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Forage yield of tetraploid bahiagrass hybrids

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Abstract - In Southern Brazil, much of the livestock activity is developed in native grasslands. Studies about forage traits, frost tolerance and nutritional value of native forage species there has been increasing with better results of productive efficiency (animal gain). The objective of this work was to evaluate forage yield of forty-five intraspecific hybrids of segregating progenies of bahiagrass (*Paspalum notatum* Flügge) from agronomic analyses under greenhouse conditions and determine the reproduction mode of selected hybrids. The hybrids had great variability in all agronomic characteristics measured. The plants with the highest total dry mass production were 17PN10P3 and 17PN29P1 (sexual reproduction), 17PN10P5, 17PN16P3 and 17PN28P4 (apomictic reproduction). The high correlation of the total dry mass with tillers number and with the root dry mass demonstrate that the hybrids can be used as forage and also to reduce the effects of soil erosion in degraded areas. The evaluations of agronomic characteristics and the determination of the mode of reproduction of the selected hybrids allowed the identification of promising genetic materials for forage yield and to soil cover, selecting them for additional stages in the breeding program.

Keywords: Apomixis. Genetic improvement. Intraspecific hybridization. *Paspalum notatum*.

Produção de forragem de híbridos tetraploides de grama forquilha

Resumo - No sul do Brasil, boa parte da atividade pecuária é desenvolvida em pastagens nativas. Estudos sobre características agrônomicas, tolerância ao frio e valor nutritivo das espécies forrageiras nativas têm aumentado, e melhores resultados de eficiência produtiva (ganho animal) são alcançados. O objetivo deste trabalho foi avaliar a produção de forragem de 45 híbridos intraespecíficos de progênies segregantes de grama-forquilha (*Paspalum notatum* Flügge), através de análises conduzidas em casa de vegetação e determinar o modo de reprodução dos híbridos selecionados. Os híbridos tiveram grande variabilidade para as características analisadas. As plantas com a mais alta produção total de matéria seca foram 17PN10P3 e 17PN29P1 (reprodução sexual), 17PN10P5, 17PN16P3 e 17PN28P4 (reprodução apomítica). A alta correlação da matéria seca total com o número de perfilhos e com a massa seca total das raízes indica que as plantas híbridas podem ser usadas como forrageiras e para recuperação de áreas degradadas, reduzindo os efeitos da erosão do solo. As avaliações das características agrônomicas e a determinação do modo de reprodução dos híbridos selecionados permitem a identificação de materiais genéticos promissores para produção de forragem e para cobertura de solo, selecionando-os para as próximas fases do programa de melhoramento.

Palavras-chave: Apomixia. Melhoramento genético. Hibridação intraespecífica. *Paspalum notatum*.

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Introduction

The Brazilian livestock is an economic activity that offers employment and income in agricultural sector. The country has a rich natural environment and the pastures are the main source of feed for the herbivores (MACHADO *et al.*, 2017).

In the Southern Brazil, most of the pastures are natives, covered almost exclusively with herbaceous species, mainly grasses (VALLS *et al.*, 2009a). However, with the advance of intensive agriculture the area of native pastures decreased (BARBOSA *et al.*, 2019). The search of natural variability occurring in forage species from native grasslands and the development of cultivars adapted to different environmental conditions with higher potential for forage yield can significantly contribute for the production systems, reducing the use of exotic forages, which in some cases presents adaptation problems (PILLAR *et al.*, 2009) and avoiding the process of degradation.

Paspalum L. genus have a prominent position in the native grasslands in the southern Brazil due to its forage value, adaptation to grazing and climate and is a source of genetic variability for use in improvement programs (VALLS *et al.*, 2009b). Among these species, *Paspalum notatum* Flüge, commonly known as 'bahiagrass', has good forage quality, high yield and persistence to grazing and trampling by animals (POZZOBON; VALLS, 1997) and can be used as a pioneering species to reduce the effects of soil erosion and degradation (BARBOSA *et al.*, 2019).

Native biotypes of bahiagrass in Rio Grande do Sul are tetraploids (4x) and reproduce by apomixis. Apomictic populations are genetically uniform due their clonal reproduction via seeds (DAURELIO *et al.*, 2004). For obtaining genetic variability for breeding programs, sexual reproduction and successive cycles of hybridization, aiming at gene recombination, are necessary (SARAIVA *et al.*, 2021). Apomictic genotypes as pollen donors and sexual genotypes as female are freely crossable (ORTIZ *et al.*, 2013) and produce offspring that segregated for reproduction mode (AGUILERA *et al.*, 2015). In bahiagrass, a race, called capim Pensacola (*P. notatum* var. *Saurae*) is sexual and diploid (2x) (QUARIN, 1992). The sexual genotype of bahiagrass was duplicated by Quarin *et al.* (2001, 2003) and Weiler *et al.* (2015) and the duplicate plants were used in intraspecific crosses schemes with 4x apomictic plants native from south of Brazil. The hybrid progenies were used in assays for agronomic evaluation (WEILER *et al.*, 2018; BARBOSA *et al.*, 2019) and exhibited heterosis for traits of interest such as forage production and resistance to cold. Once identified, the superior hybrids with traits fixed through apomixis can be selected and then established as cultivars, allowing for pasture diversification and increased productivity. Moreover, the sexual reproduction hybrids are necessary for obtaining genetic variability (SARAIVA *et al.*, 2021) in new cycles of crosses.

The aim of this research was to identify and selected new hybrids of bahiagrass most productive in greenhouse conditions, verify which plants invest the most in rhizomes as a survival criterion and to evaluate the mode of reproduction of the selected segregating hybrids.





