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ORIGINAL ARTICLE

Evaluation of meiotic abnormalities and pollen viability in aposporous and sexual tetraploid *Paspalum notatum* (Poaceae)

Maricel Podio · Lorena A. Siena · Diego Hojsgaard · Juliana Stein · Camilo L. Quarin · Juan Pablo A. Ortiz

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Abstract We analyzed anaphase I configurations and pollen viability in aposporous and sexual tetraploid ($2n = 4x = 40$) cytotypes of *Paspalum notatum*. Five natural aposporous accessions and three experimentally obtained sexual individuals were used. In addition, 16 (8 aposporous and 8 sexual) F_1 hybrids, previously classified by their mode of reproduction, were analyzed. Cytogenetic observations revealed normal and abnormal anaphase I configurations in both aposporous and sexual genotypes. Anaphase I abnormalities were mainly laggard chromosomes, chromatin bridges, and micronuclei. On average, 44.36 % of aposporous meiocytes and 29.66 % of sexual ones showed abnormal anaphase I configurations. The total numbers of normal and abnormal anaphase I were highly significantly different between aposporous and sexual strains. The pollen viability test indicated that aposporous individuals had significantly more non-viable pollen than sexual ones; a positive correlation ($r = 0.71$; $r^2 = 0.50$) between the variables was detected. Analysis of aposporous and sexual hybrids confirmed differences in the numbers of normal and abnormal anaphase I patterns in the aposporous and sexual parents. However, similar proportions of viable pollen were produced by both groups of hybrids. In this case, the variables were not correlated ($r = 0.23$; $r^2 = 0.05$). Data from this study indicated that aposporous strains had a genetic rearrangement affecting meiosis that was absent in the experimentally obtained sexual individuals and that it was transmitted to the progeny. The possible association between meiotic abnormalities and the inheritance of apospory is discussed.

Keywords Anaphase I · Apospory · Apomixis · Meiotic abnormalities · Pollen viability

M. Podio · L. A. Siena · J. Stein · J. P. A. Ortiz (✉)
Laboratorio de Biología Molecular, Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, Campo Experimental J. Villarino CC 14, S2125ZAA Zavalla, Santa Fe, Argentina
e-mail: jortiz@unr.edu.ar

M. Podio · C. L. Quarin · J. P. A. Ortiz
Instituto de Botánica del Nordeste (IBONE-CONICET), Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste, CC209, 3400 Corrientes, Argentina

D. Hojsgaard
Department of Systematic Botany, Albrecht-von-Haller Institute of Plant Sciences, Georg-August-University of Göttingen, Untere Karspüle 2, 37073 Göttingen, Germany

Introduction

Paspalum notatum Flüggé (Bahia grass) is a perennial rhizomatous forage grass native to South America and distributed from México to Argentina (Burton 1967). The species is widely naturalized in the warm and humid regions of the western hemisphere and is considered a multiploid complex, including several naturally occurring cytotypes with chromosome numbers $2n = 2x = 20$, $3x = 30$, $4x = 40$, and $5x = 50$ (Burton 1946; Tischler and Burson 1995; Gates et al. 2004). The diploid form (*P. notatum* var. *saurae* Parodi) is sexually and meiotically stable with ten bivalents at meiosis. The tetraploid cytotype, considered the typical botanical morph of the species, is apomict and self-fertile (Burton 1948; Quarin 1992). Apomixis in *P. notatum* is characterized by the presence of aposporous embryo sacs with cytological unreduced nuclei, derived from somatic cells of the nucellus after a series of mitosis. Aposporous sacs present two large polar nuclei in a widely vacuolated central cell, the egg cell, one or two

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synergids, and the absence of antipodals (Martínez et al. 2001). Embryos develop by parthenogenesis from the 2*n* egg cell, and the endosperm is formed after the fertilization of the polar nuclei (pseudogamy) (Martínez et al. 2001). Since apomicts produce viable (and reduced) pollen, the trait can be transferred to a progeny if they are used as male parents in crosses to sexual or facultative apomictic plants. Based on their chromosome-pairing behavior at meiosis and the chromosome configurations in hybrids with the diploid form, tetraploids are considered autopolyploids (Forbes and Burton 1961; Quarin et al. 1984). However, much variation in meiotic chromosome associations and pollen fertility has been detected among accessions (Dahmer et al. 2008). Completely sexual tetraploid plants have never been found in nature, although residual sexuality (i.e., the capacity of apomicts to eventually produce some descendants by a sexual process) has been frequently observed in natural accessions (Martínez et al. 2001; Espinoza et al. 2006; Rebozzio et al. 2011).

