

Note

**MICROSPOROGENESIS IN *BRACHIARIA BOVONEI*
(CHIOV.) ROBYNS AND *B. SUBULIFOLIA* (MEZ) CLAYTON
(POACEAE)**

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ABSTRACT: Some African species of *Brachiaria* have been introduced into the Americas and became the most important forage for pastures in the tropics. New cultivars can be obtained either from direct selections from the natural existing variability in the germplasm collections or from interspecific hybridizations. Polyploidy is predominant in the genus *Brachiaria* and correlated with apomixis which complicates hybridization. The objective of cytological studies underway on the *Brachiaria* germplasm collection at Embrapa Beef Cattle is to determine the chromosome number and meiotic behavior of accessions. For the breeding of *Brachiaria* species, compatible sexual and apomictic accessions need to be identified. Microsporogenesis was evaluated in two accessions of *Brachiaria bovonei* (BO01 and BO05) and one accession of *B. subulifolia* (SU01). BO01 is pentaploid ($2n = 5x = 45$), BO05 is tetraploid ($2n = 4x = 36$), and SU01 is hexaploid ($2n = 6x = 54$), derived from $x = 9$. Meiotic abnormalities typical of polyploids, characterized by precocious chromosome migration to the poles in metaphases, laggard chromosomes in anaphases, and micronuclei in telophases and tetrads, were recorded in high frequency in all the accessions generating unbalanced gametes. Both accessions of *B. bovonei* presented chromosome stickiness. The results are discussed in the view of the *Brachiaria* breeding program objectives.

Key words: *Brachiaria*, meiosis, chromosome number, polyploidy, breeding program

**MICROSPOROGÊNESE EM *BRACHIARIA BOVONEI* (CHIOV.)
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RESUMO: Algumas espécies africanas de *Brachiaria* foram introduzidas nas Américas e tornaram-se importantes pastagens nos trópicos. Novas cultivares podem ser obtidas por seleção direta da variabilidade genética existente na coleção de germoplasma ou por hibridização interespecífica. Poliploidia é predominante no gênero *Brachiaria* e correlacionada com apomixia, o que complica a hibridização. O objetivo dos estudos citogenéticos na coleção de germoplasma de *Brachiaria* da Embrapa Gado de Corte é determinar o número de cromossomos e o comportamento meiótico dos acessos. A microsporogênese foi avaliada em dois acessos de *Brachiaria bovonei* (BO01 e BO05) e um acesso de *B. subulifolia* (SU01). BO01 é pentaploide ($2n = 5x = 45$), BO05 é tetraploide ($2n = 4x = 36$), e SU01 é hexaploide ($2n = 6x = 54$), todos derivados de $x = 9$. Anormalidades meióticas típicas de poliploides, caracterizadas por migração precoce de cromossomos para os pólos em metafases, cromossomos retardatários em anáfases, e micronúcleos em telófases e tétrades foram observadas em alta frequência em todos os acessos gerando gametas geneticamente desbalanceados. Ambos os acessos de *B. bovonei* apresentaram aderências cromossômicas. Os resultados são discutidos sob o ponto de vista dos objetivos do melhoramento genético.

Palavras-chave: *Brachiaria*, meiose, número de cromossomos, poliploidia, melhoramento

INTRODUCTION

The main germplasm collections of *Brachiaria* in the world are in Colombia (International Center for Tropical Agriculture – CIAT) with 687 accessions, Ethiopia (International Livestock Center for Africa –

ILCA) with 520 accessions, and Brazil (Embrapa Beef Cattle Research Center – CNPGC) with 475 accessions. Some species, however, are poorly represented in these centers. Nine accessions of *B. bovonei* (Chiov.) Robyns and nine of *B. subulifolia* (Mez) Clayton are found in the three main germplasm col-

lections above cited (Keller-Grein et al., 1996). At CNPGC, only two accessions of *B. bovonei* and one accession of *B. subulifolia* are actually available. According to Renvoize's taxonomic classification (Renvoize et al., 1996) *B. bovonei* and *B. subulifolia* belong to group 6 based on morphological descriptors. The first presents rachis almost triquetrous while the second presents upper lemma nipped at the tip. Both species are of African origin.

