Mitotic behavior in root tips of *Brachiaria* genotypes with meiotic chromosome elimination during microsporogenesis

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ABSTRACT. Three accessions of *Brachiaria brizantha*, three of *B. humidicola*, and two interspecific hybrids between *B. ruziziensis* and *B. brizantha* were analyzed with regard to their mitotic behavior in root tips. All these genotypes revealed chromosome elimination or lack of chromosome affinity in previous analyses of microsporogenesis. Analyses of root tips showed a normal mitotic division in all accessions and hybrids, reinforcing the notion that the genetic control of meiosis is totally independent of that of mitosis. The implications of these findings for the *Brachiaria* breeding program are discussed.

Key words: *Brachiaria*; Chromosome elimination; Mitosis; Meiosis

INTRODUCTION

Cell division requires an array of complicated processes that must be executed in a spatially and sequentially controlled manner (Dewitte and Murray, 2003). The basic mechanism of mitotic cell cycle control is highly conserved among eukaryotes (Burssens et al., 1998). Progression through the cell cycle boundaries is dependent upon specific serine/threonine kinases, generally referred to as cyclin-dependent kinases, whose activity is modulated by phosphorylation/dephosphorylation events and by their association with regulatory subunits called cyclins (Shaul et al., 1996). The mitotic cell cycle encompasses four sequential ordered stages - G_1, G_2, S, and M. The first gap (G_1 phase) intercedes between the previous mitosis (M) and the entry into the next replication of DNA (S phase), whereas the second gap (G_2) separates the S phase from the subsequent M phase.

Cell cycle regulation differs in each type of organism, suggesting a fine genetic control. Cell cycle regulatory genes have been identified in several plant species (Staiger and Doonan, 1993; Jacobs, 1995; Assaad et al., 1997; Huntley and Murray, 1999). Meiosis, on the other hand, involves a combination of sequential events that result in four reduced gametes in all sexually reproducing organisms. Many meiotic genes have been reported and isolated in animals and plants (Baker et al., 1976; Golubovskaya, 1979, 1989; Shwarzacher, 2003). Although both the mitotic cell cycle and meiosis are genetically controlled, their control is independent, so that a mutation affecting the mitotic cell cycle does not necessarily affect the meiotic process and vice versa.

Cytogenetic analyses recently performed on the meiotic behavior of several accessions and interspecific hybrids of *Brachiaria* revealed chromosome elimination or lack of genome affinity during microsporogenesis (Risso-Pascotto et al., 2004, 2006a; Mendes et al., 2006; Mendes-Bonato et al., 2006a). *Brachiaria* is a genus of tropical grasses of African origin, introduced to Brazil only in the second half of the last century, but it changed beef cattle production in the country, placing Brazil as the second largest producer and first exporter of beef in the world. This study analyzed the chromosome behavior during mitosis in these genotypes to compare with the chromosome behavior observed during meiosis.

MATERIAL AND METHODS

Three accessions of *Brachiaria brizantha* (B176, B183, and B222), three of *B. humidicola* (H03, H30, and H42), and two interspecific hybrids between *B. ruziziensis* and *B. brizantha* (HB19 and HB40) were analyzed with regard to their mitotic behavior in root tips. Roots were collected from plants grown in pots in greenhouse and fixed in ethanol:acetic acid (3:1, v/v) for 24 h. Afterward, they were transferred to 70% alcohol and stored under refrigeration at 4°C until use. Prior to chromosome staining using Feulgen technique, root tips were hydrolyzed in 1 N HCl at 60°C for 10 min. After squashing, roots were stained with Schiff's reagent for 45 min, and then, macerated in a drop of 45% acetic acid. Images were photographed with Kodak Imagelink - HQ, ISO 25 in black and white film. Table 1 presents the number of cells analyzed for each mitotic phase per genotype.

RESULTS AND DISCUSSION

The mitotic behavior in root tips of accessions and hybrids was completely normal (Table 1) as illustrated in Figure 1. Precocious chromosome migration to the poles, laggard chromosomes, micronuclei, or lack of genome affinity was never observed.

Table 1. Genotypes, collection sites, and number of cells analyzed at each mitotic phase, and percentage of abnormal cells.