Exclusively sexual tetraploid plants were experimentally generated after doubling the chromosome complement of sexual diploids or by crossing a highly sexual tetraploid genotype with a naturally apomictic accession (Quarin et al. 2001, 2003). These individuals generated intra-specific tetraploid populations segregating for the mode of reproduction that were then used to study the inheritance of apospory and to characterize the genomic region responsible for the trait (Martínez et al. 2001, 2003; Stein et al. 2004). Genetic analysis showed that apospory was inherited as a dominant locus with a distorted segregation ratio (Martínez et al. 2001). In all crosses performed between sexual and aposporous individuals, a lower-than-expected number of aposporous progeny was obtained (Martínez et al. 2001; Stein et al. 2004). The excess of apospory-free (sexual) plants was attributed to a pleiotropic lethal effect with incomplete penetrance of the apospory allele, or to the partial lethality of a factor (or factors) linked to aposporous genes that causes gamete death when homozygous (Martínez et al. 2001). Experimental crosses of sexual diploids and tetraploids with an apomict triploid plant demonstrated that apospory could only be transmitted by pollen through diploid or hypodiploid gametes (Martínez et al. 2007). The presence of a lethal factor associated with the apospory allele(s) was first proposed by Nogler (1984) in *Ranunculus auricomus*, a species with aposporous apomixis. He reported that apomixis is monogenic and dominant, and it could only be transmitted in the heterozygous state through diploid or polyploid gametes (Nogler 1984). Genetic analyses with molecular markers has also revealed that the apospory locus in *P. notatum* was located in a chromosome block that showed restriction in recombination and preferential chromosome pairing (Martínez et al. 2003; Stein et al. 2004, 2007).

Cytogenetic examinations during microsporogenesis of the completely sexual tetraploid genotype Q4188 and the aposporous natural accession Q4117, parents of a mapping population segregating for apospory, showed that meiotic abnormalities characterize anaphase I of Q4117 (Stein et al. 2004). These abnormalities were attributed to genetic rearrangements in one chromosome of the apomictic parent. Because the homologous chromosomes of one bivalent frequently remained tightened beyond metaphase I or had delayed separation, a paracentric inversion located close to the centromere has been implicated in this behavior (Stein et al. 2004). Moreover, based on a comparative mapping approach, Pupilli et al. (2004) found that a translocation could be involved in the chromosome segment carrying the apomixis factors. The presence of an inversion or translocation in the apomictic parent (Q4117) could explain both the distorted segregation ratio of apospory, probably caused by differential survival of meiocytes carrying the control for the trait, and the suppression of recombination near the apospory-locus observed in the species.

The objectives of this work were: (1) to evaluate the meiotic behavior of chromosomes in anaphase I and pollen viability in natural aposporous accessions and in experimentally obtained completely sexual tetraploid plants, and (2) to determine whether meiotic abnormalities observed during microsporogenesis are transmitted to progeny associated with aposporous reproduction.

Materials and methods

Plant material

Five natural tetraploid aposporous accessions and three completely sexual experimentally generated tetraploid genotypes were used (Table 1). The mode of reproduction of each plant was previously determined by examining sectioned ovules at anthesis that had been stained with safranin and fast green, or by inspection of intact embryo sacs using interference contrast microscopy on methyl-salicylate-cleared ovaries, according to Young et al. (1979) (Table 1). In addition, 16 (8 aposporous and 8 sexual) F_1 plants derived from a cross between the parental plants Q4188 (sexual) and Q4117 (aposporous) were analyzed. These F_1 hybrids were part of a larger mapping population segregating for apospory that was previously used to determine the mode of inheritance of tetraploid races and to construct a genetic linkage map of the species at the tetraploid level (Stein et al. 2004, 2007). The mode of reproduction of each progeny had been previously determined by analyzing molecular markers completely linked to apospory and then by examining the embryo sacs at anthesis (Stein et al. 2004). All plants are kept in the live

Table 1 Identification, origin and mode of reproduction of tetraploid ($2n = 4x = 40$) accessions and experimentally obtained sexual genotypes of *Paspalum notatum*

Accession identification	Origin (collection area or source)	Mode of reproduction
Q3775	Tamaulipas, Mexico	Apomict[a]
Q3776	Villa Tunari, Chapare region, Bolivia	Apomict[a]
Q4012	Tres Lagoas, Brazil	Apomict[b]
Q4117	State of Rio Grande do Sul, Brazil	Apomict[a]
N160	Pedro Juan Caballero, Amambay, Paraguay	Apomict[b]
Q4188	Experimental	Sexual[c]
Q4205	Experimental	Sexual[c]
C4–4x	Experimental	Sexual[d]

[a] Martínez et al. (2001),
[b] Espinoza et al. (2006),
[c] Quarin et al. (2003), and
[d] Quarin et al. (2001)

germplasm collection of the Instituto de Botánica del Nordeste (IBONE), Corrientes, Argentina, and Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, Argentina.

