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Meiotic behavior of a nonaploid accession endorses x = 6 for *Brachiaria humidicola* (Poaceae)

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ABSTRACT. *Brachiaria humidicola* (Poaceae), originally from Africa, is an economically important pasture plant in tropical South America. An accession of *B. humidicola* (H038) collected from the wild African savanna (Mbeya, Tanzania) showed irregular microsporogenesis. This meiotic behavior was consistent with an allopolyploid origin. Multivalent chromosome association at diakinesis gave tri- to octavalents, associated with two nucleoli in some cells. Six non-congregated univalents in metaphase I and anaphase I, along with previous lines of evidence for x = 6 in *B. humidicola*, confirm H038 as a nonaploid accession, 2n = 9x = 54. Asynchrony in the genome during microsporogenesis also corroborated this assumption. Its putative origin could be a cross between two related species with different rhythms in meiosis. The meiotic behavior of this accession reinforces the hypothesis of the existence of a new basic chromosome number (x = 6) for *Brachiaria*. The use of this accession in the breeding of this important forage grass for the tropics is discussed.

Key words: *Brachiaria humidicola*; Basic chromosome number; Forage grass; Genome affinity; Meiosis

INTRODUCTION

Polyploidization is a key component of plant evolution. Up to 70% of flowering plants are known to be polyploid (Stebbins, 1971; Masterson, 1994), which means that the majority of angiosperms have undergone polyploidization at least once. According to Wendel (2000), genome doubling appeared in the Cretaceous and remains an active and ongoing process. Thus, many angiosperm genomes have experienced several cycles of polyploidization at various times in the past and are appropriately considered to have "paleopolyploid" genomes.

It is difficult, however, to ascertain the importance of genome doubling in the evolutionary history of flowering plants (Wendel, 2000). The origin of various types of polyploids (e.g., autopolyploid, allopolyploid, segmental allopolyploid) is pivotal to recognize its potential evolution and to correlate it with life-history attributes and ecological parameters (Harlan and deWet, 1975). According to Vanichanon et al. (2003), understanding the number of polyploidization events that have occurred in the formation of a given species, and the consequences of such events, has been a major challenge.

Polyploidy is widespread in the genus *Brachiaria* (Basappa et al., 1987; Honfi et al., 1990; Valle and Savidan, 1996; Bernini and Marin-Morales, 2001; Utsunomiya et al., 2005; Mendes-Bonato et al., 2002, 2006). An analysis of the DNA content of 435 accessions of 13 species of *Brachiaria* in the Brazilian collection, maintained at Embrapa Beef Cattle (Campo Grande, MS), revealed that only 13.04% are 2n whereas the others are polyploid, with a large predominance of tetraploids (58.13%) (Penteado et al., 2000). Meiotic behavior in some species of this germplasm collection, involving the analysis of chromosome association at diakinesis, suggested that tetraploid accessions may have arisen in different ways: autopolyploidy, segmental allopolyploidy, or allopolyploidy (Mendes-Bonato et al., 2002; Utsunomiya et al., 2005; Risso-Pascotto et al., 2006a). In *B. brizantha*, for instance, unequivocal evidence of recent allopolyploidization was detected in three accessions, derived from x = 9 (Mendes et al., 2006; Risso-Pascotto et al., 2006b). Recent studies performed on 54 accessions of *B. humidicola* revealed that 28 of them showed 2n = 54 chromosomes (Boldrini KR, unpublished data) and that one accession (H038) reinforced the evidence that this species is derived from x = 6. This paper discusses the meiotic behavior in this accession and infers about its potential use in the ongoing breeding program of *Brachiaria* to produce new superior cultivars.

