RESEARCH ARTICLE

Meiotic behaviour in three interspecific three-way hybrids between *Brachiaria ruziziensis* and *B. brizantha* (Poaceae: Paniceae)

ELENIZA DE VICTOR ADAMOWSKI[1], MARIA SUELY PAGLIARINI[1,*] and CACILDA BORGES DO VALLE[2]

[1]*Department of Cell Biology and Genetics, State University of Maringá, 87020-900 Maringá PR, Brazil*
[2]*Embrapa Beef Cattle, P.O. Box 154, 79002-970 Campo Grande MS, Brazil*

Abstract

The meiotic behaviour of three three-way interspecific promising hybrids (H17, H27, and H34) was evaluated. These hybrids resulted from the crosses between *B. ruziziensis* × *B. brizantha* and crossed to another *B. brizantha*. Two half-sib hybrids (H27 and H34) presented an aneuploid chromosome number ($2n = 4x = 33$), whereas hybrid H17 was a tetraploid ($2n = 4x = 36$), as expected. Chromosome paired predominantly as multivalents suggesting that genetic recombination and introgression of specific target genes from *B. brizantha* into *B. ruziziensis* can be expected. Arrangement of parental genomes in distinct metaphase plates was observed in H27 and H34, which have different male genitors. Hybrids H17 and H34 have the same male genitor, but did not display this abnormality. In H17, abnormalities were more frequent from anaphase II, when many laggard chromosomes appeared, suggesting that each genome presented a different genetic control for meiotic phase timing. Despite the phylogenetic proximity among these two species, these three hybrids presented a high frequency of meiotic abnormalities, mainly those related to irregular chromosome segregation typical of polyploids, H34, 69.1%; H27, 56.1% and H17, 44.9%. From the accumulated results obtained through cytological studies in *Brachiaria* hybrids, it is evident that cytogenetical analysis is of prime importance in determining which genotypes can continue in the process of cultivar development and which can be successfully used in the breeding. Hybrids with high frequency of meiotic abnormalities can seriously compromise seed production, a key trait in assuring adoption of a new apomictic cultivar of *Brachiaria* for pasture formation.

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Introduction

The pantropical genus *Brachiaria*, contains about 100 species mainly from the African continent, and is found in a wide range of habitats, from semidesert to swamps (González and Morton 2005). While grasses from this genus have been continuously exploited by the local pastoralists for millennia, the interest in species of this genus as sown and managed forage only began in 1960s. This occurred first on a limited scale in humid, coastal, tropical Australia, followed by tropical South Africa, and later in Brazil in the early 1970s (Miles *et al*. 2004). Currently, the genus *Brachiaria* is the most widely used forage grass in the South American savanna due to its physiological tolerance to low fertility acid soils of the tropics (Rao *et al*. 1996).

Brachiaria brizantha (A. Rich) Stapf (palisadegrass), *B. decumbens* Stapf (signalgrass), *B. humidicola* (Rendle) Schweick. (koroniviagrass), and *B. ruziziensis* Germain and Evrard (ruzigrass), are the most commercially exploited brachiaria grasses. Their economic importance is greatest in tropical America, where extensive adoption over the past three decades has had a revolutionary impact on the productivity of vast areas of previously underused, marginal soils (Miles *et al*. 2004). Nowadays, *Brachiaria* alone accounts for at least 85% of the cultivated pastures in Brazil (Miles and Valle 1996), covering over 50 million hectares and sustaining the largest commercial herd in the world—about 205 million.

Two cultivars, *B. decumbens* cv. Basilisk and *B. brizantha* cv. Marandu, are undoubtedly the most widely grown species not only in the Brazilian savannas but throughout the

*For correspondence. E-mail: mspagliarini@uem.br.

