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Genetic Parameters, Prediction of Gains and Intraspecific Hybrid Selection of *Paspalum notatum* Flügge for Forage Using REML/BLUP

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Abstract: Genetic improvement of native forage species is a sustainable alternative for maximizing livestock production. *Paspalum notatum* Flügge is the most important forage grass in the native grasslands of southern Brazil, with substantial potential available for further genetic improvement. The objective of this study was to quantify a range of genetic parameters and predict yield gains in a population of *P. notatum* intraspecific hybrids. Results indicated intraspecific hybrids of *P. notatum* had high magnitudes of heritability in the broad and average sense of genotype, plus high selective accuracy and genetic variation for all forage characteristics evaluated. This indicated REML/BLUP can contribute useful information for plant selection in future plant breeding programs. The genetic material studied showed high genetic variability for forage production. Analysis indicated hybrids 336, 332, 437, 132 and male parent '30N' should be included in new crosses to increase the dry matter production of *P. notatum*. Parents need to be selected from different groups in order to maximize genetic variability and heterosis. In addition, these parents must be included in diallel crosses. The results obtained in this study provide important information for the future breeding of improved *P. notatum* cultivars for commercialization.

Keywords: mixed models; multivariate analysis; parental selection; plant breeding

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1. Introduction

Native forage species in pastoral agriculture systems help to provide productive stability and conservation of natural resources and can reduce costs and risks associated with livestock production, culminating in increased sustainability of the system [1]. *Paspalum notatum* Flügge is the main constituent of natural pastures in South America [2] and is a native perennial grass [3]. In addition to being cultivated as fodder and cover all over the world [4,5], it has high forage yield [6,7] and is of wide economic importance [8]. Furthermore, when intercropped with legumes, the dry matter production of *P. notatum* was similar to when fertilized with 240 kg N ha^{-1} [9].

The first studies to identify the mode of reproduction were carried out by Burton [10]. Later, Forbes and Burton [11] performed the artificial chromosomal duplication, with colchicine, of a sexual diploid ecotype. A sexual tetraploid was obtained in Argentina through chromosomal duplication directly in the culture medium in plants [12]. In Brazil, Weiler et al. [13] were successful in chromosomal duplication from sexual tetraploids. There was the possibility of carrying out crosses to create improved hybrids for commercial

release. Some commercial cultivars were released previously in the southeastern United States and Argentina [4,14]. However, in Brazil the only cultivar available is 'Pensacola', which is diploid and has lower forage production compared with tetraploid ecotypes [8,9]. It is also less efficient in the use of nitrogen (N) compared with tetraploid ecotypes [10].

Beef cattle grazing systems in Brazil predominantly use forages which have apomixis as a form of reproduction [15]. Apomixis refers to a form of asexual reproduction through seeds, which produces progenies genetically identical to the mother plant [16]. Among the forages most used in agricultural production in this region, the generas *Panicum*, *Urochloa*, *Cenchrus* and *Paspalum* are biologically important [17]. *Paspalum notatum* has two cytotypes associated with different modes of reproduction [18], the diploid (2n = 2x = 20), which is sexual and cross-pollinating [10], and the tetraploid (2n = 4x = 40), which is apomictic for apospory [2,10,19]. In *P. notatum*, apomixis is gametophytic of the aposporic type, where embryo sacs originate from cells of the nucellus, and includes processes of apomeiosis, parthenogenesis and pseudogamy [20–24]. The most abundant cytotype is the apomictic tetraploid [2], and, consequently, in Rio Grande do Sul/Brazil, *P. notatum* ecotypes are generally tetraploid and reproduce apomictically [11]. Given the mode of reproduction, the exploitation of heterosis becomes a key element in breeding superior hybrids. Therefore, identifying progenitors with superior performance and with dominant allele frequencies for the characteristics of interest is fundamental for exploring hybrid vigor [25] because heterosis results from the accumulation of favorable dominant alleles [26].

The plant breeding process is time consuming, expensive and laborious due to the prolonged period of experimental evaluation and selection of superior genotypes before commercialization [27,28]. Therefore, the adoption of more robust and efficient statistical methodologies is essential to guide the process of genetic gain, especially in perennial species [29]. Furthermore, in plant breeding, estimates of genetic parameters that produce reliable predictions and information on genetic values are crucial for the success of the program [30]. Restricted/residual maximum likelihood/best linear unbiased prediction (REML/BLUP) is considered the standard evaluation method for perennial species. This method offers precision and the possibility to model both fixed and random effects [25,31–33], which is more informative than analysis of variance [34]. The variance components of genetic parameters are estimated via REML and genotypic values are predicted via BLUP [31]. Effectively, the BLUP presents a favorable characteristic for the shrinkage of the estimators towards the mean and reduces its variance and increases its predictive precision [35]. The REML/BLUP procedure has been widely used in annual allogamy breeding crops such as *Zea mays* (L.) [36–38] and autogamy such as *Glycine max* (L.) [39,40]. The procedure has also been applied to selection for perennial crops e.g., *Prunus persica* (L.) [41], but the approach is less common. Recently, the BLUP procedure was used to determine the specific combining ability of *Urochola decumbens* [42]. In *P. notatum*, some genetic parameters for forage characteristics have previously been estimated via REML/BLUP [25].

