



Genetic diversity of a *Paspalum notatum* Flüggé germplasm collection

Juliana Maria Fachinetto¹, Miguel Dall'Agnol^{2*}, Cleber Henrique Lopes de Souza³, Roberto Luis Weiler², Carine Simioni²

¹ Universidade Regional do Noroeste do Estado do Rio Grande do Sul, Ijuí, RS, Brazil.

² Universidade Federal do Rio Grande do Sul, Faculdade de Agronomia, Departamento de Plantas Forrageiras e Agrometeorologia, Porto Alegre, RS, Brazil.

³ Universidade Federal do Rio Grande do Sul, Programa de Pós-graduação em Zootecnia, Porto Alegre, RS, Brazil.

ABSTRACT - The objective of this study was to assess the genetic diversity among accessions of *Paspalum notatum* based on morphological marker traits and identify those morphological traits that contribute most to the variability among accessions. Fifty-three accessions were evaluated for the following traits: length, width, color, and pubescence of sheath; length, width, insertion angle, color of midrib, and pubescence of leaves; number, length, and angle of racemes; length of flowering stems; and plant growth habit (erect or prostrate). Multivariate analysis of the quantitative morphological data based on the Mahalanobis distance and UPGMA method grouped the accessions into 19 heterogeneous clusters. The relative contribution of each trait to cluster formation was obtained using the Singh method. Qualitative morphological traits were used to construct a dendrogram based on Jaccard's similarity, generating 13 clusters. Based on the morphological traits investigated, there is high genetic variation among accessions of *P. notatum*, demonstrating that these accessions have a good potential for a breeding program. Length of racemes, width of sheath, length of flowering stems, and length of leaves were found to contribute most to the variability among accessions. A number of traits contributed to a lesser degree. The set of characters used are representative of the vast phenotypic plasticity observed in *P. notatum*, a diversity which is likely to be related to the species apomictic form of reproduction and the origin of the accessions.

Key Words: forage grass, genetic improvement, multivariate analysis

Introduction

Species of the genus *Paspalum* L. are the main components of native pasture in the tropical and subtropical regions of the Americas (Sartor et al., 2009). Most species of *Paspalum* are apomictic tetraploids or sexual self-incompatible diploids (Ortiz et al., 2013). From an evolutionary point of view, the genus *Paspalum*, therefore, represents an excellent model for the investigation of the role of apomixis and polyploidy (Whitton et al., 2008; Ortiz et al., 2013). From an agronomic point of view, the reproductive mode of the species of the genus *Paspalum* is of relevance, since the development of new cultivars relies on natural genetic variation, and intra- and interspecific hybridization efforts are affected by the reproductive mode

(Adamowski et al., 2005). Among the forage grasses of the genus *Paspalum*, *Paspalum notatum* Flüggé is of great importance because of its wide distribution in the natural grasslands of the Pampa region (Boldrini et al., 2010). Favored by its growth habit, the species distribution is gradually further increasing and is considered one of the most promising native forage grasses in its region (Barreto, 1974; Canto-Dorow et al., 1996; Pozzobon and Valls, 1997). The genetic and morphological diversity of the species holds great potential for the development of new cultivars with desirable characteristics (Canto-Dorow et al., 1996; Pozzobon and Valls, 1997; Steiner, 2005; Cidade et al., 2008; Dahmer et al., 2008).

Studies of morphological variation in plants are a useful tool in genetic improvement programs and enable access to the genetic variability within a germplasm bank (Azevedo et al. 2011; Martuscello et al., 2012). In plant breeding programs, knowledge of variability and genetic diversity within a species is essential for the rational use of genetic resources (Govindaraj et al., 2015).

The development of new cultivars in *Paspalum* has been focused on identifying genotypes with high forage production, using sexual parents to generate genetic variability. In addition, these materials have been characterized in relation to their genetics and morphology

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*Corresponding author: miguel.dallagnol@ufrgs.br

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(Dahmer et al., 2008; Fachinetto et al., 2012; Pereira et al., 2012; Pereira et al. 2015). Subsequently, these materials are subjected to intra or interspecific crosses, using duplicate diploid sex plants as the female parent (Huber et al., 2016; Machado et al., 2017).

In face of the great productive potential of *P. notatum* evidenced by previous studies (Fachinetto et al., 2012), the objectives of this study were to assess the genetic variability among accessions of *P. notatum* based on morphological traits, study the genetic diversity based on morphological traits, and identify the traits that contributed most to the discrimination of accessions.

