RESEARCH

Interspecific Tetraploid Hybrids between Two Forage Grass Species: Sexual *Paspalum plicatulum* and Apomictic *P. guenoarum*

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ABSTRACT

The Plicatula group of the grass genus *Paspalum* contains about 30 species. Most are tetraploid and reproduce by apomixis, though some of them contain sexual diploid races. We crossed a sexual colchicine-induced autotetraploid plant of brownseed paspalum *(Paspalum plicatulum* Michx.) with apomictic tetraploid *P. guenoarum* Arechav., also from the Plicatula group. Crossability was 35% and the progeny showed morphological characteristics intermediate to those of the parents but resembling more the male parent. The random amplified polymorphic DNA (RAPD) analysis showed that the whole progeny amplified bands that were specific of the male parent, confirming its hybrid origin. Meiotic chromosome behavior of hybrids exhibited primarily bivalent and quadrivalent associations similar to those of the two parents. This suggests that natural tetraploid *P. guenoarum* shares the same basic chromosome set with that of autotetraploid *P. plicatulum*, and both species probably originate from the same ancestral species. Fourteen out of 23 hybrids reproduced only sexually, while nine were obligate or highly apomictic. Seed set ranged from 11 to 55% among the hybrids. Our results open the possibility of exchanging genes at the tetraploid level in breeding programs of *P. plicatulum* and *P. guenoarum*. This possibility is based on their rate of crossability, the degree of fertility among the hybrids, the segregation observed for the reproductive mode in the F_1 progeny, and the very simple procedure flow cytometry seed screen (FCSS) used to determine the reproductive mode for each hybrid.

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Abbreviations: FCSS, flow cytometry seed screen; GR, cultivar Rojas of *P. guenoarum*; 4PT, sexual induced-tetraploid *P. plicatulum*; PMC, pollen mother cell; RAPD, random amplified polymorphic DNA.

The group Plicatula is an informal botanical infrageneric category established by Chase (1929) to embrace all those species of the grass genus *Paspalum* with brown or dark olivaceous mature spikelets and shining fruits of dark brown or jet black colored surface. Plicatula is a very complex group of more than 30 species (A. Chase, unpublished manuscript, 1939). Some species are difficult to differentiate among each other due to the morphological intergradations and the wide range of variation usually observed. There are several species in the Plicatula group that are components of the natural pastures in South America, but some of them were introduced to cultivation and sown in other areas of the world. Particularly, some cultivars were released mainly in Australia, Argentina, and the United States for four species of the Plicatula group: brownseed paspalum (*P. plicatulum* Michx.), brunswickgrass (*P. nicorae* Parodi), atra paspalum (*P. atratum* Swallen), and *P. guenoarum* Arechav. (Evers and Burson, 2004). Because all these species are primarily tetraploid ($2n = 4x = 40$) and reproduce by apomixis, the released cultivars were selected from natural ecotypes collected mainly in South America and without any genetic improvement through breeding.

Rare diploid cytotypes with $2n = 2x = 20$ chromosomes have been reported for species of Plicatula that otherwise are usually

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tetraploid and apomictic. Sexually reproducing diploids were discovered for *P. compressifolium* Swallen (Quarin et al., 1996), *P. glaucescens* Hack. (Pritchard, 1962; syn. *P. yaguaronense* Henrard), *P. plicatulum* (Espinoza and Quarin, 1997), *P. lenticulare* Kunth (Espinoza et al., 2001; syn. *P. limbatum* Henrard), and *P. wrightii* Hitchc. & Chase (Martínez and Quarin, 1999; syn. *P. hydrophilum* Henrard). This rare sexual germplasm provides a possibility for improving the common apomictic tetraploid races of these species by crossing a sexual plant with an apomict, but this is contingent on doubling the chromosomes of a diploid. The relevance of this procedure has been largely recognized by several authors since Forbes and Burton (1961a, b), Acuña et al. (2009), and Quesenberry et al. (2010) doubled the chromosomes of sexual diploid bahiagrass (*P. notatum* Flügge) to cross sexual induced tetraploid plants with natural apomictic tetraploids of the same species. However, doubling chromosomes to induce sexual tetraploid plants entails an especially difficult task. In addition to the results reported by Forbes and Burton (1961a) for *P. notatum*, induced sexual tetraploids have been obtained only for three other *Paspalum* species in the last half century: *P. hexastachyum* Parodi (Quarin and Hanna, 1980), *P. simplex* Morong (Cáceres et al., 1999), and *P. plicatulum* (Sartor et al., 2009).

There are several outstanding forage grass species that belong to the Plicatula group such as *P. atratum*, *P. nicorae*, *P. plicatulum*, and *P. guenoarum* (Evers and Burson, 2004). Because all these species are tetraploid and apomictic, there has been no genetic improvement through breeding among them. The discovery of sexual diploid plants of *P. plicatulum* (Espinoza and Quarin, 1997) and the induced sexual tetraploid plants obtained from these diploids have opened the possibility for genetic improvement programs for this species (Sartor et al., 2009). Moreover, the availability of these sexual tetraploid plants of *P. plicatulum* might facilitate interspecific crosses among several species of the group at the tetraploid level. Because apomictic plants produce cytologically reduced gametes, we pollinated a sexual tetraploid plant of *P. plicatulum* with pollen of apomictic tetraploid *P. guenoarum* to investigate (i) the genomic relationship of these two species through analysis of meiotic chromosome behavior in the interspecific hybrids, (ii) the fertility of the interspecific hybrids as an indication of the feasibility for gene transfer among these species in plant breeding programs, and (iii) the proportion of apomictic hybrids in the first generation.