Materials and Methods

Plant material and crosses

The experiment was carried from November 2019 to February 2021 at Department of Forage Plants and Agrometeorology of the Agronomics School, Federal University of Rio Grande do Sul (UFRGS), located in the city of Porto Alegre, Rio Grande do Sul state, Brazil (latitude 30° 1'16.13" S and longitude 51°13'23.99" W).

The crosses were made during the summer 2017 in the greenhouse to generate a segregating bahiagrass population, following the methodology described by Burton (1948) and adapted by Machado *et al.* (2021), between sexual and apomictic tetraploid clones. The female parents were the sexual tetraploid hybrids called KC1, KD6, KD9, KF1, KF2, KF7, KF15, KM3, KM4, KM5, KM6, KN2, KN4 and the artificial polyploid WKS92, generated by Weiler *et al.* (2015). As male parents, the apomictic tetraploid hybrids called KD2, KD5, KD7, KF8, KF10, KF16, KN3, KN5, and the native ecotypes of the species, Bagual (BAG) and André da Rocha (AR). The hybrids were obtained in crosses performed in 2015 and your mode of reproduction was evaluated by Krycki, Simioni and Dall'Agnol (2016). The seeds were collected at least 21 days after pollination. The progeny from each cross was referred as a family and a code was given to identify each hybrid.

Agronomic evaluations

The hybrids (individual genotypes) were vegetative propagated in a greenhouse, generating five clonal replicates for each genotype, placed in 2.8-L pots with a commercial substrat. The clones were arranged following a randomized complete block design with four replications. After the adaptation period, the first cut was performed when the plants reached the minimum height at 25cm, leaving a residue of 5 cm from the ground height (PEREIRA *et al.*, 2011).

In the subsequent cuts, the plants with erect and prostrate growth habits were harvested when they reached an average height of 30 cm and 20 cm, respectively, leaving a residue of 5 cm from the ground height (WEILER *et al.*, 2018; BARBOSA *et al.*, 2019). Six cuts were performed in the summer and spring of 2020/2021 (two years/growth seasons). Four harvests were performed in the first year and two in the second. Before the cuts, the Plant Height (PH, cm) was measured from the soil up to height of the leaves, the Growth Habit (GH) was estimated using a 1-to-5 scale, where 1 represented the plants "prostrate", and 5 meant "erect" and was not statistically evaluated; and was counting the Tiller Number (TN).

After each cut, while still green, leaves were separated from the stem to assess the Leaf: Stem ratio (LSR). Afterwards, the green material went to the air-forced drier oven at 60°C for a minimum period of 48h or until constant weight to evaluate of the Total Dry Mass (TDM, g plant⁻¹). The variable Roots Dry Mass (RDM, g plant⁻¹) was performed at the end of experiment, after the last cut made to evaluate forage yield. The collected roots were placed to dry with forced air at 60°C for a minimum period of 72h and were subsequently weighed.

The data were submitted to analysis of variance (ANOVA) using the PROC GLM procedure in SAS





statistical software (SAS, 2004) with F test at 5% probability. When significant differences between genotypes were detected, comparison of means was performed by the Scott- Knott test at 5% probability/significance ($p \leq 0.05$) with GENES software (CRUZ, 2016). Pearson's correlation coefficient was used to test the associates among the variables LSR, TN, RDM and PH. TDM was used as a criterion for genotype selection.

Mode of reproduction

The mode of reproduction of the five selected hybrids, using TDM as a criterion for genotype selection was determined based on the observation of embryo sacs morphology of the hybrid plants. For this, inflorescences at anthesis were collected and fixed in FAA solution [40 ml 95% ethanol: 14 ml distilled water: 3 ml 40% formalin: 3 ml glacial acetic acid] for 24 h, transferred to ethanol 70% and refrigerated. Pistils were dissected out of the flowers and dehydrated with alcohol and clarified with methyl salicylate according to the method established by Young, Sherwood and Bashaw (1979) and modified by Acuña *et al.* (2007). At least 30 mature ovules were observed in each plant with an interference contrast microscope. According with the number of ovules with aposporous embryo sacs or sexual, the plants were classified as sexual or facultative apomictic. Presence of antipodal cells characterizes sexual ovules and default of antipodal cells, multiple or single embryo sacs with the egg apparatus and the central cell characterize apomictic ovules (ACUÑA *et al.*, 2009; KUMAR; SAXENA; GUPTA, 2017). Aborted or immature ovules were not counted (MARCÓN *et al.*, 2019); only embryo sacs identified as sexual or apomictic were used for classification.

Results and Discussion

From the crosses, we obtained 144 seeds that were sown in petri dishes. Individual seedlings were planted in pots, maintained in greenhouse, and 45 tetraploid hybrids of sexual and apomictic parents, corresponding to 16 families (Table 1) were evaluated for the productive potential under these conditions. Hybrids were obtained from only 31% of the seeds, due to the incidence of fungi at the time of seed germination, failures in transplanting, weakness of seedlings and adverse environmental conditions as attack by some insect or fungus in the greenhouse.

