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Short Communication

Hybrids of *Paspalum plicatulum* × *P. guenoarum*: Selection for forage yield and cold tolerance in a subtropical environment

Híbridos de Paspalum plicatulum × P. guenoarum: Selección para rendimiento de forraje y tolerancia al frío en ambiente subtropical

KARLA M. SARAIVA[1], MIGUEL DALL'AGNOL[1], EDER A. M. DA MOTTA[1], EMERSON A. PEREIRA[2], CLEBER H. L. DE SOUZA[1], CARINE SIMINONI[1], ROBERTO L. WEILER[1], MAURÍCIO M. KOPP[3], RAQUEL SCHNEIDER-CANNY[4] AND MARLON R. BARBOSA[5]

[1]*Departamento de Plantas Forrageiras e Agrometeorologia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.* ufrgs.br/agronomia
[2]*Universidade Regional do Noroeste do Estado do Rio Grande do Sul, Ijuí, RS, Brazil.* unijui.edu.br
[3]*Embrapa Pecuária Sul, Bagé, RS, Brazil.* embrapa.br/pecuaria-sul
[4]*Noble Research Institute, Ardmore, USA.* noble.org
[5]*Instituto Nacional de Investigación Agropecuaria (INIA), Tacuarembó, Uruguay.* inia.uy

Abstract

Selection of improved genotypes is important for pasture-based feeding systems in subtropical regions. Our goal was to identify hybrids of *Paspalum* with enhanced forage yield and cold tolerance across 2 sites [Bagé and Eldorado do Sul (ES)], in Rio Grande do Sul, Brazil. We evaluated 19 *P. plicatulum* × *P. guenoarum* hybrids, *P. plicatulum* genotype 4PT, *P. guenoarum* cultivars Azulão and Baio and, as Control, *Megathyrsus maximus* cv. Aruana. At both sites, the experimental design was a completely randomized block with 4 replications. Total dry mass (total-DM), leaf-DM and cold tolerance (ColdT) were recorded. At Bagé, hybrid 102069 produced higher total-DM and leaf-DM than the progenitors and cv. Aruana, while at ES, hybrids 102069 and 10308 produced higher total-DM than 4PT, Azulão and Aruana; hybrid 102069 had higher leaf-DM. At Bagé, 16 hybrids displayed ColdT similar to their progenitors and higher than Aruana, while at ES, 12 hybrids showed ColdT similar to Azulão and Baio and higher than 4PT and Aruana. This study demonstrated that hybrids of *Paspalum* with superior forage yield to their progenitors and Aruana, and hybrids with higher ColdT than 4PT and Aruana are in existence. The hybridization technique shows potential for producing alternative genotypes with higher forage yield and ColdT for sowing in subtropical regions.

Keywords: Biomass production, genetic variability, hybridization, native grasses.

Resumen

La selección de genotipos forrajeros mejorados es fundamental para garantizar la productividad y sostenibilidad de sistemas de producción animal basados en pasturas en regiones subtropicales. El objetivo de este trabajo fue identificar híbridos del género *Paspalum* con alto rendimiento de forraje y tolerancia al frío en Bagé y Eldorado do Sul, Rio Grande do Sul, Brasil. Fueron evaluados 19 híbridos de *P. plicatulum* × *P. guenoarum*, *P. plicatulum* genotipo 4PT y los cultivares (cv.) Azulão y Baio de *P. guenoarum*; como testigo se utilizó *Megathyrsus maximus* cv. Aruana. En ambos sitios se utilizó un diseño de bloques al azar, con cuatro repeticiones, para evaluar las variables producción de materia seca total (PMS-total), producción de materia seca de hojas (PMS-hojas) y tolerancia al frio (Tfrío). En Bagé, el híbrido 102069 produjo mayor PMS-total y PMS-hojas que los progenitores y el testigo. En Eldorado do Sul, los híbridos 102069

