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Microsporogenesis in *Brachiaria brizantha* (Poaceae) as a selection tool for breeding

M.S. Pagliarini[1], C.B. Valle[2], E.M. Santos[1], D.V. Mendes[1], Z.H. Bernardo[1], A.B. Mendes-Bonato[1], N. Silva[1] and V. Calisto[1]

[1]Departamento de Biologia Celular e Genética,
Universidade Estadual de Maringá, Maringá, PR, Brasil
[2]Embrapa Gado de Corte, Campo Grande, MS, Brasil

Corresponding author: M.S. Pagliarini
E-mail: mspagliarini@uem.br

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ABSTRACT. The genus *Brachiaria* comprises more than 100 species and is the single most important genus of forage grass in the tropics. *Brachiaria brizantha*, widely used in Brazilian pastures for beef and dairy production, is native to tropical Africa. As a subsidy to the breeding program underway in Brazil, cytological studies were employed to determine the chromosome number and to evaluate microsporogenesis in 46 accessions of this species available at Embrapa Beef Cattle (Brazil). Thirty-four accessions presented 2n = 36; seven had 2n = 45, and five had 2n = 54 chromosomes. Based on the higher level of chromosome association observed in diakinesis, in tetra-, penta-, and hexavalents, respectively, it was concluded that they are derived from x = 9; consequently, these accessions are tetra- (2n = 4x = 36), penta- (2n = 5x = 45), and hexaploids (2n = 6x = 54). The most common meiotic abnormalities were irregular chromosome segregation due to polyploidy. Chromosome stickiness, abnormal cytokinesis, non-congressed bivalents in metaphase I and chromosomes in metaphase II, and chromosome elimination were recorded at varying frequencies in several accessions. The mean percentage of meiotic abnormalities ranged from 0.36 to 95.76%. All the abnormalities had the potential to

affect pollen viability by generating unbalanced gametes. Among the accessions, only the tetraploid ones with less than 40% of abnormalities are suitable as pollen donors in intra- and interspecific crosses. Currently, accessions with a high level of ploidy (5 and 6n) cannot be used as male genitors in crosses because of the lack of sexual female genitors with the same levels of ploidy.

Key words: *Brachiaria brizantha*; Forage grass; Microsporogenesis; Breeding

INTRODUCTION

Brachiaria brizantha is widespread in tropical Africa, occurring in open and wooded grasslands, along margins of woodlands and thickets, and in upland grasslands. The collection sites of available germplasm cover the natural geographic range in eastern and southeastern Africa. However, considerable gaps in the collection exist for west Africa and southern tropical Africa, specially Zaire and Zambia, which are centers of diversity for this species (Valle and Pagliarini, 2009).

The *Brachiaria* collection expedition performed during 1984-1985 by the International Center for Tropical Agriculture (CIAT, Colombia), supported by Biodiversity International (ex-IPGRI; ex-IBPGR) resulted in the collection of about 800 accessions of at least 23 known species (Keller-Grein et al., 1996). Almost 50% of the collected accessions were of *B. brizantha*, reflecting its wide distribution and the focus on this species as a promising pasture grass. A total of 987 accessions of *Brachiaria* are available in the major world collections, of which 399 (40.4%) are of *B. brizantha* (Keller-Grein et al., 1996).

The systematic classification of the genus *Brachiaria* is far from adequate. Renvoize et al. (1996) allocated the known species into nine groups. *Brachiaria brizantha*, *B. decumbens*, *B. ruziziensis*, *B. dura*, *B. eminii*, and *B. oligobrachiata* were placed in Group 5. The first three species are the most important and currently used as cultivated pastures in the tropics. Species of this group show: i) elliptical oblong spikelet shape maintained as two separate groups on this basis; ii) few to several racemes, scattered along a central axis, ascending or spreading; iii) crescentic and narrow rachis; iv) large, long, ovate or oblong, and turgid spikelets; v) cuff-like lower glume, and vi) granulose upper lemma. *Brachiaria brizantha* has a crescentic rachis that is seldom more than 1 mm wide; the spikelets are born in a single row, and the glumes and lower lemma are cartilaginous in texture. It could be distinguished from the other two species by its erect, tufted habit and often much longer leaf blades (Renvoize et al., 1996).