Material and Methods

The seeds of 25 accessions of *P. notatum* were obtained from the United States Department of Agriculture (USDA). They had been collected at different locations in southern Brazil, Argentina, Uruguay, and Paraguay during the 1950s and 1970s (Figure 1) (Fachinetto et al., 2012). Twenty-seven accessions were obtained from the Departamento de Plantas Forrageiras e Agrometeorologia (DPFA) of the Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul (UFRGS) and cloned vegetatively. These had been collected at different locations in Rio Grande do Sul and São Paulo (Brasil), Uruguay, and Argentina (Figure 1) (Dahmer et al., 2008). Clones of the cultivar Pensacola and two biotypes of *Paspalum guenoarum* were used as control. In total, 55 accessions were included in the experiment in the form of seedlings, which were established in the spring of 2008. The experimental design was completely randomized with five replicates, using 10 lines with 28 plants in each, totalizing 280 plants. Plants were spaced at 1-m intervals and evaluated individually. Before the installation of the

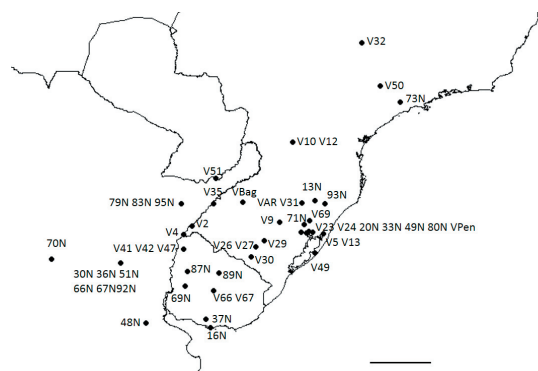
experiment, a basic fertilization was carried out according to recommendations and to the soil analysis. Subsequently, fertilizations were performed in three fractional doses of nitrogen. The experiment was irrigated by aspersion to avoid water deficit in the evaluation period.

The experimental area is located in the physiographic region of the Central Depression, whose climate is of the Cfa type, subtropical humid with hot summer, according to the Köppen classification. Average monthly temperatures range from 9 to 25 °C, with January and February as the hottest months, and June and July as the coldest months. The annual average precipitation is 1466 mm, while the monthly average is 125 mm. The soil is classified as Dystrophic Red Argisol - Pvd (Streck et al., 2002).

Morphological analyses were performed at full flowering during the summer of 2009. All measurements were taken in three replicates for all 53 accessions of *P. notatum*, except for *P. guenoarum*. The following characteristics were evaluated for each accession: length of sheath (LS; cm), width of sheath (WS; cm), color of sheath (CS; visual scores: 1- greenish, 2- violaceous), pubescence of sheath (PS; visual scores: 1- pubescent, 2- glabrous); length of leaves (LL; cm), width of leaves (WL; cm), insertion angle of leaves (IAL; grade), color of midrib of leaves (CML; visual score: 1- white; 2- greenish), pubescence of leaves (PL; visual scores: 1- pubescent, 2- glabrous); number of racemes (RN), length of raceme (LR; cm), angle between racemes (AR; grade), length of flowering stems (LFS; cm), and plant habit (PH; visual scores: 1- erect; 2- prostrate). To facilitate a standardized comparison between accessions, the measurements of sheaths and leaf blades were performed on the first leaf below the flag leaf. In the inflorescences with more than two racemes, the angulation was measured among the external racemes.

A multivariate analysis of all quantitative data was performed. In the clustering analysis, the Mahalanobis generalized distance (D^2) was used to measure dissimilarity and constructed an Unweighted Pair Group (UPGMA) dendrogram. The relative importance of the characters was determined from the non-standard average using the Singh method (Singh, 1981). Statistical analyses were carried out using the GENES software (Cruz, 2006).

Quantitative data obtained were transformed into binary matrix. To estimate the similarity among the accessions, Jaccard's coefficient was used, through the similarity of qualitative data (SIMQUAL), and clustering analysis constructed the UPGMA dendrogram using NTSYS 2.1 (Rohlf, 1998).



Scale: 300 km.

Figure 1 - Location of *Paspalum notatum* accessions.

Results

Mean leaf sheath lengths and widths ranged within and among accessions. In certain accessions, only a single leaf sheath color (violaceous or green) was found. In others, the

color of the leaf sheath showed variation. Leaf sheaths were glabrous in all accessions, with the exception of accessions V49 (completely pubescent) and V50 (variable) (Table 1).

