

**MARIANA FERRARI FELISMINO**

**Comportamento meiótico em irmãos-germanos e  
meios-irmãos entre *Brachiaria ruziziensis* x *B.*  
*brizantha* (Poaceae)**

Dissertação apresentada ao Programa de Pós-Graduação em Ciências Biológicas (área de concentração – Biologia Celular) da Universidade Estadual de Maringá, para obtenção do grau de Mestre em Ciências Biológicas.

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Dedico  
Aos meus pais.

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## APRESENTAÇÃO

Esta dissertação é composta por um artigo intitulado “**Meiotic behavior in full and half-sib hybrids between *Brachiaria ruziziensis* and *B. brizantha* (Poaceae)**” originado a partir da análise de quatro híbridos interespecíficos de *Brachiaria*, o qual será submetido à revista **GENETICS AND MOLECULAR BIOLOGY**.

# Comportamento meiótico em irmãos-germanos e meios-irmãos entre *Brachiaria ruziziensis* x *B. brizantha* (Poaceae)

*Mariana Ferrari Felismino*  
*Maria Suely Pagliarini*  
*Cacilda Borges do Valle*

**RESUMO.** O comportamento meiótico de quatro híbridos interespecíficos promissores (três sexuais: HBGC313, HBGC315, irmãos-germanos, e HBGC324; e um apomítico: HBGC325; estes últimos também irmãos-germanos, porém meios-irmãos em relação aos dois primeiros) foi avaliado. Os genitores femininos foram dois acessos sexuais tetraploidizados artificialmente de *B. ruziziensis* (R41 e R44:  $2n = 4x = 36$ ), os quais foram cruzados com um genótipo apomítico agronomicamente superior de *B. brizantha* (B140). A análise de células em diacinese revelou a presença de configurações cromossômicas multivalentes variando de um a três quadrivalentes em alguns meiócitos, sugerindo que a recombinação genética e a introgressão de genes de interesse de *B. brizantha* em *B. ruziziensis* é possível. Os quatro híbridos apresentaram diferentes frequências e tipos de anormalidades meióticas. Embora algumas anormalidades tenham sido comuns entre os híbridos, outras foram genótipo-específicas. O híbrido HBGC324 foi o mais estável em seu comportamento meiótico, podendo ser utilizado como genitor sexual em futuros cruzamentos no programa de melhoramento. Uma anormalidade relacionada à orientação do fuso (uma possível mutação *fuso divergente*) foi encontrada pela primeira vez em *Brachiaria* em dois dos híbridos analisados (HBGC313 e HBGC325). A partir de dados acumulados de estudos citogenéticos em híbridos, tornou-se óbvio que a análise citogenética é fundamental no programa de melhoramento de *Brachiaria*. A frequência e os tipos de anormalidades meióticas podem afetar a fertilidade do pólen e a produção de sementes, determinando, assim, quais genótipos podem continuar no processo de desenvolvimento de cultivares e quais podem ser usados com sucesso como genitores no programa de melhoramento para o desenvolvimento de cultivares superiores. Com base nos presentes resultados, somente o híbrido HBGC324 é recomendado para permanecer no programa.

# Meiotic behavior in full and half-sib hybrids between *Brachiaria ruziziensis* and *B. brizantha* (Poaceae)

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**Abstract.** The meiotic behavior of four interspecific promising hybrids (three sexual: HBGC313, HBGC315, which are full-sibs, and HBGC324; and one apomictic: HBGC325, which are also full-sibs, and half-sibs to the two previous ones) was evaluated. The female genitors were two artificially tetraploidized sexual accessions of *B. ruziziensis* (R41 and R44:  $2n = 4x = 36$ ), which were crossed to an agronomically superior tetraploid apomictic genotype of *B. brizantha* (B140). Analysis of cells in diakinesis revealed multivalent chromosome configurations ranging from one to three quadrivalents in some meiocytes, suggesting that genetic recombination and introgression of specific target genes from *B. brizantha* into *B. ruziziensis* can be expected. The four hybrids presented varying frequencies and types of meiotic abnormalities. Although some abnormalities were common among hybrids, others were genotype-specific. Hybrid HBGC324 was the least affected and could be used as a sexual genitor in future crosses in the breeding program. One abnormality related to spindle orientation (a putative *divergent spindle* mutation) was recorded for the first time in the two of the present hybrids (HBGC313 and HBGC325). From the expertise gathered through cytological studies on *Brachiaria* hybrids, it has become obvious that cytogenetical analysis is fundamental in breeding programs involving polyploid species. The frequencies and types of abnormalities may impact pollen fertility and potential of seed production, thus determining which genotypes can continue in the process of cultivar development and which can be successfully used as genitors in the breeding for superior forage performance. Based on the present results, only hybrid HBGC324 is recommended to remain in the program.

