RESEARCH ARTICLE

Desynapsis and precocious cytokinesis in *Brachiaria humidicola* (Poaceae) compromise meiotic division

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Abstract

The forage grass species *Brachiaria humidicola* is native to African savannas. Owing to its good adaptation to poorly drained and infertile acid soils, it has achieved wide utilization for pastures in Brazilian farms. Among the 55 accessions of *B. humidicola* analysed from the Embrapa Beef Cattle collection, one (H022), presented desynapsis and an abnormal pattern of cytokinesis in the first meiotic division. Among 28 inflorescences analysed in this accession, 12 were affected by the anomaly. In affected meiocytes, the first cytokinesis occurred in metaphase I and was generally perpendicular to a wide-metaphase plate, dividing the genome into two parts with an equal or unequal number of chromosomes. The normal cytokinesis after telophase I did not occur, and the meiocytes entered metaphase II, progressing to the end of meiosis with the occurrence of the second cytokinesis. As the first cytokinesis occurred precociously, whereas the second was normal, tetrads were formed but with unbalanced chromosome numbers in microspores. Abnormal cytokinesis occurred only in those meiocytes that underwent desynapsis after diakinesis. The implications of this abnormality in the *Brachiaria* breeding programme are discussed.

[Calisto V., Fuzinatto V. A., Message H. J., Mendes-Bonato A. B., Boldrini K. R., Pagliarini M. S. and Valle C. B. 2008 Desynapsis and precocious cytokinesis in *Brachiaria humidicola* (Poaceae) compromise meiotic division. *J. Genet.* **87**, 27–31]

Introduction

Meiosis is a specialized differentiation process that generates recombinant haploid gametes from a diploid zygote (Pankratz and Forsburg 2005). During meiosis, a single round of DNA replication is followed by two successive nuclear divisions characterizing meiosis I and II. Homologous chromosomes segregate at meiosis I, and sister chromatids separate at meiosis II (Tsubouchi and Roeder 2003). Although callose deposition during microsporogenesis always leads to the formation of four haploid microspores, the timing of cytokinesis varies among angiosperms. In general, monocotyledons undergo successive cytokinesis, in which cell plates are formed after both the first and the second meiotic divisions. In plants of this group there is a distinct dyad stage.

In higher plants, cytokinesis is a genetically controlled multistep process. At least three cellular components play important roles in its occurrence: (i) the golgi apparatus produces secretory vesicles and synthesizes the cell wall polysaccharides, (ii) golgi-derived vesicles fuse to form a cell plate, and (iii) the cytoskeleton required for phragmoplast formation and expansion controls the cell division planes. Other cellular components, including the endoplasmic reticulum, intermediate filaments and proteins such as calmodulin and myosin, may also play important roles in cytokinesis (Staelin and Hepler 1996).

Several mutants affected in cytokinesis have been reported in higher plants (Beadle 1932; Peirson *et al*. 1996; Hülskamp *et al*. 1997; Nickle and Meinke 1998; Caetano *et al*. 2001; Boldrini *et al*. 2006; Gallo *et al*. 2007). In this paper, we are reporting an original pattern of cytokinesis recorded in one accession of *Brachiaria humidicola*, an important forage-grass species for Brazilian beef production

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Keywords. *Brachiaria humidicola*; cytokinesis; desynapsis; meiosis; forage grass; breeding.

systems owing to its good adaptation to poorly drained and infertile acid soils (Keller-Grein *et al*. 1996), such as those found in swamps throughout the Brazilian savannas.

Materials and methods

Accessions of *B. humidicola* from the Embrapa Beef Cattle *Brachiaria* germplasm collection (Campo Grande, state of Mato Grosso do Sul, Brazil), collected in the wild African savannas in the mid 1980s by CIAT (Colombia), were cytologically analysed. Site characteristics of the plots in the field 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; pH4.2).

Inflorescences for meiosis studies were collected in plots with 16 plants 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. Photomicrographs were made with a Wild Leitz microscope using Kodak Imagelink – HQ, ISO 25 black and white film.

