SHORT COMMUNICATION

Physical mapping of rDNA genes corroborates allopolyploid origin in apomictic *Brachiaria brizantha*

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Abstract *Brachiaria* (Trin.) Griseb belongs to the family Poaceae, and within the genus, apomixis or sexuality is present in different accessions of the same species. The majority of *Brachiaria* species are polyploid and apomictic, making strategies for crop improvement by breeding very intricate. In spite of the high frequency of apomictic polyploids, the relationship of polyploidy and hybridization with apomixis in *Brachiaria* is still unclear. Further analysis requires detailed knowledge regarding the genomic composition of the polyploids. The present work introduces the use of fluorescent in situ hybridization (FISH) into cytogenetic analysis of *Brachiaria*. Physical mapping of heterologous rDNA sequences, associated with conventional karyotyping of the *B. brizantha* diploid sexual (BRA 002747) and the tetraploid apomictic (BRA000591) accessions, provided evidence of the latter being of allopolyploid origin. Based on our results and on previous knowledge on apomixis in *B. brizantha*, we suggest that the origin of apomixis was probably a consequence of hybridization.

Keywords Allopolyploid · Apomixis · Chromosomes · Cytogenetics · FISH · Hybridization

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Introduction

Species of the genus *Brachiaria* (Trin.) Griseb. (Poaceae) are important forage grasses in tropical and subtropical countries. In Brazil, the cultivated pasture area comprises around 115 million hectares (Karia et al. 2006), forming the main basis for cattle production. The majority of this area, from 50 to 70 million hectares, is cultivated with *Brachiaria.*

Analysis of accessions in the *Brachiaria* collection established at the International Center for Tropical Agriculture (CIAT), Colombia and duplicated at Embrapa, Brazil, indicated an evident prevalence of polyploids. The wide predominance of tetraploids is based on the basic chromosome number $x = 9$ (Sotomayor-Ríos et al. 1968; Christopher and Abrahan 1976; Thompson and Estes 1986; Basappa et al. 1987; Dujardin 1979; Valle and Glienke 1991; Valle and Savidan 1996; Bernini 1997; Penteado et al. 2000; Bernini and Marin-Morales 2001; Mendes-Bonato et al. 2002a; Risso-Pascotto et al. 2003, Utsunomiya et al. 2005). Also, it is evident that polyploidy in the genus is associated with apomixis (Valle and Savidan 1996), a natural mechanism of plant reproduction that results in the formation of seeds asexually, that retains the maternal genotype in the progeny (Hanna and Bashaw 1987, Asker and Jerling 1992; Nogler 1984). No diploid accession of this collection presented apomixis, and only one tetraploid *B. humidicola* was characterized as sexual (Valle et al. 2008).

In the most cultivated species in Brazil, *B. brizantha*, a polyploidy series, is present with reports of diploid, tetra, penta, and hexaploid accessions (Valle and Glienke 1991; Penteado et al. 2000; Mendes-Bonato et al. 2002b; Risso-Pascotto et al. 2003). Only one accession among 275 is a diploid sexual, while the remaining are polyploid

apomicts (Carnahan and Hill 1961; Valle and Glienke 1991). Despite the frequent occurrence of polyploidy in *Brachiaria*, the putative association of polyploidy and hybridization with apomixis in the genus is still unclear.

Hybridization is considered, since Ernst's work (1918, in Tucker and Koltunow 2009), as an important factor in the evolution of apomixis, because all natural apomicts are polyploid and highly heterozygous. Carman (1997, 2001, 2007) suggested that apomixis might result from the asynchronous expression of the genes involved in female reproduction in hybrids of two sexual-related ancestors. More recent analyses showing that the character is under the control of few loci suggest that apomixis may be a consequence of mutation in alleles of genes controlling sexuality or a consequence of epigenetic changes in gene regulation. Therefore, apomixis might develop through different pathways from ancestors with sexual modes of reproduction (reviewed by Koltunow and Grossniklaus 2003; Tucker and Koltunow 2009).

