

Mode of Reproduction of Colchicine-Induced *Paspalum plicatulum* Tetraploids

María E. Sartor, Camilo L. Quarin, and Francisco Espinoza*

ABSTRACT

Paspalum plicatulum Michx. is a wild forage grass species. The common races are tetraploid and apomictic, while sexual diploid representatives have been reported sporadically. Objectives of this study were to induce sexual 4x individuals from sexual diploids, determine the capacity for hybridization with other apomictic 4x species closely related with *P. plicatulum*, and thus create a tetraploid sexual material cross compatible with apomictic 4x species of the Plicatula group of *Paspalum*. Two induced tetraploid plants were recovered from germinating 2x seeds treated for 24 h with colchicine. Bivalent and quadrivalent chromosomes were the most common association at meiosis in these autotetraploids. Embryological analysis and progeny tests using molecular markers revealed that both induced tetraploids reproduced sexually. Single-seed screening by flow cytometry confirmed full sexual reproduction. These plants retained the high self-incompatibility system of the diploids, but set seed after reciprocal crosses and when crossed with pollen of apomictic 4x *P. guenoarum* Arechav. of the Plicatula group. Both sexual 4x plants constitute the foundational material for plant improvement through gene exchange and selection in apomictic 4x *P. plicatulum* and possibly in several apomictic species of the Plicatula group.

M.E. Sartor, C.L. Quarin, and F. Espinoza, Instituto de Botánica del Nordeste (IBONE-CONICET), Facultad de Ciencias Agrarias, Univ. Nacional del Nordeste, Casilla de Correo 209 (3400), Corrientes, Argentina. Received 16 May 2008. *Corresponding author (espinoza@agr.unne.edu.ar).

Abbreviations: PMC, pollen mother cell; RAPD, random amplified polymorphic DNA; T, treatment.

MOST SPECIES OF THE GENUS *Paspalum* are naturally distributed throughout the tropical and subtropical regions of the New World and are major constituents of native rangeland in those regions. Chase (1929) grouped the species related to *P. plicatulum*, characterized by spikelets with transversely wrinkled lemmas and shining dark brown antherium, under the general name of Plicatula. The center of variation for this group is central-western Brazil, eastern Bolivia, eastern Paraguay, and northeastern Argentina. The large diversity observed in natural populations makes this group an extremely variable complex of species and forms, taxonomically not yet well defined. Several species of this group are promising candidates for use as fodder grasses in warm regions. In fact, some races of *P. guenoarum* (Ramírez, 1954), *P. plicatulum* (Oram, 1990), and *P. atratum* Swallen (Kretschmer et al., 1994; Quarin et al., 1997), three species of the Plicatula group, are cultivated for grazing in different regions of the world.

Paspalum plicatulum is a perennial grass species with a wide range of natural distribution from Georgia, Florida, and west to Texas, south to Argentina, and throughout the West Indies (Chase, 1929). Tetraploidy is the most common condition for this species, as reported by many authors and for materials from different geographical origins. Exceptional diploid accessions have been reported

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(summarized in Espinoza and Quarin, 1997). The common tetraploid races reproduce by apomixis and pseudogamy and are self-compatible (Burson and Bennett, 1971). Diploids reproduce sexually and are allogamous owing to self-incompatibility (Espinoza and Quarin, 1997).

This sexual-diploid–apomictic–polyploid condition constitutes a common genetic system for a large number of *Paspalum* species (Quarin, 1992) and, indeed, for a wide range of naturally apomictic, warm-season grasses. Apomixis in the polyploids is mostly facultative, though the expression of sexuality is usually much less important than apomixis. In spite of occasional sexual reproduction of a given genotype, a fully sexual 4x plant has never been found among wild tetraploid populations of any *Paspalum* species, although facultative apomictic genotypes with different capacity to develop meiotic embryo sacs have been observed in these species (Norrman et al., 1989; Burson, 1997). These genotypes generate both maternal (via apomixis) and nonmaternal (via sexuality) offspring. This phenomenon severely compromises genetic improvement by crossing and subsequent selection at the 4x level. Crossing is difficult since the progeny arises mainly by apomixis, and a preselection is required to separate maternal from nonmaternal descendants. Therefore, the existence of fully sexual plants at the 4x level is an important prerequisite for any breeding program in these polyploid apomictic grasses. Moreover, fully sexual tetraploid individuals can then be used as female parents in crosses with naturally apomictic 4x plants, allowing genetic studies of agronomic traits (including apomixis itself).

