



Seed yield and quality of *Paspalum notatum* Flüge intraspecific hybrids

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ABSTRACT. Bahiagrass (*Paspalum notatum* Flüge) is an important forage in South America and the United States because of its high palatability, resistance to trampling and grazing, and tolerance to cold, but it exhibits low seed yield and poor seed quality. Previous studies reported improvements in forage production and nutritive value in hybrids and ecotypes; however, information about seed yield and quality in forage bahiagrass is limited. This study aimed to characterize the seed yield and quality of nine *P. notatum* intraspecific hybrids and three controls: *P. notatum* ecotypes V4 and Bagual and cultivar Pensacola. Inflorescence density, 1,000-seed weight, seed yield and germination rate decreased in year 2 influenced by weather conditions and ergot. Seed yield ranged from 139 (Pensacola) to 1,158 (Bagual) kg ha⁻¹ among all entries, where Bagual, C18, and V4 produced more than 974 kg ha⁻¹, which was approximately seven times more than Pensacola. Bagual, C18, V4, 336, C15, 225, and D3 showed germination rates greater than 83%. In conclusion, Bagual had the highest seed yield and germination rate, whereas hybrids C18 and 336 showed high seed yield, 1,000-seed weight, and germination rate in both years. Bagual, C18, and 336 should be used in future breeding programs to improve seed production traits. Our study revealed that selection for seed production traits can result in improvements in seed yield and quality in bahiagrass.

Keywords: bahiagrass; hybridization; native species; plant breeding.

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Introduction

Bahiagrass is a warm-season perennial grass with high palatability to cattle, high resistance to trampling and grazing, and great diversity for tolerance to cold (Pozzobon & Valls, 1997; Gates, Quarin, & Pedreira, 2004).

Intraspecific tetraploid bahiagrass breeding has been explored in Brazil, Argentina, and the United States. To date, intraspecific breeding efforts to improve forage traits have resulted in the selection of improved hybrids (Barbosa et al., 2019; Graminho et al., 2019) and the release of the cultivar Boyero (Urbani et al., 2017). The bahiagrass breeding program of the Federal University of Rio Grande do Sul (UFRGS) has explored tetraploid bahiagrass germplasm and reported improvements in forage production and nutritive value in native ecotypes and intraspecific hybrids (Fachinetto, Schneider, Hubber, & Dall'Agnol, 2012; Steiner et al., 2017; Machado et al., 2017; Machado, Krycki, Weiler, Simioni, & Dall'Agnol, 2021; Weiler et al., 2018; Barbosa et al., 2019; Graminho et al., 2019). Introduced species such as *Urochloa* spp. and *Megathyrsus* spp. have been used because of the availability of commercial cultivars on the market (MAPA, 2021). Both of these are less adapted to the local environmental conditions of the Pampas biome (Nabinger, Moraes, & Maraschin, 2000; Rolim, Ferreira, Schneider, & Overbeck, 2015; Andrade et al., 2019). Indeed, bahiagrass genotypes have shown great forage production and distribution and tolerance to cold and frost and are better options for use in southern Brazil (Steiner et al., 2017; Machado et al., 2017; Weiler et al., 2018; Barbosa et al., 2019; Machado et al., 2019; Graminho et al., 2019). Bahiagrass, however, has been used with relative success in Brazil, and Pensacola is the only cultivar registered and available in the country. This single cultivar had an annual market of 177 tons of seeds in 2018/2019, and the seed yield was reported to be approximately 288 kg ha⁻¹ (MAPA, 2021).

The hybrids evaluated in this study are the result of one cycle of crossing and selection in two different populations (Weiler, Dall'Agnol, Simioni, Krycki, Dahmer, & Guerra, 2017; Weiler et al., 2018; Machado et al., 2021). A16, C15, C18, and D3 originated from crossing André da Rocha and Bagual (Steiner et al., 2017) as pollen donors and Q4188, Q4205 (Quarin et al., 2003), and C4-4X (Quarin, Espinoza, Martínez, Pessino, & Bovo, 2001) were used as female genitors (Weiler et al., 2018). The reproductive mode of these hybrids was determined (Weiler et al., 2017) and they were selected for high dry matter yield and persistence and tolerance to cold based on individual plants, and their agronomic value was later demonstrated through assessments in two locations (Barbosa et al., 2019). Other sources of hybrids 225, 336, 437, 712, and 10036 are derived from crosses of the apomictic accessions 36N, 83N, V4, and 95N used as male parents with the same female parents used by Weiler et al. (2018). These hybrids were selected for high dry matter yield and their reproductive mode was characterized by Machado et al. (2021).