Brachiaria is the single most important genus of forage grasses for pastures in the tropics. Cultivars of *Brachiaria* cover large expanses of pasture in the major ecosystems of tropical America, the humid lowlands and the savannas. Besides providing a means of transforming roughage grown most commonly on soils of low fertility levels into high-quality protein for human consumption, they convey an ecological and sustainable approach to doing so (Valle and Pagliarini, 2009).

In Brazil, there are 13 registered cultivars of *Brachiaria* listed on the National Service for Cultivar Protection (www.agricultura.gov.br/sementes/cultivaresregistradas). Except for cv. Mulato II, the remainder were selected from the natural existing variability of *B. brizantha*,

B. decumbens, *B. humidicola*, and *B. ruziziensis*. *Brachiaria brizantha* cv. Marandu and *B. decumbens* cv. Basilisk are two of the most cultivated varieties in Brazil and worldwide. Cultivar Marandu was released in 1984 by Embrapa, and originated from germplasm introduced to the State of São Paulo (Brazil) from the Marondera Grasslands Research Station, in Zimbabwe (Keller-Grein et al., 1996). It is resistant to spittlebugs, but it requires soils of medium to high fertility and does not tolerate waterlogged sites. This grass provides palatable forage of nutritional quality similar to that of *B. decumbens* cv. Basilisk.

The availability of abundant high-quality seed is vital to widespread adoption of current and future *Brachiaria* cultivars. *Brachiaria* seed production varies according to geographical distribution, photoperiod, management practices, and edaphic conditions (Valle and Pagliarini, 2009). Low seed production may be due to cytological and embryological causes. In the genus, polyploidy is predominant and correlated with apomixis (Valle and Savidan, 1996) and frequently with high levels of meiotic abnormalities (Mendes-Bonato et al., 2002, 2006; Risso-Pascotto et al., 2003, 2006a, 2009a,b; Utsunomiya et al., 2005). However, apomixis is pseudogamic, which means that viable gametes are necessary to fertilize the secondary nuclei of the embryo sac to guarantee the correct endosperm development (Valle and Savidan, 1996). New cultivars can be produced by selection of accessions chosen from the natural genetic variability, generally polyploids, or by intra- or interspecific hybridization with artificially tetraploidized accessions of *B. ruziziensis*. Anyway, viable gametes will be necessary to ensure endosperm development. Thus, the best accessions will be those with a reasonably normal microsporogenesis. In the present paper, cytological studies were employed to determine chromosome number and to evaluate microsporogenesis in accessions of *B. brizantha* available at Embrapa Beef Cattle Research Center (Brazil) to aid in the selection of those to be used in the breeding of the genus.

MATERIAL AND METHODS

Forty-six accessions of *B. brizantha* available at Embrapa Beef Cattle Research Center (Campo Grande, MS, Brazil) were cytologically evaluated by light microscopy. These accessions were collected in the wild African savannas in the 1980s by CIAT (Colombia), transferred to Embrapa Genetic Resources and Biotechnology (Brazil), and after quarantine, to Campo Grande, MS. The collection sites in Africa are presented in Table 1. In Brazil, the accessions are maintained in the field, where site characteristics of cultivation at Embrapa Beef Cattle are climate type Aw: tropical humid savanna; average annual precipitation = 1526 mm; average temperature = 22°C; altitude 520 m; latitude = 20°28' S; longitude = 55°40' W; poor dark-red Latossol soil composed of 59 sand, 8% silt and 33% clay; pH 4.2.

Inflorescences for the meiotic analyses were collected in 16 clonal plants representing each accession and fixed in a mixture of 95% ethanol, chloroform and propionic acid (6:3:2) for 24 h, transferred to 70% alcohol and stored under refrigeration until use. Microsporocytes were prepared by squashing and staining with 0.5% propionic carmine. The number of meiocytes analyzed varied according to the availability of inflorescences for each accession. Photomicrographs were taken in a Wild Leitz microscope using Kodak Imagelink - HQ, ISO 25 black and white film.

The mode of reproduction of each accession was previously determined (Valle CB, unpublished data) by examination of embryo sacs in methylsalicylate-cleared ovaries using differential interference contrast microscopy (Young et al., 1979).

Table 1. Accession codes at Cenargen/Embrapa (BRA) and at Embrapa Beef Cattle (B), collection sites in Africa (country and province), DNA amount (pg), ploidy level (x) as estimated by flow cytometry, and mode of reproduction determined by differential interference contrast microscopy in each accession of *Brachiaria brizantha*.

