

Understanding the extent of phenotypic variability in accessions of *Paspalum urvillei* Steud. from the USDA NPGS

Juliano Lino Ferreira^{1,*} , Raoni Gwinner² , Leila Maria Ferreira³ , Jéferson Ferronato⁴ ,
Leandro Gonçalves Leite⁵ , Kátia Graciele Gonçalves Ferreira⁶ ,
Júlio César Rodrigues Lopes Silva⁷ , Luís Paulo dos Santos⁸ , Alisson Wilians Teixeira Silva⁹ 

¹Empresa Brasileira de Pesquisa Agropecuária, Embrapa Pecuária Sul, Rodovia BR-153, km 602,9, Vila Industrial, Zona Rural, Caixa Postal 242, CEP 96401970, Bagé, Rio Grande do Sul, Brasil

*Autor para correspondência: juliano.ferreira@embrapa.br

²Embrapa Amazônia Ocidental, Rodovia AM-010, km 29, (Estrada Manaus/Itacoatiara), Caixa Postal 319, CEP 69010-970, Manaus, Amazonas, Brasil

³Universidade Federal de Lavras, Departamento de Estatística, Câmpus Universitário, Caixa Postal 3037, CEP 37200-900, Lavras, Minas Gerais, Brasil

⁴Universidade Federal do Pampa, Avenida Maria Anunciação Gomes Godoy, 1650, CEP 96460-000, Bagé, Rio Grande do Sul, Brasil

⁵Universidade Federal de Santa Maria, Programa de Pós-graduação em Agrobiologia, Avenida Roraima, 1000, Prédio 16, sala 3256A, Cidade Universitária, Bairro Camobi, CEP 97105-900, Santa Maria, Rio Grande do Sul, Brasil

⁶Instituto de Ciências Agrárias (ICA) - UFMG - Campus Regional de Montes Claros, Avenida Universitária, 1.000, Bairro Universitário, CEP 39404-547, Montes Claros, Minas Gerais, Brasil

⁷Universidade Estadual de São Paulo (UNESP), Faculdade de Ciências Agrônômicas, Avenida Universitária, 3780, Altos do Paraíso, CEP 18610-034, Botucatu, São Paulo, Brasil

⁸Universidade Estadual de Campinas, Centro de Biologia Molecular e Engenharia Genética, Avenida Cândido Rondon, 400, Cidade Universitária, CEP 13083-875, Campinas, São Paulo, Brasil

⁹Universidade Federal de Lavras, Programa de Pós-Graduação em Biotecnologia Vegetal, Caixa Postal 3037, CEP 37200-000, Lavras, Minas Gerais, Brasil

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ABSTRACT - The objective of this study was to investigate the phenotypic variability in *Paspalum urvillei* Steud. related to twelve zootechnical traits to better understand the diversity of 41 accessions from the USDA GRIN platform from seven countries. First, we performed a structure inference based on the phenotypic data, identifying two structure groups, and the majority of Brazilian accessions belonged to the first group. Then, we performed principal component analysis and principal coordinate analysis, making Violin plots for each trait within each structure group. Next, we performed spatial clustering of the accessions and utilized an unweighted pair group method with arithmetic mean clustering. In brief, the Brazilian *P. urvillei* breeding programs may be successful by importing USDA germplasm resources from the second phenotypic structure group.

Keywords: cluster analysis, germplasm bank, forage

RESUMO - Entendendo a extensão da variabilidade fenotípica em acessos de *Paspalum urvillei* Steud. do banco de germoplasma do USDA NPGS. O objetivo deste estudo foi investigar a variabilidade fenotípica de *Paspalum urvillei* em relação a doze características zootécnicas, a fim de compreender melhor a diversidade de 41 acessos da plataforma USDA GRIN de sete países. Primeiro foi realizada uma inferência de estrutura baseada nos dados fenotípicos, identificando dois grupos estruturados, onde a maioria dos acessos brasileiros é inferida ao primeiro grupo. Em seguida, examinou-se a análise de componentes principais e análise de coordenadas principais, foram feitos gráficos de violino para cada característica dentro de cada grupo estruturado. Por fim, foi realizado o agrupamento espacial dos acessos e o método de médias não ponderadas de grupos pareados. Em suma, o programa brasileiro de melhoramento de *P. urvillei* pode alcançar sucesso importando acessos do USDA inferidos ao segundo grupo estruturado.

Palavras-chave: análise de agrupamento, banco de germoplasma, forragem

INTRODUCTION

Within the *Paspalum* genus, *P. urvillei* is the most common species in Brazil. *Paspalum urvillei* Steud. (Vaseygrass) is a native species of the subtropical fields of some South American countries, including Brazil (Burson 1979). This species is currently present on practically all continents. *P. urvillei* is a sexually reproducing perennial

plant, with the first stage of development occurring until mid-April. *P. urvillei* has a cespitose habit, with a knotty base comprised of very small (less than 1 cm) rhizomes, and ranges from 1 meter to 2.5 meters in height. Compared to other *Paspalum* species, *P. urvillei* is highly tolerant to frost. Taxonomically, this species belongs to the Dilatata group. Native grasses are of special interest for use as feed because they are already adapted to the local environment.

