

## RESEARCH NOTE

# Abnormal timing of cytokinesis in microsporogenesis in *Brachiaria humidicola* (Poaceae: Paniceae)

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### Introduction

Meiosis is controlled by a large number of genes, generally dominant, whose expression is stage-specific, site-specific and time-specific (Gottschalk and Kaul 1974, 1980a,b; Baker *et al.* 1976; Golubovskaya 1979, 1989). Among genes acting in the meiotic process, those responsible for the partitioning of the cytoplasm after nuclear division play a very important role in the formation of viable gametes. After two rounds of chromosome segregation (karyokinesis) and one simultaneous or two successive cytoplasmic divisions (cytokinesis), the final product of male meiosis in flowering plants emerges as a tetrad of haploid microspores enclosed in a callose wall. The timing of cytokinesis varies among angiosperms. In most monocot plants, cytokinesis is successive, i.e. one partitioning of the cytoplasm occurs after telophase I and another after telophase II, so that there is a distinct dyad stage. However, in most dicots, it is simultaneous and occurs after telophase II (Peirson *et al.* 1996).

Many meiosis mutations affecting the pattern of cytokinesis have been reported in higher plants (Peirson *et al.* 1996). In *Brachiaria*, a promising African genus of forage grass for the Brazilian savannas, absence of cytokinesis leading to  $2n$  microspores and binucleated or tetranucleated microspores have been reported in *B. brizantha* (Risso-Pascotto *et al.* 2003) and *B. nigropedata* (Utsunomiya *et al.* 2005). The present study details meiotic and postmeiotic abnormalities related to cytokinesis observed in one accession of *B. humidicola*.

### Materials and methods

Twentyfive accessions of *B. humidicola*, collected in the African savannas in the mid-1980s by CIAT (Colombia), from the Embrapa Beef Cattle germplasm collection (Campo

Grande, state of Mato Grosso do Sul, Brazil) were analysed cytologically. Site characteristics of the plots in Embrapa Beef Cattle Research Center in Brazil were: 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 (59% sand 8% silt 33% clay; pH 4.2).

Inflorescences for meiotic study were 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. Photomicrographs were made with a Wild Leitz microscope using Kodak Imagelink - HQ, ISO 25 black and white film.

### Results and discussion

Cytological characterization revealed one accession (H003) presenting several meiotic abnormalities, mainly owing to its polyploid condition. Chromosome number in this accession was defined as  $2n = 7x = 42$ . Two basic chromosome numbers,  $x = 7$  and  $x = 9$ , have been reported for the genus *Brachiaria* (Basappa *et al.* 1987; Bernini and Marin-Morales 2001; Mendes-Bonato *et al.* 2002, 2006; Utsunomiya *et al.* 2005). More recently, a new number,  $x = 6$ , was determined for several accessions of *B. dictyoneura* (Risso-Pascotto *et al.* 2006), a species suggested to belong to the same agamic complex as *B. humidicola* (Renvoize *et al.* 1996). The constant presence of 12 univalents in diakinesis that remained as laggard in anaphase I, and then segregated as sister chromatids, together with the fact that the remaining 30 chromosomes segregated as 15 chromosomes towards each pole in anaphase I and II, strongly suggest a different origin for this accession, probably by allopolyploidy with the parents not showing the same meiotic rhythm.

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**Keywords.** cytokinesis; forage grass; microsporogenesis; polyploidy;  $2n$  gametes; *Brachiaria humidicola*.

Abnormalities related to abnormal timing of cytokinesis were also found to be frequent in this accession. As a monocotyledonous species, *B. humidicola* was expected to present successive cytokinesis. However, a large number of meiocytes did not show the first cytokinesis after telophase I (table 1), and the second division occurred in a common cytoplasm (figure 1,a–c). The first cytokinesis occurred after telophase II, giving rise to a dyad with two binucleated microspores (figure 1,d). Following callose wall dissolution, the binucleated microspores were released (figure 1,e) and the second cytokinesis began to occur discreetly by invagination (figure 1,f–h). The furrow of invagination progressed up to the opposite side (figure 1,i), dividing the binucleated microspores into two normal uninucleated microspores (figure 1,j). A similar pattern of cytokinesis was reported in an intergeneric hybrid between *Zea mays* and *Tripsacum dactyloides* (Kindiger 1993), but affected only a few microspores and was one among several types of abnormal microspore behaviours observed. This pattern of cytokinesis is being reported for the first time in *Brachiaria*. In other *Brachiaria* species where cytokinesis was absent (Risso-Pascotto et al. 2003; Utsunomiya et al. 2005), the failure of cytokinesis occurred after telophase I or telophase II, but never in released microspores, or by invagination. For this accession of *B. humidicola*, the first cytokinesis occurred after telophase II, and the second cytokinesis, also programmed by the cell, occurred beyond that time. It is suggested that the genetic control for cytokinesis exists in these meiocytes and was activated, but it was not synchronous with karyokinesis.