Agronomic evaluations

Significant differences were observed among hybrids and families for the traits TN, PH, LSR, RDM and TDM. The averages obtained indicated variability generated by intraspecific hybridization, forming distinct groups between the evaluated genotypes (Table 2). Acuña *et al.* (2011), Zilli *et al.* (2015), Weiler *et al.* (2018) and Barbosa *et al.* (2019) registered a high level of diversity for a series of characteristics in segregating progenies of *P. notatum*, indicated heterosis for interest agronomic traits. Great variability between genotypes for forage yield was registered in *P. notatum*, *P. dilatatum* (VENUTO *et al.*, 2003), *P. leptum* (syn. *P. nicorae*), *P. guenoarum* (PEREIRA *et al.*, 2012), *P. simplex* populations (BRUGNOLI *et al.*, 2013) and in interspecific hybrid progenies obtained by crossbreeding with species of the *Plicatula* group of





Paspalum (NOVO *et al.*, 2017; MOTTA *et al.*, 2020a; 2020b).

Table 1. Identification of crosses, seed set, number and identification of hybrids of bahiagrass.

Crosses				
Sexual female	Apomictic male	Number of seeds obtained	Number of hybrids	ID Hybrid plants
KC1	KN5	4	3	17PN1P2 17PN1P3
KD6	KN5	10	6	17PN1P4 17PN2P1 17PN2P2 17PN2P3 17PN2P4 17PN2P5
KD6	KF8	7	4	17PN2P6 17PN3P1 17PN3P2 17PN3P3 17PN3P4
KD9	*BAG	3	1	17PN5P1
KF1	KF16	2	1	17PN6P1
KF1	KN5	1	1	17PN7P1
KF15	KF8	10	3	17PN8P1
KF15	KD2	6	4	17PN8P2 17PN8P3 17PN9P1 17PN9P2 17PN9P3 17PN9P4
KF15	KD7	5	5	17PN10P1 17PN10P2 17PN10P3 17PN10P4 17PN10P5
KF7	KF16	3	3	17PN14P1 17PN14P2 17PN14P3
KF2	KF8	1	1	17PN15P1
KF4	**AR	10	0	-
.	**AR	3	0	-
KM3	KD7	7	3	17PN16P1 17PN16P2 17PN16P3
KM3	KD5	3	0	-
KM4	KF10	7	0	-
KM4	KD7	10	0	-
KM4	KF8	1	0	-
KM4	KN5	1	0	-
KM4	BAG	9	2	17PN23P1 17PN23P2
KM5	KD7	2	0	-
KM6	KF16	3	1	17PN25P1
KM6	KD7	2	0	-



KN2	KD2	4	0	-
KN4	KM2	10	0	-
KN4	KF16	1	0	-
KN4	KD5	10	4	17PN28P1 17PN28P2 17PN28P3 17PN28P4
WKS92	KN3	9	3	17PN29P1 17PN29P2 17PN29P3
Total		144	45	

*BAG = Bagual ecotype, **AR = André da Rocha ecotype.

The variable TN generated four groups. The group A, with five plants (17PN10P1, 17PN10P5, 17PN23P2, 7PN25P1 and 17PN29P1) and average 15.61 tillers plant⁻¹. Pereira *et al.* (2012) evaluated *Paspalum* species and found no statistical differences for this variable among the accessions.

The variable PH determined four groups with high variation. The more erect plants, according to scale used in the variable GH, had a mean variation for PH of 34.85 cm (group A) and 32.48 cm (group B) while the plants more prostrate had a height difference of 29.93 cm (group C) and 27.75 cm (group D), showing correlation both them variables.

The LSR formed five groups. In the group A, the genotype 17PN3P1 obtained the highest ratio, 20.98, followed the plant 17PN2P1 (19.65 ratio) and 17PN2P6 (18.91 ratio). The lowest LSR indices were obtained by group E, with an average ratio of 5.09. The LSR should be one of the main selection conditions within a forage breeding program because the leaves are responsible for photosynthesis and too are the main source of nutrients for ruminants in grazing systems (RODRIGUES *et al.*, 2008).

For the RDM variable, the analyses generated seven groups. The group A had one genotype, 17PN28P4, with the largest amount of roots, 40.32g plant⁻¹ but the group B too presented an expressive amount of roots, a mean of 34.75 g plant⁻¹. The plants of these groups are 17PN2P3, 17PN14P2 and 17PN16P3.

The mean obtained for TDM indicated the most variability among the variables, forming 13 groups. In the group A, only two plants with the highest productions, 17PN28P4 (41.26 g plant⁻¹) and 17PN10P3 (41.15 g plant⁻¹), while the group B was formed for three genotypes, 17PN29P1 (39.42 g plant⁻¹), 17PN16P3 (39.32 g plant⁻¹) and 17PN10P5 (38.88 g plant⁻¹). Due to their outstanding forage yield, these five genotypes were selected in this study.

A large variability for TDM in the *Paspalum* genus was presented by Acuña *et al.* (2009). Weiler *et al.* (2018) and Barbosa *et al.* (2019) on hybrids of *P. notatum* and by Brugnoli *et al.* (2013) in *P. notatum* and *P. simplex* access. Pereira *et al.* (2012) also found variability for TDM in *P. guenoarum* and *P. lepton* (syn. *P. nicorae*) populations.

Genetic variability is important for plant breeding purposes. Modern agriculture needs fast adaptation to changing market and environmental conditions (KANDEMIR; SAYGILI, 2015). Through hybridization cycles, the variability contained in the germplasm of *Paspalum* genus is release (NOVO *et al.*, 2017) and





offers new genes to the service of plant breeding (SPILLANE; CURTIS; GROSSNIKLAUSS, 2004).

Table 2. Agronomic variables: Tiller Number (TN); Plant Height (PH); Leaf:Stem ratio (LSR); Growth Habit (GH): 1-to-5 scale (1- plants “prostrate”, and 5 plants “erect”); Root Dry Mass (RDM) and Total Dry Mass (TDM) of the hybrid genotypes of bahiagrass.

