Genetics and Molecular Biology, 29, 4, 711-714 (2006)

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Short Communication

Cytogenetic evidence for genome elimination during microsporogenesis in interspecific hybrid between *Brachiaria ruziziensis* and *B. brizantha* (Poaceae)

Andréa Beatriz Mendes-Bonato[1], Claudicéia Risso-Pascotto[1], Maria Suely Pagliarini[1] and Cacilda Borges do Valle[2]

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

Abstract

Microsporogenesis was analyzed in an interspecific hybrid between an artificially tetraploidized sexual accession of *Brachiaria ruziziensis* (R genome) and a natural apomictic tetraploid accession of *B. brizantha* (B genome). Chromosomes associated predominantly as bivalents. From this phase to the end of meiosis, chromosomes presented irregular segregation and abnormal arrangement in the metaphase plate. During metaphase I, in 27.8% of meiocytes, bivalents were distributed in two metaphase plates. In anaphase I, two distinct and typical bipolar spindles were formed. In 29.7% of pollen mother cells, one genome did not divide synchronically, with chromosomes lagging behind or not segregating at all. The second division was very irregular, resulting in polyads. Based on previous results from analysis of a triploid hybrid between these species, where the R genome was eliminated by asynchrony during meiosis, it is suggested that the laggard genome in this hybrid also belongs to *B. ruziziensis*.

Key words: *Brachiaria*, forage grass, interspecific hybridization, meiotic rhythm, microsporogenesis.

Received: June 20, 2005; Accepted: April 24, 2006.

Manipulation of genetic variability is essential for crop improvement. Naturally existing variation can be introgressed from alien sources, provided the genotypes are compatibile. The degree of genetic divergence between polyploid hybrids is displayed in chromosome pairing, which reflects genome affinity (Sundberg *et al*., 1991). In an original sense, genome analysis is the study of the pairing of chromosomes with the purpose of revealing phylogenetic relationships between two organisms. The methodology is based on the assumption that the extent of chromosome pairing in hybrids reflects the degree of relationship between the parental species (Dewey, 1982). In the widest sense, the term genome analysis covers all techniques that describe aspects of the genome, including karyotype analysis, meiotic chromosome pairing, measurements of DNA content, *in situ* hybridization, restriction enzyme analysis, genome mapping, and so forth (Seberg and Petersen, 1998). Genome analysis has been performed in several groups of plants, including economically important species. In forage grasses, genome analysis has been reported in several genera (Stebbins, 1981; Burson, 1981; Chennaveeraiah and Hiremath, 1974). In the genus *Brachiaria*, genome analysis has been only recently initiated (Risso-Pascotto *et al*., 2004).

Send correspondence to Maria Suely Pagliarini. Departamento de Biologia Celular e Genética, Universidade Estadual de Maringá, 87020-900 Maringá, PR, Brazil. E-mail: mspagliarini@uem.br.

The majority of accessions in this genus are polyploid, mainly tetraploid (2n = 4x = 36), and apomictic, and thus, breeding is complicated. Hybrids have been synthesized, however, at the Embrapa Beef Cattle Research Center by using artificially tetraploidized sexual accessions of *B. ruziziensis* as the female parent and natural apomictic tetraploids of *B. brizantha* or *B. decumbens* as the pollen parents. An extensive program of cytogenetic evaluation to characterize hybrids is in progress. Genome affinity is a pre-requisite for potential parents to produce fertile hybrids, as well as to ensure production of sufficient viable seed to allow adoption of a selected variety as a cultivar. The microsporogenesis of the hybrid Hb-19-92 between *B. ruziziensis* (genome R; 2n = 4x = 36) as the sexual female genitor and *B. brizantha* (genome B; 2n = 4x = 36) as the apomictic pollen donor, is described in this paper, focusing on the behavior of both genomes.

For meiotic studies, Hb-19-92 inflorescences were collected from a single plant growing in a tuft in the *Brachiaria* germplasm collection at Embrapa Beef Cattle (Campo Grande, MS, Brazil) and fixed in a mixture of ethanol (95%), chloroform, and propionic acid (6:3:2 v/v) for

24 h, and refrigerated (4 °C) until use. Microsporocytes (PMCs) were prepared by squashing and stained with 0.5% propionic carmine. Chromosome pairing was evaluated at diakinesis. More than 1800 microsporocytes were analyzed.