Colchicine-induced tetraploids have been obtained from sexual diploid strains in a small number of *Paspalum* species. Some induced tetraploids reproduced exclusively by sexual means (Forbes and Burton, 1961; Cáceres et al., 1999; Quarin et al., 2001), while in other cases the induced tetraploids were facultative apomictic (Quarin and Hanna, 1980; Quarin et al., 1998; Quarin et al., 2001). Therefore, in some cases duplicating the genomes apparently induces apomictic expression. The genetic basis underlying this conversion from sexuality to asexuality following a rise in the ploidy level is still not clearly understood. Consequently, the recovery of sexual 4x individuals from sexual diploids may result in unexpected complexities. Induction of polyploidy from sexual diploid plants was already used to develop ploidy-compatible, sexual plants that permit apomixis and so achieve genetic recombination in other warm-season forage grass genera, for example, *Brachiaria* (Gobbe et al., 1983) or *Panicum* (Savidan et al., 1989).

Our objective was to produce tetraploid sexual plants by doubling the chromosomes of sexual diploid *P. plicatulum* plants using colchicine. The recovery of sexual tetraploid genotypes is essential for the genetic improvement of the tetraploid races. The induced 4x sexual strains, used as female parents, would allow hybridization with naturally occurring

4x apomicts, since apomictic strains produce reduced pollen grains. Moreover, *P. plicatulum* belongs to a large group of taxonomically closely related species in which tetraploidy and apomixis are the rule. The recovery of a sexual 4x strain in *P. plicatulum* may allow gene exchange among most of these species and constitute an important base for plant improvement in these valuable forage grasses.

MATERIALS AND METHODS

Plant Material

Mature seeds from sexual diploid, open-pollinated plants of *P. plicatulum*, accession H-14, were used for chromosome doubling. The diploid level of this exceptional material was determined in a previous report (Honfi et al., 1990). Cuttings from seven individual plants of the original collection were supplied by Dr. Ana I. Honfi. Plants were potted and grown in a greenhouse at IBONE. Open-pollinated seeds were harvested from these plants. The most common condition for this species throughout the range of its natural distribution is tetraploidy and apomixis. The exceptional diploid accession H-14 was collected by Dr. Honfi at Candelaria, Misiones, Argentina.

Colchicine Treatment and Ploidy Determination

Caryopses were dissected from spikelets and placed in Petri dishes on wet Whatman paper at 27°C during 24 h to initiate germination. Following imbibition for 24 h on colchicine-free, wet paper, caryopses were transferred to similar germinators moistened with a fresh colchicine solution (0.1% w/v) plus 2% dimethylsulphoxide (DMSO) for 24 (treatment [T] 1), 48 (T2), and 72 (T3) hours. After the colchicine treatment, caryopses were washed with sterilized water and changed to Petri dishes without colchicine to allow germination to progress. Seedlings were individually transferred to pots with sterilized soil and maintained in the greenhouse. Chromosome number was determined in root-tip cells. Young roots were collected from potted plants, treated in a saturated solution of α -bromonaphthalene for 2 h and hydrolysed in 1000 mol m⁻³ HCl at 60°C for 10 min. Root tips were stained with 20 mol m⁻³ basic fuchsin, squashed with a drop of 46.6 mol m⁻³ aceto-carmin on slides and observed with a phase contrast microscope. Several rounds of chromosome counts and dissection of plants were needed to separate the tetraploid sectors in chimerical 2x/4x plants. Two tetraploid clones finally obtained were identified 4c-4x and 7d-4x, and their corresponding, isogenic diploids 4c-2x and 7d-2x.