**Key words:** *Brachiaria brizantha*, *Brachiaria ruziziensis*, cytogenetics, forage grasses, interspecific hybrids, meiotic abnormalities.



## Introduction

In the last decades, Brazil became the biggest beef cattle producer and largest exporter of beef products. Tropical climate and extensive flat terrain provide the natural conditions for year-round beef production on cultivated pastures. Brachiariagrass alone accounts for 85% of the cultivated pastures in Brazil, covering over 80 million hectares and sustaining the largest commercial herd in the world – about 205 million of heads (IBGE, 2004). The genus *Brachiaria* has great success as a forage grass in the South American savannas due to its physiological tolerance to the typical low-fertility acid soils of the tropics (Rao *et al.*, 1996). Two cultivars, *B. decumbens* cv. Basilisk and *B. brizantha* cv. Marandu are undoubtedly the most widely grown species of forage grass in the Brazilian savannas and throughout the tropics. However, the lack of options to diversify pastures worry farmers since cv. Basilisk lacks resistance to a ubiquitous family of sucking insects, the spittlebugs, and cv. Marandu, while quite resistant, requires higher soil fertility (Miles *et al.*, 1996) and has proven weakly suited for poorly-drained soils, especially in the Amazon region where spittlebugs are year-round pests (Barbosa, 2006).

Pasture renovation and intensification of beef production demand new cultivars, which can be obtained by selection from native germplasm or generated in breeding programs. The brachiariagrass breeding program was delayed because the most important accessions and cultivars under use are polyploid ( $2n = 4x = 36$ ) and apomictic (Valle and Savidan, 1996). *Brachiaria* breeding became effective only when a suitable sexual germplasm was developed in the 80s. *B. ruziziensis* (ruzigrass) is a natural, sexual diploid species. Accessions of this species were tetraploidized with colchicine in Belgium (Swenne *et al.*, 1981; Gobbe *et al.*, 1981). Fortunately, after chromosome doubling, this species maintained the sexuality necessary for hybridization with

apomictic natural tetraploid accessions. These materials have allowed apomixis to be exploited in the breeding of *Brachiaria*.

The main objective of the *Brachiaria* program in tropical America is to use the sexuality of the tetraploid ruzigrass to release the genetic diversity locked in the natural tetraploid apomictics such as *B. brizantha* (palisadegrass), and *B. decumbens* (signalgrass), to produce novel apomictic hybrid cultivars (Valle and Savidan, 1996; Miles and Valle, 1996; Miles *et al.*, 2004). In 1988, the Embrapa Beef Cattle Center initiated an extensive program based on interspecies hybridization, with the objective of determining the inheritance of apomixis and thus manipulating this character for the development of new improved hybrids. Firstly, the crosses were done between sexual accessions of *B. ruziziensis* and apomictic *B. brizantha* cv. Marandu or *B. decumbens* cv. Basilisk. Afterwards, other accessions of superior agronomic performance and resistant to spittlebugs such as B140 were used as pollen donors. Hundreds of hybrids were obtained from the crosses and 88 selected based on leafiness and regrowth ability after cuts are under agronomic evaluation. However, besides resistance to spittlebugs and adaptation to infertile soils, a new cultivar needs to have a high seed production to attend the internal market demand to renovate pastures and also for export.

Cultivars available commercially are direct selections from the natural genetic variability (Miles *et al.*, 2004), except for one interspecific hybrid (cv. 'Mulato'), which produces less than 10% viable seeds thus compromising the commercial interests of the seed industry and impairing wide adoption. Among several factors that could compromise seed viability, Hopkinson *et al.* (1996), cite interspecific hybridization *per se*, and the polyploid nature of genotypes which impair seed viability by affecting pollen fertility. Previous cytogenetical analysis performed on some *Brachiaria* hybrids produced by Embrapa Beef Cattle (Risso-Pascotto *et al.*, 2005 a; Fuzinato, 2006;

Adamowski *et al.*, in preparation) revealed different types and varying frequencies of meiotic abnormalities thus indicating possibilities of selection of genotypes for higher pollen viability and, probably, higher seed production. This paper describes the meiotic behavior of four promising hybrids between *B. ruziziensis* and *B. brizantha*, and based on types and frequencies of abnormalities, indicates which are more stable to ensure greater pollen fertility and success in hybridizations.

