













# Characterization and genetic diversity in *Paspalum notatum* Flüge accessions: Morphological and geographical distance

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Received: February 7, 2022  
Accepted: June 10, 2022

**How to cite:** 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 Tassis, G. 2022. Characterization and genetic diversity in *Paspalum notatum* Flüge accessions: Morphological and geographical distance. Revista Brasileira de Zootecnia 51:e20220015. <https://doi.org/10.37496/rbz5120220015>

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**ABSTRACT** - The objective of this work was to describe morphology and grouping of *Paspalum notatum* accessions, based on multicategorical data which discards the redundant variables for quantification of genetic diversity. We also tested the hypothesis that geographical distance was correlated with morphological divergence. In our study, multivariate analyzes successfully demonstrated the geographic and morphological variability of the *P. notatum* accessions characterized. Many of these evaluated accessions can be included in future genetic improvement programs. Based on two methodologies for discarding variables, it was possible to identify the potentially important morphological characteristics from genetic diversity studies and characterize new accessions aimed at improving forage and seed production. The methodologies used to discard variables are biometric tools that can be used successfully in future plant breeding programs, especially when a large number of traits and accessions are being evaluated. Although significant, geographic distance had a low association with morphological traits. This indicated the need to use other characteristics, such as forage and seed yield, in addition to molecular analysis. Our analyzes showed genetic variability in *P. notatum* for all the characteristics studied.

**Keywords:** cluster analysis, hierarchical method, optimization method, Tocher

## 1. Introduction

The *Paspalum* genus comprises approximately 330 species and is most commonly found in natural tropical and subtropical pastures in the Americas (Zuloaga and Morrone, 2005). The genus is native to grasslands in Rio Grande do Sul (Brazil), Uruguay, and Argentina and is recognized for its productivity and quality for grazing ruminants. Additionally, it responds to intensification managements such as fertilization and supplemental irrigation (Graminho et al., 2017; Motta et al., 2020). Thus, there has been increased interest in different *Paspalum* species to increase the productivity of native pastures and extend its use into cultivated improved pastures.

*Paspalum notatum* Flüge (Bahagrass) is a perennial grass with strong, shallow, and horizontal rhizomes consisting of short and robust internodes. The culms are simple and ascending, and the leaves have overlapping sheaths, glabrous sheaths, or sheaths with ciliated margins (Nabinger and Dall'Agnol, 2019). It is native to hot and humid regions of the Western Hemisphere and is naturally distributed from Mexico to the fields of Argentina and Uruguay (Urbani et al., 2017). Since 1936, superior varieties of *P. notatum* have been selected, evaluating hundreds of ecotypes from United States and Latin America

(Burton, 1967). The diversity found in South American ecotypes suggests that this is the center of origin (Chase, 1929; Morrone et al., 2012).

The ability of the species to survive, even under conditions of low fertility, drought, flooding, and severe continuous stocking, means *P. notatum* is an important source of feed for low-input livestock production systems (Nabinger and Dall'Agnol, 2019). However, other studies have also shown *P. notatum* responds well to fertilization and irrigation (Weiler et al., 2018; Motta et al., 2021). Fachinetto et al. (2017) reported *P. notatum* is the most common species present in the native pastures of southern Brazil, where several ecotypes have adapted to different edaphoclimatic conditions, resulting in variability in both yield and forage quality. Steiner et al. (2017) evaluated *P. notatum* accessions collected in different locations in Brazil, Uruguay, and Argentina and reported large variation in total forage production and leaf yield. Further, many of these accessions were superior to Pensacola, the sole commercial cultivar of the species available in the market, when evaluated for winter survival in southern Brazil.

The objective of this work is to describe morphology and grouping of the *P. notatum* accessions evaluated, based on multicategorical data, which discards the redundant variables for quantification of genetic diversity. We also tested the hypothesis that geographical distance is correlated with morphological divergence.

## 2. Material and Methods

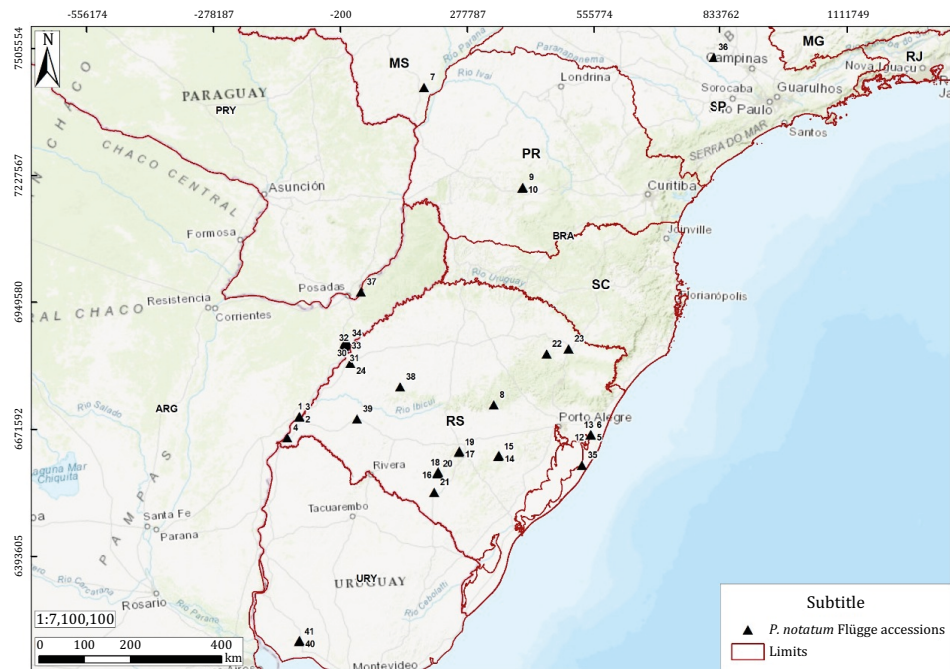
The experiment was carried out in Porto Alegre, Rio Grande do Sul, Brazil (30°04'13.1" S and 51°08'26.3" W). A total of 41 accessions were collected in different regions of the southern cone of South America, mainly in southern Brazil (Figure 1) for morphological characterization. Accessions were obtained by collecting the whole plant and later placed in pots with Carolina soil EC07® substrate.

Measurements were taken during full flowering (January and February) to standardize observations with plant phenology. The analyzed plants were similar in size and were established in 2.8-L pots (22 cm high × 22 cm diameter) with a commercial substrate and transferred outside the greenhouse with daily irrigation in the summer.

Morphological observations were made on leaf sheath, leaf blade, flowering stem, and inflorescence length. Growth habit was also quantified. Each morphological characteristic was evaluated in ten reproductive tillers from each accession. Leaf sheath width (cm), length (from source node to tip; mm), hairiness (1 = hairy, 2 = glabrous), and color (1 = green, 2 = violaceous) were quantified. For leaf blades measurements of blade length (cm), maximum width (cm), hairiness (1 = hairy, 2 = glabrous), color of the base of the central vein (1 = whitish, 2 = greenish), and angle of leaf insertion (°) were taken. All measurements were made on all the intact green leaves present on each of the 10 reproductive tillers. The number of nodes (n), internode length (cm), and the elongated racemes after floral induction were also measured on each tiller (mm). For the inflorescence, measurements included: raceme number (n), length (cm), angle (°), and spikelet length (mm). Height was measured from the base to the average height (cm) of the leaf canopy on the plant. Growth habit was classified as erect (1), decumbent (2), or prostrate (3).

