RESEARCH ARTICLE

Chromosome numbers and meiotic analysis in the pre-breeding of *Brachiaria decumbens* (Poaceae)

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Abstract

A total of 44 accessions of *Brachiaria decumbens* were analysed for chromosome count and meiotic behaviour in order to identify potential progenitors for crosses. Among them, 15 accessions presented $2n = 18$; 27 accessions, $2n = 36$; and 2 accessions, $2n = 45$ chromosomes. Among the diploid accessions, the rate of meiotic abnormalities was low, ranging from 0.82% to 7.93%. In the 27 tetraploid accessions, the rate of meiotic abnormalities ranged from 18.41% to 65.83%. The most common meiotic abnormalities were related to irregular chromosome segregation, but chromosome stickiness and abnormal cytokinesis were observed in low frequency. All abnormalities can compromise pollen viability by generating unbalanced gametes. Based on the chromosome number and meiotic stability, the present study indicates the apomictic tetraploid accessions that can act as male genitor to produce interspecific hybrids with *B. ruziziensis* or intraspecific hybrids with recently artificially tetraploidized accessions.

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Introduction

Tropical grasses for pastures represent the single most valuable resource in animal production worldwide. Native and cultivated pastures cover wide extensions of land in the tropics. However, they are dangerously composed of a few varieties derived from apomictic ecotypes, creating monocrops (Valle and Pagliarini 2009). This lack of biodiversity exposes the ecosystem to risks by exerting tremendous selection pressure on pests and/or diseases and justifies the urgency in developing and releasing new cultivars by breeding and/or selection. A number of cultivars have been released in the last 30 years. In Brazil, there are 10 registered cultivars listed on the National Service for Cultivar Protection. *Brachiaria decumbens* cv. Basilisk is the most widely used grass forage worldwide because it adapts to acid soils, is easy to manage and readily establishes from seed, but most importantly, produces good quality forage to support animal production all year long. *B. decumbens* has a narrow distribution in Africa. The *Brachiaria* collection expedition performed during 1984–85 by the International Center for Tropical Agriculture (CIAT, Cali, Colombia), supported by the Biodiversity International (ex-IPGRI; ex-IBPGR) resulted in the collection of about 800 accessions of at least 23 known species (Keller-Grein *et al.* 1996).

Seed production is an important trait to be selected if the new variety is to be adopted both nationally and abroad. However, this is not easily accomplished because the best accessions are polyploid, and polyploidy is correlated with apomixis. Although apomictic, the accessions are pseudogamic, which means that viable gametes are necessary to fertilize the secondary nuclei of the embryo-sac (Valle and Savidan 1996). Polyploidy is also correlated with abnormal chromosome segregation during meiosis, affecting pollen viability (Mendes-Bonato *et al*. 2002, 2006; Utsunomiya *et al*. 2005; Risso-Pascotto *et al*. 2006; Pagliarini *et al.* 2008). Thus, the best accessions for breeding will be those presenting high meiotic stability. In this context, in this paper we determined the chromosome number and evaluated the meiotic behaviour of 44 accessions of *B. decumbens* available at Embrapa Beef Cattle (Brazil) as a subsidy for the breeding programme.

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Material and methods

Brachiaria decumbens accessions available at Embrapa Beef Cattle Research Center (Campo Grande, Brazil) were cytologically evaluated under light microscopy. These accessions were collected in the wild African savannahs in 1980s by the International Center for Tropical Agriculture (CIAT, Cali, Colombia), transferred to Embrapa Genetic Resources and Biotechnology (Brazil), and after quarantine, to Campo Grande, MS. The collection sites in Africa are presented in table 1.