In this study the objective was to estimate a group of genetic parameters and predict yield gains for a population of *P. notatum* intraspecific hybrids. In addition, two clustering methods were used in order to verify the variability within the tested population.

2. Materials and Methods

The study was carried out in the municipality of Eldorado do Sul, Rio Grande do Sul, Brazil (lat. 30°29′26″ S, long. 51°06′42″ W, alt. 62 m a.s.l.). The climate is classified as Cfa under the Köppen classification [43] and the soil is a Dystrophic Red Argisols (Ultisols) (USDA Soil taxonomy) [44]. Soil samples (0–0.2 m) were collected and tested prior to sowing the experiment. Results showed the following chemical characteristics: clay = 15%; pH (H_2O) = 5.4; SMP index = 6.3; P (mg dm^{-3}) = 15.6; K (mg dm^{-3}) = 151.4; M.O. = 2.7%. The protocol for base and cover fertilization for perennial grasses followed the recommendation of the CQFS [45]. Urea (46% N) was applied at a rate equivalent to 160 kg N ha^{-1} [45].

A randomized complete block design with four replicates was established at the UFRGS (Federal University of Rio Grande do Sul) Experiment Station. A total of 84 genotypes of *P. notatum* were evaluated. Clones were transplanted into the field with an on-the-square spacing of 1.0 m within and between rows. All genotypes tested were transplanted simultaneously. Three genotypes, 'C44X' [12], 'Q4188' and 'Q4205' [46], were tetraploid sexual genotypes sourced from the Instituto de Botánica del Nordeste (IBONE-UNNE), Corrientes, Argentina. Seven tetraploid apomitic genotypes ('30N', '36N', '48N', '70N', '83N', '95N' and 'V4') were sourced from collections originally made in South America by the United States Department of Agriculture (USDA). The ploidy level of male parents was determined according to Fachineto et al. [47] and the reproduction mode according to Machado et al. [48]. The determination of the ploidy level was performed from the gametic chromosome number, using pollen mother cells analyzed in young inflorescences collected.

In addition, 74 intraspecific hybrids were evaluated (Table 1). These were a result of crosses between female sexual plants (from IBONE-UNNE) and apomitic male genotypes (from the USDA germplasm bank).

Table 1. Identification of male (♂4x) and female (♀4x) parents used to develop intraspecific hybrids of *Paspalum notatum*.

♂(4x)	Origin	♀(4x) *	Hybrids
30N	Santa Fé—Argentina	Q4188	121; 221; 321; 421; 521; 621; 721; 821; 921; 1021;1121
36N	Santa Fé—Argentina	C44X Q4205	112; 212; 312; 412; 512; 612; 712; 812;912 132; 232; 332; 432; 532; 632;732
83N	Corrientes—Argentina	C44X Q4188	115; 215; 315; 415; 515; 615 125; 225; 325; 425; 525; 625; 725; 825; 925
95N	Corrientes—Argentina	C44X Q4205 Q4188	116; 216; 316; 416 136; 236; 336; 436; 536; 636; 736; 836; 936; 1036; 1136; 1636 126; 226; 326; 426; 526; 626; 726; 826; 926; 1026; 1126
V4	Barra do Quaraí/RS—Brazil	Q4205	137; 237; 337; 437; 537

* All female parents originated from IBONE-UNNE, Corrientes, Argentina.

Hybrids were created according the methodology described by Burton [10] and later adapted by Weiler et al. [49]. The genotypes from USDA used in these crosses were selected in a previous evaluation for forage production [47]. In the mother plants, before the anthesis, rooted culms bearing inflorescences were collected and placed in a jar with water. These culms were placed in an artificial fog chamber with a high level of air humidity to avoid the anthers from dehiscence. In the morning of the next day, the flowers were emasculated, using sharp pointed tweezers to remove the anthers. Non-flowering spikelets were eliminated from the inflorescence. Fresh pollen from the apomictic parent was collected in paper envelopes and later dusted on emasculated inflorescences of the sexual plants. After pollination, the inflorescences were bagged and labeled. Seeds were collected at least 21 days after pollination. The progeny from each cross was referred as a family, and a number was given to identify each hybrid (Figure 1).