Improving seed production in native warm-season perennial grass is a challenge because these are not domesticated plants, and seed production is extremely important because it is the last step to cultivar release (Rios et al., 2020). In addition, small differences in productive potential and seed quality represent large gains in the economic viability of these materials, and their evaluation is a critical step to consider in the release of cultivars. This study aimed to characterize the seed production and quality of tetraploid bahiagrass hybrids and compared them to Pensacola and tetraploid ecotypes native to Brazil.

Material and methods

Germplasm

The evaluated germplasm included nine *P. notatum* intraspecific hybrids and three controls, namely, the *P. notatum* ecotypes 'V4' and 'Bagual' and the cultivar Pensacola (Table 1).

Site description

This study was carried out at the Agronomic Experimental Station of UFRGS, Rio Grande do Sul State (RS), Brazil (30°05' S, 51°40' W, 46 m a.s.l.). The soil at the experimental site is a dystrophic Ultisol. The climate in the region is subtropical humid Cfa according to the Köppen classification (Kuinchtner & Buriol, 2001). Irrigation was supplied until soil saturation. Therefore, the experiment consisted of a group of twelve *P. notatum* Flügge genotypes (Table 1) arranged in a randomized complete block design (RCBD), with three replications evaluated for two years.

Table 1. List of genotypes included in the agronomic evaluation for seed yield and quality and origin, genealogies and reproductive mode.

Genotype	Source and determination of reproductive mode	Origin	Reproductive mode
A16	Weiler et al. (2017, 2018)	Q4188 x André da Rocha	Facultative apomictic
C15	Weiler et al. (2017, 2018)	Q4205 x André da Rocha	Apomictic
C18	Weiler et al. (2017, 2018)	Q4205 x André da Rocha	Sexual
D3	Weiler et al. (2017, 2018)	Q4205 x Bagual	Apomictic
225	Machado et al. (2021)	Q4188 x 83N	Apomictic
336	Machado et al. (2021)	Q4205 x 95N	Sexual
437	Machado et al. (2021)	Q4205 x V4	Sexual
712	Machado et al. (2021)	C4-4X x 36N	Facultative apomictic
10036	Machado et al. (2021)	Q4205 x 95N	Facultative apomictic
Bagual	Planalto region, RS (Brazil)	Native	Apomictic
V4	Barra do Quaraí, RS (Brazil)	Native	Apomictic
Pensacola	Diploid Commercial cultivar	United States	Sexual

Crop management and data collection

Soil samples were taken at depths of 0 to 20 cm and analyzed in the Soil Analysis Laboratory of UFRGS (Table 2). The pH in the experimental area was corrected to 5.5 using 1,600 kg ha⁻¹ of filler limestone and was fertilized with 180 kg ha⁻¹ of P₂O₅ (triple superphosphate) and 200 kg ha⁻¹ of K₂O (KCl). To keep the pH at 5.5, 3,734 kg ha⁻¹ and 8,800 kg ha⁻¹ of dolomite limestone was applied in years 1 and 2, respectively. Phosphorus and potassium were also applied to compensate for nutrient extraction due to the forage harvests throughout the year: 180 kg ha⁻¹ of P₂O₅ in each year (triple superphosphate) and 210 and 150 kg ha⁻¹ of K₂O (muriate of potash) in years 1 and 2.

Table 2. Chemical characteristics of the soil before and after starting the experiment.

Year	Clay	pH H ₂ O	OM	P	K	H+Al	Al	Ca	Mg	CEC	Base sat.	Al sat.
	g kg ⁻¹		g kg ⁻¹	mg dm ⁻³				cmol _c dm ⁻³			%	
*	180	4.9	15.0	5.5	58	2.5	0.6	1.0	0.1	5.7	27	18.2
1	170	4.6	14.0	7.9	69	6.2	0.9	1.0	0.4	7.7	20	36.3
2	210	3.9	12.0	13.0	114	13.7	1.0	0.7	0.4	15.0	8	44.2

*Soil analysis before starting the experiment (2015); ¹Soil analysis during the first year (2016/17); ²Soil analysis during the second year (2017/18) of the experiment.