Inflorescences for meiotic studies were collected from individual plants growing in the field, fixed in a mixture of ethanol 95%, chloroform and propionic acid (6:3:2) during

Table 1. Origin, (country and province), latitude and longitude of collection sites in Africa, DNA amount and ploidy level, and mode of reproduction of different accession of *B. decumbens.*

Code at cenargen	Code at Embrapa Beff Cattle	Origin				DNA amount (pg) (ploidy level)*	Mode of reproduction**
		Country	Province	Latitude	Longitude		
BRA4430	D04	Kenya	Siaya	0° 1′ 0″ S	34° 21′ 0″ E	1.00 (2*x*)	Sexual
BRA4448	D05	Kenya	Siaya	0° 4′ 60″ N	34° 16′ 60″E	1.00 (2*x*)	Sexual
BRA4456	D06	Kenya	Siaya	0° 6 0″ N	34° 31′ 0″ E	1.00 (2*x*)	Sexual
BRA4596	D18	Rwanda	Kigali	2° 13′ 0″ S	30° 13′ 0″ E	1.00 (2*x*)	Sexual
BRA4600	D19	Rwanda	Kigali	2° 16′ 60″ S	30° 1′ 0″ E	1.00 (2*x*)	Sexual
BRA4618	D20	Rwanda	Kibungo	1° 55′ 0″ S	30° 19′ 60″E	1.00 (2*x*)	Sexual
BRA4626	D21	Rwanda	Kibungo	2° 16′ 0″ S	30° 37′ 0″ E	1.00 (2*x*)	Sexual
BRA4634	D22	Rwanda	Kibungo	2° 21′ 0″ S	30° 46′ 60″ E	1.00 (2*x*)	Sexual
BRA4651	D24	Rwanda	Kibungo	2° 6′ 0″ S	30° 33′ 0″ E	1.00 (2*x*)	Sexual
BRA4707	D28	Rwanda	Byumba	1° 25′ 60″ S	30° 34′ 0″ E	1.00 (2*x*)	Sexual
BRA4715	D29	Rwanda	Byumba	1° 24′ 0″ S	30° 24′ 0″ E	1.00 (2*x*)	Sexual
BRA4723	D30	Rwanda	Byumba	1° 19′ 60″ S	30° 18′ 0″ E	1.00 (2*x*)	Sexual
BRA4740	D32	Rwanda	Byumba	1° 30′ 0″ S	30° 10′ 60″E	1.00 (2*x*)	Sexual
BRA4758	D33	Rwanda	Kigali	1° 54′ 0″ S	30° 10′ 60″E	1.00 (2*x*)	Sexual
BRA4774	D34	Rwanda	Butare	2° 21′ 0″ S	29° 54′ 0″ E	1.00 (2*x*)	Sexual
BRA4782	D35	Rwanda	Butare	2° 28′ 60″ S	29° 52′ 60″E	1.00 (2*x*)	Sexual
BRA4766	D40	Rwanda	Gitarama	2° 10′ 60″ S	29° 57′ 0″ E	1.00 (2*x*)	Sexual
BRA5657	D80	Burundi	–	–	–	1.00 (2*x*)	Sexual
BRA4413	D03	Kenya	Trans Nzoia	1° 3′ 0″ S	34° 55′ 60″ E	2.31 (4*x*)	Apomictic
BRA4472	D07	Kenya	South Nyanza	1° 7′ 0″ S	34° 33′ 0″ E	2.16 (4*x*)	Apomictic
BRA4481	D08	Kenya	Nandi	0° 13′ 0″ N	35° 18′ 0″ E	2.29 (4*x*)	Apomictic
BRA4499	D09	Kenya	Nakuru	0° 16′ 60″ S	36° 1′ 0″ E	2.05 (4*x*)	Apomictic
BRA4511	D11	Kenya	Trans Nzoia	1° 4′ 0″ N	34° 54′ 0″ E	2.03 (4*x*)	Apomictic
BRA4529	D12	Kenya	Trans Nzoia	1° 4′ 60″ N	34° 49′ 60″ E	2.14 (4*x*)	Apomictic
BRA4545	D14	Kenya	Nandi	0° 21′ 0″ N	35° 3′ 0″ E	2.25 (4*x*)	Apomictic
BRA4561	D15	Burundi	Rutana	4° 1′ 0″ S	30° 4′ 60″ E	2.26 (4*x*)	Apomictic
BRA4588	D17	Burundi	Kirundo	2° 31′ 0″ S	30° 10′ 60″ E	2.14 (4*x*)	Apomictic
BRA4421	D36	–	–	–	–	2.60 (4*x*)	Apomictic
BRA4464	D37	Kenya	South Nyanza	0° 54′ 0″ S	34° 31′ 60″ E	2.13 (4*x*)	Apomictic
–	D53	–	–	–	–	2.10 (4*x*)	Apomictic
BRA0116	D59	–	–	–	–	2.28 (4*x*)	Apomictic
BRA1961	D61	–	–	–	–	2.09 (4*x*)	Apomictic
BRA1058	D62	Uganda	–	–	–	2.08 (4*x*)	Apomictic
BRA1988	D68	–	–	–	–	2.09 (4*x*)	Apomictic
BRA1970	D69	–	–	–	–	2.06 (4*x*)	Apomictic
BRA1996	D70	–	–	–	–	2.09 (4*x*)	Apomictic
BRA1392	D76	–	–	–	–	–	Apomicitc
BRA0060	D77	–	–	–	–	2.08 (4*x*)	Apomictic
BRA7609	D79	–	–	–	–	2.10 (4*x*)	Apomictic
BRA7731	D82	–	–	–	–	2.36 (4*x*)	Apomictic
BRA4502	D10	Kenya	Uasin Gishu	0° 15′ 1″ N	35° 25′ 0″ E	2.65 (5*x*)	Apomictic
BRA4537	D13	Kenya	Trans Nzoia	1° 3′ 0″ N	34° 51′ 0″ E	2.48 (5*x*)	Apomictic
BRA4421	D36	Kenya	Trans Nzoia	1° 7′ 0″ N	35° 4′ 0″ E	2.60 (5*x*)	Apomictic
BRA0191	D58	–	–	–	–	2.68 (5*x*)	Apomictic
BRA7552	D71	–	–	–	–	2.62 (5*x*)	Apomictic