Cytogenetic and pollen viability analysis

Young inflorescences were collected and fixed in 3:1 ethyl alcohol:acetic acid for at least 24 h at room temperature, transferred to 70 % (v/v) aqueous alcohol solution and stored at −20 °C until use, according to Quarin and Burson (1983). Paternal meiocytes (PMCs) were prepared by squashing the anthers and staining with 45 % acetic carmine. Anaphase I configurations were determined in at least 30 meiocytes per plant. Chromosome numbers of F_1 plants were determined at metaphase I and anaphase I. Permanent slides were prepared using Venetian turpentine.

Pollen viability was estimated by staining pollen grains at anthesis with Alexander's reagent (Alexander 1980). Pollen grains stained purple were considered potentially viable, and those pale-green or non-colored were considered non-viable. A minimum of 900 mature pollen grains, from at least five flowers per plant, were scored. All observations were carried out using a light transmission microscope.

Comparisons of the numbers of normal and abnormal anaphase I configurations as well as viable and non-viable pollen between sexual and aposporous individuals were evaluated by using chi-square tests. The relationship between the proportions of abnormal anaphases and non-viable pollen was evaluated using a simple correlation coefficient (r). Proportions were arcsin transformed before analysis. P values lower than 0.05 were considered significant. The statistical package Infostat (http://www.infostat.com.ar) was used to analyze data.

Cytoembryological analysis of F_1 hybrids

The reproductive behavior of each F_1 plant was quantitatively evaluated by cytoembryological observations as described in Martínez et al. (2001). Inflorescences were collected at anthesis and fixed in FAA (70 % ethanol, glacial acetic acid, formaldehyde, 90:5:5) for 24–48 h. Pistils were dehydrated in a tertiary butyl alcohol series and embedded in paraffin. Samples were sectioned at 12–15 µm and stained with safranin and fast green. Observations were carried out with a light transmission microscope. Individuals with ovules bearing only a single meiotic embryo sac (of the *Polygonum* type), characterized by the egg apparatus (egg cell and two synergids), a large two-nucleated central cell, and a mass of proliferated antipodals at the chalazal end, were registered as sexual (non-aposporous). In contrast, hybrids were classified as apomictic when at least some ovules contained one or several embryo sacs characterized by two large polar nuclei in a widely vacuolated central cell, the egg cell, and one or two synergids and by the absence of antipodals. The proportion of aposporous embryo sacs in each plant was determined by analyzing 25–30 ovules per plant.

Results

Anaphase I configurations and pollen viability in aposporous and sexual tetraploid *Paspalum notatum*

Anaphase I configurations during microsporogenesis of tetraploid *P. notatum* were analyzed in five natural aposporous accessions and three experimentally obtained sexual tetraploid plants. In total 335 and 124 meiocytes from aposporous and sexual individuals, respectively, were scored (Table 2). On average, 55.6 and 70.3 % of the PMCs from aposporous and sexual plants, respectively, showed normal anaphase I configurations with homologous chromosomes migrating toward opposite poles (Fig. 1a). However, meiotic abnormalities, such as lagging chromosomes, early chromatid separation of lagging univalents, lagging homologous chromosome pairs due to delayed separation, and chromatin bridges between bivalents, were observed (Fig. 1b–f). The overall percentages of meiocytes with anaphase I abnormalities were 44.4 % in aposporous and 29.7 % in sexual individuals (Table 2). At telophase I,

Table 2 Anaphase I configurations, pollen viability and mode of reproduction of aposporous and sexual tetraploid *Paspalum notatum*

