Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Meiotic behavior in nonaploid accessions of *Brachiaria humidicola* (Poaceae) and implications for breeding

K.R. Boldrini[1], E.V. Adamowski[1], N. Silva[1], M.S. Pagliarini[1] and C.B. Valle[2]

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

Corresponding author: M.S. Pagliarini
E-mail: mspagliarini@uem.br

Genet. Mol. Res. 10 (1): 169-176 (2011)
Received July 29, 2010
Accepted December 1, 2010
Published February 1, 2011
DOI 10.4238/vol10-1gmr990

ABSTRACT. *Brachiaria humidicola* is a grass adapted to seasonally swampy grasslands in Africa; two cultivars, 'common' and Llanero, are widely used in Brazilian pastures. New cultivars are in great demand in order to diversify current production systems to achieve improved quality and yield. Cytological analyses of 55 accessions of this species available from the Embrapa Beef Cattle germplasm collection revealed that 27 are apomictic and have 2n = 54 chromosomes. Chromosome pairing as bi- to nonavalent associations at diakinesis indicated a basic chromosome number in this species of x = 6, as found in other closely related *Brachiaria* species. Thus, these 27 accessions are nonaploid (2n = 9x = 54). Abnormalities were found in the meiosis of these accessions, at variable frequencies. The most common abnormalities were those related to irregular chromosome segregation, which led to unbalanced gamete formation; but chromosome stickiness, cell fusion, and absence of cytokinesis were also recorded. Although some accessions have a low frequency of meiotic abnormalities, ensuring potentially good

pollen viability, these cannot be used in hybridization due to a lack of sexual accessions with the same ploidy level.

Key words: *Brachiaria*; Breeding; Forage grass; Hybridization; Meiosis

INTRODUCTION

Brachiaria is a grass genus of the family Poaceae, comprising about 100 species, mostly of African origin. Only a few species are used as forage pastures but represent the most important forage resource in the tropics, covering large areas of pasture in major ecosystems of Latin America. In Brazil, at least 50% of the humid lowlands in the savannas are occupied by *Brachiaria*, an area of about 50 million hectares (Macedo, 2005). Cattle farming in Brazil are based almost solely on cultivated pastures of *Brachiaria* cultivars. These cultivated pastures are often established on low- to medium-fertility soils. Attributes such as adaptation to shade, drought-water logging, low fertility and high aluminum soils, tolerance to heavy defoliation, strong regrowth, good seed production, and apomixis, which renders pastures uniform, are associated with the widespread use and success of *Brachiaria* species in production systems worldwide (Valle and Pagliarini, 2009).

In Brazil, there are 13 registered cultivars listed in the National Service for Cultivar Protection. *Brachiaria decumbens* cv. Basilisk and *B. brizantha* cv. Marandu are the most widely used, because they are easy to manage and readily establish from seed. *Brachiaria humidicola* is a species represented by two cultivars in Brazilian pastures: 'Common' *B. humidicola* and Llanero. More recently, a new cultivar was registered and protected but has not yet been commercialized - cv. BRS Tupi (http://extranet.agricultura.gov.br/php/proton/cultivarweb/detalhe_cultivar.php?codsr=391). These cultivars are better adapted to low-fertility and poorly drained soils (Argel and Keller-Grein, 1996) but are generally of lower nutritive value to cattle.