BRA..., Brazilian germplasm code; D..., code at EBC; *ploidy level previously determined by Penteado *et al.* (2000). ** Mode of reproduction determined by Valle *et al.* (2008).

24 h and stored under refrigeration. Anthers containing cells in meiosis were isolated from flowers and slides containing cells spreads were obtained after squashing and staining with 0.5% propionic carmine. Images were obtained using Kodak Imagelink, HQ, ISO 25 black and white film (Rochester, USA).

The ploidy level was previously determined by flow cytometry (Penteado *et al.* 2000). The mode of reproduction of each accession was also previously determined (Valle *et al.* 2008) by examination of embryo-sacs using interference contrast microscopy on methylsalicilate-cleared ovaries according to the methodology of Young *et al.* (1979).

Results and discussion

The previous study of the mode of reproduction (Valle *et al.* 2008) revealed that the diploid accessions reproduce

Table 2. Percentage of cells with meiotic abnormalities in each meiotic phase of different accession of *B. decumbens*.

Code	Chr. No.	Ploidy level	N° of PMCs	Percentage of cells with meiotic abnormalities								
				Met I*	Ana I	Tel I	Pro II	Met II	Ana II	Tel II	Tet	Mean
D04	$2n = 18$	2x	866	3.17	28.57	9.37	7.69	5.61	2.85	1.09	5.14	7.93
D05	$2n = 18$	2x	1013	5.02	9.61	5.19	0.00	3.07	18.98	3.80	8.95	6.82
D06	$2n = 18$	2x	1123	1.98	4.76	10.81	0.00	0.93	9.45	2.27	6.64	4.60
D18	$2n = 18$	2x	773	0.00	2.50	5.40	0.00	0.00	4.30	0.90	2.60	1.96
D19	$2n = 18$	2x	1288	0.00	0.90	0.00	0.00	1.52	3.63	0.00	0.58	0.82
D20	$2n = 18$	2x	962	3.18	2.85	1.11	0.00	1.29	0.00	4.73	4.37	2.19
D21	$2n = 18$	2x	1023	0.00	9.09	0.00	0.00	0.00	7.80	2.22	2.22	2.05
D24	$2n = 18$	2x	1004	8.91	6.16	6.13	2.08	7.35	6.00	0.96	4.06	5.17
D28	$2n = 18$	2x	642	0.00	11.40	17.80	0.00	0.00	7.50	1.60	1.80	5.00
D29	$2n = 18$	2x	920	0.83	4.00	6.38	1.50	3.22	5.45	0.00	0.40	2.06
D30	$2n = 18$	2x	668	11.66	7.57	8.33	3.03	12.50	0.00	0.00	4.62	5.98
D32	$2n = 18$	2x	1142	0.00	3.90	0.80	0.00	0.00	6.10	0.53	0.00	1.40
D33	$2n = 18$	2x	1004	0.76	1.33	0.00	0.00	2.38	6.66	1.63	1.33	1.59
D34	$2n = 18$	2x	1168	0.00	6.40	1.40	0.00	0.00	1.80	1.60	2.30	1.68
