

RESEARCH NOTE

Chromosome number and meiotic behaviour in *Brachiaria jubata* (Gramineae)

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The production of new genetic variability through hybridization has been undertaken in *Brachiaria*, an important forage grass genus of African origin widely used in pastures in the Brazilian tropics. Although the genus comprises about 100 species, only a few of them present favourable agronomic attributes and are explored. In the genus *Brachiaria*, the majority of species and accessions are polyploid and apomictic, which makes breeding through hybridization more complex. Sexuality has been found in diploids and eventually among tetraploids with normal meiosis. *Brachiaria jubata* presents good forage value and adaptation to riverbanks and lake margins, and is therefore relevant to pasture systems in the tropics. Furthermore, the most interesting form of resistance to spittlebugs—antibiosis—was identified in one accession of *B. jubata*. Thus its interest in the Brazilian breeding programme lies in the possibility of using hybridization to transfer high resistance to this insect to susceptible accessions. This paper reports chromosome numbers and meiotic behaviour in 21 accessions of this species as a tool in selecting promising ones for crossing. Only one accession was found to be diploid; the others were tetraploid. Microsporogenesis was normal in the diploid accession, while among the tetraploid ones, irregular chromosome segregation leading to unbalanced gamete formation was common but variable. Pollen fertility among tetraploid accessions was high.

Cytogenetic studies were carried out on accessions of *Brachiaria jubata* from the *Brachiaria* germplasm collection at Embrapa Beef Cattle kept in the field in Campo Grande (state of Mato Grosso do Sul, Brazil), which comprises 475 accessions of 15 species collected in Africa in the mid-1980s by CIAT (Colombia). Inflorescences were collected and

fixed in a mixture of ethanol 95%, chloroform and propionic acid (6:3:2) for 24 h, transferred to 70% alcohol and stored under refrigeration until use. Microsporocytes (PMCs) were prepared by squashing and staining with 0.5% propionic carmine. Chromosome countings and chromosome associations were recorded in 20 PMCs at diakinesis and meiotic phases were evaluated in over 1000 cells in inflorescences collected from four plants of each accession. Pollen viability was analysed by staining fresh dehiscent anthers also with 0.5% propionic-carmine.

Table 1 presents the results of cytological evaluations. Only one accession showed diploid chromosome number (BRA005312), $2n = 2x = 18$. The remaining 20 accessions are tetraploid ($2n = 4x = 36$). The available literature indicates a prevalence of tetraploidy in the genus. A polyploid series—diploidy to hexaploidy—was reported for *B. brizantha*, the species most studied (Carnahan and Hill 1961; Sotomayor-Ríos *et al.* 1970; Basappa *et al.* 1987; Valle and Glienke 1991; Valle and Miles 1994; Bernini and Marin-Morales 2001; Mendes-Bonato *et al.* 2002). Pentaploidy was reported in few accessions (Letteriello *et al.* 1999; Penteadó *et al.* 2000). *B. jubata* is a species still very little studied despite reports of good forage value and adaptation to a stressful environment such as lake and river borders. Diploidy was also reported by Kammacher *et al.* (1973), whereas tetraploidy was reported by Kammacher *et al.* (1973) and Olorode (1975). Polyploidy is very common in grasses. According to Stebbins (1956), the occurrence of natural polyploidy among grasses is about 70%. Basic chromosome numbers $x = 7$ and $x = 9$ have been generally accepted for the genus *Brachiaria*, with most species presenting chromosome numbers in multiples of 9 (Basappa *et al.* 1987), as was the case with the present accessions of *B. jubata*.

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Table 1. Accession code, chromosome number (2n), number of PMCs analysed, and percentage of abnormal cells in meiosis.