Apomixis segregation analysis in *Brachiaria* showed that this character is under the control of a single dominant locus, whereas sexuality is recessive; the suggested genotype for tetraploid apomicts in *Brachiaria* is *Aaaa* and for diploid sexuals, *aa* (Valle et al. 1994; Miles and Escandon 1997). The absence of the apomictic character in induced tetraploid plants of diploid sexual *B. brizantha* and *B. ruziziensis* (Swenne et al. 1981; Gobbe et al. 1981, 1982; Araujo et al. 2005) corroborates the hypothesis that apomixis in the genus depends mainly on different gene profiles (*A*) which probably originated through hybridization and/or mutation. Additionally, analysis of meiotic behavior indicated the presence of more than one genome within pentaploid accessions of *B. brizantha* (Mendes 2004; Mendes et al. 2006), in *B. ruziziensis* (Risso-Pascotto et al. 2005), and in *B. humidicola* (Boldrini et al. 2006).

The major objective of the present work was a cytogenetic analysis of *B. brizantha* diploid sexual (BRA 002747) and tetraploid apomictic accessions (BRA000591) aimed at elucidating the origin of polyploidy in the apomictic accession. Therefore, as a new approach in *Brachiaria* chromosome analysis, we have introduced the use of fluorescent in situ hybridization (FISH) for physical mapping of heterologous 5S- and 18S-5.8S-25S rDNA sequences. The rDNA loci have been shown previously to provide useful chromosome markers to differentiate genomes in allotetraploids, such as *Brassica juncea* (Maluszynska and Hasterok 2005) or *Arachis hypogaea* (Seijo et al. 2004). In particular, the number and localization of 5S rDNA loci are often a distinguishing feature between even closely related genomes, as has been shown by ecotype-specific polymorphisms in *Arabidopsis thaliana* (Fransz et al. 1998). Here, we demonstrate that in situ hybridization of rDNA probes in combination with

conventional karyotyping was effective in determining the allopolyploid origin of the apomictic *B. brizantha* accession. Based on our findings and on the previously published data on *B. brizantha* as specified earlier, we suggest that the probable origin of apomixis was through hybridization.

Materials and methods

Plant material

Plants of *B. brizantha* diploid ($2n = 2x = 18$) sexual accession BRA-002747, tetraploid ($2n = 4x = 36$), apomictic accession BRA-000591 (cv. Marandu) were maintained in the experimental field at Embrapa Genetic Resources and Biotechnology, Brasília-DF, Brazil. Stolons of these plants were transferred to plastic bags containing sterile soil and kept in the greenhouse for 2–3 weeks.

Chromosome preparation

After this time, root tips of 5 mm length were isolated from the germinating roots of at least five plants around 8:30 am, when meristematic activity was high. The root tips were washed in distilled water and treated with 0.002 M 8-hydroxyquinoline for 1 h at room temperature and 2 h at 4°C. The material was fixed in freshly prepared 3:1 ethanol-glacial acetic acid for 12 h and maintained in 70% ethanol at 4°C. Samples were washed in $1\times$ enzyme buffer (4 mM citric acid, 6 mM sodium citrate, pH 4.8), and incubated in an enzyme mixture consisting of 11.9% pectinase (Sigma P2401 from *Rhyzopus sp.*) and 7% cellulase (Sigma C1184 from *Aspergillus niger*) at 37°C for 3 h. The roots tips were subsequently washed two times in $1\times$ enzyme buffer for 15 min each. Root meristems were dissected on histological slides in 45% acetic acid, dried, and treated by Feulgen reaction (Feulgen and Rossenbeck 1924) or incubated with Chromomycin A_3 (CMA_3) or 4′,6-diamidino-2-phenylindole (DAPI). For FISH analysis, excised meristems were squashed in a drop of 45% acetic acid. Cover slips were removed after freezing the slides in liquid nitrogen. The preparations were stained for 30 min with 2 μg/ml DAPI to enable quality testing using fluorescence microscopy.