D35	$2n = 18$	2x	890	2.17	3.77	2.19	0.00	0.00	2.46	2.27	1.98	1.91
D03	$2n = 36$	4x	1251	2.08	49.69	50.44	30.76	21.10	62.90	77.68	69.96	45.58
D07	$2n = 36$	4x	986	4.24	55.38	23.71	25.40	12.17	64.86	26.04	38.06	31.23
D08	$2n = 36$	4x	972	12.93	60.00	39.13	21.80	23.70	83.10	78.80	60.60	47.50
D09	$2n = 36$	4x	1159	9.23	58.75	50.53	3.74	2.81	80.00	68.18	37.45	38.83
D10	$2n = 36$	4x	922	10.40	85.50	86.50	78.20	22.00	91.10	71.80	72.70	64.70
D11	$2n = 36$	4x	865	24.50	84.30	44.90	38.40	61.50	92.50	93.10	87.50	65.83
D12	$2n = 36$	4x	1155	4.59	77.70	41.00	8.75	23.00	90.90	87.40	74.10	50.93
D13	$2n = 36$	4x	881	1.88	44.40	20.00	12.90	10.40	56.40	29.00	36.60	24.06
D14	$2n = 36$	4x	1275	4.90	71.00	22.40	0.51	2.85	57.60	36.60	29.50	28.17
D15	$2n = 36$	4x	870	5.94	57.10	21.20	25.40	12.50	57.50	27.60	54.80	29.43
D17	$2n = 36$	4x	1432	4.48	89.12	91.07	5.52	14.45	96.40	84.24	33.97	53.84
D22	$2n = 36$	4x	1253	3.30	88.10	27.80	10.40	7.60	93.50	85.70	93.90	51.28
D36	$2n = 36$	4x	1045	0.96	14.81	0.00	3.12	2.77	10.00	49.60	66.06	18.41
D37	$2n = 36$	4x	1001	5.11	75.50	24.10	12.50	18.70	84.30	64.60	77.90	45.30
D40	$2n = 36$	4x	1230	5.40	61.40	37.70	7.60	5.20	85.50	54.60	94.70	44.01
D58	$2n = 36$	4x	1365	3.06	57.20	37.20	26.50	3.37	82.20	58.60	59.30	40.90
D59	$2n = 36$	4x	1419	4.70	86.80	66.90	25.30	15.20	96.10	95.30	97.10	60.90
D61	$2n = 36$	4x	1368	3.32	80.80	68.05	25.50	8.50	88.50	92.90	89.90	57.10
D62	$2n = 36$	4x	1015	1.19	78.60	57.60	27.08	4.90	87.90	90.80	94.30	55.20
D68	$2n = 36$	4x	1276	3.57	70.60	54.00	32.00	11.70	86.90	91.90	88.60	54.90
D69	$2n = 36$	4x	1426	2.10	50.50	17.40	26.40	2.47	69.00	54.70	49.30	33.90
D70	$2n = 36$	4x	1482	1.52	57.80	28.40	17.40	14.20	87.30	82.50	87.30	47.05
D76	$2n = 36$	4x	1267	11.80	51.60	30.10	35.70	46.90	94.00	90.80	42.20	50.30
D77	$2n = 36$	4x	1599	4.36	79.20	52.80	23.80	10.70	85.00	78.50	79.90	51.70
D79	$2n = 36$	4x	1016	4.30	86.80	14.70	3.20	3.90	81.80	46.40	57.40	37.30
D80	$2n = 36$	4x	1459	7.40	45.60	17.90	23.40	7.75	42.80	37.40	47.90	28.76
D82	$2n = 36$	4x	1251	2.80	66.90	28.60	14.30	1.60	70.20	65.70	64.40	39.30
D53	$2n = 45$	5x	1749	3.77	87.60	67.50	48.50	16.60	98.50	96.80	100.00	64.90
D71	$2n = 45$	5x	1223	30.30	58.10	49.00	28.20	23.90	96.60	91.10	97.90	59.38