Genotype (ID)	TN (tillers plant ⁻¹)	PH (cm)	LSR	GH	RDM (g plant ⁻¹)	TDM (g plant ⁻¹)
17PN1P2	13.50 b	32.44 b	13.00 c	4	27.78 c	26.51 h
17PN1P3	14.00 b	35.43 a	12.65 c	4	28.01 c	33.76 e
17PN1P4	12.25 b	31.54 b	15.68 b	3	27.86 c	27.82 h
17PN2P1	12.83 b	32.83 b	19.65 a	4	16.65 f	26.76 h
17PN2P2	8.88 d	35.74 a	13.76 c	5	11.42 g	24.94 i
17PN2P3	10.42 c	34.58 a	14.27 c	3	35.35 b	33.34 e
17PN2P4	11.66 c	35.27 a	9.85 d	4	23.49 d	33.95 e
17PN2P5	10.50 c	30.03 c	13.85 c	4	14.48 g	27.51 h
17PN2P6	11.41 c	28.34 d	18.91 a	3	12.62 g	20.50 l
17PN3P1	10.83 c	32.42 b	20.98 a	4	24.73 d	30.80 f
17PN3P2	11.41 c	35.38 a	12.36 c	4	23.81 d	32.55 e
17PN3P3	12.41 b	31.41 b	11.97 c	3	29.12 c	36.06 d
17PN3P4	7.58 d	29.28 c	12.08 c	3	15.40 g	23.33 j
17PN4P1	9.08 d	28.40 d	14.68 c	2	23.83 d	30.37 g
17PN5P1	8.33 d	27.15 d	13.58 c	2	30.04 c	21.81 k
17PN6P1	12.33 b	30.03 c	7.55 d	3	27.28 c	33.74 e
17PN7P1	11.98 b	34.18 a	10.43 d	4	27.36 c	27.86 h
17PN8P1	12.08 b	28.32 d	16.23 b	2	20.12 e	26.71 h
17PN8P2	7.50 d	26.69 d	3.61 e	2	15.97 f	18.21 m
17PN8P3	8.91 d	26.80 d	6.56 e	2	11.86 g	21.18 l
17PN9P1	13.00 b	28.76 d	7.47 d	2	17.09 f	32.47 e
17PN9P2	11.33 c	29.81 c	10.28 d	2	24.92 d	29.57 g
17PN9P3	12.16 b	29.68 c	12.27 c	2	28.70 c	33.29 e
17PN9P4	11.08 c	30.05 c	8.53 d	2	27.44 c	33.64 e
17PN10P1	14.83 a	29.03 c	11.58 c	2	28.93 c	35.19 d
17PN10P2	13.16 b	27.54 d	8.17 d	1	21.58 e	27.19 h
17PN10P3	12.92 b	34.31 a	12.48 c	3	28.67 c	41.15 a
17PN10P4	10.25 c	32.69 b	10.27 d	3	26.54 c	33.52 e
17PN10P5	16.33 a	34.22 a	7.35 d	3	30.40 c	38.88 b
17PN14P1	11.83 b	34.78 a	11.36 c	4	29.88 c	26.77 h
17PN14P2	13.83 b	34.98 a	14.15 c	4	32.65 b	31.00 f
17PN14P3	9.92 c	35.53 a	10.57 d	4	24.49 d	29.64 g
17PN15P1	8.58 d	32.76 b	4.34 e	4	10.74 g	19.70 l
17PN16P1	13.00 b	31.83 b	8.03 d	3	23.89 d	30.22 g
17PN16P2	13.33 b	32.38 b	3.61 e	3	17.95 f	28.50 g
17PN16P3	12.08 b	36.26 a	10.15 d	3	36.26 b	39.32 b
17PN23P1	9.75 c	33.43 b	6.36 e	3	18.45 f	34.85 d
17PN23P2	15.75 a	33.87 a	8.46 d	3	20.64 e	37.64 c
17PN25P1	15.50 a	32.77 b	6.52 e	2	28.81 c	36.18 d
17PN28P1	11.41 c	30.28 c	8.39 d	1	31.02 c	37.58 c





17PN28P2	8.41 d	27.78 d	4.64 e	2	20.40 e	24.08 j
17PN28P3	10.42 c	31.14 c	11.50 c	1	28.12 c	37.81 c
17PN28P4	11.00 c	32.63 b	16.76 b	2	40.32 a	41.26 a
17PN29P1	15.67 a	34.02 a	7.20 d	3	24.48 d	39.42 b
17PN29P2	10.66 c	34.27 a	10.14 d	3	27.56 c	37.75 c
17PN29P3	10.83 c	33.12 b	7.49 d	3	24.79 d	35.43 d
Média	11.63	31.83	10.86		24.39	31.08
Cv%	12.41	5.82	20.93		10.73	3.54

Means preceded by different uppercase letters in the column don't differ by the Scott-Knott test ($P < 0.05$).

The results of Pearson's correlation among the variables are presented on Table 3, considering all the genotypes and means of the analyzed variables.

Table 3. Coefficients of phenotypic correlation for agronomic traits to Total Dry Mass (TDM) in bahiagrass hybrids: Tiller Number (TN), Plant Height (PH), Leaf: Stem ratio (LSR), Growth Habit (GH), Root Dry Mass (RDM) and Total Dry Mass (TDM).

	TN	PH	LSR	RDM	TDM	GH
TN	1	0.330*	-0.013 ns	0.348*	0.546*	0.039 ns
PH		1	0.086 ns	0.334*	0.483*	0.686*
LSR			1	0.181 ns	-0.024 ns	0.265 ns
RDM				1	0.660*	-0.176 ns
TDM					1	-0.145 ns
GH						1

ns = values no significant; *value significant (t test) at $p \leq 0.05$.