Morphological analyses

Metaphase chromosomes from nine cells from each accession were analyzed. Based on average length of the chromosomes, the arms ratio (long arm/short arm of individual chromosomes), the position of the centromere, the presence of satellites, and the karyotypes of both accessions of *B. brizantha* were constructed.

In situ hybridization

Probes were labeled either with digoxigenin-11-dUTP or biotin-11-dUTP (Roche) by random primed labeling. Probes used were pTa71 containing the 18S-5.8S-25S rDNA genes from *Triticum aestivum* (Gerlach and Bedbrook 1979) and pTA794, which has the complete 5S rDNA gene of *T. aestivum* (Gerlach and Dyer 1980). Pretreatment, hybridization, washing, and detection procedures essentially followed protocols of Schwarzacher and Heslop-Harrison (2000). Denatured DNA probes (100 ng/ml) were mixed in a hybridization solution containing 50% (v/v) formamide, 10% (w/v) dextran sulfate, 0.125 mM EDTA, 0.125% (w/v) SDS, and 1 μg of salmon sperm DNA. The chromosomes and DNA were denatured together by incubating the slides with hybridization/probe mix in a humid chamber at 78–80°C for 10 min, floating in a heated water bath. Subsequently, the temperature in the chamber was slowly brought to 37°C, and the slides were incubated for 16 h at this temperature. Hybridization sites were detected by sheep anti-digoxigenin conjugated to fluorescein isothiocyanate (anti-dig-FITC; green fluorescence) and Cy3-streptavidin (red fluorescence). FITC signals were amplified using anti-sheep antibody conjugated to fluorescein (Vector Laboratories). Chromosomes were counterstained with DAPI. Slides were observed with Zeiss Axiophot epifluorescence microscope using appropriate filters. Microphotographs were taken on Kodak Ultra ISO 400 color print film, digitalized and edited using only Adobe Photoshop functions (brightness, cropping, overlay), which affect the whole image equally.

Results

A general karyogram based on CMA$_3$ and DAPI staining of somatic metaphase chromosomes of the two accessions of *B. brizantha*, the diploid sexual and the tetraploid apomictic is shown in Fig. 1. Chromosomes are paired and ordered according to their size. This analysis confirmed the presence of 18 and 36 chromosomes in the diploid sexual

and tetraploid apomictic, respectively, with a basic chromosome number of $x = 9$. A more detailed picture was achieved when analyzing Feulgen-stained chromosomes with regard to the length of the chromosomes (μm), the arms ratio (long arm/short arm of individual chromosomes), and the centromere position. Results of this analysis are shown in Tables 1 and 2, and represented as ideograms in Fig. 2.

In the diploid accession, five pairs of homologous chromosomes were submetacentric (SM), three metacentric (M) and only one subtelocentric (ST), following the classification of Levan et al. (1964) (Figs. 1, 2). The shortest pair of chromosomes was 2.4 μm long, designated pair IX, and the ratio of the short to long arm length in this pair was 1.38. The longest pair (I) of this accession was 5.15 μm long, and the ratio of the arms was 4.13 in this pair (Table 1). One pair of satellite chromosomes could be identified in chromosome pair VIII. The satellites were easier to identify after in situ hybridization of the 18S-5.8S-25S rDNA probe, as delineated later (Fig. 5b). Almost all pairs of homologous chromosomes in the tetraploid accession showed the centromere in the median region, except for the pairs III and XIV, which were submetacentric (SM; Table 2). The shortest pair was 2.2 μm long, and the longest was 5.6 μm. Chromosome pair VIII showed a secondary constriction and a satellite, which was more clearly visible after DAPI staining (Fig. 5a). The average length in the pair I of the diploid accession (5.15 μm) was similar to those determined in pairs I and II of the tetraploid accessions (5.6 and 5.2 μm). Similarly, pair II (4.85 μm) was similar to pairs III and IV (4.85 and 4.75 μm), pair III (4.55 μm) to pairs V and VI (4.55 and 4.3 μm) and so forth up to the pair IX.