Analysis of Meiosis

Inflorescences of induced tetraploid plants and their isogenic diploid clones were fixed in 3:1 solution (v/v) of absolute ethanol:acetic acid for 24 h and stored in 70% (v/v) ethanol at 4°C. Pollen mother cells (PMCs) were stained with aceto-carmin and observed with a phase contrast microscope.

Mode of Reproduction

The method of reproduction was determined by observing megasporogenesis and embryo sac development of induced 4x plants and their isogenic 2x clones. Inflorescences at different

stages of development were fixed in an FAA solution (10% ethanol, glacial acetic acid, and 37% formaldehyde in the ratio 18:1:1, v/v). Ovaries were isolated from flowers, dehydrated in a tertiary butyl alcohol series, embedded in paraffin and sectioned 12 µm thick. Then the material was stained in a safranin-fast green staining series and observed under bright-field microscopy.

Additionally, the method of reproduction of the induced 4x plants was confirmed by progeny tests and by flow cytometric seed screen following the methods described by Matzk et al. (2000).

For progeny tests two populations (22 plants each) were obtained by crossing: 7d-4x × 4c-4x, and 4c-4x × *P. guenoarum* cv. Rojas (apomictic tetraploid). Molecular markers (random amplified polymorphic DNA [RAPDs]) were used to analyze the progenies in relation to the genotype of the maternal 4x plant. The rationale was that specific bands of the induced 4x, maternal plants would segregate in their progeny if the plants had reproduced sexually, while a banding pattern in any individual progeny plant identical with that of the maternal parent would indicate an apomictic origin. Genomic DNA extraction and RAPD amplification were performed as indicated in Daurelio et al. (2004). Decamers used belonged to the University of British Columbia series 5 and 8.

Flow cytometry seed screen, following the methods described by Matzk et al. (2000), was also used on mature seeds from both induced, tetraploid plants to determine the relative DNA content within the embryo and the endosperm tissues, and so to confirm their reproduction mode. Since in this species of *Paspalum* apomixis is apospory followed by pseudogamy, the embryo/endosperm relative DNA content has different values when the seed is formed by sexual or by apomictic processes. This is because in most apomictic species of *Paspalum*, aposporous embryo sacs have two unreduced polar nuclei in the central cell. Then, if 2C is the DNA content of the embryo, the endosperm should be 3C in a seed produced sexually, while in a seed formed by apomixis, the embryo/endosperm DNA content ratio will

be 2C/5C. The central cell of the aposporous embryo sac has two unreduced (2n) polar nuclei, while the sperm nucleus (n) comes from meiosis. By cross-pollinations between both induced 4x plants, 20 freshly harvested caryopses from each induced 4x plant 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 DAPI staining buffer were 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 caryopsis) was used to determine fertility following self- or cross-pollination for each of the two induced 4x plants. Self-pollination was achieved by confining inflorescences within glassine bags before anthesis. For cross-pollination, fresh pollen was collected from different 4x genotypes or from other *Paspalum* species and dusted on stigmata at anthesis.

RESULTS AND DISCUSSION

Caryopses Treated with Colchicine

Effects of colchicine treatment of caryopses are indicated in Table 1. All seeds germinated after colchicine treatment, but most of them produced deformed, nonviable seedlings. Polyploidization was observed in only five of the 12 plants that survived. Following T1 (24 h), four plants were mixoploid (contained both 2x and 4x sectors). However, after several rounds of plant dissection followed by chromosome counting it was possible to recover two fully tetraploid plants (4%), which represented the foundational colchicine-induced tetraploid clones (generation C₀). These clones were identified as 4c-4x and 7d-4x. Diploid plants arising from these mixoploid seedlings (identified as 4c-2x and 7d-2x) were maintained to be used as controls in further experiments. These plants (4c-2x and 7d-2x) are the diploid clones isogenic to the induced autotetraploid 4c-4x and 7d-4x plants, respectively. Additionally, self-pollination of plant 7d-4x, produced a few seeds that were sufficient to establish the first generation of colchicine-induced tetraploids (generation S₁), identified as 7d-4xC1.