Burton (1940) found that *P. urvillei* had $2n=4x=40$ (IIJJ) allotetraploid which was subsequently confirmed by De Wet (1954). This species is a C4 grass with a high rate of photosynthesis (Rua *et al.* 2010). *P. urvillei* is also a well-known undesirable plant in fields and disturbed environments (Randall 2017). Generally, disturbed environments are mainly caused by development, anthropic erosion, climate, natural fire disturbances and invasive species.

In forage breeding, it is imperative to identify the most important phenotypic traits to increase plant production. Therefore, the evaluation of trait occurrences and differences in a population is the key to determining potentially useful crosses among accessions. Although the majority of studies focus on genetic diversity with molecular markers, it is also useful for plant breeders to consider the morphological diversity of traits of interest. For example, Tuhina-Khatunm *et al.* (2015) show the importance of determining genetic variation based on morphological traits. Recent articles published by Martuscello *et al.* (2012), Pereira *et al.* (2012), Rocha *et al.* (2017) and Kaur *et al.* (2018) showed the importance of genetic variability and detected high variability in forage species based on zootechnical traits. In this way, some biometric strategies, including data processing using principal component analysis, principal coordinate analysis, and group structure approaches, stand out as useful options for understanding trait relationships in a population.

Principal coordinate analysis and principal component analysis are statistical methods usually applied in population genetics studies to recognize structures in the distribution of genetic variation and genealogical interpretation across a set of individuals of a particular species. The object of both methodologies is to create a graphical plot approximating, as closely as possible, original dissimilarities. Normally, the results of these two approaches correspond closely, especially when there are few or no missing data. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) (Sokal and Michener 1958) is an accurate methodology for creating a phylogenetic tree based on a distance matrix. This technique is widely used in biometry with the purpose of comprehending genetic diversity and systematic approaches. In this study, we used a Violin plot. This plot is a histogram used to useful interpreting ranges, medians and shape variabilities. The Violin plot shape is linked to the frequency of the values.

Therefore, the objective of this study was to assess the phenotypic variability in an international germplasm bank of *P. urvillei* from the Germplasm Resources Information Network (GRIN), a plant germplasm platform from the United States Department of Agriculture (USDA), based on agronomic characteristics and to use these data to determine possibilities for parent selection. Important clues should be recognized to guide choices about which germplasm resources should be imported to Brazil for use in *P. urvillei* breeding programs.

MATERIALS AND METHODS

Free access to the data used in this study is available at the Germplasm Resources Information Network (GRIN) platform of the National Plant Germplasm System (NPGS) of the USDA. The data comprise 12 variables of zootechnical interest: foliage height, plant height, plant width, foliage distribution, leaf length, leaf width, stem size, tillers, maturity, foliage amount, seed production, and winter survival. The accession morpho-agronomic data were evaluated concomitantly in two USDA NPGS locations in the US state of Georgia in the cities of Griffin and Byron, during the period from September 1989 to November 2003. The field plot size was 1.5 m x 6 m, with a 0.5 m distance between plants. Four replicates were performed with a randomized complete block design. The results of this platform are presented as one value comprising the means of all years and sites for each accession.

The traits were evaluated in 41 accessions, originating from 7 countries (Table 1): Brazil (1), Uruguay (2), Argentina (3), South Africa (4), Bolivia (5), Mexico (6), and Swaziland (7). Of these, 21 accesses originated from Brazil.

First, we used the raw quantitative data to analyze the correlation between quantitative variables and their main parameters. The multivariate analysis of simple correlations between the accessions and the determination of relative importance of certain characteristics was performed using the software Genes (Cruz 2013).

An altitude-based pattern in the studied accessions was estimated with Google Earth Pro (2018). A spatial cluster analysis was performed with R software (R Core Team - 2013) (Fig. 1) using the `hclust(dist(x))` function - mode = complete, with hierarchical dendrogram clustering using the squared Euclidean distance.

With the purpose of performing a multivariate approach combining qualitative and quantitative data, we converted quantitative data on phenotypic variables into categorized classes, with a limit of 1 standard deviation. The qualitative variables were codified based on the original scale given by the USDA. In this way, the completed multivariate analysis of the categorical data. First, we used Structure v.2.3.4 software (Evanno *et al.* 2005) to screen and infer the phenotypically structured variation. This program was run with a burn-in length of 10,000, 100,000 MCMC reps after burn-in and 111 independent runs. According to the Structure program developer, it would require at least 20 independent runs to infer a suitable genetic structure. The Structure Harvester program (Earl & von Holdt 2012) was used to determine the best phenotypic-genetic structure. Moreover, Shannon informational diversity statistics, Principal Coordinate Analysis (PCoA), and Mantel Tests between phenotype distance matrices and geographic matrix distance were performed using GenAlex v.6.503 (Peakall & Smouse 2012). We also performed an analysis of the most important summary statistics related to phenotype

Table 1. Description of *Paspalum urvillei* accessions of USDA germplasm.