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tropics. This rapid expansion of their acreage did not occur without problems: both cultivars have significant limitations. The first one lacks resistance to a ubiquitous family of sucking insects, the spittlebugs (Homoptera: Cercopidae); the second, while resistant, requires higher soil fertility and does not tolerate waterlogged soils (Miles *et al*. 1996; Barbosa 2006). Brazilian pastures are severely degraded because of inadequate fertilization and mismanagement. Renovation of pastures and intensification of production practices demand new cultivars. Eight cultivars are presently commercialized by a dynamic seed industry dominated by Brazilian companies, and seven of these cultivars are in direct selections from naturally occurring germplasm collected in Africa (Miles *et al*. 2004). All the cultivars are polyploids ($2n = 4x = 36$) and apomictic that held back the initiation of brachiaria grass breeding programmes until suitable sexual germplasm was developed in the mid 1980s (Valle and Savidan 1996).

To increase the genetic variability in the genus hoping to generate new cultivars for pasture diversification, an extensive programme based on intraspecies and interspecies hybridization was undertaken at the Embrapa Beef Cattle Center in 1988, with the objective of determining the inheritance of apomixis and thus manipulating this character for the development of new improved hybrids (Valle and Savidan 1996). At first, hybridizations were between sexual *B. ruziziensis* and apomictic *B. brizantha* or *B. decumbens*. A great number of hybrids were obtained and some are under agronomic evaluations. Some interesting sexual hybrids were selected to be crossed to some of the superior ecotypes of the paternal species. The *B. ruziziensis/B. decumbens/B. brizantha* complex provides a wealth of genetic variation for the introgression of derived genes of interest, such as for spittlebugs resistance and nutritive value, among others. This paper describes the meiotic behaviour of three three-way hybrids obtained between *B. ruziziensis* and *B. brizantha*, and compares the types and frequencies of abnormalities that may affect pollen viability and seed production, thus determining their use as cultivars or genitors in the breeding programme.

Material and methods

Cytological studies were carried out on three three-way interspecific hybrids. The original female genitor in these hybrids was an artificially tetraploidized sexual accession of *B. ruziziensis* (R50, $2n = 4x = 36$), which was crossed to apomictic *B. brizantha* cv. Marandu (M), ($2n = 4x = 36$). One F1 sexual hybrid obtained S13 (R50 × M/27), was then pollinated by *B. brizantha*-B132 to produce hybrid H27, and by *B. brizantha*-B166 to produce both H17 and H34. Thus the hybrids H17 and H34 are full sibs and half sibs to hybrid H27.

The hybrids were produced by controlled pollination in the greenhouse at Embrapa Beef Cattle Center (Campo Grande, State of Mato Grosso do Sul, Brazil). These three 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 95% ethanol, chloroform and propionic acid (6 : 3 : 2 v/v) for 24 h and stored under refrigeration until use. Microsporocytes were prepared by squashing and staining with 0.5% propionic carmine. More than 2100 pollen mother cells (PMCs) were analysed in each hybrid. Images were photographed with Kodak Imagelink - HQ, ISO 25 black and white film.

Results and discussion

Conventional cytological analyses revealed one hybrid (H17) with $2n = 4x = 36$ chromosomes as its genitors and the two others from the cross between sexual F_1 hybrid S13 and two different apomicitic *B. brizantha* (H27 and H34) with an abnormal chromosome number, $2n = 4x = 33$ (figure 1,a, b). The original female progenitor in the three hybrids was an obligate sexual accession (R50) artificially tetraploidized with colchicine in Belgium (Gobbe *et al*. 1981; Swenne *et al*. 1981). These materials have allowed apomixis to be exploited in the breeding of *Brachiaria*. In the second generation of the crosses, the sexual S13 hybrid was crossed to two different apomictic ecotypes of *B. brizantha*, chosen for superior performance in agronomic trials: B166 and B132.