Correspondence: E.A.M. da Motta, Departamento de Plantas Forrageiras e Agrometeorologia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 7712, Porto Alegre, RS, Brazil. Email: edermotta87@yahoo.com.br

y 10308 presentaron mayores PMS-total, mientras que el híbrido 102069 produjo más PMS-hojas que el genotipo 4PT, el cv. Azulão y el testigo. En Bagé, 16 híbridos presentaron Tfrío similar a los progenitores pero mayor que el testigo, En Eldorado do Sul, 12 híbridos mostraron Tfrío similar a los cvs. Azulão y Baio pero superior al genotipo 4PT y al testigo. El estudio permitió identificar híbridos de *Paspalum* con rendimiento de forraje superior a los progenitores y el testigo cv. Aruana, e híbridos con mayor Tfrío que el genotipo 4PT y el testigo. La técnica de hibridación tiene potencial para obtener genotipos con mayor rendimiento de forraje y tolerancia al frío para regiones subtropicales.

Palabras clave: Adaptación climática, gramíneas nativas, hibridación, producción de biomasa, variabilidad genética.

Introduction

The production of cultivars adapted to subtropical edapho-climatic conditions with higher potential for biomass production than existing cultivars has been the objective of forage breeding programs throughout the southern region of Brazil. Motta et al. (2016) suggested that the genetic variability among species belonging to the genus *Paspalum* in natural ecosystems in South America represented a broad and important germplasm source to be exploited, with the possibility of increasing the efficiency of production systems and recovery of degraded pasture areas.

The genus *Paspalum* comprises many diverse native species with forage attributes for animal production and adaptability to different ecosystems (Novo et al. 2016). These characteristics demonstrate the potential for use in breeding programs and the establishment of cultivated pastures (Motta et al. 2017). However, the predominant reproductive mode for *Paspalum* species is apomixis (Acuña et al. 2009) and therefore, sexual reproduction is necessary for obtaining genetic variability. With the attainment of tetraploid sexual *P. plicatulum* plants (Sartor et al. 2009), the exploitation of the segregating populations through breeding between apomictic tetraploids and a sexual progenitor was made possible. The progeny resulting from this breeding segregate into sexual and apomictic individuals. When selected and identified, the apomictic hybrids may be used in field assays for agronomic evaluation and then established as cultivars. Alternatively, the selected sexual hybrids may be used as female progenitors for further breeding (Aguilera et al. 2011). The main reason for using hybridization in apomictic species is to fix superior breeds through apomixis (Zilli et al. 2015).

Temperature is a conditioner of vegetative development because damage by low temperatures may result in growth reduction, leaf lesions and basic functional disorders (Taiz and Zeiger 2013). The southern region of Brazil is characterized by the occurrence of low temperatures and frosts in the winter, which may impair forage supply to grazing animals since natural and some cultivated pastures are susceptible to these conditions, making little if any growth during this time.

Hybridization performed by the Forage Breeding Group (FBG) of the Federal University of Rio Grande do Sul (UFRGS), using the apomictic *P. guenoarum* cultivars Azulão and Baio (as pollen donors) and the sexual tetraploid genotype 4PT of *P. plicatulum*, produced *Paspalum* interspecific hybrids that may have a significant positive impact on animal production in southern Brazil. Field assays are necessary to determine the forage production traits of these novel genetic resources, to provide a basis for selection of superior genetic material and consequently, the establishment of novel cultivars for subtropical conditions. Therefore, the goal of this research was to identify new hybrids of *Paspalum* with enhanced forage yield and cold tolerance across different edaphoclimatic regions located in Rio Grande do Sul state, Brazil.