Plant ID	Plant #	Taxonomy	Country	City
PI 164065	1	<i>P. urvillei</i>	Brazil (1)	Florianópolis
PI 202046	2	<i>P. urvillei</i>	Argentina (2)	Buenos Aires
PI 203747	3	<i>P. urvillei</i>	Brazil (1)	São Gabriel
PI 203749	4	<i>P. urvillei</i>	Uruguay (3)	Rivera
PI 203752	5	<i>P. urvillei</i>	Brazil (1)	Porto Alegre
PI 204237	6	<i>P. urvillei</i>	Uruguay (3)	Rivera
PI 276255	7	<i>P. urvillei</i>	Uruguay (3)	Montevideo
PI 283025	8	<i>P. urvillei</i>	Uruguay (3),	Tacuarembó
PI 304040	9	<i>P. urvillei</i>	Brazil (1)	Uruguaiana
PI 304041	10	<i>P. urvillei</i>	Brazil (1)	Vacaria
PI 304042	11	<i>P. urvillei</i>	Brazil (1)	Vacaria
PI 304043	12	<i>P. urvillei</i>	Brazil (1)	Vacaria
PI 304044	13	<i>P. urvillei</i>	Brazil (1)	Vacaria
PI 304045	14	<i>P. urvillei</i>	Brazil (1)	Montenegro
PI 304047	15	<i>P. urvillei</i>	Brazil (1)	Uruguaiana
PI 304048	16	<i>P. urvillei</i>	Brazil (1)	Vacaria
PI 304050	17	<i>P. urvillei</i>	Brazil (1)	Uruguaiana
PI 310253	18	<i>P. urvillei</i>	Brazil (1)	Uruguaiana
PI 310255	19	<i>P. urvillei</i>	Brazil (1)	Tupancireta
PI 310257	20	<i>P. urvillei</i>	Brazil (1)	Pelotas
PI 310258	21	<i>P. urvillei</i>	Brazil (1)	Pelotas
PI 310263	22	<i>P. urvillei</i>	Brazil (1)	Pelotas
PI 310266	23	<i>P. urvillei</i>	Brazil (1)	Julio de Castilho
PI 364984	24	<i>P. urvillei</i>	South Africa (4)	Limpopo
PI 365131	25	<i>P. urvillei</i>	Swaziland (6)	Malkerns
PI 202296	26	<i>P. urvillei</i>	Argentina (2)	Buenos Aires
PI 300079	27	<i>P. urvillei</i>	South Africa (4)	East London
PI 303957	28	<i>P. urvillei</i>	Brazil (1)	Uruguaiana
PI 304146	29	<i>P. urvillei</i>	Mexico (5)	Lagos-Aquascalientes
PI 404506	30	<i>P. urvillei</i>	Brazil (1)	Vacaria
PI 404885	31	<i>P. urvillei</i>	Uruguay (3)	Paysandu
PI 404886	32	<i>P. urvillei</i>	Uruguay (3)	Tranqueras
PI 462304	33	<i>P. urvillei</i>	Uruguay (3)	Cerro Largo
PI 462305	34	<i>P. urvillei</i>	Uruguay (3)	Cerro Largo
PI 462306	35	<i>P. urvillei</i>	Uruguay (3)	Salto
PI 509008	36	<i>P. urvillei</i>	Brazil (1)	Ivoti
PI 509013	37	<i>P. urvillei</i>	Argentina (2)	Villa Maria
PI 509014	38	<i>P. urvillei</i>	Argentina (2)	Olivia
PI 509015	39	<i>P. urvillei</i>	Argentina (2)	Arroyito
PI 509016	40	<i>P. urvillei</i>	Argentina (2)	Buenos Aires
PI 509017	41	<i>P. urvillei</i>	Bolivia (7)	Villa Montes

information, including the main category frequency, trait diversity, polymorphism information content (PIC) (based on the class of each phenotype of a determined trait – treated like alleles), a coancestry matrix between the inferred populations and an UPGMA phylogenetic tree based on the shared allele distance using PowerMarker version 3.25 (Liu & Muse 2005). Principal Component Analysis (PCA) was performed with R software. Finally, we created Violin plots (Hintze & Nelson 1998) with the R software package ggplot2.

In the PCA and PCoA graphs, we used the accession identification numbers described in Table 1. In the Violin plots, only the inferred populations were identified for each trait.

RESULTS

Considering all accessions of *P. urvillei*, there were significant simple correlations among the quantitative variables of foliage height versus plant height (0.56413**), foliage height versus leaf length (0.36138**), plant height versus plant width (0.56926**), plant height versus leaf length (0.4136**), plant width versus leaf length (0.36416**) and leaf length versus leaf width (0.26618*). Considering the correlations within each inferred population, we had only a single significant correlation in population 1: plant height versus plant width (0.6891**), while in population 2, we had four significant correlations: foliage height versus plant height (0.7168**), plant height versus leaf width (0.5708*), plant width versus maturity (0.6428*), and leaf length versus leaf width (0.5537*).