Accession code at Cenargen	Accession code at Embraba Beef Cattle	Country	Province	DNA amount (pg)*	Ploidy level*	Mode of reproduction
BRA-006297	B003	South Africa	-	-	-	Apomictic
BRA -002062	B032	Kenya	Rift Valley	2.38	4x	Apomictic
BRA-002143	B031	Kenya	Rift Valley	-	6x	Apomictic
BRA-002160	B038	Kenya	Rift Valley	2.95	6x	Apomictic
BRA-002119	B039	Malawi	Southern	1.91	4x	Apomictic
BRA-002321	B044	-	-	2.31	4x	Apomictic
BRA-000167	B046	-	-	-	-	Apomictic
BRA-003051	B055	Ethiopia	Gamo Gofa	2.62	5x	Apomictic
BRA-003310	B065	Ethiopia	Ilubabor	2.64	5x	Apomictic
BRA-003344	B068	Ethiopia	Ilubabor	2.58	5x	Apomictic
BRA-003506	B074	Ethiopia	Welega	2.74	5x	Apomictic
BRA-003514	B075	Ethiopia	Gojjam	2.75	5x	Apomictic
BRA-003611	B076	Ethiopia	Gonder	2.83	6x	Apomictic
BRA-003638	B078	Ethiopia	Gonder	2.80	6x	Apomictic
BRA-003646	B079	Ethiopia	Gonder	2.25	4x	Apomictic
BRA-003727	B080	Kenya	Bungoma	2.34	4x	Apomictic
BRA-003735	B081	Kenya	Bungoma	2.88	6x	Apomictic
BRA-003743	B082	Kenya	Bungoma	2.28	4x	Apomictic
BRA-003760	B083	Kenya	Siaya	2.38	4x	Apomictic
BRA-003778	B084	Kenya	Siaya	2.12	4x	Apomictic
BRA-003816	B085	Kenya	South Nyanza	2.01	4x	Apomictic
BRA-003883	B086	Kenya	Nandi	2.20	4x	Apomictic
BRA-003913	B088	Kenya	Kwale	2.29	4x	Apomictic
BRA-003956	B090	Kenya	Trans Nzoia	2.27	4x	Apomictic
BRA-004049	B093	Kenya	Kwale	2.27	4x	Apomictic
BRA-004146	B095	Zimbabwe	Kadoma	2.19	4x	Apomictic
BRA-004219	B096	Zimbabwe	Mutasa	2.39	4x	Apomictic
BRA-004227	B097	Zimbabwe	Umtali	2.37	4x	Apomictic
BRA-004235	B098	Zimbabwe	Umtali	2.01	4x	Apomictic
BRA-004316	B099	Burundi	Makamba	2.05	4x	Apomictic
BRA-002968	B120	Ethiopia	Sidamo	2.78	5x	Apomictic
BRA-002992	B122	Ethiopia	Sidamo	2.68	5x	Apomictic
BRA-003042	B123	Ethiopia	Gamo Gofa	2.18	4x	Apomictic
BRA-003221	B134	Ethiopia	Kaffa	2.86	6x	Apomictic
BRA-003255	B136	Ethiopia	Kaffa	2.76	5x	Apomictic
BRA-003590	B153	Ethiopia	Gonder	2.44	4x	Apomictic
BRA-003921	B167	Kenya	Kwale	2.00	4x	Apomictic
BRA-004081	B173	Zimbabwe	Shamva	2.32	4x	Apomictic
BRA-004375	B183	Rwanda	Kibungo	2.59	5x	Apomictic
BRA-003522	B188	Ethiopia	Gojjam	2.36	4x	Apomictic
BRA-004006	B193	Kenya	Uasin Gishu	2.70	5x	Apomictic
BRA-004278	B210	Zimbabwe	Kwekwe	2.07	4x	Apomictic
BRA-003026	B219	Ethiopia	Sidamo	2.21	4x	Apomictic
BRA-003654	B225	Ethiopia	Gojjam	2.13	4x	Apomictic
BRA-004201	B254	Zimbabwe	Inyanga	2.91	6x	Apomictic
BRA-007579	B296	Burundi	Gitega	2.75	5x	Apomictic

*Determined by flow cytometry (Penteado et al., 2000).

