

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

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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 $2 \mathrm{n}=36$; seven had $2 \mathrm{n}=45$, and five had $2 \mathrm{n}=54$ 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- $(2 n=4 x$ $=36)$, penta- $(2 \mathrm{n}=5 \mathrm{x}=45)$, and hexaploids $(2 \mathrm{n}=6 \mathrm{x}=54)$. The most common meiotic abnormalities were irregular chromosome segregation due to polyploidy. Chromosome stickiness, abnormal cytokinesis, noncongressed 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 6 n ) 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 $=1526 \mathrm{~mm}$; average temperature $=22^{\circ} \mathrm{C}$; altitude 520 m ; latitude $=20^{\circ} 28^{\prime} \mathrm{S}$; longitude $=55^{\circ} 40^{\prime} \mathrm{W}$; 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 | 4 x | Apomictic |
| BRA-002143 | B031 | Kenya | Rift Valley | - | 6 x | Apomictic |
| BRA-002160 | B038 | Kenya | Rift Valley | 2.95 | 6 x | Apomictic |
| BRA-002119 | B039 | Malawi | Southern | 1.91 | 4 x | Apomictic |
| BRA-002321 | B044 | - | - | 2.31 | 4 x | Apomictic |
| BRA-000167 | B046 | - | - | - | - | Apomictic |
| BRA-003051 | B055 | Ethiopia | Gamo Gofa | 2.62 | 5 x | 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 | 6 x | Apomictic |
| BRA-003638 | B078 | Ethiopia | Gonder | 2.80 | 6 x | Apomictic |
| BRA-003646 | B079 | Ethiopia | Gonder | 2.25 | 4 x | Apomictic |
| BRA-003727 | B080 | Kenya | Bungoma | 2.34 | 4 x | Apomictic |
| BRA-003735 | B081 | Kenya | Bungoma | 2.88 | 6 x | Apomictic |
| BRA-003743 | B082 | Kenya | Bungoma | 2.28 | 4 x | Apomictic |
| BRA-003760 | B083 | Kenya | Siaya | 2.38 | 4 x | Apomictic |
| BRA-003778 | B084 | Kenya | Siaya | 2.12 | 4 x | Apomictic |
| BRA-003816 | B085 | Kenya | South Nyanza | 2.01 | 4 x | Apomictic |
| BRA-003883 | B086 | Kenya | Nandi | 2.20 | 4 x | Apomictic |
| BRA-003913 | B088 | Kenya | Kwale | 2.29 | 4 x | Apomictic |
| BRA-003956 | B090 | Kenya | Trans Nzoia | 2.27 | 4 x | Apomictic |
| BRA-004049 | B093 | Kenya | Kwale | 2.27 | 4 x | Apomictic |
| BRA-004146 | B095 | Zimbabwe | Kadoma | 2.19 | 4 x | Apomictic |
| BRA-004219 | B096 | Zimbabwe | Mutasa | 2.39 | 4 x | Apomictic |
| BRA-004227 | B097 | Zimbabwe | Umtali | 2.37 | 4 x | Apomictic |
| BRA-004235 | B098 | Zimbabwe | Umtali | 2.01 | 4 x | Apomictic |
| BRA-004316 | B099 | Burundi | Makamba | 2.05 | 4 x | Apomictic |
| BRA-002968 | B120 | Ethiopia | Sidamo | 2.78 | 5 x | Apomictic |
| BRA-002992 | B122 | Ethiopia | Sidamo | 2.68 | 5 x | Apomictic |