Results and discussion

The *B. humidicola* collection at Embrapa Beef Cattle is represented by 60 accessions. Fiftyfive were cytologically analysed. One accession (H022) presented an abnormal meiotic behaviour characterized by a distinct pattern of cytokinesis in the first meiotic division. Among 28 inflorescences analysed in this accession, 12 were affected by the anomaly. Among 2567 meiocytes analysed from among several slides encompassing the different stages of meiosis in the affected inflorescences, 16.82% presented abnormal cytokinesis. H022 is polyploid ($2n = 54$), as are some other accessions analysed in this *Brachiaria* species (Boldrini K. R. and Adamovski unpublished data). Although the basic chromosome number has not yet been correctly determined in this accession, there are some evidences for $x = 6$, as reported in *B. dictyoneura*, a species closely related to *B. humidicola* (Risso-Pascotto *et al*. 2006a).

Polyploidy has been largely reported in the genus, with a prevalence of tetraploids (Mendes-Bonato *et al*. 2002, 2006a; Utsunomiya *et al*. 2005; Risso-Pascotto *et al*. 2006b). *Brachiaria* polyploids are generally predisposed to meiotic abnormalities because of the occurrence of irregular chromosome segregation in both divisions. Precocious chromosome migration to the poles in metaphase and laggard chromosomes in anaphase, which lead to micronuclei formation in telophase, are the most common meiotic abnormalities found in polyploids. In H022, these abnormalities were also generally recorded among the analysed inflorescences (figure 1a–c), but in low frequency.

The differentiating characteristic in this accession was the pattern of cytokinesis. A precocious cytokinesis in metaphase I divided the meiocyte into two cells of equal or unequal sizes (figure 1,f–k). In the majority of meiocytes, the plane of cytokinesis was perpendicular to the metaphase plate separating the chromosome set into two parts with equal or unequal numbers of chromosomes (figure 1,f–h). In a few meiocytes, however, the plane of cytokinesis was parallel to the metaphase plate and divided the meiocyte into an anucleated cell and a cell containing the entire chromosome set (figure 1,j–l).

Those meiocytes that were prone to undergo precocious cytokinesis could be identified before the initiation of this process. In these, a wide-metaphase plate (figure 1,e) differentiated them from the normal ones (figure 1,a), where the metaphase plate was restricted to the centre of meiocytes. In the normal and affected meiocytes, diakinesis was characterized by many bivalents and many multivalents which remained as such till metaphase I in the normal ones. In the affected meiocytes, however, chromosome associations were disrupted at the end of the diakinesis, separating the chromosomes by desynapsis (figure 1,d). Thus, the 54 univalent chromosomes were aligned, forming a wide-metaphase plate.

Chromosome pairing is a genetically controlled phenomenon involving a series of major genes responsible for several events occurring in the synapsis process. Mutations in these genes have been widely reported in many plant species and most of them are controlled by single recessive genes (Baker *et al*. 1976; Golubovskaya 1979, 1989; Koduru and Rao 1981). Mutations affecting the synapsis process could be divided into 'asynapsis', a term that denotes lack of chromosome pairing during late prophase I, and 'desynapsis', which denotes the falling apart of the synapsed homologues owing to their inability to generate or retain chiasmata (Koduru and Rao 1981). In the present accession synapsis occurred forming bivalents and multivalents, but it was disrupted precociously separating the chromosomes into univalents. Although several putative meiotic mutations have been reported in the *Brachiaria* genus (Mendes-Bonato *et al*. 2001, 2003, 2004, 2006b; Risso-Pascotto *et al*. 2002, 2003a,b, 2005; Junqueira Filho *et al*. 2003; Mendes-Vieira *et al*. 2005; Boldrini *et al*. 2006; Gallo *et al*. 2007), synapsis mutants were never recorded.

Although there is no clear explanation for the relationship of desynapsis with cytokinesis, only those cells affected by desynapsis underwent precocious cytokinesis. During mitosis in higher plants, a cortical ring of microtubules, called 'the preprophase band', marks the site at which the cell plate will be formed, determining the division pattern (Staelin and Hepler 1996). On the other hand, lack of preprophase band in meiosis, and the division pattern is determined by 'spore domains'. Cytokinesis-defective mutants have been characterized in several species of higher plants (Beadle 1932; Peirson *et al*. 1996;