Seeds from the parents and the F1 generation were incubated on Germest paper lined petri dishes, for germination under controlled temperature and day length in a germination chamber: 8 h of light at 30 °C and 16 h of darkness at 20 °C. Afterwards, the seedlings were kept in honeycomb trays until they had five fully expanded leaves. Seedlings with four tillers were then selected and transplanted into pots filled with substrate Carolina Soil™, a commercial substrate, composed of peat, vermiculite, organic residue and limestone. The evaluations were carried out in two growing seasons: (15 March and 26 April 2013) and (12 November 2013; 17 December 2013; 9 January 2014; 2 February 2014). The plants were cut to a 5 cm residual height when they reached an average height of 20 cm to quantify dry matter yield. Samples were sorted into morphological components (leaf blades, stem and inflorescences) then dried in an oven at 60 °C, until constant weight.

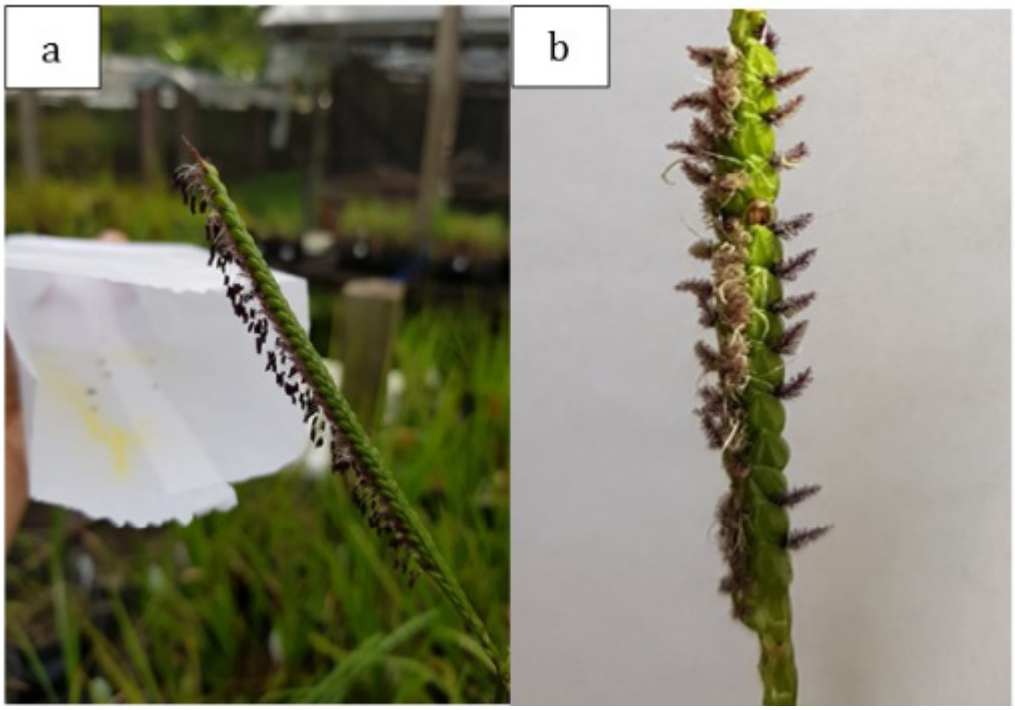

Figure 1. Phases of intraspecific crossing in *Paspalum notatum* Flügge. (**a**) Male parent and pollen collected; (**b**) female inflorescence pollinated after emasculation.

Plant height (PH, cm), tiller population density (TPD, tillers plant^{-1}), accumulated total dry mass (ATDM, kg DM plant^{-1}), accumulated leaf dry mass (ALDM, kg DM plant^{-1}), accumulated stem dry mass (ASDM, kg DM plant^{-1}) and accumulated inflorescence dry mass (AIDM, kg DM plant^{-1}) were measured. Non-destructive observations were taken prior to cutting at each date. PH was measured from the soil surface to the average bend of the leaves and then the total number of tillers on each plant were counted to determine TPD. The ATDM, ALDM, ASDM and AIDM yields are the total accumulated DM of each component summed across the entire evaluation period. The leaf:stem ratio (LSR) was calculated from ALDM and ASDM.

Estimates of variance components and genetic parameters were obtained using SELEGEN-REML/BLUP software [30] following the REML/BLUP procedure. The genetic statistical model used considered a randomized block design in one location and one year, according to the model below:

$$y = Xr + Zg + e \quad (1)$$

where y is the data vector, r is the vector of replicate effects (assumed to be fixed), g is the vector of genotypic effects (assumed to be random), e is the error vector (random) and X and Z are the incidence matrices.