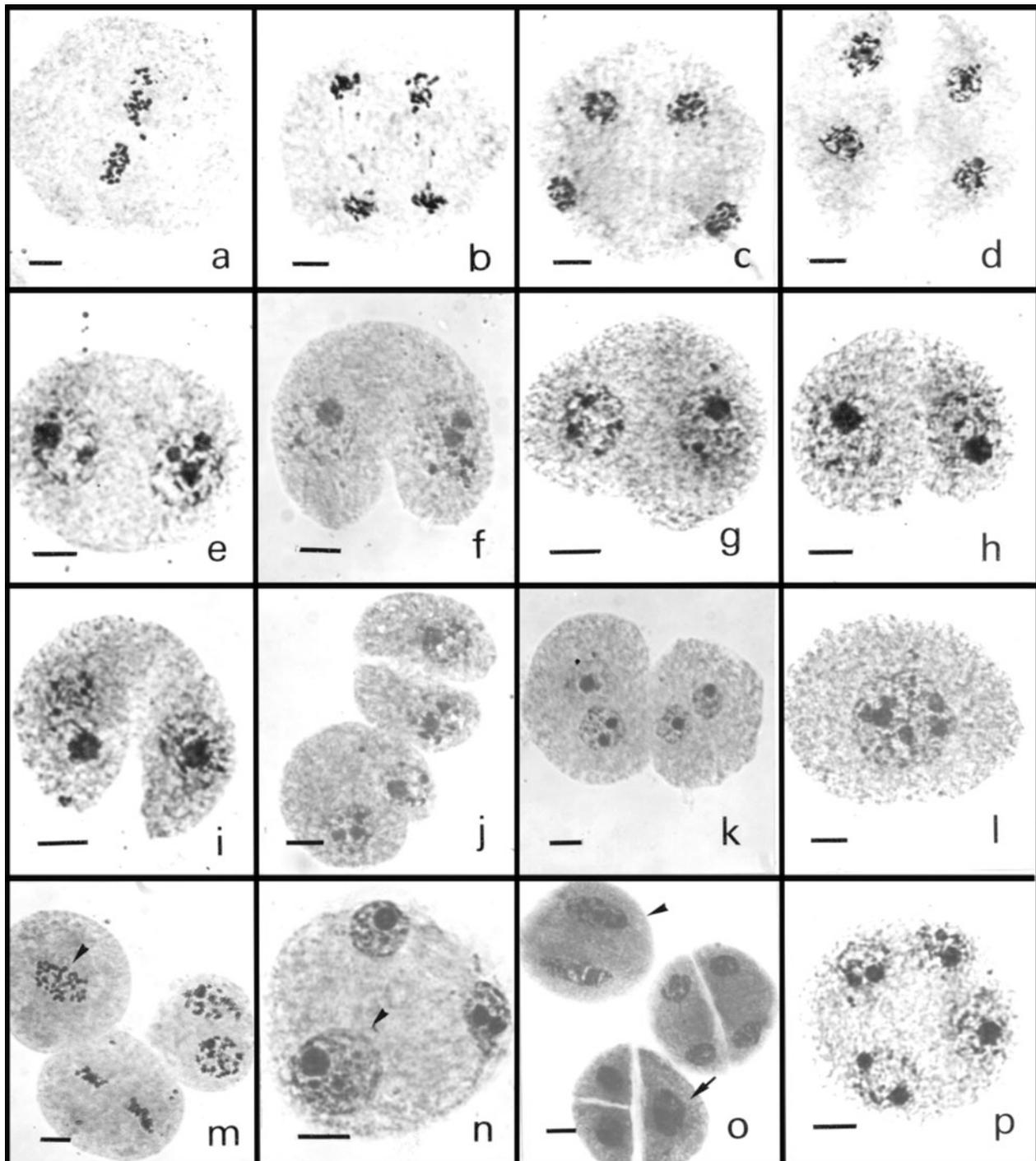
The absence of the first and/or the second cytokinesis in H003 also predisposes it to the occurrence of other abnormal-

ities in the second division. Meiocytes that underwent only one cytokinesis gave rise to binucleated dyads (figure 1,k). Some meiocytes that did not undergo the first cytokinesis regrouped the two chromosome sets (figure 1,m), originating a restitutional nucleus. Tripolar spindles in anaphase II were detected in a considerable number of meiocytes and gave rise again to restitutional nucleus in telophase II (figure 1,n). When these cells underwent cytokinesis, they gave rise to triads with one uninucleated ( $2n$ ) and two binucleated microspores (figure 1,o). However, if these cells did not undergo any cytokinesis, they originated trinucleated microspores with one  $2n$  nucleus. On the other hand, in those meiocytes that lack both cytokineses, a tetranucleated microspore was formed (figure 1,p). Among a large number of microspores analysed, more than 60% underwent some aspect of irregular cytokinesis, including late but programmed cytokinesis, and total absence of one or both cytokineses.