## **Material and Methods**

Cytological studies were carried out on four interspecific hybrids between *B. ruziziensis* and *B. brizantha*. Two female genitors were utilized: both were two artificially tetraploidized sexual accessions of *B. ruziziensis* (R41 and R44:  $2n = 4x = 36$ ), which were crossed to a natural apomictic genotype: *B. brizantha* (B140), ( $2n = 4x = 36$ ). All four hybrids are related through the male genitor: B140. Hybrids HBGC313 and HBGC315 are full-sibs; hybrids HBGC324 and HBGC325 are also full-sibs, and half-sibs to the two previous ones. Table 1 shows the hybrids, their genitors, and mode of reproduction, previously determined by examination of embryo-sacs using interference contrast microscopy on methylsalicylate-cleared ovaries (Young *et al.*, 1979). The hybrids were produced by artificial pollination in the greenhouse at Embrapa Beef Cattle (Campo Grande, State of Mato Grosso do Sul, Brazil) in 1991-92. These hybrids have excellent phenotypes from the forage standpoint and are under small plot agronomical evaluation.

Inflorescences for meiotic studies were collected from individual plants under free growth in the field and fixed in a mixture of ethanol 95%, chloroform, and

propionic acid (6:3:2 v/v) during 24 hours and stored under refrigeration until use. Microsporocytes were prepared by squashing and stained with 0.5% propionic carmine. More than 2300 pollen mother cells (PMCs) were analyzed in each hybrid. Images were photographed with Kodak Imagelink – HQ, ISO 25 black and white film.

Table 1. Origin of the hybrids and mode of reproduction.

Hybrid	Female genitor	Male genitor	Plant	Mode of reproduction <sup>1</sup>
HBGC313	R41	B140	13	Sexual
HBGC315	R41	B140	18	Sexual
HBGC324	R44	B140	4	Sexual
HBGC325	R44	B140	5	Apomictic

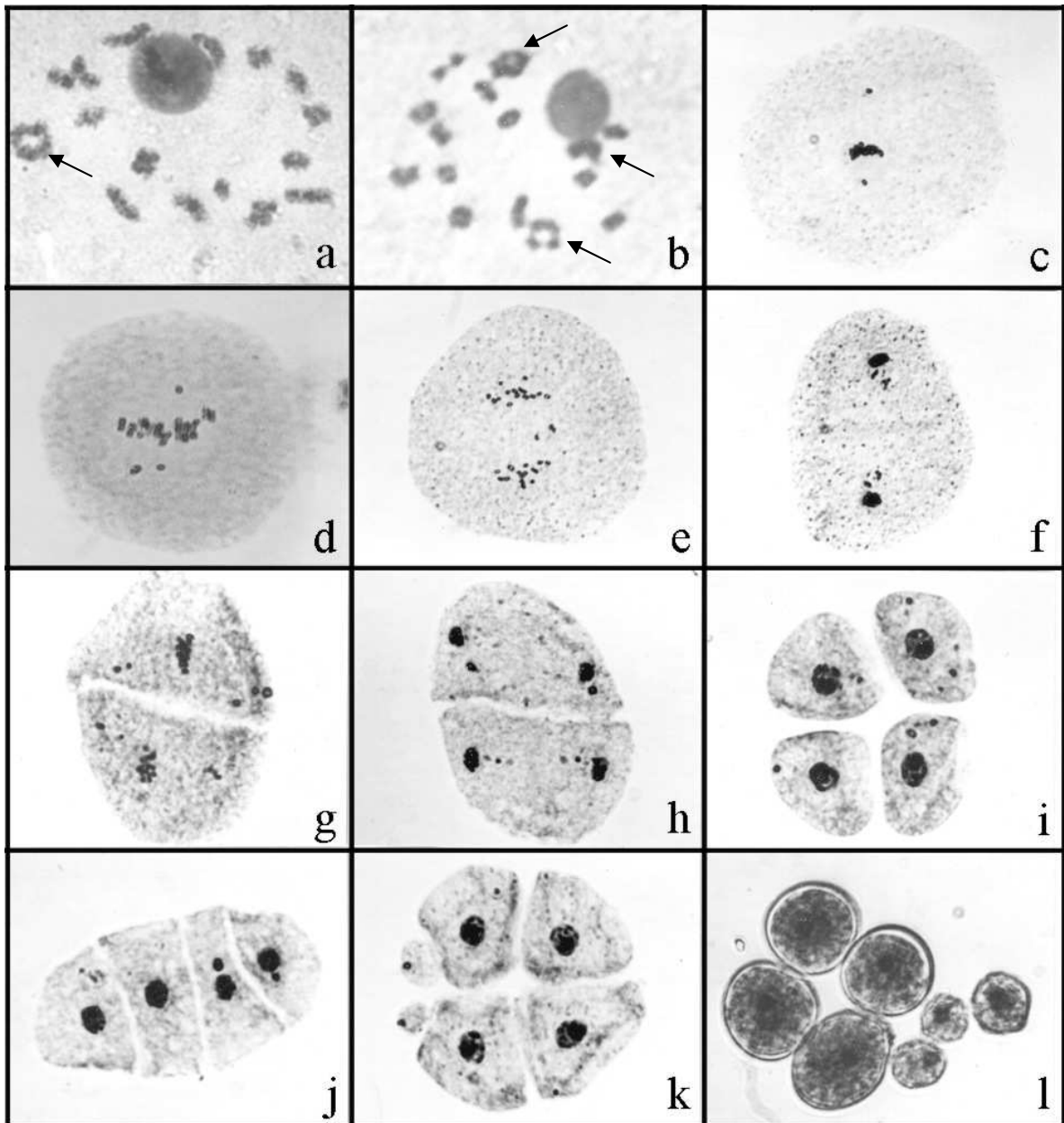
<sup>1</sup>Determined by microscopic examination of cleared ovaries