The majority of *Brachiaria* species is polyploid (Basappa et al., 1987; Valle & Savidan, 1996; Mendes-Bonato et al., 2002, 2006; Utsunomiya et al., 2005; Risso-Pascotto et al., 2006a). The analysis of DNA content by flow cytometry indicated that polyploidy in the CNPGC collection ranged from $4n$ to $7n$ (Penteado et al., 2000). The tetraploid level largely predominates in this collection (58%). Some species, such as *B. bovonei*, were not evaluated by flow cytometry, but among those analyzed, including *B. subulifolia*, the authors recommended a confirmation of ploidy level by chromosome counting. Thus, besides determining the chromosome number in both species, this study also presents data about their meiotic behavior as a contribution to the *Brachiaria* breeding program.

MATERIAL AND METHODS

Two accessions of *B. bovonei* (BO01 and BO05) and one accession of *B. subulifolia* (SU01) available at Embrapa Beef Cattle Research Center (Campo Grande, MS, Brazil) were evaluated. Both accessions were collected in wild East African savannas in the 1980s by the International Center for Tropical Agriculture (CIAT, Colombia), and then, transferred to Embrapa Genetic Resources and Biotechnology Center - Cenargen (Brazil). After quarantine they were transferred to Campo Grande where they are kept in plots in the field. The site characteristics of cultivation in Campo Grande, Mato Grosso do Sul, Brazil are: climate type Aw: tropical humid savanna; average annual precipitation = 1,526 mm; average temperature = 22°C; altitude 520 m; 20° 28' S; 55° 40' W; Oxisol (590 g kg⁻¹ sand; 80 g kg⁻¹ silt; 330 g kg⁻¹ clay; pH = 4.2).

Inflorescences for meiotic study 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 hours, transferred to 70% alcohol and stored under refrigeration until use. Microsporocytes were prepared by squashing and staining with 0.5% propionic carmine. Photomicrographs were taken in a Wild Leitz microscope using Kodak Imagelink - HQ, ISO 25 black and white film.

RESULTS

Chromosome counting of both species at diakinesis and anaphase I revealed that BO01 accession is pentaploid ($2n = 5x = 45$) (Figure 1a), BO05 is tetraploid ($2n = 4x = 36$) (Figure 2a), and SU01 is hexaploid ($2n = 6x = 54$), derived from $x = 9$. The meiotic behavior of these accessions was evaluated and several meiotic abnormalities were recorded (Table 1). The most common abnormalities in the three accessions were those related to irregular chromosome segregation due to polyploidy (Figure 1 and Figure 2). In the pentaploid accession of *B. bovonei* (BO01) (Figure 1b) and in the hexaploid accession SU01, several multivalent chromosomes associations were observed. However, in the tetraploid accession of *B. bovonei* (BO05) chromosomes associated predominantly as bivalents, and few quadrivalents were recorded at diakinesis (Figure 2a). Precocious chromosome migration to the pole in metaphase I (Figure 1c), and metaphase II (Figure 1g and Figure 2e), laggards in anaphase I (Figure 1f, g and Figure 2b, c) and anaphase II (Figure 1h and Figure 2f), leading to micronuclei formation in telophase I (Figure 1e and Figure 2d) and telophase II (Figure 1i and Figure 2g) were recorded in high frequency in the three accessions. The meiotic fate of telophase I and II micronuclei varied among accessions. In BO01, some micronuclei of telophase I were eliminated as microcytes at the end of the first division in 50% of meiocytes (Figure 1f). The same behavior was found after the second division when micronuclei were eliminated as microcytes in the tetrad (Figure 1k, l). In this accession, however, the majority of micronuclei remained in the tetrad (Figure 1j, k, l). In BO05, micronuclei remained inside the microspores in all the tetrads (Figure 2h, i). Micronuclei and microcytes in tetrads were also observed in SU01. Chromosome stickiness was another meiotic abnormality detected in both accessions of *B. bovonei*.

DISCUSSION

Two basic chromosome numbers have been reported along decades for several species in the genus *Brachiaria*, $x = 7$ and $x = 9$ (Basappa et al., 1987; Bernini & Marin-Morales, 2001; Valle & Savidan, 1996; Mendes-Bonato et al., 2002, 2006; Utsunomiya et al., 2005). However, recently, a new basic chromosome number, $x = 6$, was reported for *B. dictyoneura* (Risso-Pascotto et al., 2006b). There is a wide prevalence of polyploid forms in the genus *Brachiaria*, with a predominance of tetraploid accessions within species (Mendes-Bonato et al., 2002, 2006; Utsunomiya

Table 1 - Meiotic abnormalities in *Brachiaria bovonei* and *B. subulifolia*.