In tropical America, the main objective of the *Brachiaria* breeding is to use the sexuality of the tetraploid ruzigrass to release the genetic diversity locked in the natural tetraploid apomictics, signalgrass and palisadegrass, to produce novel apomictic hybrid cultivars (Valle and Savidan 1996; Miles *et al*. 2004). Results of experimental hybridization in Brazil showed that, sexual and apomictic plants occur in approximately equal proportions among hybrids, indicating a monogenic inheritance of apomixes (Miles and Valle 1996; Savidan 2000). Superior apomictic hybrids are desirable in the breeding programme since their traits are fixed by apomixis, and homogeneous improved permanent pastures can, thus, be established. On the other hand, superior sexual hybrids to be used as female genitors in crosses are needed to broaden the genetic base and to introgress more desirable genes into the gene pool.

All the original progenitors in these hybrids were tetraploid ($2n = 4x = 36$). It was not possible to detect with conventional staining from which genitor the aneuploidy ($2n = 4x = 33$) arose in two hybrids but apparently it is related to the S13 mother plant, since the aneuploid hybrids had different male genitors. Considering that: (i) female gametes are more tolerant to aneuploidy than male gametes (Sybenga 1992; Singh 1993), (ii) severe meiotic abnormalities were found in tetraploidized accessions of *B. ruziziensis* (R50) used as female genitor in the original cross, seriously compromising pollen viability (Risso-Pascotto *et al*. 2005a), and (iii) in a previous study, the genome of *B. ruziziensis* was delayed during meiosis in relation to the genome