## Results and Discussion

The *B. ruziziensis*/*B. brizantha*/*B. decumbens* complex provides a wealth of genetic variation for the introgression of derived genes of interest, such as resistance to spittlebugs and nutritive value, among others. Gene introgression is possible through meiotic recombination. Analysis of diakinesis configurations in the four hybrids revealed multivalent chromosome configurations ranging from one to three quadrivalents in some meiocytes (Fig. 1 a, b). In the majority of meiocytes, however, chromosome paired as 18 bivalents of the same genome. Chromosome pairing in hybrids is used as a method of assessing genomic relationship between species (Alonso and Kimber, 1981) and also provides an important starting point in alien introgression programs (Gale and Miller, 1987).

The degree of differentiation between hybridizing taxa can be estimated not only by analysis of chromosome pairing, but also by analyzing meiotic abnormalities (Rieseberg *et al.*, 2000). Despite the phylogenetic proximity between *B. ruziziensis* and *B. brizantha* (Reinvoize *et al.*, 1996), the four hybrids presented different frequencies and types of meiotic abnormalities (Table 2). Although some abnormalities were common among hybrids, others were genotype-specific. Irregular chromosome segregation was recorded among the four hybrids in different frequencies. These were characterized by precocious chromosome migration to the poles in metaphase I (Fig. 1 c, d) and metaphase II (Fig. 1 g); laggard chromosomes in anaphase I (Fig. 1 e) and anaphase II, leading to micronuclei formation in telophase I (Fig. 1 f), telophase II (Fig. 1 h) and in tetrads (Fig. 1 i to k), or generating microcytes in tetrads (Fig. 1 k, l). Hybrid HBGC324 was the least affected one; among 3051 meiocytes analyzed, only 18.2% showed meiotic abnormalities. Irregular chromosome segregation was the most common meiotic abnormality recorded in *Brachiaria* hybrids analyzed (Lutts *et al.*, 1991; Risso-Pascotto *et al.*, 2005 a; Fuzinato, 2006; Adamowski *et al.*, in preparation). Such abnormalities are due to interspecies hybridization and to the polyploid condition of the hybrids.

Other meiotic abnormalities found in some of these hybrids, such as chromosome stickiness, abnormal cytokinesis, and absence of cytokinesis were also recorded in other *Brachiaria* hybrids (Risso-Pascotto *et al.*, 2005 a; Fuzinato, 2006; Adamowski *et al.*, in preparation) and have also been widely reported among accessions of different species of *Brachiaria* (Mendes-Bonato *et al.*, 2001 a b, 2002 a b; Risso-Pascotto *et al.*, 2005 a b; Utsunomiya *et al.*, 2005), suggesting that the genes controlling the character are in the gene pool of the genus. One abnormality, however, was recorded for the first time in two



**Figure 1.** Aspects of irregular chromosome segregation in the four interspecific hybrids. a, b) Diakinesis with 16 bivalents and one quadrivalent (arrow) (a), and 12 bivalents and 3 quadrivalents (arrows) (b) (Magnification 1000X). c, d) Metaphase I with precocious chromosome migration to the poles. e) Anaphase I with laggard chromosomes. f) Telophase I with micronuclei. g) Metaphase II with precocious chromosome migration to the poles. h) Telophase II with micronuclei. i) Isobilateral tetrad with micronuclei in all microspores. j) Linear tetrad with micronuclei. k) Tetrad with microcytes and micronuclei. l) Pollen grains with different sizes. (Magnification 400X)