Phases	Abnormalities	BO01		BO05		SU01	
		2n = 5x = 45		2n = 4x = 36		2n = 6x = 54	
		No. of PMCs analyzed	No. of abnormal PMCs	No. of PMCs analyzed	No. of abnormal PMCs	No. of PMCs analyzed	No. of abnormal PMCs
Metaphase I	Precocious chromosome migration	210	108	126	25	150	60
	Chromosome stickiness		17		19		-
Anaphase I	Laggard chromosomes	127	79	112	31	136	108
	Chromosome stickiness		2		8		-
Telophase I	Micronuclei	164	48	152	12	140	86
	Chromosome stickiness		9		-		14
Prophase II	Micronuclei	198	97	113	61	125	61
	Chromosome stickiness		29		-		-
	Microcytes		17		-		-
Metaphase II	Precocious chromosome migration	216	46	134	56	155	102
	Chromosome stickiness		19		3		-
	Microcytes		23		-		-
Anaphase II	Laggard chromosomes	157	122	99	41	120	96
	Chromosome stickiness		13		4		-
	Microcytes		9		-		-
Telophase II	Micronuclei	233	176	172	94	134	109
	Microcytes		35		-		-
Tetrad	Micronuclei	160	136	87	58	159	130
	Microcytes		11		-		-
	Micronuclei and microcytes		21		-		-
Total		1465	1017 (69.42%)	995	412 (41.42%)	1119	766 (68.45%)

et al., 2005; Risso-Pascotto et al., 2006a). Among several species studied, only a few hexaploid accessions ($2n = 6x = 54$) were recorded and only in *B. brizantha* (Mendes-Bonato et al., 2002).

The abnormalities observed in the accessions under analysis were recorded in other polyploid accessions of different species of *Brachiaria* (Mendes-Bonato et al., 2002, 2006; Utsunomiya et al., 2005; Risso-Pascotto et al., 2006a). The meiotic fate of micronuclei in the genus *Brachiaria* seems to be genotype-specific. Accessions of different species and within species behaved differently in relation to micronuclei fate (Mendes-Bonato et al., 2002, 2006; Utsunomiya et al., 2005; Risso-Pascotto et al., 2006a), but unbalanced gametes are formed either way.

Chromosome stickiness observed in *B. bovonei* has been frequently reported in different species of the genus *Brachiaria* (Mendes-Bonato et al., 2001a, b; Utsunomiya et al., 2005; Risso-Pascotto et al., 2005). Chromosome stickiness is characterized by intense

chromosome clustering during any phase of the cell cycle. Chromosomes start to assemble at prophase I or metaphase I, frequently form congregations and do not orientate correctly on the equatorial plate. They display failure or irregular anaphase I disjunction, while chromosome fragmentation occurs from prophase I onwards, resulting in male and female sterility (Rao et al., 1990) Although chromosome stickiness is a cytological phenomenon that has been recorded for almost a century, adequate explanations are still lacking.

The abnormalities related to irregular chromosome segregation recorded in these accessions are typical of polyploids. However, the frequency could be affected by environmental factors. In *Brachiaria*, the environmental effect in the meiotic process was demonstrated in an interspecific hybrid between *B. ruziziensis* and *B. brizantha* cultivated in three distinct years (Fuzinato et al., 2008). Several meiotic abnormalities were recorded and varied in type and frequency in the three years of collection. Studies developed in many