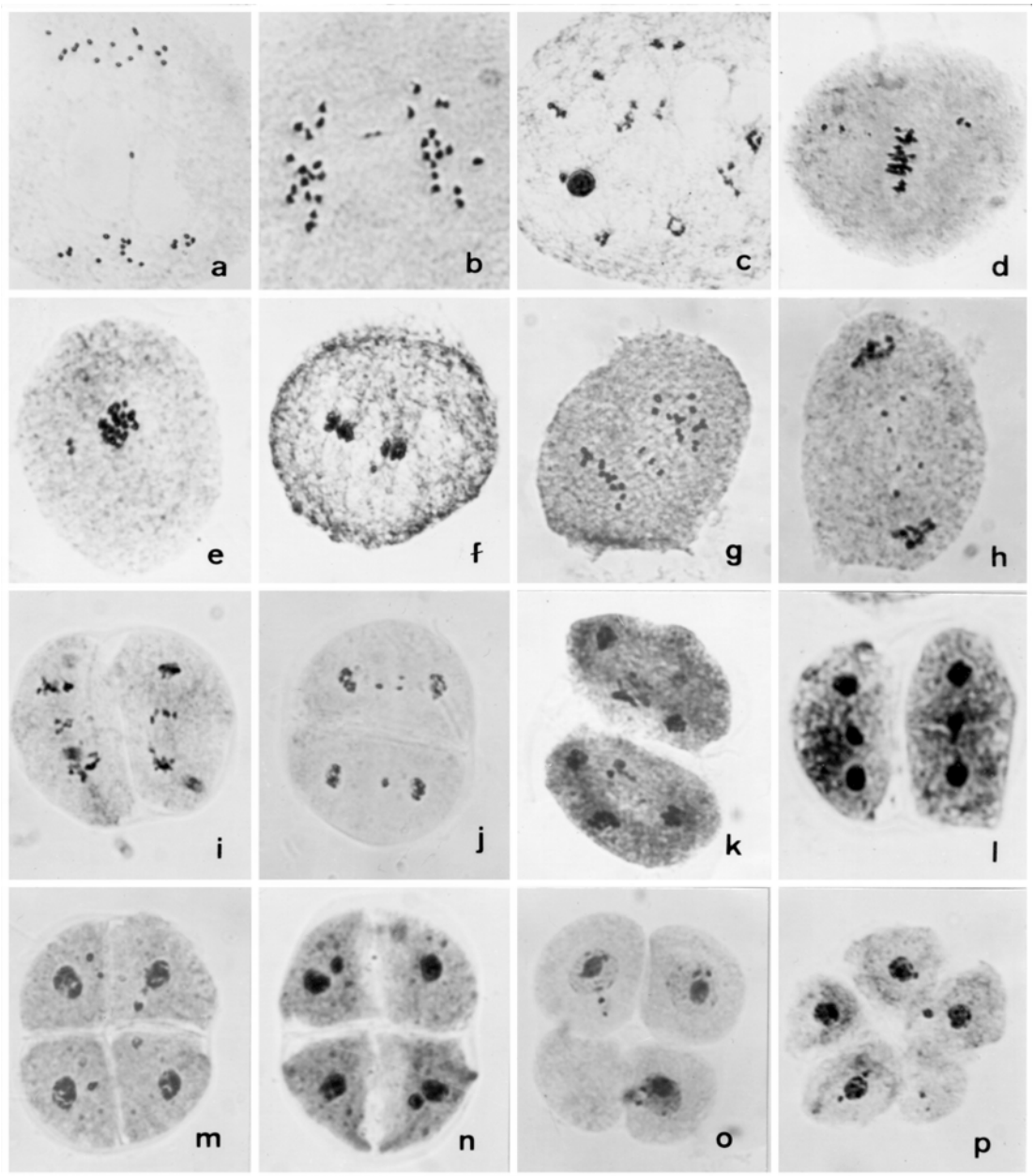

Figure 1. Some aspects of meiotic behaviour in hybrids H34 and H27 of *Brachiaria* with $2n = 4x = 33$ chromosomes. *(a, b)* Two anaphases I with 16 chromosomes in each pole and a laggard. In *b* the laggard is undergoing sister-chromatid segregation. *(c)* Diakinesis with several multivalents. *(d)* Metaphase I with precocious chromosome migration to the poles. *(e)* Polar view of metaphase I with a noncongregated bivalent. *(f)* Metaphase I with two distinct plates. *(g)* Anaphase I with three laggard chromosomes. *(h)* Early telophase I with small micronuclei. *(i)* Anaphase II with laggard chromosomes. *(j)* Telophase II with small micronuclei. *(k, l)* Telophase II with large micronuclei. *(m, n)* Tetrads with small and large micronuclei, respectively. *(o)* Tetrad with an anucleated microspore and *(p)* tetrad with micronuclei and microcyte.

of *B. brizantha* (Risso-Pascotto *et al*. 2004), we suggest that ruzigrass R50 and later S13 was probably the genitor that provided the aneuploid gamete, despite the fact that hybrid H17 did not display it.

In diakinesis, the three hybrids presented a high frequency of multivalent chromosome pairing (figure 1c), showing that the species are closely related and that the gene introgression is possible among them through meiotic recombination. Chromosome pairing in hybrids is used as a method of assessing genomic relationships between species (Alonso and Kimber 1981). In addition, it also provides an important starting point in alien introgression programmes (Gale

and Miller 1987). Based on the morphological characters, Renvoize *et al*. (1996), showed that *B. brizantha, B. ruziziensis*, and *B. decumbens* belongs to the same taxonomic group. The phylogenetic proximity among them was reinforced by molecular markers (Suárez 1994). The meiotic pairing of chromosomes in the hybrids suggests that the *B. ruziziensis* and *B. brizantha*, form a coherent gene pool or agamic complex, once ploidy barriers are overcome (Lutts *et al*. 1991).

According to Rieseberg *et al*. (2000), the degree of differentiation between hybridizing taxa can be estimated not only by analyses of chromosome pairing behaviour, but also by estimating and analysing meiotic abnormalities. Despite this phylogenetic proximity among species, the three hybrids presented a high frequency of meiotic abnormalities (table 1).

Table 1. Meiotic abnormalities in the three interspecific three-way hybrids of *Brachiaria*.