**Table 2.** Frequencies of meiotic abnormalities in the four hybrids of *Brachiaria*

Phase	Abnormalities	HBGC313 R41 x B140/13		HBGC315 R41 x B140/18		HBGC324 R44 x B140/4		HBGC325 R44 x B140/5	
		Sexual		Sexual		Sexual		Apomictic	
		No. of PMCs <sup>1</sup> analyzed	No. of abnormal PMCs	No. of PMCs analyzed	No. of abnormal PMCs	No. of PMCs analyzed	No. of abnormal PMCs	No. of PMCs analyzed	No. of abnormal PMCs
<b>MI*</b>	Precocious migration	<b>486</b>	140	<b>652</b>	134	<b>753</b>	54	<b>776</b>	83
<b>AI</b>	Laggard chromosomes	<b>169</b>	110	<b>257</b>	152	<b>278</b>	62	<b>209</b>	38
	Chromosome stickiness		8		-		-		-
	Divergent spindle		16		-		-		82
<b>TI</b>	Micronuclei	<b>460</b>	254	<b>396</b>	222	<b>515</b>	47	<b>394</b>	55
	Chromosome stickiness		14		-		12		-
	Abnormal cytokinesis		-		-		19		-
	Divergent spindle		5		-		-		70
<b>PII</b>	Micronuclei	<b>449</b>	267	<b>235</b>	138	<b>305</b>	57	<b>482</b>	35
	Chromosome stickiness		13		-		-		-
	Absence of cytokinesis		8		-		-		14
	Abnormal cytokinesis		-		-		19		-
	Divergent spindle		19		-		-		341
<b>MII</b>	Precocious migration	<b>177</b>	58	<b>187</b>	47	<b>311</b>	50	<b>216</b>	10
	Abnormal cytokinesis		-		-		9		-
	Divergent spindle		14		-		-		192
<b>AII</b>	Laggard chromosomes	<b>28</b>	11	<b>69</b>	48	<b>93</b>	34	<b>26</b>	2
	Divergent spindle		4		-		-		22
<b>TII</b>	Micronuclei	<b>151</b>	88	<b>203</b>	109	<b>94</b>	21	<b>34</b>	3
	Chromosome stickiness		2		-		-		-
	Divergent spindle		14		-		-		26
<b>T</b>	Micronuclei	<b>537</b>	281	<b>1148</b>	670	<b>702</b>	142	<b>237</b>	86
	Microcytes		16		1		12		13
	Polyads		24		-		18		23
<b>Total (%)</b>		<b>2457</b>	1366 (55.6)	<b>3147</b>	1521 (48.3)	<b>3051</b>	556 (18.2)	<b>2374</b>	1095 (46.1)

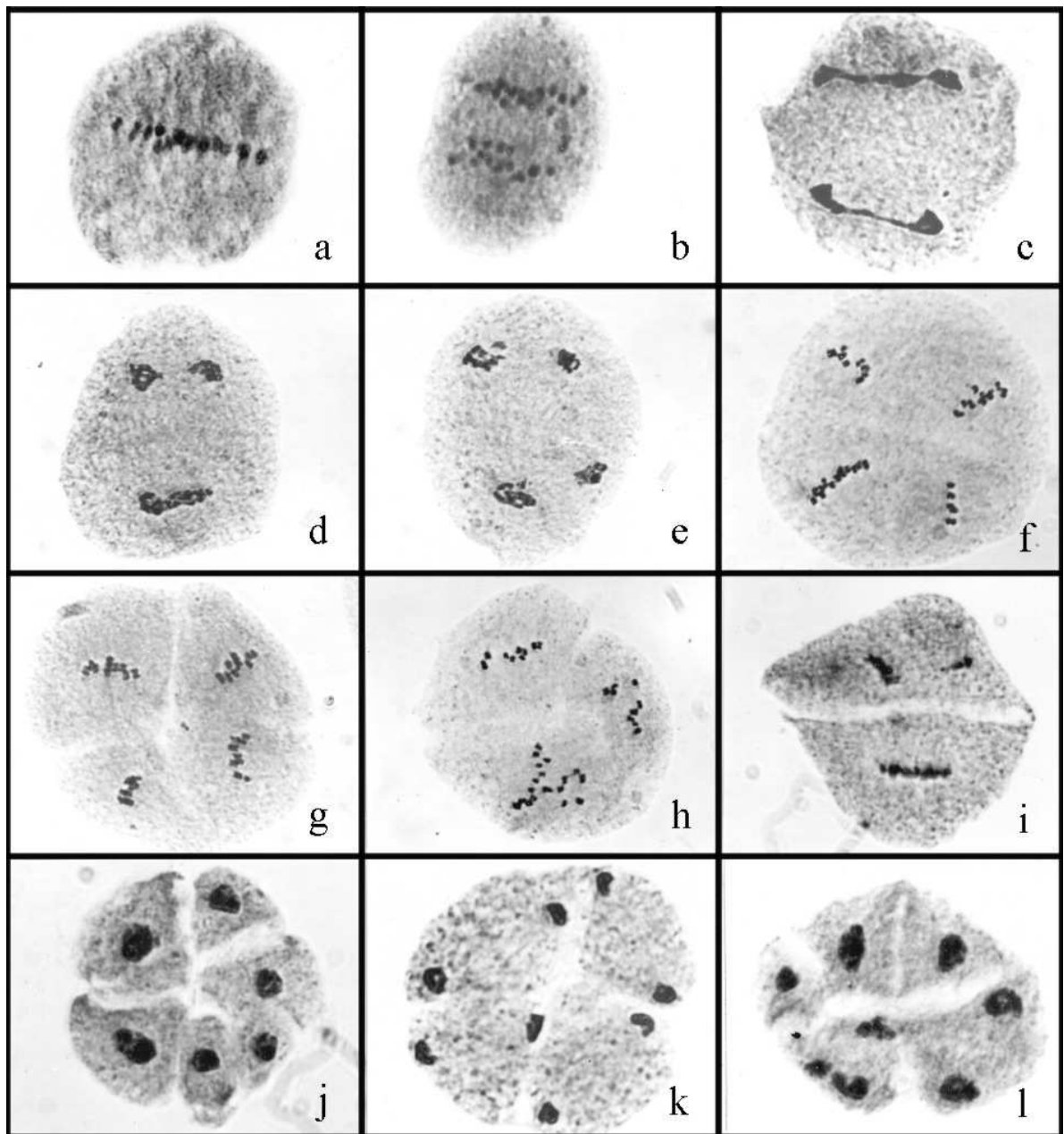
<sup>1</sup>PMCs: Pollen mother cells.