Accession	2n	No. PMCs	Percentage of abnormal PMCs								Pollen fertility	
			Met I	Ana I	Tel I	Pro II	Met II	Ana II	Tel II	Tetrad	(%)	
BRA005312	18	1140	3.66	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.70	96.09
BRA005207	36	1220	5.56	6.03	7.19	4.35	15.95	8.00	2.86	4.97	93.98	93.98
BRA005215	36	1168	14.74	24.36	15.65	9.59	7.38	9.92	7.84	5.99	88.25	88.25
BRA005177	36	1174	8.57	5.83	5.93	4.62	10.06	8.93	5.63	7.60	86.86	86.86
BRA005363	36	1371	21.74	21.25	12.99	14.81	16.83	15.00	10.81	8.23	88.42	88.42
BRA005487	36	1166	29.89	22.12	26.66	9.87	10.74	13.63	7.84	8.64	–	–
BRA006211	36	1299	9.94	10.95	10.24	8.23	11.05	8.19	8.15	9.59	–	–
BRA005401	36	1523	12.42	18.38	13.21	8.06	16.77	16.37	17.47	12.11	90.79	90.79
BRA005355	36	1577	13.23	10.62	10.98	8.00	16.87	11.12	17.84	14.28	88.40	88.40
BRA005495	36	1230	16.47	18.88	16.28	13.87	19.65	23.68	18.71	16.37	86.29	86.29
BRA005533	36	1220	7.60	10.15	6.12	5.04	8.86	11.03	12.03	16.66	84.27	84.27
BRA004332	36	1435	15.66	17.86	15.29	15.38	8.27	9.78	12.59	16.67	90.40	90.40
BRA005452	36	1175	7.74	7.80	5.97	3.12	10.06	21.81	15.17	16.88	87.73	87.73
BRA005371	36	1382	25.62	38.42	28.96	26.87	31.87	25.90	21.19	21.58	87.21	87.21
BRA005223	36	1378	27.13	39.89	17.01	7.79	21.24	24.82	18.94	23.67	88.24	88.24
BRA005509	36	1223	19.14	25.19	13.89	14.58	29.65	27.92	24.31	23.81	90.68	90.68
BRA005274	36	1332	8.84	27.12	17.92	15.33	22.73	23.93	25.30	32.67	89.58	89.58
BRA002348	36	1380	36.96	43.27	51.63	29.37	34.15	39.08	31.10	33.49	91.39	91.39
J42	36	1358	33.55	26.16	9.49	13.66	25.71	16.77	8.44	13.92	88.87	88.87
J37	36	1305	20.80	16.66	27.72	18.06	34.09	33.33	26.09	21.94	–	–
J40	36	1262	8.27	18.58	16.47	13.77	15.00	16.67	24.32	23.98	–	–

In the single diploid accession identified (BRA005312), a low frequency of irregular chromosome segregation was observed in metaphase I; the remaining phases of meiosis were perfectly normal as expected for a sexual material that depends on regular meiosis for seed formation (table 1). Among tetraploids, however, irregular chromosome segregation was common but variable among accessions. In six tetraploid accessions, less than 10% of the tetrads were abnormal; in seven others, abnormal tetrads did not exceed 17%, and in the remaining seven, up to 33.5% of tetrads were abnormal. The most common abnormalities included precocious chromosome migration to the poles in both metaphases (figure 1, b&f) and laggards in metaphases (figure 1, c&g), leading to the formation of micronuclei in telophases (figure 1d) and tetrads (figure 1h). Similar observations were also reported in polyploid accessions of other *Brachiaria* species (Sotomayor-Ríos *et al.* 1970; Basappa *et al.* 1987; Valle *et al.* 1989; Mendes-Bonato *et al.* 2002; Risso-Pascotto *et al.* 2003). The most common cytological consequence of irregular chromosome segregation during meiosis is the formation of unbalanced microspores that culminate in sterile pollen grains. Fusion between two cells was recorded in six of the 21 accessions analysed (Mendes-Bonato *et al.* 2003). However, meiosis continued regularly in these cells and normal microspores were produced. Pollen viability in fresh pollen grains was very high considering the ploidy level of the accessions. Pollen fertility in the diploid accession was 96%, whereas in the tetraploid ones it ranged from 84.27 to 93.98%. Apomictic *Brachiaria* accessions are pseudogamic, i.e. the polar nuclei are necessary for proper endosperm formation and seed fill. Thus, pollen fertility plays an important role in the dissemination and success of the genotype.

The diploid or polyploid condition of the accessions was also demonstrated by chromosome associations at diakinesis (table 2). In the diploid accession, chromosomes paired as bivalents, while in the tetraploid ones univalents, bivalents, trivalents and quadrivalents were recorded (figure 1a). Bivalents predominated; univalents and quadrivalents were found in low frequencies, while trivalents were rare and recorded only in a few accessions. Stebbins (1947) classified polyploids into autopolyploids, allopolyploids, autoallopolyploids, and segmental allopolyploids. In autopolyploids, all genomes are identical and homologous chromosomes have equal opportunities to pair at meiosis. When pairing starts at different sites, multivalents are formed. However, the maintenance of the multivalence till metaphase I will depend on its frequency and chiasma localization. In segmental allopolyploids, the genomes are not identical. As they result from hybridization of closely related diploid species followed by the doubling of the chromosome numbers, many bivalents and a few multivalents are formed. Low frequency of quadrivalents in tetraploid accessions has been reported in *B. brizantha* (Mendes-Bonato *et al.* 2002) and that was interpreted as a result of segmental allopolyploidy. Although low multivalent frequency is an argument frequently used in advocating segmental allopolyploidy, Sybenga (1996) pointed out that this character is not necessarily a reliable indication of limited pairing affinity, and thus of homology, because even true autopolyploids may form quadrivalents with frequencies substantially lower than the theoretically possible. To date, the origin of tetraploidy in *Brachiaria* species and accessions remains unknown. This genus needs adequate taxonomic revision and genome characterization would certainly substantiate this process.