To quantify the genetic diversity among the accessions, the Gower (1971) distance was obtained based on multicategorical (morphological) data. Geographic distances were estimated using the linear distance method from the GPS point associated with the collection point of each accession. We performed the Mantel Z test (Mantel, 1967) at 5% error probability, using the morphological and geographic distance matrices to verify whether the morphological variations between locations were the result of isolation by distance. Coefficients of correlation magnitudes were classified according to Silveira et al. (2021).

Subsequently, for both distances, the original Tocher (Rao, 1952) and sequential Tocher (Vasconcelos et al., 2007) optimization clustering methods were applied. In addition to the analysis by the hierarchical unweighted pair group method with arithmetic means (UPGMA), data is presented using a dendrogram.



V - Valls, J.F.M.; CN - Nabinger, C.; MD - Dall'Agnol, M.; St - Steiner, M.G.; nn - not numbered; RS - Rio Grande do Sul, Brazil; SC - Santa Catarina, Brazil; MS - Mato Grosso do Sul, Brazil; PR - Paraná, Brazil; SP - São Paulo, Brazil; AR - Argentina; UY - Uruguay.

Accessions number, collector, and site: (1; V 14244 A; Uruguiana, RS); (2; V 14244 E; Uruguiana, RS); (3; V 14244 H; Uruguiana, RS); (4; V 14310; Barra do Quaraí, RS); (5; V 14326; Capivari do Sul, RS); (6; V 14327; Capivari do Sul, RS); (7; V 14614; Itaquiraí, MS); (8; V 14783; Vale do Sol, RS); (9; V 14287; Candói, PR); (10; V 14282; Candói, PR); (11; V 14865; Capivari do Sul, RS); (12; V 14869; Capivari do Sul, RS); (3; V 14870; Capivari do Sul, RS); (14; MD nn; Encruzilhada do Sul, RS); (15; MD nn; Encruzilhada do Sul, RS); (16; MD nn; Lavras do Sul, RS); (17; MD nn; Caçapava do Sul, RS); (18; MD nn; Lavras do Sul, RS); (19; MD nn; Caçapava do Sul, RS); (20; MD nn; Lavras do Sul, RS); (21; MD nn; Bagé, RS); (22; MD nn; André da Rocha, RS); (23; MD nn; Vacaria, RS); (24; MD nn; São Borja, RS); (25; CN nn; Santo Tomé, AR); (26; CN nn; Santo Tomé, AR); (27; CN nn; Santo Tomé, AR); (28; CN nn; Santo Tomé, AR); (29; CN nn; Santo Tomé, AR); (30; CN nn; Santo Tomé, AR); (31; CN nn; Santo Tomé, AR); (32; CN nn; Santo Tomé, AR); (33; CN nn; Santo Tomé, AR); (34; CN nn; Santo Tomé, AR); (35; MD nn; Mostardas, RS); (36; CN nn; Piracicaba, SP); (37; AM nn; Posadas, AR); (38; St nn; Santiago, RS); (39; V 14931; Alegrete, RS); (40; MD nn; Colônia, UY); (41; MD nn; Colônia, UY).

**Figure 1** - Collection sites of the 41 *P. notatum* Flüege accessions evaluated.

The importance of morphological characteristics was evaluated through the methodology of Singh (1981). This looks at the total dissimilarity observed, for each characteristic, estimated through the participation of the components of the generalized Mahalanobis distance ( $D^2$ ). Principal component analysis (PCA) was used to eliminate descriptors that have less importance in the set of characteristics analyzed, using the criterion of Jolliffe (1972, 1973), which discards variables with greater weight in the last components of lesser importance. The criteria for discarding the main components were 80%. These methodologies were used to verify similarities between the variables of lower participation according to the Singh (1981) method and the variables discarded by the PCA analysis.

### 3. Results

The longest ( $P < 0.05$ ) leaf sheaths ( $6.7 \pm 1.5$  cm) were observed in accession 27 (Santo Tomé), while the shortest ( $2.8 \pm 1.1$  cm) were observed in accession 24 (São Borja). Accession 37 (Posadas) had the widest ( $P < 0.001$ ) leaf sheath ( $1.3 \pm 0.3$  cm), and the narrowest was from accessions 12 (Capivari do Sul), 20 (Lavras do Sul), and 32 (Santo Tomé). Accessions 2, 9, 10, 11, 13, 14, 24, 25, 28, 29, 36, and 41 had the greenest ( $P < 0.05$ ) sheath color. Most of the accessions had glabrous sheaths (2.0), while accession 36 (Piracicaba) had hairy sheaths (1.0). Accession 34 (Santo Tomé) had the longest ( $P < 0.05$ ) leaf blades ( $31.2 \pm 6.6$  cm), and accession 24 (São Borja) had the shortest ( $6.8 \pm 1.4$  cm). Accessions 8 (Vale do Sol) and 37 (Posadas) had the widest ( $P < 0.001$ ) leaf blades ( $1.1 \pm 0.2$  cm), while the narrowest leaves were observed in accessions 1, 2, 12, and 20 ( $0.5 \pm 0.1$  cm). The maximum leaf blade insertion angle was observed in accession 12 (Capivari do Sul), and the lowest insertion angle was observed in accession 6 (Capivari do Sul) ( $25.0 \pm 16.7$  degrees). Accessions 5, 7, 10, 13, 16, 17, 20, 21, 22, 24, 29, and 34 had a leaf blade central vein with a greenish base (2.0), while accessions 2 and 11 had a whitish base (1.0) (Table 1).

**Table 1** - Morphological characteristics measured in *P. notatum* Flüggé accessions collected in the southern cone of South America