MATERIAL AND METHODS

Of the 54 already studied accessions of *B. humidicola* from the Embrapa Beef Cattle germplasm collection, which comprises about 60 accessions, one accession, H038, was cytologically analyzed and is discussed here. This accession was collected in the wild African savannas (Mbeya, Tanzania) by the International Center for Tropical Agriculture (CIAT), in Colombia, in the 1980s. Site characteristics of the plots in the field in Embrapa Beef Cattle Research Center at Campo Grande, State of Mato Grosso do Sul (Brazil), where the accession was cultivated, were: 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 (59% sand; 8% silt; 33% clay; pH 4.2).

Inflorescences for meiotic study were collected in a plot with 16 plants and they were 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. Photomicrographs were made with a Wild Leitz microscope using Kodak Imagelink - HQ, ISO 25 black and white film.

RESULTS

A total of 1474 meiocytes were analyzed for H038. Counts at diakinesis and anaphase I revealed the presence of 2n = 54 chromosomes. Meiocytes with one and two nucleoli of different sizes were found in pachytene (Figure 1a) and diakinesis (Figure 1b,c). In diakinesis, chromosomes associated as bivalents and multivalents. When in multivalents, from tri- to octavalents and rare nonavalents were recorded among meiocytes (Figure 1b to f). Univalents were also found in this phase.

The most common meiotic abnormalities were irregular chromosome segregations associated with one laggard genome (Table 1). In metaphase I, the meiocytes exhibited six univalent chromosomes not congregated at the metaphase plate (Figure 1g). In anaphase I, while most of the genome initiated the chromosome migration to the poles, these six univalents moved to the metaphase plate (Figure 1h). These six univalents underwent sister-chromatid segregation (Figure 1i,j) when most of the genome was already in late anaphase I. The majority of univalents, however, reached the poles in time to be included in the telophase nuclei. In the second division, the meiotic behavior was the same. Some micronuclei were found in cells in prophase II. In metaphase II, the six segregated chromatids remained non-congregated in the metaphase plate and in anaphase II they remained again as laggard (Figure 1k). In telophase II, those chromatids that did not reach the poles in time to be included in the nuclei originated micronuclei that remained in the microspores of the tetrad (Figure 1l).

Table 1. Percentage of meiotic abnormalities observed in the accession H038 related to the laggard genome.

Phase	No. of cells analyzed	No. of cells with the abnormality present (%)
Metaphase I	225	Six non-congregated univalents at metaphase plate 68 (30.2%)
Anaphase I	111	Six laggard univalents 37 (33.3%)
Telophase I	142	Micronuclei 9 (6.3%)
Prophase II	154	Micronuclei 8 (5.2%)
Metaphase II	152	Non-congregated sister chromatids 34 (22.4%)
Anaphase II	34	Six laggard sister chromatids 11 (32.3%)
Telophase II	140	Micronuclei 9 (6.4%)
Tetrad	716	Micronuclei 99 (13.8%)

DISCUSSION

The meiotic behavior of the accession H038 was similar to that observed in two other accessions of *B. humidicola*: H003 (Boldrini KR, unpublished data) and H030 (Boldrini et