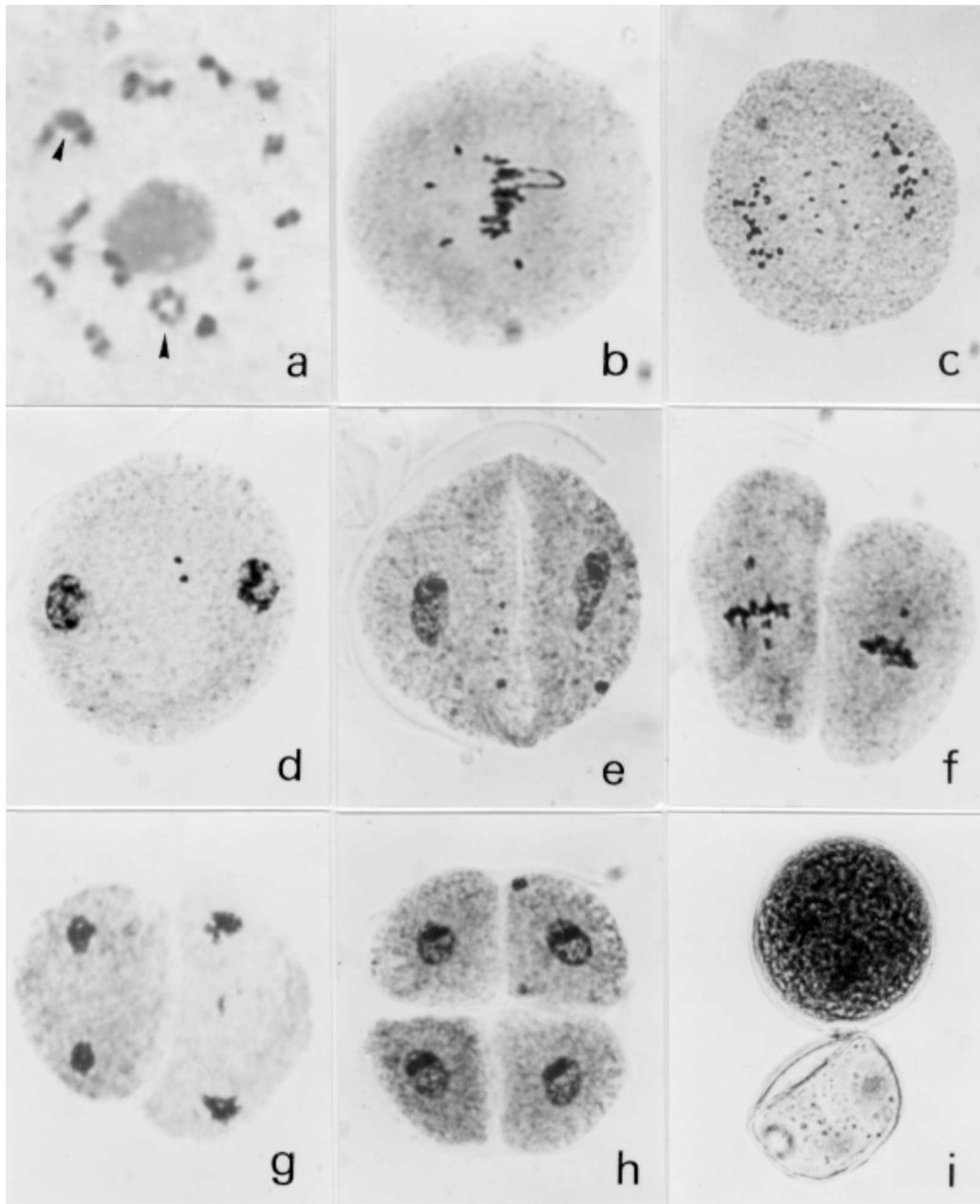


Figure 1. Aspects of microsporogenesis in tetraploid accessions of *B. jubata*. (a) Diakinesis with 14 II and 2 IV (arrowheads) (1000 \times). (b) Metaphase I with precocious chromosome migration to the poles. (c) Anaphase I with laggards. (d) Telophase I with micronuclei. (e) Prophase II with micronuclei in both cells. (f) Metaphase II with precocious chromosome migration to the poles. (g) Late anaphase II with laggard. (h) Tetrad with micronuclei in one microspore. (i) Fertile (dark) and sterile pollen grains (400 \times).