		H34 S13 × B166/17 $2n = 4x = 33$		H27 S13 × B132/8 $2n = 4x = 33$		H17 S13 × B166/1 $2n = 4x = 36$	
Phases	Abnormalities	No. of PMCs analysed	No. of abnormal PMCs(%)	No. of PMCs analysed	No. of abnormal PMCs(%)	No. of PMCs analysed	No. of abnormal PMCs(%)
Metaphase I	Precocious chromosome migration	519	46 (8.9)	427	46 (10.8)	396	15 (3.8)
	Noncongregated bivalents		43 (8.3)		-		-
	Two metaphase plates		152 (29.3)		85 (19.9)		6 (1.5)
	Cytomictic channels		95 (18.3)		15 (3.5)		-
Anaphase I	Few laggard chromosomes	367	53 (14.4)	183	38 (20.8)	142	27 (19.0)
	Laggard genome		246 (67.0)		78 (42.6)		-
	Cytomictic channels		20 (5.5)		-		-
	Chromosome stickiness		-		-		5 (3.5)
Telophase I	Small micronuclei	349	78 (22.3)	456	83 (18.2)	251	22 (8.8)
	Large micronuclei		84 (24.1)		22 (4.8)		2 (0.8)
	Cytomictic channels		23 (6.6)		1 (0.2)		-
	Irregular shape of nucleus		46 (13.2)		30 (6.6)		-
	Chromosome stickiness		-		-		7 (2.8)
Prophase II	Small micronuclei	278	44 (15.8)	262	83 (31.7)	405	35 (8.6)
	Large micronuclei		35 (12.6)		-		-
	Irregular shape of nucleus		101 (36.3)		2 (0.8)		-
	Chromosome stickiness		-		-		23 (5.7)
Metaphase II	Precocious chromosome migration	296	70 (23.7)	444	34 (7.7)	283	24 (8.5)
	Irregular metaphase plate		6 (2.0)		6 (1.4)		-
	Irregular cytokinesis		-		-		4 (1.4)
	Chromosome stickiness		-		-		10 (3.5)
Anaphase II	Irregular spindles	337	8 (2.4)	376	1 (0.3)	356	-
	Laggard genome		190 (56.4)		268 (71.3)		-
	Laggard chromosomes		81 (24.0)		84 (22.3)		303 (85.1)
	Irregular cytokinesis						4 (1.1)
Telophase II	Small micronuclei	308	48 (15.6)	341	106 (31.1)	259	173 (66.8)
	Large micronuclei		153 (49.7)		52 (15.2)		-
	Irregular shape of nucleus		1 (0.3)		144 (42.2)		-
	Irregular cytokinesis						4 (1.5)
Tetrad	Small micronuclei	540	193 (35.7)	534	458 (85.8)	755	601 (79.6)
	Large micronuclei		210 (38.9)		49 (9.2)		-
	Micronuclei and microcytes		43 (8.0)		12 (2.3)		12 (1.6)
Total		2994	2069 (69.1%)	3023	1697 (56.1%)	2847	1277 (44.9%)

Although some abnormalities were common among hybrids, others occurred solely or in two of the hybrids. Abnormalities recorded ranged from precocious chromosome migration to the poles in metaphase I and II (figure 1d), noncongressed bivalents at metaphase plate (figure 1e), genomes arranged in two distinct metaphase plates (figure 1f), some laggard chromosomes (figure 1g,i), or an entire laggard genome at anaphase I and II, small micronuclei (figure 1h, j) or large micronuclei at telophase I and II (figure 1k and l), and tetrads (figure 1m–o). Tetrads with micronuclei in microspores (figure 1m–o), with anucleate microsporores (figure 1o), or with microcytes (figure 1p) were also recorded among meiotic products. Precocious chromosome migration to the poles, laggard chromosomes, and micronuclei have been frequently reported among polyploid accessions of *Brachiaria* (Mendes-Bonato *et al*. 2001, 2002a,b, 2006a; Utsunomiya *et al*. 2005) and also among hybrids (Risso-Pascotto *et al*. 2005b) from the collection of Embrapa Beef Cattle.