Materials and Methods

The study was conducted from December 2012 to April 2014 in 2 regions of Rio Grande do Sul (RS) state, Brazil: 'Depressão Central' and 'Campanha'. The Depressão Central experimental area is located within the Agronomic Experimental Station (AES) of the UFRGS, located in Eldorado do Sul, RS, Brazil (30°06' S, 51°41' W; 32 masl). The soil is classified as a typical Dystrophic Red Argisol (Streck et al. 2008) and had the following characteristics during the experiment: clay content - 22.0%; pH in water - 5.5; SMP index - 6.5; P - 8.9 mg/dm^3; K - 105 mg/dm^3; OM - 1.5%; exchangeable Al - 0.0 cmol$_c$/dm^3; and effective CEC - 6.2 cmol$_c$/dm^3. According to Köppen's classification, this region has a humid subtropical Cfa climate with warm summers. The mean maximum temperature is 30.2 °C in January (hottest month), while mean minimum temperature is 8.5 °C in June (coldest month). Mean annual rainfall is approximately 1,450 mm.

The Campanha experimental area is located within Embrapa Pecuária Sul, located in Bagé, RS, Brazil (31°25' S, 54°07' W; 212 masl). The soil is classified as a typical Orbicular Hypochromic Luvisol (Streck et al. 2008) and had the following characteristics: clay content - 24.6%; pH in water - 5.7; SMP index - 6.0; P - 7.9 mg/dm^3; K - 45 mg/dm^3; OM - 1.5%; exchangeable Al - 0.0 cmol$_c$/dm^3; and effective

CEC - 7.9 $cmol_c/dm^3$. The regional climate, following Köppen's classification, is also humid subtropical Cfa with warm summers. Mean maximum temperature is 29.7 °C in January (hottest month), while mean minimum temperature is 7.9 °C in July (coldest month). Mean annual rainfall is approximately 1,260 mm.

Maximum and minimum temperatures and rainfall were monitored at both sites and are presented in Figure 1. At both sites, the soil was fertilized according to technical indications for perennial warm season grasses (CQFS 2004). Urea, triple superphosphate and potassium chloride were applied to supply 200 kg N/ha, 100 kg P/ha and 80 kg K/ha.

Hybrids used in this experiment were obtained in 2010 through breeding by the FBG of the Department of Forage Plants and Agrometeorology (DFPA) of the UFRGS. Hybridization was performed in greenhouses using apomictic *Paspalum guenoarum* cvv. Azulão and Baio as male progenitors (pollen donors) and the sexual ecotype 4PT of *P. plicatulum* as the female progenitor. Azulão and Baio originate from the subtropical and temperate regions of southern Brazil, Argentina and Paraguay (Steiner et al. 2017), while ecotype 4PT was collected in northeastern Argentina and had its chromosome number duplicated, resulting in the generation of a sexual tetraploid plant (Sartor et al. 2009). The progeny obtained from this breeding were evaluated (individual plants) in the field to identify and select genotypes with highest forage production.

This study evaluated the hybrids 10202, 1020104, 102084, 102080, 1020133, 102058 and 102069, resulting from crosses between *P. plicatulum* genotype 4PT and *P. guenoarum* cv. Azulão, and the hybrids 103063, 10308, 103042, 103040, 103061, 103077, 103087, 103093, 103031, 103020, 103084 and 103037 resulting from crosses between *P. plicatulum* ecotype 4PT and *P. guenoarum* cv. Baio. *P. guenoarum* cvv. Azulão and Baio and *P. plicatulum* genotype 4PT, as well as *Megathyrsus maximus* cv. Aruana, were included in the evaluation for comparison. The latter was used as Control because it is high yielding and is widely cultivated in southern Brazil.

At both sites, a completely randomized block design with 4 replicates was adopted. The experimental unit was composed of a 1.0 m row containing 5 plants spaced 20 cm apart. Inter-row spacing was 0.80 m and spacing between blocks was 1.5 m, giving a total area of 149.6 m^2 (17.6 × 8.5 m). The experiment was established using seedlings obtained from tussocks collected in the AES during April 2012. Seedlings were grown in plastic packages (300 ml), containing commercial substrate, inside DFPA's greenhouses where they remained until field transplanting in October 2012 at both sites. In Eldorado do Sul, evaluations started in December 2012, while in Bagé they started in January 2013. Evaluations were performed at harvesting, when 80% of the genotypes had leaves with an average length of 35–40 cm, leaving 10 and 15 cm of stubble height for *Paspalum* and Aruana, respectively. After harvest and for morphological evaluation, each sample was sorted into leaves, stems and inflorescences, which were then placed in a forced-air oven at 65 °C until a constant weight was reached.