Following treatments of 48 and 72 h (T2 and T3), no 4x plants were recovered.

Table 1. Induction of 4x plants from 2x caryopses in *Paspalum plicatulum* by colchicine treatment (T).

Colchicine treatments [†]	Treated and germinated caryopses (no.)	Identification of surviving plants	Chromosome numbers		Identification of recovered 4x plants
			First count	Last count [‡]	
T1 = 24h	50	T1-1	20	20	
		T1-2	20	20	
		T1-3	20 + 40	20	
		T1-4	20 + 40	20	4c-2x
				40	4c-4x
		T1-5	20 + 40	20	
		T1-6	20	20	
		T1-7	20 + 40	20	7d-2x
		40	7d-4x		
T2 = 48h	50	T2-1	20	20	
		T2-2	20	20	
T3 = 72h	50	T3-1	20	20	
		T3-2	60 [§]		

[†]Colchicine solution (0.1% w/v) plus dimethylsulphoxide (DMSO; 2%).

[‡]After plant division by sectors.

[§]Plant died before flowering.

Surprisingly, the derived plant T3-2 presented $2n = 60$ chromosomes in the three root tips that were analyzed. Unfortunately, this plant died before a new round of chromosome counting was accomplished. It could have arisen from duplication of a triploid ($2n = 30$) embryo, which occurs rarely in natural diploid populations.

Polyploid plants are often larger and have larger organs than their diploid relatives (Stebbins, 1971), a general phenomenon commonly known as the “giga effect” of polyploidization. This type of morphological variation is often observed among natural diploid and tetraploid plants of some *Paspalum* species. However, when comparing natural diploid accession H-14 with the induced tetraploid plants their general morphological aspects appeared very similar. The exception was spikelet size: diploid spikelets averaged 2.69 mm long (standard error [SE] = 0.02) × 1.59 mm wide (SE = 0.03), and induced tetraploids averaged 3.22 mm long (SE = 0.04) × 1.96 mm wide (SE = 0.01) (Fig. 1). Thus, spikelet size is an informative characteristic to differentiate induced $4x$ from $2x$ sectors in chimerical plants at flowering.

Meiosis in Induced and Noninduced Plants

Meiotic chromosome configurations at diakinesis and metaphase I of *P. plicatulum* involving three induced tetraploid plants: 4c-4x, 7d-4x, and 7d-4xC1, and two diploids: 4c-2x (isogenic to 4c-4x) and 7d-2x (isogenic to 7d-4x) are shown in Table 2. Meiotic chromosome behavior was regular in both diploid plants, showing 10 bivalents (Fig. 2A). These results agreed with those observed in the original diploid plant for this species (accession H-14) used as source of mature seeds for chromosome doubling (Espinoza and Quarin, 1997). On the other hand, most chromosomes of the induced $4x$ plants associated as bivalents and quadrivalents at diakinesis and metaphase of the first meiotic division (Fig. 2B). Univalents and trivalents were occasionally observed. Tetraploidy is the most common condition in naturally occurring populations of *P. plicatulum*. However, diploid races have been reported occasionally (Fernandes et al., 1974; Davidse and Pohl, 1972; Honfi et al., 1990). The existence of $2x$ and $4x$ races suggests a logical autopoloid origin of tetraploids. Chromosome pairing at meiosis may substantiate this assumption if tetraploid races usually show quadrivalent association. However, the published data are somewhat conflicting on this subject. Pohl and Davidse (1971) observed in several $4x$ accessions of *P. plicatulum* from Costa Rica that the most common configuration was 12 II + 4IV, and the authors concluded that this might indicate a recent segmental allopolyploid origin. A similar conclusion of segmental genome homology was stated by Burson and Bennett



Figure 1. Spikelets of diploid (left) and induced tetraploid plants (right) of *Paspalum plicatulum*. Bar = 1 mm.