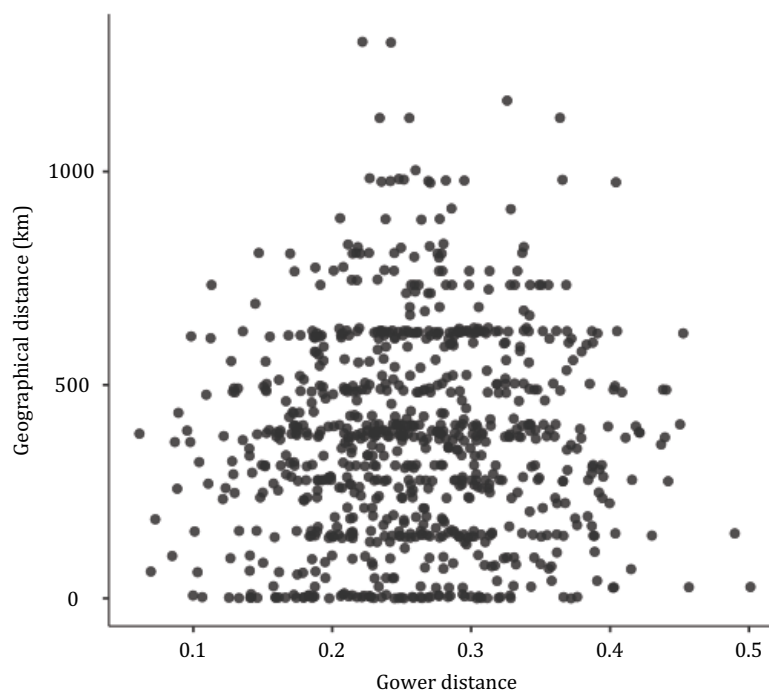
Accession	Sheath				Leaf blade					Reproductive tiller			Inflorescence			
	SL (cm)	SW (cm)	SC	SH	LL (cm)	LW (cm)	LIA (°)	LCVC	LH	NN	IL (cm)	NR	RL (cm)	SPL (mm)	RA (°)	PH
1	5.5	0.6	1.7	2.0	15.1	0.5	37.0	1.8	2.0	3.5	6.1	3.1	7.7	3.0	47.5	3.0
2	3.9	0.6	2.0	2.0	16.4	0.5	54.0	1.0	2.0	3.1	5.9	2.0	6.9	3.0	18.5	3.0
3	6.3	0.6	1.7	2.0	16.9	0.7	30.0	1.1	1.9	3.0	9.9	2.6	7.6	3.0	46.0	1.0
4	4.6	0.8	1.7	2.0	21.0	1.0	43.0	1.6	1.6	2.9	10.0	2.1	13.1	4.0	26.0	2.0
5	3.5	0.7	1.7	1.8	14.2	0.9	43.5	2.0	1.3	2.6	7.4	2.0	7.4	3.0	42.5	3.0
6	3.0	0.7	1.7	1.2	15.6	0.8	25.0	1.6	1.0	3.0	6.5	2.0	7.9	3.0	39.0	2.0
7	4.1	0.8	1.8	1.7	17.0	0.8	41.5	2.0	2.0	3.0	6.3	2.0	8.2	3.0	34.2	2.0
8	5.5	1.0	1.4	1.9	14.9	1.1	49.0	1.9	1.0	2.9	6.5	2.0	8.1	4.0	59.0	3.0
9	4.9	0.7	2.0	1.1	14.5	0.8	40.5	1.9	2.0	2.6	6.5	2.0	6.0	3.0	28.0	1.0
10	3.3	0.7	2.0	2.0	18.2	0.8	34.5	2.0	2.0	2.7	6.7	2.0	7.2	3.0	18.4	2.0
11	5.2	0.9	2.0	2.0	20.6	0.8	33.0	1.0	2.0	1.9	8.3	2.0	8.2	4.0	37.5	3.0
12	3.2	0.5	1.6	2.0	17.7	0.5	62.0	1.4	2.0	3.1	7.6	2.0	7.8	3.0	14.5	3.0
13	4.1	0.7	2.0	2.0	15.5	0.6	41.5	2.0	1.1	2.8	6.2	2.0	7.2	3.0	26.8	3.0
14	4.4	0.8	2.0	1.1	14.8	0.8	36.7	1.9	2.0	2.9	5.5	2.0	6.4	2.0	34.5	1.0
15	3.4	0.7	1.9	2.0	13.2	0.7	33.5	1.7	2.0	2.7	6.0	2.0	6.2	3.0	17.2	3.0
16	5.2	0.6	1.7	2.0	14.8	0.7	42.5	2.0	2.0	2.7	7.7	2.1	7.1	4.0	21.8	1.0
17	3.3	0.7	1.9	2.0	15.1	0.7	38.0	2.0	2.0	2.8	6.1	2.0	5.4	3.0	24.7	2.0
18	3.8	0.7	1.9	2.0	15.1	0.6	29.5	1.9	2.0	2.3	6.5	2.0	6.0	3.0	22.5	2.0
19	3.5	0.6	1.6	2.0	14.0	0.6	48.0	1.7	2.0	2.3	8.2	2.0	5.3	3.0	19.0	2.0
20	4.2	0.5	1.9	2.0	11.3	0.5	44.0	2.0	2.0	3.2	4.4	2.0	5.8	3.0	21.2	3.0
21	3.8	0.8	1.6	2.0	16.5	0.8	51.5	2.0	2.0	2.1	9.0	2.1	8.2	3.0	15.1	1.0
22	3.0	0.7	1.7	2.0	14.2	0.6	37.0	2.0	2.0	2.5	5.9	2.0	5.2	3.0	17.0	3.0
23	4.4	0.8	1.6	2.0	17.4	0.8	43.0	1.9	2.0	2.6	5.5	2.0	7.7	3.0	13.2	1.0
24	2.8	0.8	2.0	2.0	6.8	0.7	55.5	2.0	2.0	2.4	4.2	2.0	4.3	3.0	13.5	2.0
25	3.7	0.7	2.0	2.0	22.1	0.9	40.0	1.4	2.0	2.8	5.1	2.0	7.4	3.0	21.2	1.0
26	4.3	0.8	1.9	2.0	27.2	0.8	38.0	1.2	2.0	3.0	9.0	2.1	10.3	3.0	31.2	1.0
27	6.7	0.9	1.0	2.0	28.4	0.8	25.5	1.4	2.0	3.0	8.9	2.0	11.0	4.0	41.5	3.0
28	4.8	0.8	2.0	2.0	21.6	0.8	30.0	1.3	2.0	3.1	8.8	2.3	10.7	4.0	44.0	3.0
29	3.9	0.8	2.0	2.0	26.5	0.8	35.5	2.0	2.0	3.0	7.5	2.0	9.0	3.0	38.0	1.0
30	3.9	0.8	1.2	2.0	19.9	0.8	32.0	1.3	2.0	2.9	8.8	2.0	9.2	3.0	19.5	3.0
31	4.4	0.7	1.2	2.0	28.5	0.8	39.5	1.6	2.0	3.1	8.4	2.1	11.5	3.0	48.0	3.0
32	4.2	0.5	1.3	2.0	12.3	0.6	45.5	1.9	2.0	3.0	8.3	2.1	10.1	3.0	35.5	2.0
33	3.8	0.6	1.3	2.0	15.3	0.8	52.0	1.8	2.0	3.1	8.2	2.1	9.7	4.0	34.8	2.0
34	5.8	0.8	1.4	2.0	31.2	0.9	33.5	2.0	1.0	2.9	11.1	2.0	12.9	3.0	46.5	2.0
35	3.8	0.7	1.4	2.0	22.3	0.8	34.0	1.5	1.7	3.0	9.0	2.1	9.9	3.0	44.0	3.0
36	4.9	0.7	2.0	1.0	14.0	0.8	46.0	1.9	2.0	3.1	6.4	2.1	8.5	3.0	32.5	3.0
37	5.2	1.3	1.3	1.9	11.8	1.1	56.0	1.5	2.0	2.2	8.2	2.0	9.0	3.0	37.0	2.0
38	5.4	0.8	1.5	2.0	19.3	0.9	39.0	1.7	2.0	2.7	9.7	2.0	11.6	3.0	21.5	2.0
39	3.0	0.6	1.4	2.0	11.6	0.8	51.5	1.9	2.0	2.5	9.2	2.0	8.8	4.0	46.0	3.0
40	3.2	0.6	1.6	2.0	11.3	0.7	46.0	1.5	1.7	3.1	7.1	2.3	8.0	4.0	28.0	3.0
41	4.2	0.7	2.0	2.0	12.6	0.8	44.0	1.3	1.9	2.4	8.9	2.2	7.9	4.0	28.0	3.0
Grand mean	4.2	0.7	1.7	1.9	17.2	0.8	41.0	1.7	1.9	2.8	7.4	2.1	8.2	3.0	30.6	2.2

SL - sheath length; SW - sheath width; SC - sheath color (1 = green, 2 = violaceous); SH - sheath hairiness (1 = hairy, 2 = glabrous); LL - leaf blade length; LW - leaf blade width; LIA - leaf blade insertion angle; LCVC - leaf blade central vein color (1 = whitish, 2 = greenish); LH - leaf blade hairiness (1 = hairy, 2 = glabrous); NN - number of nodes; IL - internode length; NR - number of racemes; RL - raceme length; SPL - spikelet length; RA - raceme angle; PH - plant habit (1 = erect, 2 = decumbent, 3 = prostrate).