All the characters measured in leaf blade were variables among accessions. The midrib color of leaves ranged from

Table 1 - Morphologic characteristics of the of *Paspalum notatum* accessions

Accession	LS (cm)	WS (cm)	LL (cm)	WL (cm)	IAL (score)	LR (cm)	NR	AR (score)	LFS	CS	PS	CML	PL	PH
	a±SD										Visual score			
Pensacola	7.0±1.4	0.9±0.1	13.4±1.4	0.7±0.1	46.7±7.6	10.1±1.9	2	40.0±32.8	29.9±5.5	1-2	2	1	2	2
André da Rocha	6.2±0.7	0.9±0.1	10.8±1.1	0.9±0.2	40.0±20.0	8.0±0.4	2-3	61.7±47.3	18.3±3.2	1-2	2	1	2	1-2
Bagual	9.4±2.8	1.0±0.1	14.2±4.1	0.9±0.3	33.3±5.8	12.6±2.0	2	55.0±5.0	39.3±2.3	2	2	1-2	2	2
13N	9.3±2.5	0.8±0.1	17.5±7.0	0.5±0.1	30.7±5.1	9.9±2.3	2	51.0±24.6	34.2±8.5	1-2	2	1	2	1
16N	6.5±0.3	1.0±0.2	10.6±2.5	0.8±0.1	34.7±1.5	8.6±0.2	2	49.0±6.1	27.6±1.9	1-2	2	1	2	1
17N	5.6±1.0	1.0±0.2	12.5±3.0	1.0±0.1	55.0±30.4	8.8±0	2	58.3±5.8	28.2±3.7	1	2	1	2	1
20N	7.1±4.3	0.7±0.1	6.0±2.0	0.7±0.1	50.0±30.0	6.3±1.0	2	85.0±33.6	25.0±2.5	1	2	1-2	2	1
30N	9.5±0.5	0.9±0.3	17.7±0.6	0.9±0.3	45.0±22.9	13.9±1.5	2	70.0±0	46.9±1.6	1	2	1	2	1
33N	5.9±0.9	1.0±0	14.1±1.4	0.9±0.1	41.7±16.1	9.6±0.2	2	21.7±22.5	18.3±5.4	1	2	1	2	2
36N	7.9±0.8	1.1±0.1	18.1±1.4	1.0±0.1	45.0±8.7	11.6±1.3	2	56.7±10.4	37.7±2.6	2	2	1	2	1
37N	7.1±3.5	0.6±0.1	8.6±1.0	0.4±0.1	26.7±11.5	7.3±1.7	2	35.0±10.0	20.2±8.3	1-2	2	1-2	2	1-2
48N	11.0±0.5	1.0±0.1	19.5±2.6	1.0±0.1	38.3±7.6	14.2±1.4	2	38.3±10.4	42.2±4.0	1-2	2	1	2	1-2
49N	7.3±1.5	1.0±0	12.0±3.0	0.9±0.1	33.3±11.5	9.3±0.1	2	65.0±35.0	23.5±7.0	2	2	1-2	2	2
51N	6.0±0.9	1.0±0.1	11.6±1.2	0.9±0.1	35.7±9.3	9.2±1.1	2	76.7±42.5	25.9±7.5	1-2	2	1	2	1
66N	10.6±1.4	0.9±0.2	19.4±0.6	0.8±0.2	38.3±15.3	12.8±0.4	2	38.3±17.6	40.6±2.3	2	2	1	2	1
67N	11.4±1.1	0.8±0.1	18.0±2.7	0.6±0.1	30.0±13.2	11.9±1.3	2	40.0±10.0	34.9±10.2	2	2	1	2	1
69N	5.7±0.9	0.8±0.2	8.2±1.0	0.7±0.1	45.7±9.3	6.7±0.4	2	48.3±15.3	23.2±3.1	1	2	1-2	2	1
70N	9.4±0.5	1.2±0.1	22.6±2.8	1.2±0.1	30.0±5.0	10.7±1.9	2-3	63.3±43.1	38.0±2.7	1	2	1	2	1
71N	6.1±1.1	1.0±0.2	10.4±5.4	0.8±0.2	38.3±7.6	6.8±1.7	2-3	30.0±17.3	22.4±6.6	1	2	1-2	2	2
73N	6.7±1.8	1.3±0.1	14.4±1.9	1.5±0.1	43.3±14.4	10.4±0.6	2	45.0±39.7	22.3±1.5	1-2	2	2	1-2	2
79N	5.0±1.1	0.8±0.1	8.2±1.2	0.7±0.1	65.0±21.8	6.9±0.7	2	45.0±8.7	23.0±3.1	1	2	2	2	1
80N	6.8±1.5	1.0±0	12.8±1.9	1.0±0.1	45.0±5.0	9.2±0.2	2	50.0±10.0	27.2±8.0	1-2	2	1	2	1-2
83N	13.7±1.9	0.9±0.1	17.6±0.6	0.6±0.1	25.0±13.2	10.9±0.4	2	45.0±21.2	37.6±2.0	1-2	2	1	2	1
87N	4.8±0.5	0.4±0	4.8±0.5	0.7±0.1	30.0±17.3	6.1±0.6	2-5	41.7±17.6	21.8±1.6	1	2	2	2	1
