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Registration of 'Boyero UNNE' Bahiagrass

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Abstract

'Boyero UNNE' (Reg. No. CV-5, PI 676021) bahiagrass (*Paspalum notatum* Flüggé) was registered with the National Register of Plant Cultivars and the National Register of Property for Plant Cultivars, Ministry of Agriculture, Livestock Exploitations, and Fisheries of Argentina, Resolution no. 276 in August 2012 (Reg. No. 3213) and released by the National University of the Northeast (UNNE), Faculty of Agricultural Sciences (FCA), Corrientes, Argentina. Boyero UNNE is a tetraploid, highly apomictic F_1 hybrid developed between an experimentally obtained female parent, reproducing sexually, and an apomictic, wild bahiagrass genotype. It is the first registered cultivar of a tetraploid apomictic *P. notatum* developed by breeding through a sexual × apomictic hybridization scheme, exploiting apomixis and plant selection in the F_1 progeny. The new cultivar has a more upright growing habit than tetraploid bahiagrass cultivars currently in use or wild tetraploid biotypes. It can produce approximately 19% more forage dry matter than its apomictic male parent, largely exceeding (20–51%) the wild bahiagrass type currently found in natural pasturelands of northeastern Argentina, and it produced 4 to 26% more than the cultivar Argentine at three locations in Florida, USA.

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Common bahiagrass (*Paspalum notatum* Flüggé) is a perennial grass native to warm, humid regions of the western hemisphere, naturally distributed from Mexico to central Argentina and Uruguay. It has been introduced and planted extensively in the southeastern states of the United States. The species has two botanical varieties: the typical variety characterized by broad leaves, which spreads slowly through stout rhizomes with short internodes, and *P. notatum* var. *saurae* Parodi, which is taller, spreads faster, has longer and narrower leaf blades, and is more cold tolerant (Gates et al., 2004). The species is multiploid, with naturally occurring chromosome races (cytotypes) from diploid ($2n = 20$) to pentaploid ($2n = 50$). The diploid cytotype, which corresponds to *P. notatum* var. *saurae*, reproduces by sexual means and is allogamous, due to self-incompatibility (Burton, 1955). It is often referred to as Pensacola bahiagrass in the United States. The typical botanical variety includes polyploid cytotypes. Triploid and pentaploid cytotypes are extremely rare in nature. The most common species cytotype is tetraploid. Several tetraploid biotypes have long been recognized among strains introduced to the United States from different tropical regions of the Western Hemisphere (Burton, 1946). The most popular cultivars are 'Common', 'Argentine', 'Paraguay', 'Paraguay 22', and 'Wilmington' (Gates et al., 2004). Because all naturally occurring tetraploid bahiagrass types are apomictic, the breeding method was restricted to selection of naturally occurring, superior biotypes. The possibility of using conventional breeding methods was reexamined when Burton and Forbes (1961) and Forbes and Burton (1961a, 1961b) obtained sexual tetraploid plants by colchicine treatment of diploids. These sexual tetraploids were crossed with different natural apomictic tetraploid types to produce fertile hybrids, segregating by reproductive mode. The results demonstrated that sexual × apomictic crossing is a reliable method of plant

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Abbreviations: AFLP, amplified fragment length polymorphism; AS, aposporous embryo sac; CONICET, National Scientific and Technical Research Council, Argentina; FCA, Faculty of Agricultural Sciences; FCSS, flow cytometric seed screening; IBONE, Botanical Institute of Northeastern Argentina; SS, meiotic (sexual) embryo sac; S+AS, one meiotic plus one or more aposporous sac; UNNE, National University of the Northeast; WTB, wild type bahiagrass.

breeding at the tetraploid level. However, no tetraploid bahiagrass germplasm developed through crossbreeding has previously been registered as a commercial cultivar.

Most bahiagrass biomass is concentrated at the soil surface, due to its strong and dense rhizome network. A large proportion of this network develops superficially at the soil surface, that is, below the level at which grazing occurs (Gates et al., 2004). The aboveground biomass consists mainly of leaves and inflorescences that develop at flowering time from the tips of rhizome branches.

The objective of the breeding work that resulted in the development of 'Boyero UNNE' (Reg. No. CV-5, PI 676021) bahiagrass was to create a new apomictic cultivar with a higher seasonal forage yield and a more upright growing habit than existing tetraploid cultivars or wild bahiagrass types used in pastures.