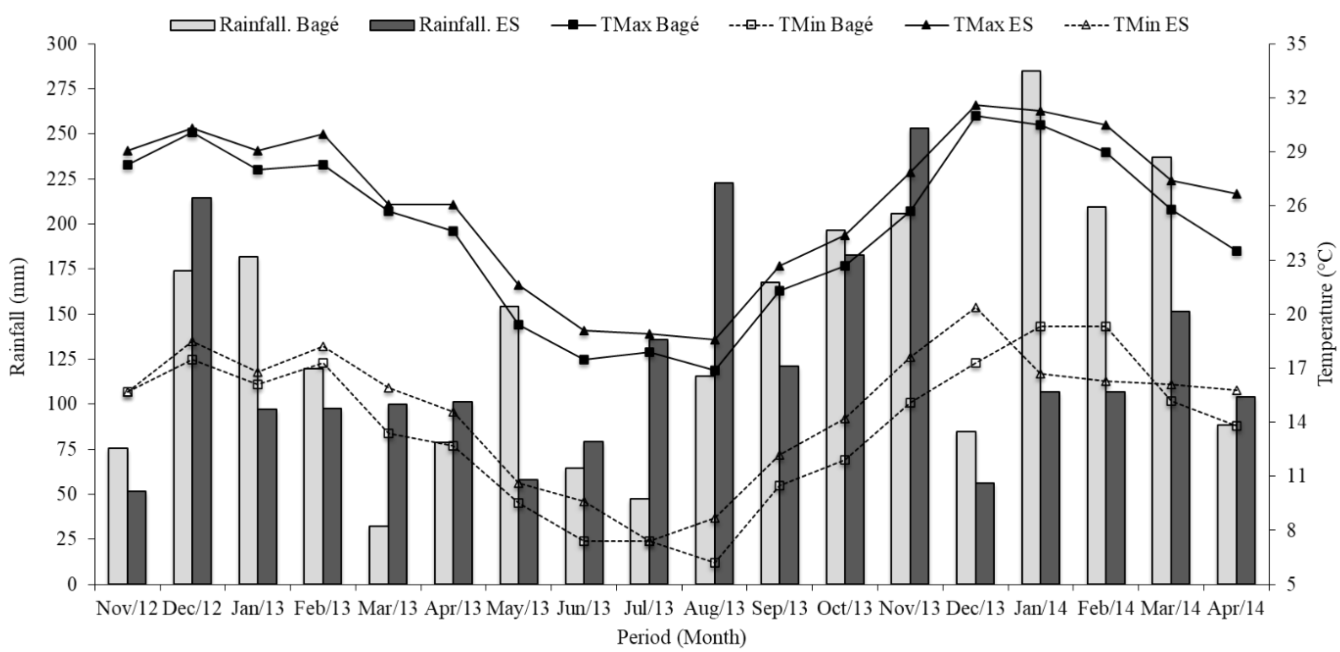

Figure 1. Mean maximum (TMax) and minimum (TMin) temperatures and rainfall recorded between November 2012 and April 2014 in Bagé and Eldorado do Sul (ES), Rio Grande do Sul, Brazil.

The following agronomic characters were evaluated: total dry mass (total-DM) and leaf-DM. After frost events during winter in 2013, visual scores were allocated for cold tolerance (ColdT). Rating system was 1–5, with 1 (many dead leaves) being the lowest and 5 (few dead leaves) being the highest tolerance rating (Acuña et al. 2009; Motta et al. 2017).

At each site, total-DM and leaf-DM data were submitted to analysis of variance involving an F test using the PROC GLM procedure in SAS software (SAS 2001). When differences among genotypes were detected, a means comparison was performed using Tukey's test. The GENES software was used for the ColdT data analysis (Cruz 2007) and when genotype differences were detected, a means comparison was performed using the Scott-Knott test. All differences refer to significance at P<0.05.