RESULTS AND DISCUSSION

Among the 46 accessions of *B. brizantha* analyzed (Table 2), 34 accessions had 2n = 36 chromosomes, while seven showed 2n = 45, and five, 2n = 54. Based on the higher level of chromosome association observed at diakinesis in tetra- (Figure 1a), penta-, and hexavalents, it was concluded that they are derived from x = 9; thus, they are tetra- (2n = 4x = 36), penta- (2n = 5x = 45), and hexaploids (2n = 6x = 54). The same basic chromosome number (x = 9) was recorded for this species by other authors (see Valle and Pagliarini, 2009). Diploidy (2n = 2x = 18) was reported in only a few accessions (Basappa et al., 1987; Mendes-Bonato et al., 2002), but tetraploids always prevail (see Valle and Pagliarini, 2009). In attempt to aid the *Brachiaria*

breeding program underway at Embrapa Beef Cattle and to determine the ploidy level of the accessions of different species to initiate an interspecific hybridization program, DNA content was measured by flow cytometry (Penteado et al., 2000). The ploidy level scored among 222 accessions of *B. brizantha* revealed that two accessions (0.9%) were 2x; 157 (70.7%) were 4x; 41 (18.5%) were 5x, and 22 (9.9%) were 6x, with DNA content ranging from 1.32 to 3.17 pg. The data obtained from the present cytogenetic studies diverge somewhat from those obtained by flow cytometry (Table 1): i) five accessions 4x (B065, B068, B122, B136, and B269) were interpreted as 5x; ii) one accession 4x (B134) was interpreted as 6x; iii) one accession 6x (B193) was interpreted as 5x, and iv) two accessions 6x (B038 and B078) were interpreted as 5x. These results showed that flow cytometry and quantity of DNA cannot always be used to infer ploidy levels and that cytological study remains the best means of evaluating chromosome number in a species. Furthermore, it allows for examining chromosome behavior during meiosis with all of its implications in determining potential fertility problems.

Table 2. Chromosome number, ploidy level, number of cells analyzed, percentage of abnormal cells in each meiotic phase and mean percentage of meiotic abnormalities in each accession of *Brachiaria brizantha.*