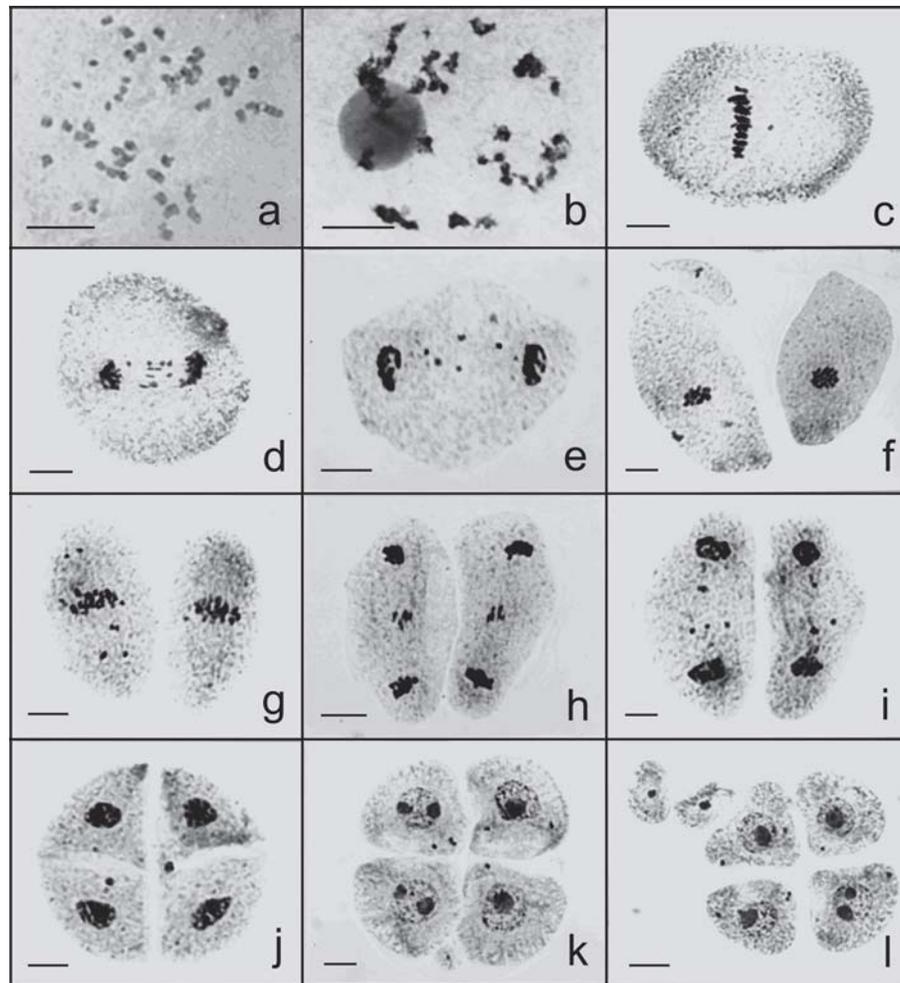


Figure 1 - Aspects of microsporogenesis in BO01 ($2n = 5x = 45$). a) Anaphase I showing 45 segregated chromosomes. b) Diakinesis with several multivalents. c) Metaphase I with precocious chromosome migration to the poles. d) Anaphase I with laggards. e) Telophase I with micronuclei. f) Metaphase II with a micronuclei and a microcyte. g) Metaphase II with precocious chromosome migration to the poles. h) Anaphase II with laggards. i) Telophase II with micronuclei. j) Tetrad with micronuclei in two microspores. k, l) Tetrads with micronuclei and microcytes. Scale bars = 19 μm .

species of higher plants revealed that the reproductive processes appear to be much more sensitive to abiotic stresses than vegetative growth (Sato et al., 2002). Meiosis is the most sensitive stage in the life cycle of seed plants (Saini, 1997; Porch & Jahn, 2001; Erickson & Markhart, 2002). Among the abiotic stresses capable of inducing abnormalities during meiosis, Sun et al. (2004) cited water availability, temperature, nutrient levels, light, soil salinity, and pollution.

In *Brachiaria* and several others forage grass, polyploidy is closely associated with asexual reproduction – apomixis. Studies on the reproductive biology of the *Brachiaria* germplasm collection at Embrapa Beef Cattle revealed that the accessions under analysis are apomictic (Valle & Savidan, 1996). Polyploidy, in general, disturbs meiotic process leading to unviable gamete formation. In the absence of gametes, reproduction is asexual. However, in *Brachiaria*, apomixis is

pseudogamic (Valle & Savidan, 1996), that means that viable male gametes are necessary to fertilize the secondary nucleus of embryo sac to develop the endosperm of the seed. Seed set was not yet evaluated in the present accessions.