Plant height showed the largest range between the minimum and maximum values, with accession PI 164065 having the highest values. Conversely, the maturity time showed the lowest variability, with accession PI 202296 having the shortest maturity time. The mean, maximum and minimum values of each parameter were as follows:

foliage height of 10.53, 140 and 20 cm, plant height of 17.28, 240 and 30 cm, plant width of 14.12, 240 and 40 cm, leaf length of 47.16, 64, and 28 cm, leaf width of 1.62, 2.1 and 0.6 cm, and maturity times of 115, 145 and 75 days, respectively.

Among all of the characteristics, the highest relative importance was observed for the foliage amount (27.096%), leaf length (15.393%), and plant width (13.282%). The lowest relative importance values were found for plant height (2.681%) and seed production (2.351%). The highest values were found for plant height versus plant width and foliage height versus plant height.

The amount of foliage varied within each population. In population I, 80% of the accessions were in the medium category, whereas in population II, 75% were classified in the abundant category. Because this variable is of special interest for forage breeders, it is important to understand its distribution pattern.

Table 2 shows the summary statistics of the evaluated variable (trait) provided by PowerMarker software version 3.25. Stem size and tiller number were repeatedly of a single type. Therefore, there was little variability in these traits compared to the other traits. Consequently, these traits showed the lowest values for trait diversity and polymorphisms among individuals. In contrast, winter survival had the largest values of trait diversity and polymorphisms among individuals.

In terms of altitude-based patterns, 12 accessions were from altitudes higher than 400 meters. Of these, seven were from sites approximately 1000 meters high or higher.

The spatial clustering separated the accessions into two main groups: one comprising the three accessions from Africa and the other comprising all the accessions from the Americas (Fig. 1). Note that accession PI 304146 belonged to the American spatial cluster but clustered slightly far from its counterparts.

Table 2. Summary statistics of evaluated variables of *Paspalum urvillei* from USDA germplasm accessions.

Variable	Main category frequency	Trait diversity	PIC
Plant width	0.439	0.659	0.595
Foliage height	0.585	0.603	0.565
Plant height	0.537	0.627	0.576
Leaf length	0.317	0.736	0.688
Leaf width	0.561	0.616	0.571
Maturity	0.537	0.664	0.635
Foliage distribution	0.854	0.256	0.233
Stem size	0.951	0.093	0.089
Tillers	0.951	0.093	0.089
Foliage amount	0.585	0.485	0.368
Seed production	0.805	0.330	0.300
Winter survival	0.268	0.829	0.809
Mean	0.616	0.499	0.460

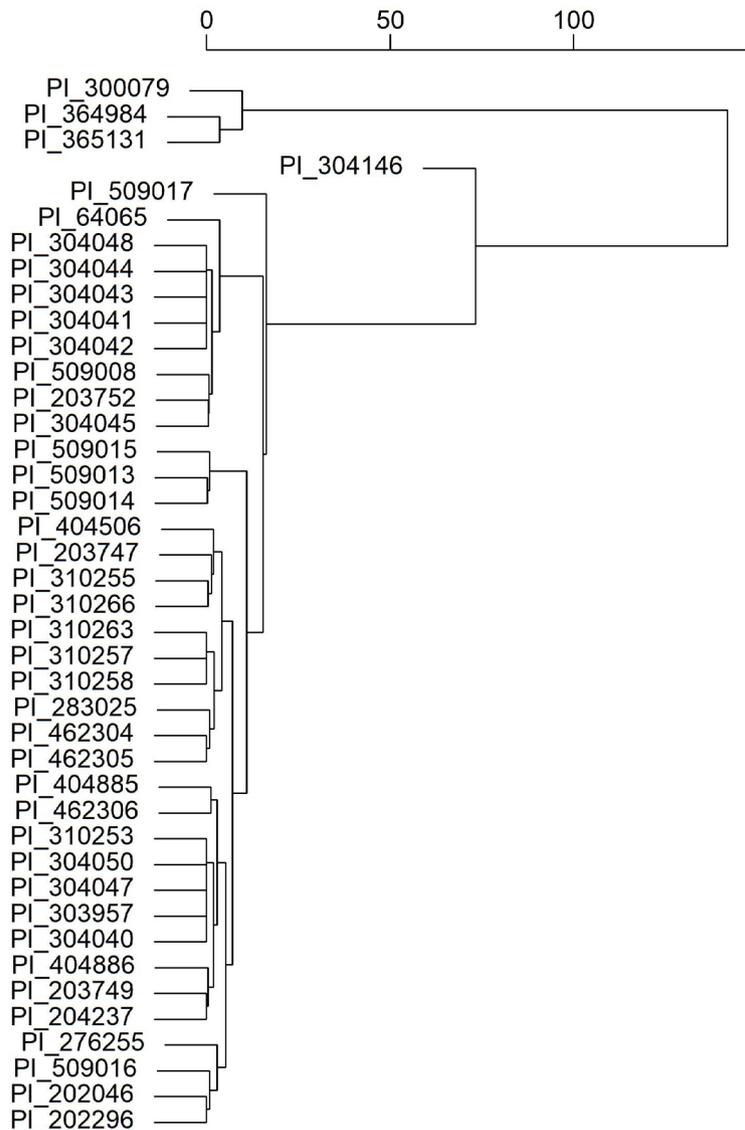


Fig. 1. Spatial clustering of *Paspalum urvillei* accessions from USDA germplasm.