Significative correlations occurs between PH and GH ($r = 0.686$), PH and TN ($r = 0.330$), PH and RDM ($r = 0.334$), PH and TDM ($r = 0.483$), TDM and RDM ($r = 0.660$), TDM and TN ($r = 0.546$), RDM and TN ($r = 0.348$), with varying magnitudes. The dates suggest that selection for Plant Height, Tiller Number and Root Dry Mass could lead to an increase of the Total Dry Mass (TDM).

The variable TN represents the capacity a plant has in maintaining dense coverage over the soil, decreasing the incidence of invaders plants (Motta *et al.*, 2016) and the RDM indicating reserve substances stored in the rhizomes. The discrimination of hybrids genotypes based on these correlations can be useful to direct plants with greater dry mass of roots and prostrate growth habit. Moreover, the high correlation of the variable TDM with TN and RDM demonstrate that the hybrids genotypes can be used as forage and also to reduce the effects of soil erosion in degraded areas.

The variables TDM and LSR were not correlated with each other, indicating that Leaf: Stem ratio alone does not explain the amount of total dry mass produced. This negative correlation was unexpected. These data suggested that when selecting better genotypes according to TDM production, genotypes with a high proportion of stems will also be selected.



The significant correlations coefficients of agronomic traits with total dry mass might be useful for indirect selective criteria in bahiagrass improving strategies (Motta *et al.*, 2016). Total dry matter is a trait of easier selection and measurement and indirectly select leaf production, identifying superior genotypes with agility and economy in the selection of forage species (Pereira *et al.*, 2017).

Phenotypic correlation coefficients of traits associated with forage production in genotypes from *Paspalum* hybrids from an interspecific cross (*Paspalum plicatulum* x *P. guenoarum*) lead to Motta *et al.* (2016) revealed significative correlations of the total dry matter with leaf dry matter ($r = 0.98$), number of tillers ($r = 0.82$), plant coverage diameter ($r = 0.80$), plant height ($r = 0.30$) and stem dry mass ($r = 0.95$). Pereira *et al.* (2017) observed traits that simultaneously showed high correlation and heritability in apomictic species of the genus *Paspalum* as total dry mass and leaf dry mass ($r = 0.95$).

High correlation of the total dry mass with leaf dry mass ($r = 0.93$), number of tillers ($r = 0.78$), survival and regrowth following the winter ($r = 0.49$) was observed by Weiler *et al.* (2018) in intraspecific tetraploid hybrids of *Paspalum notatum*. Barbosa *et al.* (2019) evaluated the 28 most productive plants in terms of herbage accumulation and production of leaves selected by Weiler *et al.* (2018) and observed positive correlations between the components of forage production. Total dry mass displayed correlation with leaf dry mass ($r = 0.84$), stem dry mass ($r = 0.79$), inflorescence dry mass ($r = 0.76$), plant height (0.60).

Barros *et al.* (2021) evaluate the correlations between ecotypes of *Andropogon lateralis*, native forage specie of Rio Grande do Sul state at two defoliation frequencies and three cutting heights. Total dry mass showed significant correlation with leaf dry matter ($r = 0.77$), vegetative tillers ($r = 0.40$), total tillers ($r = 0.53$), stem dry matter ($r = 0.85$) and reproductive tillers ($r = 0.45$). When selecting one of these resources, the other is also indirectly being favored.

Our findings and those presented by the aforementioned authors suggest a decrease in labor and evaluation times is possible when correlation studies are applied to a large number of genotypes within the breeding program.

The potential use of the plants 17PN16P3 and 17PN28P4 with higher forage yield, prostrate growth habit and great production of roots dry mass may serve to recover degraded natural pastures, if they are genotypes adapted to low fertility environments, and as a forage option during the favorable growth season for the species.

Mode of reproduction

The selected plants were classified as sexual or facultative apomictic. Based on embryo sac observations, three selected hybrid plants (17PN10P5, 17PN16P3 and 17PN28P4) were classified as facultative apomictic because their ovules contained unreduced embryo sacs and reduced embryo sacs and two hybrid plants were classified as sexual (17PN10P3 and 17PN29P1) because their ovules had only reduced embryo sacs (Table 4; Figure 1). Abnormal, atrophied and sterile ovaries was found in almost all plants (Table 4).

The reproduction mode in hybrids varied sexual to apomictic because the progenies segregate for mode





of reproduction. The apomixis is a tetrasomic monogenic inheritance conditioned by a single dominant Mendelian factor and the sexuality is a recessive character (MARTÍNEZ *et al.*, 2001). This genetic model for the inheritance of apomixis in grasses is the most widely accepted (ACUÑA *et al.*, 2011; MARTÍNEZ *et al.*, 2001; OZIAS-AKINS; VAN DIJK, 2007; STEIN *et al.*, 2004). Progenies segregating for the mode of reproduction in hybrid of the *Paspalum* genus are widely reported (MARTINEZ *et al.*, 2001; FORTES *et al.*, 2004; STEIN *et al.*, 2004; ACUÑA *et al.*, 2009; 2011; AGUILERA *et al.*, 2011; 2015; ZILLI *et al.*, 2015; NOVO *et al.*, 2016; WEILER *et al.*, 2017; KRYCKI *et al.*, 2020; MACHADO *et al.*, 2021).

Table 4. Classification for reproduction mode in selected hybrids of bahiagrass based on embryo sacs observations.