Results and Discussion

There was significant variability among genotypes for the characters total-DM, leaf-DM and ColdT. In Bagé, hybrid 102069 had highest (P<0.05) total-DM among the evaluated genotypes, accumulating 652 g/row (Table 1). The forage yield of this hybrid was 76, 93, 76 and 115% greater than that of Baio, Azulão, Aruana and 4PT, respectively. There were few significant differences in total DM for the remaining hybrids and cultivars. At Eldorado do Sul, hybrid 102069 (623 g/row) also had the highest (P<0.05) total-DM but was not significantly different (P>0.05) from hybrid 10308 (567 g/row) (Table 1). There were no significant differences in total-DM for the remaining hybrids and cultivars.

These total-DM values for 102069 and 10308 are important because they exceeded those of Azulão and Baio, which produced more than 15 t DM/ha across different environments (Pereira et al. 2012). They also outyielded Aruana, which was selected as Control because it is a perennial grass used widely for ruminant feeding in RS, Brazil, with productivity between 13 and 24 t DM/ha (Motta et al. 2017); it also has good ColdT among tropical pasture species. Our findings indicate that interspecific hybridization produced hybrid vigor for total-DM, which exceeded those of Azulão, Baio and 4PT, which were used as progenitors.

Table 1. Accumulated total dry mass (Total-DM, g/row) and leaf dry mass (Leaf-DM, g/row) production, and leaf percentage (Leaf %) for *Paspalum* hybrids and their progenitors and *Megathyrsus maximus* cv. Aruana, at two sites in Rio Grande do Sul, Brazil.

Genotype	Bagé	Eldorado do Sul	Bagé		Eldorado do Sul	
	Total-DM		Leaf-DM	Leaf %	Leaf-DM	Leaf %
102069	652a	623a	351a	54	371a	60
10308	430b	567ab	288ab	67	337ab	59
1020133	421bc	481bcd	282ab	67	279abc	58
103084	422bc	479bcd	288ab	68	306abc	64
103061	414bc	480bcd	296ab	71	323abc	67
103087	415bc	470bcd	283ab	68	275abc	59
102080	412bc	466bcd	269ab	65	283abc	61
103031	370bcd	474bcd	247b	67	315abc	66
103063	401bcd	421d	241b	60	234c	56
102084	403bcd	414d	280ab	69	236c	57
103037	345bcde	458cd	241b	70	273abc	60
103093	338bcde	456cd	235b	70	247bc	54
103020	351bcde	441d	269ab	77	279abc	63
103042	364bcd	427d	279ab	77	262bc	61
cv. Baio	371bcd	415d	239b	64	283abc	68
cv. Azulão	337bcde	449cd	248b	74	266bc	59
cv. Aruana	370bcd	410d	247b	67	235c	57
103040	357bcd	417d	263ab	74	295abc	71
1020104	328cde	445cd	229b	70	271bc	61
10202	346bcde	425d	229b	66	275abc	65
103077	351bcde	415d	243b	69	255bc	61
4PT	303de	393d	229b	76	250bc	64
102058	255e	419d	204b	80	270bc	64
Mean	381	454	260	69	279	62

Within columns, means followed by the same letters do not differ by Tukey test at P<0.05.

As for total-DM, leaf-DM for 102069 exceeded those of Azulão, Baio, 4PT and Aruana at Bagé, but differed from only Azulão, 4PT and Aruana at Eldorado do Sul. Many of the hybrids produced similar leaf-DM as 102069 at both locations (Table 1). Results showed that leaf proportion of hybrid 102069 was 54 and 60% of total-DM at Bagé and Eldorado do Sul, respectively. These values are similar to those reported by Huber et al. (2016), who evaluated interspecific *Paspalum* hybrids and observed that this component's percentage ranged between 60 and 68%. Leaf percentage is an important consideration when selecting genotypes for animal feeding, because leaf is the most nutritionally valuable morphological component of a pasture-based production system. Relative amounts of leaf and stem in available forage impacts significantly on nutritive value as well as ingestive behavior of cattle (Fernandes et al. 2014).