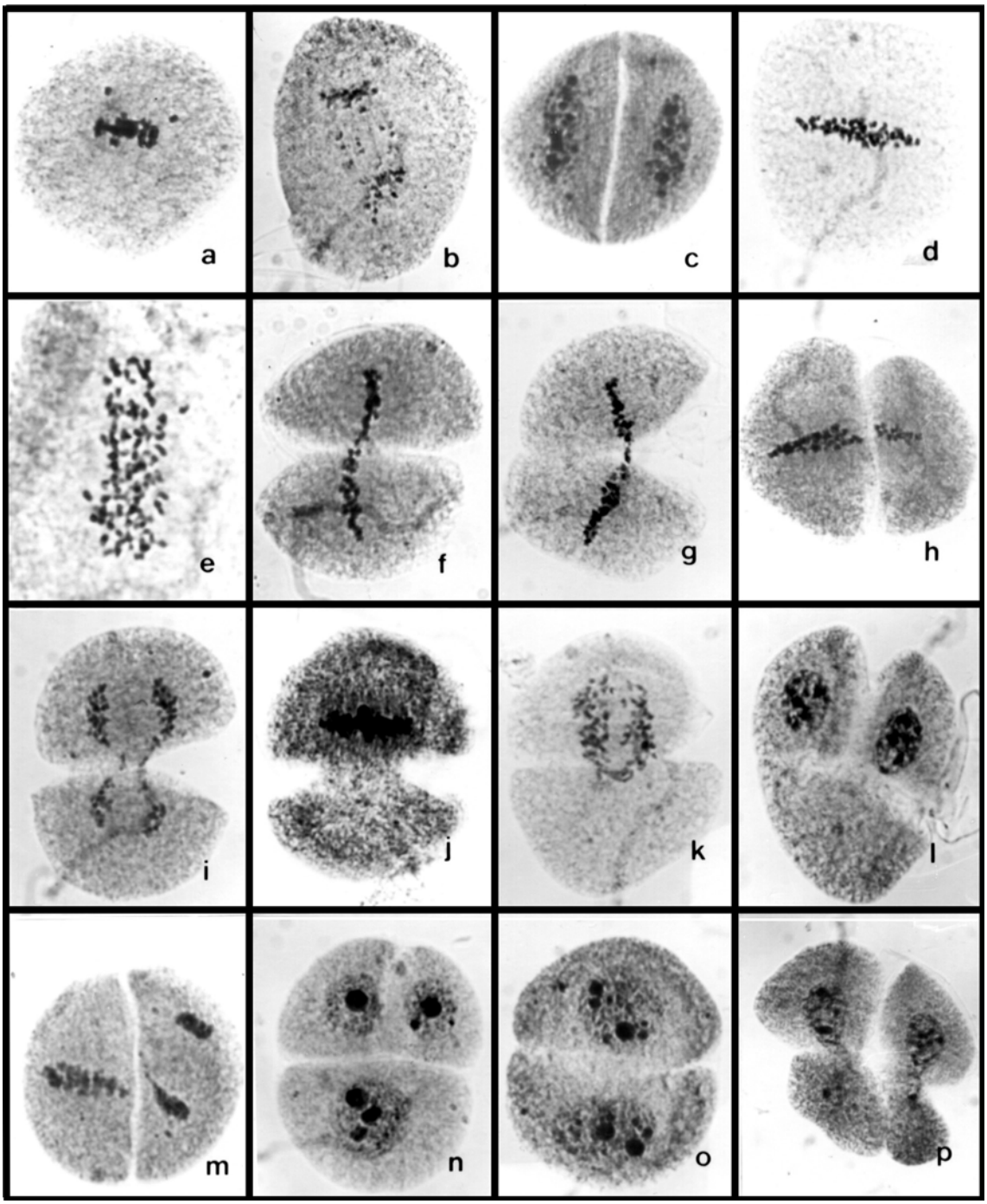

Figure 1. *a–c*, Normal meiocytes in metaphase I (a), anaphase I (b), and prophase II(c); observe the small and dense metaphase in a, and chromosome with precocious migration to the poles; laggards in b and small micronuclei in c. d, Precocious metaphase I with univalents migrating to the metaphase plate. e, Metaphase I in the affected meiocyte; compare the extension and density of metaphase plate in a and e. *f–h*, Precocious cytokinesis in metaphase I dividing the meiocytes into two cells with equal and unequal numbers of chromosomes. i, Anaphase II in meiocyte divided precociously. *j–l*, meiocytes in which the precocious cytokinesis separated one anucleate cell. m, Asynchrony in the second division. *n–p*, Triad (n), dyad (o) and tetrad (p) with abnormal cytokinesis.