The experiment was planted in November 2015 using rooted tillers from adult plants collected in field nurseries and transplanted into pure stands in ten rows (10 cuttings row⁻¹) with a plant-to-plant distance of 25 cm and a row-to-row distance of 30 cm (6 m² plot size), with three replicates. The experiment was managed from October 2016 to May 2017 (year 1) and from September 2017 to May 2018 (year 2). Plots were mowed at 10 cm stubble height during the growing season once the canopy reached 25 cm (forage yield data not shown). Mowing was performed on October 31st and November 24th, 2016 in year 1 and on September 26th, October 31st, and November 30th, 2017 in year 2. After each mowing, nitrogen was supplied at 40 kg ha⁻¹ of N (ammonium sulfate). Other N fertilization was carried out at the beginning of internode elongation on December 15th, 2016 (Year 1) and December 21st, 2017 (Year 2), totaling 200 kg ha⁻¹ of N, which is recommended for obtaining high yield (SBCS, 2016). After seed harvest, all plots were kept mowed under the same mowing management until dormancy of the plants in winter (forage yield data not shown).

Response variables were measured in two samples obtained with a fixed 0.25 m² quadrat (0.5 x 0.5 cm) placed randomly within each experimental unit. All the inflorescences within the quadrat were harvested at 10 cm above the ground when 50% of inflorescences had straw coloring and signs of seed shattering. All entries were harvested only once per year. In year 1, entries were harvested on February 8th, 2017, and in year 2, entries were harvested on February 8th, 2018, except entry 336, which was harvested on February 19th, 2018 and entry A16, which was harvested on March 21st, 2018. From the samples, the inflorescence density per area (ID), number of racemes per inflorescence (NRI), and number of seeds per raceme (NSR) were calculated.

Seed head samples were dried in a forced-air oven at 30°C for 72h and then manually threshed. The total weight of the threshed florets (filled + empty) was recorded as seed yield (SY, kg ha⁻¹). Empty florets and other inert material were removed by blowing the seeds in a forced-air seed blower (South Dakota Seed Blower), obtaining the pure seed yield (PSY, kg ha⁻¹) and the percentage of seed set (SS). In this fraction, the 1,000-seed weight (TSW) was calculated from the average weight of eight subsamples of 100 filled florets multiplied by 10 (Brasil, 2009).

For germination (Germ) tests, four replications of 100 seeds per sample were placed onto 0.2% KNO₃ solution-moistened filter paper in a plastic germination box (Brasil, 2009). Plates were kept moist for 28 days under controlled conditions in a growth chamber: 16h of light at 30°C and 8h of darkness at 20°C. The results were expressed as a percentage of normal seedlings obtained on day 28.

The viable seed rate (VSR) was estimated through the tetrazolium test, carried out with four replications of 50 seeds per plot. Seeds were first presoaked in water for 16h at 25°C, then conditioned at 30°C and exposed to 1% tetrazolium 2,3,5-triphenyl chloride solution for 24h (Brasil, 2009) and then analyzed.

The incidence of *Claviceps paspali* (ergot) was scored based on symptom levels and visually rated using a 1 to 5 scale: 1 = no visible honeydew or sclerotia; 2 = < 10%; 3 = 11–50%; 4 = 51–80%; and 5 = > 80% of the seed head completely covered with honeydew and/or sclerotia (Rios, Blount, Mackowiak, Kenworthy, & Quesenberry, 2015).

Data analysis

All variables were analyzed using linear models in R (R Development Core Team, 2018) to fit ANOVA using the package *agricolae* (Mendiburu, 2019). The model considered blocks, genotypes and year as fixed effects, and residuals as random effects. The Scott–Knott test was used to separate treatments means at $p \leq 0.05$ using the package *agricolae* (Mendiburu, 2019). Additionally, correlations between traits were determined using the package *stats* (R Development Core Team, 2018) and plotted using the package *ggcorrplot* (Kassambara, 2019).