\*MI: metaphase I; AI: anaphase I; TI: telophase I; PII: prophase II; MII: metaphase II; AII: anaphase II; TII: telophase II; T: tetrad.

of the present hybrids (HBGC313 and HBGC325). This abnormality had been widely reported in maize (Clark, 1940; Staiger and Cande, 1990; Shamina *et al.*, 2000) and was called *divergent spindle (dv)*. Two hybrids (HBGC313 and HBGC325) were affected by this putative mutation. This abnormality affects the orientation of spindle fibers (Figure 2). In normal microsporocytes, spindle converges to form focused poles, while the bivalents occupy a small portion in the center of the metaphase plate and telophase nuclei are spherical as illustrated in Figure 1 (c-l).

In microsporocytes affected by the putative *dv* mutation, bivalents were regularly co-oriented at metaphase plate, although they were distantly positioned and spread over the equatorial plate (Fig. 2 a). As the spindle fiber did not converge into focused poles in anaphase I the segregated chromosomes remained parallel and failed to converge at the poles (Fig. 2 b). As a consequence, telophase I nuclei were fusiform (Fig. 2 c) depending on the distance of chromosomes at the poles, or the genome was grouped into two (Fig. 2 d, e) or more micronuclei of different sizes in each pole. After this phase, the first cytokinesis occurred dividing the meiocyte into as many cells as the number of telophase nuclei and/or micronuclei. The second meiosis progressed normally. The number of chromosomes in each metaphase plate was variable depending on the size of telophase I micronuclei (Fig. 2 f to i). Sister-chromatids normally segregated in anaphase II, giving rise to tetrads, hexads (Fig. 2 j) or octads (Fig. 2 k, l), depending on the number of nuclei and/or micronuclei formed in the first division. The percentage of affected cells by *dv* varied between the two hybrids; HBGC325 was much more affected than HBGC313. Considering that this abnormality occurred in two hybrids with different female genitor (HBGC313 - R41, and HBGC325 – R44), but with the same male parent, *B. brizantha* (B140), it is possible that this putative mutation is in the male genitor gene pool.





**Figure 2.** Aspects of the phenotypic expression of divergent spindle. a) Metaphase I with a broad metaphase plate. b) Anaphase I with non-convergent chromosomes to the poles. c) Telophase I with two elongated nuclei. d, e) Telophase I with bipartite nuclei. f, g) Metaphase II with four metaphase plates with different numbers of chromosomes in each one resulting from bipartition of both telophase I nuclei. h, i) Metaphase II with three metaphase plates with different numbers of chromosomes resulting from bipartition of one telophase I nucleus. j) Telophase II with six nuclei resulting from bipartition of one telophase I nucleus. k, l) Telophase II with eight nuclei resulting from bipartition of both telophase I nuclei. (Magnification 400X)



This assumption, however, would seriously compromise the use of *B. brizantha* (B140) as male genitor in the hybridization program because this abnormality compromises pollen viability by fractionating the genome. This ecotype, however, has excellent agronomic traits such as vigor, leafiness, digestibility, and palatability. Besides it is one of the most resistant ecotypes to pastures spittlebugs, therefore further exploration of its hybrids should be pursued in order to determine the frequency of occurrence of this abnormality when hybridized with *B. ruziziensis*.