Hülskamp *et al*. 1997; Nieckle and Meinke 1998; Caetano *et al*. 2001; Boldrini *et al*. 2006). Among these mutants, one reported in maize (Caetano *et al*. 2001) presented a similar pattern of precocious cytokinesis occurring in metaphase I. However, in that situation, the two resulting cells underwent cytokinesis after telophase I and telophase II, originat-

ing polyads as meiotic products. In H022, the precocious cytokinesis in metaphase I hindered the first cytokinesis after telophase I. These cells, with a typical aspect of metaphase II (figure 1,f–h), received the signal to enter the second division initiating the sister-chromatid segregation in anaphase II (figure 1,i, k), following to the end of meiosis (figure 1,l, m), and undergoing the second cytokinesis after telophase II (figure 1,p). As the meiocytes of H022 underwent only two cytokineses, the meiotic products ended up as regular tetrads. Abnormal cytokinesis was recently reported in another accession of *B. humidicola* (Boldrini *et al*. 2006) in which the first cytokinesis occurred after telophase II, generating dyads with binucleate microspores. These initiated the second cytokinesis by invagination after being released from the callose wall, giving rise to normal microspores.

After precocious cytokinesis, the meiocytes entered anaphase II, but in higher number, and the second meiosis was asynchronous in the two sister cells (figure,1 m). Generally, one cell was in metaphase II and the other in telophase II. This phenomenon allowed the formation of triads with two *n* microspores and one 2*n* microspore (figure,1 n). The second cytokinesis did not always occur. In those cells, when the precocious cytokinesis occurred, a dyad of unreduced microspores was formed (figure,1 o). In a total of 497 meiotic products analysed, 16.30% were triads and 10.66% dyads. Since the plane of the first precocious cytokinesis exceeded the metaphase plate containing univalents, meiocytes were divided into two cells with equal or unequal number of chromosomes, thus giving rise to cells unbalanced in their genetic content. In the genus *Brachiaria*, the majority of accessions and species are polyploid, mainly tetraploid (Valle and Savidan 1996; Mendes-Bonato *et al*. 2002, 2006a; Utsunomiya *et al*. 2005). Absence of cytokinesis generating balanced 2*n* gametes by different cytological mechanisms has been reported in *B. brizantha* (Risso-Pascotto *et al*. 2003a), and in *B. humidicola* (Gallo *et al*. 2007) These could have played an important role in the evolution of polyploidy in the genus. The mechanism reported in H022, on the other hand, fractionates the chromosome set and generates unbalanced gametes, leading to pollen abortion and sterility.

The cause of the abnormality reported here is not known. *Brachiaria* is a wild genus from African savannas that has been domesticated only a few years ago. The Brazilian breeding programme was initiated in the year 1980s. Cytological characterization of the myriad of accessions in a dozen species of the Embrapa Beef Cattle collection has shown many abnormalities in the meiotic (Mendes-Bonato *et al*. 2001, 2002, 2003, 2004, 2006a,b; Risso-Pascotto *et al*. 2002, 2003a,b, 2005, 2006b; Mendes-Vieira *et al*. 2005) and postmeiotic processes (Junqueira Filho *et al*. 2003; Mendes-Bonato *et al*. 2004), which could be caused by putative mutations. These abnormalities are recurrent among accessions of several *Brachiaria* species collected in different years and under different environmental conditions. The abnormality reported here, affecting chromosome pairing and cytokinesis, could also represent a putative mutation in the *Brachiaria* gene pool.

The cultivated pastural area covered by *Brachiaria* today exceeds 60 million hectares in Brazil and new cultivars are in great demand to diversify production. There are only two apomictic cultivars of *B. humidicola* available commercially in Brazil and Latin America. To impact cattle production in this region, large quantities of good seeds are needed over several years. In this genus, polyploidy is correlated with apomixis (apospory of *Panicum* type), but for seed development, the secondary nuclei of the embryo sac need to be fertilized by a male gamete—pseudogamy (Alves *et al*. 2001). Therefore, fertile viable pollen are a requirement for proper seed fill and a normal meiotic process is required to produce the hybrids as well as in the hybrids themselves or accessions released as cultivars. An understanding of the meiotic process is pivotal to further research on reproduction, fertility, genetics and breeding, and in plants may have significant implications for crop production (Armstrong and Jones 2003). Abnormalities such as one reported for H022 impaired the correct development of meiosis, affecting pollen development in almost 50% of the inflorescences analysed and seriously compromising its use as a genitor and potential as a new cultivar.

Acknowledgements

Authors are grateful to UNIPASTO for financial support.

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Received 31 July 2007, in revised form 17 September 2007; accepted 8 October 2007
Published on the Web: 5 February 2008