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Forage yield of tetraploid bahiagrass hybrids

Ilson Ghellar Júnior1, Julio Antoniolli1, Karine Cristina Krycki1, Mariana de Oliveira Lima1, Roberto Luis Weiler1, André Pich Brunel1, Carine Simioni1*, Miguel Dall’Agnol1

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 caraterísticas agronômicas, 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 progêñes 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 agronômicas 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.

1 Departamento de Plantas Forrageiras e Agrometeorologia, Universidade Federal do Rio Grande do Sul. *Corresponding Author: carine.simioni@ufrgs.br.
Introduction

The Brazilian livestock in 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* Flügge, 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 their 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 \([40 \text{ ml } 95\% \text{ ethanol}: 14 \text{ ml distilled water: } 3 \text{ ml } 40\% \text{ formalin: } 3 \text{ ml glacial acetic acid}]\) 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 \(P. \text{notatum}\), indicated heterosis for interest agronomic traits. Great variability between genotypes for forage yield was registered in \(P. \text{notatum}\), \(P. \text{dilatatum}\) (VENUTO et al., 2003), \(P. \text{lepton}\) (syn. \(P. \text{nicorae}\)), \(P. \text{guenooarum}\) (PEREIRA et al., 2012), \(P. \text{simplex}\) populations (BRUGNOLI et al., 2013) and in interspecific hybrid progenies obtained by crossbreeding with species of the \(Plicatula\) group of
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           |
| 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                        | -                |
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 prostate 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, 40.32 g 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. leptot* (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

|       |       |    |    |        |
|-------|-------|----|----|---------|
| 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.
offers new genes to the service of plant breeding (SPIELLANE; 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 c              | 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         |
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 | 0.145 ns |
| TDM  | 1   | 0.145 ns | -0.176 ns | 1 |
| GH   | 1   | 0.145 ns | -0.176 ns | 1 |

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

Significant 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 prostate 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 (MARTÍNEZ 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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ORCID

Ilson Ghellar Júnior https://orcid.org/0000-0001-9751-6043
Julio Antonioli https://orcid.org/0000-0002-2918-3790
Karine Cristina Krycki https://orcid.org/0000-0003-0053-4486
Mariana de Oliveira Lima https://orcid.org/0000-0002-0123-0989
Roberto Luis Weiler https://orcid.org/0000-0002-1063-6374
André Pich Brunes https://orcid.org/0000-0002-2430-8232
Carine Simioni https://orcid.org/0000-0001-5471-1850
Miguel Dall’Agnol https://orcid.org/0000-0001-5471-1850

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