Accession	Meiocytes analyzed	Meiocytes with normal anaphase (%)	Meiocytes with abnormal anaphase				Pollen viability (%)	Ovules bearing AES (%)
			Total (%)	Laggard chromosomes	Chromatin bridges	Micronuclei		
Q3775[a]	93	62 (66.7)	31 (33.3)	16	2	13	73.6	87.0[c]
Q3776[a]	32	14 (43.8)	18 (56.2)	11	1	6	Nd	85.0[c]
Q4012[a]	126	67 (53.2)	59 (46.8)	12	18	29	61.2	100.0[d]
Q4117[a, g]	40	24 (60.0)	16 (40.0)	9	–	7	64.4	92.0[c]
N160[a]	44	24 (54.5)	20 (45.5)	6	6	8	59.6	50.0[d]
Q4188[b, g]	40	28 (70.0)	12 (30.0)	7	–	5	77.6	0.0[e]
Q4205[b]	40	30 (75.0)	10 (25.0)	8	1	1	61.2	0.0[e]
C4–4x[b]	44	29 (66.0)	15 (34.0)	14	1	–	79.1	0.0[f]

AES Aposporous embryo sacs

[a] Aposporous, [b]sexual embryo sacs, [c–f]according to Martínez et al. (2001), Espinoza et al. (2006), Quarin et al. (2001, 2003), respectively [g]Parents of the F_1 population segregating for apospory developed by Stein et al. (2004)

both aposporous and sexual plants had chromosomes grouped at the poles, although micronuclei were detected. Some micronuclei were remarkably small, suggesting that they comprised chromosome fragments (Fig. 1g, h). In particular, aposporous individuals Q3775 and Q4012 showed the highest numbers of laggard chromosomes and micronuclei, and accession Q4012 had the most PMCs with chromatin bridges (Table 2). Laggard chromosomes were also observed in sexual plants, but only two chromatin bridges were detected in the whole group (Table 2). Interestingly, the laggard chromosomes in sexual genotypes were dispersed in the cytoplasm, but numerous laggards in the aposporous strains were face-to-face and located centrally in the cell, indicating retarded separation of a bivalent (Fig. 1i). The proportions of abnormal meiocytes in Q4117 and Q4188 were similar to values reported previously by Stein et al. (2004), indicating that this meiotic behavior is characteristic of each genotype.

The total numbers of meiocytes with normal and abnormal anaphases I in aposporous and sexual individuals were significantly different ($\chi^2 = 6.55$; $P = 0.01$). These results indicated that the aposporous strains might contain a genetic rearrangement affecting meiosis that was absent in the experimentally obtained sexual genotypes.

Because chromosome rearrangements could compromise pollen viability (Quillet et al. 1995; Madlung et al. 2005), viable pollen production was quantitatively evaluated in both groups of plants. A total of 7,677 and 6,201 pollen grains were scored in the aposporous and sexual plants, respectively. On average, 64.7 % of pollen from aposporous accessions and 72.6 % of pollen from sexual genotypes was viable (Table 2). The total number of viable and non-viable pollen grains was highly significantly different between aposporous and sexual groups ($\chi^2 = 115$; $P < 0.001$). The correlation between meiotic abnormalities and pollen viability was evaluated by estimating the correlation coefficient (r) between the frequency of meiocytes with abnormal anaphase I and the frequency of non-viable pollen. The variables were positively correlated in aposporous accessions ($r = 0.71$; $r^2 = 0.50$) and negatively correlated ($r = -0.93$; $r^2 = 0.86$) in sexual genotypes.

Analysis of F_1 hybrids derived from Q4188 × Q4117

To examine the anaphase I configurations of hybrids with different modes of reproduction, a group of 16 F_1 (8 sexual and 8 apomicts) plants derived from a cross between the completely sexual genotype Q4188 (used as pistillate-parent) and the apomictic accession Q4117 (used as pollen-donor) were analyzed. Meiotic behavior at metaphase I showed the typical chromosomes associations of autopolyploids, as univalents, bivalents, trivalents, and quadrivalents (Fig. 2a, b and c). The chromosome counts of each F_1 plant in metaphase I or anaphase I confirmed the presence of 40 chromosomes in each hybrid (Fig. 2a–f).

The reproductive mode of each F_1 hybrid was corroborated by quantitatively evaluating the ovules carrying aposporous and meiotic embryo sacs. Aposporous hybrids averaged 80.65 % ovules with aposporous embryo sacs (range 65–95 %), while sexual plants always had ovaries with a single *Polygonum*-type embryo sac (Table 3).