The occurrence of genomes arranged in two metaphase plates was previously reported in a hybrid between *B. ruziziensis* and *B. brizantha* (Mendes-Bonato *et al*. 2006). The arrangement of parental genomes in distinct-metaphase plates suggests that these genomes are not able to congregate in a single plate, reflecting differences in spindle organization among species. In this analysis, a large number of meiocytes with genomes arranged into two metaphase plates in metaphase I was observed for the two aneuploid hybrids. Laggard genomes in anaphase I and II were also observed in high frequencies. Asynchrony during meiosis of both parental genomes were reported before in a triploid hybrid ($2n = 3x = 27$) between *B. ruziziensis* ($2n = 2x = 18$) and *B. brizantha* ($2n = 4x = 36$), where the female genome (ruzigrass) always remained behind in relation to the male genitor (palisadegrass) and ended up being eliminated in microspores of the tetrad (Risso-Pascotto *et al*. 2004). However, the meiotic behaviour of two other tetraploid hybrids previously analysed between these species did not show chromosome elimination, although other meiotic abnormalities, including mainly those related to irregular chromosome segregation were recorded in high frequency, compromising pollen viability (Risso-Pascotto *et al*. 2005b).

Among the three triple hybrids analysed, the meiotic behaviour of H17 ($2n = 4x = 36$) was distinct from the other two. H17 presented a quite regular meiosis I (table 1) with a few meiocytes with irregular chromosome segregation. In this hybrid, an explosion of meiotic irregularities arose at anaphase II, when a large number of meiocytes presented laggard chromosomes, but not involving an entire genome as found in two other hybrids. This is an interesting aspect, because it shows that the meiotic behaviour in hybrids is not only genotype dependent but also probably dependent on specific compatibility between genitors. H17 and H34 are full sibs and while in H34 one genome remained as laggard in anaphase I and II, and was eliminated into several large micronuclei at the end of both meiotic divisions, in H17 chromosome elimination occurred from anaphase II, and a few eliminated chromosomes gave rise to small micronuclei. This behaviour suggests that each genome presented a different genetic control for meiotic phase timing. In H17, this difference between the parental genomes appeared only in anaphase II.

Other meiotic abnormalities were recorded among the three hybrids. One of them is an irregular shape of the telophase nucleus observed in the half-sib hybrids H34 and H27. In these, the delayed ascension of one genome to the poles, gave rise to abnormal nuclei. The laggard genome was not always in time to be included in the nucleus, to originate the expected spherical telophase nucleus. Abnormal nucleus shape in both meiotic divisions was recently reported in two pentaploid accessions of *B. brizantha*, also presenting a laggard genome (Mendes-Bonato *et al*. 2006). Irregular cytokinesis dividing the meiocytes into abnormal microsporocyte sizes, or microcytes, recorded in the three hybrids is very common in *Brachiaria* microsporogenesis and was reported in *B. brizantha* (Mendes-Bonato *et al*. 2002b), *B. decumbens* (Mendes-Bonato *et al*. 2002a) and *B. nigropedata* (Utsunomiya *et al*. 2005). Cytomictic channels connecting meiocytes in all phases of the first division occurred in H34 and H27. Cytomictic channels are indispensable for cytomixis, i.e. for the chromosome transfer among meiocytes. Although true cytomixis has been reported in *B. nigropedata* (Utsunomiya *et al*. 2004), there was no evidence of chromosome transfer among meiocytes in the present hybrids. Chromosome stickiness was observed in some meiocytes of the H17 hybrid. This abnormality has been largely reported among species and accessions of the *Brachiaria* genus (Mendes-Bonato *et al*. 2001,b; Risso-Pascotto *et al*. 2005b; Utsunomiya *et al*. 2005).