Currently available commercial *Brachiaria* cultivars bring about enormous economic and social impacts. Nevertheless they also display some compromising deficiencies (Miles et al., 1996), and thus there is a need to search for new ecotypes and to develop superior hybrids through breeding. A *Brachiaria* breeding program is underway at Embrapa Beef Cattle Research Center in Brazil where extensive efforts have been made in the characterization of the germplasm collection in order to identify potential genitors and produce viable hybrids (Valle and Pagliarini, 2009). The studies reported here on cytology and mode of reproduction of accessions and hybrids have created new opportunities and challenges in the improvement of this genus. *Brachiaria* breeding, however, is not easy because the majority of accessions are polyploid and apomictic (Valle and Savidan, 1996). Until two decades ago, genetic improvement of *Brachiaria* depended entirely on selection among naturally existing genotypes. The determination of mode of reproduction of the Brazilian *Brachiaria* germplasm colletion (Valle and Savidan, 1996) and the cytogenetic studies involving the determination of ploidy levels (Utsunomiya et al., 2005; Mendes-Bonato et al., 2002, 2006; Risso-Pascotto et al., 2003, 2006a, 2009), the basic chromosome number (Mendes-Bonato et al., 2002, 2006; Risso-Pascotto et al., 2006b, 2009; Boldrini et al., 2009a,b), and the meiotic behavior (Utsunomiya et al., 2005; Mendes-Bonato et al., 2002, 2006; Risso-Pascotto et al., 2003, 2006a, 2009) have allowed us to select the best accessions for intra- and interspecific hybridization.

The objective was to produce and identify apomictic plants with desirable combinations of traits not found in accessions of the natural *Brachiaria* germplasm. *Brachiaria* hybridization was finally accomplished through the use of some obligate sexual tetraploid accessions of *B. ru-*

ziziensis, obtained by colchicine treatment of natural sexual diploids (Gobbe et al., 1981; Swenne et al., 1981) crossed with natural compatible tetraploid apomictic compatible species of the same taxonomic group (Group 5; Renvoize et al., 1996) such as *B. brizantha* and *B. decumbens*. The discovery of a polyploid (2n = 36) and sexual accession (H031) among the *B. humidicola* accessions (Valle and Glienke, 1991) in the germplasm bank at Embrapa Beef Cattle allowed us to produce for the first time intraspecific hybrids between H031 and an apomictic accession (H016) with the same chromosome number. Plants of the F_1 generation are under agronomic evaluation and some promising hybrids have been identified. Thus, the objective of this research was thus to analyze the meiotic behavior of the *B. humidicola* germplasm collection available at Embrapa Beef Cattle to help in the breeding program and to confirm the data for chromosome number and ploidy level previously obtained by flow cytometry (Penteado et al., 2000).

MATERIAL AND METHODS

Fifty-five accessions of *B. humidicola* available at Embrapa Beef Cattle Research Center (Campo Grande, MS, Brazil) were cytologically evaluated. These accessions were collected in the wild African savannas in the 1980s by the International Center for Tropical Agriculture (CIAT, Colombia), and transferred to Embrapa Genetic Resources and Biotechnology (Brazil) and after quarantine, to Campo Grande. The collection sites in Africa are presented in Table 1. They include Tanzania, Zimbabwe, and South Africa. Geographic, climatic, and edaphic characteristics of collecting sites are: latitude range: 20°17'S-11°21'N; altitude (m.a.s.l.): 560-2375; annual rainfall (mm): 630-1900; number of dry months (No.): 2-7, and soil pH: 4.0-7.0 (Keller-Grein et al., 1996).

Table 1. Accession codes and collection sites of *Brachiaria humidicola* in Africa.