Conventional cytological studies under light microscopy revealed chromosomes at diakinesis associated predominantly as bivalents (Figure 1a). Among 20 meiocytes analyzed, in 13 only bivalents were observed; in five, one quadrivalent was found; and in two, two quadrivalents were recorded. From this phase to the end of meiosis, chromosomes presented irregular segregation and abnormal arrangement in the metaphase plate (Table 1). In 27.8% of the meiocytes, the chromosomes were distributed in two metaphase plates, with nine bivalents in each (Figure 1b). In anaphase I, two typical bipolar spindles were formed (Figures 1c and 1d). In some cases (Figure 1c), nine chromosomes could be counted migrating to each pole. In the cells with two metaphase plates, chromosome segregation was quite regular in each genome (Figure 1e), with only a

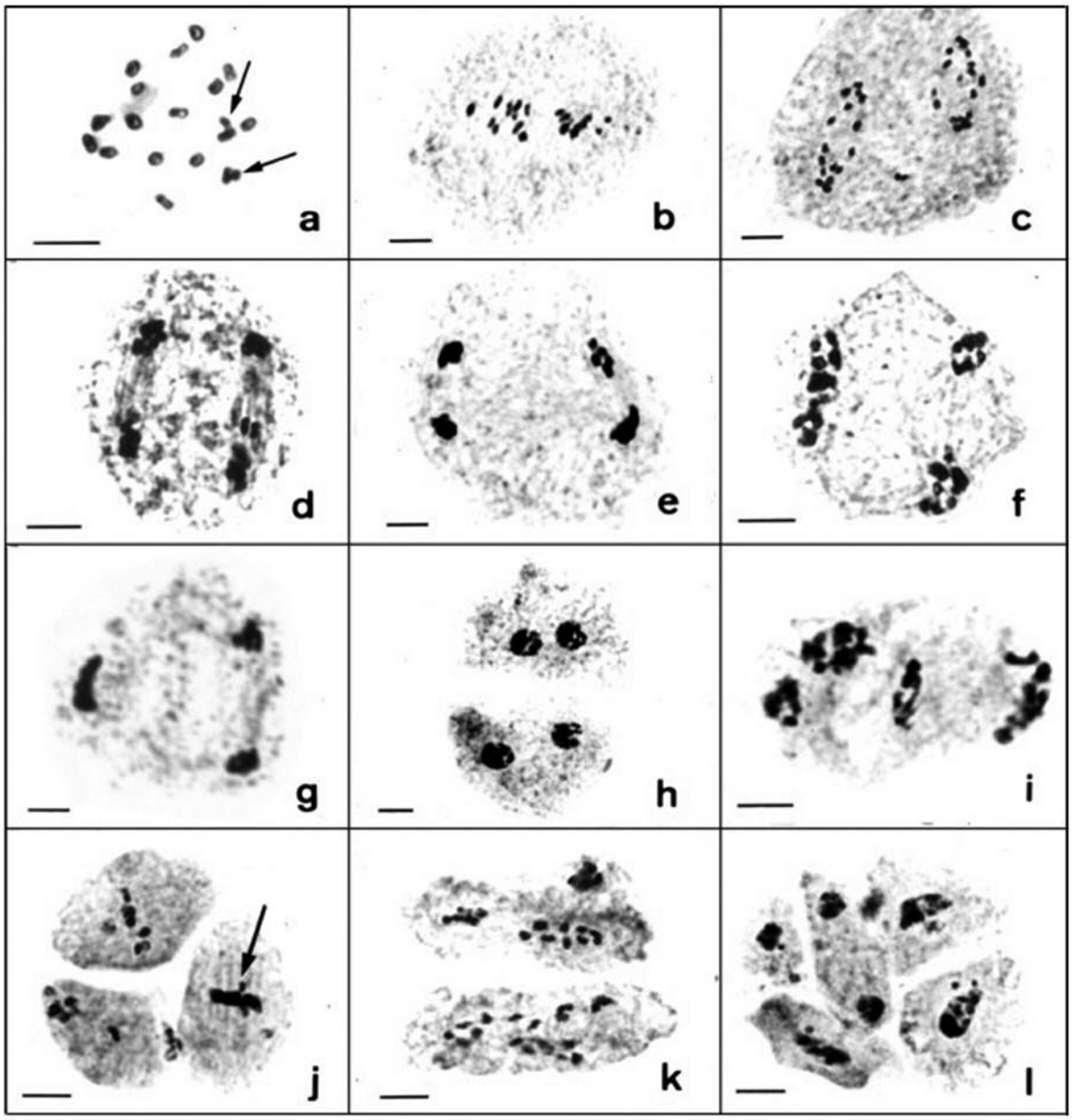

Figure 1 - Chromosome behavior during microsporogenesis in a hybrid between *B. ruziziensis* (R) and *B. brizantha* (B); (a) diakinesis with 17 II and 2 I (arrows); (b) metaphase I with B and R genomes arranged in two metaphase plates; (c, d) anaphase I with two distinct spindles (in c, note that nine chromosomes are migrating to the poles in each spindle); (e) telophase I with both genomes properly segregated; (f, g) trinucleate telophase I with only one segregated genome, the other remained non-segregated; (h, i) early and late prophase II with normal genome segregation; (j) late prophase II after normal chromosome segregation (the arrow indicates a cell with a restitution nucleus; (k) irregular chromosome distribution in anaphase II; (l) polyad with differently sized nuclei and microspores. (Bars = 1 μm).

Table 1 - Meiotic abnormalities recorded in the hybrid, related to chromosome segregation and abnormal genome orientation in the metaphase plate.

Phase	N. of cells analyzed	N. of abnormal cells	Abnormalities n. of cells with each abnormality (%)
Metaphase I	314	266	Precocious chromosome migration to the poles - 192 (72.2%)
			Two metaphase plates - 74 (27.8%)
Anaphase I	251	128	Laggard chromosomes - 90 (70.3%)
			Asynchrony between genomes - 38 (29.7%)
Telophase I	274	163	Micronuclei - 48 (29.4%)