(1971), though they observed predominantly bivalent chromosome pairing and an occasional quadrivalent, in several $4x$ *P. plicatulum* accessions. Fernandes et al. (1974) observed irregular meiotic chromosome behavior with mainly bivalent and quadrivalent associations (maximum of 7 quadrivalents per cell) in Brazilian $4x$ accessions and inferred an autotetraploid origin for $4x$ *P. plicatulum*. The number of quadrivalent chromosome associations that we observed in our induced autotetraploid material was similar to the range observed by Pohl and Davidse (1971), Davidse and Pohl (1974), and Fernandes et al. (1974) in naturally occurring $4x$ material from a wide range of geographic locations. Furthermore, the average number of quadrivalents per cell (2.8) of one of our induced $4x$ plants (4c-4x) was less than that reported for some natural tetraploid races (Burson and Bennett, 1971). This similarity in meiotic behavior between induced autotetraploids and natural tetraploids suggests that the naturally occurring tetraploid races of *P. plicatulum* originated by autopoloidy. The low number of quadrivalents reported by Burson and Bennett (1971) for several introduced tetraploid accessions (range 0.17 to 1.5 per cell) may be attributed to personal interpretation of the observed chromosome associations or may be indicative of a higher degree of diploidization

Table 2. Meiotic chromosome associations at diakinesis and metaphase I in pollen mother cells (PMCs) for colchicine-induced $4x$ plants of *Paspalum plicatulum* and the original isogenic $2x$ clones. I, univalents; II, bivalents; III, trivalents; IV, quadrivalents.

Plant number	$2n$	PMCs no.	Chromosome associations									
			Average per PMC				Range per PMC					
			I	II	III	IV	I	II	III	IV		
4c-2x	20	60		10.0								
7d-2x	20	75		10.0								
4c-4x	40	53	0.06	14.2	0.06	2.8	(0-1)	(8-18)	(0-1)	(1-7)		
7d-4x	40	71	0.4	10.12	0.22	4.66	(0-3)	(4-18)	(0-2)	(1-9)		
7d-4xC1	40	46	0.4	9.43	0.21	5.02	(0-2)	(2-16)	(0-2)	(1-9)		

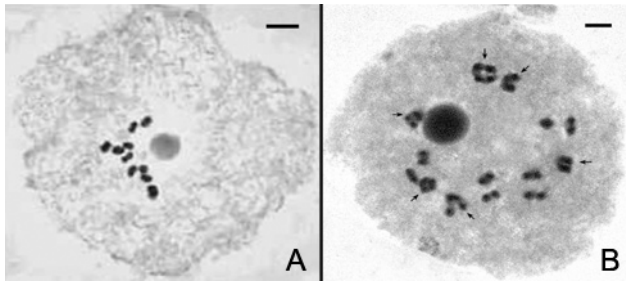


Figure 2. Meiotic chromosome associations at diakinesis of *Paspalum plicatulum*. (A) noninduced 2x plant showing 10 bivalents; (B) colchicine-induced 4x plant indicating eight bivalents plus six quadrivalents (arrows). Bar = 10 µm.

in those particular tetraploid accessions. On the basis of reported cytological analyses, autopolyploidy has been suggested as the most likely origin of those apomictic tetraploid *Paspalum* species that have sexual diploid co-specific races (Norrman et al., 1989; Quarin, 1992). Autopolyploidy was also confirmed by genetic analyses, on the basis of tetrasomic inheritance, in *P. simplex* Morong (Pupilli et al., 1997) and in *P. notatum* Flügge (Stein et al., 2004).