The mixed model equations for the prediction of r and g are equivalent to:

$$\begin{bmatrix} X'X & X'Z \\ Z'X & Z'Z \end{bmatrix} \begin{bmatrix} r \\ g \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \end{bmatrix} \quad (2)$$

The significance of random effects was obtained through deviance analysis (ANADEV) by the restricted maximum likelihood method, via LRT (likelihood-ratio test). Deviances were obtained following the method of Resende [50], using the model with and without the respective effects, subtracting the deviance obtained in the complete model from the model without the effect and compared with the chi-square (χ^2) value with a degree of freedom. The block factor, considered fixed effect, was tested by Snedecor's F test. The

genetic parameters via REML for phenotypic (σ_p^2), genotypic (σ_g^2) and environmental (σ_e^2) variance by the EM (expectation-maximization) algorithm are specified by the formulas:

$$\sigma_e^2 \left[y'y - g'X'y - g'Z'y \right] / [N - r\,(x)] \quad (3)$$

$$\sigma_g^2 \left[g'g + \sigma_e^2\, tr\, C^{22} \right] / N_g \quad (4)$$

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2 \quad (5)$$

where the number of random elements (genotypes) is N_g, the sum of the elements of the diagonal matrix (matrix operator) is tr, the total number of data is N, the number of independent linear columns is X and C^{22} is determined by the formula:

$$\begin{bmatrix} C^{11} & C^{12} \\ C^{21} & C^{22} \end{bmatrix} = \begin{matrix} X'X & X'Z \\ Z'X & Z'Z + A^{-1}\left(\sigma_g^2 + \sigma_e^2\right) \end{matrix}^{-1} \quad (6)$$

Heritability in the broad sense (h_g^2) and selective accuracy ($\hat{r}_{gg}$), genetic variation coefficient (CV_g), residual variation coefficient (CV_{res}) and relative variation coefficient (CV_r) were estimated as follows:

$$h_g^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2} \quad (7)$$

$$\hat{r}_{gg} = (h_g^2)^{1/2} \quad (8)$$

$$CV_g(\%) = \frac{100\sqrt{\sigma_g^2}}{\overline{m}} \quad (9)$$

$$CV_{res}(\%) = \frac{100\sqrt{\sigma_e^2}}{\overline{m}} \quad (10)$$

$$CV_r(\%) = \frac{CV_g}{CV_{res}} \quad (11)$$

Genetic divergence among the hybrids was evaluated by Tocher's clustering method and Unweighted Pair Group Method using Arithmetic averages (UPGMA), by the matrix of genetic distances of Mahalanobis [50]. The predicted values were obtained from the variance and covariance matrix of these genetic values as follows: $D_{ii'}^2 = \delta' G \delta$, where $D_{ii'}^2$ is the Mahalanobis distance between genotypes i and i'; G is the matrix of genotypic variance and covariance; δ [d_1, d_2, ... d_j], being $d_j = Y_{ij} - Y_{i'j}$; and Y_{ij} where mean of the i-th genotype in relation to the j-th variable.

All analyses were performed using the SELEGEN-REML/BLUP genetic-statistical computational application [30] and GENES [51] to obtain multivariate analyzes.

3. Results

In all characteristics studied, the LRT for genotypic effects was significant ($p < 0.01$) (Table 2). The highest estimates of genetic ($\hat{\sigma}_g^2$) and phenotypic ($\hat{\sigma}_p^2$) variance were for ALDM and TPD. The results indicated there was potential for selection gains within the studied germplasm. For all characteristics there was high genetic variability (CV_g), which ranged from a minimum of 27.8 (PH) to a maximum of 78.7 (ASDM). Additionally, high heritability values (h_g^2) were identified within the population studied. High values of these genetic parameters led to high average heritability of genotypes (h_{mc}^2) and high selective accuracy ($\hat{r}_{gg}$) with all values close to 1.0. This indicated strong genetic control in the studied characteristics in addition to the potential for selection among intraspecific hybrids. The relative variation coefficient (CV_r) of all characteristics studied exceeded 1.0. This was a particularly important result because heritability can vary under different environmental conditions, between years and across evaluated characteristics. Therefore, this parameter is of great importance to assist in decision making within the breeding program.

Table 2. Verisimilitude values (LRT) of deviance analysis (ANADEV) and estimates of genetic parameters (individual REML) for characteristics quantified for intraspecific hybrids of *P. notatum* Flügge.

Parameters	ATDM (kg DM Plant^{-1})	ALDM (kg DM Plant^{-1})	ASDM (kg DM Plant^{-1})	AIDM (kg DM Plant^{-1})	LSR	PH (cm)	TPD (Tillers Plant^{-1})
DEV $_{genotype}$	3518.7	3176.5	2786.2	2306.4	604.31	1418	2960.1
DEV $_{complete\ model}$	2275.1	1803.9	1352.7	1042.5	199.27	351.43	1176.9
LRT (χ^2)	1243.64 **	1372.53 **	1433.47 **	1263.93 **	405.04 **	1066.60 **	1783.22 **
LI h^2 (%)	0.6939	0.6954	0.696	0.6942	0.5895	0.6902	0.6971
LS h^2 (%)	1.2976	1.2995	1.3	1.2979	1.1544	1.2923	1.3019
$\hat{o}^2_g$	13,917.2	4972.7	1535.6	361.29	1.8772	24.755	2596.6
$\hat{o}^2_{res}$	59.716	12.688	3.0655	1.4284	0.2758	0.2175	1.27
$\hat{o}^2_p$	13.977	4985.4	1538.7	362.72	2.1529	24.972	2597.9
h^2_g	0.9957	0.9975	0.998	0.9961	0.8719	0.9913	0.9995
h^2_{mc}	0.9989	0.9994	0.9995	0.999	0.9646	0.9978	0.9999
$\hat{r}_{gg}$	0.9995	0.9997	0.9998	0.9995	0.9821	0.9989	0.9999
CV_g (%)	64.656	65.597	78.706	73.344	51.781	27.866	49.857
CV_{res} (%)	4.2352	3.3134	3.5165	4.6117	19.846	2.6118	1.1026
CV_r	15.266	19.797	22.382	15.904	2.6091	10.669	45.217
Grand mean	182.46	107.501	49.789	25.916	2.646	17.855	102.21