Results

Yield components

The genotype by year interaction was significant at $p \leq 0.001$ for inflorescence density (ID). ID varied among genotypes in the two years, but V4, 336, and 225 presented higher ID in both years. C15, Bagual, 712,

C18, and 10036 presented high ID in year 1 but it decreased in year 2. D3 and Pensacola had lower ID in both years, and A16 had higher ID than Pensacola and D3 in year 1 but did not differ from them in year 2.

Only the main effect of genotype was statistically significant at $p \leq 0.001$ for the number of racemes per inflorescence (NRI). The genotypes V4, 437, 10036, and Pensacola exhibited a higher NRI due to a higher incidence of inflorescences with three or four racemes; although significant, this result has a low biological meaning (Table 3). For the number of seeds per raceme (NSR), only the main effect of genotype was statistically significant at $p \leq 0.001$. The NSR ranged from 12 to 29 seeds per raceme. Bagual, C18, A16, D3, 336 had the highest NSR and Pensacola had the lowest NSR (Table 3).

The genotype by year interaction was statistically significant at $p \leq 0.001$ for 1,000-seed weight (TSW). TSW ranged between 1.67 and 4.03 g in year 1 and 1.77 to 3.5 in year 2 (Table 3). C18 and 437 had the highest TSW in both years, whereas Pensacola had the lowest TSW. Bagual and V4 had lower TSW than C18 and C437 in year 1 but were similar to them in year 2, as well as to 336, 10036, and 712.

Table 3. Means of inflorescence density (ID), racemes per inflorescence (NRI), seeds per raceme (NSR), thousand seed weight (TSW), seed yield (SY), and pure seed yield (PSY) for nine hybrids of *Paspalum notatum* and three controls: Pensacola, V4, and Bagual, evaluated in Eldorado do Sul, RRio Grande do Sul State, Brazil during 2017 and 2018.

Genotype	ID		NRI	NSR	TSW		SY		PSY	
	Year 1	Year 2			Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
	Inflorescences m ⁻²				g		kg ha ⁻¹			
Bagual	349 a	214 b	2.00 b	29 a	3.63 b	3.21 a	1158 a	562 a	832 a	356 a
C18	281 a	108 b	2.01 b	27 a	4.03 a	3.50 a	975 a	313 b	609 b	205 b
V4	345 a	272 a	2.04 a	19 b	3.79 b	3.34 a	1029 a	583 a	549 b	328 a
336	284 a	328 a	2.01 b	24 a	3.37 c	3.17 a	682 b	510 a	546 b	401 a
C15	356 a	169 b	2.00 b	18 b	3.08 d	2.78 b	820 b	236 b	490 b	143 b
437	235 b	269 a	2.05 a	22 b	4.02 a	3.32 a	744 b	652 a	453 b	361 a
A16	225 b	223 b	2.02 b	27 a	3.05 d	2.81 b	863 b	652 a	444 b	271 a
10036	265 a	207 b	2.05 a	20 b	3.43 c	3.33 a	794 b	491 a	431 b	215 b
225	299 a	272 a	2.01 b	22 a	3.04 d	2.93 b	702 b	558 a	425 b	348 a
712	293 a	171 b	2.02 b	18 b	3.19 d	3.22 a	613 b	355 b	379 b	183 b
D3	106 c	210 b	2.01 b	25 a	3.41 c	3.01 b	339 c	480 a	205 c	250 b
Pensacola	160 c	196 b	2.06 a	12 c	1.67 e	1.77 c	139 c	173 b	64 c	103 b

*Means followed by same letters are grouped by the Scott–Knott test ($P < 0.05$) within a given column.

The genotype by year interaction was statistically significant at $p \leq 0.001$ for seed yield (SY). In year 1, SY ranged from 139 to 1,158 kg ha⁻¹, where Bagual, C18, and V4 had the highest yield, which was approximately seven times higher than that of Pensacola and D3 (Table 3). In year 2, SY ranged from 173 to 652 kg ha⁻¹, and there was less variation among genotypes. Bagual, V4, 336, 437, A16, 10036, 225, and D3 had higher SY than C18, C15, 712, and Pensacola. The Bagual and V4 genotypes stood out in the superior SY groups in both years, while Pensacola showed the lowest SY in both years.