Mode of Reproduction

Embryological studies on induced tetraploid and isogenic diploid plants confirmed that all reproduced sexually and that both megasporogenesis and megagametogenesis were normal. Products of meiotic divisions presented a linear tetrad of megaspores. A meiotic embryo sac developed from the functional megaspore after 3 rounds of mitotic divisions to form the egg apparatus (egg cell + two synergids), the binucleated central cell, and three antipodal cells. Additional rounds of mitotic divisions form a mass of antipodal cells, which are typical of most sexual reproducing grasses (Fig. 3). A total of 109 or 85 mature ovules were analyzed for induced plants 4c-4x or 7d-4x, respectively. All of them presented the typical meiotic embryo sac, and the same feature was observed in the 2x plants 4c-2x and 7d-2x, isogenic to induced 4x plants

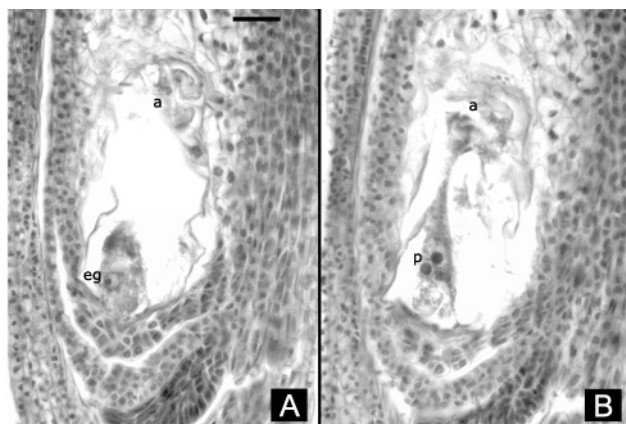


Figure 3. Meiotic embryo sac in the colchicine-induced *Paspalum plicatulum* plant 4c-4x. Two consecutive sections of the same mature ovule showing: (A) the egg cell (eg) and antipodal cells (a); (B) vacuolated central cell with two polar nuclei (p) and antipodal cells (a). Bar = 20 µm.

4c-4x and 7d-4x. These findings suggest that the mode of reproduction was preserved after the induction of polyploidy. A similar behavior had already been observed for induced tetraploid plants of other *Paspalum* species (Burton and Forbes, 1960; Cáceres et al., 1999), though facultative apomictic tetraploids were produced by chromosome doubling of sexual diploid *P. hexastachyum* Parodi (Quarin and Hanna, 1980). Similarly, one completely sexual tetraploid and two facultative apomictic plants were obtained for *P. notatum* after chromosome doubling of different diploid genotypes (Quarin et al., 2001). Considering these previous results, additional experiments were outlined to confirm that reproduction of induced 4x plants of *P. plicatulum* was exclusively sexual.

Random amplified polymorphic DNA-based progeny tests for both C_0 induced plants, 4c-4x and 7d-4x, were used to corroborate their modes of reproduction. Informative oligonucleotides in parental plants were identified. Forty oligonucleotides were analyzed and only two failed to amplify products. Of the remaining 38, 20 were polymorphic between the parents. Finally, five primers (BC710, BC711, BC726, BC730, and BC744) that generated a total of eight male parent bands and produced clear and reproducible patterns were selected to perform the molecular studies on the progenies. In addition, absence of monomorphic bands in the progeny was also considered, since it would indicate occurrence of recombination. Fingerprints obtained with the selected primers revealed that neither progeny (22 individuals from each induced 4x plant) contained any individual with a genotype identical to its maternal parent. Therefore, exclusive sexual reproduction was demonstrated for both induced 4x plants. To avoid the possible inaccuracy that might occur if some B_{III} hybrid ($2n + n$) was produced owing to fertilization in some occasional aposporous embryo sac, chromosome counting was performed on each of the 22 individuals in each of the two progenies. All proved to be tetraploids ($2n = 40$) and hence all products of $n + n$ fertilization processes. These results confirm the sexual reproductive behavior for these induced 4x plants. Since one progeny arose from an interspecific cross—induced tetraploid *P. plicatulum* 4c-4x \times *P. guenoarum* cv. Rojas—these results indicated that the entire progeny originated from interspecific hybridization. *Paspalum guenoarum* is a member of the group Plicatula established by Chase (1929) as an informal taxonomic subgeneric category within *Paspalum*. The Plicatula group consists of several apomictic tetraploid species, most of which are superior feed supplies for cattle in native American Tropics rangeland. Our results suggest that it may eventually be feasible to access some or even all of the genetic variation contained in the Plicatula group species in future plant breeding programs.