Accumulated total dry mass (ATDM), accumulated leaf dry mass (ALDM), accumulated stem dry mass (ASDM), accumulated inflorescence dry mass (AIDM), leaf:stem ratio (LSR), plant height (PH), tiller population density (TPD). Confidence interval at 5% probability with inferior (LI) and superior (LS) limits, genotypic variance ($\hat{o}^2_g$), residual variance ($\hat{o}^2_{res}$), phenotypic variance ($\hat{o}^2_p$), individual heritability in the broad sense (h^2_g), average heritability of genotype (range) (h^2_{mc}), selective accuracy ($\hat{r}_{gg}$), genetic variation coefficient (CV_g), residual variation coefficient (CV_{res}) and relative variation coefficient (CV_r). ** significant at $p < 0.01$, by the chi-square test (χ^2) with 1 degree freedom.

Based on the BLUP methodology 15 superior intraspecific hybrids were then selected to quantify forage characteristics. These hybrids represented 17.8% of the total germplasm evaluated (Table 3) and were chosen because their predicted breeding values were higher than the grand mean for all parameters evaluated. For ATDM, ALDM, ASDM, AIDM, LSR, PH, TPD and LS, new averages ($\overline{X}_{new}$) were 458.70 kg DM plant^{-1}, 270.87 kg DM plant^{-1}, 144.7855 kg DM plant^{-1}, 67.6287 kg DM plant^{-1}, 5.98:1, 27.37 cm and 207.72 tillers per plant^{-1}, respectively. The genetic value for ATDM and ALDM revealed that intraspecific hybrids 336, 332, 437, 132 and male parent '30N' were superior to other genotypes and hybrids. Genetic gains ranged from 201.1 to 399.51 kg DM plant^{-1} for ATDM and 223.4 to 347.1 kg DM plant^{-1} for ALDM, which raised the population mean.

The LSR of hybrids 1026, 525, 225 and female parents 'Q4188' and 'Q4205' showed superior values within the selected population (Table 3). Genetic gains ranged from 2.33 to 4.49. For TPD, genetic gains ranged from 81.6 to 145.8 tillers plant^{-1}. Hybrids 137, 216, 132, 332 and male parent '48N', were superior to other evaluated material. Based on the most important forage characteristics, the aforementioned intraspecific hybrids were identified as potential parents in new crosses aiming at greater genetic gain for forage production of *P. notatum*.

The Tocher optimization method identified eight distinct groups of intraspecific hybrids (Table 4). Group I contained the highest concentration of hybrids (64.3%). This demonstrated that these hybrids are more related and have less genetic variation among them. The highest average ATDM was 514.1 kg DM plant^{-1} (Group V), 501.9 kg DM plant^{-1} (Group VII), 327.3 kg DM plant^{-1} (Group VI) and 305.9 kg DM plant^{-1} (Group II). Group VI had the highest average ALDM (238.1 kg DM plant^{-1}) and Group V had the highest average TPD (192.7 tillers plant^{-1}). The constituent genotypes of Groups V, VI, VII and II can be included as parents in future crosses.

Table 3. Predicted genotypic effect (g), genotypic value (u + g), genetic gains (gain) and new average ($\overline{X}_{new}$) for characteristics studied in intraspecific hybrids of *P. notatum* Flügge.