Pure seed yield (PSY) was significantly influenced by the genotype by year interaction at $p \leq 0.001$. The native ecotype Bagual had the highest PSY in year 1, while most hybrids and the native ecotype V4 had intermediate yield and were only superior to D3 and Pensacola. In year 2, hybrids 336, 437, A16, and 225 did not differ significantly from Bagual and V4, whereas the other genotypes had lower SY and did not differ from each other.

Only the genotype effect was significant for seed set (SS). Entry 336 exhibited the highest SS, followed by Bagual and C18. Genotypes 225, C15, V4, 437, and 712 presented medium SS but higher than A16, Pensacola, and 10036, which presented the lowest SS.

Seed viability

The genotype by year interaction was statistically significant at $p \leq 0.01$ for germination (Germ). Germ ranged from 15 to 59% in year 1, showing great variability among genotypes (Table 4). Hybrids 336 and C18 and native ecotype V4 presented the highest Germ in year 1. The other genotypes did not differ from each other. In year 2, Pensacola had the highest Germ, while 10036 and D3 had significantly higher Germ among the hybrids.

The viable seed rate (VSR) was significantly influenced only by the main effect of genotype at $p \leq 0.001$. Higher VSR was observed in Bagual, C18, V4, 336, C15, 225, and D3, for which the values ranged up to 83% (C18) (Table 4). The VSR of the other genotypes ranged between 45 and 58%, except for A16, which exhibited the lowest VSR (29%).

Table 4. Means of seed set (SS), germination (Germ), viable seed rate (VSR), weed infestation rate (Weed) and incidence of ergot (*Claviceps paspali*) for nine hybrids of *Paspalum notatum* and three controls: Pensacola, V4 and Bagual evaluated in Eldorado do Sul, Rio Grande do Sul State, Brazil during 2017 and 2018.

Genotype	SS	Germ		VSR	Weed		Ergot
		Year 1	Year 2		Year 1	Year 2	
Bagual	68 b	15 b	3 c	71 a	8 c	8 b	1.5 b
C18	64 b	44 a	5 c	83 a	8 c	5 b	0.8 b
V4	54 c	43 a	34 b	63 a	6 c	2 b	2.7 a
336	80 a	59 a	11 c	76 a	8 c	5 b	1.5 b
C15	60 c	27 b	16 c	72 a	13 c	16 b	1.0 b
437	58 c	30 b	15 c	45 b	25 b	6 b	3.7 a
A16	46 d	26 b	4 c	29 c	5 c	16 b	3.9 a
10036	50 d	26 b	28 b	57 b	17 b	12 b	3.0 a
225	61 c	17 b	17 c	70 a	17 b	8 b	1.3 b
712	56 c	18 b	14 c	58 b	23 b	9 b	2.8 a
D3	56 c	30 b	35 b	74 a	28 b	38 a	1.0 b
Pensacola	49 d	34 b	56 a	51 b	68 a	37 a	2.7 a

*Means followed by the same letters are grouped by the Scott-Knott test ($p < 0.05$) within a given column.

Weed infestation and ergot

The genotype by year interaction was statistically significant at $p \leq 0.001$ for weed infestation (Weed). Weed was higher in Pensacola in year 1 and year 2, except compared to that of D3. Other genotypes had a lower infestation that ranged from 8 to 28% in years 1 and 2 to 16% in year 2.

Ergot was not present in year 1. In year 2, there was an incidence of ergot, and genotypes A16, 437, 10036, 712, Pensacola, and V4 had infestation levels that ranged from 2.7 to 3.9, reaching up to approximately 50% of the seed head completely covered with honeydew and/or sclerotia (Table 4). Other genotypes had disease manifestation levels below 10%.

Correlation

Pearson correlations (data not shown) varied between traits across years; however, most traits presented similar trends in both years. Pure seed yield (PSY) had positive moderate to high correlations with ID, TSW, and SS but had negative correlations with NRI in year 1 and Weed in both years. In general, Weed was negatively correlated with all traits except NRI in year 1 and Germ in year 2. Thousand seed weight had a positive moderate correlation with NSR, SY, and SS in year 1 but only with NSR and SY in year 2. The viable seed rate was negatively correlated with ergot.