MATERIALS AND METHODS

Plant Material and Crosses

A sexual induced-tetraploid *P. plicatulum* (4PT) plant was used as the female parent for interspecific hybridization. This material was propagated by cuttings from the 4c-4x plant induced by colchicine from a sexual diploid cospecific plant (Sartor et al., 2009). The pollen donor was the apomictic tetraploid cultivar Rojas of *P. guenoarum* (GR). Before the onset of flowering inflorescences of 4PT were isolated with glassine bags. The following morning, fresh pollen was collected from GR, the glassine bag was removed from the inflorescence of 4PT showing some spikelets at anthesis, and the GR pollen was dusted on exposed stigmas. Flowering occurs progressively and downward from the upper racemes in the inflorescences and takes 4 to 6 d to complete anthesis in all the spikelets of one inflorescence. Thus, pollination was conducted for several days in the same inflorescence, removing the glassine bag each morning and then covering it again to avoid contamination with undesired pollen. The pollinated inflorescences remained in the bags for approximately 1 mo and were harvested and then the filled spikelets were separated from empty spikelets.

Morphological Features

The following inflorescence components were measured for three sexual and three apomictic hybrid plants and for their male and female parents: inflorescence axis length, mean number of racemes per inflorescence, and distal and basal raceme lengths. Measurements were performed on inflorescences at the time of anthesis in cultivated plants growing in the field. Three inflorescences were utilized for each genotype. In addition, leaf blade widths and lengths were determined for the second stem leaf below the base of the inflorescence. Weight of 1000 caryopses was assessed for these hybrid plants and their parents.

Random Amplified Polymorphic DNA Analysis

Genomic DNA was extracted from fresh leaves as described by Dellaporta et al. (1983) and the analysis was conducted essentially as in Daurelio et al. (2004). Briefly, 40 decamers from the University of British Columbia (Vancouver, BC, Canada) (Set 8) were screened to find polymorphism between both parents. Each random amplified polymorphic DNA (RAPD) amplification was performed in a volume of 25 μL containing 30 ng primer, 20 ng genomic DNA, 1x Taq polymerase reaction buffer (Promega Corporation, Madison, WI), 15 μm each deoxyribonucleotide triphosphates (dNTPs), 1.5 mM magnesium chloride, and 1 U Taq polymerase (Promega). Amplification products were run in 5% polyacrylamide gels, electrophoresed in Tris-borate-EDTA (TBE) 1x, and stained by using Silver Staining System from Promega.

Meiosis

Young inflorescences of the male parent and of four selected hybrids were fixed in a 5:1 solution (v/v) of absolute ethanol:lactic acid for 24 h and stored in 70% ethanol at 4°C. Pollen mother cells (PMCs) were stained with 2% aceto-carmine (2%) and cells at diakinesis and metaphase I were observed with a conventional optical microscope to study the meiotic chromosome associations in apomictic tetraploid *P. guenoarum* and its interspecific hybrids with sexual tetraploid *P. plicatulum*.

Mode of Reproduction

Samples of mature seeds from pollen donor and F_1 hybrid plants were analyzed by flow cytometry seed screen (FCSS), following the methods described by Matzk et al. (2000), to determine the