Thirty-one accessions had a leaf blade hairiness value of 2.0 (hairless), whereas accessions 6 (Capivari do Sul), 8 (Vale do Sol), and 34 (Santo Tomé) had the hairiest (1.0) leaf blades (Table 1). The highest ( $P<0.001$ ) number of nodes ( $3.5\pm 0.7$ ) was observed in accession 1 (Uruguiana), and the lowest ( $1.9\pm 0.3$ ) in accession 11 (Capivari do Sul). The greatest internode length ( $11.1\pm 2.8$  cm) was from accession 34 (Santo Tomé), and the shortest ( $4.2\pm 1.5$  cm) in accession 24 (São Borja). Accession 1 (Uruguiana) had  $3.1\pm 0.6$  racemes ( $P<0.001$ ), a further 27 accessions had 2.0 racemes. The greatest raceme length ( $13.1\pm 1.2$  cm) was observed in accession 4 (Barra do Quaraí), and the shortest ( $4.3\pm 0.5$  cm) in accession 24 (São Borja). Ten accessions had a spikelet length of 4 mm, while accession 14 (Barra do Quaraí) displayed the shortest spikelet length (2 mm). The highest raceme angle was observed in accession 8 (Vale do Sol), with values of  $59^\circ$  and the lowest in accession 23 (Vacaria), with values of  $13.2^\circ$  (Table 1). A total of 19 accessions had a prostrate habit (3), 13 had a decumbent habit (2), and 9 were erect (1). The accessions evaluated showed a growth habit closest to the decumbent habit (Table 1).

### 3.1. Matrix distance association

The mantel test (correlation) between Gower distance (morphological multicategorical data) and geographic distance (linear distance) was significant ( $P<0.05$ ), but explained little of the observed variability ( $r = 0.06$ ) (Figure 2). These results indicated that geographic distance between the accessions explained very little the morphological differentiation and indicated that genetic divergence was not associated with geographical distance. Therefore, low equivalence between the clustering methods was expected.



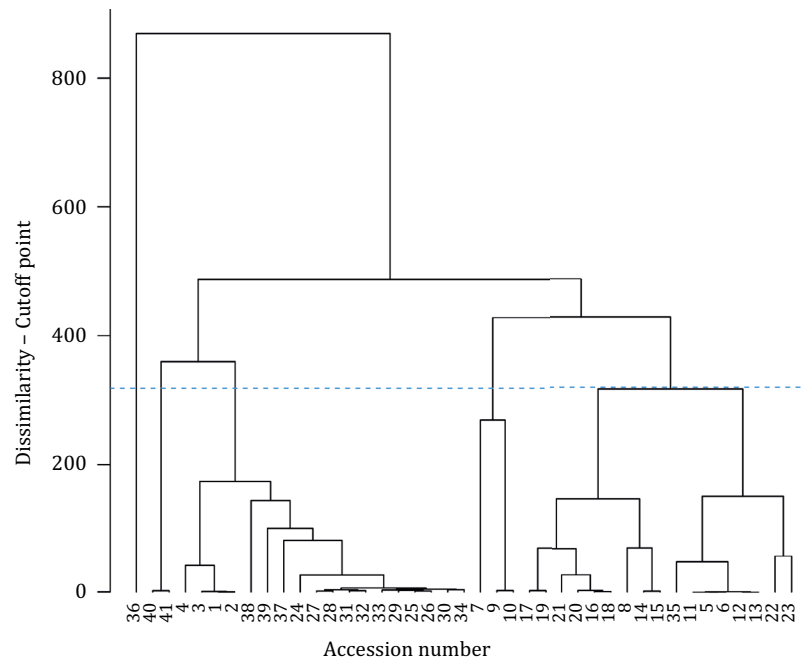
**Figure 2** - Graphic dispersion of the correlation between geographic and genetic distances (Gower) in 41 accessions of *P. notatum* Flüge ( $P<0.05$ ).

### 3.2. Geographical distance and divergence

Tocher optimization (Table 2) and the UPGMA hierarchical clustering methods were then applied (Figure 3). In contrast to the Gower distance obtained through multicategorical data, the geographic distance successfully placed the 41 accessions into three groups (Table 2). In addition, there was no difference in clustering by the original or sequential Tocher methods. The results obtained showed

**Table 2** - Composition of the three groups formed by the original and sequential Tocher optimization methods in *P. notatum* Flüge, based on the geographical distance matrix

Group	Original and sequential Tocher	
	Accession	
I	1, 2, 3, 4, 32, 31, 28, 27, 26, 25, 33, 29, 34, 30, 24, 39, 37, 38, 18, 20, 16, 21, 19, 17, 8, 15, 14, 40, 41, 22, 7, 10, 9, 23	
II	5, 6, 11, 12, 13, 35	
III	36	



Cophenetic correlation index: 0.85.

**Figure 3** - Dendrogram of genetic dissimilarity among 41 accessions of *P. notatum* Flüge, obtained by the UPGMA method, based on the geographical distance matrix.

a greater concentration of accessions in Group I (81%), which was similar to the results obtained based on the Gower distance, with highest concentration of accessions in Group I for both methods (original and sequential).

Grouping due to geographic distances (Figure 1) was expected because of the genetic divergence among populations and the geographic distance that separates the populations. This is represented through a matrix of geographic distances and represents a measurable and modifiable scale, which makes it possible to compare and measure the correlation between the genetic and geographic matrices of distance. However, the accessions that make up group II had a smaller geographic distance, as they were all sourced from the adjacent municipalities of Capivari do Sul and Mostardas (Table 2). Accession 36 (Piracicaba) was allocated to group III, which represented the furthest geographic distance.

Using Mojena's (1977) criterion, based on geographic distance, the UPGMA hierarchical clustering method separated the studied accessions into five groups. Group I (accessions 4, 3, 1, 2, 38, 39, 37, 24, 27, 28, 31, 32, 33, 29, 25, 26, 30, and 34) retained 44% of accessions. A further 42% were allocated to group II (accessions 17, 19, 21, 20, 16, 18, 8, 14, 15, 35, 11, 5, 6, 12, 13, 22, and 23), 7% to group III (accessions 7, 9, and 10), 5% to group IV (accessions 40 and 41), and 2% to group V (accession 46). Accessions 36 and 41 were the most divergent to each other, while accessions 1 and 2 were the most similar.

### 3.3. Morphological distance and divergence

The Tocher optimization method (Table 3) and the UPGMA hierarchical clustering method (Figure 4) were then analyzed with the Gower matrix. The traditional Tocher method identified 13 groups (Table 3). The first group included the majority of accessions (17), which represented 41% of total accessions evaluated. Group II included eight accessions, or 19% of the total accessions. With the sequential Tocher analysis, it was possible to reduce the number of groups from 13 to six. Group I was consistent with the original Tocher analysis. However, accessions allocated to group II increased and accounted for 31% of the accessions studied (Table 3). Our results demonstrated greater efficiency by the sequential Tocher method in grouping accessions with greater dissimilarity, compared with the original Tocher method.