Genotype	Accession	Collection site in Africa (Origin/suborigin)	Mitotic phase				No. of cells analyzed	% of abnormal cells
			PM	M	A	T		
B. brizantha	B176	Zimbabwe/Bindura	24	74	48	55	201	0
	B183	Ruanda/Kibungo	3	13	13	7	36	0
	B222	Ethiopia/Kaffa	52	51	19	25	147	0
B. humidicola	H03	Ethiopia/Sidamo	13	10	8	10	41	0
	H30	Zimbabwe/Hwange	8	9	3	8	28	0
	H42	Zimbabwe/Goromonzi	41	78	47	43	209	0
Hybrids	HB19	-	64	369	322	190	945	0
	HB40	-	48	389	164	147	748	0

PM: pro-metaphase; M: metaphase; A: anaphase; T: telophase.
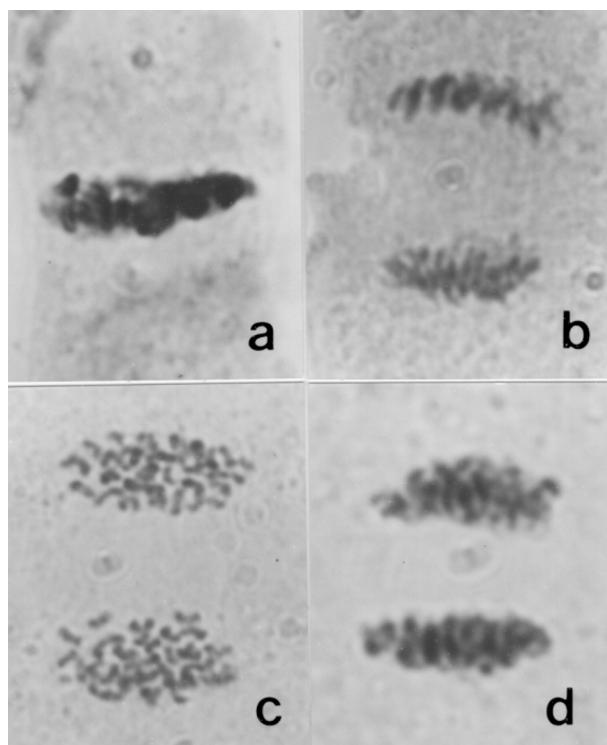
Figure 1. Aspects of normal mitosis in root tips of the *Brachiaria* hybrid HB40 (2n = 4x = 36). **a.** Metaphase. **b.** Anaphase. **c.** Early telophase. **d.** Telophase (Magnification: 1000X).

The main objective of this investigation was to contrast the mitotic and the meiotic behavior in genotypes that presented chromosome elimination or lack of genome affinity during microsporogenesis detected in a previous cytogenetic analysis. The accessions B183 and B222 of *B. brizantha* (Mendes et al., 2006), the accessions H03, H30, and H42 of *B. humidicola* (Boldrini KR, unpublished data) and the hybrid HB40 (Risso-Pascotto et al., 2004) showed chromosome elimination during microsporogenesis. Chromosome elimination was found to be caused by asynchronous meiotic rhythm, i.e., the parental genomes did not take the same time in each meiotic phase, thus the laggard genome was always eliminated from the main telophase nuclei. Chromosome elimination occurred in natural accessions of *B. brizantha* and *B. humidicola* collected in the African savannas in the middle 1980s and maintained in the field at the Embrapa Beef Cattle *Brachiaria* germplasm collection, suggesting they are recent allopolyploids. B183 and B222 are pentaploid (2n = 5x = 45), derived from x = 9, the most common basic chromosome number in the genus *Brachiaria* (Basappa et al., 1987; Valle and Savidan, 1996; Bernini and Marin-Morales, 2001; Utsunomiya et al., 2005; Mendes-Bonato et al., 2002, 2006b). In these accessions, nine univalents were eliminated by asynchronous meiotic rhythm (Mendes et al., 2006). In the *B. humidicola* accessions (H03, H30, and H42), meiotic behavior was similar, also suggesting recent allopolyploidization. However, H03 and H30 accessions are heptaploid (2n = 7x = 42) and H42 is nonaploid (2n = 9x = 54) (Boldrini KR, unpublished data), derived from x = 6, a new basic chromosome number reported for the genus and found in *B. dictyoneura* (Risso-Pascotto et al., 2006b), a species closely related to *B. humidicola*. In H03 and H42, six univalents remained behind in relation to the other genome during microsporogenesis and were eliminated; however, in H30, 12 univalents behaved as laggards and were eliminated. The same meiotic behavior was also found in the interspecific triploid hybrid HB40 (2n = 3x = 27) resulting from a cross between a sexual diploid accession of *B. ruziziensis* (2n = 2x = 18) and an apomictic tetraploid accession of *B. brizantha* (2n = 4x = 36), as pollen donor. In this hybrid, the nine univalent chromosomes of *B. ruziziensis* were eliminated also by asynchronous meiotic rhythm (Risso-Pascotto et al., 2004).