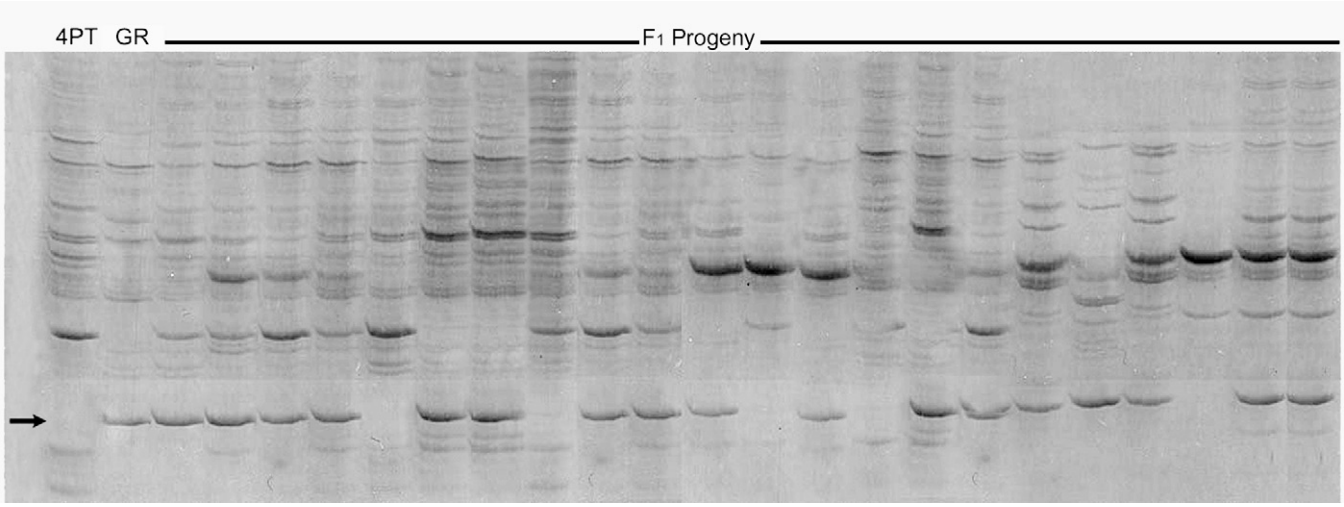

Figure 1. Silver stained polyacrylamide gel visualizing random amplified polymorphic DNA (RAPD) amplification patterns for the parents and their progeny using BC710 primer. Arrow indicates a band specific to the male cultivar Rojas of *P. guenoarum* (GR) parent used to determine the hybrid origin of the progeny. 4PT, sexual induced-tetraploid *P. plicatulum*.

reproductive pathways based on the relative embryo:endosperm DNA content. Since in these species of *Paspalum* apomixis is apospory followed by pseudogamy, the embryo:endosperm relative DNA content has different values when the seed is formed sexually or through an apomictic pathway. This is because in most apomictic species of *Paspalum*, aposporous embryo sacs have two unreduced (2*n*) polar nuclei in the central cell. The 2C value of the embryo and the 3C value of the endosperm indicate that seed has been produced sexually, as a result of double fertilization: the reduced (*n*) egg cell and the central cell (which has two *n* polar nuclei), each by one of the *n* sperm cells brought through the pollen tube. Thus, the relative embryo:endosperm DNA content is 2C:3C in a sexually formed seed. In contrast, an embryo:endosperm DNA content ratio of 2C:5C denotes the seed is formed by apospory followed by pseudogamy. The embryo is formed by parthenogenesis of an unreduced egg cell (2*n* + 0), while the endosperm arises from pseudogamy that involves the central cell (carrying two unreduced polar nuclei) fertilized by one reduced sperm cell of the pollen tube. The FCSS involved the analyses of bulked seeds in groups of 5 or 10. Additionally, when the bulked seeds analysis showed histogram peaks suggesting that some of them originated by sexuality and others by apomixis, the analysis was amplified and repeated seed by seed to establish the proportions that were formed by sexuality or by apomixis.

Following open pollination, 15 to 50 freshly harvested caryopses from each hybrid plant and male parent were analyzed. Bulks of five caryopses were chopped with a razor blade in 0.5 mL of buffer for cell extraction. After 2 min of incubation, the cell suspension was filtered through a 30 μm mesh into a sample tube. Later, 1.5 mL of fluorescent stain 4α,6-diamidino-2-phenylindole (DAPI) staining buffer was added. The buffer and stain are included in the proprietary Partec P kit (CyStain UV precise P; Partec, Münster, Germany). Suspensions were passed through a flow cytometer (Partec Ploidy Analyzer PA-II) with the detector operating at 355 nm. At least 3000 nuclei were counted for each sample. Data were analyzed using PA-II Partec FloMax software. No internal standard was added to the mature seed sample because we were only interested in the presence and the relative positions of the peaks to infer the reproductive pathway(s) present in these samples.

Fertility

Seed set (the percentage of florets forming caryopses) was examined following self- and cross-pollination for some of the sexual and apomictic hybrid plants, as well as for the male parent. Self-pollination and cross pollination was achieved by confining inflorescences within glassine bags before and after anthesis, respectively.

Statistical Analysis

Morphological features data were analyzed using PROC GLM of PC SAS (SAS Institute, 2004). When significant differences among genotypes were detected for one morphological trait, the Tukey test was used for mean separations. All differences refer to significance at $\alpha = 0.05$.

RESULTS

A total of 85 spikelets of 4PT were dusted with pollen of GR. Over 35% of the spikelets filled and consequently 30 seeds were sown in flats of stream-sterilized soil, which produced 23 seedlings. The seedlings were first transplanted in small pots into a glasshouse and then transplanted to a field nursery.

The RAPD analyses were conducted for both parents and for the whole progeny of 23 individuals. A total of 40 oligonucleotides were assayed in female and male parent plants. These primers produced satisfactory amplification profiles, but only three decamers (BC710, BC726, and BC744) were taken into account for progeny analyses. These three decamers were selected because they amplified clear bands that were specific to the male parent. All 23 individuals amplified these bands specific to the male GR parent, indicating that all were interspecific hybrids (Fig. 1).

Considerable variability was identified among the hybrids for several morphological characteristics. When selected characteristics differed between parents, the hybrids produced intermediate values to those of the parents, though some hybrids exceeded the parental values (Table 1). Inflorescences of 4PT consisted of three to four racemes per

Table 1. Morphological features of a colchicine induced tetraploid cytotype of *Paspalum plicatulum* and tetraploid *P. guenoarum* and six interspecific hybrids.