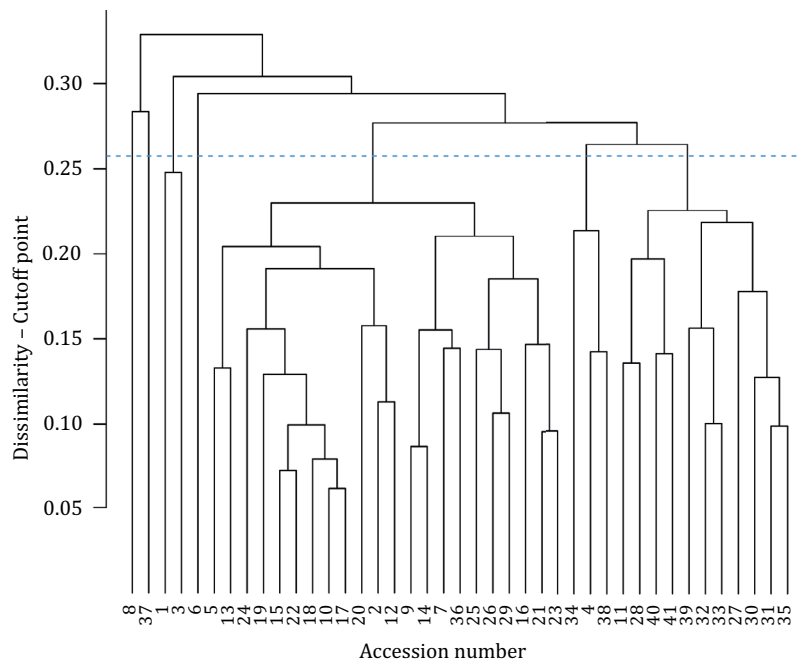
Subsequently, the Mojena criteria (1977) was applied. The UPGMA hierarchical clustering method separated the accessions into seven groups (Figure 4). Group I (accessions 5, 13, 24, 19, 15, 22, 18, 10, 17, 20, 2, 12, 9, 14, 7, 36, 26, 29, 16, 21, and 23) represented 51 % of the accessions evaluated; group II (accessions 11, 28, 40, 41, 39, 32, 33, 27, 30, 31, and 35) accounted for a further 26%; group III (accessions 34, 4 and 38) accounted for 7%; group IV (accessions 1 and 3) had 4% to the total accessions; and the remaining 2% were allocated to group VI (accession 37) and VII (accession 8). Accessions 24 and 27 were found to be the most divergent, while accessions 10 and 17 were the most similar.

### 3.4. Importance of the characteristics evaluated

Based on Singh's (1981) methodology, the relative contribution of observed morphological characteristics to genetic divergence ranged from 3.01 to 15.1% for sheath width (SW) and plant habit (PH), respectively (Figure 5). Analysis showed that eight characteristics accounted for a total of 64.7%

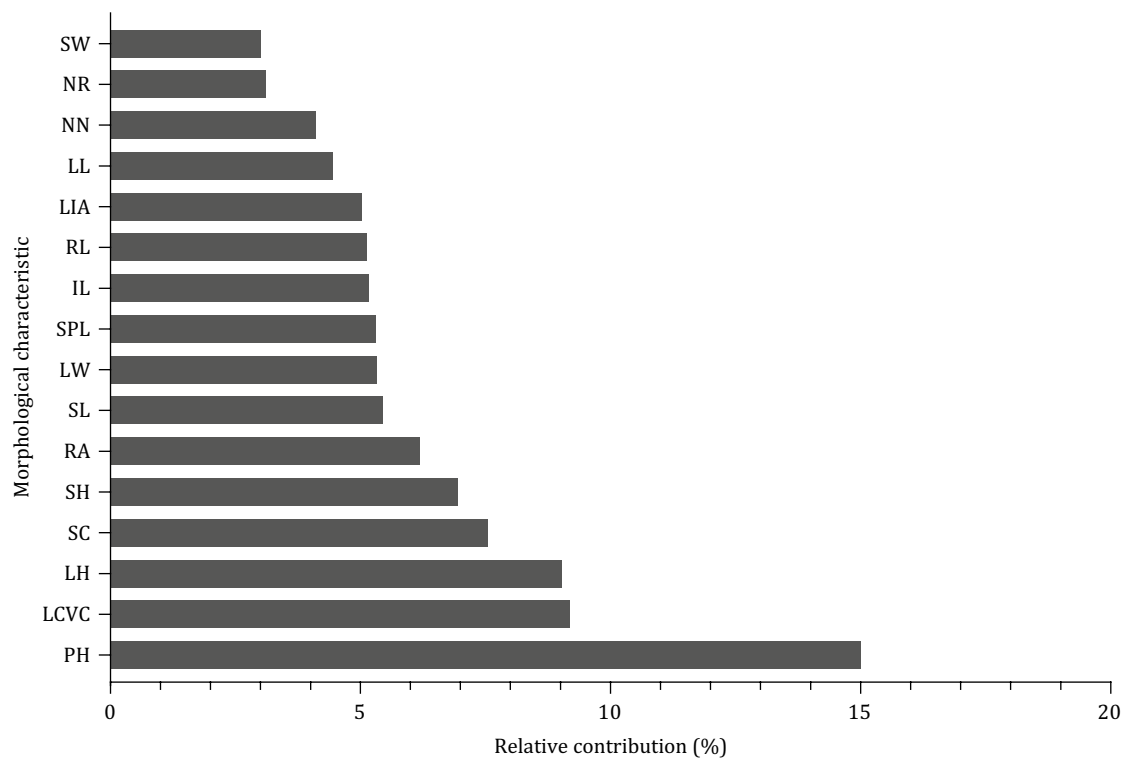
**Table 3** - Composition of groups formed by the original and sequential Tocher optimization methods in *P. notatum* Flüge, based on the Gower distance matrix

Group	Original Tocher grouping	
	Accession	
I	10, 17, 18, 15, 22, 19, 24, 20, 7, 23, 13, 25, 16, 21, 29, 9, 32	
II	31, 35, 30, 28, 27, 26, 38, 33	
III	2, 12, 40, 41	
IV	14, 36	
V	5, 39	
VI	8	
VII	6	
VIII	4	
IX	11	
X	37	
XI	1	
XII	34	
XIII	3	
	Sequential Tocher grouping	
I	10, 17, 18, 15, 22, 19, 24, 20, 7, 23, 13, 25, 16, 21, 29, 9, 32	
II	31, 35, 30, 28, 27, 26, 38, 33, 4, 41, 11, 39, 40	
III	2, 12, 36, 5, 1	
IV	6, 14	
V	8, 34, 37	
VI	3	



Cophenetic correlation index: 0.68.

**Figure 4** - Dendrogram of genetic dissimilarity of 41 accessions of *P. notatum* Flüge, obtained by the UPGMA method, based on the Gower distance matrix, which evaluated 16 characteristics.



SW - sheath width; NR - number of racemes; NN - number of nodes; LL - leaf blade length; LIA - leaf blade insertion angle; RL - raceme length; IL - internode length; SPL - spikelet length; LW - leaf blade width; SL - sheath length; RA - raceme angle; SH - sheath hairiness; LH - leaf blade hairiness; LCVC - leaf blade central vein color; PH - plant habit.