The coancestry analysis value of the two inferred populations was 0.1895. This value is consistent with the 0.189 obtained by the diversity analysis of the population with the Shannon informational diversity statistics approach.

The Mantel test concerning the correlation of phenotypic traits and geographic coordinates had a value of 0.187%, with a 0.0349% coefficient of determination, which is considered very low. The Mantel test showed limited correlations between geographic distance and phenotype. This finding may be because few genotypes were used in this study.

The best phenotypic structure separated the *P. urvillei* accessions into two groups (Fig. 2). These inferred groups were designed populations I and II. The Uruguayan accessions PI 203749, PI 204237, and PI 276255 grouped together with the Rio Grande do Sul accessions, forming practically the same group. The other group was formed

by accessions from Argentina, Uruguay, Mexico and South Africa. The accession PI 283025 (Uruguay) had less than 80% membership to any specific group.

The two-dimensional dispersion showed a high degree of variability with a well-defined structure (Figs. 3, 4). The diversity between accessions reached a value as high as 0.917, with an average value of 0.501. The first PCA component explained 88.09% of the variability. Conversely, in the PCoA, the first three axes explained 46.13% of the variability. These two approaches clustered almost all of the accessions, with the y-axis forming a linear border between the two structure groups.

The UPGMA tree of only 4 accessions (PI 202296, PI 462304, PI 462305, and PI 462306) supported the PCoA and PCA analyses. The highlighted accession on the UPGMA tree was PI 303957, which clustered separately from all other genotypes, comprising a single group (Fig. 5).

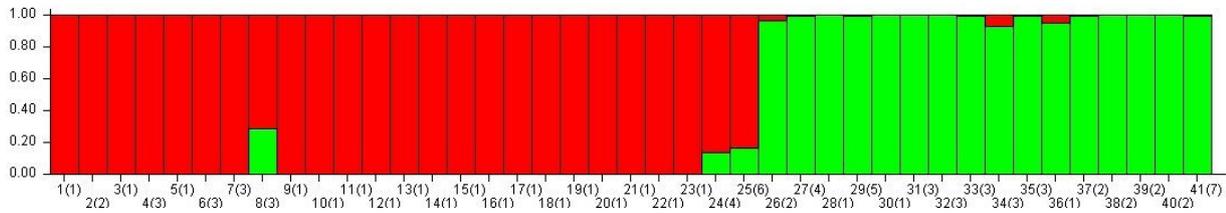


Fig. 2. The phenotypic structure of *Paspalum urvillei* accessions from USDA germplasm.

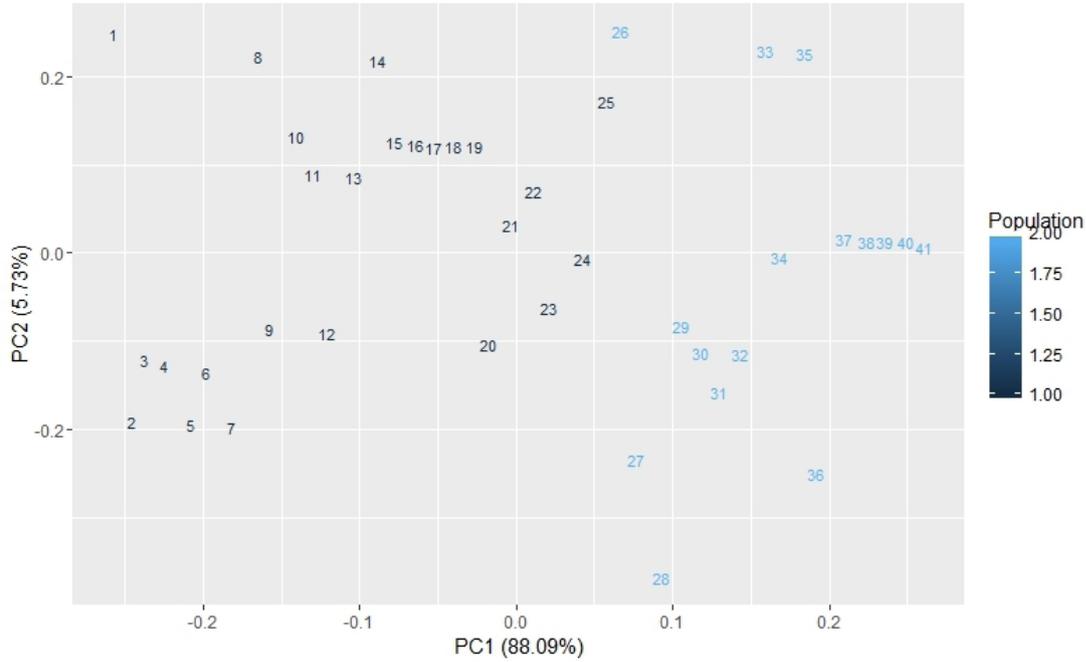


Fig. 3. Principal Component Analysis of *Paspalum urvillei* accessions from USDA germplasm.