The localization of ribosomal genes through FISH using heterologous sequences for 5S and 18S-5.8S-25S rDNA as probes is shown in Figs. 3–5. In the diploid accession (Fig. 3a, b), the 5S rDNA probe was observed in the interstitial region of only one pair of chromosomes. One large 18S-5.8S-25S locus was detected mainly on the satellites of the chromosome pair, which displays a secondary constriction (chromosome pair VIII in Fig. 1, 2). This site

Fig. 1 *Brachiaria brizantha* metaphase chromosomes. **a** Diploid sexual BRA-002747 (CMA$_3$ staining). **b** Tetraploid apomictic BRA-000591 (DAPI staining). *Scale bar* 3 μm
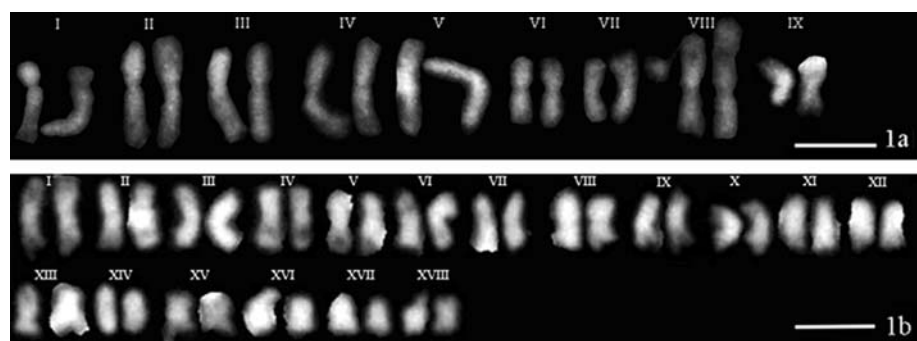
Table 1 Diploid sexual accession of *B. brizantha* BRA002747 ($2n = 2x = 18$)

Pair of chromosomes	I		II		III		IV		V		VI		VII		VIII		IX	
Chromosomes	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Cell 1	6.0	6.0	5.8	5.5	5.7	5.7	5.5	5.5	5.1	5.1	4.1	4.1	3.8	3.8	3.7	3.7	3.4	3.3
Cell 2	4.8	4.8	4.7	4.7	4.7	4.7	4.1	4.1	3.8	3.8	3.6	3.5	3.5	3.5	3.4	3.3	2.9	2.6
Cell 3	5.1	5.1	5.1	5.1	4.7	4.7	4.5	4.5	3.9	3.9	3.6	3.6	3.5	3.5	3.3	3.3	2.6	2.4
Cell 4	5.5	5.5	5.5	5.5	4.9	4.9	4.5	4.5	4.1	3.8	3.7	3.7	3.4	3.4	3.0	2.8	2.6	2.4
Cell 5	4.6	4.5	4.3	4.1	4.1	4.1	4.1	3.8	3.6	3.4	2.6	2.5	2.6	2.4	2.2	2.2	1.9	1.7
Cell 6	5.3	5.3	4.2	4.2	4.1	3.9	3.8	3.6	3.8	3.5	3.5	3.5	3.2	2.6	2.6	2.6	2.0	2.0
Cell 7	4.4	4.1	4.1	4.1	3.8	3.8	3.5	3.5	3.5	3.2	3.0	3.0	2.9	2.9	2.6	2.5	2.3	2.3
Cell 8	5.8	5.8	5.1	5.1	5.2	5.1	4.6	4.6	4.1	4.1	3.6	3.6	3.4	3.2	3.3	3.0	2.7	2.4
Cell 9	5.5	5.2	4.6	4.6	4.3	4.3	4.3	4.3	3.9	3.9	3.6	3.6	3.6	3.6	3.2	3.0	2.4	2.2
$\overline{X}$	5.20	5.10	4.80	4.70	4.60	4.50	4.30	4.20	3.90	3.80	3.40	3.40	3.30	3.20	3.00	2.90	2.50	2.30
σ	0.44	0.46	0.44	0.53	0.46	0.58	0.53	0.58	0.20	0.27	0.41	0.34	0.31	0.45	0.45	0.43	0.43	0.41
Length $\overline{X}$ of the pair	5.15		4.75		4.55		4.25		3.85		3.40		3.25		2.95		2.40	
Arms length ratio	4.13		1.87		2.33		2.00		1.11		1.96		2.50		1.02		1.38	
Centromere position	ST		SM		SM		SM		M		SM		SM		M		M	