89N	5.0±2.1	0.8±0.1	8.7±1.2	0.7±0.2	35.0±0	6.4±0.7	2-4	52.3±52.3	20.2±4.6	2	2	1	2	1
92N	11.8±2.2	0.8±0.1	24.6±1.4	0.6±0.1	23.3±7.6	14.7±0.4	2	43.3±2.9	45.0±6.9	1-2	2	1	2	1
93N	6.3±1.8	0.7±0.1	6.5±1.2	0.6±0.1	23.3±17.6	6.4±0.2	2	40.0±5.0	21.4±0.6	2	2	2	2	1
95N	7.6±2.1	1.0±0.1	20.3±4.3	0.9±0.1	60.0±15.0	12.2±1.4	2	60.0±8.7	39.7±1.5	2	2	1	2	1
V2	6.2±0.9	0.7±0.1	15.7±3.6	0.6±0.1	23.3±12.6	8.9±0.6	2	35.0±5.0	29.4±1.0	1-2	2	1	2	1
V4	8.1±1.9	1.2±0.1	18.3±1.3	1.1±0.2	55.0±20.0	13.2±0.9	2-3	18.3±5.8	33.0±8.1	1-2	2	1	2	1
V5	7.2±0.2	0.8±0.2	8.4±2.3	0.8±0.1	41.7±7.6	7.7±0.6	2	93.3±40.1	27.5±1.6	1	2	1-2	1-2	1-2
V9	8.0±4.4	0.8±0.1	12.6±3.6	0.8±0.2	36.7±23.1	9.6±3.1	2	45.0±14.1	23.2±13.9	1	2	1-2	2	1-2
V10	5.6±1.2	1.0±0.2	11.3±5.0	1.0±0.1	41.7±20.2	8.1±1.0	2	53.3±24.7	22.0±6.3	2	2	1	2	1
V12	9.4±0.8	0.9±0.1	12.3±2.8	0.6±0.1	36.7±5.8	9.9±2.2	2-3	23.3±5.8	30.7±4.6	2	2	1	2	1
V13	7.2±0.7	0.9±0.1	11.1±1.9	1.0±0.1	35.0±13.2	7.1±0.3	2-4	41.7±5.8	21.7±0.4	1	2	2	1	1
V23	5.4±0.3	0.8±0.1	7.3±1.4	0.6±0.1	26.7±10.4	6.6±1.1	2	28.3±7.6	22.1±3.2	2	2	1	2	2
V24	9.9±2.1	0.7±0.1	6.9±4.3	0.6±0.2	15.0±13.2	8.1±0.6	2	30.7±16.8	22.2±0.4	1	2	1-2	2	1
V26	5.7±0.5	0.7±0.1	8.1±0.5	0.5±0.1	25.0±5.0	6.2±0.3	2	38.3±12.6	22.7±0.2	2	2	1	2	2
V27	5.5±1.8	0.8±0.1	6.9±3.1	0.7±0.1	18.3±15.3	6.2±0.3	2-3	38.3±23.1	20.0±1.3	2	2	1-2	2	2
V29	7.4±1.6	1.0±0.1	14.3±2.4	1.0±0.1	31.7±10.4	9.1±1.3	2	43.3±15.3	22.4±1.9	1-2	2	1	2	1
V30	5.5±0.8	0.9±0.1	10.0±1.6	0.8±0.1	53.3±32.1	7.1±1.1	2	46.7±5.8	25.0±2.8	1-2	2	1	2	2
V31	6.8±3.0	1.0±0.1	10.2±1.8	0.8±0.3	35.0±13.2	8.4±1.7	2	33.3±15.3	23.5±6.6	1	2	1-2	2	2
V32	6.0±1.7	1.0±0.2	13.8±4.4	0.9±0.1	33.3±23.6	8.5±0	2	20.0±0	15.2±0	1-2	2	1-2	2	2
V35	4.4±0.6	1.0±0	9.9±1.6	1.0±0.1	50.0±7.1	7.5±0.5	2	40.0±0	17.4±0	1	2	1-2	2	2
V41	6.1±1.8	1.1±0.1	14.4±1.2	1.0±0.1	30.0±17.3	9.8±1.2	2	42.5±3.5	29.0±6.3	1-2	2	1	2	2
V42	8.6±2.9	1.0±0.1	11.0±5.8	0.8±0.2	30.0±18.0	9.9±1.9	2	70.3±4.5	30.4±3.8	1	2	1-2	2	1
V47	3.6±0.5	0.8±0.1	6.6±1.3	0.7±0.2	38.3±2.9	5.9±0.9	2	36.7±2.9	14.9±3.1	1	2	1	2	2
V49	6.7±0.6	0.7±0.1	6.2±4.7	0.6±0.2	23.3±15.3	5.3±1.7	2	55.0±18.0	16.2±5.5	1	1	2	1	2
V50	5.7±1.5	1.1±0.1	9.5±0.6	1.2±0.4	45.0±8.7	8.2±1.2	2	38.3±41.6	15.8±10.9	1	1-2	2	2	2
V51	7.7±0.9	0.9±0.1	15.5±2.6	0.8±0.1	55.0±26.5	10.5±1.5	2	70.0±13.2	34.9±4.1	1	2	1	2	1
V66	6.1±0.9	1.1±0.1	10.5±1.4	1.0±0.2	46.7±15.3	9.2±0.2	2	50.0±5.0	24.8±5.9	2	2	1-2	1-2	2
V67	6.4±1.7	1.0±0.1	14.4±3.6	0.9±0.1	43.3±2.9	8.9±1.8	2	33.3±7.6	26.5±0.6	1-2	2	1	2	1
V69	5.2±0.9	0.8±0.1	7.9±3.4	1.0±0.6	46.7±20.2	6.7±0.6	2	36.7±5.8	22.8±2.7	1	2	1-2	2	1