* Met I, metphase I; Ana I, anaphase I; Tel I, telophase I; Pro II, prophase II; Met II, metaphase II; Ana II, anaphase II; Tel, telophase II; Tet, tetrad. PMCs, pollen mother cells.

sexually while the polyploid are apomictic (table 1). Among the 44 accessions cytologically analysed, 15 presented $2n = 18$; 27 accessions, $2n = 36$; and 2 accessions, $2n = 45$ chromosomes (table 2). The number of diploid accessions ($2n = 2x = 18$) was higher than that observed in other *Brachiaria* species. Diploidy in *B. decumbens* was reported in some occasions (Valle and Glienke 1991) and tetraploidy predominated (Pritchard 1967; Ndikumana 1985; Basappa *et al.* 1987). In an attempt to first subsidize the *Brachiaria* breeding programme underway at Embrapa Beef Cattle, Penteado *et al.* (2000) determined the ploidy level of the accessions using flow cytometry (table 1). The ploidy level scored among 51 accessions of *B. decumbens* revealed that 23 accessions (45.1%) were $2x$; 23 (45.1%), $4x$; and five (9.8%), $5x$; with DNA contents ranging from 1.00 in the diploid accessions to 2.68 pg in the polyploid ones (table 1). The data obtained from the present cytogenetic studies (table 2) diverge, in part, from those obtained from flow cytometry: (i) three accessions $4x$ (D022, D040, and D080) were considered diploid ($2x$) by flow cytometry;