Methods

Breeding History

Boyero UNNE is an apomictic, tetraploid bahiagrass hybrid derived from a short background of crosses contributing to its pedigree (Fig. 1). It was developed from crosses between Q3664 and Q4117 made with the aid of an artificial fog chamber at the National University of the Northeast (UNNE), Faculty of Agricultural Sciences (FCA), Corrientes, Argentina. Blooming spikelets from Q3664 were emasculated inside the chamber immediately after anthesis and before the anthers dehisced. Pollen collected from Q4117 was dusted on the emasculated spikelets. The process was repeated every morning until anthesis occurred in all spikelets of the inflorescence. Pollinated inflorescences were covered with glassine bags until harvest.

Q3664 is a white-stigma bahiagrass plant that originated from a cross between a colchicine-induced tetraploid sexual plant (PT-2) and a wild, apomictic tetraploid strain bearing white stigmas (WSB), made by Burton and Forbes (1961), and followed by further cycles of open-pollination at Tifton, GA. In 1978, a vigorous plant was selected and named SWSB (sexual white stigma bahiagrass) because of its stigma color and because segregation was observed in its progeny. This genotype was then introduced to Argentina in 1979 as a vegetative propagule. Embryological analyses and progeny tests mediated by molecular markers revealed that this genotype was a facultative apomictic plant with a high level (>70%) of sexual reproduction, and it was renamed Q3664 (Quarin et al., 1984; Ortiz et al., 1997). Despite some capacity for apomictic reproduction, Q3664 is a suitable female parent for breeding programs using a sexual × apomictic scheme because it reproduces mainly sexually and it has a useful recessive marker (white stigmas).

The male parent Q4117 is an apomictic tetraploid strain with a low residual capacity for sexual reproduction (Ortiz et al., 1997; Martínez et al., 2001). It has purple stigmas and is native to the state of Rio Grande do Sul, Brazil. It was introduced to FCA, Corrientes, Argentina, in the 1970s as Guaiba no. 673 from the Agronomic Experimental Station, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.

The Q3664 × Q4117 cross produced seed from which 122 progeny were recovered. Because white stigma is a recessive trait of the facultative apomictic female parent, 15 plants of the progeny bearing white stigmas were judged to have originated either by parthenogenesis or by occasional self-pollination and therefore were discarded. The remaining 107 plants had purple stigmas and were assumed to be of hybrid origin.

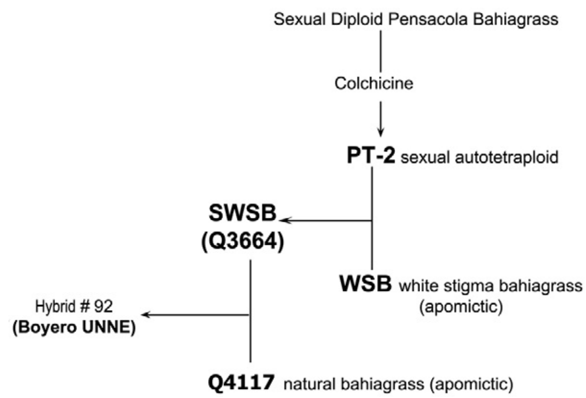

Fig. 1. Pedigree of Boyero UNNE. WSB (white stigma bahiagrass) and Q4117 are apomictic tetraploid bahiagrass accessions, representing two wild types of the species. SWSB (sexual white stigma bahiagrass) is a selected genotype that originated from a controlled hybridization process followed by further cycles of open-pollination.

Reproductive Mode

The 107 hybrids were transplanted to the field, and their reproductive mode was determined through cytoembryological studies. Mature ovules collected at anthesis were sequentially sectioned and stained with safranin-fast green and observed with a transmitted light microscope. Structure and cellular organization clearly differentiate sexual (meiotic) from aposporous embryo sacs. Fifty-five ovules per plant were examined. Of the hybrids, 81 exclusively exhibited ovules with a meiotic embryo sac and were classified as sexual, while those hybrids having ovules with aposporous embryo sacs were classified as apomictic. The reproductive mode of three hybrids remained undetermined due to their limited flowering. The vast majority of the mature ovules in apomictic hybrids had ovaries bearing one or more often two, three, or even several aposporous embryo sacs (AS). A few ovaries had one sexual plus one or more aposporous sacs (S+AS); or even some ovules with just one sexual sac (SS). Considering the proportion of ovaries having aposporous sacs (ovaries with AS or S+AS), the embryological expression of the apomictic trait exceeded 80% in the majority of the 23 apomictic hybrids.