Species or hybrids	Inflorescence axis length (cm)	SD	Number of racemes per inflorescence	SD	Upper raceme length (cm)	SD	Lower raceme length (cm)	SD	Leaf blade width (cm)	SD	Leaf blade length (cm)	SD	Seed weight (g)†
P. plicatulum (S)‡	7.86 d§	1.09	3.33 b	0.57	10.56 a	1.28	10.43 cd	2.30	0.66 b	0.11	32.66 bc	8.01	2.66
P. guenoarum (A)¶	20.20 ab	2.35	8.33 a	2.88	6.13 a	2.65	17.46 ab	3.33	1.63 a	0.15	51.50 a	4.97	2.39
Hybrids													
01 (S)	10.20 cd	1.55	4.00 b	0.00	7.66 a	0.86	7.80 d	1.12	0.90 b	0.10	28.80 c	1.20	3.78
02 (S)	13.66 bcd	1.27	4.33 ab	0.57	7.53 a	1.36	11.63 bcd	1.75	1.00 b	0.17	35.43 abc	5.95	4.19
06 (S)	17.50 abc	3.67	4.66 ab	0.57	11.43 a	1.60	14.70 abc	2.98	1.20 ab	0.26	47.00 ab	7.69	3.94
12 (A)	18.76 ab	1.06	5.00 ab	0.00	11.06 a	0.11	17.76 ab	0.25	1.13 ab	0.35	45.00 abc	4.35	2.66
20 (A)	19.90 ab	0.88	4.66 ab	0.57	9.03 a	1.77	16.53 abc	1.53	0.76 b	0.15	46.23 abc	6.33	2.30
22 (A)	22.10 a	5.10	6.33 ab	3.21	11.20 a	2.81	20.73 a	2.21	1.03 b	0.25	50.06 ab	7.65	3.20

†Weight of 1000 hulled seeds.

‡S, sexual plant.

§Values within a column followed by the same letter are not significantly different at the $\alpha = 0.05$ probability level according to Tukey test.

¶A, apomictic plant.

inflorescence axis that averaged 7.86 cm in length while in *P. guenoarum* the inflorescences contained 5 to 10 racemes and the axis averaged 20.2 cm in length. The racemes of 4PT were all of similar lengths while those of *P. guenoarum* had pyramidal truncate inflorescences. The inflorescences of six hybrids were analyzed and the mean axis length ranged from 10.2 to 22.1 cm and the mean number of racemes per inflorescence was variable but intermediate to the parents. Hybrids displayed considerable variation for inflorescence shape and raceme length. There were inflorescences that resembled the female parent (i.e., hybrid #01) and others that had more pyramidal inflorescences, though no one of them showed the clear difference in raceme length observed between the upper and the lower raceme in the male parent (Table 1). The mean leaf blade width was 0.66 cm for the female parent and 1.63 cm for the male parent. The values of leaf blade width obtained from six hybrids were intermediate to the parents and varied from 0.76 to 1.20 cm. The mean leaf length was also variable among the same six hybrids and intermediate to the parents with the exception of hybrid #01 that had leaves shorter than the mother plant. Judged by the whole phenotypic appearance, each descendant resembled more the male than the female parent, confirming its hybrid origin. In most hybrids, the weight of 1000 hulled seed exceeded the values of either parent.

During diakinesis and first metaphase of meiosis in *P. guenoarum*, the chromosomes were present mainly as bivalents and quadrivalents (Table 2, Fig. 2A). Meiosis was analyzed in the microsporocytes of four hybrid plants. Meiotic chromosome configurations at diakinesis and first metaphase were similar to those in the parental species with primarily bivalent and quadrivalent associations, with occasional univalents and trivalents (Fig. 2B).

The flow cytometric analysis indicated that 14 of the 23 hybrids formed seed exclusively through a sexual process (Table 3). The embryo was formed by a reduced egg cell fertilized by a similarly reduced sperm cell ($n + n$), and the endosperm arose from the second fertilization event involving the two polar haploid nuclei of the meiotic embryo sac and the second sperm cell of the pollen tube [$(n + n) + n$]. Therefore the histogram of these sexual hybrids showed a high 2C peak for the embryo and a smaller 3C peak for the endosperm (Fig. 3A). The remaining nine hybrids inherited the apomictic reproductive mode from *P. guenoarum*. They produced seed through apospory followed by parthenogenesis (embryo, $2n + 0$) and pseudogamy (endosperm, $5n$). Because the aposporous embryo sac contained two unreduced polar nuclei ($2n$), and pollen had reduced sperm cells (n), pseudogamy generated a $5n$ endosperm [$(2n + 2n) + n$]. Thus the histogram of these apomictic hybrids showed an embryo:endosperm DNA content ratio of 2C:5C (Fig. 3B).

Table 2. Meiotic chromosome configurations of tetraploid *Paspalum plicatulum* and *P. guenoarum* and their interspecific hybrids at diakinesis and metaphase I.

Species or hybrid	Number of plants	Number of PMC†	Average and range () per cell			
			I	II	III	IV
P. plicatulum (S)‡§	1	53	0.06 (0–1)	14.20 (8–18)	0.06 (0–1)	2.80 (1–7)
P. guenoarum (A)¶	1	21	1.38 (0–6)	12.33 (7–18)	0.09 (0–1)	3.38 (1–6)
4PT × GR hybrids	4	38	1.65 (0–6)	12.26 (4–18)	0.50 (0–2)	3.07 (1–7)

†PMC, pollen mother cells.