Accession code at Embrapa Beef Cattle	Accession code at Cenargen (Embrapa)	Country of collection in Africa	Municipality of collection	Province	Latitude	Longitude
H006	BRA004863	Zimbabwe	Harare	Harare	18° 1' 0'' S	30° 33' 0'' E
H008	BRA004901	Zimbabwe	Harare	Harare	17° 10' 0'' S	31° 4' 0'' E
H010	BRA004952	Zimbabwe	Inyanga	Inyanga	18° 16' 59'' S	32° 43' 0'' E
H012	BRA004979	Zimbabwe	Inyanga	Inyanga	18° 8' 59'' S	31° 49' 0'' E
H014	BRA005045	Zimbabwe	Masvingo	Masvingo	19° 58' 59'' S	30° 46' 0'' E
H021	BRA004871	Zimbabwe	Harare	Harare	17° 52' 59'' S	30° 43' 0'' E
H022	BRA004910	Zimbabwe	Koroi	Urungwe	16° 31' 59'' S	29° 34' 0'' E
H023	BRA004928	Zimbabwe	Koroi	Lomagundi	16° 41' 59'' S	29° 46' 0'' E
H024	BRA004936	Zimbabwe	Macheke	Makoni	18° 10' 59'' S	31° 58' 0'' E
H025	BRA004987	Zimbabwe	Watsomba	Mutasa	18° 40' 59'' S	32° 37' 59'' E
H028	BRA005053	Zimbabwe	Mvuma	Chilimanzi	19° 26' 59'' S	30° 37' 59'' E
H029	BRA005061	Zimbabwe	Main Camp	Hwange	18° 46' 59'' S	27° 0' 0'' E
H033	BRA006164	Tanzania	Iringa	Iringa	7° 46' 0'' S	35° 25' 59'' E
H035	BRA006131	Tanzania	Kibao	Iringa	8° 25' 0'' S	35° 19' 59'' E
H036	BRA006149	Tanzania	Kibao	Iringa	8° 34' 59'' S	35° 8' 59'' E
H037	BRA006157	Tanzania	James Corner	Iringa	8° 31' 0'' S	35° 4' 0'' E
H038	BRA006165	Tanzania	Njombe	Iringa	9° 5' 59'' S	34° 49' 0'' E
H042	BRA004855	Zimbabwe	Makumbi	Goromonzi	17° 40' 0'' S	31° 16' 59'' E
H044	BRA004995	Zimbabwe	Stapleford	Umtali	18° 49' 59'' S	32° 31' 59'' E
H101	BRA007650	South Africa	-	Zululand	-	-
H106	BRA000540	South Africa	Zululand	-	-	-
H107	BRA001929	Commercial	-	-	-	-
H108	BRA001937	Commercial	-	-	-	-
H112	BRA002208	Unknown	-	-	-	-
H121	BRA006076	Tanzania	Sao Hill	Iringa	8° 20' 59'' S	35° 17' 59'' E
H124	BRA005932	Tanzania	Sao Hill	Iringa	8° 5' 59'' S	35° 25' 59'' E
H127	-	-	-	-	-	-

In Brazil, the accessions are maintained in the field, where site characteristics of cultivation at the Embrapa Beef Cattle are: 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 soil composed of 59% sand, 8% silt and 33% clay; pH = 4.2.

Inflorescences for the meiotic study were collected in 16 clonal plants representing each accession and 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. The number of meiocytes analyzed varied from 766 to 1756, according to the availability for each accession. Photomicrographs were taken in a Wild Leitz microscope using Kodak Imagelink - HQ, ISO 25 black and white film.

The mode of reproduction of each accession had been previously determined (Valle, 1990) by examination of embryo sacs using interference contrast microscopy on methyl salicylate-cleared ovaries (Young et al., 1979).

RESULTS AND DISCUSSION

The cytological analyses under light microscopy revealed that there are 27 accessions with 2n = 54 chromosomes in the *B. humidicola* germplasm collection. The basic chromosome numbers commonly reported for the genus *Brachiaria* are x = 7 and x = 9, with a predominance of the latter (Basappa et al., 1987; Valle and Savidan, 1996; Bernini and Marin-Morales, 2001; Utsunomiya et al., 2005; Mendes-Bonato et al., 2002, 2006; Risso-Pascotto et al., 2003, 2009). However, a new basic chromosome number, x = 6, was recently reported for *B. dictyoneura* (Risso-Pascotto et al., 2006b), a species belonging to the same taxonomic group of *B. humidicola* (Renvoize et al., 1996). In *B. dictyoneura*, all the accessions available at Embrapa Beef Cattle showed 2n = 4x = 24 chromosomes. Evidence obtained from accessions with odd levels of ploidy (7x or 9x) in *B. humidicola* (Boldrini et al., 2009a,b, 2010), the presence of hexavalents in accessions with 2n = 36 chromosomes, and the incidence of octa- and nonavalents in the present accessions (Figure 1a, b) corroborate the occurrence of a basic chromosome number x = 6 in *B. humidicola*. Thus, the accessions studied here are nonaploid (2n = 9x = 54).