Anaphase I analyses were performed on 322 and 275 meiocytes from aposporous and sexual hybrids, respectively (Table 3). Aposporous F_1 plants averaged 72.0 % abnormal anaphases I, while sexual plants averaged 41.5 % (Table 3). Interestingly, aposporous F_1 progeny had more abnormalities than either their aposporous progenitor (Q4117) or the other natural accessions evaluated in this work. The total numbers of normal and abnormal anaphase I configurations were highly significantly different between

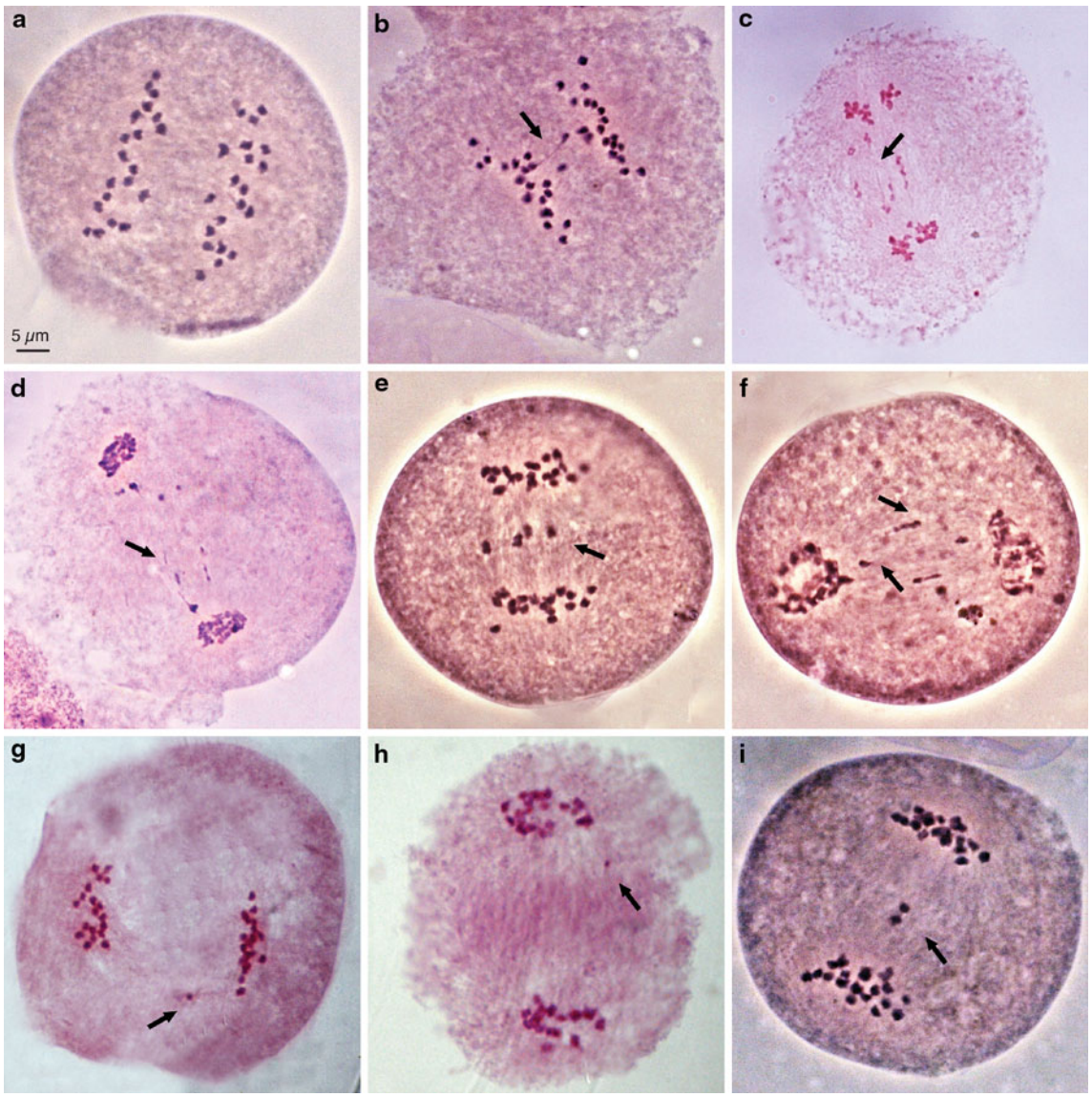

Fig. 1 Meiotic behavior in aposporous and sexual tetraploid *Paspalum notatum*. **a** Anaphase I in sexual genotype Q4188 showing normal chromosome segregation (20:20); **b** anaphase I with a chromatin bridge between bivalents (*arrow*) in apomictic accession Q4117; **c** and **d** early telophase I with laggard chromosomes and chromatin bridges (*arrow*) in apomictic accession Q4012; **e** and **f** anaphase I and early telophase I with laggard chromosomes (*arrow*) in apomictic accession Q3776; **g** and **h** meiocytes at early telophase I showing micronucleus (*arrow*) in apomictic accession Q3775; **i** anaphase I with two laggard chromosomes located face-to-face in the central part of the cell in the apomictic accession Q3775