| BRA-003042 | B123 | Ethiopia | Gamo Gofa | 2.18 | 4 x | Apomictic |
| BRA-003221 | B134 | Ethiopia | Kaffa | 2.86 | 6 x | Apomictic |
| BRA-003255 | B136 | Ethiopia | Kaffa | 2.76 | 5 x | Apomictic |
| BRA-003590 | B153 | Ethiopia | Gonder | 2.44 | 4 x | Apomictic |
| BRA-003921 | B167 | Kenya | Kwale | 2.00 | 4 x | Apomictic |
| BRA-004081 | B173 | Zimbabwe | Shamva | 2.32 | 4 x | Apomictic |
| BRA-004375 | B183 | Rwanda | Kibungo | 2.59 | 5 x | Apomictic |
| BRA-003522 | B188 | Ethiopia | Gojjam | 2.36 | 4 x | Apomictic |
| BRA-004006 | B193 | Kenya | Uasin Gishu | 2.70 | 5 x | Apomictic |
| BRA-004278 | B210 | Zimbabwe | Kwekwe | 2.07 | 4 x | Apomictic |
| BRA-003026 | B219 | Ethiopia | Sidamo | 2.21 | 4 x | Apomictic |
| BRA-003654 | B225 | Ethiopia | Gojjam | 2.13 | 4 x | Apomictic |
| BRA-004201 | B254 | Zimbabwe | Inyanga | 2.91 | 6 x | 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 $2 \mathrm{n}=$ 36 chromosomes, while seven showed $2 n=45$, and five, $2 n=54$. 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- $(2 n=4 x=36)$, penta- $(2 n$ $=5 x=45)$, and hexaploids $(2 n=6 x=54)$. The same basic chromosome number $(x=9)$ was recorded for this species by other authors (see Valle and Pagliarini, 2009). Diploidy ( $2 \mathrm{n}=2 \mathrm{x}=$ 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 $2 \mathrm{x} ; 157(70.7 \%)$ were 4 x ; $41(18.5 \%)$ were 5 x , and $22(9.9 \%)$ were 6 x , 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 5 x ; ii) one accession 4 x (B134) was interpreted as 6 x ; iii) one accession 6 x (B193) was interpreted as 5x, and iv) two accessions 6 x (B038 and B078) were interpreted as 5 x . 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 | 2 n | Ploidy | No. of cells | \% of abnormal cells |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  | M I | A I | T I | P II | M II | A II | T II | Tetrad | Mean |
| B003 | $2 \mathrm{n}=36$ | 4 x | 1194 | 15.62 | 69.68 | 32.88 | 28.00 | 42.18 | 55.36 | 36.62 | 40.66 | 40.13 |
| B032 | $2 \mathrm{n}=36$ | 4 x | 862 | 20.17 | 46.76 | 19.53 | 11.65 | 11.43 | 42.10 | 41.86 | 10.28 | 25.47 |
| B039 | $2 \mathrm{n}=36$ | 4 x | 1135 | 27.86 | 47.36 | 30.55 | 64.02 | 65.38 | 93.24 | 83.24 | 77.37 | 61.12 |
| B044 | 2n-36 | 4 x | 1097 | 5.69 | 39.64 | 2.56 | 10.56 | 27.75 | 44.18 | 49.12 | 63.20 | 30.34 |
| B046 | $2 \mathrm{n}=36$ | 4 x | 1186 | 61.36 | 79.02 | 87.50 | 95.65 | 98.10 | 100.0 | 97.22 | 78.53 | 87.17 |
| B065 | $2 \mathrm{n}=36$ | 4 x | 1136 | 73.83 | 100.0 | 98.59 | 100.0 | 93.65 | 100.0 | 100.0 | 100.0 | 95.76 |