			Two nuclei in both poles - 90 (55.2%)
			One restitution nucleus- 25 (15.4%)
Prophase II	232	180	Micronuclei - 62 (34.4%)
			Binucleated cells - 108 (60%)
			One uninucleated and one binucleated cell - 10 (5.6%)
Metaphase II	182	108	Abnormal chromosome disposition in the plate - 72 (66.7%)
			Precocious chromosome migration to the poles - 31 (28.7%)
			Triads - 5 (4.6%)
Anaphase II	139	89	Laggard chromosomes - 81 (91%)
			Triads - 8 (9%)
Telophase II	182	145	Micronuclei - 110 (75.9%)
			Triads - 15 (24.1%)
Tetrad	246	203	Micronuclei in micro-spores - 95 (46.8%)
			Triads 9 (4.4%)
			Pentads - 13 (6.4%)
			Hexads - 81 (39.9%)
			Microcytes - 5 (2.5%)

few laggards being observed in some cells (Figure 1c and d). In those cells with a single metaphase plate, however, there was a predominance of irregular chromosome segregation. Table 1 shows the frequency of cells with precocious chromosome migration to the poles in metaphase I and II, laggard chromosomes in anaphase I and II, and micronuclei in the telophase of the first and second divisions. These irregularities contributed to the formation of abnormal meiotic products, including a high frequency of micronuclei in microspores, triads, and polyads.

Another interesting aspect observed in the first division was that the two genomes were not synchronized in anaphase I. In 29.7% of the cells in anaphase, while one genome underwent normal chromosome migration to the poles, the other lagged behind (Figure 1f) or did not segregate at all (Figure 1g); a trinucleate telophase I with one restitution nucleus was formed in these cases. In those cells where chromosome segregation occurred regularly, a tetranucleate telophase I was observed (Figure 1 h, i). Consequently, the second division was very irregular. In pollen mother cells with a non-segregated genome, a cell with a dense metaphase plate containing 2n chromosomes could be seen (Figure 1j), whereas the sister cell underwent normal cytokinesis. Disorderly chromosome distribution in anaphase II was frequently found (Figure 1k). Polyads with differently sized nuclei and microspores were formed (Figure 1l). The seed set was not yet evaluated in the hybrid.