‡S, sexual plant.

§Data from Sartor et al. (2009).

¶A, apomictic plant.

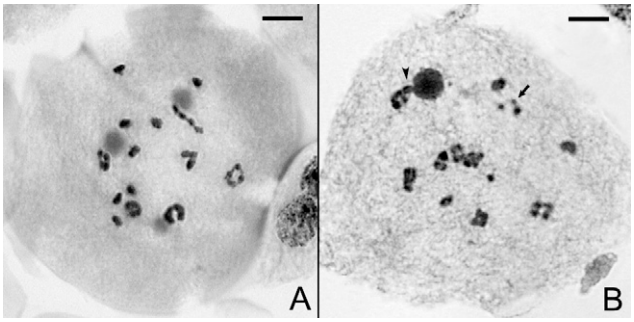

Figure 2. Meiotic chromosome associations in *Paspalum guenoarum* and its interspecific hybrids with *P. plicatulum*. A) Diakinesis in *P. guenoarum* with eight bivalents and six quadrivalents. B) Diakinesis in a *P. plicatulum* × *P. guenoarum* hybrid with one univalent, four bivalents, one trivalent, and seven quadrivalents. The arrow head points to a bivalent overlapped with a quadrivalent, and the arrow indicates the trivalent. Bar = 5 µm.

Some of the nine apomictic hybrids showed some ability for sexual reproduction. In addition, rare hexaploid seeds were recovered from four of the apomictic hybrids. Hexaploids arose when fertilization ($2n + n$) occasionally occurred in an aposporous embryo sac of tetraploid hybrids.

An important variation was observed for seed set of hybrid plants (Table 4). The proportion of spikelets containing a caryopsis ranged from 11.3 to 55.0% in open pollination conditions for the 11 hybrids that were analyzed. More than 17,000 mature spikelets of these 11 hybrids were considered and seed set averaged 31.3%. A total of 6627 spikelets from five different hybrids were self-pollinated and the seed set averaged 9.5%, with a range of 0.3 to 23.6%.

DISCUSSION

Based on the phenotypic comparisons of selected morphological traits, which resembled more the male than the female parent, and because the female parent was completely self-sterile (Sartor et al., 2009), the 23 descendants from *P. plicatulum* × *P. guenoarum* were classified as interspecific hybrids. Additionally, RAPD analysis showed that the whole progeny amplified those bands that were specific to the male parent, indicating the hybrid origin of the 23 descendants. The selected morphological traits were significantly different between the induced autotetraploid plant of *P. plicatulum* and *P. guenoarum* with the exception of the upper raceme length in their inflorescences. Most hybrids showed morphological characteristics intermediate to the parents. However, most values observed in the hybrids were not significantly different from the values recorded for the male parent *P. guenoarum*, but they were significantly different, in most cases, from the values obtained for 4PT female parent. The exception was the hybrid #01, which showed morphological characteristics that were not significantly different from 4PT but differed from male parent. These results have a singular significance for plant improvement programs in view of the fact that it is possible to generate an array of morphological options by crossing *P. plicatulum* and *P. guenoarum* at tetraploid level.

Table 3. Reproductive behavior of 23 interspecific hybrids determined by flow cytometry on mature seeds.

Hybrid	Number of seed	Proportion of seeds originated by mean of		
		Sexuality ($n + n$) (%)	Apomixis ($2n + 0$) (%)	B_{III}† ($2n + n$) (%)
1	45	100	0	0
2	40	100	0	0
3	40	0	100	0
4	35	100	0	0
5	35	100	0	0
6	35	100	0	0
7	20	100	0	0
8	25	100	0	0
9	35	0	100	0
10	35	100	0	0
11	35	100	0	0
12	15	0	93.33	6.67
13	40	2.5	95	2.5
14	45	100	0	0
15	35	100	0	0
16	15	13.33	80	6.67
17	45	100	0	0
18	35	100	0	0
19	45	100	0	0
20	15	0	100	0
21	15	20	80	0
22	36	0	97.22	2.78
23	15	13.30	86.70	0

†B_{III}, hybrids originated through the fertilization of an unreduced female gamete ($2n$) with a reduced male gamete (n).

Meiotic chromosome associations in the GR showed a higher mean of quadrivalents (3.38 per cell with a maximum of six per cell) than previously reported for other accessions of the same species. Pritchard (1970) mentioned an average of 0.82 quadrivalent per cell in a tetraploid accession from Paraguay and suggested that *P. guenoarum* might have originated from hybridization at the species level. Burson and Bennett (1971a) reported a mean of 1.84 with a maximum of five quadrivalents per cell; this would indicate that the species was either a segmental allotetraploid or an autotetraploid. The authors proposed that since the species showed a low frequency of quadrivalents and no pollen mother cell had 10 quadrivalents, it was probably a segmental allopolyploid. This would imply a hybrid origin of two genomically related species. Based on chromosome associations observed by Burson and Bennett (1971a) and in our own results, we suggest that *P. guenoarum* likely was originated by autoploidy rather than by interspecific hybridization. The chromosome

Table 4. Seed set of *Paspalum plicatulum* × *P. guenoarum* hybrids.