	ATDM (kg DM Plant^{-1})					ALDM (kg DM Plant^{-1})					ASDM (kg DM Plant^{-1})				
Order	**Hybrid**	**g**	**u + g**	**Gain**	$\overline{X}_{new}$	**Hybrid**	**g**	**u + g**	**Gain**	$\overline{X}_{new}$	**Hybrid**	**g**	**u + g**	**Gain**	$\overline{X}_{new}$
1	336	399.51	581.97	399.51	581.97	336	239.64	347.14	239.64	347.14	437	130.86	180.65	130.86	180.65
2	332	332.58	515.04	366.05	548.51	332	226.72	334.22	233.18	340.68	V4	117.15	166.94	124.01	173.79
3	437	319.52	501.98	350.54	533.00	132	171.29	278.80	212.55	320.05	336	111.68	161.47	119.90	169.69
4	132	262.96	445.42	328.64	511.10	437	155.89	263.40	198.39	305.89	221	96.12	145.90	113.95	163.74
5	30N	206.79	389.25	304.27	486.73	30N	108.63	216.13	180.43	287.94	332	79.61	129.40	107.08	156.87
6	221	200.32	382.79	286.95	469.41	236	103.66	211.16	167.64	275.14	515	70.68	120.47	101.02	150.80
7	236	188.31	370.77	272.86	455.32	221	102.77	210.27	158.37	265.87	236	66.33	116.12	96.06	145.85
8	137	168.76	351.22	259.84	442.31	95N	99.84	207.34	151.05	258.56	116	60.28	110.07	91.59	141.38
9	95N	168.08	350.54	249.65	432.11	725	99.00	206.50	145.27	252.77	30N	49.69	99.48	86.93	136.72
10	515	162.79	345.25	240.96	423.42	137	91.42	198.92	139.89	247.39	137	48.64	98.43	83.10	132.89
11	V4	148.26	330.72	232.54	415.00	926	82.56	190.06	134.67	242.18	132	43.18	92.97	79.47	129.26
12	48N	143.36	325.82	225.10	407.56	V4	68.46	175.96	129.16	236.66	70N	42.64	92.43	76.40	126.19
13	216	121.01	303.47	217.10	399.56	636	67.07	174.57	124.38	231.88	337	42.48	92.27	73.79	123.58
14	926	98.23	280.69	208.61	391.07	225	62.94	170.44	119.99	227.49	316	40.70	90.49	71.43	121.22
15	337	95.81	278.27	201.09	383.55	721	59.34	166.84	115.95	223.45	48N	40.26	90.05	69.35	119.14

	AIDM (kg DM Plant^{-1})					LSR					PH (cm)				
Order	**Hybrid**	**g**	**u + g**	**Gain**	$\overline{X}_{new}$	**Hybrid**	**g**	**u + g**	**Gain**	$\overline{X}_{new}$	**Hybrid**	**g**	**u + g**	**Gain**	$\overline{X}_{new}$
1	132	47.84	73.76	47.84	73.76	Q4188	4.49	7.14	4.49	7.14	437	13.35	31.20	13.35	31.20
2	30N	47.81	73.73	47.83	73.74	1026	4.09	6.73	4.29	6.94	525	11.74	29.60	12.54	30.40
3	336	47.62	73.53	47.76	73.67	525	4.07	6.71	4.22	6.86	332	8.93	26.78	11.34	29.19
4	48N	46.40	72.32	47.42	73.33	225	3.39	6.03	4.01	6.65	115	8.15	26.01	10.54	28.40
5	515	39.64	65.55	45.86	71.78	Q4205	3.07	5.72	3.82	6.47	636	8.13	25.98	10.06	27.91
6	212	35.92	61.83	44.20	70.12	1136	2.68	5.33	3.63	6.28	621	7.63	25.48	9.65	27.51
7	316	32.89	58.81	42.59	68.50	921	2.49	5.14	3.47	6.11	926	7.50	25.36	9.35	27.20
8	437	32.17	58.09	41.29	67.20	1636	2.31	4.96	3.32	5.97	336	6.77	24.63	9.03	26.88
9	95N	32.10	58.02	40.27	66.18	532	1.63	4.28	3.14	5.78	1136	6.51	24.36	8.75	26.60
10	70N	28.97	54.89	39.14	65.05	721	1.37	4.02	2.96	5.61	132	6.15	24.01	8.49	26.34
11	137	28.03	53.94	38.13	64.04	725	1.26	3.90	2.81	5.45	1036	6.13	23.98	8.27	26.13
12	116	27.51	53.43	37.24	63.16	536	1.14	3.79	2.67	5.31	936	6.01	23.86	8.08	25.94
13	332	25.67	51.58	36.35	62.27	825	1.10	3.74	2.55	5.19	836	5.95	23.80	7.92	25.77
14	415	24.26	50.17	35.49	61.40	912	0.98	3.62	2.43	5.08	1021	5.88	23.73	7.77	25.63
15	236	17.66	43.58	34.30	60.21	936	0.97	3.61	2.34	4.98	537	5.56	23.41	7.63	25.48

	TPD (Tillers Plant^{-1})				
Order	**Hybrid**	**g**	**u + g**	**Gain**	$\overline{X}_{new}$
1	137	146	248	146	248
2	216	126	228	136	238
3	132	104	206	125	227
4	332	102	204	119	222
5	48N	91	194	114	216
6	725	87	189	109	212
7	726	85	188	106	208
8	95N	73	175	102	204
9	321	69	171	98	200
10	336	66	168	95	197
11	36N	63	165	92	194
12	926	55	157	89	191
13	436	53	156	86	188
14	V4	52	155	84	186
15	221	52	154	82	184

Accumulated total dry mass (ATDM), accumulated leaf dry mass (ALDM), accumulated stem dry mass (ASDM), accumulated inflorescence dry mass (AIDM), leaf: stem ratio (LSR), plant height (PH), tiller population density (TPD).