Metaphase cells from root meristems showing the length of the chromosomes (μm), the arms length ratio (long arm/short arm of individual chromosomes), and the centromere position

M metacentric, *SM* submetacentric, *ST* subtelocentric

corresponds to the nucleolar-organizing region (NOR). In the tetraploid accession, three pairs of 5S rDNA sites were detected using the 5S rDNA probe (Fig. 4b, d), whereas the 18S-5.8S-25S rDNA sequence was detected in two pairs of chromosomes (Figs. 4d, 5b). Figure 5b clearly shows that there is only one pair with active sites of 18S-5.8S-25S rDNA. The other locus is on the terminal region of a chromosome pair lacking satellites, which is inactive. The 18S-5.8S-25S rDNA and the 5S rDNA sites of the diploid accessions were also mapped in the ideograms (Fig. 2).

Discussion

This work confirms that combining conventional karyotyping and fluorescent in situ hybridization (FISH) facilitates ideogram construction and interpretation of origins of polyploidy in *Brachiaria* accessions.

In the tetraploid apomictic accession, the ratio of the length of the arms was very similar for all pairs, suggesting structural stability. Metacentric chromosomes were prevalent in this accession as well as found in other tetraploid accessions (Bernini and Marin-Morales 2001); however, chromosomes were longer than those previously described. On the other hand, the majority of the chromosomes of the diploid accession analyzed were SM and longer than those described by Bernini and Marin-Morales (2001) as being metacentric. This is an indication of some phylogenetic distance between this accession and other diploids. It could also be a result of bulky chromosome structural alterations

in this accession. The presence of secondary constrictions in chromosome pair VIII was also described for the diploid sexual accession R134 of *B. ruziziensis* (Bernini and Marin-Morales 2001), suggesting the conservation of this cytogenetic character along species differentiation. A relationship in the length of the chromosomes of the diploid and the tetraploid accession was observed but with different centromere positions.

Further evidence indicating the allotetraploid origin of the *B. brizantha* apomictic accession BRA000591 was provided using FISH with 5S and 18S-5.8S-25S rDNA probes, thus corroborating previous suggestion of allopolyploidy (Mendes-Bonato et al. 2002a, Mendes et al. 2006). The most striking difference between the two accessions is the presence of only one locus of 5S rDNA in the diploid and three loci in the tetraploid accession. This was verified in metaphase as well as in interphase nuclei (data not shown). In the case of tetraploidization through chromosome doubling, two loci would have been expected. Furthermore, hybridization of the 18S-5.8S-25S rDNA probe identified one active NOR site in the diploid accession and two loci in the tetraploid, but only one active on a chromosome pair with secondary constrictions. This indicates a phenomenon described as nucleolar dominance. According to this phenomenon, hybrids or allopolyploids develop nucleoli on chromosomes of only one of the two parents (for review see Pikaard 2000).