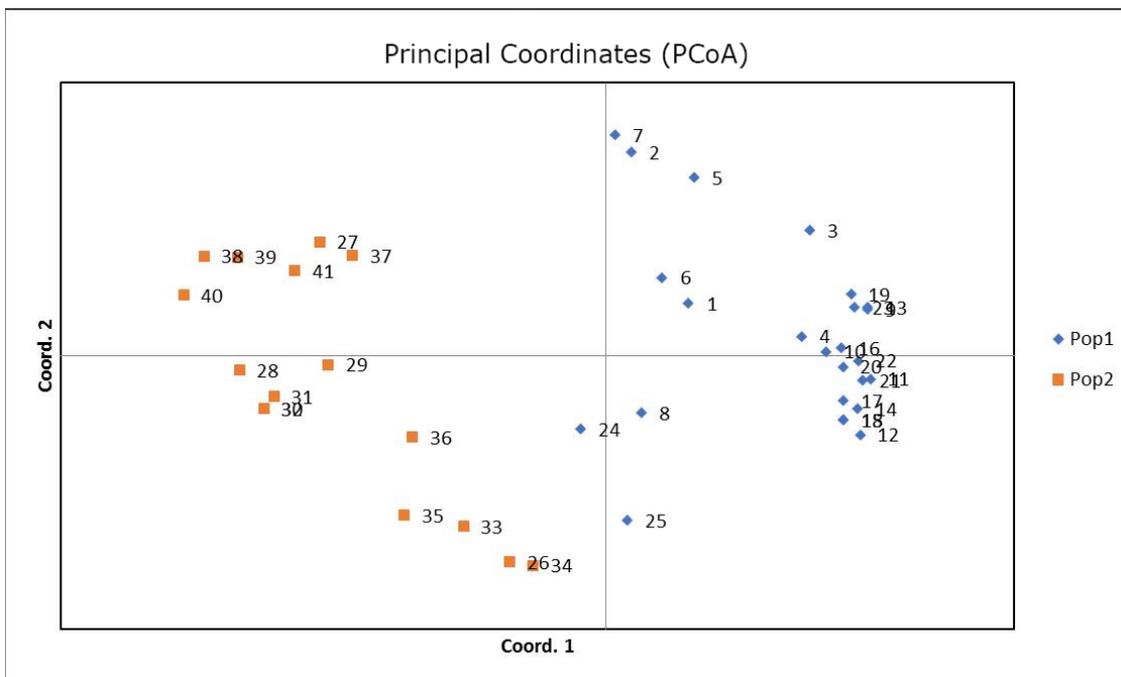


Fig. 4. Principal Coordinate Analysis of *Paspalum urvillei* accessions from USDA germplasm.

