the PH character. Considering this and the associated genetic correlations, it is suggested that indirect selection via PH could lead to increased forage yield. Multivariate analysis methods have demonstrated their effectiveness in identifying superior genotypes, and based on the results obtained, it is recommended to use two or more multivariate techniques in studies of genetic diversity and/or for the selection of superior genotypes.

The use of REML/BLUP is a powerful tool in perennial forage plant improvement programs, as it allows for the estimation of genetic parameters and the identification of superior genotypes through predicted genetic values. Based on the BLUP values, the hybrids D3, D16, C17, C2, and B17 were identified as superior for forage production, and they could be incorporated into breeding programs for future crosses aimed at direct selection for this trait.

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