Further analyses were conducted to estimate the actual level of apomictic reproduction in the hybrid that was finally used to develop the Boyero UNNE cultivar. This was accomplished using flow cytometric seed screening (FCSS), as described by Matzk et al. (2000), and a progeny test performed with amplified fragment length polymorphism (AFLP) markers. The FCSS was conducted as a seed-by-seed analysis to determine the proportion of seed formed from aposporous versus sexual sacs. The genome size was established in each seed for the embryo and the endosperm tissue in terms of C value, that is, the DNA content of the whole unreplicated reduced chromosome complement. As the seed develops by apospory + parthenogenesis + pseudogamy (apomixis), the relative DNA content in the embryo nuclei is 2C, while the endosperm is 5C. This is because the embryo is parthenogenetic, while the endosperm is a product of syngamy of two unreduced polar nuclei ($2n + 2n$) and a reduced sperm nucleus (n). Otherwise, if a seed developed by a double-fertilization event involving a meiotic embryo sac, the embryonic nuclei would have a 2C DNA content ($n + n$). The endosperm nuclei have a 3C value, as a result of a triple fusion: two reduced polar nuclei of the central cell and a single reduced sperm cell.

A set of comparative progeny tests with AFLP markers was conducted to estimate the genetic/genotypic variation of Boyero UNNE, its apomictic male parent, Q4117, and a sexual plant, Q4205. Genotype Q4205 is a 100% sexual tetraploid germplasm line, selected among progeny of the self-pollinated plant Q3664 (Quarin et al., 2003). Progenies of Boyero UNNE, Q4117, and Q4205 were obtained from seed produced under open pollination. The AFLP analyses were conducted according to the same protocol followed by Rebozzio et al. (2011).

Evaluation Experiments

Because the main aim was to obtain vigorous apomictic hybrids with upright growing habit, only the 23 hybrids classified as apomictic were considered for initiating the selection process. A first selection for superior plant type was made during the first spring–summer season by visually judging the growing habit and plant vigor. Those apomictic hybrids with more erect culms were selected first, and then the most vigorous among them, showing long leaves and forming tufts with longer diameter, were chosen. This resulted in the selection of nine hybrids for further evaluations. Approximately 200 seeds from each of the nine selected apomictic hybrids, the original male parent Q4117, and the local tetraploid population of wild-type bahiagrass (WTB) were germinated in a seed tray with sterilized soil. Germination produced enough seedlings to establish the trials. Seedlings were transplanted to 100-cm^3 pots to increase plant size prior to transplanting in field trials.

The first of two replicated forage clipping trials was established in 2001 at two locations in northern Argentina, where the nine selected hybrids were evaluated. A second replicated trial was planted in 2008 at three locations in the United States in Florida, but this trial only evaluated three of the most promising hybrids from the 2001 trial.

Clipping Trials in Argentina

Trials were conducted at Corrientes in an experimental field of UNNE FCA, on an Udipsamment alphic soil, and at Juan J. Castelli, Chaco province, in a private farm on an Oxic Haplustoll soil. A complete randomized block design with three replications was used in both locations to evaluate the nine apomictic F_1 hybrids, the male parent Q4117, and WTB. All plots were established from 11 plants, representing a hybrid line, transplanted in a single 2-m row at a distance of 0.2 m between plants and 1 m between rows. The plots were established on 12 and 20 Oct. 2001 at Corrientes and Chaco, respectively. All plots were fertilized with 150 kg ha^{-1} of 15–34.1–18.1 ($N–P_2O_5–K_2O$) in one application at the beginning of the study. Weeds were removed from the plots by a hoe during the first year. The plots were harvested three times per year, at the end of spring, summer, and fall seasons. A 0.5-m-long strip was cut with scissors in the middle of each plot, to 5-cm stubble height. The harvested forage was immediately weighed, and a subsample was oven-dried at 60°C to determine dry matter yield. Data were analyzed using Di Rienzo–Guzmán–Casanoves test (Di Rienzo et al., 2002) at the $P = 0.05$ significance level (Di Rienzo et al., 2014). The entire area was mowed following sampling to maintain plot uniformity. Weeds were manually removed with a hoe after each forage harvest and at the end of winter in 2002 and 2003. The Chaco location was abandoned after the first year due to plant losses caused by severe climatic conditions that included excessive rainfall followed by a long period of drought and high temperatures.