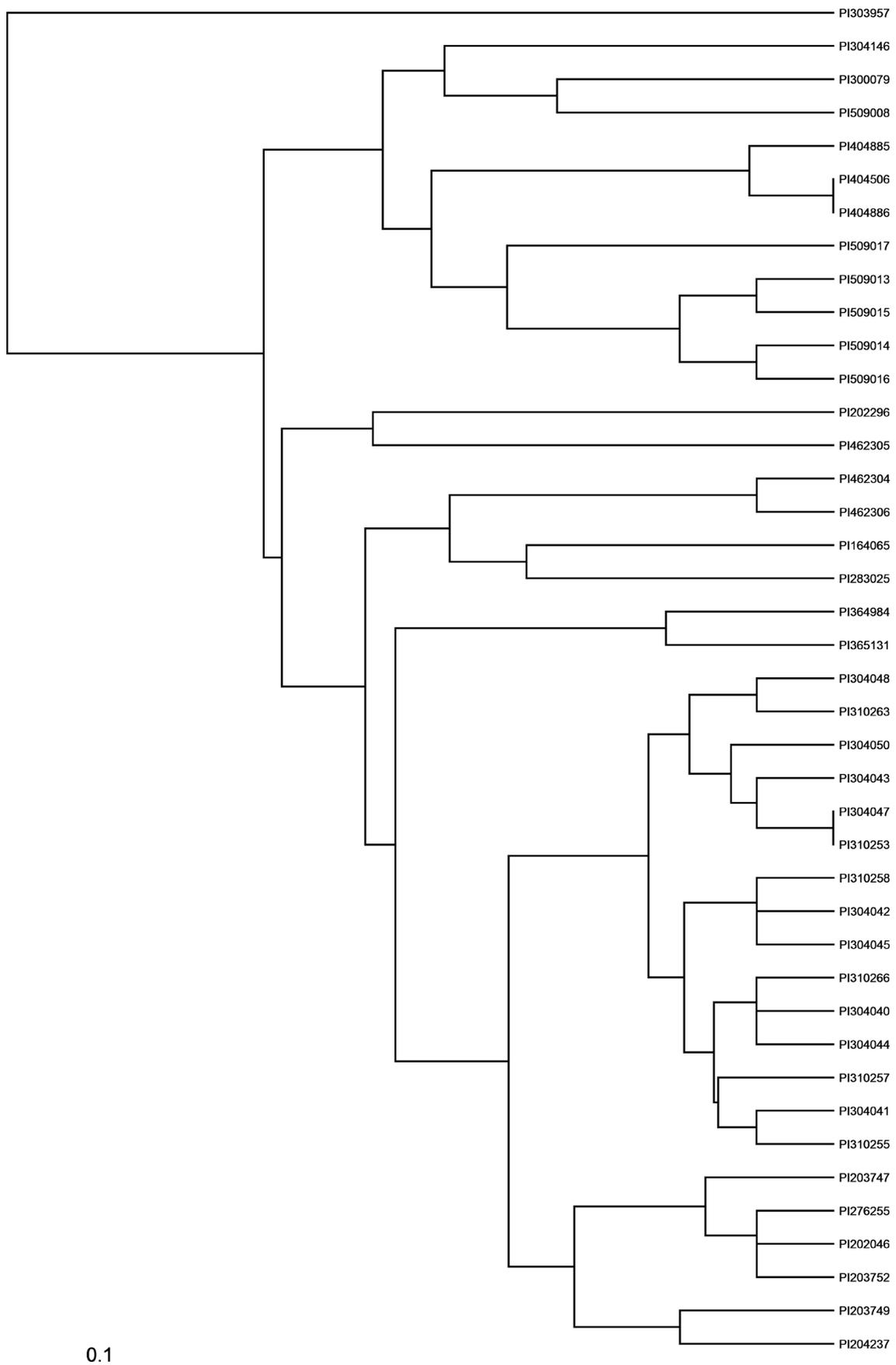


Fig. 5. UPGMA of *Paspalum urvillei* accessions from USDA germplasm.

DISCUSSION

Our phenotypic data allowed the detection of two structural groups. Sawasato *et al.* (2008), using 7 SSR primers in a panel of 64 Brazilian accessions of *P. urvillei*, detected seven structure groups. This difference in the number of groups may be due to these authors using primers from *Zea mays* L., *Sorghum bicolor*, *Trifolium repens*, and *Lolium multiflorum* L. In addition, the limited number of primers generated 28 polymorphic bands. Conversely, these authors grouped high altitude accessions in Brazil, whereas in our study, individuals from these regions were clustered into two groups. Furthermore, these SSR targets may not be related to functional parts of the *P. urvillei* genome.

Our value of 0.1895 in the coancestry matrices of the two inferred populations shows that the accessions analyzed in this study are not closely related, as evidenced by the diversity among the genotypes, which is consistent with their mean diversity values.

The Mantel test showed limited correlations between geographic distance and the phenotypic data. This could be due to the few genotypes used in this study.

The separation of the two structure groups was consistent using both PCoA and PCA. This congruence is also expected when few variables are analyzed with no lost data (Mohammadi & Prasanna 2003), which occurred in the present work. This fact also reinforces the clustering of the accessions into two groups.

The summary statistics are presented in Table 2, and the Violin plot is useful for exploring the data. Winter survival showed the lowest frequency, which was accompanied by high gene diversity for this variable. The low frequency of the main category of this trait probably likely resulted in a smoother pattern in both populations. In the same way, leaf width was also variable between the two structure groups.

Observing the Violin plot approach (Fig. 6), we can see that the variable for winter survival had approximately the same shape in the two groups, being well scattered among the several classes. This fact highlights the known tolerance of *P. urvillei* to cold temperatures in addition to the high diversity detected in these evaluated accessions.

Interestingly, the Brazilian accessions PI 303957, PI 404506, and PI 509008 were included in structure group 2. This information shows that there are differences between