ID	SES	AES	AbES	T	MR
17PN10 – P3	19	-	13	32	*sex
17PN10 – P5	16	18	01	35	**fac apo
17PN16 – P3	08	18	06	32	fac apo
17PN28 – P4	11	12	07	30	fac apo
17PN29 – P1	19	-	17	36	sex

ID (identification); SES: sexual embryo sacs; AES: apomictic embryo sacs; AbES: aborted or immature embryo sacs; T (total number of ovaries analyzed); *sex=sexual; **fac apo = facultative apomictic.



Figure 1. Morphology of embryo sacs of bahiagrass tetraploids hybrids. (a) Sexual ovary with antipodes (arrow) and egg cell. (b) Aposporous ovary with multiple sacs (arrows). Scale: 10 µm.



The hybridization strategy between sexual and apomictic genotypes results in widening genetic variability allowing the selection of new genotypes. The determination of the mode of reproduction in segregating progenies obtained by crosses is essential. Based by the reproductive mode, the plants are separated in two groups: superior apomictic are identified and directly multiplied for testing, selection, and commercial release as cultivars, maintaining superior genotypes as clones of seeds, while sexual hybrids may be used in further crosses in the breeding programs (MILES, 2007; SIMIONI; VALLE, 2009; KANDEMIR; SAYGILI, 2015; WEILER *et al.*, 2017).

The results presented here identified variability for agronomic traits in the hybrids studied, creates a substantial opportunity to select news genotypes with superior agronomic performance to be indicated for new steps within the breeding program.

Using TDM as criterion for genotype selection and based on significant and positive correlation from TDM with PH, RDM and TN, the following hybrid genotypes was selected: the sexual 17PN10P3 and 17PN29P1 that can be used to obtain new elite recombinants in crosses schemes, and the apomictic 17PN10P5, 17PN16P3 and 17PN28P4 that will be able to participate in new stages for a future process of releasing cultivars for use as a forage plant.

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Conflict of interest

The authors declare that the research was conducted in the absence of any potential conflicts of interest.

Ethical statements

The authors confirm that the ethical guidelines adopted by the journal were followed by this work, and all authors agree with the submission, content and transfer of the publication rights of the article to the journal. They also declare that the work has not been previously published nor is it being considered for publication in another journal.

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References

ACUÑA, C. A. *et al.* Reproductive characterization of bahiagrass germplasm. **Crop Science**, v. 47, n. 4, p. 1711-1717, 2007. DOI: <https://doi.org/10.2135/cropsci2006.08.0544>.

ACUÑA, C. A. *et al.* Bahiagrass tetraploid germplasm: reproductive and agronomic characterization of segregating progeny. **Crop Science**, v. 49, n. 2, p. 581-588, 2009. DOI: <https://doi.org/10.2135/cropsci2008.07.0402>.

ACUÑA, C. A. *et al.* Tetraploid bahiagrass hybrids: breeding technique, genetic variability and proportion of heterotic hybrids. **Euphytica**, v. 179, n. 2, p. 227-235, 2011. DOI: <https://doi.org/10.1007/s10681-010-0276-y>.

AGUILERA, P. M. *et al.* Interspecific tetraploid hybrids between two forage grass species. Sexual *Paspalum plicatum* and apomictic *P. guenoarum*. **Crop Science**, v. 51, n. 4, p. 1544-1550, 2011. DOI: <https://doi.org/10.2135/cropsci2010.10.0610>.

AGUILERA, P. M. *et al.* Inheritance of aposporous apomixis in interspecific hybrids derived from sexual *Paspalum plicatum* and apomictic *Paspalum guenoarum*. **Crop Science**, v. 55, n. 5, p. 1-10, 2015. DOI: <https://doi.org/10.2135/cropsci2014.11.0770>.

BARBOSA, M. R. *et al.* Herbage accumulation of bahiagrass hybrids in two different environments in southern Brazil. **Pesquisa Agropecuária Gaúcha**, v. 25, n. 1/2, p. 58-69, 2019. DOI: <https://doi.org/10.36812/pag.2019251/258-69>.

BARROS, T. *et al.* Dissimilarity between *Andropogon lateralis* ecotypes under different defoliation frequencies and heights. **Ciência Rural**, v. 53, n. 3, 2022. DOI: <https://doi.org/10.1590/0103-8478cr20201079>.





BRUGNOLI, E. A. *et al.* Diversity in diploid, tetraploid, and mixed diploid–tetraploid populations of *Paspalum simplex*. **Crop Science**, v. 53, n. 4, p. 1509-1516, 2013. DOI: <https://doi.org/10.2135/cropsci2012.08.0497>.

BURTON, G. W. Artificial fog chamber facilitates *Paspalum* emasculation. **Journal American Society Agronomy**, v. 40, p. 281-282, 1948.

CRUZ, C. D. Genes Software – extended and integrated with the R, Matlab and Selegen. **Acta Scientiarum Agronomy**, v. 38, n. 4, p. 547-552, 2016. DOI: <https://doi.org/10.4025/actasciagron.v38i4.32629>.

DAURELIO, L. D. *et al.* Genetic diversity in sexual diploid and apomictic tetraploid populations of *Paspalum notatum* situated in sympatry or allopatry. **Plant Systematics and Evolution**, v. 244, p. 189–199, 2004. DOI: <https://doi.org/10.1007/s00606-003-0070-6>.

FORTES, N. B. *et al.* Segregación de la apomixis en *Paspalum notatum* a partir de cruzamientos entre una planta sexual autotetraploide inducida y un padre apomítico. In: REUNIÓN DE COMUNICACIONES CIENTÍFICAS Y TECNOLÓGICAS, 2004, Corrientes, Argentina. **Resúmenes [...]**. Corrientes: Universidad Nacional del Nordeste, 2004.

