Ciência Rural, Santa Maria, v.47: 07, e20161049, 2017 http://dx.doi.org/10.1590/0103-8478cr20161049
ISSNe 1678-4596
ANIMAL PRODUCTION

Forage characters of different *Paspalum* species in Rio Grande do Sul: a meta-analysis

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ABSTRACT: *The objective of this study was to evaluate, through meta-analysis, the forage characteristics of various species of the genus Paspalum and to use them to select the best ecotypes that can be used in artificial hybridization as parents and hybrids for pasture production and natural pasture recovery systems. Data were obtained from studies conducted by the Department of Forage Plants and Agrometeorology of the Universidade Federal do Rio Grande do Sul. Database comprised tests conducted with ecotypes and/or hybrids of Paspalum spp. in plots for evaluating total dry mass production, leaf dry mass production, and stem dry mass production by means of cuts. Total dry mass production, which included leaves and stems, differed between the ecotypes and hybrids. Hybrid H12 was the most divergent of all evaluated accessions. The greatest genetic divergence occurred due to dry mass production. Hybrids showed high total dry mass production, comprised mainly of leaves. Hybrid H12 and the accession of Paspalum lepton 28E were identified as the most dissimilar based on the generalized Mahalanobis distance using Tocher's method. Total dry mass production is the characteristic that most contributed to the detection of genetic variability.*
Key words: *apomixis, hybridization, plant breeding, natural pasture, variability.*

Caracteres forrageiros de diferentes espécies do gênero *Paspalum* no Rio Grande do Sul: uma meta-análise

RESUMO: *O objetivo deste trabalho foi avaliar, por meio de meta-análise, a variabilidade dos caracteres forrageiros de espécies do gênero Paspalum e utilizá-los para selecionar os melhores ecótipos para serem utilizados em hibridações artificiais como genitores e híbridos para serem empregados em sistemas de produção a pasto e recuperação de pastagens naturais. Os dados foram obtidos a partir ensaios, do Departamento de Plantas Forrageiras e Agrometeorologia da Universidade Federal do Rio Grande do Sul. A base de dados foi composta por ensaios conduzidos com ecótipos e/ou híbridos do gênero Paspalum, em parcelas, avaliando por meio de cortes a produção de massa seca total, de folhas e colmos. Houve diferença entre ecótipos/híbridos para produção de matéria seca total, de folhas e colmos. O híbrido H12 foi o mais divergente dos acessos avaliados. A maior divergência genética ocorreu devido à produção de massa seca. Os híbridos apresentam elevada produção de massa seca total, sendo esta composta principalmente por folhas. O método de Tocher utilizando a distância generalizada de Mahalanobis identifica o híbrido H12 e o acesso de Paspalum lepton 28E como os mais dissimilares. A produção de massa seca total é o caractere que mais contribui para a detecção da variabilidade genética.*
Palavras-chave: *apomixia, hibridação, melhoramento genético, pastagem natural, variabilidade.*

INTRODUCTION

Grasses of the genus *Paspalum* are the most important forage plants used in pastures in South America (NOVO et al., 2015). In particular, Rio Grande do Sul has soil and climate conditions that enable species of the genus *Paspalum* to adapt well ecologically, resulting in increased forage production.

Several studies have highlighted that species of the genus *Paspalum*, being native, have advantages over exotic plants and high inter- and intraspecific variabilities in forage characteristics (REIS et al., 2010; PEREIRA et al., 2011; PEREIRA et al., 2012). As a result, there is great potential for exploiting these species in genetic improvement programs for using them as forage plants (REIS et al., 2010). Identifying the characteristics of species favorable to productive exploitation; will therefore, enable the selection of superior materials as cultivars.

Received 11.25.16 Approved 03.30.17 Returned by the author 05.05.17
CR-2016-1049.R1

Apomixis is the predominant reproduction method in polyploid species of the genus *Paspalum*. However, it hinders genetic recombination as well impairs cultivar protection because of legal regulations (PEREIRA et al., 2012). However, hybridization, when one of the parents exhibits sexual reproduction, can result in variability and allows the selection of progenies with immediate fixation of characteristics of interest due to apoximis (ACUÑA et al., 2009).