The results obtained from the cytological analysis of these three triple hybrids revealed that meiotic behaviour varies among genotypes, and seems to be both species and genotype-specific. The three hybrids were differentially affected by meiotic abnormalities in the percentage of affected cells (H34, 69.1%; H27, 56.1%, and H17, 44.9%) and also in types of abnormalities. Knowledge accumulated from analyses of meiotic behaviour in *Brachiaria* hybrids by our group have shown that *B. ruziziensis*, the unique obligate sexual species in this agamic complex has incompatibilities in sharing the same cellular space with *B. brizantha* when used as female genitor in crosses. Fortunately, this behaviour seems to be genotype-specific, since not all hybrids produced compromising abnormalities. Apomictic hybrids depend on central cell fertilization by viable pollen to develop the endosperm, assure seed set, proper seed germination, and pasture establishment. Thus, microsporogenesis needs to be fairly normal. For sexual hybrids to be used in future crosses, meiosis has to proceed normally so as not to carry defects further into progenies. Thus, promising hybrids produced in the *Brachiaria* breeding programme must be selected based on

their meiotic behaviour besides presenting good agronomic characteristics.

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References

Alonso L. C. and Kimber G. 1981 The analysis of meiosis in hybrids. II. Triploid hybrids. *Can. J. Genet. Cytol*. **23**, 221–234.

Barbosa R. A. 2006 Morte de pastos de Braquiarias. Embrapa Gado de Corte. Campo Grande, MS.

Gale M. D. and Miller T. E. 1987 The introduction of alien genetic variation into wheat. In: *Wheat Breeding: its Scientific Basis*, (ed. Chapman & Hall), pp. 173–210. Lupton FGH, London.

Gobbe J., Swenne A. and Louant B. P. 1981 Diploides naturels et autotetraploides induits chez *Brachiaria ruziziensis* Germain et Evrard: criteres d'identification. *Agron. Trop*. **36**, 339–346.

González A. M. T. and Morton C. M. 2005 Molecular and morphological phylogenetic analysis of *Brachiaria* and *Urochloa* (Poaceae). *Mol. Phylogenet. Evol*. **37**, 36–44.

Lutts S., Ndikumana J. and Louant B. P. 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 D. V., Boldrini K. R., Mendes-Bonato A. B., Pagliarini M. S. and Valle C. B. 2006 Cytological evidence of natural hybridization in *Brachiaria brizantha* Stapf (Gramineae). *Bot. J. Linn. Soc*. **150**, 441–446.

Mendes-Bonato A. B., Pagliarini M. S., Valle C. B. and Penteado M. I. O. 2001a Meiotic instability in invader plants of signal grass *Brachiaria decumbens* Stapf (Gramineae). *Acta Sci*. **23**, 619–625.

Mendes-Bonato A. B., Pagliarini M. S., Valle C. B. and Penteado M. I. O. 2001b A severe case of chromosome stickiness in pollen mother cells of *Brachiaria brizantha* (Hochst) Stapf (Gramineae). *Cytologia* **66**, 287–291.

Mendes-Bonato A. B., Pagliarini M. S., Forli F., Valle C. B. and Penteado M. I. O. 2002a Chromosome number and microsporogenesis in *Brachiaria brizantha* (Gramineae). *Euphytica* **125**, 419–425.

Mendes-Bonato A. B., Junqueira Filho R. G., Pagliarini M. S., Valle C. B. and Penteado M. I. O. 2002 b 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 A. B., Pagliarini M. S., Risso-Pascotto C. and Valle C. B. 2006a Chromosome number and meiotic behavior in *Brachiaria jubata* (Gramineae). *J. Genet*. **85**, 83–87.

Mendes-Bonato A. B., Risso-Pascotto C., Pagliarini M. S. and Valle C. B. 2006b Cytogenetic evidence for genome elimination during microsporogenesis in interspecific hybrid between *Brachiaria ruziziensis* and *B. brizantha* (Poaceae). *Genet. Mol. Biol*. **29**, 711–714.

Miles J. W. and Valle C. B. 1996 Manipulation of apomixis in *Brachiaria* breeding. In: *Brachiaria*: *Biology, Agronomy, and Improvement* (ed. Miles J. W., Maass B. L. and Valle C. B), pp. 164–177. CIAT/Embrapa, Cati, Columbia.