aposporous and sexual F_1 hybrids ($\chi^2 = 73.0$; $P < 0.001$). This outcome suggested that aposporous hybrids probably inherited the genetic rearrangement observed in the apomictic parent, and therefore they present an increment in the number of meiotic abnormalities with respect to sexual hybrids.

Pollen viability of F_1 plants was scored in 10,516 and 13,556 pollen grains from aposporous and sexual hybrids, respectively. On average, aposporous and sexual hybrids produced 76.3 and 76.6 % viable pollen, respectively. The differences between groups in the total numbers of viable and non-viable pollen were non-significant ($\chi^2 = 1.8$; $P < 0.179$). The proportions of abnormal anaphases I and non-viable pollen were not significantly correlated in either aposporous ($r = 0.23$, $r^2 = 0.05$) or sexual ($r = -0.68$; $r^2 = 0.46$) hybrids.

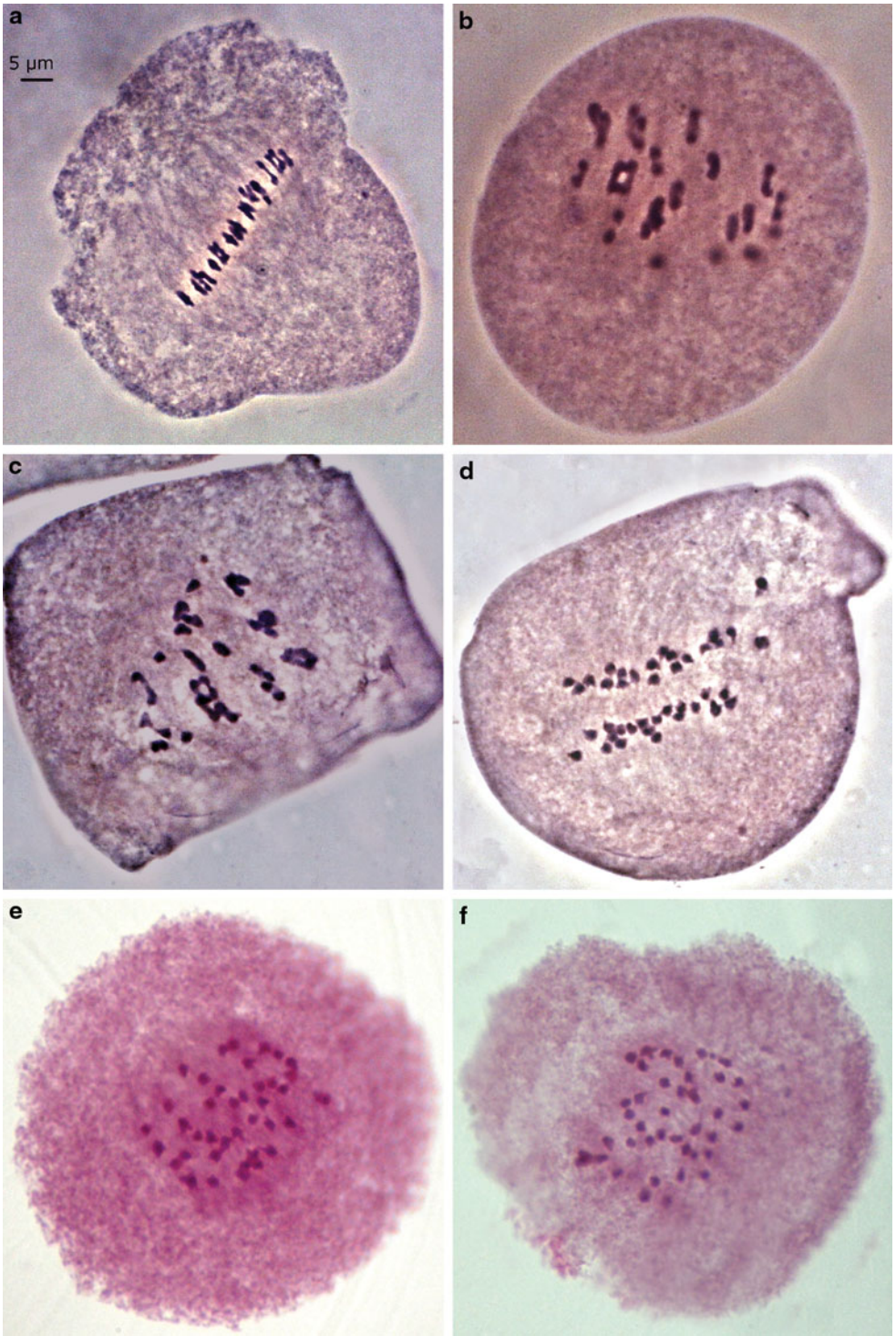

Fig. 2 Cytogenetic analysis of F_1 hybrids of *Paspalum notatum*. **a** Metaphase I showing 40 chromosomes arranged in bivalent and multivalent configurations aligned in the equatorial region in apomictic hybrid 112; **b** and **c** metaphase I with chromosomes associated as univalents, bivalents, trivalents, and quadrivalents (hybrid 112); **d** early anaphase I in sexual hybrid 77 showing 40 chromosomes at the equatorial region and 20 chromosomes moving to each cell pole (*laterally squashed cell*); **e**, **f** anaphases I from the F_1 plants 19 (aposporous) and 1 (sexual) showing 40 chromosomes, respectively (*pole to pole squashed cells*)

Table 3 Analysis of anaphase I configurations, pollen viability, and percentage of aposporous embryo sac in *Paspalum notatum* F_1 hybrids

Hybrid	Meiocytes analyzed	Normal anaphase (%)	Abnormal anaphase				Pollen viability (%)	Ovules bearing AES[c] (%)