Meta-analyses combined the results of several studies to create a reproducible and quantifiable summary of data, thereby improving the statistical power in studies on the effects of treatments and improving precision in the estimation and size of the effect (LOVATTO et al., 2007). An evaluation of variability in the expression of forage characteristics of interest and of the genetic diversity of ecotypes and hybrids of different species of the genus *Paspalum* by means of a meta-analysis enables the comparison of data from different trials, thus increasing the number of ecotypes, hybrids, and repetitions evaluated. It also provided information for the selection of superior plants as well as the formation of new elite recombinants. Therefore, the objective of this study was to evaluate, through a meta-analysis, the forage characteristics of species of the genus *Paspalum* and to use them to select the best ecotypes for use in artificial hybridization as parents and hybrids in pasture production and natural pasture recovery systems.

MATERIALS AND METHODS

Data were obtained from studies conducted between 2002 and 2014 by the Department of Forage Plants and Agrometeorology of the Universidade Federal do Rio Grande do Sul, in which the forage characteristics of ecotypes and hybrids of the genus *Paspalum* were evaluated.

As criteria for inclusion in the database, the studies had to have been conducted in Rio Grande do Sul with ecotypes and/or hybrids of the genus *Paspalum* in plots, free from animal grazing for two agricultural years and total dry mass production (TDMP), leaf dry mass production (LDMP), and stem dry mass production (SDMP) had to be determined by means of cuts and expressed as kg of DM per hectare per year (kg DM $ha^{-1}year^{-1}$). TDMP, LDMP, and SDMP were calculated by the sum of the cuts performed in each agricultural year. Different years and locations were treated as repetitions.

Because only a few trials reported the leaf:stem ratio (LSR) and harvest index (HI), we calculated these variables using the following formulae: LSR = LDMP/SDMP (PEREIRA et al., 2012; PEREIRA et al., 2015; MOTTA et al., 2017) and HI = LDMP/TDMP (PEREIRA et al., 2012), respectively.

Data from six studies were used in the database: SANTOS (2005), STEINER (2005), SAWASATO (2007), TOWNSEND (2008), PEREIRA et al. (2012), and MOTTA et al. (2017) The studies produced 135 observations on the following species: *Paspalum notatum*, ecotypes André da Rocha and Bagual, and the commercial cultivar 'Pensacola'; *P. guenoarum*, ecotypes Azulão, Baio, and the cultivar 'Rojas'; *P. urvillei*: accessions collected in Rio Grande do Sul, in the municipalities of André da Rocha, Eldorado do Sul, and Bagé; *P. lepton*: accessions collected in Rio Grande do Sul, in the municipalities of Alegrete (26A and 26D) and Rosário do Sul (28B, 28C, and 28E); *P. denticulatum* and *P. pauciciliatum*. Two different species belonging to other genera, *Panicum maximum* Jacq. cv. 'Aruana' and the species popularly known as giant missionary grass (*Axonopus catharinensis* Valls), were used as controls.

Genotypes and artificial hybrids productivity data were also evaluated at the premises of the Instituto de Botánica del Nordeste (IBONE), which is based at the School of Agrarian Sciences in the province of Corrientes in Argentina. The *P. guenoarum* ecotype 'Rojas' was used as the male parent, and the *P. plicatulum* sexual genotype 4c-4x was used as the female parent. The chromosomal set of the sexual genotype was duplicated with colchicine (SARTOR et al., 2009) to enable cross-breeding between the different species and promote the production of fertile offspring. Hybrids evaluated (H12, H13, H20, and H22) were selected for their higher TDMP, as described by PEREIRA et al. (2015) and MOTTA et al. (2017).

After the normality test, the data were analyzed using a mixed model that considered ecotypes/hybrids as fixed effects and experiments and repetitions as random effects. Nine variance and covariance matrix models were tested as follows: variance component (VC), compound symmetry (CS), unstructured (UN), first-order autoregression [AR(1)], heterogeneous first-order autoregression [ARH(1)], heterogeneous compound symmetry (CSH), first-order autoregression with moving averages [ARMA (1,1)], and first-order ante-dependence [ANTE(1)]. Selection of the best matrix model was based on the Bayesian Information Criterion. When differences were detected, the means were compared using the LSMEANS procedure with 10% probability. These procedures were performed using the SAS statistical package, version 9.2 (SAS, 2002).

Multivariate analysis was performed with the GENES Program (CRUZ, 2007) using the generalized distance of Mahalanobis (D^2) to estimate the existing genetic dissimilarity between the ecotypes and hybrids. Tocher's method was used as the technique for grouping the evaluated materials. Finally, to define the total variability observed, the magnitude of the relative contribution of the variables (Sj) was determined by Singh's model (SINGH, 1981).