Diploid ( $2n$ ) microspores resulting from abnormal cytokinesis have been reported in *B. brizantha* (Risso-Pascotto et al. 2003) and *B. nigropedata* (Utsunomiya et al. 2005). Diploid ( $2n$ ) gametes might have contributed to the evolutionary history of the genus *Brachiaria*. In this genus, the majority of species are polyploid, mainly tetraploid (Valle and Savidan 1996; Penteado et al. 2000; Mendes-Bonato et al. 2002, 2006; Utsunomiya et al. 2005). The origin of polyploidy is not yet well known. However, evidence from conventional cytological studies points to the hypothesis that some polyploid accessions originated by autotetraploidy, segmental allotetraploidy (Mendes-Bonato et al. 2002, 2006; Risso-Pascotto et al. 2003; Utsunomiya et al. 2005) and, in some cases, by allopolyploidy (Mendes et al. 2006).

**Table 1.** Percentage of cells with abnormal cytokinesis in the accession H003 of *B. humidicola*.

Phase	No. of cells analysed/ No. of abnormal cells	Abnormalities (%)
Prophase II	104/91	Absence of cytokinesis 91 (87.5)
Metaphase II	274/164	Absence of cytokinesis 164 (58.8)
Anaphase II	184/132	Absence of cytokinesis 100 (54.3) Tripolar spindle 32 (17.8)
Telophase II	564/268	Absence of cytokinesis 184 (32.6) Restitutional nucleus 84 (14.9)
Meiotic products	450/438	Absence of cytokinesis 16 (3.6) Uninucleated dyad 10 (2.2) Binucleated dyad 373 (82.9) Triads 30 (6.8) Monads 9 (2.1)
Microspores	2234/1403	Binucleated 557 (24.9) Initial cytokinesis 222 (9.9) Advanced cytokinesis 607 (27.2) Trinucleated 10 (0.4) Tetranucleated 17 (0.8)



**Figure 1.** Aspects of abnormal cytokinesis in the accession H003 of *B. humidicola*. (a to c) Phases of meiosis II with absence of the first cytokinesis: metaphase II (a), anaphase II (b), and telophase II (c). (d) Telophase II presenting the first cytokinesis. (e) Binucleated microspore released from the dyad. (f to j) Progressive stages of the second cytokinesis by invagination in binucleated microspores. Observe that in j one microspore is initiating the cytokinesis whereas the other has just completed it. (k) Dyad with two binucleated microspores. (l) Mononucleated *n* microspore resulted from the second cytokinesis. (m) Meiocytes in the second division without the first cytokinesis (prophase II and metaphase II). Observe that in one meiocyte in metaphase II, the two chromosome sets were rejoined owing to absence of cytokinesis, resulting in a restitutional nucleus (arrowhead). (n) Trinucleated telophase II with a restitutional nucleus (arrowhead) resulting from tripolar spindle orientation. (o) Products of meiosis with different genetic constitution: a binucleated dyad, a triad with a restitutional nucleus in one microspore (arrow), and a future dyad with restitutional nucleus in both microspores (arrowhead). (p) Tetranucleated microspore that resulted from absence of both cytokineses. (Scale bar = 1  $\mu$ m)

According to Veilleux (1985) and Bretagnolle and Thompson (1995),  $2n$  gametes play an important role in evolution of higher plants, and even more so in grasses, where polyploidy is widely reported (de Wet 1986; Hunziker and Stebbins 1986).

Among *Brachiaria* species available at Embrapa Beef Cattle Research Center, the *B. humidicola* germplasm collection is represented by 60 accessions (Valle and Savidan 1996). Determination of ploidy level by flow cytometry showed that all of them are polyploid, with the ploidy level ranging from  $4n$  to  $7n$  (Penteado et al. 2000). Owing to their adaptation to poorly drained and infertile acid soils (Keller-Grein et al. 1996), such as those found in the Brazilian 'pantanal' region, some promising apomictic accessions of this species are under careful agronomic and grazing evaluation in the hope of selecting new cultivars. Other accessions, however, may be used in intraspecific or interspecific hybridization as pollen donors. In this genus, polyploidy is correlated with apomixis, but for seed development the secondary nuclei of the embryo sac need to be fertilized by a male gamete (pseudogamy). The occurrence of late cytokinesis, per se, did not affect pollen viability because the meiotic product, although lately formed, was characterized by four  $n$  microspores. However, despite some abnormalities due to polyploidy recorded in this accession during microsporogenesis, pollen fertility was high. Less than 5% of the tetrads presented micronuclei in microspores. Accessions with high frequency of meiotic abnormalities due to polyploidy which severely impair pollen viability need to be discarded in the breeding programme. This accession, despite high pollen fertility, might also be discarded because of its high level of ploidy and its basic chromosome number  $x = 6$ . The hybridization programme in the *Brachiaria* genus involves crosses only between tetraploid ( $2n = 4x = 36$ ) parents, generally derived from  $x = 9$ .

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Received 16 September 2005; in revised form 4 April 2006