Polyploid accessions, in general, display a high frequency of meiotic abnormalities that impair pollen fertility. However, apomictic polyploid accessions of *Brachiaria* need viable male gametes to fertilize the secondary nucleus of the embryo sac (pseudogamic apomixis) to guarantee endosperm development (Valle and Savidan, 1996). In the 27 nonaploid accessions under analysis, the percentage of abnormal cells ranged from 14.52 to 60.0% (Table 2). Only three accessions showed less than 20% of abnormal cells. In 13 accessions, the abnormal cells ranged from 20.0 to 40.0%, and in 11, from 40.0 to 60.0%. These results show that good pollen viability can be found in several accessions.

Chromosome pairing at diakinesis showed different associations, from bi- to nonavalents (Figure 1a, b). The frequency of meiotic abnormalities among accessions is considerably variable (Table 2). In these accessions, the abnormalities were characterized by irregular chromosome segregation, non-oriented bivalents at the metaphase plate, chromosome stickiness, cell fusion, and absence of cytokinesis leading to dyad and triad formation. The predominance of irregular chromosome segregation, typical of polyploids, was characterized by precocious chromosome migration to the poles in metaphases (Figure 1h), laggard chromosomes in anaphases (Figure 1c to f, i), leading to the formation of micronuclei in telophases (Figure 1g, j),

tetrads (Figure 1k), and polyads (Figure 1l). These abnormalities have been reported in polyploid accessions of other *Brachiaria* species (Utsunomiya et al., 2005; Mendes-Bonato et al.,