Clipping Trials in the United States

A second phase of replicated forage clipping trials included three of the most promising hybrids selected in Argentina and the tetraploid cultivar, Argentine, used as a check cultivar. The experiment was performed at three locations in Florida (Gainesville, Live Oak, and Quincy). Seasonal forage yields were recorded in 2007 and 2008 at Gainesville. Forage yield data were also obtained from plots cultivated at Live Oak and Quincy in 2008.

Seed from hybrids F_1 49, F_1 65, Boyero UNNE (= F_1 92), and Argentine were sown in 13 Mar. 2006 using a sterile germination mix. Seedlings were transplanted to seedling flats with multiple cells and then into a field at Gainesville, on a loamy, siliceous, subactive, thermic, Arenic Endoaquult soil, on 15 May 2006. Forty seedlings were transplanted into each plot (2 m by 3 m). Five replications of each pure-stand plot were planted without alleys in a randomized complete block design. In July 2006, plots were fertilized with 500 kg ha^{-1} of 16–4–8 ($N–P_2O_5–K_2O$). Plots were harvested using a sickle bar mower, leaving a 5-cm stubble height. A 2.35- by 0.7-m strip was cut in the middle of each plot, the forage collected, weighed, and a subsample (approximately 700 g) immediately taken after harvest. The subsample was weighed and the material dried at 60°C for 48 h prior to reweighing to determine dry mass. Plots were harvested on 4 May 2007 and every 4 wk during the rest of the 2007 growing season. With the exception of the last harvest of each year, plots were fertilized with 286 kg ha^{-1} of 21–7–14 ($N–P_2O_5–K_2O$) following each cutting. In 2008, plots were harvested for the first time on 13 May and every 4 wk during the growing season. Plots were fertilized with 375 kg ha^{-1} of 16–4–8 ($N–P_2O_5–K_2O$) following each cutting. Although the fertilizer type was different in 2007 and 2008, the amount of N applied after each cutting was the same in both years.

Seeds germinated in March 2007 at Gainesville were transplanted into a field located at Live Oak, FL, on 9 May 2007. The soil at this location is classified as a thermic, coated Typic Quartzipsamment. Four blocks (rows) were planted, containing four plots (1.2 m by 1.2 m) separated by a 1-m alley, as a randomized complete block design. Each plot contained 36 seedlings of each genotype, and genotypes were randomized within each block. Plots were fertilized with 530 kg ha^{-1} of 34–0–0 ($N–P_2O_5–K_2O$) and 100 kg ha^{-1} of 0–0–60 ($N–P_2O_5–K_2O$) during 2007. A 70-cm-wide strip was cut across each plot on 15 May 2008, leaving a 5-cm stubble height. Plots were harvested every 4 wk and fertilized with 376 kg ha^{-1} of 16–4–8 ($N–P_2O_5–K_2O$) following each cutting for the remainder of the growing season.

Seedlings from the three hybrids and Argentine were also planted in Quincy, FL, on 10 May 2007 in a fine-loamy, kaolinitic, thermic Typic Kandiudult soil. Forty seedlings were transplanted into each plot (2 m by 3.2 m). Five replications of each pure-stand plot were planted in a randomized complete block design. In 2008, plots were first harvested on 13 May and then harvested, as repetitive measures, every 4 wk. On 22 May, plots were fertilized with 171 kg ha^{-1} of 35–0–0 (N–P_2O_5–K_2O) and 28 kg ha^{-1} of 0–0–60 (N–P_2O_5–K_2O). On 13 June, plots were fertilized with 67 kg N ha^{-1}, 20 kg P ha^{-1}, and 42 kg K ha^{-1}. Plots were harvested again on 11 July and fertilizer was applied with 376 kg of 16–4–8 (N–P_2O_5–K_2O).