In the accession B176 of *B. brizantha*, meiotic behavior was distinct. This accession is a hexaploid (2n = 6x = 54), derived from x = 9 (Risso-Pascotto et al., 2006a). In this accession, chromosomes were arranged in two metaphase plates in the first meiotic division. In anaphase I, only nine univalents migrated from each plate to the opposite pole in an angle to form a typical tripolar spindle and, therefore, a restitutional nucleus. The remainder of the chromosomes stayed on the metaphase plate in the first division. After cytokinesis, the restitutional nucleus was eliminated as a microcyte, and the second division proceeded normally for the remainder of the genome. This hexaploid accession could have originated from the chromosome doubling of a triploid derived from species that did not display the same behavior for spindle organization.

In the tetraploid (2n = 4x = 36) interspecific hybrid HB19, the meiotic behavior of *B. ruziziensis* and *B. brizantha* genomes was typical of a lack of genome affinity (Mendes-Bonato et al., 2006a). In the first meiotic division of this hybrid, the nine bivalents of *B. ruziziensis* organized their metaphase plate while the nine bivalents of *B. brizantha* organized a distinct metaphase plate, both in the same cytoplasm. Each chromosome set segregated in its own spindle in anaphase I, forming four telophase nuclei.

This anomalous chromosome behavior observed during microsporogenesis in these accessions and hybrids was not detected during mitosis, reinforcing the notion that the genetic control of meiosis and mitosis is totally independent. Chromosome elimination in mitosis has been widely documented in interspecific hybrids in the early mitosis of embryo development.

The well-analyzed examples were crosses between *Hordeum vulgare* and *H. bulbosum* (Davies, 1974) and crosses between other *Hordeum* species (Jorgensen and Bothmer, 1988; Linde-Laursen and Bothmer, 1993). Other examples of somatic chromosome elimination in interspecific hybrids have been observed in *Nicotiana* (Gupta, 1969) and *Solanum* (Clulow et al., 1991).

Chromosome elimination in interspecific hybrids is a powerful tool in breeding programs. Differential chromosome elimination has facilitated the production of additional lines, while total elimination of one genome permits the formation of haploids. In the genus *Brachiaria*, the majority of species are polyploidy, mainly tetraploid (Valle and Savidan, 1996; Penteado et al., 2000; Utsunomiya et al., 2005; Mendes-Bonato et al., 2002, 2006a) and polyploidy is closely associated with apomixis (Valle and Savidan, 1996). The embryo emerges from a nucellus somatic cell, containing only the maternal genome. However, apomixis is pseudogamic in this genus, i.e., for seed formation, a viable male gamete needs to fertilize the secondary nucleus of the embryo sac restoring the chromosome proportion found in sexual plants (3n endosperm: 2n embryo) for correct endosperm development. Until now, only tetraploid accessions with few meiotic abnormalities have been used as male genitors in the hybridization program. Accessions with other ploidy levels have been generally discarded from the *Brachiaria* breeding program. From the present investigation and considering the species cited, it is suggested that chromosome elimination in *Brachiaria* occurs only in meiosis, independent of the ploidy level. Thus, the somatic tissues of these plants are homogeneous in their chromosome constitution since chromosome elimination does not seem to occur in mitosis. It is possible that an accession with chromosome elimination during microsporogenesis, but with a good agronomic trait as a forage grass, and independent of its ploidy level, could be used to create a new cultivar without affecting its overall somatic performance. A crucial condition for the new cultivar to be adopted, however, is good seed production, and that may require adequate behavior during meiosis to assure seed fill.

ACKNOWLEDGMENTS

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