| B068 | $2 \mathrm{n}=36$ | 4 x | 706 | 7.03 | 32.44 | 8.95 | 7.62 | 3.10 | 40.00 | 14.30 | 2.50 | 14.49 |
| B079 | $2 \mathrm{n}=36$ | 4 x | 1336 | 2.51 | 60.25 | 26.28 | 27.70 | 10.57 | 53.70 | 44.00 | 69.13 | 36.76 |
| B080 | $2 \mathrm{n}=36$ | 4 x | 1492 | 4.85 | 62.70 | 50.23 | 58.97 | 31.85 | 82.50 | 72.15 | 61.55 | 53.10 |
| B082 | $2 \mathrm{n}=36$ | 4 x | 1323 | 11.33 | 65.34 | 65.00 | 56.14 | 67.32 | 61.10 | 90.36 | 87.32 | 62.98 |
| B083 | $2 \mathrm{n}=36$ | 4 x | 1483 | 15.85 | 72.16 | 37.58 | 24.26 | 53.89 | 69.59 | 78.51 | 94.46 | 55.78 |
| B084 | $2 \mathrm{n}=36$ | 4 x | 1310 | 8.57 | 78.37 | 14.17 | 36.79 | 39.30 | 39.52 | 65.88 | 82.64 | 45.65 |
| B085 | $2 \mathrm{n}=36$ | 4 x | 1356 | 8.02 | 59.89 | 54.24 | 69.69 | 38.51 | 52.00 | 83.09 | 57.74 | 52.89 |
| B086 | $2 \mathrm{n}=36$ | 4 x | 1180 | 5.55 | 72.14 | 81.25 | 79.19 | 29.82 | 56.97 | 70.00 | 64.83 | 57.46 |
| B088 | $2 \mathrm{n}=36$ | 4 x | 1093 | 17.06 | 44.80 | 17.26 | 4.30 | 11.11 | 33.33 | 24.42 | 22.28 | 21.82 |
| B090 | $2 \mathrm{n}=36$ | 4 x | 768 | 0.00 | 0.00 | 0.00 | 0.00 | 1.92 | 0.00 | 0.00 | 1.03 | 0.36 |
| B093 | $2 \mathrm{n}=36$ | 4 x | 1080 | 5.83 | 31.85 | 3.66 | 3.73 | 4.34 | 37.11 | 45.05 | 50.69 | 22.78 |
| B095 | $2 \mathrm{n}=36$ | 4 x | 573 | 5.70 | 49.25 | 11.65 | 4.54 | 18.60 | 0.00 | 14.28 | 27.53 | 18.79 |
| B096 | $2 \mathrm{n}=36$ | 4 x | 1146 | 24.46 | 32.98 | 12.82 | 13.79 | 6.42 | 32.67 | 21.92 | 4.58 | 18.70 |
| B097 | $2 \mathrm{n}=36$ | 4 x | 899 | 8.09 | 18.96 | 47.05 | 5.97 | 2.80 | 52.83 | 60.00 | 72.50 | 33.53 |
| B098 | $2 \mathrm{n}=36$ | 4 x | 1179 | 10.86 | 43.80 | 36.72 | 1.90 | 13.84 | 48.64 | 48.88 | 45.27 | 31.23 |
| B122 | $2 \mathrm{n}=36$ | 4 x | 1219 | 10.08 | 98.50 | 72.73 | 47.30 | 25.00 | 100.0 | 100.0 | 98.04 | 68.96 |
| B099 | $2 \mathrm{n}=36$ | 4 x | 1236 | 33.58 | 71.24 | 67.11 | 93.24 | 58.48 | 61.64 | 73.62 | 77.51 | 67.05 |
| B123 | $2 \mathrm{n}=36$ | 4 x | 1186 | 1.94 | 62.50 | 10.40 | 5.11 | 3.50 | 72.46 | 81.21 | 84.36 | 40.19 |
| B134 | $2 \mathrm{n}=36$ | 4 x | 1140 | 71.90 | 98.60 | 90.28 | 97.83 | 79.14 | 100.0 | 100.0 | 95.90 | 91.71 |
| B136 | $2 \mathrm{n}=36$ | 4 x | 1153 | 62.07 | 99.32 | 87.41 | 79.86 | 84.14 | 100.0 | 96.60 | 87.35 | 87.09 |
| B153 | $2 \mathrm{n}=36$ | 4 x | 1109 | 34.48 | 88.65 | 80.77 | 80.15 | 82.73 | 84.89 | 96.53 | 100.0 | 81.03 |
| B167 | $2 \mathrm{n}=36$ | 4 x | 1398 | 72.22 | 63.79 | 63.34 | 37.58 | 60.01 | 68.24 | 69.91 | 75.54 | 63.83 |
| B173 | $2 \mathrm{n}=36$ | 4 x | 1085 | 41.61 | 77.54 | 91.97 | 99.26 | 68.97 | 80.00 | 96.92 | 85.92 | 80.27 |
| B188 | $2 \mathrm{n}=36$ | 4 x | 1185 | 43.67 | 68.03 | 50.32 | 56.93 | 70.95 | 71.32 | 79.31 | 86.62 | 65.89 |