KANDEMİR, N.; SAYGILI, I. Apomixis: new horizons in plant breeding. **Turkish Journal of Agriculture and Forestry**, v. 39, p. 549-556, 2015. DOI: <https://doi.org/10.3906/tar-1409-74>.

KRYCKI, K. C.; SIMIONI, C.; DALL'AGNOL, M. Cytoembryological evaluation, meiotic behavior and pollen viability of *Paspalum notatum* polyploidized plants. **Crop Breeding and Applied Biotechnology**, v. 16, n.4, p. 282-288, 2016. DOI: <http://dx.doi.org/10.1590/1984-70332016v16n4a43>.

KRYCKI, K.C.K. *et al.* Reproductive analyses of intraspecific *Paspalum notatum* Flüggé hybrids. **Crop Breeding and Applied Biotechnology**, v. 20, n.1, 2020. DOI: <https://doi.org/10.1590/1984-70332020v20n1a14>.

KUMAR, S.; SAXENA, S.; GUPTA, M. C. Marker-assisted screening of breeding populations of an apomictic grass *Cenchrus ciliaris* L. segregating for the mode of reproduction. **Crop Breeding and Applied Biotechnology**, v. 17, p. 10-17, 2017. DOI: <https://doi.org/10.1590/1984-70332017v17n1a2>.

MACHADO, J. M. *et al.* Reproduction mode and apospory expressivity of selected hybrids of *Paspalum notatum* Flüggé. **Journal of Plant Breeding and Crop Science**, v. 13, n. 2, p. 58-63, 2021. DOI: <https://doi.org/10.5897/JPBCS2021.0948>.





MACHADO, J. M. *et al.* Agronomic evaluation of *Paspalum notatum* Flüggé under the influence of photoperiod. **Revista Brasileira de Zootecnia**, v. 46, n. 1, p. 8-12, 2017. <http://dx.doi.org/10.1590/S1806-92902017000100002>.

MARCÓN, F. *et al.* Genetic distance and the relationship with heterosis and reproductive behavior in tetraploid bahiagrass hybrids. **Molecular Breeding**, v. 39, n. 6, [art.] 89, p. 1-13, 2019. DOI: <https://doi.org/10.1007/s11032-019-0994-3>.

MARTINEZ, E. J. *et al.* Inheritance of apospory in bahiagrass, *Paspalum notatum*. **Hereditas**, v. 135, n. 1, p. 19-25, 2001. DOI: <https://doi.org/10.1111/j.1601-5223.2001.00019.x>.

MILES, J. W. Apomixis for cultivar development in tropical forage grasses. **Crop Science**, v. 47, n. 53, 2007. DOI: <https://doi.org/10.2135/cropsci2007.04.0016IPBS>.

MOTTA, E.A.M. *et al.* Forage performance of *Paspalum* hybrids from an interspecific cross. **Ciência Rural**, v. 46, n. 6, p. 1025–103, 2016. DOI: <http://dx.doi.org/10.1590/0103-8478cr20150232>.

MOTTA, E.A.M. *et al.* Agronomic performance of interspecific *Paspalum* hybrids under nitrogen fertilization or mixed with legumes. **Agrosystems, Geosciences & Environment**, v. 3, n. 1, 2020a. DOI: <https://doi.org/10.1002/agg2.20127>.

MOTTA, E.A.M. *et al.* Nutritive value and herbage mass in hybrids of *Paspalum plicatulum* × *Paspalum guenoarum* fertilized with nitrogen or in mixture with temperate legumes. **Grassland Science**, v. 66, n. 4, p. 261–270, 2020b. DOI: <https://doi.org/10.1111/grs.12280>.

NOVO, P. E. *et al.* Interspecific hybrids between *Paspalum plicatulum* and *P. oteroi*: a key tool for forage breeding. **Scientia Agricola**, v. 73, n. 4, p. 356-362, 2016. DOI: <http://dx.doi.org/10.1590/0103-9016-2015-0218>.

NOVO, P. E. *et al.* Hybridization and heterosis in the Plicatula group of *Paspalum*. **Euphytica**, v. 213, [art.] 198, 2017. DOI: <https://doi.org/10.1007/s10681-017-1983-4>.

ORTIZ, J. P. A. *et al.* Harnessing apomictic reproduction in grasses: what we have learned from *Paspalum*. **Annals of Botany**, v. 112, n. 5, p. 767-787, 2013. DOI: <https://doi.org/10.1093/aob/mct152>.

OZIAS-AKINS, P.; VAN DIJK, P. J. Mendelian genetics of apomixis in plants. **Annual Review Genetics**, v. 41, p. 509-537, 2007. DOI: <https://doi.org/10.1146/annurev.genet.40.110405.090511>.





PEREIRA, E. A. *et al.* Produção agronômica de uma coleção de acessos de *Paspalum nicorae* Parodi. **Revista Brasileira de Zootecnia**, v. 40, n. 3, p. 498-508, 2011. DOI: <https://doi.org/10.1590/S1516-35982011000300006>.

PEREIRA, E.A. *et al.* Variabilidade genética de caracteres forrageiros em acessos do gênero *Paspalum* em diferentes ambientes. **Pesquisa Agropecuária Brasileira**, v. 47, n. 10, p. 1533-1540, 2012. DOI: <https://doi.org/10.1590/S0100-204X2012001000017>.