Accession	2n	Ploidy	No. of cells	% of abnormal cells								
				M I	A I	T I	P II	M II	A II	T II	Tetrad	Mean
B003	2n = 36	4x	1194	15.62	69.68	32.88	28.00	42.18	55.36	36.62	40.66	40.13
B032	2n = 36	4x	862	20.17	46.76	19.53	11.65	11.43	42.10	41.86	10.28	25.47
B039	2n = 36	4x	1135	27.86	47.36	30.55	64.02	65.38	93.24	83.24	77.37	61.12
B044	2n - 36	4x	1097	5.69	39.64	2.56	10.56	27.75	44.18	49.12	63.20	30.34
B046	2n = 36	4x	1186	61.36	79.02	87.50	95.65	98.10	100.0	97.22	78.53	87.17
B065	2n = 36	4x	1136	73.83	100.0	98.59	100.0	93.65	100.0	100.0	100.0	95.76
B068	2n = 36	4x	706	7.03	32.44	8.95	7.62	3.10	40.00	14.30	2.50	14.49
B079	2n = 36	4x	1336	2.51	60.25	26.28	27.70	10.57	53.70	44.00	69.13	36.76
B080	2n = 36	4x	1492	4.85	62.70	50.23	58.97	31.85	82.50	72.15	61.55	53.10
B082	2n = 36	4x	1323	11.33	65.34	65.00	56.14	67.32	61.10	90.36	87.32	62.98
B083	2n = 36	4x	1483	15.85	72.16	37.58	24.26	53.89	69.59	78.51	94.46	55.78
B084	2n = 36	4x	1310	8.57	78.37	14.17	36.79	39.30	39.52	65.88	82.64	45.65
B085	2n = 36	4x	1356	8.02	59.89	54.24	69.69	38.51	52.00	83.09	57.74	52.89
B086	2n = 36	4x	1180	5.55	72.14	81.25	79.19	29.82	56.97	70.00	64.83	57.46
B088	2n = 36	4x	1093	17.06	44.80	17.26	4.30	11.11	33.33	24.42	22.28	21.82
B090	2n = 36	4x	768	0.00	0.00	0.00	0.00	1.92	0.00	0.00	1.03	0.36
B093	2n = 36	4x	1080	5.83	31.85	3.66	3.73	4.34	37.11	45.05	50.69	22.78
B095	2n = 36	4x	573	5.70	49.25	11.65	4.54	18.60	0.00	14.28	27.53	18.79
B096	2n = 36	4x	1146	24.46	32.98	12.82	13.79	6.42	32.67	21.92	4.58	18.70
B097	2n = 36	4x	899	8.09	18.96	47.05	5.97	2.80	52.83	60.00	72.50	33.53
B098	2n = 36	4x	1179	10.86	43.80	36.72	1.90	13.84	48.64	48.88	45.27	31.23
B122	2n = 36	4x	1219	10.08	98.50	72.73	47.30	25.00	100.0	100.0	98.04	68.96
B099	2n = 36	4x	1236	33.58	71.24	67.11	93.24	58.48	61.64	73.62	77.51	67.05
B123	2n = 36	4x	1186	1.94	62.50	10.40	5.11	3.50	72.46	81.21	84.36	40.19
B134	2n = 36	4x	1140	71.90	98.60	90.28	97.83	79.14	100.0	100.0	95.90	91.71
B136	2n = 36	4x	1153	62.07	99.32	87.41	79.86	84.14	100.0	96.60	87.35	87.09
B153	2n = 36	4x	1109	34.48	88.65	80.77	80.15	82.73	84.89	96.53	100.0	81.03
B167	2n = 36	4x	1398	72.22	63.79	63.34	37.58	60.01	68.24	69.91	75.54	63.83
B173	2n = 36	4x	1085	41.61	77.54	91.97	99.26	68.97	80.00	96.92	85.92	80.27
B188	2n = 36	4x	1185	43.67	68.03	50.32	56.93	70.95	71.32	79.31	86.62	65.89
B210	2n = 36	4x	1160	42.14	78.72	64.79	41.72	75.52	80.89	71.43	65.07	65.04
B219	2n = 36	4x	1224	28.33	98.08	86.42	97.22	88.27	98.26	100.0	99.39	87.00
B225	2n = 36	4x	1561	75.94	69.82	60.31	62.80	78.57	84.90	67.27	61.31	70.12
B296	2n = 36	4x	1160	73.97	94.59	100.0	100.0	97.16	92.54	97.14	97.50	94.11
B038	2n = 45	5x	1693	23.60	79.79	48.83	42.10	32.98	63.85	42.21	87.20	52.57
B055	2n = 45	5x	1194	25.68	94.48	71.88	64.67	66.67	97.80	100.0	100.0	77.65
B074	2n = 45	5x	1545	36.97	87.62	72.12	12.57	47.88	82.89	81.12	96.88	64.75
B075	2n = 45	5x	989	20.07	61.26	67.69	48.64	65.65	60.00	62.20	53.00	54.81
B078	2n = 45	5x	1338	10.81	60.29	7.01	47.65	79.41	99.09	80.83	82.42	58.43
B120	2n = 45	5x	1152	54.30	99.35	90.91	90.0	90.07	99.28	100.0	100.0	90.48
B183	2n = 45	5x	1189	56.39	99.28	80.15	81.48	91.55	99.30	100.0	100.0	88.52
B031	2n = 54	6x	1733	69.60	79.66	84.38	76.17	88.06	78.21	76.78	88.44	80.16
B076	2n = 54	6x	1579	30.18	57.61	25.53	33.33	27.69	92.38	51.49	91.56	51.22
B081	2n = 54	6x	806	46.61	93.00	40.74	11.36	55.00	10.34	77.66	66.99	50.21
B254	2n = 54	6x	1252	19.33	68.96	48.17	41.67	41.66	66.31	71.14	73.13	53.80
B193	2n = 54	6x	1161	43.83	82.58	80.00	73.79	66.43	68.10	90.13	90.10	74.37