	Hybrid plants (no.)	Spikelets analyzed (no.)	Mean and range of spikelets with caryopses (%)
Self–pollinated	5	6627	9.5 (0.3– 23.6)
Open–pollinated	11	17376	31.3 (11.3- 55.0)

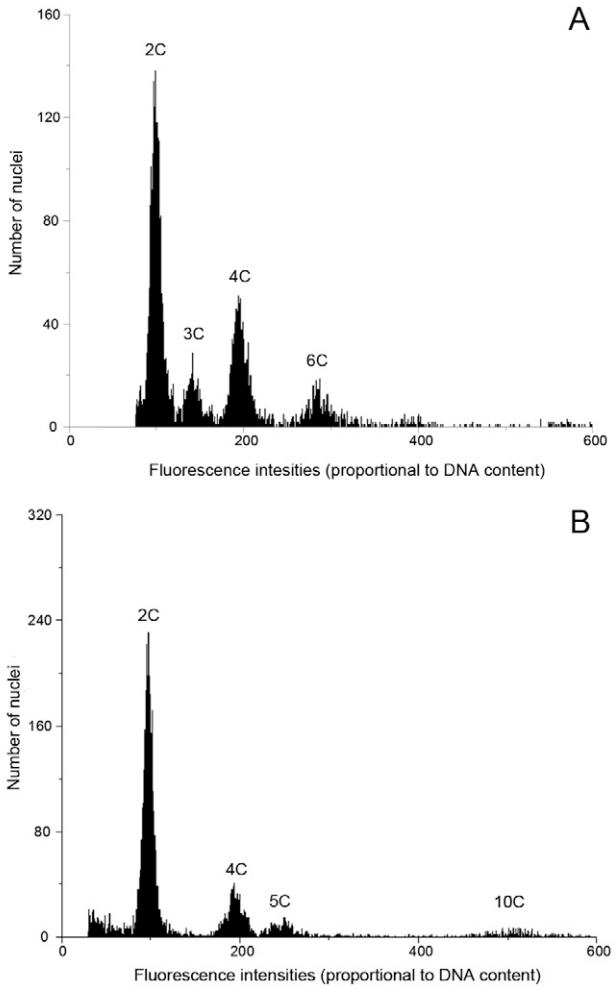

Figure 3. Flow cytometry histograms generated from seeds of two *Paspalum plicatulum* × *P. guenoarum* interspecific hybrids. A) Sexual hybrid showing a high 2C peak for the embryo and a smaller 3C peak for the endosperm. B) Apomictic hybrid with embryo:endosperm DNA content ratio of 2C:5C. Both histograms showed additional peaks produced by embryo and endosperm nuclei at G2 stage of the cell cycle.

behavior reported by Sartor et al. (2009) for the newly acquired autotetraploid *P. plicatulum* through colchicine treatment of a diploid biotype supports our proposition. The 4PT plant averaged 2.8 quadrivalents per cell with a maximum of seven per cell, while we observed in the GR a higher average of quadrivalents per cell (3.38) and six quadrivalents as a maximum. The data of Sartor et al. (2009) means that all 40 chromosomes would not necessarily associate and form 10 quadrivalents. Moreover, tetraploid *P. guenoarum* averaged an even higher number of quadrivalents per cell than was observed in the experimentally obtained autotetraploid *P. plicatulum*. This strongly suggests that *P. guenoarum* might have originated by autoploidy.

The chromosome associations in the *P. plicatulum* × *P. guenoarum* tetraploid hybrids indicated that the two species are autotetraploids and share the same basic genome complement. The feasibility of interspecific hybridization, the chromosome behavior of the hybrids at meiosis, and the degree of fertility observed among them open new possibilities for plant improvement through breeding and gene transfer between these species.

Since Saura (1941) counted $2n = 40$ chromosomes in *P. plicatulum*, many authors have reported the same chromosome number for several accessions, indicating that tetraploidy is the rule (Gates et al., 2004). However, some diploids ($2n = 20$) have been occasionally reported since Brown (1950) discovered a diploid cytotype. Tetraploid races are all apomictic (Pritchard, 1970; Bashaw et al., 1970; Burson and Bennett, 1971b), while diploids reproduce sexually (Espinoza and Quarin, 1997). On the contrary, only apomictic tetraploid biotypes are known for *P. guenoarum* (Pritchard, 1970; Burson and Bennett, 1971a) and no diploid plants have ever been discovered for this species. Our results indicate that it is possible to bypass the bottleneck that limits the lack of sexual biotypes in *P. guenoarum* for plant improvement through breeding. Chromosome doubling of sexual diploid *P. plicatulum* made it possible to cross sexual and apomictic *P. plicatulum* at the tetraploid level, but it also implied the possibility for crossing *P. plicatulum* with apomictic *P. guenoarum* at the tetraploid level (Sartor et al., 2009).