The observed cophenetic correlation coefficient demonstrated an adequate relationship between the distance matrix and the generated dendrogram (Figure 2). The UPGMA clustering method found 10 homogeneous groups which were heterogeneous to the others identified. These results differed to those found by the Tocher method (Table 3). Both methods demonstrated the presence of high genetic variability within the intraspecific hybrids studied (Table 3; Figure 2). The first two groups contained 71% of the hybrids studied, with Group I containing 46% of all hybrids, followed by Group II (Figure 2) with a

further 25%. For the ATDM characteristic, groups VI, VII and VIII had the highest average yields of 514.1, 501.9 and 356.7 kg DM plant^{-1}, respectively. Groups VI (320.1 kg DM plant^{-1}), VII (263.4 kg DM plant^{-1}) and IV (20.65 kg DM plant^{-1}) had superior ALDM compared with the other groups identified. LSR was higher for Groups V (6.3), III (6.1) and IV (3.9).

Table 4. Composition of groups formed by the Tocher optimization method for evaluated intraspecific hybrids of *P. notatum* Flügge, based on the average genetic Euclidean distance matrix.

Group	Hybrids
I	232; 325; 126; 1121; 326; 526; 826; 1126; 626; 416; 426; 615; 412; 632; 925; 812; 521; 315; 625; 425; 212; 712; 112; 432; 512; 215; 226; 536; 612; 121; 125; 912; 83N; 312; 736; 825; 821; 237; 732; C44X; 421; 1036; 436; 537; 1021; 415; 136; 836; 36N; 726; 115; 936; 721; 1636
II	636; 926; 621; 337; 321; 316; 236; 515; 70N; 116; 95N; 30N; 48N; 221; V4
III	225; 921; Q4188; Q4205; 725
IV	1026; 1136; 525
V	332; 336; 132
VI	137; 216
VII	437
VIII	532

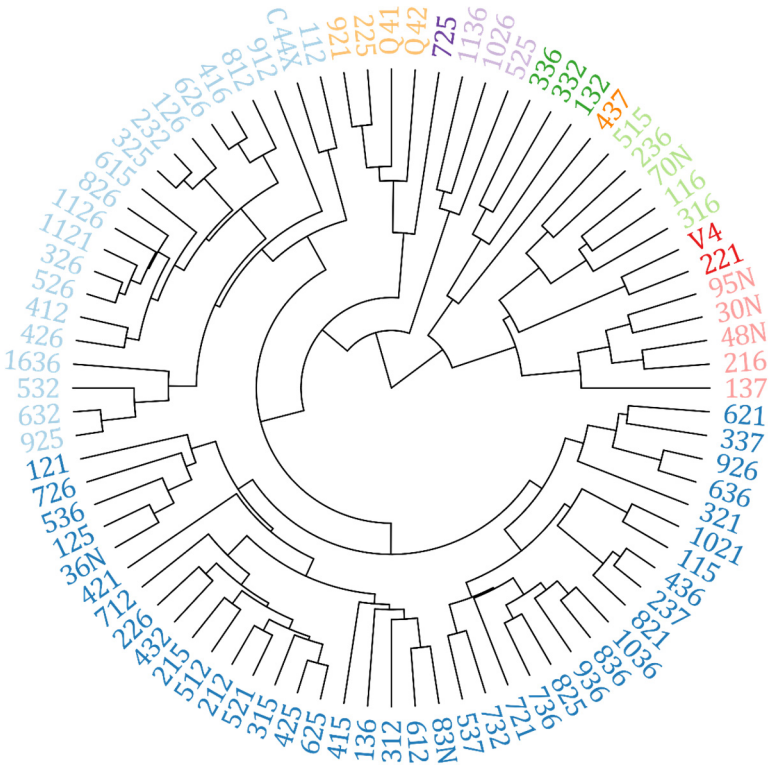

Figure 2. Dendrogram of genetic dissimilarity among 74 intraspecific hybrids and eight parents of *P. notatum* Flügge, obtained by the UPGMA method, based on the average genetic Euclidean distance matrix considering the yield and morphological characteristics evaluated. Cophenetic correlation index = 0.76. Group I (blue); Group II (light blue); Group III (orange); Group IV (purple); Group V (light purple); Group VI (green); Group VII (orange); Group VIII (light green); Group IX (red) and Group X (light red).