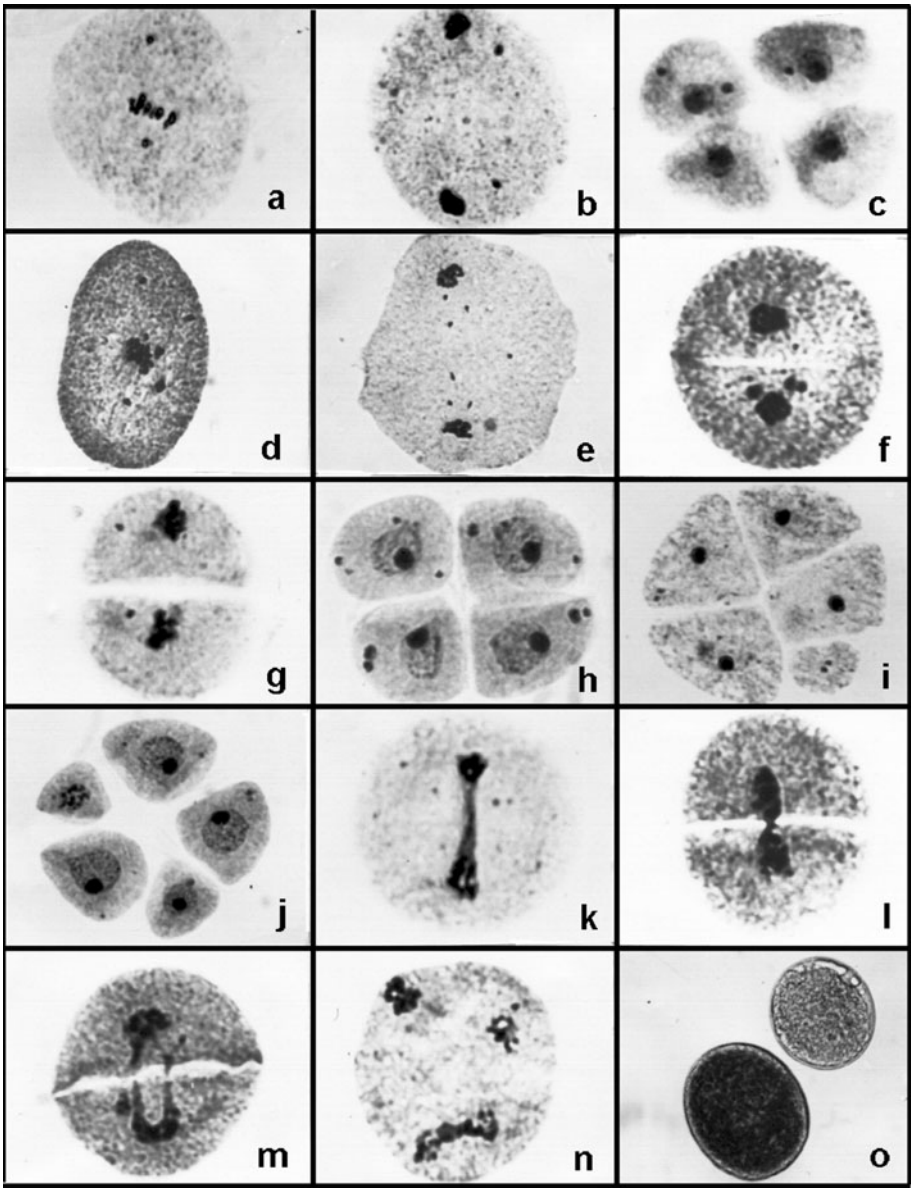

Figure 1. Meiotic behaviour in diploid and polyploid accessions of *B. decumbens*. (a–c) Diploid meiocytes with precocious chromosome migration to the poles in metaphase I (a), micronuclei in telophase I (b), and micronuclei in microspores of the tetrad (c). (d–h) Tetraploid meiocytes with precocious chromosome migration to the poles in metaphase I (d) and II (g), laggards in anaphase I (e), micronuclei in prophase II (f), and microspores of the tetrad (h). (i–j) Polyads of tetraploid accessions with microcytes and micronuclei. (k–n) Different aspects of chromosome stickiness. (o) Fertile (dark) and sterile pollen grains

(ii) one accession 5*x* (D053) was interpreted as 4*x*; and (iii) four 4*x* accessions (D010, D013, D036, and D058) were interpreted as 5*x*. These results demonstrate the importance of cytological studies, which remain the best methodology to evaluate the chromosome number in a species.

Among the diploid accessions, the rate of meiotic abnormalities was low, ranging from 0.82% to 7.93% (table 2). The abnormalities were those related to irregular chromosome segregation in both meiotic divisions (figure 1, a–c). A low rate of chromosome stickiness and abnormal cytokinesis were also recorded in these accessions. Similar meiotic abnormalities were also recorded in diploid accessions of *B. brizantha* (Mendes-Bonato *et al.* 2002) and *B. ruziziensis* (Pagliarini *et al.* 2008). In the 27 tetraploid accessions, the rate of meiotic abnormalities ranged from 18.41% to 65.83% (table 2). In the two pentaploid accessions, the rate of abnormalities was similar to that found in tetraploid accessions.

In tetraploid and pentaploid accessions, the most common meiotic abnormalities were precocious chromosome migration to the poles in metaphases (figure 1, d&g), laggards in anaphases (figure 1e), leading to micronuclei formation in telophases, prophase II (figure 1f), and tetrads (figure 1h). Micronuclei were also eliminated as microcytes (figure 1, i&j), affecting pollen viability (figure 1o). The same behaviour of chromosomes was reported in polyploid accessions of *B. brizantha* (Mendes-Bonato *et al.* 2002), *B. nigropedata* (Utsunomiya *et al.* 2005), *B. jubata* (Mendes-Bonato *et al.* 2006), *B. dictyoneura* (Risso-Pascotto *et al.* 2006), and *B. ruziziensis* (Pagliarini *et al.* 2008). Chromosome stickiness was also recorded in the polyploid accessions (figure 1, k–n). Chromosome stickiness has been recorded in different *Brachiaria* species (Mendes-Bonato *et al.* 2001a,b; Utsunomiya *et al.* 2005). All these abnormalities can compromise pollen viability by generating unbalanced gametes. Although apomictic, the polyploid accessions of *Brachiaria* are pseudogamic, which means that viable pollen is necessary to fertilize the secondary nuclei of the embryo-sac to guarantee endosperm development.