The risks associated with extensive *Brachiaria* monoculture in Brazil are obvious. The two most cultivated varieties in the Brazilian savannas—*B. brizantha* cv. Marandu and *B. decumbens* cv. Basilisk—lack certain characteristics, and this makes them vulnerable. New cultivars are urgently needed and interspecific hybridization is under way as a means of introgressing desirable genes from wild species into these two widely cultivated varieties. *Brachiaria* has only

been successfully hybridized in the past decade when the first interspecific crosses were made. Difficulties that delayed the process included differences in ploidy level among accessions and compatible species and reproduction by apomixis (Valle *et al.* 1993).

Regardless of an adequate germplasm base, the breeding programme demands a knowledge of the reproduction mode, chromosome number and meiotic behaviour

Table 2. Accession code, chromosome number and types of chromosome association at diakinesis, and average associations per cell.

Accession	2n	Average association/cell				Chromosome variation			
		I	II	III	IV	I	II	III	IV
BRA005312	18	0.00	9.00	0.00	0.00	–	(9)	–	–
BRA005207	36	0.65	15.09	0.05	0.85	–	(12–18)	(0–1)	(0–3)
BRA005215	36	1.35	15.11	0.20	1.60	(0–3)	(09–18)	(0–1)	(0–3)
BRA005177	36	0.75	14.65	0.25	1.30	(0–3)	(09–18)	(0–1)	(0–3)
BRA005363	36	1.00	14.75	0.00	1.35	(0–4)	(10–18)	–	(0–3)
BRA005487	36	0.00	15.00	0.00	1.50	–	(14–16)	–	(1–2)
BRA006211	36	0.10	15.85	0.00	1.05	(0–2)	(14–18)	–	(0–2)
BRA005401	36	0.10	15.85	0.00	1.05	(0–2)	(14–18)	–	(0–2)
BRA005355	36	0.00	14.00	0.00	2.00	–	(10–18)	–	(0–4)
BRA005495	36	1.10	14.40	0.30	1.30	(0–4)	(11–18)	(0–1)	(0–3)
BRA005533	36	0.00	13.00	0.00	2.50	–	(10–18)	–	(0–4)
BRA004332	36	0.00	14.10	0.00	1.95	–	(10–18)	–	(0–4)
BRA005452	36	1.25	13.75	0.15	1.70	(0–4)	(10–18)	(0–1)	(0–4)
BRA005371	36	0.10	12.05	0.00	2.05	(0–2)	(10–18)	–	(0–4)
BRA005223	36	0.50	13.90	0.00	1.00	(0–4)	(13–18)	–	(0–2)
BRA005509	36	1.15	14.60	0.15	1.30	(0–8)	(10–18)	(0–1)	(0–3)
BRA005274	36	0.60	16.03	0.00	0.70	(0–2)	(13–18)	–	(0–2)
BRA002348	36	1.70	14.00	0.15	1.40	(0–6)	(12–18)	(0–1)	(0–3)
J42	36	0.30	13.06	0.10	2.05	(0–3)	(09–18)	(0–1)	(0–9)
J37	36	0.00	15.00	0.00	1.50	–	(12–18)	–	(0–3)
J40	36	1.11	15.22	0.11	0.83	(0–3)	(13–18)	(0–1)	(0–2)

within and among compatible species in order to direct the crosses (Valle and Savidan 1996). Natural diploids of *Brachiaria* always reproduce sexually, while polyploids are essentially apomictic, especially those with irregular meiosis. In *B. jubata*, some tetraploid accessions under analysis showed a low frequency of meiotic irregularities. An investigation about mode of reproduction in these accessions (C. B. Valle, unpublished data) showed that the diploid (BRA005312) and the tetraploid accession BRA005401 have sexual reproduction. All the others were apomictic. Pollen fertility was high but seed production of *B. jubata* in this collection has been consistently low, which might be related to abnormal meiosis in the megagametophyte. Since irregularities are not affecting pollen fertility, the course of investigation should focus on the female apparatus. Diploid accessions are rare and the more valuable parental material is polyploid, thus hybridization in *Brachiaria* requires sexual tetraploid females. Tetraploid apomictic accessions with high pollen fertility could be used as pollen donors if other desirable traits have been ascertained, when highly productive apomictic hybrids are desired. Taking this into account, the sexual diploid accession needs to be artificially tetraploidized before crossing but the sexual tetraploid can be immediately used. In the tetraploid apomictic accessions under analysis pollen fertility was very high, which makes them potentially useful as pollen donors in crosses.

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