RESULTS AND DISCUSSION

There was a difference in TDMP between the ecotypes/hybrids (P = 0.0645; Table 1). The 'Aruana' (*Panicum maximum*) and 'Rojas' (*P. guenoarum*) cultivars had higher TDMP, with an average of 20467kg of DM $ha^{-1}year^{-1}$, which was 51% greater than that observed in ecotypes with lower TDMP, which averaged 9997kg of DM $ha^{-1}year^{-1}$ (Table 1). For *P. guenoarum* ecotypes Azulão and Baio and the *P. lepton* accessions, TDMP was intermediate at 12463kg of DM $ha^{-1}year^{-1}$ (Table 1). The evaluated hybrids had an average TDMP of 19255kg of DM $ha^{-1}year^{-1}$, similar to the production observed in superior and intermediate materials (Table 1). These results demonstrate the high potential of the materials evaluated under the soil and climatic conditions of Rio Grande do Sul. Although some of these materials were commercial cultivars, the native genotypes never underwent any genetic improvement but had high forage yields.

Even though hybridization was performed with parents of different species, TDMP of the hybrids and of their male parent *P. guenoarum* Rojas was similar, with no significant gains in productivity being confirmed. This may indicate genetic proximity between the parents because according to PATERNIANI & CAMPOS (2005), hybrid vigor occurs when the genetic distance between parents is high.

TDMP of 'Pensacola' was lower than the averages observed in the hybrids (97%), *P. guenoarum* ecotypes (60%), *P. lepton* accessions

Table 1 - Total dry mass production (TDMP), leaf dry mass production (LDMP), and stern dry mass production (SDMP) in kg of DM per hectare per year of ecotypes/hybrids of the genus *Paspalum*, *Panicum maximum* Jacq. cv. 'Aruana', and *Axonopus catharinensis* evaluated in Rio Grande do Sul (2002-2014).

Ecotype	TDMP	LDMP	SDMP
Panicum maximum Aruana	20364a	12459b	6887a
Axonopus catharinensis	10442c	6627cd	2624c
P. guenoarum Azulão	12651b	8339c	3202b
P. guenoarum Baio	12956b	8211c	3472b
P. guenoarum Rojas	20569a	15023a	4885ab
P. notatum André da Rocha	10080c	6392cd	3111b
P. notatum Bagual	10716c	6845cd	3103b
P. notatum Pensacola	9764c	6502cd	1819c
P. lepton 26A	12407b	8154c	2126c
P. lepton 26D	12279b	8404c	1933c
P. lepton 28B	13139b	8182c	2820b
P. lepton 28C	12185b	8284c	2062c
P. lepton 28E	11622b	7361c	2153c
P. urvillei (André da Rocha)	10430c	7169cd	2620c
P. urvillei (Bagé)	9806c	7323cd	2228c
P. urvillei (Eldorado do Sul)	9746c	7185cd	2022c
P. denticulatum	8010c	4868d	1154c
P. pauciciliatum	10977c	5626d	3261b
Hybrids			
H12	19424ab	15135a	3648b
H13	19700ab	14751ab	3595b
H20	19115ab	12821ab	3042b
H22	18782ab	14789ab	3107b
P	0.0645	0.0307	0.0864

Averages followed by the same letters in the columns did not differ according to the LSMEANS test at 10% probability.

(27%), *P. notatum* ecotypes (6.5%), and *P. urvillei* accessions (2.3%). This comparison is important because the 'Pensacola' cultivar and *P. atratum* cv. 'Pojuca' are the only cultivars with seeds available in the Brazilian market.

Differences in LDMP were observed between the ecotypes/hybrids (P = 0.0307; Table 1). LDMP was higher in *P. guenoarum* cv. 'Rojas' and in hybrid H12, with an average of 15079kg of DM $ha^{-1}year^{-1}$ (Table 1). The other hybrids H13, H20, and H22 had LDMP similar to that of the two high-productivity materials and cultivar 'Aruana', which had intermediate leaf production (Table 1). The *P. denticulatum* and P. *pauciciliatum* ecotypes yielded lower LDMP, which was expected, as they also had lower TDMP (Table 1).

Leaf production is a very important characteristic because leaves are responsible for photosynthesis (RODRIGUES et al., 2008); they are the preferred structures consumed by animals and have the highest nutritional quality (BRATTI et al., 2009). According to PEREIRA et al. (2011), it is essential that selection for increased leaf production and reduced stem production be targeted in forage plant improvement, as showed by hybrid H12, which had LDMP of 132; 7.1%,. This value was higher than that of the commercial cultivar 'Pensacola' as well as the average leaf production of the hybrids.