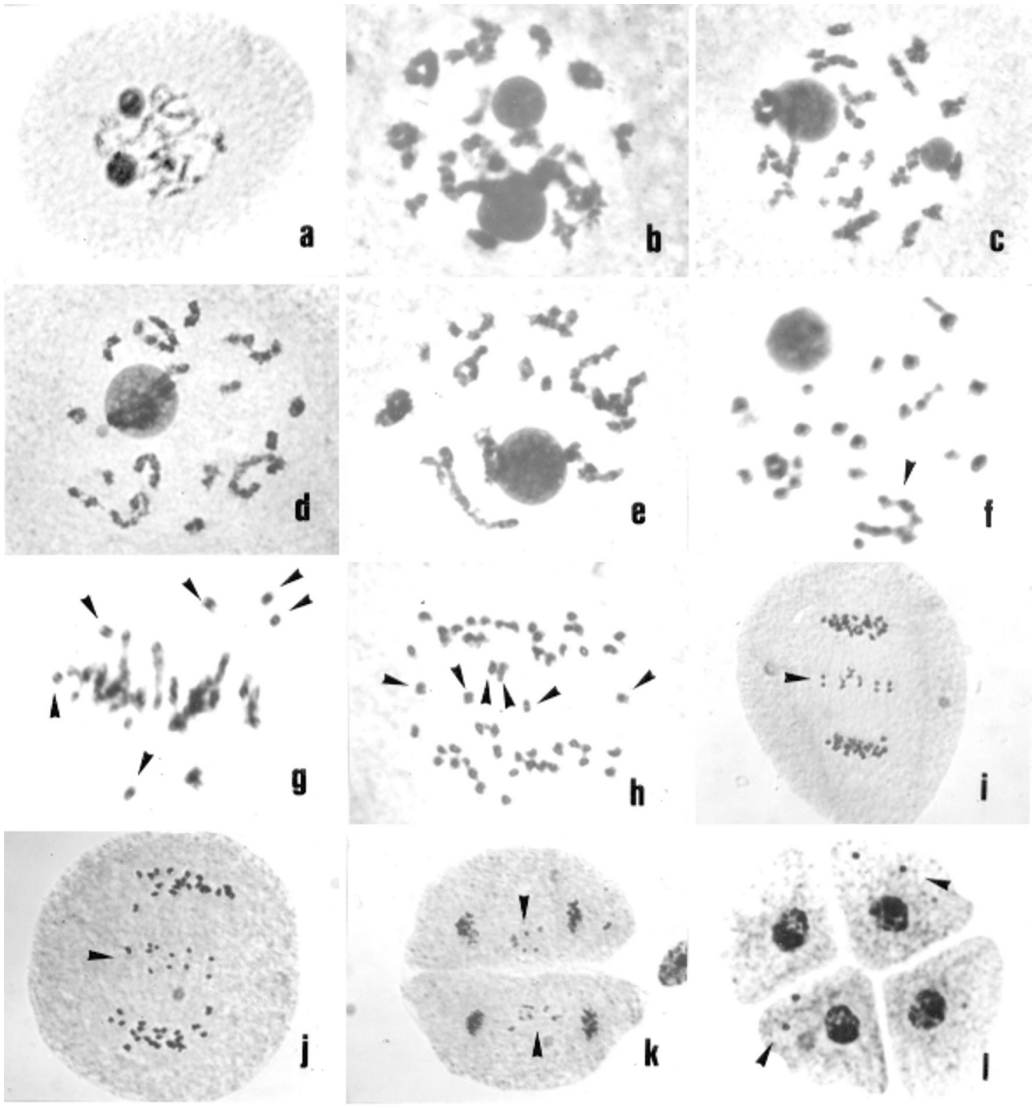

Figure 1. Aspects of microsporogenesis in H038**. a.** Meiocyte in pachytene with two nucleoli of different sizes. **b,c.** Diakinesis with two nucleoli of different sizes and several multivalent chromosome associations. **d-f.** Diakinesis with several multivalents involving different amounts of chromosomes. In *f* it is possible to distinguish a nonavalent chromosome association (arrowhead). **g.** Meiocyte in metaphase I with six non-congregated univalent chromosomes at metaphase plate (arrowheads). **h.** Early anaphase I with six univalent chromosomes reaching the metaphase plate (arrowheads) and the main segregated genome migrating to the poles. **i,j.** Anaphases I with the main genome reaching the poles and the laggard genome undergoing sister chromatid segregation (arrowheads). **k.** Late anaphase II with six laggard sister chromatids in each cell (arrowheads). **l.** Tetrad with micronuclei in three microspores (arrowheads).

al., 2009), both with 2n = 42 chromosomes. In H003, twelve univalents were found to be asynchronous with the main genome, while in accession H030, six univalents showed this behavior. For both accessions, an allopolyploid origin was proposed, based on chromosome numbers derived from x = 6. The data on the meiotic behavior of H003 and H030 ascertained that they are heptaploids derived from x = 6.