The objective of cytological studies underway on the *Brachiaria* germplasm collection at Embrapa Beef Cattle is to determine the chromosome number of accessions, mode of reproduction, and meiotic behavior. For the breeding of *Brachiaria* species, compatible sexual and apomictic accessions need to be identified. Interploid crosses were attempted without success (Ferguson & Crowder, 1974; Hacker, 1988) but once ploidy barriers were removed, several interesting hybrids were produced. Hybridization is being done among some rare sexual tetraploid accessions, mainly of *B. ruziziensis* artificially tetraploidized by colchicine in Belgium (Svenne et al., 1981; Gobbe et al., 1981) and more recently of a

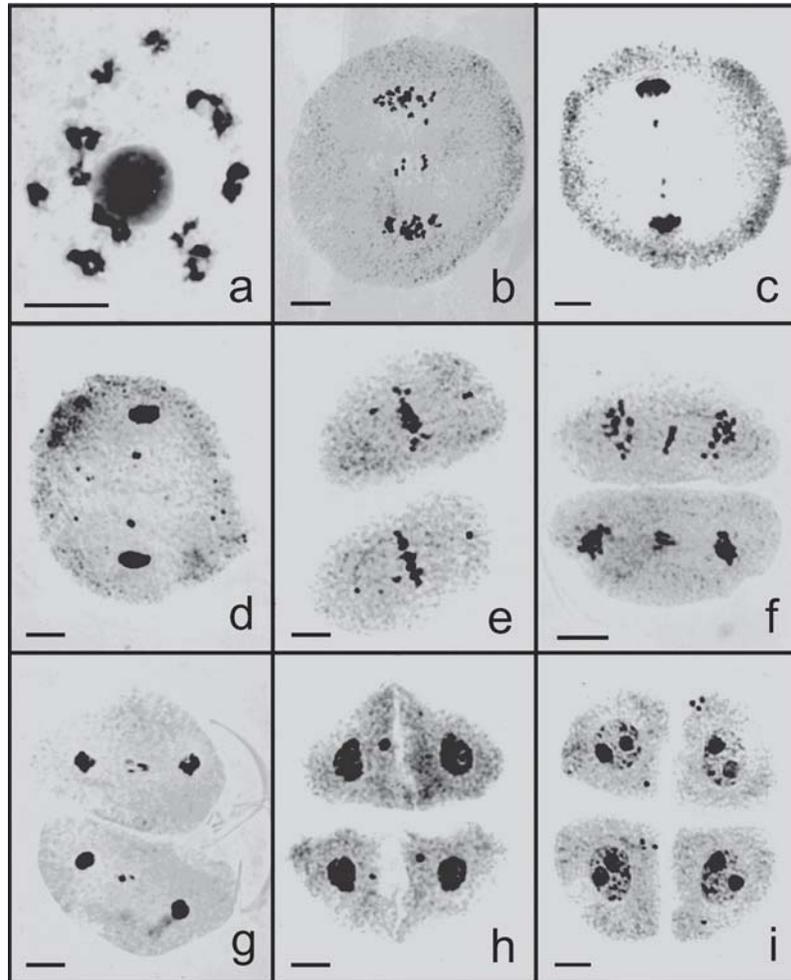


Figure 2 - Aspects of microsporogenesis in BO05 ($2n = 4x = 36$). a) Diakinesis with 16 bivalents and one quadrivalent. b, c) Anaphase I with several laggards. d) Telophase I with micronuclei. e) Metaphase II with precocious chromosome migration to the poles. f) Anaphase II with laggards. g) Telophase II with micronuclei. h) Tetrad with micronuclei in three microspores. i) Tetrads with micronuclei in the four microspores. Scale bars = 19 μ m.

natural tetraploid sexual *B. humidicola*, with apomictic tetraploid accessions as pollen donors. For an apomictic tetraploid accession to act as a male genitor, meiotic stability is required to guarantee endosperm development and to produce normal tetraploid hybrid progeny, with both sexual and apomictic reproduction. From the cytogenetic data obtained in the present analysis, none of the three accessions analyzed could be recommended for interspecific hybridization because of high meiotic instability (BO01 = 69.42%, BO05 = 41.42%, and SU01 = 68.45%) and also because of the ploidy level detected in BO01 (5x) and SU01 (6x). Phylogenetic studies also need to be carried out with these species to determine compatibility with agronomic important species. Cytogenetic studies need to continue in the germplasm *Brachiaria* collection to find ideal accessions for the breeding program, either for intraspecific or interspecific hybridization.

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