The F_1 progeny segregated for reproductive mode. However, the small number of hybrids in this progeny rendered impossible any genetic analysis regarding the control of apomixis in these species. Notwithstanding, the observed 14:9 sexual:apomictic ratio might be in concordance with the 1:1 ratio observed in other apomictic grass species such as Guinea grass [*Megathyrsus maximus* (Jacq.) B. K. Simon & S. W. L. Jacobs] (Savidan, 1975) or in *Brachiaria* species (Valle and Glenke, 1993). These authors proposed a monogenic control of reproductive mode with apospory dominant to sexuality based on 1:1 sexual:apomictic segregation ratio in progenies of a sexual colchicine-induced autotetraploid plant crossed by a natural apomictic tetraploid plant. A similar genetic model was proposed for *Paspalum notatum* though, in that case, the analysis indicated a clear distorted segregation against apospory, suggesting that the control may have had some pleiotropic lethal effect with incomplete penetrance (Martínez et al., 2001). Aside from determining a precise model for inheritance of apospory, our results open the possibility to exchange genes in a breeding program of *P. plicatulum* and *P. guenoarum* at the tetraploid level. This possibility is based on the crossability between the two species (35%), the degree of fertility among the hybrids (up to 55%), the segregation observed for the reproductive mode in the F_1 progeny, and the very simple procedure (FCSS) to determine the reproductive mode of each hybrid. Breeding practices may involve the creation of a large hybrid population, as proposed by Hanna (1995). The apomictic segregants could be identified easily by FCSS using a small seed sample of each hybrid. Because our analyses indicated that the hybrids could be classified as fully sexual, apomictic, or facultative apomictic, the FCSS analysis of 10 seeds per hybrid (seed by seed) may

be sufficient to classify each plant by its reproductive mode. Once the apomictic hybrids are identified, they could enter field trials for agronomic evaluation and increase to cultivar status. Otherwise, the sexual hybrids could be evaluated and selected to be used as female parents in new breeding attempts. Because our results suggest a monogenic control of reproductive mode with apospory dominant to sexuality, as it was proposed for other grass species such as *Megathyrsus maximus* (Jacq.) B. K. Simon & S. W. L. Jacobs (Savidan, 1975), *Brachiaria* species (Valle and Glenke, 1993), and *Paspalum notatum* (Martínez et al., 2001), sexuality should be conditioned by the homozygous recessive genotype. This brings the possibility to synthesize a tetraploid sexual breeding population from first generation hybrids formed by crossing the 4PT plant with a variety of apomictic tetraploid accessions of *P. guenoarum*. This breeding scheme was proposed for *Brachiaria* breeding programs by Miles (2007). Briefly, the procedure implies a synthetic tetraploid population that would remain fully sexual generation after generation and could be handled by simple mass selection methods. Since the goal for breeding programs would be the release of only apomictic cultivars, the creation of a synthetic tetraploid sexual breeding population represents a starting step, to be followed by crossing of this population to one or more elite apomicts (*P. plicatulum* or *P. guenoarum*) to generate hybrid populations from which to extract superior apomictic segregants to be developed to cultivar status. We expect that other apomictic tetraploid species of the Plicatula group of *Paspalum* may be involved in this interspecific breeding scheme. This would depend on the crossability and the degree of fertility in the hybrids to be obtained by crossing a synthetic tetraploid sexual population with other apomictic tetraploid species of the Plicatula group.

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References

Acuña, C.A., A.R. Blount, K.H. Quesenberry, K.E. Kenworthy, and W.W. Hanna. 2009. Bahiagrass tetraploid germplasm: Reproductive and agronomic characterization of segregating progeny. Crop Sci. 49:581–588. doi:10.2135/cropsci2008.07.0402

Bashaw, E.C., A.W. Hovin, and E.C. Holt. 1970. Apomixis, its evolutionary significance and utilization in plant breeding. p. 245–248. *In* M.J.T. Norman (ed.) Proc. 11th Int. Grasslands Congr., Surfers Paradise, QLD, Australia. 13–23 Apr. 1970. University of Queensland Press, St. Lucia. QLD, Australia.

Brown, W.V. 1950. A cytological study of some Texas gramineae. Bull. Torrey Bot. Club 77:63–76. doi:10.2307/2482267

Burson, B.L., and H.W. Bennett. 1971a. Chromosome numbers, microsporogenesis, and mode of reproduction of seven *Paspalum* species. Crop Sci. 11:292–294. doi:10.2135/cropsci1971.0011183X001100020038x

Burson, B.L., and H.W. Bennett. 1971b. Meiotic and reproductive behavior of some introduced *Paspalum* species. J. Mississippi Acad. Sci. 17:5–8.

Cáceres, M.E., F. Pupilli, C.L. Quarin, and S. Arcioni. 1999. Feulgen-DNA densitometry of embryo sacs permits discrimination between sexual and apomictic plants in *Paspalum simplex*. Euphytica 110:161–167. doi:10.1023/A:1003772623703

Chase, A. 1929. The North American species of *Paspalum*. Contr. U. S. Natl. Herb. 28:1–310.