a - average; SD - standard deviation; LS - length of sheath; WS - width of sheath; LL - length of leaves; WL - width of leaves; IAL - insertion angle of leaves; LR - length of racemes; NR - number of racemes; AR - angle between the racemes; LFS - length of flowering stems; CS - color of sheath (1- greenish, 2- violaceous); PS - pubescence of sheath (1- pubescent, 2- glabrous); CML - color of midrib of leaves (1- white, 2- greenish); PL - pubescence of leaves (1- pubescent, 2- glabrous); PH - plant habit (1- erect, 2- prostrate).

full white to full green, with variation in some accessions. In most accessions, the leaf blades were glabrous, two accessions (V13 and V49) had pubescent leaves only, and there was variation in three accessions (Table 1).

In most accessions, the inflorescence consisted of two racemes, although three, four, and up to five racemes were observed in some accessions (V13, 70N, 87N, and 89N). Length of raceme, angle between racemes, and length of the flowering stems also ranged within and among accessions (Table 1).

Twenty-nine accessions had an erect habit, 18 had a prostrate habit, and six showed variation (Table 1).

The highest variance among the evaluated characteristics was seen for the angle between racemes (AR $s^2 = 244.48$), with maximum and minimum mean values of 93.33 and 18.33°, respectively (Table 2). This trait accounted for 53.65% of the total variance observed. The insertion angle of the leaves (IAL $s^2 = 116.94$), which ranged from 65.0 to 15.0°, contributed 25.65% to the total variance. The length of flowering stems (LFS $s^2 = 63.60$) presented values between 46.9 and 14.9 cm and contributed 14% of the total variance. These three characteristics alone accounted for 93.26% of the total variance of the nine traits evaluated in the present study.

The clustering analysis using UPGMA method based on the Mahalanobis distance divided the studied accessions into 19 groups (Figure 2). In all groups, there was a wide variation in the characteristics studied. The greatest difference was observed between accessions 87N and 70N (109.89). The smallest difference was observed between accessions V26 and 93N (0.99).

It is important to consider the contribution of each of the assessed traits to the variability among accessions (Table 3). Here, the traits that contributed the most were length of racemes (21.59%), width of sheath (20.09%), length of flowering stems (18.70%), and length of leaves (16.61%). Together, these traits accounted for 76.99% of

the variability among accessions. Jaccard's similarity index obtained to qualitative morphological characters was 0.88 and formed 13 groups (Figure 3). Seven groups were formed by a single accession, while the others grouped accessions with Jaccard's similarity equal to 1.

Discussion

Morphological characterization, one of the most traditional forms of germplasm characterization, helps to describe the genetic diversity existing within a plant species. The outcomes of the morphological analysis can then be linked to environmental conditions and to the results of cytological, agronomic, and genetic analyses (Borner et al., 2006). Multivariate analysis techniques can be used to evaluate the differences among accessions and to select the descriptors that best allow to discriminate among the accessions in a germplasm bank (Azevedo et al., 2011; Santos et al., 2011).

Previous work on the morphological characterization of *P. notatum* has shown the polymorphic nature of this species and a subdivision of the species into varieties has been suggested (Parodi, 1948; Rosengurt et al., 1970). However, others maintain that there are only two true varieties, *P. notatum* var. *saurae* (a diploid sexual) and *P. notatum* var. *notatum* (an apomictic tetraploid), and that all other observed forms should be classified as biotypes (Canto-Dorow et al., 1996).