**Figure 5** - Relative contribution of multicategorical morphological characteristics to the genetic dissimilarity of 41 accessions of *P. notatum* Flüge.



of the genotype discrimination. Specifically, individual contributions were from PH (15.1%), leaf blade central vein color (LCVC; 9.18%), leaf blade hairiness (LH; 9.02%), sheath color (SC; 7.54%), sheath hairiness (SH; 6.95%), raceme angle (RA; 6.19%), sheath length (SL; 5.44%), and leaf blade width (LW; 5.33%) (Figure 5). Based on Singh's (1981) methodology, the use of these eight characteristics would be sufficient to quantify the genetic variability of the accessions studied. This method considers that the greater the variability found for a specific characteristic, the greater its importance in relation to the others. The remaining eight morphological characteristics had a smaller contribution and accounted for 35.3% of the total divergence.

To evaluate the importance of the measured characteristics, principal components analysis (PCA) was applied. This allowed us to discriminate among characteristics of greater weight, in the components with lower contribution in variability. The first seven principal components explained 80.4% of the total data variation among all observed characteristics (Table 4). Nine principal components presented eigenvalues ( $\lambda_j$ ) lower than 0.7 (Table 4) and allowed variables of greater weight to be discarded in these components of lesser importance. Subsequently, it was concluded that variables SC, SH, spikelet length (SPL), SL, RA, leaf blade length (LL), LW, and raceme length (RL) could be discarded in genetic diversity studies, as they contribute little to the discrimination among accessions (Table 4).

**Table 4 - Principal components (PC), estimates of variances (eigenvalue  $\lambda_j$ ), percentage of variance explained by components (importance %), and accumulated variance (% accumulated) of *Paspalum notatum* Flüge accessions**

PC	$\lambda_j$	Importance (%)	% accumulated	Highlight	Recomendation <sup>1</sup>
PC1	4.25	26.54	26.53	RL	
PC2	2.26	14.13	40.66	SW	
PC3	1.85	11.53	52.19	SH	
PC4	1.53	9.57	61.76	LH	
PC5	1.17	7.33	69.09	NR	
PC6	1.02	6.39	75.48	LIA	
PC7	0.78	4.90	80.38	LCVC	
PC8	0.69	4.30	84.67	SC	Discard
PC9	0.57	3.57	88.24	SH	Discard
PC10	0.54	3.36	91.61	SPL	Discard
PC11	0.42	2.62	94.22	SL	Discard
PC12	0.35	2.16	96.38	SC	Discard
PC13	0.29	1.81	98.19	RA	Discard
PC14	0.13	0.82	99.09	LL	Discard
PC15	0.09	0.57	99.57	LW	Discard
PC16	0.07	0.42	100.00	RL	Discard

<sup>1</sup> According to the criterion of Jolliffe (1972).

RL - raceme length; SW - sheath width; SH - sheath hairiness; LH - leaf blade hairiness; NR - number of racemes; LIA - leaf blade insertion angle; LCVC - leaf blade central vein color; SC - sheath color; SH - sheath hairiness; SPL - spikelet length; SL - sheath length; SC - sheath color; RA - raceme angle; LL - leaf blade length; LW - leaf blade width; RL - raceme length.

## 4. Discussion

The results obtained here show there was a low association between geographic and morphological distance. The Tocher optimization method (Table 2), based on geographic distance, grouped the accessions of group II, mainly because they are closer accessions and present greater similarity (Lamas et al., 2022). Therefore, it is expected that other factors may be determining the distribution of genetic variability of the observed morphological characteristics. Accessions from different geographic locations can generate genetic structuring through the discontinuity of landscapes (Manel et al., 2003), in addition to preventing gene flow, which is the main factor responsible for the genetic homogeneity between subpopulations (Petit and Excoffier, 2009). Furthermore, it is

important to note that geographic variation is also responsible for imposing different environmental conditions on populations, causing different selective pressures (Lemos et al., 2012). Therefore, the estimation of the population genetic structure makes it possible to determine how genetic variability is distributed within a species (Fajardo et al., 2016). In addition to the information on geographic distribution and the correlation with morphology, forage and seed production, molecular tools (Cidade et al., 2013; Garcia et al., 2013; Weiler et al., 2017), and genetic maps (Stein et al., 2007) become important when the objective is to discriminate accessions and select for characters by multivariate techniques.

Breeding is highly dependent on the genetic diversity available within the species under evaluation (Sant'Anna et al., 2021). In our study, it was possible to characterize and estimate the genetic diversity among 41 accessions of *P. notatum* Flügge, studied using characteristics of multicategory origin (Table 1). Based on the Gower distance, the original (Rao, 1952) and sequential Tocher (Vasconcelos et al., 2007) clustering methods showed divergences in the accession discrimination. Specifically, there was a reduction in the number of clusters by the sequential method compared with the original (Table 3). Based on these results, it can be inferred that there is genetic variability among the 41 accessions based on the morphological characteristics studied, because the grouping of accessions with greater dissimilarity was performed more efficiently by the sequential Tocher method than by the original Tocher method (Vasconcelos et al., 2007).

The Tocher (original and sequential) and hierarchical (UPGMA) methods showed large concentrations of genotypes in groups I and II, regardless of the distance used (geographic or morphological). It was expected that accessions from different regions, microclimates, and natural selection would result in heterogeneous morphological characteristics and allow differentiation and clustering for breeding purposes (Barros et al., 2022). The large number of accessions in a single cluster demonstrate that they are more closely related and have less genetic variation among themselves (Kaur et al., 2018). This shows that morphological characterization is a valuable source of information for breeding programs (Boczkowska et al., 2014), in addition to being commonly used to assess genetic divergence among accessions (Barros et al., 2022). However, they have limitations to estimate genetic diversity (Kaur et al., 2018). Therefore, information based on geographic, physiological, and molecular distances should be complemented. When the intention is to identify regions with high potential, we recommend collection of superior accessions based on agronomic characteristics. In addition, the concomitant use of different clustering methods (optimization and UPGMA) confers greater efficiency in the discrimination of accessions in terms of their genetic distances (Nardino et al., 2017) and are widely used in studies of genetic divergence (Sant'Anna et al., 2021; Silveira et al., 2022a).

By analyzing the relative contribution of characteristics to genetic divergence using the Singh (1981) method, it is possible to classify the characteristics with the greatest contribution to total genetic divergence. It has the additional benefit of eliminating the characteristics with the least contribution (Perini et al., 2018; Silveira et al., 2022b). The PCA is used to characterize accessions from germplasm banks for different plant species (Smýkal et al., 2017; Maeda et al., 2018; Hashemi and Khadivi, 2020). It is also used to identify the characteristics of higher or lower contribution and/or to discard characteristics with less relevance (Silveira et al., 2019; Jesus et al., 2022). As expected, our study showed there were divergences between the methodologies for selecting and/or discarding characteristics. Therefore, for greater safety when choosing to eliminate a characteristic, it is recommended to use more than one disposal methodology (Costa et al., 2018). When the accessions were evaluated based on Singh's method (1981), it showed characteristics of minor relative importance for genetic divergence: spikelet length (SPL), internode length (IL), RL, leaf blade insertion angle (LIA), LL, number of nodes (NN), number of racemes (NR), and SW (Figure 5).