The ColdT evaluation of the genotypes revealed the existence of significant genetic variability for this characteristic at both locations (Table 2). At Bagé, all hybrids (P<0.05) were more tolerant of cold weather than the Control, Aruana; however, when compared with the progenitors Azulão, Baio and 4PT, only hybrids 103077, 102080 and 103037 had lower tolerance. At Eldorado do Sul, Aruana again had the lowest (P<0.05) ColdT among the tested genotypes, whereas 12 hybrids plus Azulão and Baio were most tolerant. Azulão and Baio are known to persist through winter and are considered tolerant of the lower temperatures and frosts that occur in subtropical environments (Fachinetto et al. 2012). The large number of hybrids tolerant of cold weather indicates the transmission of this characteristic from the ecotypes to the progeny. Of all available cultivars of *M. maximus*, Aruana is considered to be among the most tolerant of cold conditions (Corrêa 2002). The higher ColdT displayed by hybrids, when compared with Aruana, agrees with results obtained by Motta et al. (2017), who also observed higher ColdT among *Paspalum* progenies, when compared with the commercial cultivar. This result suggests that use of hybridization between ecotypes adapted to subtropical environments may produce progeny with higher levels of resistance to cold conditions. The ColdT characteristic is essential in subtropical regions, because cold-tolerant ecotypes may have higher persistence and higher herbage allowance for grazing animals during winter along with more rapid regrowth in spring.

Paspalum species with desirable agronomic characteristics obviously exist in natural ecosystems and represent a pool of genetic material for use in pasture breeding programs to enhance ruminant production. The results obtained in this study demonstrate that there are *Paspalum* interspecific hybrids with total-DM and leaf-DM superior to those from Azulão, Baio and 4PT used as progenitors, as well as from Aruana, across both sites. The fact that several hybrids showed ColdT similar to that of Azulão and Baio and considerably higher than that of Aruana, indicates that these hybrids should not suffer from cold temperatures during winter to a greater degree than the existing ecotypes. Hybridization techniques can be used to obtain superior genotypes and facilitate the breeding process in the attainment of novel perennial forage cultivars with higher yields and ColdT in subtropical weather than the existing ecotypes.

Table 2. Means for cold tolerance in *Paspalum* hybrids and their progenitors and *Megathyrsus maximus* cv. Aruana, at two sites in Rio Grande do Sul, Brazil.

Genotype	Cold tolerance score[1]	
	Bagé	Eldorado do Sul
102069	3.5a	3.1a
10308	3.2a	3.5a
1020133	3.3a	3.1a
103084	3.4a	3.5a
103061	2.6a	2.4b
103087	3.2a	3.2a
102080	2.5b	2.3b
103031	3.5a	3.6a
103063	2.8a	2.5b
102084	3.5a	3.1a
103037	2.2b	2.4b
103093	3.5a	3.4a
103020	3.1a	2.2b
103042	2.9a	2.3b
cv. Baio	4.1a	3.7a
cv. Azulão	3.7a	3.6a
cv. Aruana	1.5c	1.1c
103040	3.2a	3.3a
1020104	3.3a	3.3a
10202	3.2a	3.5a
103077	2.4b	2.5b
4PT	2.8a	2.1b
102058	3.1a	3.1a
Mean	3.1	2.9

Within columns, means followed by the same letter do not differ by Scott-Knott test at P<0.05.
[1]Scoring scale from 1 (many dead leaves) to 5 (few dead leaves).

Conclusions

We conclude that hybridization can be an alternative tool for obtaining genotypes with higher forage yield and ColdT for subtropical regions. Based on the combination of superior total-DM and leaf-DM, as well as ColdT at 2 sites, hybrids 102069, 10308, 1020133, 103084, 103061, 103087, 103031 and 102080 hybrids are recommended for further evaluation, such as seed production, response to fertilizer application and animal performance.

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(Note of the editors: All hyperlinks were verified 10 January 2021.)

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