Harvested forage from Gainesville, Live Oak, and Quincy were analyzed as repeated measures using Mixed Procedure (SAS version 9.2; SAS Institute, 2011). Locations, genotypes, and harvest dates were considered fixed, and replicates were considered random. When significant treatment effects ($P = 0.05$) were found, the minimum significant difference (MSD) among means was calculated using the Waller–Duncan test. The data reported here are part of a larger experiment that consisted of a total of 12 apomictic bahiagrass clones at Gainesville and 13 entries at Live Oak and Quincy. The overall experimental error was used to determine significant differences.

Characteristics and Discussion

Boyero UNNE bahiagrass was registered with the National Register of Plant Cultivars and the National Register of Property for Plant Cultivars, Argentinian National Seed Institute (INASE), Ministry of Agriculture, Livestock Exploitations, and Fisheries of Argentina, Resolution no. 276, on 28 Aug. 2012 (Reg. No. 3213). Boyero UNNE was released by UNNE FCA, Corrientes, Argentina, to provide livestock producers with a higher-yielding forage cultivar with an upright growing habit (Fig. 2) as an alternative to wild bahiagrass types. Tetraploid bahiagrass is a valuable, widespread indigenous grass species on native pasturelands in South America; however, it is low yielding as a pasture forage grass. Boyero UNNE is also an alternative for Argentine, the most popular tetraploid bahiagrass cultivar, because Boyero UNNE has significantly higher forage production during the spring and total annual forage accumulation than Argentine. Boyero UNNE's more erect growing habit facilitates its utilization for mowing and hay production. Boyero UNNE originated by hybridization of a facultative apomictic tetraploid bahiagrass cytotype with a high rate of sexual reproduction, pollinated with a highly apomictic tetraploid strain, followed by selection. All tetraploid bahiagrass cultivars released in the United States and internationally resulted from selection of naturally occurring, superior types from introduced germplasm. Boyero UNNE is the first registered cultivar of tetraploid apomictic *P. notatum* that was developed by breeding through a sexual × apomictic hybridization scheme, thereby exploiting apomixis and plant selection in the F_1 progeny.

Forage Yield in Argentina

During the first year, all hybrids and controls produced approximately twice as much forage at Corrientes than at Chaco, probably due to inclement weather conditions—heavy rainfall followed by high temperatures and deficient soil moisture—at Chaco (Table 1). There were no significant differences among genotypes in the trials conducted at Chaco. However, at Corrientes, three hybrids— F_1 49, F_1 65, and Boyero UNNE—showed significantly higher forage yields compared with controls and the other hybrids during the first season. There was a drastic and progressive decrease in forage yield at Corrientes throughout the three consecutive trial periods from 2001 to 2004. The yield decrease was expected as a consequence of stand age effects and because the minimum amount of fertilizer (N–P–K) was added only at the beginning of the study (2001) to hasten establishment. Because fertilizer addition on native pasturelands is an unusual practice in subtropical Argentina, the decision was made to avoid any supplementary fertilization after the pasture was established. In this way, the hybrids were compared with the WTB control under conditions similar to current regional practices. Hybrid yields differed during the second evaluation period (2002–2003) at Corrientes. Seven out of nine hybrids performed significantly better than the local WTB, and F_1 49, F_1 65, and Boyero UNNE were again among the superior hybrids, although none of them showed significantly higher forage yield than the male parent (Q4117). During the third year, all hybrids produced significantly more forage than the WTB. Four hybrids, including F_1 65 and Boyero UNNE, also had significantly greater yields than the male parent Q4117. Six out of nine hybrids exhibited higher forage production (3-yr mean) than WTB (control), but they were not significantly different than male parent Q4117. Even so, F_1 49, F_1 65, and Boyero UNNE had 3-yr mean yields that clearly exceeded Q4117 forage production by more than 1 t ha^{-1}.