The abnormalities caused by this putative gene (*dv*) added to irregular chromosome segregation due to polyploidy, always present in *Brachiaria* hybrids, are enough to discard a hybrid from the breeding program. In HBGC313, the percentage of abnormal meiocytes was 55.6%, while in HBGC325 was 46.1%. Hybrid HBGC315, also showed a high percentage of abnormalities (54.6%), although characterized only by irregular chromosome segregation. The more stable hybrid among the four analyzed was HBGC324, with 18.2% of abnormal cells and that reproduces sexually.

Crosses between sexual and apomictic plants segregate 1:1 (Valle and Savidan, 1996), indicating a simple inheritance of apomixis in *Brachiaria* as was determined for *Panicum maximum* (Savidan, 2000). Among the four selected promising hybrids, only one (HBGC325) is apomictic, which may allow for some seed production since megasporogenesis is independent of meiosis (Araújo *et al.*, 2000). These plants, however, are pseudogamous (Alves *et al.*, 2001), thus microsporogenesis will be affected by whatever abnormalities are present, and pollen fertility may be impaired.

At the moment, the *Brachiaria* breeding program aims at producing apomictic hybrids since whatever traits are selected will breed true, i.e., will not segregate, which contributes to establishing homogeneous improved pastures. However, superior sexual hybrids need to continue in the program, acting as female genitors in polycross blocks

in order to pyramidize desirable alleles, introgress desirable genes and also to broaden the genetic base of the program. Among the four hybrids analyzed, the sexual HBGC313 and the apomictic HBGC325 should be discarded from the program because of the high frequency of the putative mutation *dv* which seriously compromises pollen fertility. From the two remaining sexual hybrids, only HBGC324 could continue in the program because it has shown high meiotic stability. As a matter of fact, this hybrid is the most stable from the material already analyzed in the Embrapa Beef Cattle breeding program.

Hybrids must produce a good amount of viable seeds, besides good overall dry matter production and nutritive value, in order to be widely utilized and adopted in production systems. Due to pseudogamy, the desirable superior apomictic hybrids need viable pollen grains to fertilize the secondary nucleus of the embryo sac and thus ensure normal and vigorous endosperm development and plenty of seed set.

## References

- Adamowski EV, Pagliarini MS and Valle CB. Meiotic behavior in three interspecific backcross hybrids between *Brachiaria ruziziensis* and *B. brizantha* (Poaceae: Paniceae). (in preparation).
- Alonso LC and Kimber G (1981) The analysis of meiosis in hybrids. II. Triploid hybrids. Can J Genet Cytol 23: 221-234.
- Alves ER, Carneiro VTC and Araújo ACG (2001) Direct evidence of pseudogamy in an apomictic *Brachiaria brizantha* (Poaceae). Sex Plant Reprod 14: 207-212.
- Araújo ACG, Mukhambetzhannov S, Pozzobon MT, Santana EF and Carneiro VTC (2000) Female gametophyte development in apomictic and sexual *Brachiaria brizantha* (Poaceae). Rev Cytol Biol Veget – Le Botaniste 23: 13-28.

- Barbosa RA (2006) Morte de pastos de Braquiárias. Embrapa Gado de Corte, Campo Grande, MS, 206 p.
- Clark FJ (1940) Cytogenetic studies of divergent meiotic spindle formation in *Zea mays*. Am J Bot 27: 547-559.
- Fuzinato VA (2006) Avaliação da microsporogênese em híbridos sexuais de *Brachiaria* (Poaceae). Dissertação. Mestrado em Genética e Melhoramento. Universidade Estadual de Maringá. 55p.
- Gale MD and Miller TE (1987) The introduction of alien genetic variation into wheat. In Lupton FGH (ed.). Wheat Breeding: its Scientific Basis. Chapman & Hall, London pp 173-210.
- Gobbe J, Swenne A and Louant BP (1981) Diploïdes naturels et autotétraploïdes induits chez *Brachiaria ruziziensis* Germain et Evrard: critères d'identification. Agron Trop 36: 339-346.
- Hopkinson JM, Souza FHD, Diulgheroff S, Ortiz A, Sánchez M (1996) Reproductive physiology, seed production, and seed quality of *Brachiaria*. In Miles JW, Maass BL and Valle CB (eds) *Brachiaria: Biology, Agronomy, and Improvement*. CIAT/Embrapa, Colômbia/Brasília, pp 124-140.
- IBGE-Instituto Brasileiro de Geografia e Estatística ([www.ibge.gov.br](http://www.ibge.gov.br)) Pesquisa Pecuária. Atualizado em 2004.
- Lutts S, Ndikumana J, Louant BP (1991) Fertility of *Brachiaria ruziziensis* in interspecific crosses with *Brachiaria decumbens* and *Brachiaria brizantha*: meiotic behavior, pollen viability, and seed set. Euphytica, 57: 267-274.
- Mendes-Bonato AB, Pagliarini MS, Valle CB do and Penteadó M.I.O (2001 a) A severe case of chromosome stickiness in pollen mother cells of *Brachiaria brizantha* (Hochst) Stapf (Gramineae). Cytologia 66, 287-291.
- Mendes-Bonato AB, Pagliarini MS, Valle CB do and Penteadó MIO (2001 b) Meiotic instability in invader plants of signal grass *Brachiaria decumbens* Stapf (Gramineae). Acta Scient 23: 619-625.