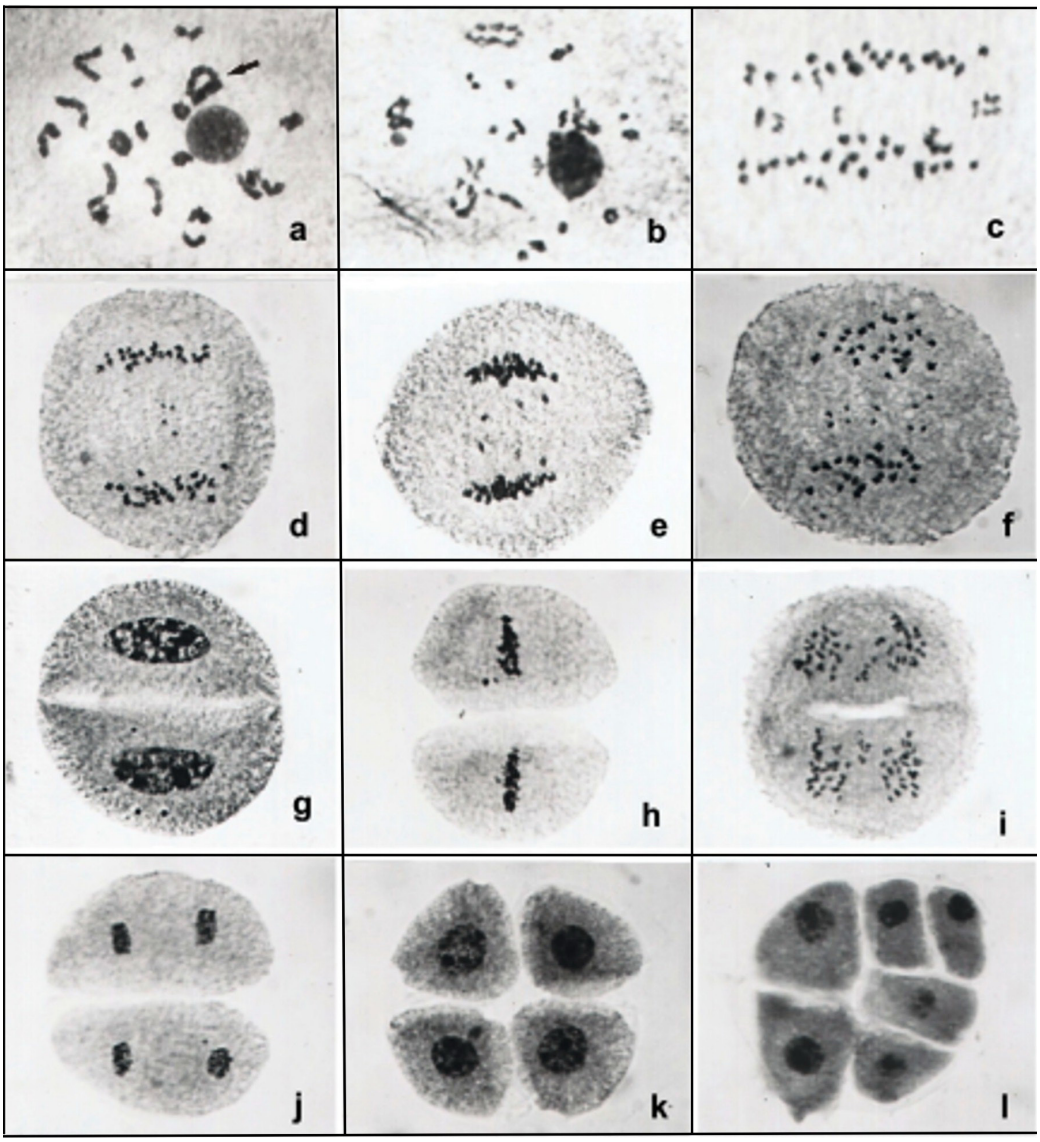

Figure 1. Aspects of microsporogenesis in nonaploid accessions of *Brachiaria humidicola*. **a.b.** Microsporocytes in diakinesis showing multiple forms of chromosome association, from bi- to nonavalents. **c.** Anaphase I with 54 segregated chromosomes. **d.e.f.** Anaphase I with different number of laggard chromosomes. **g.** Telophase I with small micronuclei. **h.** Metaphase II with precocious chromosome migration to the pole. **i.** Anaphase II with laggard chromosomes. **j.** Telophase II with a small micronucleus in one cell. **k.** Tetrad with a micronucleus in one microspore. **l.** Polyad of microspores (magnification 400X).

Table 2. Mode of reproduction, number of cells analyzed, and percentage of cells with meiotic abnormalities in the nonaploid accessions (2n = 9x = 54) of *Brachiaria humidicola*.