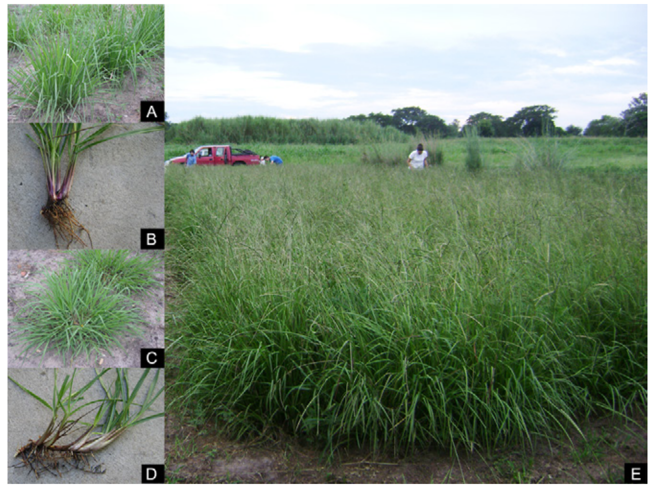

Fig. 2. Bahiagrass, *Paspalum notatum*. (A–B) Erect growing habit of Boyero UNNE. (C–D) Prostrate growing habit of wild-type bahiagrass (WTB). (E) Hand-harvesting seed of Boyero UNNE in an experimental stand at National University of the Northeast, Faculty of Agricultural Sciences (UNNE FCA), Corrientes, Argentina.

Forage Yield in Florida

Gainesville

Forage yields of four bahiagrass genotypes varied significantly among seasons in 2007 and 2008, with the greatest forage yields observed in early to mid-summer (Table 2). The largest genotypic differences were observed in the spring of both

Table 1. Mean dry matter yield through spring-summer-fall trial periods of nine apomictic F1 hybrids, the apomictic male parent (Q4117) and a local wild type bahiagrass (WTB) at Corrientes, Argentina, from 2001 to 2004, and at J.J. Castelli, Chaco province, Argentina, first season (2001–2002).

Entry†	Chaco	Corrientes			
	2001–2002	2001–2002	2002–2003	2003–2004	3-yr mean‡
	Mg ha^{-1}				
F_1 49	7.30a§	18.24a	6.43a	2.17b	8.95a
Boyero UNNE	6.45a	17.13a	7.13a	2.50a	8.92a
F_1 65	7.21a	15.56a	6.33a	2.57a	8.49a
F_1 48	7.09a	14.94b	7.16a	2.63a	8.25a
F_1 64	6.70a	14.87b	7.77a	2.07b	8.24a
F_1 29	5.61a	13.68b	6.27a	2.70a	7.55a
Q4117	6.98a	15.03b	5.67a	1.73b	7.48a
F_1 53	6.99a	14.28b	4.07b	1.77b	6.70b
F_1 39	6.69a	12.90b	5.23a	1.90b	6.68b
WTB	5.39a	12.00b	4.03b	1.43c	5.82b
F_1 83	6.65a	10.94b	3.93b	1.87b	5.58b

† Entries are ordered from highest to lowest yield using the 3-yr mean.

‡ For entries tested over three consecutive years at Corrientes.

§ Means in a column followed by different letters are significantly different ($P = 0.05$), Di Rienzo–Guzmán–Casanoves test.

Table 2. Seasonal and annual forage mass accumulation of three apomictic bahiagrass hybrids and Argentine as a check cultivar, grown at Gainesville, FL.

Entry†	2007				2008			
	Spring	Summer	Fall	Total	Spring	Summer	Fall	Total
	Mg ha^{-1}							
Boyero UNNE	4.55a‡	8.35a	0.71a	13.60a	3.19a	9.53a	2.48a	15.20a
F_1 65	4.28a	8.35a	0.85a	13.47a	3.56a	9.26a	2.92a	15.74a
Argentine	1.12b	9.40a	0.37b	10.89b	1.88b	10.22a	2.53a	14.64a
F_1 49	2.17b	8.08a	0.44ab	10.69b	2.47ab	8.41a	2.89a	13.77a

† Entries are ordered from highest to lowest annual forage yield for 2007.

‡ Different letters indicate significant differences ($P = 0.05$), Waller–Duncan means separation procedure.

years, with most hybrids producing more forage than Argentine. For example, Boyero UNNE yielded 4.1 times more forage than Argentine in spring 2007 and 1.7 times more in spring 2008. However, hybrids did not differ from Argentine during the summer of both years. Argentine accumulated less annual forage yield than Boyero UNNE and F_1 65 in 2007, but no hybrids produced more than Argentine in 2008.