Miles J. W., Maass B. L. and Valle C. B. 1996 *Brachiaria*: *Biology, Agronomy, and Improvement*. CIAT/Embrapa, Colombia.

Miles J. W., Valle C. B., Rao I. M. and Euclides V. P. B. 2004 *Brachiariagrasses*. In: *Warm-Season (C4) Grasses* (ed. L. Sollenberger), pp. 745–760. ASA, CSSA, SSSA, Madison.

Rao I. M., Kerridge P. C. and Macedo M. C. M. 1996 Nutritional requeriments of *Brachiaria* and adaptation to acid soils. In: *Brachiaria: Biology, Agronomy, and Improvement* (ed. Miles J. W., Maass B. L. and Valle C. B), pp. 53–71. Colombia: CIAT/Embrapa, Cati, Colombia.

Renvoize S. A., Clayton W. D. and Kabuye C. H. S. 1996 Morphology, taxonomy, and natural distribution of *Brachiaria* (Trin.) Griseb. In: *Brachiaria: Biology, Agronomy, and Improvement* (ed. Miles J. W., Maass B. L. and Valle C. B), pp. 1–15. CIAT/Embrapa, Cati, Colombia.

Rieseberg L. H., Baird S. J. E. and Gardner K. A. 2000 Hybridization, introgression, and linkage evolution. *Plant Mol. Biol*. **42**, 205–224.

Risso-Pascotto C., Pagliarini M. S., Valle C. B. and Jank L. 2004 Asynchronous meiotic rhythm as the cause of selective chromosome elimination in an interspecific *Brachiaria* hybrid. *Plant Cell Rep*. **22**, 945–950.

Risso-Pascotto C., Pagliarini M. S. and Valle C. B. 2005a Multiple spindles and cellularization during microsporogenesis in an artificially induced tetraploid accession of *Brachiaria ruziziensis* (Gramineae). *Plant Cell Rep*. **23**, 522–527.

Risso-Pascotto C., Pagliarini M. S. and Valle C. B. 2005b Meiotic behavior in interspecific hybrids between *Brachiaria ruziziensis* and *Brachiaria brizantha* (Poaceae). *Euphytica* **145**, 155–159.

Savidan Y. H. 2000 Apomixis: genetics and breeding. *Plant Breed. Rev*. **18**, 13–86.

Singh R. J. 1993 *Plant cytogenetics*. CRC Press. Boca Raton Ann Arbor, London, Tokio.

Suárez M. M. 1994 Relaciones filogeneticas entre seis especies del gênero *Brachiaria* utilizando marcadores moleculares RAPD. BSc thesis, Depto de Biologia, Faculdad de Ciências Bâsicas, Pontifîcia Universidad Javeriana, Santafê de Bogotâ, DC, Colômbia.

Swenne A., Louant B. P. and Dujardin M. 1981 Induction par la colchicine de formes autotétraploïdes chez *Brachiaria ruziziensis* Germain et Evrard (Graminee). *Agron. Trop*. **36**, 134–141.

Sybenga J. 1992 *Cytogenetics in plant breeding*. Springer-Verlag. Berlin.

Utsunomiya K. S., Pagliarini M. S. and Valle C. B. 2004 Chromosome transfer among meiocytes in *Brachiaria nigropedata* (Ficalho & Hiern) Stapf (Gramineae). *Cytologia* **69**, 395–398.

Utsunomiya K. S., Pagliarini M. S. and Valle C. B. 2005 Microsporogenesis in tetraploid accessions of *Brachiaria nigropedata* (Ficalho & Hiern) Stapf (Gramineae). *Biocell* **29**, 295–301.

Valle C. B. and Savidan Y. H. 1996 Genetics, cytogenetics, and reproductive biology of *Brachiaria*. In: *Brachiaria: Biology, Agronomy, and Improvement* (ed. Miles J. W., Maass B. L. and Valle C. B), pp. 147–163. CIAT/Embrapa, Cati, Colombia.

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