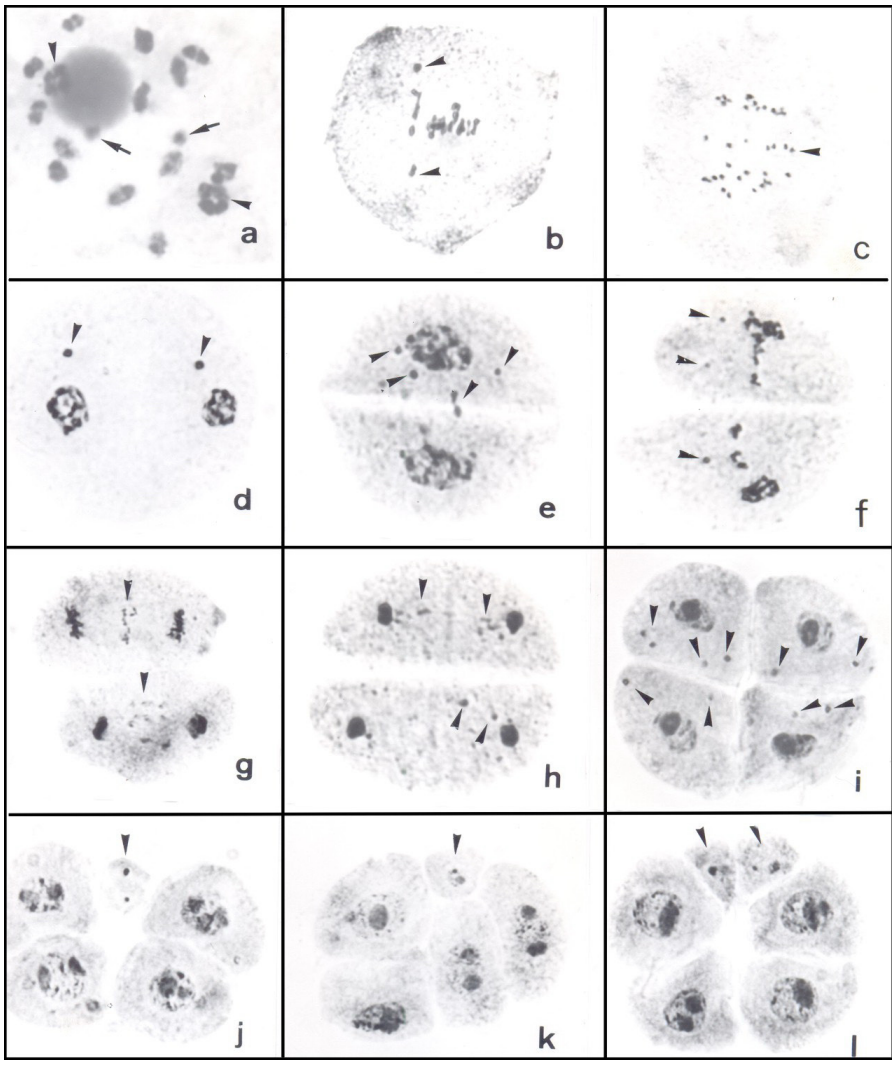

Figure 1. Some aspects of irregular chromosome segregation during microsporogenesis in *Brachiaria brizantha*. **a.** Meiocyte (2n = 4x = 36) in diakinesis showing two quadrivalents (arrowheads), two univalents (arrows), and bivalents. **b.** Metaphase I with precocious chromosome migration to the poles. **c.** Anaphase I with laggards. **d.** Telophase I with micronuclei. **e.** Prophase II with micronuclei. **f.** Metaphase II with precocious chromosome migration to the poles. **g.** Anaphase II with laggards. **h.** Telophase II with micronuclei. **i.** Tetrad with several micronuclei. **j. k. l.** Tetrads with microcytes. (400X).

As polyploids, these accessions displayed meiotic abnormalities related to irregular chromosome segregation during both meiotic divisions along with other types of abnormalities. The mean percentage of meiotic abnormalities ranged from 0.36 to 95.76%. The most common meiotic abnormalities in the accessions were precocious chromosome migration to the poles in metaphases (Figure 1b, f), laggards in anaphases (Figure 1c, g), leading to micronucleus formation in telophases (Figure 1d, h) in prophase II (Figure 1e), and tetrads (Figure

1i). Micronuclei were also eliminated as microcytes (Figure 1j, k, l). The same type of behavior was reported in polyploid accessions of *B. brizantha* (Mendes-Bonato et al., 2002; Risso-Pascotto et al., 2003), *B. nigropedata* (Utsunomiya et al., 2005), *B. jubata* (Mendes-Bonato et al., 2006), *B. dictyoneura* (Risso-Pascotto et al., 2006a), *B. dura* (Risso-Pascotto et al., 2009a), and *B. bovonei* and *B. subulifolia* (Risso-Pascotto et al., 2009b). Irregular chromosome segregation compromises pollen fertility by producing unbalanced microspores.