Live Oak and Quincy

A significant location effect and a significant interaction between location and genotypes were observed with the 2008 forage yield data collected at Live Oak and Quincy. There was also a significant seasonal effect and an interaction between seasons and genotypes (Tables 3 and 4). Spring forage yield varied greatly among genotypes at Live Oak and Quincy. However, Boyero UNNE was the highest yielding at both locations, producing 1.9 times more than Argentine at Live Oak, and 2.4 times more at Quincy. No differences were observed among genotypes for summer forage yield at either location. Hybrid yield differences from the fall season were observed only at Live Oak, where the three hybrids produced more than Argentine (Table 3). Annual forage accumulation was greater for Boyero UNNE and F_1 49, in comparison to Argentine at Live Oak and Quincy (Tables 3 and 4).

The Selected Hybrid and Its Reproductive Mode

As mentioned above, the expression of the apomictic trait as assessed by embryological studies exceeded 80% in the

Table 3. Seasonal forage mass accumulation of three apomictic bahiagrass hybrids and Argentine as a check cultivar grown at Live Oak, FL, 2008.

Entry†	Spring	Summer	Fall	Total
	Mg ha^{-1}			
Boyero UNNE	2.29a‡	9.84a	1.15b	13.28a
F_1 49	1.35b	9.30a	1.67a	12.31ab
F_1 65	2.10a	8.35a	1.27b	11.72bc
Argentine	1.21b	8.95a	0.82c	10.99c

† Entries are ordered from highest to lowest total annual yield.

‡ Different letters indicate significant differences ($P = 0.05$), Waller–Duncan means separation procedure.

Table 4. Seasonal forage mass accumulation of three apomictic bahiagrass hybrids and Argentine as a check cultivar grown at Quincy, FL, 2008.

Entry†	Spring	Summer	Fall	Total
	Mg ha^{-1}			
F_1 49	2.12a‡	6.51a	1.85a	9.72a
Boyero UNNE	2.06a	6.31a	1.36a	8.82ab
F_1 65	1.37b	6.35a	1.53a	8.68bc
Argentine	0.86c	5.70a	1.60a	7.69c

† Entries are ordered from highest to lowest total annual yield.

‡ Different letters indicate significant differences ($P = 0.05$), Waller–Duncan means separation procedure.

majority of the original 23 F_1 apomictic hybrids. Particularly, in Boyero UNNE, 86% of its mature ovules had exclusively AS, 7% had a single SS, and 7% were mixed ovules bearing S+AS. Therefore, the possibility of seed development by apomixis varied from 86 to 93%, whereas the possibility of sexual seed development varied from 7 to 14% because 7% of mixed ovaries may develop either by apomixis or by sexuality. This indicates that Boyero UNNE was a highly apomictic genotype, bearing some potential for sexual reproduction. Additional analyses were undertaken to further address genetic stability, including FCSS and a progeny test assisted by AFLP molecular markers. The aims were to determine the proportions of seeds formed by apomixis versus sexuality (flow cytometry), and the real possibility of genetic segregation in its progeny (progeny test). The FCSS analysis showed that 83% of Boyero UNNE seeds originated by apospory, parthenogenesis, and pseudogamy, and 17% sexually. An AFLP progeny test estimated the genetic/genotypic variation of Boyero UNNE, its male parent Q4117, and a sexual tetraploid germplasm line, Q4205. Previous reports based on cytoembryological studies had indicated that Q4205 was a 100% sexual reproducing genotype (Quarin et al., 2003) and that Q4117 was a highly apomictic genotype with a potential for sexual reproduction of 3.7 to 8% (Martínez et al., 2001). The possibility of sexual reproduction of Boyero UNNE varied from 7 to 14%, as indicated above. In the progeny tests, a total of 197 AFLP markers were obtained with two primer combinations. From these, only 57 resulted to be polymorphic (28.93%). Polymorphic AFLP-amplified bands were observed among the nine-plant progeny of Q4205, and each plant represented a different genotype. Pairwise genetic distances among individuals was estimated for the Jaccard's dissimilarity coefficient (1-S). Also, a dendrogram (Fig. 3) was constructed using an unweighted pair-group method with arithmetical average (UPGMA) cluster analysis. An estimate of the confidence limits for the grouping produced by the dendrogram was obtained by performing 999 bootstrap resampling with PAST statistical program (Hammer et al., 2001). Neither Q4117 nor Boyero UNNE progenies showed genetic variation in relation to their mother plants. A unique genotype was observed for Q4117 and its 14-plant progeny. Similarly, the specific genotype of Boyero UNNE was repeated in its 12-plant progeny. Therefore, very rare, new genotypes are expected from seed propagation of Boyero UNNE. Although the residual sexuality observed at embryological stages (7–14%) may be in concordance with the observed amount of sexual seed development (17%), the occurrence of a sexually derived plant should be quite infrequent. In fact, phenotypically off-type plants were visually observed in less than 1% of the cases, in a large space-planted plot of Boyero UNNE. These results are in agreement with previous reports that showed a dramatic decrease of achieved sexual reproduction in the progeny when compared to the degree of residual sexuality estimated at embryological stages of a facultative apomictic *Paspalum* species. For example, the degree of sexual reproduction for three facultative apomictic accessions of *P. notatum* had been estimated to vary from approximately 15 to 95%, according to cytoembryological studies (Rebozzio et al., 2011). However, 95 to 100% of the individuals of their progenies had the corresponding maternal genotype, that is, the same fingerprint pattern as estimated by AFLP markers. A similar and even more dramatic increase of effective apomictic origin was observed in adult progenies of different facultative apomictic accessions of *P. malacophyllum* when compared to data obtained by cytoembryology or in mature seeds by FCSS (Hojsgaard et al., 2013).