Differences in SDMP were observed between the ecotypes/hybrids (P = 0.0463; Table 1). SDMP was higher for the cultivar 'Aruana', with 6887kg of DM ha^{-1} $year^{-1}$, which was 30% higher than that observed for *P. guenoarum* 'Rojas'. The cv. 'Rojas' was the second highest producer of stems and was also similar to the *P. guenoarum* ecotypes Azulão and Baio, *P. notatum* André da Rocha and Bagual, and *P. lepton* accession 28B (Table 1). We reported that 33%, 24%, and 17% of TDMP of 'Aruana', *P. guenoarum*, and the hybrids, respectively, were composed of stems, which indicated that the hybrids brought benefits not only by increasing TDMP but also by improving the structural characteristics of the plants, resulting in a lower proportion of components that are less important for livestock grazing.

There were no differences between the ecotypes/hybrids in either the LSR (P = 0.3580) or HI (P = 0.7108), with averages of 4.1 and 0.50, respectively, and these data were used to estimate the Mahalanobis distance, Tocher clustering, and relative contribution to variability.

The measurements of genetic dissimilarity demonstrated a high magnitude (0.11-107.2), which indicated a broad range of genetic variability among the ecotypes/hybrids (Table 2). Combinations of hybrids H12, H20, and H13 with *P. lepton* accession 28E and of hybrid H12 with *P. lepton* accession 26A had the highest divergence, with distances of 107.2, 101.2, 95.8, and 96.1, respectively.

In general, genotypes which present greater distance also have a greater genetic distance among them. Thus, the estimate of genetic dissimilarity becomes very important because when combined with knowledge about the performance of parents, it provides an alternative to cross-breeding by indicating genetic contributions with high combining capacities (VIEIRA et al., 2005)

In relation to the maximum distance obtained between all possible combinations of the ecotypes evaluated, we observed that most genotypes exhibited the maximum distance when combined with hybrid H12, which indicated that it was the most divergent of the materials evaluated. Thus, hybrid H12 can be used as a parent in future hybridization, because according to PEREIRA et al. (2015), genotypes that are genetically distant offer possibilities for new crosses targeting the formation of elite recombinants.

The smallest distances were observed between the *P. urvillei* accessions from Bagé and Eldorado do Sul (D^2 = 0.11), between the *P. guenoarum* ecotypes Azulão and Baio (D^2 = 0.15), and between the *P. lepton* accessions 26D and 28C (D^2 = 0.30). Smaller distance between the ecotypes *P. guenoarum* Azulão and Baio may be because they are of the same species and that the evaluated agronomic characteristics are similar. Although, cultivar 'Rojas' belongs to the same species, it had greater TDMP, LDMP, and SDMP, which may explain why its values of genetic dissimilarity were close to those of the Azulão and Baio ecotypes. For *P. urvillei (*Bagé and Eldorado do Sul) and *P. lepton* (26D and 28C), the smaller distances may have occurred because they are the same ecotypes, although they were harvested in different regions.

Cluster analysis using Tocher's method resulted in the formation of three distinct groups (Table 3). There were 16 ecotypes in Group I, representing 72% of the materials evaluated, which indicated that crosses among these ecotypes, when viable, could reduce the possibility of obtaining superior genotypes. Groups II and III were composed of 5 and 1 ecotypes/hybrids, representing 23% and 5% of the evaluated materials, respectively (Table 3).

Formation of groups is important for targeting potential new hybrid combinations because they should be based on the magnitude of their

Table 2 - Estimates of the Mahalanobis distances (D^2) based on five variables[(1)] in the ecotypes and hybrids of *Paspalum*, *Panicum maximum* Jacq. cv 'Aruana,' and *Axonopus catharinensis* evaluated in Rio Grande do Sul (2002–2014).