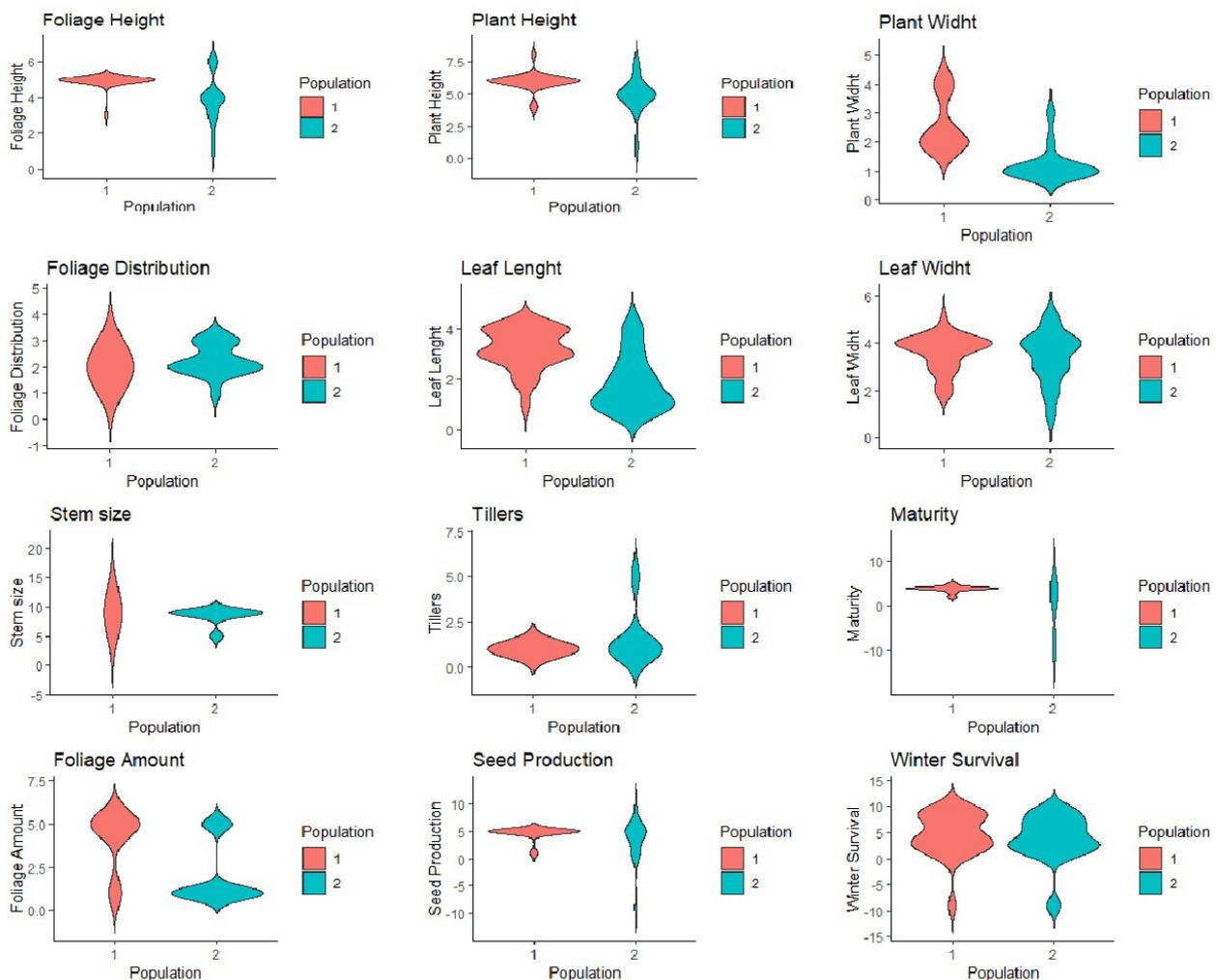


Fig. 6. Violin plot of *Paspalum urvillei* accessions from USDA germplasm.

these genotypes and the other accessions from Brazil, providing evidence for potential heterotic combination in Brazilian. The African accessions PI 364984 and PI 365131 were placed together in the first structure group. These accessions were placed together with spatial clustering as well. Although the accessions share geographic proximity, this does not always mean that they share the same genetic structure. In the present work, PI 404506 had 4 other counterparts from Vacaria city, even though those accessions were from the other population. It seems that in this specific circumstance, the spatial cluster did not correlate with the phenotypic cluster. One explanation is that in nature, spatial clusters do not necessarily imply the same genetic structure due to a series of factors, such as mutations.

In the present study, 90% of the *P. urvillei* accessions were from the southern part of South America, which was expected because this species is native to subtropical South America (Burson 1992). Although *P. urvillei* is known to be tolerant to cold temperatures, nine accessions (six from group I and three from group II) showed complete death in winter. This detail might be related to the USDA GRIN evaluation environment (Georgia State, USA).

An interesting observation is that population 1 had only one significant correlation between variables at $p < 0.01$, whereas population 2 presented 4 significant correlations, one at $p < 0.01$ and the other three at $p < 0.05$. Significant correlations in population 1 were not present in population 2. This finding also confirmed the differences between the two populations. This finding exhibited the same direction as that in the Violin plots (Fig. 6), where each structured population had a dissimilarity pattern with a dense shape, highlighting the differences between the two inferred populations. The overall high correlation between plant height and plant width, to a certain degree, was expected.

Stem size showed nearly no variability. Only two accessions from population II were in the medium stem size category, and all others were categorized as having a large stem size. Based on this observation, we decided to focus on specific traits with more interesting variability.

Currently, there is a demand for more practical plant breeding approaches. With this method, it is important to recognize useful parameters as well as to understand statistical language in a comprehensive way. From this perspective, the present work combined many techniques to investigate the variability of *P. urvillei* in a broad range of accessions from the USDA NPGS GRIN. In our study of these data, the use of multiple approaches was very useful and revealed remarkable information.

From these data, we propose that there are real possibilities to increase the value of *P. urvillei* used in Brazilian forage breeding, especially with the guided introduction of USDA accessions matching the inferred second phenotypic structure group, since the majority of Brazilian accessions belonged to the first structure group.

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