The analysis of polyploid hybrids is important in taxonomic and evolutionary studies of plants. According to Chapman and Kimber (1992) and King et *al*. (1999), the use of meiotic analysis to examine genome relationships has a number of practical advantages over other methods used to distinguish individual genomes and chromosomes in a hybrid either morphologically or using cytological techniques such as C-banding, fluorescent *in situ* hybridization (FISH) or genomic *in situ* hybridization (GISH). The analysis of microsporogenesis is less expensive and the results are of relevance to breeding work. The demonstration that two genomes are sufficiently similar to pair at meiosis is direct evidence that genes may be exchanged between them by recombination. In the present hybrid, based on the frequency of chromosome association, the probability of inter-genomic recombination is low since multivalents were rare. Their arrangement in two distinct metaphase plates with nine bivalents in each one suggests that chromosome pairing occurred within genomes, i.e., the eighteen chromosomes of the R genome paired in nine bivalents, and the eighteen chromosomes of the B genome did the same. Such a bivalent disposition in the metaphase plate was never reported in hybrids between other accessions of the *Brachiaria* species under analysis or in other interspecific hybrids of other genera of higher plants. In interspecific hybrids of other species of plants, in general, chromosomes of both genomes were reported to align themselves in a single metaphase plate.

The production of interspecific hybrids between certain plant species is followed by the selective elimination of chromosomes of one parent during the first few days of embryonic development. Well-analyzed examples are crosses of *Hordeum vulgare* and *H. bulbosum* (Davies, 1974) and crosses between other *Hordeum* species (Jorgensen and Bothmer, 1988; Linde-Laursen and Bothmer, 1993). Few reports in the literature have described chromosome elimination during sporogenesis. In plants, such an elimination has been reported in *Paspalum*, a genus of forage grass

closely related to *Brachiaria* (Adamowski *et al*., 1998), and in an interspecific triploid hybrid between *Brachiaria ruziziensis* (sexual, 2n = 2x = 18) and *B. brizantha* (apomictic, 2n = 4x = 36) recently analyzed by Risso-Pascotto *et al.* (2004). In the latter, and in the hybrid under analysis, the species involved in the hybridization are the same, but the hybrids resulted from crossings between different accessions. In the triploid hybrid of *B. ruziziensis* x *B. brizantha* (Risso-Pascotto *et al.,* 2004), the genome of *B. ruziziensis* was eliminated by asynchrony during meiosis, i.e., the nine unpaired univalent chromosomes did not follow the normal segregation of the nine bivalents of *B. brizantha*. They lagged behind during both meiotic divisions and were finally excluded from the telophase nuclei. A similar behavior occurred in 29.7% of the PMCs in the hybrid under analysis, although in distinct spindles. While one genome reached the poles, the other lagged behind or did not segregate at all. Based on the results of the triploid hybrid between these species, where chromosomes of the *B. ruziziensis* genome lagged and were eliminated (Risso-Pascotto *et al*., 2004), one can speculate that in the present hybrid the genome that remained behind also belonged to *B. ruziziensis* (R genome).

Despite its importance as a forage grass, detailed studies of genomic affinity in the genus *Brachiaria* have not been undertaken so far. Analyses of genetic relationships in the genus, based on morphological characters (Renvoize *et al.,* 1996) or molecular markers (Thome *et al.,* 1996), and the relative easiness of obtaining fertile interspecific hybrids between tetraploid accessions confirm the phylogenetic proximity among *B. ruziziensis, B. decumbens,* and *B. brizantha*. However, cytological studies of the genus are scarce. Karyotype studies performed on accessions of five *Brachiaria* species showed that even though there are morphological similarities and crossing ability, they display distinct karyotype characteristics (Bernini and Marin-Morales, 2001). Meiotic analyses of hybrids among these species have demonstrated distinct behavior, depending on which parents are used in the crosses. In that context, genome affinity seems to be closely related to the genetic background of the genotypes involved. In both *Brachiaria* species involved, there are accessions with distinct morphological, phenological and agronomic characteristics, suggestive of a different genetic diversity. Hybrids must produce a good amount of viable seeds to be widely utilized as forage in production systems. Cytological analyses become an essential screening tool in breeding programs, determining the hybrid fate in the cultivar development process. Although fertility was not yet evaluated, the meiotic abnormalities observed in both meiotic divisions, culminating in 82.5% of abnormal tetrads, allow to suggest that this hybrid will not advance to the cultivar status.

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Associate Editor: Marcelo Guerra