Other meiotic abnormalities were recorded. i) Non-congressed bivalents in metaphase I (Figure 2a, b) and non-congressed chromosomes in metaphase II (Figure 2c) were seen in accession B046. A total of 39.81% of meiocytes were affected in metaphase I and 60.78% in metaphase II. Among tetrads, 78.53% showed micronuclei (Figure 2d). These abnormalities were reported in *B. brizantha*, but in lower frequencies (Mendes-Bonato et al., 2002). According to Nicklas and Ward (1994), these abnormalities can be related to a defective kinetochore. ii) Several accessions showed chromosome stickiness with different degrees of severity. Chromosome stickiness is characterized by intense chromosome clustering during any phase of meiosis and compromises pollen viability by breaking the chromosomes at any point after bridge formation (Figure 2e-h). Although many studies have reported the occurrence of chromosome stickiness, the primary cause and the biochemical basis for this abnormality are still unknown. Chromosome stickiness has been recorded in different *Brachiaria* species (Mendes-Bonato et al., 2001a,b; Utsunomiya et al., 2005). iii) Abnormal nucleolus disintegration was also found in some accessions. Here, the nucleolus underwent a normal pattern of disorganization. After diakinesis, the nucleolus was disorganized into several micronucleoli in metaphase I (Figure 2i) and II. After this phase, they were rejoined into micronucleoli of bigger sizes (Figure 2j, k), until complete normal nucleolus formation at prophase II and tetrad (Figure 2l). This abnormality was recorded in several accessions, but did not compromise pollen viability, because a normal nucleolus was formed in the tetrads and pollen grains. Abnormal nucleolus disintegration was reported in several accessions (Risso-Pascotto et al., 2002) and hybrids (Fuzinatto et al., 2007, 2008) of *Brachiaria*. iv) A particular mechanism of abnormal cytokinesis was recorded in accession B039 leading to the formation of some 2n microspores (Figure 2i-l). In the affected cells, the first cytokinesis did not occur after telophase I, but occurred during metaphase II, and was initiated in the middle of the cell (Figure 2m). In some meiocytes, the metaphase plates were very close and rejoined leading to the formation of a restitutional nucleus (Figure 2n-p). This abnormality was reported in accessions of *B. decumbens*, *B. humidicola*, and *B. dura* (Gallo et al., 2007). 2n gametes have an important function in breeding programs. In *Brachiaria*, an attempt was made with 2n gametes to introduce sexuality into the hexaploid *B. humidicola* complex. Hybrids were produced and need to be analyzed for ploidy level and mode of reproduction once they flower (see Valle and Pagliarini, 2009). v) Chromosome elimination was recorded in the pentaploid (2n = 5x = 45) accession B038. In this accession, nine univalent chromosomes remained as laggards in anaphase I (Figure 2q) and anaphase II (Figure 2s), while 18 segregated chromosomes migrated to the poles in anaphase I. In the majority of meiocytes, this genome remained outside the telophase nuclei (Figure 2r) and was eliminated as micronuclei in tetrads (Figure 2t). Chromosome elimination in two pentaploid accessions of *B. brizantha*, caused by asynchrony between the two parental genomes, was also reported by Mendes et al. (2006). Similar behavior was recorded in hepta- and nonaploid accessions of *B. humidicola*, derived from x = 6 (Boldrini et al., 2009a,b, 2010). The asynchrony between parental genomes and the elimination of one genome in micronuclei

in microspores strongly suggests that some accessions with odd level of ploidy are derived from hybridization. Evidence of allopolyploidy in *B. brizantha* was also provided by Risso-Pascotto et al. (2006b).