The establishment of a FISH protocol for localization of multiple rDNA sequences provides a novel approach for viewing genomic organization and chromosome

Table 2 Tetraploid apomictic accession of *B. brizantha* BRA000591 ($2n = 4x = 36$)

Pair of chromosomes	I		II		III		IV		V		VI		VII		VIII		IX	
Chromosomes	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Cell 1	6.1	6.1	3.9	3.7	3.4	3.4	3.4	3.3	3.4	3.2	3.1	3.0	3.0	2.9	3.0	2.9	2.9	2.8
Cell 2	4.2	4.1	4.1	4.0	3.9	3.8	3.7	3.6	3.5	3.5	3.4	3.2	3.2	3.2	3.1	3.0	2.9	2.9
Cell 3	5.5	5.3	5.1	4.8	4.7	4.6	4.6	4.6	4.4	4.4	4.3	4.1	3.7	3.5	3.4	3.3	3.5	3.3
Cell 4	6.3	6.2	6.1	5.9	5.9	5.5	5.5	5.2	5.4	5.2	5.2	5.1	4.7	4.7	4.6	4.5	4.6	4.6
Cell 5	5.9	ND	5.6	5.3	5.3	5.2	5.3	5.2	4.9	4.7	4.8	4.5	4.5	4.4	3.5	3.2	3.0	3.3
Cell 6	5.8	5.7	5.6	5.3	5.5	5.5	5.3	5.3	5.3	5.3	5.0	4.8	4.8	4.6	4.6	4.4	4.1	4.1
Cell 7	6.0	6.0	6.0	5.8	5.3	5.3	5.3	5.0	5.1	5.1	4.8	4.4	4.5	4.3	4.5	4.2	4.1	4.1
Cell 8	ND	ND	6.0	5.9	5.8	5.5	5.8	5.5	5.5	5.4	5.5	5.2	5.0	4.7	4.8	4.3	4.7	4.7
Cell 9	6.2	ND	5.8	5.5	4.8	ND	4.6	4.6	4.4	4.3	4.3	4.0	3.8	3.8	3.8	3.6	3.8	3.6
$\overline{X}$	5.70	5.50	5.30	5.10	4.90	4.80	4.80	4.70	4.60	4.50	4.40	4.20	4.10	4.00	3.80	3.70	3.70	3.70
σ	0.63	0.72	0.78	0.76	0.80	0.78	0.78	0.72	0.74	0.75	0.73	0.72	0.68	0.64	0.61	0.68	0.63	0.68
Length $\overline{X}$ of the pair	5.60		5.20		4.85		4.75		4.55		4.30		4.05		3.75		3.70	
Arms length ratio	1.60		1.00		1.97		1.25		1.21		1.56		1.65		1.25		1.66	
Centromere position	M		M		SM		M		M		M		M		M		M	

Pair of chromosomes	X		XI		XII		XIII		XIV		XV		XVI		XVII		XVIII	
Chromosomes	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Cell 1	2.8	2.7	2.5	2.5	2.5	2.5	2.6	2.5	2.4	2.3	2.3	2.3	2.3	2.3	2.0	2.0	2.0	1.9
Cell 2	2.9	2.9	2.7	2.6	2.6	2.6	2.5	2.5	2.5	2.5	2.6	2.3	2.3	2.3	2.2	2.2	2.0	2.0
Cell 3	3.7	3.3	3.2	3.1	3.2	3.1	3.1	3.0	3.0	3.0	2.8	2.5	2.3	2.3	2.3	2.2	2.3	2.2
Cell 4	4.3	4.0	4.1	3.9	4.1	3.7	4.0	3.8	3.4	3.3	3.3	3.3	3.3	3.2	2.7	2.6	2.5	2.3
Cell 5	3.1	3.0	3.0	3.0	3.1	2.9	2.9	2.8	2.8	2.7	2.8	2.7	2.9	2.5	2.3	2.3	2.2	2.1
Cell 6	4.1	3.9	3.7	3.7	3.8	3.5	3.6	3.3	3.4	2.4	3.0	3.0	2.7	2.7	2.6	2.5	2.2	1.9
Cell 7	4.1	4.0	4.0	3.9	3.9	3.6	3.6	3.3	3.4	3.2	3.3	3.3	3.2	3.1	2.6	2.5	ND	ND
Cell 8	4.6	4.6	4.5	3.9	4.1	3.9	3.9	3.9	3.8	ND	3.6	3.4	3.3	3.2	3.0	2.9	2.4	2.1
Cell 9	3.7	3.7	3.7	ND	3.6	3.5	3.2	3.0	3.1	2.5	3.0	2.9	2.7	2.5	2.6	2.4	2.4	2.3
$\overline{X}$	3.70	3.50	3.00	3.30	3.40	3.20	3.20	3.10	3.00	2.70	2.90	2.80	2.70	2.60	2.40	2.40	2.10	2.10
σ	0.63	0.59	0.70	0.55	0.50	0.44	0.51	0.47	0.44	0.39	0.38	0.40	0.40	0.37	0.29	0.24	0.22	0.15
Length $\overline{X}$ of the pair	3.60		3.30		3.30		3.15		2.95		2.85		2.65		2.40		**2.10**	
Arms length ratio	1.70		1.00		1.03		1.06		1.77		1.03		1.00		1.36		1.00	
Centromere position	M		M		M		M		SM		M		M		M		M	