Accession	Mode of reproduction	No. PMCs	Phases							
			M I	A I	T I	P I	M II	A II	T II	Tetrad	Mean
H006	Apomictic	1845	33.20	55.13	0.00	0.00	34.06	39.14	0.00	0.00	14.52
H008	Apomictic	1313	53.02	55.00	11.77	3.90	28.98	36.00	29.05	12.90	28.40
H010	Apomictic	1055	57.69	74.63	16.03	8.33	40.30	87.30	41.79	23.88	43.05
H012	Apomictic	1414	43.57	75.00	10.63	37.20	47.72	71.14	36.14	52.01	46.74
H014	Apomictic	1352	67.90	65.49	8.22	30.38	31.52	55.55	6.96	3.38	35.28
H021	Apomictic	826	35.82	62.24	20.86	9.00	50.36	100.00	15.74	25.68	31.72
H022	Apomictic	1285	37.28	37.50	10.08	6.66	13.84	33.33	4.25	30.07	27.93
H023	Apomictic	850	26.89	59.83	21.23	18.55	43.13	53.84	34.02	26.97	31.64
H024	Apomictic	1270	10.87	70.14	1.96	9.75	18.60	51.62	2.15	7.90	20.00
H025	Apomictic	1062	65.69	88.25	19.70	3.68	45.52	82.40	22.73	16.54	42.84
H028	Apomictic	1227	23.03	78.75	3.42	3.94	20.00	87.50	8.80	5.10	27.54
H029	Apomictic	1055	62.60	46.90	21.80	35.60	76.90	58.40	38.60	42.90	48.05
H033	Apomictic	1067	54.20	60.30	22.70	8.00	45.80	65.30	44.10	59.90	44.80
H035	Apomictic	1198	45.22	54.03	12.05	7.28	35.75	48.35	15.56	9.77	26.87
H036	Apomictic	1072	83.58	74.60	45.56	21.01	56.33	77.60	46.66	20.57	52.70
H037	Apomictic	1193	43.30	74.20	46.50	37.00	69.30	83.00	62.10	71.10	60.00
H038	Apomictic	1155	46.66	84.68	6.33	5.20	18.43	32.25	6.43	20.30	26.32
H042	Apomictic	1087	38.80	72.00	44.55	29.00	44.60	52.60	43.20	54.30	47.75
H044	Apomictic	1396	25.32	76.14	13.58	9.10	17.75	16.67	12.09	16.31	20.00
H101	Apomictic	766	54.33	66.40	3.62	2.16	47.70	50.95	1.55	3.49	46.47
H106	Apomictic	1074	58.33	77.90	24.63	8.88	55.07	79.55	37.04	30.66	46.18
H107	Apomictic	1111	57.64	55.00	13.42	7.50	33.95	45.67	6.29	18.63	27.18
H108	Apomictic	1229	34.65	63.20	0.66	0.00	38.77	58.62	0.00	1.12	23.35
H112	Apomictic	1086	70.37	76.00	6.16	4.26	46.15	78.74	20.29	25.00	39.50
H121	Apomictic	1756	55.73	82.47	44.87	23.00	55.60	58.70	11.40	69.62	49.94
H124	Apomictic	1342	40.52	68.49	16.55	30.45	22.94	63.36	27.66	32.14	35.61
H127	Apomictic	1022	25.88	58.26	21.06	5.61	18.12	36.43	13.45	10.44	24.36

PMCs = pollen mother cells; MI, MII = metaphase I and II; AI, AII = anaphase I and II; TI, TII = telophase I and II; PI = polyad I.

2002, 2006; Risso-Pascotto et al., 2003, 2006a, 2009) and also in polyploid hybrids derived from *B. ruziziensis* crossed with *B. brizantha* or *B. decumbens* (Risso-Pascotto et al., 2005; Fuzinatto et al., 2007; Adamowski et al., 2008; Felismino et al., 2010). Non-oriented bivalents, recorded at low frequency, have rarely been reported in the genus *Brachiaria*. These abnormalities led to unbalanced gamete formation, affecting pollen viability. Chromosome stickiness, recorded at high frequency in H002, had been reported in several accessions of *Brachiaria* (Mendes-Bonato et al., 2001a,b; Utsunomiya et al., 2005) and in interspecific hybrids (Risso-Pascotto et al., 2005; Fuzinatto et al., 2007; Adamowski et al., 2008; Felismino et al., 2010). Cell fusion was recorded in *B. decumbens* (Mendes-Bonato et al., 2001a) and in *B. humidicola* (Boldrini et al., 2006a). The absence of cytokinesis, observed at low frequency in the present accessions, is also a common abnormality among *Brachiaria* accessions (Utsunomiya et al., 2005; Risso-Pascotto et al., 2003, 2006a; Boldrini et al., 2006b; Gallo et al., 2007).

Breeding of *B. humidicola* is warranted to increase nutritive value, seed and overall dry matter production, decrease seed dormancy and susceptibility to spittlebugs. The main focus has been directed to the hexaploid germplasm pool of this species since the sole sexual accession (H031) in this collection is a hexaploid (2n = 6x = 36). Hybridization was accomplished and some promising hybrids are under agronomic evaluation (Valle et al., 2009). The progeny segregated in a 1:1 proportion in sexual and apomictic hybrids (Zorzatto et al., 2010). Apomixis is desirable in the breeding program, since it fixes the selected interesting agronomic characteristics through the

production of clonal seeds. The embryo is not fertilized but viable pollen is necessary to fertilize the central cell in order to result in a viable endosperm (pseudogamy). Thus, good male meiotic stability is desired to ensure seed development. The apomictic hybrids will breed true and selected ones are candidates for cultivar development whereas the superior sexual hybrids become part of the next recombination cycle in the program.