Additionally, flow cytometry was used to determine the proportional DNA content of embryo and endosperm nuclei as an indication of the pathway of reproduction. As expected, seed samples of the two induced 4x plants yielded a

high 2C peak for the embryo and a smaller 3C peak corresponding to the endosperm. Additional 4C and 6C peaks were produced by embryo and endosperm nuclei at the G2 stage of the cell cycle (Fig. 4). This ratio characterizes sexual reproduction in seed plants (Matzk et al., 2000). The 2C embryo peak and the 3C endosperm peak are consistent with fertilization of the egg cell and the central cell (bearing two nuclei) of reduced embryo sacs with reduced male gametes. These findings corroborate that both induced 4x plants, 4c-4x and 7d-4x, are fully sexual.

Fertility

Seed set on induced (4c-4x, 7d-4x) and noninduced (4c-2x, 7d-2x) plants was analyzed for both self- and cross-pollination (Table 3). Seed set from self-pollination was extremely low (0.8 and 4.5%) in the two noninduced plants (4c-2x and 7d-2x). These results were similar to those observed in natural diploid accession H-14 plants (2.2%) as reported by Espinoza and Quarin (1997). A similar result was observed in the induced 7d-4x (C₀) plant with a very low seed set (3.6%). On the other hand, the autotetraploid plants, 4c-4x (C₀) and 7d-4xC1, failed to develop seed following self-pollination. Additional mature self-pollinated spikelets were analyzed in the following summer season, and absolute self-sterility was confirmed for the induced 4x plant 4c-4x.

When diploid plants were crossed reciprocally, seed set of 59.2% (on 4c-2x) or 48.3% (7d-2x) was obtained. The isogenic tetraploids 4c-4x and 7d-4x set seed after reciprocal crosses, though their fertility (24.7 or 11.4, respectively) was much lower than that of their isogenic diploid counterparts. The induced 4c-4x plant was further evaluated for seed set following pollination with each two different accessions of *P. guenoarum*, a species that belongs to the same group Plicatula as *P. plicatulum*. When the pollen donor was *P. guenoarum* cv. Rojas or accession Q4108, seed was formed by 35.1, or 17.9%, of spikelets, respectively (Table 3). Moreover, seed set was assessed on some individual interspecific F₁ hybrids, and it ranged between 28 and 55% (data not shown). These results indicated that it will be feasible to obtain F₂ and backcross populations in future breeding programs or genetic studies involving at least these two Plicatula group species and perhaps others.