Two basic chromosome numbers, x = 7 and x = 9, have been reported for the genus *Brachiaria*, with a large prevalence of x = 9 (Basappa et al., 1987; Honfi et al., 1990; Valle and Savidan, 1996; Bernini and Marin-Morales, 2001; Utsunomiya et al., 2005; Mendes-Bonato et al., 2002, 2006). However, recently, a new basic chromosome number, x = 6, was determined for several accessions in the germplasm collection of *B. dictyoneura* at Embrapa (Risso-Pascotto et al., 2006c), a species belonging to the same taxonomic group and closely related to *B. humidicola*, according to Renvoize et al. (1996). Thus, from the data of the meiotic behavior of H003 and H030, it was assumed that they were heptaploid derived from x = 6.

Brachiaria humidicola H038, however, exhibited multivalent chromosome association at diakinesis, from tri- to nonavalents, associated with the presence of two nucleoli in some cells. Also, six non-congregated univalents in the metaphase plate in metaphase I, and six laggard univalents in anaphase I were observed, which underwent sister chromatid segregation. All this evidence suggests that this is a nonaploid accession, 2n = 9x = 54, derived from x = 6. It could have originated from a crossing between two related species with a different rhythm in meiosis. The presence of 24 segregated chromosomes in each pole in anaphase I suggests that one genitor, probably the male, was an apomictic accession with 2n = 8x = 48 chromosomes, which contributed with unreduced gametes. The female genitor could be a sexual accession with 2n = 2x =12. Apomixis is widespread among the polyploid accessions of *Brachiaria* (Valle and Savidan, 1996), and unreduced gametes due to different mechanisms have also been reported in the genus, including *B. humidicola* (Boldrini et al., 2006). It is widely accepted that in nature auto and allopolyploids arise by the union of unreduced gametes (Harlan and deWet, 1975; Bretagnolle and Thompson, 1995; Ramsey and Schemske, 1998).

Analyses of the meiotic behavior in several accessions and species of *Brachiaria* have revealed that this genus is under continuous evolution and crosses between related species have contributed to the origin of new allopolyploid accessions. Evidence of allopolyploidy obtained from asynchronous meiotic rhythm, similar to that described in H038, has been provided in another accession of *B. humidicola* (Boldrini et al., 2009) and also in two pentaploid accessions (2n = 5x = 45) of *B. brizantha*, a species derived from x = 9 (Mendes et al., 2006). In these accessions, a genome with nine chromosomes behaved as laggard during both meiotic divisions. Other evidence of allopolyploidy in this species was provided in a hexaploid accession (2n = 6x = 54) where the genomes were arranged in two distinct metaphase plates, and from each plate, a genome with nine univalents migrated to an opposite and convergent pole, forming a trinucleated telophase I (Risso-Pascotto et al., 2006b).

Polyploidization is both an ancient and an ongoing process (Wendel, 2000). *Brachiaria* is a genus of African origin where polyploidy, from 4x to 9x, derived from x = 6, 7, and 9 has been widely reported (Basappa et al., 1987; Honfi et al., 1990; Valle and Savidan, 1996; Bernini and Marin-Morales, 2001; Utsunomiya et al., 2005; Mendes-Bonato et al., 2002, 2006; Risso-Pascotto et al., 2006b). The highest chromosome number in the genus (2n = 10x = 90) was reported in *B. bovonei* (Spies and Du Plessis, 1986), and 2n = 8x = 72 in some other species (Takeota, 1965; Reeder, 1967), but when and how the events of polyploidization occurred

have not been clarified. In angiosperm, according to Wendel (2000), recent polyploidization events have been superimposed on these more ancient genome doubling events, often followed by additional rounds of "diploidization". Only the most recent genome duplications are likely to be classically recognized as constituting "polyploid speciation" events. The present accession of *B. humidicola*, with 2n = 9x = 54, might have undergone several rounds of polyploidization in the past until the recent event of allopolyploidization. Otto and Whitton (2000) and Hegarty and Hiscock (2005) suggested that polyploidization may be the most common mechanism of sympatric speciation in plants and that hybrid speciation (allopolyploidization) is an important evolutionary phenomenon. Putative genitors for this accession could be an apomictic male with 2n = 8x = 48 and a sexual female with 2n = 2x =12, but these have never been reported until now in cytogenetic studies. As the non-domesticated *Brachiaria* accession under analysis was collected in the wild African savannas, the existence of accessions with these chromosome numbers in their center of origin cannot be ruled out.