Discussion

Improving seed production in a native warm-season perennial grass such as bahiagrass is a challenge because similar to other tropical forages, this species presents non domesticated traits such as dehiscent seeds (Valle, Jank, & Resende, 2009). In this study, we evaluated seed production and seed quality in a group of tetraploid hybrids resulting from one cycle of crossing between native ecotypes from Rio Grande do Sul, Brazil, and artificially duplicated sexual diploids (Weiler et al., 2017; 2018; Machado et al., 2021). These tetraploid hybrids were compared with Pensacola, a sexual diploid cultivar, and V4 and Bagual, both native tetraploid and apomict ecotypes. These genotypes exhibited variability in seed production and seed quality traits, and the results from this study will serve as a basis for selecting breeding lines with improved seed yield for releases and for crosses in breeding programs.

In segregating progenies for the mode of reproduction, sexual reproduction hybrids are necessary for obtaining genetic variability (Saraiva et al., 2021) in new cycles of crosses, while facultative apomicts produce seeds through both sexual and apomictic means. The sexual reproduction percentage varies by environmental conditions (Kandemir & Saygili, 2015). Therefore, facultative apomictic hybrids can be used in breeding programs since the level of apomictic expression among hybrids can be evaluated. It is preferable to release highly apomictic genotypes as cultivars because they maintain their heterosis and desired agronomic traits through clonal reproduction via seeds and produce uniform progenies and stability in successive reproductive cycles (Acuña et al., 2019).

Most response variables related to seed yield components exhibited significantly better performance in the first production year because the environmental conditions were more favorable for plant development.

These results can be explained by the lower rainfall with high average temperatures during the months of December 2017 to April 2018 of the second year, covering the entire period from flower bud differentiation to seed filling. In addition to climatic conditions, sward age has been reported as a factor with a strong effect on *Paspalum* seed yield. Chadhokar and Humphreys (1973) reported that *Paspalum plicatum* Michx. produced a higher seed set during the first year of seed production. Adjei, Mislevy, and Chason (1992) and Gates and Burton (1998) found a year effect and interaction with other factors on the seed yield of diploid bahiagrass, and Rios et al. (2020) found that for bahiagrass, most of the response variables, except seed weight, exhibited significantly better performance during the first year of production. These contrasting results, observed by different authors and in this study, indicate that observed variations in seed yield in the first year of cultivation are more strongly influenced by climatic conditions than factors related to pasture age. Despite the strong effect of year, it was possible to determine the seed yield potential and seed quality of these hybrids and to select breeding lines with improved traits compared to Pensacola.

The most important component to predict seed yield is inflorescence density, which is determined by the number of reproductive units per area (Humphreys, 1981). ID was highly correlated with SY in both years, exhibiting its influence in determining SY. For instance, Bagual presented high ID and SY in both years, while C18 in year 2 had low ID but increased NSR and TSW, suggesting some compensation effect due to the reduction in ID. In contrast, C15 showed the highest ID in both years, but there was no evidence of compensation of other seed yield components for SY between years. In general, the second year resulted in a lower ID for all genotypes, except for 336, 437, D3, and Pensacola. A similar result was observed for the cultivar Argentine, which in year 2 showed a significant reduction in ID (Rios et al., 2020).

In most bahiagrass accessions, the inflorescence consists of two racemes, although three, four, and up to five racemes are occasionally observed (Fachinetto, Dall'Agnol, Souza, Weiler, & Simioni, 2017). NRI should not be a trait of great importance in bahiagrass breeding to predict seed production because differences among genotypes were very small and genotypes with the highest NRI were not those with the highest SY. The NRI was negatively correlated with SY (-0.44) and with PSY (-0.5) in year 1 and was not related to these variables in year 2. The number of seeds per raceme and 1,000-seed weight showed a significant and positive correlation with SY and PSY. These features were emphasized by Lopes et al. (2019) for presenting high heritability (H^2 above 0.80) in hybrids of *P. plicatum* Michx. x *P. guenoarum* Arech.