- Mendes-Bonato AB, Junqueira Filho RG, Pagliarini MS, Valle CB do and Pentead MIO (2002 a) Unusual cytological patterns of microsporogenesis in *Brachiaria decumbens*: abnormalities in spindle and defective cytokinesis causing precocious cellularization. *Cell Biol Int* 26: 641-646.
- Mendes-Bonato AB, Pagliarini MS, Forli F, Valle CB do and Pentead MIO (2002 b) Chromosome number and microsporogenesis in *Brachiaria brizantha* (Gramineae). *Euphytica* 125: 419-425.
- Miles J and Valle CB do (1996) Manipulation of apomixis in *Brachiaria* breeding. . In Miles JW, Maass BL and Valle CB (eds) *Brachiaria: Biology, Agronomy, and Improvement*. CIAT/Embrapa, Colômbia/Brasília, pp 164-177.
- Miles JW, Maass BL and Valle CB do (1996) *Brachiaria: Biology, Agronomy, and Improvement*. CIAT/Embrapa, 288 p.
- Miles JW, Valle CB do, Rao IM and Euclides VPH (2004) *Brachiariagrasses*. In: Moser LE, Burson BL, Sollenberger LE (ed). *Warm-Season (C4) Grasses*. ASA, CSSA, SSSA, Madison. Pp 745-760.
- Rao IM, Kerridge PC and Macedo MCM (1996) Nutritional requirements of *Brachiaria* and adaptation to acid soils. In Miles JW, Maass BL and Valle CB (eds) *Brachiaria: Biology, Agronomy, and Improvement*. CIAT/Embrapa, Colômbia/Brasília, pp 53-71.
- Renvoize SA, Clayton WD and Kabuye CHS (1996) Morphology, taxonomy, and natural distribution of *Brachiaria* (Trin.) Griseb. In Miles JW, Maass BL and Valle CB (eds) *Brachiaria: Biology, Agronomy, and Improvement*. CIAT/Embrapa, Colômbia/Brasília, pp 1-15.
- Rieseberg LH, Baird SJE and Gardner KA (2000) Hybridization, introgression, and linkage evolution. *Plant Mol Biol* 42:205-224.
- Risso-Pascotto C, Pagliarini MS, Valle CB do (2005 a) Meiotic behavior in interspecific hybrids between *Brachiaria ruzizensis* and *Brachiaria brizantha* (Poaceae). *Euphytica*, 145: 155-159.

- Risso-Pascotto C, Pagliarini MS and Valle CB do (2005b) Multiple spindles and cellularization during microsporogenesis in an artificially induced tetraploid accession of *Brachiaria ruziziensis* (Gramineae). *Plant Cell Rep* 23: 522-527.
- Savidan YH (2000) Apomixis: Genetics and breeding. *Plant Breed Rev* 18: 13-86.
- Shamina N, Dorogova N and Trunova S (2000) Radial spindle and the phenotype of the maize meiotic mutant, *dv*. *Cell Biol Int* 24: 729-736.
- Staiger CJ and Cande WZ (1990) Microtubule distribution in *dv*, a maize meiotic mutant defective in the prophase to metaphase transition. *Dev Biol* 138: 231-242.
- Swenne A, Louant BP and Dujardin M (1981) Induction par la colchicine de formes autotétraploïdes chez *Brachiaria ruziziensis* Germain et Evrard (Graminée). *Agron. Trop.* 36: 134-141.
- Utsunomiya KS, Pagliarini MS and Valle CB do (2005) Microsporogenesis in tetraploid accessions of *Brachiaria nigropedata* (Ficalho & Hiern) Stapf (Gramineae). *Biocell*, 29: 295-301.
- Valle CB do and Savidan YH (1996) Genetics, cytogenetics, and reproductive biology of *Brachiaria*. . In Miles JW, Maass BL and Valle CB (eds) *Brachiaria: Biology, Agronomy, and Improvement*. CIAT/Embrapa, Colômbia/Brasília, pp.147-163.
- Young BA, Sherwood RT and Bashaw EC (1979) Cleared-pistyl and thick-sectioning techniques for detecting aposporous apomixis in grasses. *Can J Bot* 57: 1668-1672.

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