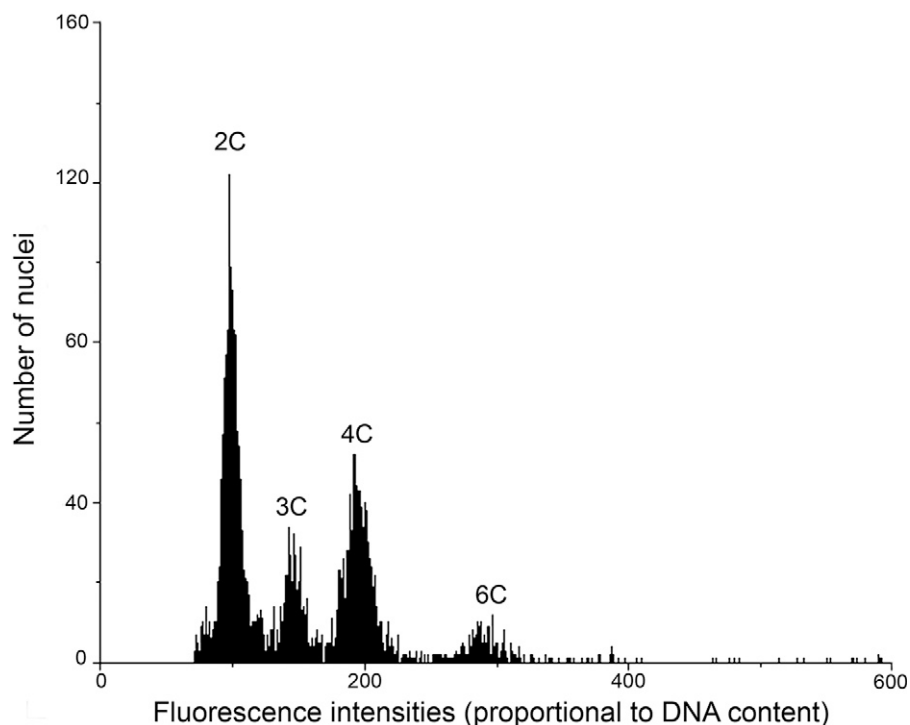


Figure 4. Flow cytometry peaks indicating an embryo to endosperm DNA content ratio of 2:3, demonstrating the occurrence of sexually formed seeds in induced 4c-4x plants. Coefficients of variation: 2C = 6.5%; 3C = 6.6%; 4C = 4.9%; 6C = 4.5%.

CONCLUSIONS

This study describes the development of the first tetraploid genotypes of *P. plicatulum* that reproduce exclusively by sexual means. In the wild, tetraploid and apomictic *P. plicatulum* is the most common and widely distributed, though rare sexual diploid strains have been reported. Sexual tetraploid individuals have never been found in natural

Table 3. Seed set (proportion of spikelets that formed caryopses) following self- or cross-pollination in noninduced (2x) and induced (4x) plants of *Paspalum plicatulum*.

Pollination procedure	2n	Scored spikelets (no.)	Spikelets with caryopses (no.)	Seed set
Self-pollination				
4c-2x	20	976	8	0.8
7d-2x	20	595	27	4.5
4c- 4x (2005)	40	1331	0	0.0
4c- 4x (2006)	40	2461	0	0.0
7d-4x	40	504	18	3.6
7d-4xC1	40	490	0	0.0
Cross-pollination				
Intraspecific crosses				
4c-2x × 7d-2x	20	579	343	59.2
7d-2x × 4c-2x	20	951	459	48.3
4c-4x × 7d-4x	40	1535	379	24.7
7d-4x × 4c-4x	40	1035	118	11.4
Interspecific crosses				
4c-4x × <i>P. guenoarum</i> cv. Rojas (4x)	40	555	195	35.1
4c-4x × <i>P. guenoarum</i> Q4108 (4x)	40	251	45	17.9

populations of either *P. plicatum* or other tetraploid species of the Plicatula group that are apomictic, in other words, *P. guenoarum*, *P. compressifolium* Swallen, *P. nicorae* Parodi, *P. atratum* Swallen, or *P. glaucescens* Hack. Most species of the Plicatula group are valuable forage grasses of native grasslands in South America. Some 4x apomictic species have been brought into cultivation in different regions of the world, in other words, *P. atratum* (Kretschmer et al., 1994; Quarin et al., 1997), *P. guenoarum* (Ramírez, 1954), and *P. plicatum* (Oram, 1990). Tetraploid apomictic *Paspalum* spp. usually produce fully functional, reduced pollen following meiotic cell divisions. The sexual tetraploids *P. plicatum* plants obtained in this study open the way for plant breeding programs involving hybridizations, gene exchange, plant selection, and manipulation of apomixis, accessing the genetic variation contained in naturally occurring apomictic tetraploid *P. plicatum* races and probably several other tetraploid apomictic species in the Plicatula group. The exploitation of these newly acquired sexual tetraploid clones of *P. plicatum* will facilitate new approaches in the study of the biology and genetics of the Plicatula group, including studies of the inheritance of apomixis.

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