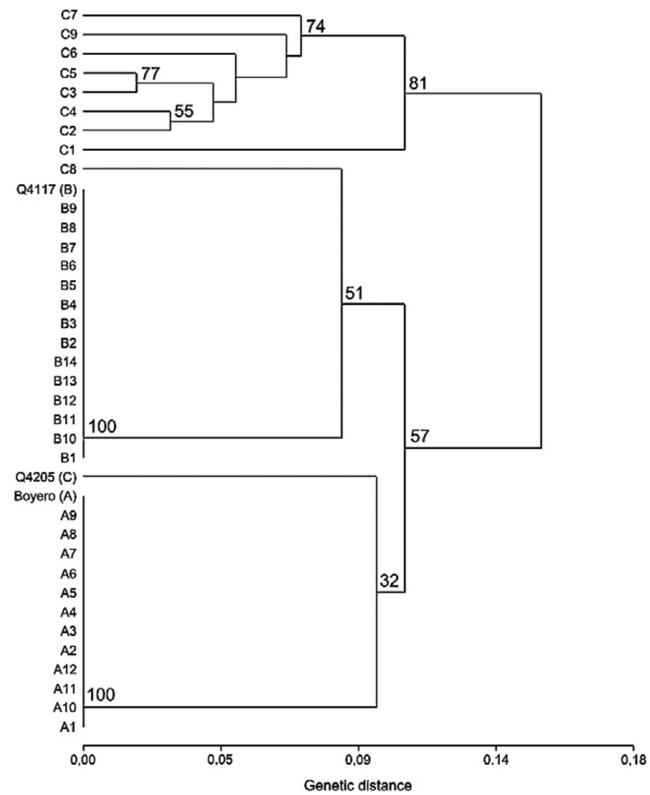

Fig. 3. Dendrogram originated by the amplified fragment length polymorphism analyses of three progenies from three different bahiagrass genotypes whose reproductive mode had been analyzed by embryological techniques or flow cytometric seed screening: Q4205 (designated as C) previously classified as completely sexual , while Q4117 (designated as B) and Boyero UNNE (designated as A), both previously classified as facultative apomictics. Dendrogram shows that the whole progeny of Q4205 originated effectively by sexuality, whereas the progenies of Q4117 and Boyero UNNE originated exclusively by apomixis.

Clearly, there is a biological advantage of the apomictic with respect to the sexual pathway to develop an adult descendant in facultative apomictic *Paspalum* species. These previous and present results suggest that the reproductive mode of Boyero UNNE results in extremely effective genetic stability.

Availability

Breeder and foundation seed will be produced and maintained by Universidad Nacional del Nordeste, Facultad de Ciencias Agrarias, Corrientes, Argentina (UNNE FCA). Certified seed will be produced and commercialized in New Zealand by PGG Wrightson Seeds Limited, 57 Waterloo Rd., Christchurch, New Zealand, and in South America by PGG Wrightson Seeds, Cno. Máximo Santos 4900, Montevideo, Uruguay, CP 12400 (www.wrightsonpas.com.uy) in agreement with UNNE FCA. Seed of Boyero UNNE has been deposited in the US National Plant Germplasm System, where it will be available on expiration of the PVP in August 2032.

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