| B210 | $2 \mathrm{n}=36$ | 4 x | 1160 | 42.14 | 78.72 | 64.79 | 41.72 | 75.52 | 80.89 | 71.43 | 65.07 | 65.04 |
| B219 | $2 \mathrm{n}=36$ | 4 x | 1224 | 28.33 | 98.08 | 86.42 | 97.22 | 88.27 | 98.26 | 100.0 | 99.39 | 87.00 |
| B225 | $2 \mathrm{n}=36$ | 4 x | 1561 | 75.94 | 69.82 | 60.31 | 62.80 | 78.57 | 84.90 | 67.27 | 61.31 | 70.12 |
| B296 | $2 \mathrm{n}=36$ | 4 x | 1160 | 73.97 | 94.59 | 100.0 | 100.0 | 97.16 | 92.54 | 97.14 | 97.50 | 94.11 |
| B038 | $2 \mathrm{n}=45$ | 5 x | 1693 | 23.60 | 79.79 | 48.83 | 42.10 | 32.98 | 63.85 | 42.21 | 87.20 | 52.57 |
| B055 | $2 \mathrm{n}=45$ | 5 x | 1194 | 25.68 | 94.48 | 71.88 | 64.67 | 66.67 | 97.80 | 100.0 | 100.0 | 77.65 |
| B074 | $2 \mathrm{n}=45$ | 5 x | 1545 | 36.97 | 87.62 | 72.12 | 12.57 | 47.88 | 82.89 | 81.12 | 96.88 | 64.75 |
| B075 | $2 \mathrm{n}=45$ | 5 x | 989 | 20.07 | 61.26 | 67.69 | 48.64 | 65.65 | 60.00 | 62.20 | 53.00 | 54.81 |
| B078 | $2 \mathrm{n}=45$ | 5 x | 1338 | 10.81 | 60.29 | 7.01 | 47.65 | 79.41 | 99.09 | 80.83 | 82.42 | 58.43 |
| B120 | $2 \mathrm{n}=45$ | 5 x | 1152 | 54.30 | 99.35 | 90.91 | 90.0 | 90.07 | 99.28 | 100.0 | 100.0 | 90.48 |
| B183 | $2 \mathrm{n}=45$ | 5 x | 1189 | 56.39 | 99.28 | 80.15 | 81.48 | 91.55 | 99.30 | 100.0 | 100.0 | 88.52 |
| B031 | $2 \mathrm{n}=54$ | 6 x | 1733 | 69.60 | 79.66 | 84.38 | 76.17 | 88.06 | 78.21 | 76.78 | 88.44 | 80.16 |
| B076 | $2 \mathrm{n}=54$ | 6 x | 1579 | 30.18 | 57.61 | 25.53 | 33.33 | 27.69 | 92.38 | 51.49 | 91.56 | 51.22 |
| B081 | $2 \mathrm{n}=54$ | 6 x | 806 | 46.61 | 93.00 | 40.74 | 11.36 | 55.00 | 10.34 | 77.66 | 66.99 | 50.21 |
| B254 | $2 \mathrm{n}=54$ | 6 x | 1252 | 19.33 | 68.96 | 48.17 | 41.67 | 41.66 | 66.31 | 71.14 | 73.13 | 53.80 |
| B193 | $2 \mathrm{n}=54$ | 6 x | 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 $(2 n=4 \mathrm{x}=36)$ 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; RissoPascotto 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 (MendesBonato 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 21). 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 2 n 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). 2 n 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 $(2 n=5 x=45)$ 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 heptaand nonaploid accessions of $B$. humidicola, derived from $\mathrm{x}=6$ (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 RissoPascotto 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 6 n ), 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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