			Total (%)	Laggard chromosomes	Chromatin bridges	Micronuclei		
9[a]	33	8 (24.2)	25 (75.8)	9	4	12	78.0	90.0
19[a]	94	18 (19.1)	76 (80.9)	48	1	27	78.1	80.0
23[a]	32	8 (25.0)	24 (75.0)	11	0	13	66.1	85.0
27[a]	58	24 (41.4)	34 (58.6)	7	4	23	78.1	80.0
40[a]	13	6 (46.2)	7 (53.8)	1	1	5	–	70.0
74[a]	39	10 (25.6)	29 (74.3)	22	2	5	73.7	65.0
86[a]	19	6 (31.6)	13 (60.4)	5	3	5	84.7	80.0
112[a]	34	4 (11.8)	30 (88.2)	15	8	7	75.8	95.0
1[b]	53	25 (47.1)	28 (52.8)	26	2	0	75.0	0.0
5[b]	6	6 (100)	0 (0.0)	0	0	0	70.7	0.0
47[b]	51	30 (58.8)	21 (41.2)	16	2	3	75.5	0.0
55[b]	6	1 (16.7)	5 (83.3)	4	0	1	82.1	0.0
77[b]	11	5 (45.5)	6 (54.5)	6	0	0	77.6	0.0
83[b]	33	14 (42.4)	19 (57.6)	11	2	6	83.6	0.0
88[b]	109	81(74.3)	28 (25.7)	16	3	9	69.3	0.0
108[b]	6	5 (83.3)	1 (16.7)	1	0	0	79.2	0.0

At least 25 ovules per plant were analyzed

[a] Aposporous, [b]sexual, and [c]*AES* aposporous embryo sacs

Discussion

In this work, we quantitatively evaluated meiotic abnormalities in anaphase I and pollen viability in five natural aposporous accessions and three experimentally obtained sexual genotypes of tetraploid *P. notatum*. Moreover, sexual and aposporous hybrids were also evaluated for these traits to determine the extent to which meiotic abnormalities were associated with the mode of reproduction. The natural aposporous accessions had more meiotic abnormalities and lower pollen viability than the experimentally obtained sexual genotypes. Interestingly, in aposporous accessions, the variables were positively correlated, indicating that meiotic abnormalities could be responsible for the lower pollen viability observed in these plants. Similar meiotic behavior and lower pollen viability were described in tetraploid apomictic *Brachiaria brizantha* Stapf and *B. decumbens* Stapf (Mendes-Bonato et al. 2002a, b).