The 1,000-seed weight varied among the genotypes. Pensacola had the lowest value (1.67 to 1.77 g), approximately half the weight of the tetraploid genotypes, but higher than that reported by Adjei et al. (1992) of 1.02 g in 1,000 seeds. Gates et al. (2004) explained that this difference occurs because tetraploids have larger cells and morphological structures than diploids. Among the tetraploids, the lowest value was obtained for A16 and C15, while the highest was obtained for C18 and 437. In this study, weak correlations were observed between TSW and seed quality traits, unlike other studies that reported significant correlations between seed size and germination in diploid ($r = 0.91$; Adjei et al., 1992), tetraploid ($r = 0.44$; Zilli et al., 2019) and forage and turf-type bahiagrass ($r = 0.6$; Rios et al., 2020). However, a high negative correlation was estimated between TSW and weeds in year 1 (-0.71) and in year 2 (-0.58) because competition for light, water and nutrients has a strong negative effect on seed development. There was a strong year effect on TSW, which is also explained by the different environmental conditions (Table 3). In year 1, flower filling was favored, resulting in a higher TSW for almost all genotypes. In year 2, higher temperatures during flower filling resulted in lighter caryopses. A similar result was found for dallisgrass (*Paspalum dilatatum* Poir.), where low temperatures (21/16°C) resulted in the highest percentage of florets with caryopses and highest caryopsis weights (Pearson & Shah, 1981).

Bagual, V4 and some breeding lines had higher seed yield (SY) and pure seed yield (PSY) than Pensacola. In this study, Pensacola had seed yields of 139 and 173 kg ha⁻¹ in years 1 and 2, respectively. These values are lower than those reported in the literature, where the SY of Pensacola is approximately 550 kg ha⁻¹ (Gates & Burton, 1998). The low SY of Pensacola in this study should be related to differences in the crop management. However, the SY and PSY of the breeding lines were equivalent or superior to the seed yield reported for Pensacola, especially Bagual, which achieved SY values greater than 1,000 kg ha⁻¹ in year 1. It is important to note that Bagual, V4, and the breeding lines are native germplasm from the same region of this study and are therefore better adapted to the local ecological and climatic conditions than Pensacola.

Several studies have been carried out to establish management for seed production in bahiagrass, and defoliation and nitrogen fertilization management have achieved successful and satisfactory results (Adjei et al., 1992; Adjei, Mislevy, & Chason, 2000; Gates et al., 2004; Rios et al., 2020). For example, under

fertilization management, Pensacola, Tifton 9, and Recurrent Restricted Phenotypic Selection (RRPS) Cycle 18 produced similar results at the best treatment, with seed yields of approximately 500 kg ha⁻¹ (Gates & Burton, 1998). In this study, some genotypes exhibited an SY above 500 kg ha⁻¹ in both years as a result of the genetic merit of the genotypes and crop management. Although these are very high yields, further studies are necessary to verify this SY under farm conditions, but these results indicate a potentially economically viable seed production in these genotypes.

In the Brazilian forage seed market, bahiagrass cultivars compete with the most common species, such as *Urochloa* spp. and *Megathyrus* spp., which have a seed yield of approximately 350 kg ha⁻¹ (Pizarro, Hare, Mutimura, & Changjun, 2013; Andrade, Thomas, & Ferguson, 1983; Canto et al., 2016). Bagual, C18, 336, V4, and C15 produced between 700 and 1,100 kg ha⁻¹ and therefore would be an excellent option for native improved cultivars for farmers in the subtropical zone in Brazil.

Seed quality was studied by germination and tetrazolium tests. In the germination test, seeds were not scarified, and the low germination rates are the result of many seeds being in dormancy. There was a strong year effect on germination rate due to the different environmental conditions presented. In year 1, when temperatures were milder, the percentage of germinated seeds was greater for most genotypes, except for Pensacola. The hybrids C18 and 336 and the ecotype V4 were those with the highest germination in year 1. In year 2, all tetraploid genotypes showed low germination due to many dormant seeds, as confirmed by the tetrazolium test. Higher temperatures and lower rainfall may cause a large percentage of hard seeds. Pearson and Shah (1981) found 30% germination in dallisgrass seeds when produced at milder temperatures (21/16°C) and only 4.9% when produced at higher temperatures (30/25°C). In windmill grass (*Chloris cucullata* Bisch.) and *C. subdolichostachya* Muell., Herrera et al. (2008) found that dormancy was greater in seeds harvested in dry years (2003 and 2005) than in the rainy year (2004), in line with the results found in this study. The occurrence of weeds significantly interfered with the percentage of seed germination in year 2 (-0.6) since competition may have further reduced the water supply.