For PCA (Table 4), the characteristics RL, SW, SH, LH, NR, LIA, and LCVC were identified as discardable, based on the criterion of Jolliffe (1972, 1973). Based on both analyses, it is suggested that the characteristics RL, SW, and LIA be eliminated from evaluation in future studies. Results reported here emphasize the importance of using both methodologies when deciding which

characteristics to quantify in characterization and/or genetic divergence studies for species within the *Paspalum* genus.

This study demonstrated morphological variation of characteristics and accessions based on multivariate analyses. Divergence results showed that many of the accessions evaluated here are suitable for inclusion in future breeding programs. These can be used to characterize the forage potential and seed production of the species, which has been the main obstacle in the development of new *Paspalum* hybrids.

## 5. Conclusions

There is a low level of correlation, despite being significant, between geographical and morphological distances in genetic divergence, indicating that it is necessary to use forage and seed production characteristics, in addition to molecular analyses. This will help accurately determine the possible existence of geographical and genetic variability between accessions of the genus *Paspalum*. The methodologies used to discard variables are biometric tools that can be used in plant improvement programs, particularly when a large number of characteristics and accessions are being evaluated. There is genetic variability in *P. notatum* for all morphological characteristics analyzed. The superior access can be used in new combinations between apomictic and sexual plants to create superior hybrids.

## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

Conceptualization: M.G. Steiner, R.L. Weiler, A. Mills, M. Dall'Agnol, C. Nabinger and D.C. Silveira. Formal analysis: D.C. Silveira. Funding acquisition: M. Dall'Agnol. Investigation: M.G. Steiner, R.L. Weiler, A.P. Brunes, M. Dall'Agnol, E.A.M. Motta, R. Sampaio and G. Tassis. Methodology: M.G. Steiner, R.L. Weiler, A.P. Brunes, M. Dall'Agnol, E.A.M. Motta, R. Sampaio and G. Tassis. Project administration: R.L. Weiler, A.P. Brunes, M. Dall'Agnol, E.A.M. Motta, R. Sampaio and G. Tassis. Resources: M. Dall'Agnol. Supervision: M.G. Steiner, A.P. Brunes, M. Dall'Agnol, C. Nabinger and G. Tassis. Validation: R. Sampaio. Visualization: R.L. Weiler, A. Mills and E.A.M. Motta. Writing-original draft: R.L. Weiler, A. Mills and D.C. Silveira. Writing-review & editing: D.C. Silveira.

## References

- Barros, T.; Weiler, R. L.; Machado, J. M.; Silveira, D. C.; Brunes, A. P.; Mills, A.; Simioni, C. and Dall'Agnol, M. 2022. Dissimilarity between *Andropogon lateralis* ecotypes under different defoliation frequencies and heights. *Ciência Rural* 52:e20201079. <https://doi.org/10.1590/0103-8478cr20201079>
- Boczkowska, M.; Nowosielski, J.; Nowosielska, D. and Podyma, W. 2014. Assessing genetic diversity in 23 early Polish oat cultivars based on molecular and morphological studies. *Genetic Resources and Crop Evolution* 61:927-941. <https://doi.org/10.1007/s10722-014-0087-4>
- Burton, G. W. 1967. A search for the origin Pensacola Bahia grass. *Economic Botany* 21:379-382. <https://doi.org/10.1007/BF02863165>
- Chase, A. 1929. The North American species of *Paspalum*. *Contributions from the United States National Herbarium* 28:1-310.
- Cidade, F. W.; Vigna, B. B. Z.; Souza, F. H. D.; Valls, J. F. M.; Dall'Agnol, M.; Zucchi, M. I.; Souza-Chies, T. T. and Souza, A. P. 2013. Genetic variation in polyploid forage grass: Assessing the molecular genetic variability in the *Paspalum* genus. *BMC Genetics* 14:50. <https://doi.org/10.1186/1471-2156-14-50>
- Costa, M. I. A.; Melo, L. A.; Ferreira, S. C. and Matsuo, E. 2018. Morfometria reprodutiva e diversidade genética em cultivares de soja. *Nucleus* 15:207-215.

- Fachinetto, J. M.; Dall'Agnol, M.; Souza, C. H. L.; Weiler, R. L. and Simioni, C. 2017. Genetic diversity of a *Paspalum notatum* Flüge germplasm collection. *Revista Brasileira de Zootecnia* 46:714-721. <https://doi.org/10.1590/S1806-92902017000900002>
- Fajardo, C. G.; Vieira, F. A. and Molina, W. F. 2016. Conservação genética de populações naturais: Uma revisão para Orchidaceae. *Biota Amazônia* 6:108-118.
- Garcia, M.; Vigna, B. B. Z.; Sousa, A. C. B.; Jungmann, L.; Cidade, F. W.; Toledo-Silva, G.; Francisco, P. M.; Chiari, L.; Carvalho, M. A.; Karia, C. T.; Faleiro, F. G.; Godoy, R.; Dall'Agnol, M.; Pagliarini, S. S.; Souza, F. H. D.; Souza-Chies, T. T.; Jank, L.; Resende, R. M. S.; Valle, C. B.; Zucchi, M. I. and Souza, A. P. 2013. Molecular genetic variability, population structure and mating system in tropical forages. *Tropical Grasslands* 1:25-30. [https://doi.org/10.17138/TGFT\(1\)25-30](https://doi.org/10.17138/TGFT(1)25-30)
- Gower, J. C. 1971. A general coefficient of similarity and some of its properties. *Biometrics* 27:857-874. <https://doi.org/10.2307/2528823>
- Graminho, L. A.; Dall'Agnol, M.; Potter, L.; Lopes, R.; Simioni, C. and Weiler, R. L. 2017. Forage characters of different *Paspalum* species in Rio Grande do Sul: a meta-analysis. *Ciência Rural* 47:e20161049. <https://doi.org/10.1590/0103-8478cr20161049>
- Hashemi, S. and Khadivi, A. 2020. Morphological and pomological characteristics of white mulberry (*Morus alba* L.) accessions. *Scientia Horticulturae* 259:108827. <https://doi.org/10.1016/j.scienta.2019.108827>
- Jesus, O. N.; Lima, L. K. S.; Soares, T. L.; Silva, L. N.; Santos, I. S.; Sampaio, S. R. and Oliveira, E. J. 2022. Phenotypic diversity and alternative methods for characterization and prediction of pulp yield in passion fruit (*Passiflora* spp.) germplasm. *Scientia Horticulturae* 292:110573. <https://doi.org/10.1016/j.scienta.2021.110573>
- Jolliffe, I. T. 1972. Discarding variables in a principal component analysis. I: Artificial data. *Journal of the Royal Statistical Society: Series C (Applied Statistics)* 21:160-173. <https://doi.org/10.2307/2346488>
- Jolliffe, I. T. 1973. Discarding variables in a principal component analysis. II: Real data. *Journal of the Royal Statistical Society: Series C (Applied Statistics)* 22:21-31. <https://doi.org/10.2307/2346300>
- Kaur, R.; Kapoor, R.; Vikal, Y. and Kaur, K. 2018. Assessing genetic diversity in dual purpose oat (*Avena sativa* L.) cultivars based on morphological and quality traits. *International Journal of Current Microbiology and Applied Sciences* 7:1574-1586. <https://doi.org/10.20546/ijcmas.2018.705.187>
- Lamas, C. Y.; Urtasun, M. M.; Giamminola, E. M.; Pratta, G. R.; Caruso, G. B.; Morandini, M. N. and de Viana, M. L. 2022. Fruit and seed characterization of wild populations of a traditional Andean crop: *Solanum betaceum* Cav. (Solanaceae) in the Argentinian Yungas. *Genetic Resources and Crop Evolution* 69:231-244. <https://doi.org/10.1007/s10722-021-01223-3>
- Lemos, C. A.; Vagenas, D. N. F. and Vaz-Dos-Santos, A. M. 2012. Uso de técnicas moleculares para análise da variação geográfica em clupeídeos: revisão de metodologias, síntese de conceitos e aplicabilidade. *Revista Ceciliania* 4:94-97.
- Maeda, H.; Akagi, T. and Tao, R. 2018. Quantitative characterization of fruit shape and its differentiation pattern in diverse persimmon (*Diospyros kaki*) cultivars. *Scientia Horticulturae* 228:41-48. <https://doi.org/10.1016/j.scienta.2017.10.006>
- Manel, S.; Schwartz, M. K.; Luikart, G. and Taberlet, P. 2003. Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution* 18:189-197.
- Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* 27 (2 Part 1):209-220.
- Mojena, R. 1977. Hierarchical grouping methods and stopping rules: an evaluation. *Computer Journal* 20:359-363. <https://doi.org/10.1093/comjnl/20.4.359>
- Morrone, O.; Aagesen, L.; Scatagliani, 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 *Panicaceae* (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.; Rios, E. F.; Souza, C. H. L.; Weiler, R. L.; Brunas, A. P.; Simioni, C.; Ávila, M. R.; Neto, D.; Felix, L. D. and Santos, T. N. 2020. Agronomic performance of interspecific *Paspalum* hybrids under nitrogen fertilization or mixed with legumes. *Agrosystems, Geosciences & Environment* 3:e20127. <https://doi.org/10.1002/agg2.20127>
- Motta, E. A. M.; Graminho, L. A.; Dall'Agnol, M.; Pötter, L.; Nabinger, C.; Souza, C. H. L.; Krycki, K. C.; Santos, T. N.; Weiler, R. L. and Ávila, M. R. 2021. Response of Bahiagrass hybrids to nitrogen fertilization or mixture with legumes. *Revista Brasileira de Zootecnia* 50:e20210015. <https://doi.org/10.37496/rbz5020210015>
- Nabinger, C. and Dall'Agnol, M. 2019. Guia para reconhecimento de espécies dos Campos Sulinos. IBAMA, Brasília.
- Nardino, M.; Baretta, D.; Carvalho, I. R.; Follmann, D. N.; Ferrari, M.; Pelegrin, A. J.; Szarecki, V. J.; Konflanz, V. A. and Souza, V. Q. 2017. Divergência genética entre genótipos de milho (*Zea mays* L.) em ambientes distintos. *Revista de Ciências Agrárias* 40:164-174. <https://doi.org/10.19084/RCA16013>
- Perini, J. L.; Zeffa, D. M.; Freiria, G. H.; Novais, P. S. and Prete, C. E. C. 2018. Diversidade genética entre acessos de soja tipo alimento com base no algoritmo de Gower. *Colloquium Agrariae* 14:47-57.
- Petit, R. J. and Excoffier, L. 2009. Gene flow and species delimitation. *Trends in Ecology & Evolution* 24:386-393. <https://doi.org/10.1016/j.tree.2009.02.011>