Ecotype/hybrid	Greatest D^2	Most distant ecotype/hybrid	Smallest D^2	Closest Ecotype/hybrid	Average D^2
Panicum maximum Aruana	71.11	Pni28E	13.8	PgRojas	40.0
Axonopus catharinensis	66.90	H12	1.13	Ppauc	21.7
P.guenoarum Azulão	52.43	H12	0.15	PgBaio	19.7
P. guenoarum Baio	52.60	H12	0.15	PgAz	19.4
P. guenoarum Rojas	87.60	Pni28E	2.60	H13	44.9
P. notatum André da Rocha	78.70	H12	2.90	PnBagual	30.0
P. notatum Bagual	66.51	H12	2.90	PnAR	24.0
P. notatum Pensacola	80.42	H12	3.80	Pdenticulatum	26.7
P. lepton 26A	96.10	H12	0.53	Pni28C	31.8
P. lepton 26D	92.40	H12	0.30	Pni28C	28.6
P. lepton 28B	95.60	H12	0.53	Pni26A	30.5
P. lepton 28C	95.50	H12	0.30	Pni26D	30.6
P. lepton 28E	107.21	H12	0.65	Pni26A	32.2
P. urvillei (André da Rocha)	77.60	H12	0.30	PuBagé	25.8
P. urvillei (Bagé)	77.14	H12	0.11	PuES	24.9
P. urvillei (Eldorado do Sul)	75.00	H12	0.11	PuBagé	23.4
P. denticulatum	76.90	H12	1.90	Axocath	20.3
P. pauciciliatum	71.32	H12	1.13	Axocath	21.5
H12	107.20	Pni28E	34.8	H22	70.0
H13	95.80	Pni28E	0.57	H22	49.8
H20	101.24	Pni28E	1.17	H22	54.0
H22	90.51	Pni28E	0.57	H13	46.3

[(1)]Total dry mass production (TDMP); Leaf dry mass production (LDMP); Stem dry mass production (STDM); Leaf: stem ratio (LSR); Harvest index (HI).

dissimilarity and the potential of parents. However, in addition to being dissimilar, parents should combine medium and high variability with regard to the characteristics being improved (ALMEIDA et al., 2011).

Analysis of the relative contribution to total variability showed that 78.8% of the genetic divergence occurred as a result of TDMP, whereas LSR, LDMP, SDMP, and HI had a contribution of only 21.2% (Table 4). PEREIRA et al. (2015) analyzed the agronomic ecotypes and hybrids of the genus *Paspalum* (individual plants) and reported that the relative variability contribution of TDMP to genetic divergence was 46.2%, whereas the other seven evaluated variables accounted for 53.8% of the variability observed.

The fact that TDMP makes the greatest contribution to variability indicates that it was the

Table 3 - Breakdown of the genetic dissimilarity of ecotypes and hybrids of the genus *Paspalum*, *Panicum maximum* Jacq. cv 'Aruana', and *Axonopus catharinensis* evaluated using Tocher's cluster analysis (Rio Grande do Sul: 2002–2014).

Groups	Clustering
I	*P. urvillei* (Bagé), *P. urvillei* (Eldorado do Sul), *P. urvillei* (André da Rocha), *P. lepton* 28B, *P. pauciciliatum, P. lepton* 28C, *P. lepton* 26D, *P. lepton* 26A, *P. lepton* 28E, *P. denticulatum , Axonopus catharinensis, P. notatum* Pensacola, *P. guenoarum* Baio, *P. guenoarum* Azulão, *P. notatum* Bagual, *P. notatum* André da Rocha
II	H13, H20, H22, *P. guenoarum* Rojas, *Panicum maximum* Jacq. cv Aruana
III	H12

Table 4 - Relative contribution to the variability of characteristics associated with the forage potential of ecotypes and hybrids of the genus *Paspalum, Panicum maximum* Jacq. cv. 'Aruana', and *Axonopus catharinensis* based on the generalized Mahalanobis distance (Rio Grande do Sul: 2002–2014).

Variable	Sj(1)	Sj(%)(2)
Total dry mass production (TDMP)	6395	79
Leaf dry mass production (LDMP)	637	8
Stem dry mass production (SDMP)	274	3
Leaf:stem ratio (LSR)	648	8
Harvest index (HI)	163	2

most effective variable for expressing the variability among genotypes and demonstrated its importance as a component in the selection of genotypes with superior forage characteristics. ALVES et al. (2003) reported the importance of determining the relative contribution of characteristics to reduce the number of evaluations necessary to determine genetic variability, given that only characteristics that effectively contributed to this variability can be measured. Smaller contribution to the genetic variability by botanical separation and structural components, when compared to TDMP, suggested that the measurements could be discontinued, because it required excessive labor and time in return for a relatively small contribution to variability.

CONCLUSION

The hybrids exhibited high TDMP, composed mostly of leaves, with a smaller proportion of stems than that observed in the ecotypes with similar or higher TDMP. The *P. guenoarum* genotypes Rojas, Baio, and Azulão stood out in terms of TDMP and could be used in future crosses. Tocher's method using generalized Mahalanobis distance allowed us to identify hybrid H12 and *P. lepton* accession 28E as the most dissimilar and the *P. urvillei* accession from Bagé and Eldorado do Sul as the most similar. TDMP is the characteristic that contributes most to the detection of genetic variability.

ACKNOWLEDGEMENTS

We would like to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and SulPasto for the financial support and human resources.

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