The diversity of the forms found in this study is much greater than what has been described for the species so far. Our results indicate that there is variability for all morphological characters analyzed and in particular for the angle between the racemes, the insertion angle of the leaves, and the length of flowering stems (Table 2). We also found variation in the number of racemes. It should be stressed that this is one of the traits used to characterize the *Notata* group of the genus *Paspalum* (Chase, 1929). Variation in this trait has been found to be most common in *P. notatum* var. *saurae* (Canto-Dorow et al., 1996).

Table 2 - Descriptive statistics of the quantitative morphological characteristics

Variable	Variance	Average	Maximum	Minimum
LS	4.15	7.19	13.7	3.6
WS	0.03	0.91	1.27	0.4
LL	21.06	12.37	24.6	4.8
WL	0.03	0.83	1.47	0.4
IAL	116.94	37.83	65.0	15.0
LR	5.42	9.02	14.68	5.28
NR	0.02	2.06	3.0	2.0
AR	244.48	47.15	93.33	18.33
LFS	63.60	26.95	46.9	14.9

LS - length of sheath; WS - width of sheath; LL - length of leaves; WL - width of leaves; IAL - insertion angle of leaves; LR - length of racemes; NR - number of racemes; AR - angle between the racemes; LFS - length of flowering stems.

Table 3 - Relative contribution of characters to divergence obtained from the Singh method (Singh, 1981)

Variable	Value (%)
Length of sheath	5.71
Width of sheath	20.09
Length of leaves	16.61
Width of leaves	2.78
Insertion angle of leaves	4.34
Number of racemes	4.35
Length of racemes	21.59
Angle between the racemes	5.83
Length of flowering stems	18.70

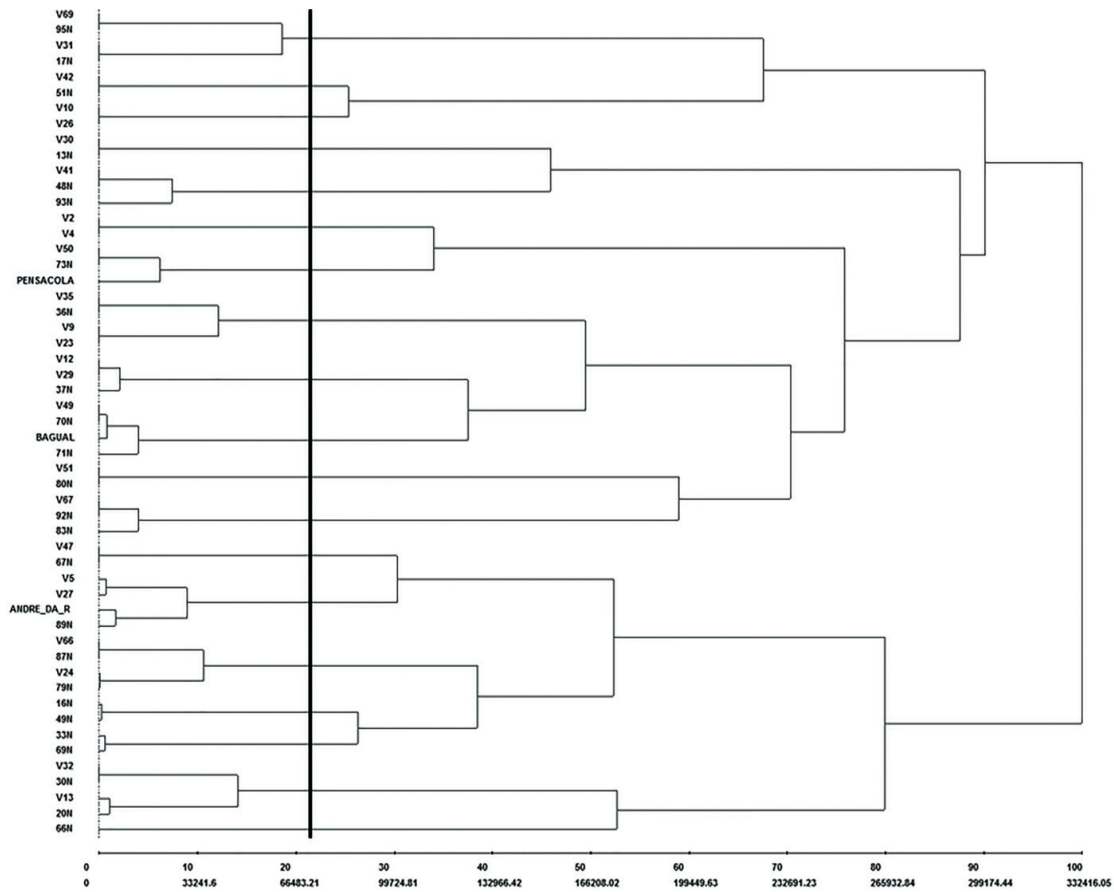


Figure 2 - Relationships among *Paspalum notatum* accessions based on quantitative traits obtained by Mahalanobis distance.