To produce viable gametes, allopolyploids must behave effectively as diploids during meiosis, so that only identical chromosomes (homologous) pair (Moore, 2002). Rounds of polyploidization, followed by diploidization and stabilization of meiotic features leading to pairs of homologous chromosomes, have been a major component of genome evolution in plants (Jannoo et al., 2004). The objective of the cytogenetic studies in the *Brachiaria* genus is to select compatible accessions with the same ploidy level to produce intra- and interspecific hybrids. The apomictic accessions can act as pollen donors to create new varieties well adapted to the edaphoclimatic conditions of the tropics. In *B. humidicola*, especially, accessions with 2n = 36 with a regular meiosis are desirable in order to cross with a sexual one, also 2n = 36, recently found in the germplasm collection of this species. Viable hybrids have been produced at the hexaploid level (2n = 36) and are under agronomic evaluation aiming at cultivar release. The common cultivar of *B. humidicola* largely used as pasture in waterlogged or poorly drained sites is 2n = 54, but no compatible sexual counterpart has been identified in order to breed at this ploidy level. Unfortunately, accessions of allopolyploid origin, showing irregular meiosis, such as H038, should not be used in the breeding program. On the other hand, since these accessions are apomictic, seed is formed without fertilization of the embryo and some viable pollen may be enough to guarantee endosperm formation after fertilization of the central cell. Further testing of pollen viability should be conducted before discarding an accession such as H038 from the breeding program.

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REFERENCES

Basappa GP, Muniyamma MS and Chinnappa CC (1987). An investigation of chromosome numbers in the genus *Brachiaria* (Poaceae: Paniceae) in relation to morphology and taxonomy. *Can. J. Bot.* 65: 2297-2309.

Bernini C and Marin-Morales MA (2001). Karyotype analysis in *Brachiaria* (Poaceae) species. *Cytobios* 104: 157-171.

Boldrini KR, Pagliarini MS and do Valle CB (2006). Abnormal timing of cytokinesis in microsporogenesis in *Brachiaria humidicola* (Poaceae: Paniceae). *J. Genet.* 85: 225-228.

Boldrini KR, Micheletti PL, Gallo PH, Mendes-Bonato AB, et al. (2009). Origin of a polyploid accession of *Brachiaria humidicola* (Poaceae: Panicoideae: Paniceae). *Genet. Mol. Res.* 8: 888-895.

Bretagnolle F and Thompson JD (1995). Tansley review No. 78. Gametes with the somatic chromosome number: mechanisms of their formation and role in the evolution of autopolyploid plants. *New Phytol.* 129: 1-22.

Harlan JR and deWet JMJ (1975). On Ö. Winge and a prayer: the origins of polyploidy. *Bot. Rev.* 41: 361-390.

Hegarty MJ and Hiscock SJ (2005). Hybrid speciation in plants: new insights from molecular studies. *New Phytol.* 165: 411-423.

Honfi A, Quarín CL and Balls JFM (1990). Estudios cariológicos en gramineas sudamericanas. *Darwiniana* 30: 87-94.

Jannoo N, Grivet L, David J, D'Hont A, et al. (2004). Differential chromosome pairing affinities at meiosis in polyploid sugarcane revealed by molecular markers. *Heredity* 93: 460-467.

Masterson J (1994). Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. *Science* 264: 421-424.