- Rao, C. R. 1952. Advanced statistical methods in biometric research. John Wiley & Sons, New York.
- Sant'Anna, I. C.; Cruz, C. D.; Gouvêa, L. R. L.; Scaloppi Junior, E. J.; Freitas, R. S. and Gonçalves, P. S. 2021. Genetic diversity analyses of rubber tree genotypes based on UPOV descriptors. *Industrial Crops and Products* 165:113416. <https://doi.org/10.1016/j.indcrop.2021.113416>
- Silveira, D. C.; Machado, J. M.; Faccioli, M. W. F.; Tolfo, A. M. and Eich, C. 2019. Diversidade genética de acessos de feijão crioulo na região Noroeste do Rio Grande do Sul. *Pesquisa Agropecuária Gaúcha* 25:133-146. <https://doi.org/10.36812/pag.2019251/2133-146>
- Silveira, D. C.; Pelissoni, M.; Buzatto, C. R.; Scheffer-Basso, S. M.; Ebone, L. A.; Machado, J. M. and Lângaro, N. C. 2021. Anatomical traits and structural components of peduncle associated with lodging in *Avena sativa* L. *Agronomy Research* 19:250-264. <https://doi.org/10.15159/AR.21.001>
- 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. 2022a. 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>
- Silveira, D. C.; Basso, S. M. S.; Ebone, L. A.; Caverzan, A.; Machado, J. M.; Schaeffer, A. H.; Folchini, J. A. and Lângaro, N. C. 2022b. Morphological traits of stem to indirect selection of resistance to lodging in *Avena sativa* L. *Journal of Crop Science and Biotechnology* 25:39-50. <https://doi.org/10.1007/s12892-021-00111-7>
- Singh, D. 1981. The relative importance of characters affecting genetic divergence. *Indian Journal Genetic Plant Breeding* 41:237-245.
- Smykal, P.; Hradilová, I.; Trněný, O.; Brus, J.; Rathore, A.; Bariotakis, M.; Das, R. R.; Bhattacharyya, D.; Richards, C.; Coyne, C. J. and Pirintzos, S. 2017. Genomic diversity and macroecology of the crop wild relatives of domesticated pea. *Scientific Reports* 7:17384. <https://doi.org/10.1038/s41598-017-17623-4>
- Stein, J.; Pessino, S. C.; Martínez, E. J.; Rodriguez, M. P.; Siena, L. A.; Quarin, C. L. and Ortiz, J. P. A. 2007. A genetic map of tetraploid *Paspalum notatum* Flügge (bahiagrass) based on single-dose molecular markers. *Molecular Breeding* 20:153-166. <https://doi.org/10.1007/s11032-007-9083-0>
- Steiner, M. G.; Dall'Agnol, M.; Nabinger, C.; Scheffer-Basso, S. M.; Weiler, R. L.; Simioni, C.; Schifino-Wittmann, M. T. and Motta, E. A. M. 2017. Forage potential of native ecotypes of *Paspalum notatum* and *P. guenoarum*. *Anais da Academia Brasileira de Ciências* 89:1753-1760. <https://doi.org/10.1590/0001-3765201720160662>
- Urbani, M. H.; Acuña, C. A.; Doval, D. W.; Sartor, M. E.; Galdeano, F.; Blount, A. R.; Quesenberry, K. H.; Mackowiak, C. L. and Quarin, C. L. 2017. Registration of 'Boyero UNNE' Bahiagrass. *Journal of Plant Registrations* 11:26-32. <https://doi.org/10.3198/jpr2016.04.0021crc>
- Vasconcelos, E. S.; Cruz, C. D.; Bhering, L. L. and Resende Júnior, M. F. R. 2007. Método alternativo para análise de agrupamento. *Pesquisa Agropecuária Brasileira* 42:1421-1428. <https://doi.org/10.1590/S0100-204X2007001000008>
- 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. and 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>
- Zuloaga, F. O. and Morrone, O. 2005. Revisión de las especies de *Paspalum* para América del Sur austral (Argentina, Bolivia, sur de Brasil, Chile, Paraguay y Uruguay). Missouri Botanical Garden Press, Saint Louis, MO.