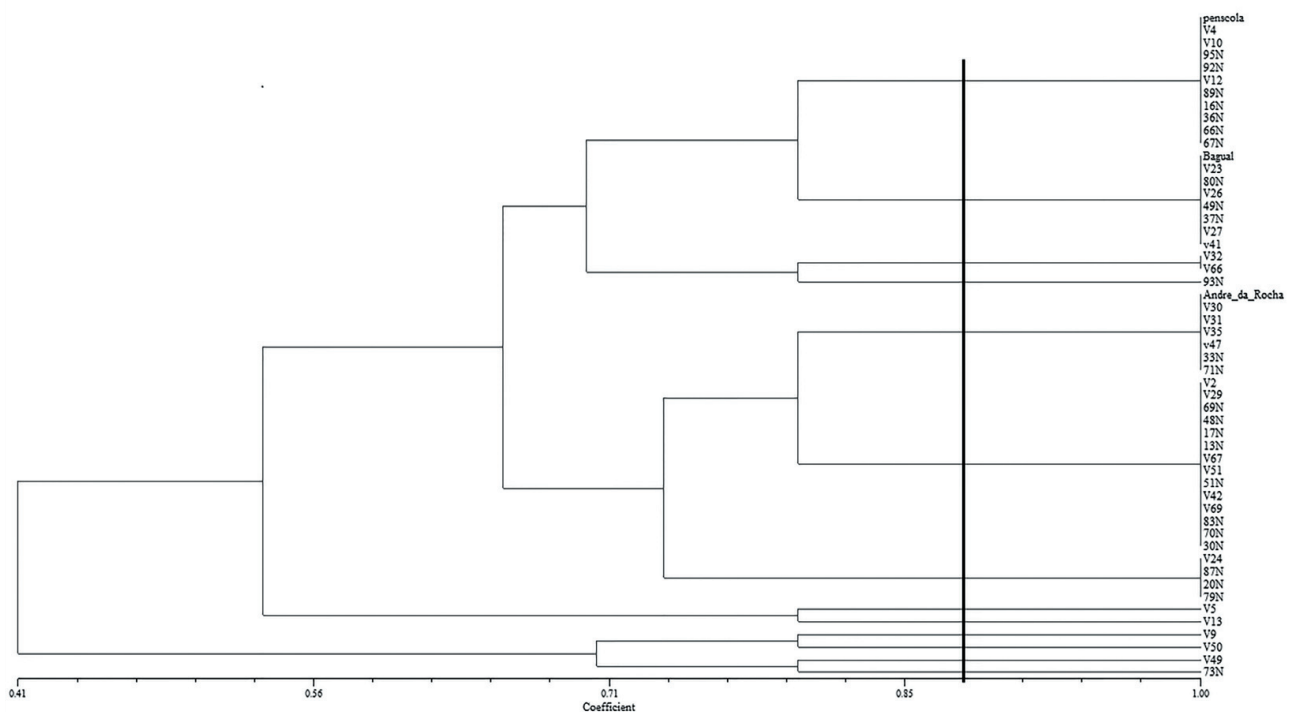


Figure 3 - Relationships among *Paspalum notatum* accessions based on qualitative traits obtained by Jaccard's similarity.

The morphological groups found in this study showed varying degrees of correspondence to previously described classifications. Barreto (1974) described four “forms” of *P. notatum* based on aspect, vigor, size, and hairiness of the leaves; height of the stems; number and length of the racemes; and size and color of the spikelets. The relationship between those “forms” and the groups found in this study is not straightforward, since Barreto (1974) included only plants from the state of Rio Grande do Sul in his analysis, and the traits on which he based his classification were different from those used in our study.