Seed dormancy may be one of the contributing causes of slow establishment in many warm-season grasses and is a recurrent feature in *Paspalum* species, and it can be divided into two main components: physical dormancy due to the seed coat and physiological embryo dormancy (Adkins, Bellairs, & Loch, 2002). The seed coat component could be because of the fact that external covering structures and/or caryopsis coats limit water uptake by the caryopsis (Simpson, 2007), and in some panicoid grasses such as bahiagrass, these structures are even more likely to affect germination because the coleorhiza must emerge through a rigid lemma by opening a hinged flap termed the germination lid (Haar, Van Aelst, & Dekker, 2014). In this case, the tetrazolium test is a better alternative to verify viability, as the spikelet section facilitates water uptake, and the observation takes place through the reaction of salt with living tissues that become red. Unlike germination, which was strongly influenced by the environment, particularly with respect to dormancy, the viable seed rate was not influenced by the year factor (Table 4). The Bagual, C18, V4, 336, C15, 225, and D3 genotypes showed the highest VSR, ranging from 63 to 83%, all above the rate of 40% required by Brazilian legislation for seed marketing (Brasil, 2008). Hybrid A16 had the worst result (29%), being the only one below the rate required by law for marketing, which is probably due to the high occurrence of ergot in this genotype (approximately 50%).

In addition, the differences in seed yield and quality between years had weeds and ergot as limiting factors. The higher temperatures at anthesis resulted in a high incidence of ergot, as warm days favor the development of the disease (Rios et al., 2015), drastically reducing seed yield and quality. The incidence of ergot increases as stigmas of unfertilized ovaries remain receptive and become more prone to infection by the ergot pathogen (Burton & Lefebvre, 1948). Facultative apomictic and sexual tetraploids spend more time starting embryo development than highly apomictic plants (Hojsgaard, Martínez, & Quarin, 2013), suggesting that these genotypes would be more vulnerable to ergot. We did not find this association, but genetic variability exists for this trait, indicating that it can be improved through breeding (Rios et al., 2015). The low incidence of ergot was observed in five hybrids, namely, 225, C18, C15, D3, and 336, the last four coming from the same female parent (Q4205), indicating that this genotype could increase disease resistance, which needs to be further investigated.

Seed production and 1,000-seed weight were the yield components most negatively affected by weed infestation. Weed control is an indispensable operation in fields for seed production, and weed incidence provides valuable information about competition for light and nutrients and persistence for the breeding program (Nieto, Brondo, & Gonzalez, 1968). In general, the native ecotypes and breeding lines, except D3,

showed a lower weed infestation rate than Pensacola in both years, suggesting better soil covering and prevention of weed emergence (Table 4). Genotypes persisting in these conditions will need less herbicides and labor, resulting in economic benefits to farmers (Van Heemst, 1985).

The breeding lines and native ecotypes evaluated in this study presented satisfactory seed production and quality traits under unfavorable environmental conditions than those of the controls. Moreover, crop management has a large role in seed production and should be more thoroughly studied for hybrids considered for release. The selection for seed production traits in tetraploid bahiagrass reveals that improved genotypes with high seed production and high seed quality can be achieved.

Conclusion

The nine hybrids evaluated in the current study showed variability in seed yield component traits. Inflorescence density was the most important component for seed yield in the bahiagrass genotypes. However, C15 had a high number of inflorescences but low seed production, suggesting that other seed components are necessary for seed production. Among apomictic genotypes, Bagual had the highest seed yield and viable seed rate, whereas the sexual hybrids C18 and 336 showed great potential for seed production due to high seed yield, 1,000-seed weight and viable seed rate. Hybrids C18 and 336 and ecotype Bagual should be used in new cycles of crosses to improve seed production with high seed quality.

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