So far, no sexual accessions have been identified with 2n = 54 chromosomes to allow intraspecific hybridization at this ploidy level, which includes the commercial cultivars Common and Llanero. Both of them are apomictic nonaploids (2n = 9x = 54) and produce reasonable quantities of seed and forage. Dormancy and seed quality are, however, constant issues among producers and seed improvement. An approach to introduce sexuality into the hexaploid *B. humidicola* complex includes hybridization of the sexual genotype to an apomictic that produces 2n gametes identified cytologically (Gallo et al., 2007). Hybrids have not yet been produced due to the lack of flowering synchrony between the progenitors.

The usual habitat of *B. humidicola* in Africa comprises seasonally swampy grassland (Keller-Grein et al., 1996). In Brazil, the development of new cultivars adapted to waterlogged ecosystems and combining good forage quality to yield is in high demand. Another interesting aspect that needs investigation is nitrification inhibition, which has been identified in some ecotypes of this species. This ability could greatly benefit tropical systems in avoiding N loss from water tables and the atmosphere (Subbarao et al., 2007). Additional germplasm acquisition is still warranted for *B. humidicola*, because some African regions such as most of Southern Africa, Nigeria and Sudan have not been visited (Keller-Grein et al., 1996). According to Valle and Pagliarini (2009), further exploration of sites where the sexual polyploid ecotype (H031) was collected should produce important novel variations for breeding purposes, in both sexual and apomictic forms of *B. humidicola*.

ACKNOWLEDGMENTS

Research supported by UNIPASTO.

REFERENCES

Adamowski EV, Pagliarini MS and Valle CB (2008). Meiotic behavior in three interspecific three way hybrids between *Brachiaria ruziziensis* and *B. brizantha* (Poaceae: Paniceae). *J. Genet.* 87: 33-38.

Argel PJ and Keller-Grein G (1996). Regional Experience with *Brachiaria*: Tropical America - Humid Lowlands. In: *Brachiaria*: Biology, Agronomy, and Improvement (Miles JW, Maass BL and Valle CB, eds.). CIAT/EMBRAPA, Cali, 205-221.

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 Valle CB (2006a). Cell fusion and cytomixis during microsporogenesis in *Brachiaria humidicola* (Poaceae). *South Afr. J. Bot.* 72: 478-481.

Boldrini KR, Pagliarini MS and do Valle CB (2006b). 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. (2009a). Origin of a polyploid accession of *Brachiaria humidicola* (Poaceae: Panicoideae: Paniceae). *Genet. Mol. Res.* 8: 888-895.

Boldrini KR, Pagliarini MS and Valle CB (2009b). Meiotic behavior of a nonaploid accession endorses x = 6 for *Brachiaria humidicola* (Poaceae). *Genet. Mol. Res.* 8: 1444-1450.

Boldrini KR, Pagliarini MS and Valle CB (2010). Evidence of natural hybridization in *Brachiaria humidicola* (Rendle) Schweick. (Poaceae: Panicoideae: Paniceae). *J. Genet.* 89: 91-94.

Felismino MF, Pagliarini MS and Valle CB (2010). Meiotic behavior of interspecific hybrids between artificially