Daurelio, L.D., F. Espinoza, C.L. Quarin, and S.C. Pessino. 2004. Genetic diversity in sexual diploid and apomictic tetraploid populations of *Paspalum notatum* situated in sympatry or allopatry. Plant Syst. Evol. 244:189–199. doi:10.1007/s00606-003-0070-6

Dellaporta, S.L., J. Wood, and J.B. Hicks. 1983. A plant DNA minipreparation: Version II. Plant Mol. Biol. Rep. 1:19–21. doi:10.1007/BF02712670

Espinoza, F., and C.L. Quarin. 1997. Cytoembryology of *Paspalum chaseanum* and sexual diploid biotypes of two apomictic *Paspalum* species. Aust. J. Bot. 45:871–877. doi:10.1071/BT96055

Espinoza, F., M.H. Urbani, E.J. Martínez, and C.L. Quarin. 2001. The breeding systems of three *Paspalum* species with forage potential. Trop. Grassl. 35:211–217.

Evers, G.W., and B.L. Burson. 2004. Dallisgrass and other *Paspalum* species. p. 681–713. *In* L.E. Moser, B.L. Burson, and L.E. Sollenberger (ed.) Warm-season (C4) grasses. Agron. Monogr. 45. ASA, CSSA, SSSA, Madison, WI.

Forbes, I., and G.W. Burton. 1961a. Induction of tetraploidy and a rapid field method of detecting induced tetraploidy in Pensacola bahiagrass. Crop Sci. 1:383–383. doi:10.2135/cropsci1961.0011183X000100050032x

Forbes, I., and G.W. Burton. 1961b. Cytology of diploids, natural and induced tetraploids, and intraspecies hybrids of Bahiagrass, *Paspalum notatum* Flügge. Crop Sci. 1:402–406. doi:10.2135/cropsci1961.0011183X000100060006x

Gates, R.N., C.L. Quarin, and C.G.S. Pedreira. 2004. Bahiagrass. p. 651–680. *In* L.E. Moser, B.L. Burson, and L.E. Sollenberger (ed.) Warm-season (C4) grasses. ASA, CSSA, SSSA, Madison, WI.

Hanna, W.W. 1995. Use of apomixis in cultivar development. Adv. Agron. 54:330–350.

Martínez, E.J., and C.L. Quarin. 1999. Citoembriología y comportamiento reproductivo de un citotipo diploide de *Paspalum hydrophilum* y sus híbridos con *P. palustre* (Poaceae, Paniceae). (In Spanish, with English abstract.) Darwiniana 37:243–251.

Martínez, E.J., M.H. Urbani, C.L. Quarin, and J.P. Ortiz. 2001. Inheritance of apospory in bahiagrass, *Paspalum notatum*. Hereditas 135:19–25. doi:10.1111/j.1601-5223.2001.00019.x

Matzk, F., A. Meister, and I. Schubert. 2000. An efficient screen for reproductive pathways using mature seeds of monocots and dicots. Plant J. 21:97–108. doi:10.1046/j.1365-313x.2000.00647.x

Miles, J.W. 2007. Apomixis for cultivar development in tropical forage grasses. Crop Sci. 47:238–249. doi:10.2135/cropsci2007.04.0016IPBS

Pritchard, A.J. 1962. The cytology and reproduction of *Paspalum yaguaronense* Henr. Aust. J. Agric. Res. 13:206–211. doi:10.1071/AR9620206

Pritchard, A.J. 1970. Meiosis and embryo sac development in *Urochloa mosambisensis* and three *Paspalum* species. Aust. J. Agric. Res. 21:649–652. doi:10.1071/AR9700649

Quarin, C.L., M.T. Pozzobon, and J.F.M. Valls. 1996. Cytology and reproductive behavior of diploid, tetraploid and hexaploid germplasm accessions of a wild forage grass: *Paspalum compressifolium*. Euphytica 90:345–349. doi:10.1007/BF00027486

Quarin, C.L., and W.W. Hanna. 1980. Effect of three ploidy levels on meiosis and mode of reproduction in *Paspalum hexastachyum*. Crop Sci. 20:69–75. doi:10.2135/cropsci1980.0011183X002000010016x

Quesenberry, K.H., J.M. Dampier, Y.Y. Lee, R.L. Smith, and C.A. Acuña. 2010. Doubling the chromosome number of bahiagrass via tissue culture. Euphytica 175:43–50. doi:10.1007/s10681-010-0165-4

Sartor, M.E., C.L. Quarin, and F. Espinoza. 2009. Mode of reproduction of colchicine-induced *Paspalum plicatulum* tetraploids. Crop Sci. 49:1270–1276. doi:10.2135/cropsci2008.05.0270

SAS Institute. 2004. SAS/STAT user's guide release. Release 9.0. SAS Institute, Cary, NC.

Saura, F. 1941. Cariología de algunas especies del género *Paspalum*. (In Spanish.) Inst. Genét. Fac. Agron. Vet. Universidad de Buenos Aires. 2:41–48.

Savidan, Y. 1975. Hérédité de l'apomixie. Contribution à l'étude de l'hérédité de l'apomixie sur *Panicum maximum* Jacq. (analyse des sacs embryonnaires). (In French.) Cah. O.R.S.T.O.M. Sér. Biol. 10:91–95.

Valle, C.B., and C. Glenke. 1993. Towards defining the inheritance of apomixis in *Brachiaria*. Apomixis Newsl. 6:24–25.