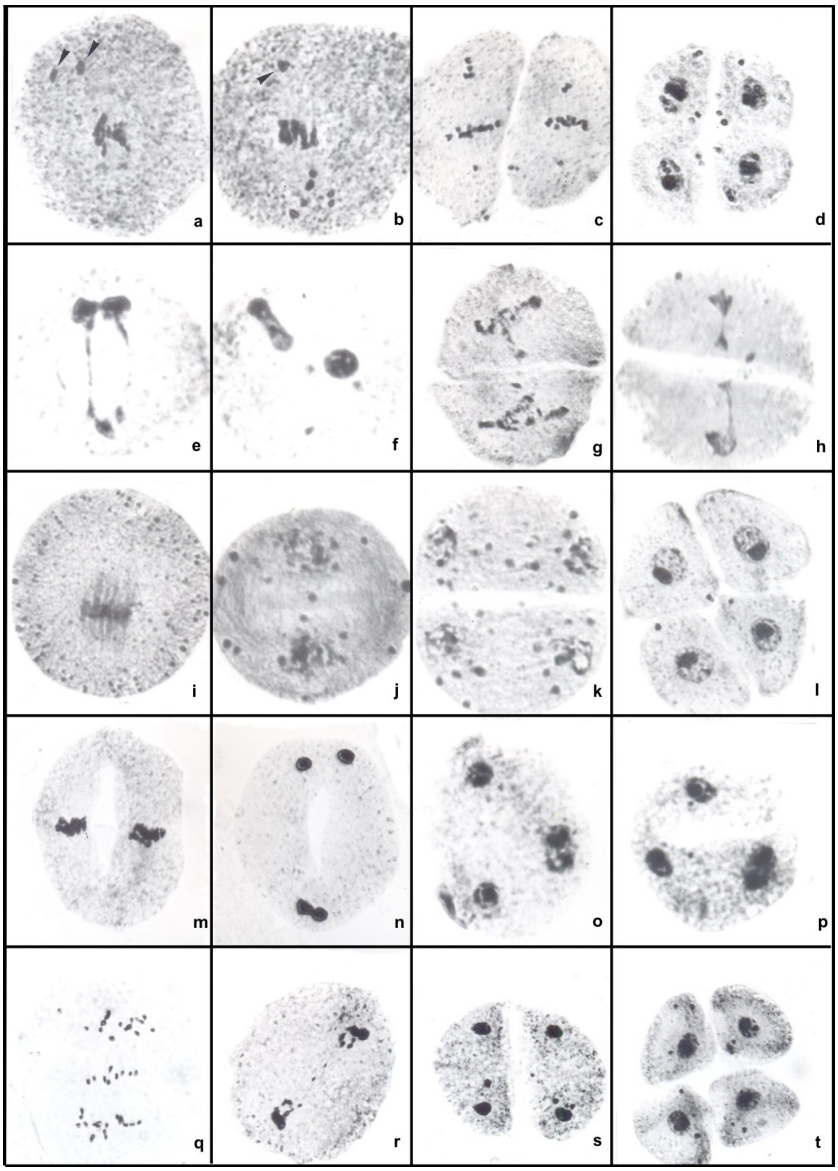

Figure 2. Some aspects of meiotic abnormalities recorded in *Brachiaria brizantha* accessions. **a. b.** Metaphase I with non-congressed bivalents (arrowheads). **c.** Metaphase II with non-congressed chromosomes. **d.** Tetrad with micronuclei and microcytes. **e. f. g. h.** Meiocytes with chromosome stickiness. **i. j. k. l.** Aspects of abnormal nucleolus disintegration. **m. n. o. p.** Abnormal cytokinesis leading to the formation of a restitutional nucleus. **q. r. s. t.** Asynchrony in meiosis in the pentaploid accession B038. Note the laggard genome with 9 univalents in q (400X).

The results obtained in the present study show that among the 46 apomictic accessions analyzed, 34 are tetraploid, but due to different kinds of meiotic abnormalities, not all of them can be used as male genitors in crosses before accessing fertility and potential seed productivity. Among them, the percentage of abnormal cells ranged from 0.36 to 95.75%. At least 12 accessions, with less than 40% abnormal cells, could be considered for crosses depending on their agronomic characteristics. Among the penta- and hexaploid accessions, meiotic abnormalities were always frequent, i.e, greater than 50%. Accessions with high levels of ploidy (5 and 6n), have not been used as male genitors in crosses due to a lack of compatible female genitors, i.e., of the same ploidy level. One pentaploid accession, not listed here (B178), has been released as cultivar Xaraés by Embrapa in 2003 (Valle et al., 2004), because of its high dry matter production and rapid regrowth after grazing. Seed production is adequate due to apomixis (meiosis is bypassed), and thus, it already covers millions of hectares of pastures in Brazil alone. Such accessions, depending on their meiotic stability and fertility could be used directly as candidates for cultivar development. Once agronomic value, seed production and animal performance have been assessed, these can be released as new cultivars for pastures.

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