tetraploidized sexual *Brachiaria ruziziensis* and tetraploid apomictic *B. brizantha* (Poaceae). *Sci. Agric.* 67: 191-197.
Fuzinatto VA, Pagliarini MS and Valle CB (2007). Microsporogenesis in sexual *Brachiaria* hybrids (Poaceae). *Genet. Mol. Res.* 6: 1107-1117.
Gallo PH, Micheletti PL, Boldrini KL, Risso-Pascotto C, et al. (2007). 2n gamete formation in the genus *Brachiaria* (Poaceae: Paniceae). *Euphytica* 154: 255-260.
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.
Keller-Grein G, Maass BL and Hanson J (1996). Natural Variation in *Brachiaria* and Existing Germplasm Collections. In: *Brachiaria*: Biology, Agronomy, and Improvement (Miles JW, Maass BL and Valle CB, eds.). CIAT/EMBRAPA, Cali, 17-42.
Macedo MCM (2005). Pastagens no Ecossistema Cerrado: Evolução das Pastagens para o Desenvolvimento Sustentável. In: Reunião Anual da Sociedade Brasileira de Zootecnia. A Produção Animal e o Foco no Agronegócio. Anais, Goiânia, 56-84.
Mendes-Bonato AB, Pagliarini MS, Valle CB and Penteado MIO (2001a). 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, Silva N and Valle CB (2001b). Meiotic instability in invader plants of signal grass *Brachiaria decumbens* Stapf (Gramineae). *Acta Scient.* 23: 619-625.
Mendes-Bonato AB, Pagliarini MS, Forli F, Valle CB, et al. (2002). Chromosome number 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.
Miles JW, Maass BL and Valle CB (1996). *Brachiaria*: Biology, Agronomy, and Improvement. CIAT/Embrapa, Cali.
Penteado MIO, Santos ACM, Rodrigues IF, Valle CB, 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.
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 Mendes-Bonato AB (2003). Chromosome number and microsporogenesis in pentaploid accession of *Brachiaria brizantha* (Gramineae). *Plant Breed.* 122: 136-140.
Risso-Pascotto C, Pagliarini MS and Valle CB (2005). Meiotic behavior in interspecific hybrids between *Brachiaria ruziziensis* and *Brachiaria brizantha* (Poaceae). *Euphytica* 145: 155-159.
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, Pagliarin MS and Valle CB (2006b). A new basic chromosome number for the genus *Brachiaria* (Trin.) Griseb. (Poaceae: Panicoideae: Paniceae). *Genet. Res. Crop Evol.* 53: 7-10.
Risso-Pascotto C, Pagliarini MS and Valle CB (2009). Microsporogenesis in *Brachiaria bovonei* (Chiov.) Robyns and *B. subulifolia* (Mez.) Clayton (Poaceae). *Sci. Agric.* 66: 691-696.
Subbarao GV, Rondon M, Ito O, Ishikawa T, et al. (2007). Biological nitrification inhibition (BNI) - Is it a widespread phenomenon? *Plant Soil* 294: 5-18.
Swenne A, Louant BP and Dujardin M (1981). Induction par la colchicine de formes autotétraploïdes chez *Brachiaria ruziziensis* Germain et Evrard (Gramínée). *Agron. Trop.* 36: 134-141.
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 (1990). Coleção de Germoplasma de Espécies de *Brachiaria* no CIAT: Estudos Básicos Visando ao Melhoramento Genético. Embrapa Gado de Corte, Campo Grande, 33.
Valle CB and Glienke C (1991). New sexual accession in *Brachiaria. Apomixis Newsl.* 7: 42-43.
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.
Valle CB and Pagliarini MS (2009). Biology, Cytogenetics, and Breeeding of *Brachiaria*. In: *Brachiaria*: Biology, Agronomy, and Improvement (Miles JW, Maass BL and Valle CB, eds.). CIAT/EMBRAPA, Cali, 103-151.
Valle CB, Resende RMS, Chiari L, Jank L, et al. (2009). Agronomic Evaluation of *Brachiaria humidicola* Hybrids. In: Simpósio Internacional Sobre Melhoramento de Forrageiras. Embrapa Gado de Corte, Campo Grande.
Young BA, Sherwood RT and Bashaw EC (1979). Cleared-pistil and thick-sectioning techniques for detecting aposporous apomixis in grasses. *Can. J. Bot.* 57: 1672-1688.
Zorzatto C, Chiari L, Bitencourt GA, Valle CB, et al. (2010). Identification of a molecular marker linked to apomixis in *Brachiaria humidicola* (Poaceae). DOI: 10.1111/j.1439-0523.2010.01763.x. *Plant Breed.* 129: 734-736.