PEREIRA, E. A. *et al.* Genetic gain in apomictic species of the genus *Paspalum*. **Revista Ceres**, v. 64, p. 60-67, 2017. DOI: <https://doi.org/10.1590/0034-737X201764010009>.

PILLAR, V. P. *et al.* (ed.). **Campos sulinos: conservação e uso sustentável da biodiversidade**. Brasília: Ministério do Meio Ambiente, 2009. 403 p.

POZZOBON, M. T.; VALLS, J. M. Chromosome number in germplasm accessions of *Paspalum notatum* (Gramineae). **Brazilian Journal Genetics**, v. 20, n. 1, p. 29-34, 1997. DOI: <https://doi.org/10.1590/S0100-84551997000100006>.

QUARIN, C. L. The nature of apomixis and its origin in Panicoid grasses. **Apomixis Newsletter**, v. 5, p. 7-15, 1992.

QUARIN, C. L. Registration of Q4188 and Q4205, sexual tetraploid germoplasm of bahiagrass. **Crop Science**, v. 43, n. 2, p. 745-746, 2003. DOI: <https://doi.org/10.2135/cropsci2003.7450>.

QUARIN, C. L. *et al.* A rise of ploidy level induces the expression of apomixis in *Paspalum notatum*. **Sexual Plant Reproduction**, v. 13, p. 243-249, 2001. DOI: <https://doi.org/10.1007/s004970100070>.

RODRIGUES, R. C. *et al.* Produção de massa seca, relação folha/colmo e alguns índices de crescimento do *Brachiaria brizantha* cv. Xaraés cultivado com a combinação de doses de nitrogênio e potássio. **Revista Brasileira de Zootecnia**, v. 37, n. 3, p. 394-400, 2008. DOI: <https://doi.org/10.1590/S1516-35982008000300003>.

SARAIVA, K. M. *et al.* Hybrids of *Paspalum plicatulum* × *P. guenoarum*: Selection for forage yield and cold tolerance in a subtropical environment. **Tropical Grasslands-Forrajes Tropicales**, v. 9, n. 1, p. 138-143, 2021. DOI: [https://doi.org/10.17138/tgft\(9\)138-143](https://doi.org/10.17138/tgft(9)138-143).





SAS. Statistical Analysis System. SAS STATISTICAL SOFTWARE. SAS/STAT User's guide: statistics. Cary: **SAS Institute Inc.**, 2004. 1 CD-ROM.

SIMIONI, C.; VALLE, C. B do. Chromosome duplication in *Brachiaria* (A. Rich.) Stapf allows intraspecific crosses. **Crop Breeding and Applied Biotechnology**, v. 9, p. 328-334, 2009.

SPILLANE, C.; CURTIS, M. D.; GROSSNIKLAUS, U. Apomixis technology development – virgin births in farmers' fields? **National Biotechnology**, v. 22, n. 6, p. 687–691, 2004. DOI: <https://doi.org/10.1038/nbt976>.

STEIN, J. *et al.* Tetraploid races of *Paspalum notatum* show polysomic inheritance and preferential chromosome pairing around the apospory -controlling locus. **Theoretical Applied Genetics**, v. 109, p. 186–191, 2004. DOI: <https://doi.org/10.1007/s00122-004-1614-z>.

VALLS, J. F. M. *et al.* *Ex situ* management of plant genetic resources. In: MARIANTE, A.S.; SAMPAIO, M.J.A.; INGLIS, M.C.V. (Ed.). **The state of Brazil's plant genetic resources: second national report: conservation and sustainable utilization for food and agriculture**. Brasília: Embrapa Technological Information, 2009a. p. 65-79.

VALLS, J. F. M. *et al.* Patrimônio florístico dos campos: potencialidades de uso e a conservação de seus recursos genéticos. In: PILLAR, V. P. *et al.* (Ed.). **Campos sulinos: conservação e uso sustentável da biodiversidade**. Brasília: Ministério do Meio Ambiente, 2009b. p. 139-154.

VENUTO, B. C. *et al.* Forage yield, nutritive value, and grazing tolerance of Dallisgrass Biotipes. **Crop Science**, v. 43, n. 1, p. 295-301, 2003. DOI: <https://doi.org/10.2135/cropsci2003.2950>

WEILER, R. L. *et al.* Chromosome doubling in *Paspalum notatum* var. *saure* (cultivar Pensacola). **Crop Breeding and Applied Biotechnology**, v. 15, n. 2, p. 106-111, 2015. DOI: <http://dx.doi.org/10.1590/1984-70332015v15n2n19>.

WEILER, R. L. *et al.* Determination of the mode of reproduction of bahiagrass hybrids using cytoembryological analysis and molecular markers. **Revista Brasileira de Zootecnia**, v. 46, n. 3, p. 185-191, 2017. DOI: <http://dx.doi.org/10.1590/S1806-92902017000300002>.

WEILER, R. L. *et al.* Intraspecific tetraploid hybrids of *Paspalum notatum*: agronomic characterization of segregating progeny. **Scientia Agricola**, v. 75, p. 36-42, 2018. DOI: <https://doi.org/10.1590/1678-992x-2016-0354>.





YOUNG, B. A.; SHERWOOD, R. T.; BASHAW, E. C. Cleared-pistil and thick-sectioning techniques for detecting aposporous apomixis in grasses. **Canadian Journal of Botany**, v. 57, p. 1668-1672, 1979.

ZILLI, A. L. *et al.* Heterosis and expressivity of apospory in tetraploid Bahiagrass hybrids. **Crop Science**, v. 55, n. 3, p. 1189–1201, 2015. DOI: <https://doi.org/10.2135/cropsci2014.10.0685>.