Canto-Dorow et al. (1996) defined four biotypes of *P. notatum* based on the length and width of the leaves, presence or absence of hair, size of the spikelets, and number of veins in lemma I: biotype A leaf blades that are both long and wide; biotype B, with long and narrow leaf blades; biotype C, with short and narrow leaf blades; and biotype D, which is characterized by pubescent leaf blades. Steiner (2005) classified 41 accessions into six morphological groups. The traits that most contributed to the formation of groups were length and width of the leaves, length of the racemes, and spikelets. Finally, Cidade (2006) performed a morphological characterization based on eight traits in 95 accessions of *P. notatum*, which resulted in the formation of eight groups. The traits that most contributed to the divergence among accessions in that study were height of the flowering stem, length and width of the leaf blades, and length of the racemes. The differences in the groups obtained in this study in relation to those reported by other authors are probably due to the use of larger number and different characters than those used in this study. In identifying 13 and 19 morphologic groups based on qualitative and quantitative data, respectively, in our morphological analysis of *P. notatum*, we report an intraspecific diversity that is greater than that found by any other authors. This is probably due to the inclusion of the older accessions obtained from the USDA, which resulted in greater geographical coverage and, hence, in a more comprehensive collection. Our results show that the recovery of material from germplasm seed banks can be an excellent strategy to increase the genetic variability in breeding programs. This increase is of particular importance for the success of breeding programs in apomictic species, such as *P. notatum*.

Apomixis is an alternative route of plant reproduction, in which parent plants produce genetically identical offspring via seeds. In agriculture, apomixis is desirable because it guarantees the perpetuation of superior genotypes by self-seeding without loss of hybrid vigor (Ortiz et al., 2013). In apomictic species, low diversity indices within each

accession are expected (Dall’Agnol and Schifino-Wittmann, 2005). This low genetic diversity within accessions can be observed in an analysis of discrete morphological traits with two or three possible outcomes, such as color and pubescence of the sheath, color of the midrib, pubescence of the leaves, and plant habit. Most accessions showed no variation in these traits, allowing to restrict the analysis to a few individuals per accession. Moreover, the plants originating from UFRGS (coded as V) are clones, whose only source of variation is somaclonal, thereby, further reducing the possibility of variation within accessions (Rodrigues, 2008; Majesky et al., 2015). In the plant seeds obtained from the USDA (coded as N), on the other hand, some level of variation within accessions can be expected, since apomixis is facultative in most tetraploids of the genus *Paspalum* (Sartor et al., 2011), and a degree of genetic diversity is therefore possible in apomictic *Paspalum* species (Sartor et al., 2013; Majesky et al., 2015).

We have previously published an agronomic characterization and persistence analysis of these accessions, showing a high dry matter production (Fachinetto et al., 2012). Of the 52 accessions of *P. notatum* included in the study, 44 exceeded the forage production of the Pensacola cultivar, while showing persistence at the climatic conditions (winter) of the region where the experiment was conducted. The four accessions found to be most productive in our 2012 study (30N, 48N, 95N, and V4) were found in the present study to belong to different morphological groups (Figures 2 and 3).

When breeding facultative apomicts, the choice of genetically distant parents can help preserve the genetic variability and maximize heterosis, while the morphological characterization allows to select parents that combine desirable characteristics. In the accessions studied, those with bigger leaf blades and an erect habit have a higher dry matter production.

The groups identified in this study did not show a clear relation to the places of origin and accessions collected in the same or in nearby regions were spread over different groups. Conversely, the accessions in each group originated from a range of locations. This allows the selection of the morphological group with the most relevant features for the purposes of a breeding program, while at the same time maintaining the genetic variability of the material.

Those traits that contributed most to the variability among accessions are those that deserve most attention in the selection of materials for a breeding program. In special, the length of leaves, which contributes with 16.61% to formation of groups, is closely related to forage

production and plant habit. The plant habit could thus be used to select accessions of a forage grass. An erect habit is an adaptation to the competition for light and erect plants tend to achieve a greater height (Bernardes, 1987). Plants with an erect habit can therefore be expected to have a greater forage production. In the case of *P. notatum*, the height is, in general, composed of the length of leaves and sheaths. However, plants with a prostrate habit may be better adapted to heavy grazing; they can therefore be used in continuous grazing systems and are better at colonizing new habitats (Zimmer, 1994).

In this context, it is of great relevance that the native region of *P. notatum*, the Pampa region, is under great threat of human urbanization and economic activities (Pillar et al., 2009). This greatly endangers the biodiversity of this biome, as well as the persistence of native species. The conservation of genetic diversity can be assisted by maintaining species populations in germplasm banks (Ferreira, 2006). The addition of this type of material to our study has increased the level of genetic diversity found above previously observed levels, thereby highlighting the importance of this type of conservation strategy.

Conclusions

There is a wide genetic variability among accessions of *Paspalum notatum* for morphological traits, which shows great relevance of this information in a program of genetic improvement of *Paspalum notatum*. The genetic similarity found among accessions can help the breeders to define the best parental for crosses, in which accessions of different clusters should be preferred.

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