Most abnormal anaphases I detected in aposporous strains were characterized by laggard chromosomes placed face-to-face in the middle of the cell and by chromatin bridges, confirming previous observations reported by Stein et al. (2004). The presence of chromatin bridges with accompanying fragments is indirect evidence for the existence of inversions (Brown 1972), so the significantly higher number of meiotic abnormalities in aposporous accessions could be due to a chromosomal structural aberration that is absent in the sexual genotypes analyzed. Because the tetraploid accessions studied here were widely distributed, this genetic structure appears to be preserved in individuals throughout the geographic distribution of the species.

The lack of the chromosomal rearrangement in the tetraploid sexual genotypes could be explained by the origin of these plants. Q4188 originated from a cross between genotypes Q3664 × Q3853. Parent Q3664 originated from a cross between a sexual tetraploid plant (PT-2), induced by colchicine treatment of a sexual diploid biotype (*P. notatum* var. *saurae*), and a highly sexual white-stigma Bahia grass strain (WSB) (Quarin et al. 2003). Plant Q4205 was a selected selfing progeny of plant Q3664 (Quarin et al. 2003). In contrast, C4–4*x* was a novel autotetraploid obtained in vitro from a colchicine-treated callus generated from young inflorescences of a natural sexual diploid genotype (Quarin et al. 2001). Thus, they may not have received the chromosome segment carrying the rearrangement (inversion or translocation) present in the natural tetraploid apomictic accessions.

Analysis of the F_1 progeny corroborated the differences between aposporous and sexual hybrids in their numbers of meiocytes carrying normal and abnormal anaphase I configurations. Aposporous F_1 hybrids had more abnormalities than sexual hybrids or their apomictic progenitor. This fact could be due to fusion of the female and male genomes during hybrid formation that decreased the probability of a

regular chromosome pairing. Similar results were obtained in inter-specific hybrids between sexual *B. ruziziensis* Germain and Evrard and tetraploid apomictic *B. brizantha* (Felismino et al. 2010).

Pollen analyses in natural accessions and sexual genotypes supported the idea that meiotic aberrations in aposporous strains could affect pollen viability. However, these aberrations were not maintained in aposporous hybrids, which had similar proportions of viable pollen to sexual hybrids, although they had more abnormal anaphase I configurations. Moreover, no significant correlation was detected between meiotic abnormalities and pollen viability in hybrids. Mendes-Bonato et al. (2009) also reported a lack of correlation between meiotic abnormalities and pollen viability in *B. brizantha.* These results might indicate that, in addition to the meiotic behavior, other factors determine viable pollen production in these hybrids.

In agreement with previous work on the features and inheritance of apospory in *P. notatum* (Martínez et al. 2001, 2003; Stein et al. 2004), our current data support the idea that one chromosome rearrangement could carry the determinant(s) for apospory. This rearrangement certainly could affect chromosome pairing during meiosis and thus be responsible for the abnormal anaphase I and telophase I configurations and lower pollen viability, as was detected in the apomictic individuals analyzed. A chromosome structure of this type could also explain the segregation distortion and the lack of recombination near the apospory locus detected in the species (Martínez et al. 2003; Stein et al. 2004). Other studies on cytogenetic and molecular mapping in *P. notatum* have shown that apomicts have structural rearrangements (Pupilli et al. 2004; Stein et al. 2004, 2007), which most likely caused the observed abnormalities and consequently unviable gametes. Recently, several molecular markers that were completely linked to apospory were detected in accessions from different geographic origins, indicating that this genomic region is highly conserved in natural biotypes (Rebozzio et al. 2012).

Accordingly, the transmission of apospory by pollen in *P. notatum* could only occur in meiocytes that received the chromosome carrying apospory that had recombined in sectors outside of the apospory-block (and thus retained this specific chromosome-segment intact). The recombinant products of crossing over within the apospory-block would become non-viable, as recombination events involving inversions (or even particular types of translocations) generate unviable gametes (Brown 1972). Evidence supporting this hypothesis is still lacking in *P. notatum*, but in situ hybridization (FISH) experiments carried out with BAC clones containing sequences associated with apospory in *P. simplex* showed that the apospory-controlling locus was located adjacent to a chiasma on two chromatids of an unbound arm of one chromosome in a bivalent group (Calderini et al. 2006). Similar analyses would be necessary to provide information about the chromosome location of the apospory block in *P. notatum* and determine whether the chromosome segment carrying apospory is actually involved in the genetic rearrangements affecting meiosis of aposporous individuals.

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