4. Discussion

The significance for genetic effects (Table 2) indicated the existence of genetic variability and the possibility of yield gains through targeted selection criteria [52,53]. Genetic variance ($\hat{o}^2_g$) was positive and non-zero values demonstrate greater genetic variation and chances of

production gains with selection [54] and relative coefficient of variation values (CV_r) greater than 1.0 indicated a benefit in selection [55]. Heritability values (h_g^2) were >0.50, which is considered high, mainly because it indicates how much of the genotypic variation is of genetic origin [31]. Therefore, it is clear that the variance is a particular case of covariance of a variable with itself. Covariance plays a fundamental role in selection as the association and similarity between parents and children is of interest to the breeder and guarantees progress in selection and can be measured by covariance. The narrow sense heritability for forage and seed yield was previously also identified as high [25]. However, in this study the correlation values were lower. These values indicated a high probability of success in the selection of hybrids with increased potential forage yields. Selective accuracy ($\hat{r}_{gg}$) is the correlation between the true genotypic value of the genotypes and the estimated/predicted value [56]. High values of $\hat{r}_{gg}$ (close to 1) indicate the experimental design was adequate and the results obtained were reliable (Table 2). The quality of the genotypic assessment should be based on $\hat{r}_{gg}$ because they provide more accurate inferences of the genetic value of the hybrids evaluated [53]. It is important to note that selective accuracy ($\hat{r}_{gg}$) is not associated with the genetic merit of the genotypes studied [57]. The set of information generated through the estimation of genetic parameters within the population assists the breeder in selection decisions [30]. Estimating genetic parameters via REML and prediction of genotypic values via BLUP offers a more robust and efficient statistical methodology in genetic improvement [26,29,34]. Thus, the data obtained here demonstrate high potential to produce genetic gains in the forage production traits studied in future breeding selections.

Breeding programs need to prioritize crossing genotypes with high average values for desirable traits [58]. The formation of several groups (Table 4) demonstrated genetic divergence between the genotypes studied. This means there will be numerous alternatives for crosses among the groups identified [59]. These authors emphasized that the analysis of genetic divergence simplifies and facilitates the use of germplasm in future crosses. Similar to other studies of genetic divergence, the Tocher method used here also showed a trend towards the formation of a larger group and genotypes isolated in other groups [60–62]. Multivariate analyses, such as Tocher (Table 4) and UPMG (Figure 2), are powerful tools to assist in the characterization [63] and discrimination of genotypes [64,65] and should be extensively used in genetic diversity studies [66–68]. Understanding genetic diversity is one of the fundamental steps within genetic improvement programs, with the multivariate analysis approach being the most commonly used [69]. Generally, when there is a large amount of data, such as morphological, physiological, biochemical and molecular data, multivariate analyses are used with emphasis on the selection and preservation of genotypes with potential for use within the breeding program [69,70]. The use of dendrograms (Figure 2) to graphically describe the clustering method requires the cophenetic correlation coefficient to exceed 0.70 [63]. Crosses between elite genotypes with complementary characteristics is desirable to obtain hybrids with enough genetic variability to outperform the parents [71]. Crosses between hybrids assigned to the same group are not interesting, mainly because of low variability and the non-exploitation of the existing potential diversity (Table 4; Figure 2). Similarly, the formation of groups with many genotypes can be a limiting factor in the choice of parents for breeding programs because of the proximity between the genotypes within the group [72]. Thus, a combination of desirable characteristics and low genetic similarity is required to increase the probability of exploitation of heterosis [8].

This study has shown intraspecific hybrids of *P. notatum* had high magnitudes of heritability in the broad and average sense of genotype, plus high selective accuracy and genetic variation for all forage characteristics evaluated. This indicated there is potential to select superior hybrids using the REML/BLUP method in future plant breeding forages programs. The genetic material studied included hybrids with high genetic variability for forage production. In the next stage of the breeding program, the selected hybrids (Table 3; Figure 2) can be included in new crosses with female parents that have high genetic value. These parents will be selected from different groups to maximize genetic variability and

heterosis. In addition, these parents must be included in diallel crosses mainly, aiming to select the best parents capable of generating new productive populations, mainly aiming at the general combining ability, which refers to the ability of a parent to produce progenies with a given performance when crossed with a number of other parents, or even in relation to specific combination ability, which refers to the performance of a specific combination. This will allow the results to be confirmed by BLUP and multivariate analysis (Tocher and UPGMA) because these analyses are predictive in nature. Based on diallel crosses, parameters such as heterosis, heterobeltiosis and combining ability (general and specific) can be estimated. The advances obtained with the study of a promising species such as *P. notatum* will future favor the availability of commercial cultivars for the purpose of use in intensive livestock production systems and in the recovery of degraded areas. In addition, it will also contribute to the conservation of natural grassland areas in South America.

5. Conclusions

REML-estimated genetic parameters in combination with optimization via multivariate analysis can identify superior genetic material which allows the selection of superior *P. notatum* forage hybrids for pastoral systems.

Multivariate analyses are indispensable tools in plant breeding. They create divergent groups for a characteristic of interest, each of which contains a range of homogeneous genotypes and enables selection of the best parents from each group.

The new averages identified for characteristics of interest via BLUP indicated intraspecific hybrids 336, 332, 437, 132 and male parent '30N' can be used in new crosses to increase the dry matter production of *P. notatum*.

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