Moore G (2002). Meiosis in allopolyploids - the importance of 'Teflon' chromosomes. *Trends Genet.* 18: 456-463.

Mendes DV, Boldrini KR, Mendes-Bonato AB, Pagliarini MS, et al. (2006). Cytological evidence of natural hybridization in *Brachiaria brizantha* Stapf (Gramineae). *Bot. J. Linn. Soc.* 150: 441-446.

Mendes-Bonato AB, Pagliarini MS, Forli F, Valle CB, et al. (2002). Chromosome numbers and microsporogenesis in *Brachiaria brizantha* (Gramineae). *Euphytica* 125: 419-425.

Mendes-Bonato AB, Risso-Pascotto C, Pagliarini MS and Valle CB (2006). Chromosome number and meiotic behaviour in *Brachiaria jubata* (Gramineae). *J. Genet.* 85: 83-87.

Otto SP and Whitton J (2000). Polyploid incidence and evolution. *Annu. Rev. Genet.* 34: 401-437.

Penteado MIO, Rodrigues IF, Valle CB, Seixas MAC, et al. (2000). Determinação de Poliploidia e Avaliação da Quantidade de DNA Total em Diferentes Espécies de Gênero *Brachiaria*. Boletim de Pesquisa, 11. Embrapa Gado de Corte, Campo Grande, 19.

Ramsey J and Schemske DW (1998). Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annu. Rev. Ecol. Syst.* 29: 467-501.

Reeder JR (1967). Notes on Mexican grasses. VI. Miscellaneous chromosome numbers. *Bull. Torrey Bot. Club* 94: 1-17.

Renvoize SA, Clayton WD and Kabuye CHS (1996). Morphology, Taxonomy, and Natural Distribution of *Brachiaria* (Trin.) Griseb. In: *Brachiaria*: Biology, Agronomy, and Improvement (Miles JW, Maass BL and Valle CB, eds.). CIAT/EMBRAPA, Cali, 1-15.

Risso-Pascotto C, Pagliarini MS and Valle CB (2006a). Microsporogenesis in *Brachiaria dictyoneura* (Fig. & De Not.) Stapf (Poaceae: Paniceae). *Genet. Mol. Res.* 5: 837-845.

Risso-Pascotto C, Mendes DV, Silva N, Pagliarini MS, et al. (2006b). Evidence of allopolyploidy in *Brachiaria brizantha* (Poaceae: Paniceae) through chromosome arrangement at metaphase plate during microsporogenesis. *Genet. Mol. Res.* 5: 797-803.

Risso-Pascotto C, Pagliarini MS and Valle CB (2006c). A new basic chromosome number for the genus *Brachiaria* (Trin.) Griseb. (Poaceae: Panicoideae: Paniceae). *Genet. Res. Crop Evol.* 53: 7-10.

Spies JJ and Du Plessis H (1986). Chromosome studies on African plants. I. *Bothalia* 16: 87-88.

Stebbins GL (1971). Chromosomal Evolution in Higher Plants. Edward Arnold, London.

Takeota T (1965). Chromosome numbers of some East African grasses. *Am. J. Bot.* 52: 864-869.

Utsunomiya KS, Pagliarini MS and do Valle CB (2005). Microsporogenesis in tetraploid accessions of *Brachiaria nigropedata* (Ficalho & Hiern) Stapf (Gramineae). *Biocell* 29: 295-301.

Valle CB and Savidan Y (1996). Genetics, Cytogenetics, and Reproductive Biology of *Brachiaria*. In: *Brachiaria*: Biology, Agronomy, and Improvement (Miles JW, Maass BL and Valle CB, eds.). CIAT/EMBRAPA, Cali, 147-163.

Vanichanon A, Blake NK, Sherman JD and Talbert LE (2003). Multiple origins of allopolyploid *Aegilops triuncialis*. *Theor. Appl. Genet.* 106: 804-810.

Wendel JF (2000). Genome evolution in polyploids. *Plant Mol. Biol.* 42: 225-249.