The main objective of the *Brachiaria* breeding programme is to select apomictic tetraploid accessions with regular meiosis to be crossed to either artificially tetraploidized *B. ruziziensis* obtained from chromosome doubling in Belgium (Gobbe *et al.* 1981; Swenne *et al.* 1981) and available at Embrapa Beef Cattle germplasm collection or to other tetraploidized *B. decumbens* (Simioni and Valle 2009). This survey indicated which apomictic tetraploid accessions of this germplasm collection are potential genitors to act as pollen donors for such breeding programme either to produce intraspecific or intraspecific hybrids. Among the 27 tetraploid accessions analysed, at least 10, with less than 40% of abnormal meiotic cells, could be used as male genitors as long as they display promising agronomic characteristics.

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References

Basappa G. P., Muniyamma M. S. and Chinnappa C. C. 1987 An investigation of chromosome numbers in the genus *Brachiaria* (Poaceae: Paniceae) in relation to morphology and taxonomy. *Can. J. Bot.* **65**, 2297–2309.

Gobbe J., Swenn A. and Louant B. P. 1981 Diploides naturals et autotétraploides induits chez *Brachiaria ruziziensis* Germain et Evrard: critères d'identification. *Agron. Trop.* **36**, 339–346.

Keller-Grein G., Maass B. L. and Hanson J. 1996 Natural variation in *Brachiaria* and existing germoplasma collections. In *Brachiaria: biology, agronomy, and improvement* (ed. J. W. Miles, B. L. Maass and C. B. Valle), pp. 17–42. CIAT/Embrapa, Cali, Colombia.

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. 2002 Chromosome number and microsporogenesis in *Brachiaria brizantha* (Gramineae). *Euphytica* **125**, 419–425.

Mendes-Bonato A. B., Pagliarini M. S., Risso-Pascotto C. and Valle C. B. 2006 Chromosome number and meiotic behavior in *Brachiaria jubata* (Gramineae). *J. Genet.* **85**, 83–88.

Ndikumana J. 1985 Etude de l'hybridation entre espèces apomictiques et sexuées dans le genre *Brachiaria*. Dissertation, Université Catholique de Louvain, Louvain, Belgium.

Pagliarini M. S., Pascotto C., Souza-Kaneshima A. M. and Valle C. B. 2008 Analysis of meiotic behavior in selecting potentail genitors among diploid and artificially induced tetraploid accessions of *Brachiaria ruziziensis* (Poaceae). *Euphytica* **164**, 181–187.

Penteado M. I. O., Santos A. C. M., Rodrigues I. F., Valle C. B., Seixas M. A. C. and Esteves A. 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, MS, Brazil.

Pritchard A. J. 1967 Apomixis in *Brachiaria decumbens* Stapf. *J. Aust. Agric. Sci.* **33**, 264–265.

Risso-Pascotto C., Pagliarini M. S. and Valle C. B. 2006 Microsporogenesis in *Brachiaria dictyoneura* (fig. & de not.) Stapf (Poaceae: Paniceae). *Genet. Mol. Res.* **5**, 837–845.

Simioni C. and Valle C. B. 2009 Chromosome duplicaction in *Brachiaria* (A.Rich.) Stapf allows intraspecific crosses. *Crop Breed. Appl. Biotechnol.* **9**, 328–334.

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

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

Valle C. B. and Savidan Y. 1996 Genetics, cytogenetics, and reproductive biology of *Brachiaria*. In *Brachiaria: biology, agronomy,*

and improvement (ed. J. W. Miles, B. L. Maass and C. B. Valle). pp. 147–163. CIAT/Embrapa, Cali, Colombia.

Valle C. B. and Pagliarini M. S. 2009 Biology, cytogenetics, and breeeding of *Brachiaria*. In *Genetic resources, chromosome engineering, and crop improvement* (ed. R. J. Singh), pp. 103–151. CRC Press, Boca Raton, USA.

Valle C. B., Simioni C., Resende R. M. S. and Jank C. 2008 Melhoramento genético de *Brachiaria*. In *Melhoramento de forrageiras tropicais* (ed. R.M.S. Resende, C. B. Valle and L. Jank), pp. 13–53. Embrapa Gado de Corte, Campo Grande, Brazil.

Valle C. B. and Glienke C. 1991 New sexual accessions in *Brachiaria*. *Apomixis Newsl*. **3**,11–13.

Young B. A., Sherwood R. T. and Bashaw E. C. 1979 Cleared-pistyl and thick- sectioning techniques for detecting aposporous apomixis in grasses. *Can. J. Bot.* **57**, 1668–1672.

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