ND not determined, *M* metacentric, *SM* submetacentric, *ST* subtelocentric

Fig. 2 *B. brizantha* metaphase chromosomes spread after Feulgen reaction and ideograms. **a** Diploid sexual BRA-002747. **b** Tetraploid apomictic BRA-000591. In the ideograms, sites of 5S rDNA are labeled *green* and those of 18S-5.8S-25S rDNA *red*. The three 5S rDNA sites in BRA-000591 could not be assigned distinctly. *Scale bar* (micrographs) 6 μm (**a**) and 20 μm (**b**); *scale bar* (ideograms) 2 μm
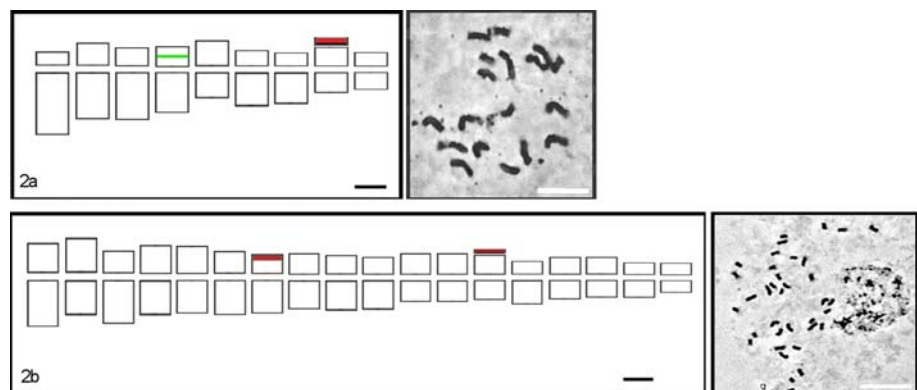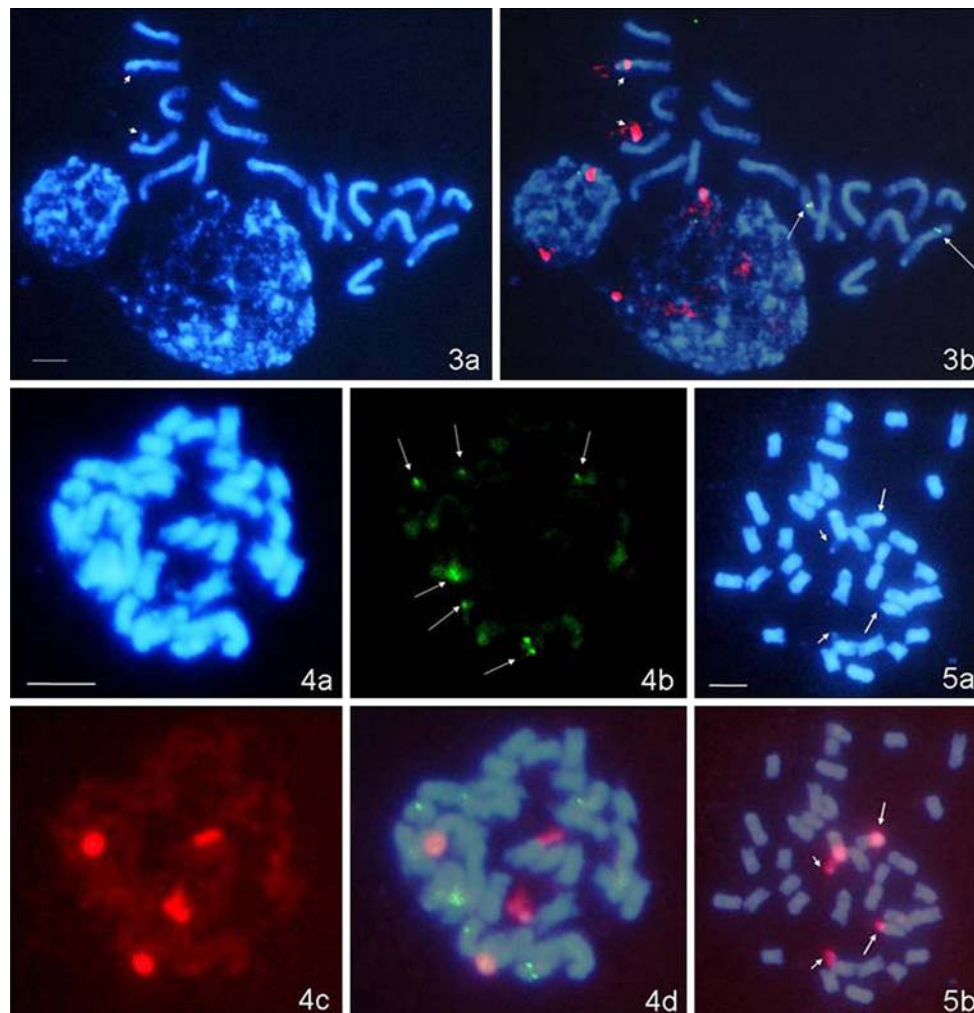
Figs. 3–5 Fluorescent in situ hybridization (FISH) in metaphase chromosomes of *B. brizantha* using 5S (*green*) and 18S-5.8S-25S (*red*) rDNA probes. **3** Diploid sexual BRA-002747; **3a** DAPI staining; **3b** detection of one pair of sites of 5S rDNA (*arrows*) and one pair of sites of 18S-5.8S-25S rDNA mainly on the satellite of another chromosome (*arrowhead*) after double hybridization. **4**, **5** Tetraploid apomitic BRA-000591. **4a**, **5a** DAPI staining; **4b**, **d** three pairs of 5S rDNA sites; **4c**, **b**, **5b** two pairs of 18S-5.8S-25S rDNA sites. In **5a**, **b** the chromosome pair with secondary constriction is marked with *short arrows*, the other pair with *long arrows*. *Bars* 5 μm

structure in *Brachiaria*, also opening up new perspectives with regard to physical mapping of apomixis-related DNA sequences in the absence of large segregating populations and genetic polymorphisms yet unavailable for the genus.

Previous knowledge on *B. brizantha* suggests that apomixis expression depends on a different gene allele (*A*) (Swenne et al. 1981; Gobbe et al. 1981, 1982; Araujo et al. 2005), single and dominant (Valle et al. 1994; Miles and Escandon 1997), the meiosis profile (Mendes 2004; Risso-Pascotto et al. 2005, Mendes-Bonato et al. 2006, Boldrini et al. 2006), and current results are strong evidence of allopolyploidization in apomicts. These data corroborate the hypothesis that the origin of apomixis in *Brachiaria* is through hybridization, as suggested for many other systems (Carman 1997, 2001, 2007).

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