A Severe Case of Chromosome Stickiness in Pollen Mother Cells of *Brachiaria brizantha* (Hochst.) Stapf (Gramineae)

Andréa Beatriz Mendes-Bonato¹, Maria Suely Pagliarini¹,*, Cacilda Borges do Valle² and Maria Isabel de Oliveira Penteado³

¹ Department of Cell Biology and Genetics, State University of Maringá, 87020–900 Maringá-Paraná, Brazil
² Embrapa Beef Cattle, C.P. 154, 79002–970 Campo Grande-MS, Brazil
³ Embrapa, Secretary of Intellectual Property, Pq. Est. Biologica, 70770–901 Brasilia, DF, Brazil

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Summary Among 25 accessions of *Brachiaria brizantha* in the Embrapa Beef Cattle collection, one accession (GC 1113/95) presented a severe case of chromosome stickiness in meiosis, impairing normal chromosome segregation. Accession was tetraploid (2n = 4x = 36) with chromosomes pairing in bivalents and few quadrivalents at diakinesis. Stages of prophase I were normal and chromosome stickiness became evident from metaphase I persisting to microspore stage. Bridges of different thickness were formed in anaphase I and II by chromosomes that did not separate. Some of them even persisted until telophase stages. The precise causes of chromosome stickiness could not be ascertained, but genetic factors might be controlling the phenomenon, since only this accession cultivated on Brazilian savannas under the same environmental conditions of the 24 other accessions presented the abnormality.

Key words *Brachiaria brizantha*, Grasses, Chromosome stickiness, Microsporogenesis.

The genus *Brachiaria*, native of the East African savannas, includes several species of agronomic importance in natural and introduced pastures throughout the tropical lowland. These species are *B. mutica*, *B. decumbens*, *B. brizantha*, *B. humidicola* and *B. ruziensis*. The economic importance of *Brachiaria* grasses in Brazil is well established. Two species are being widely cultivated as pastures in Brazilian acid soil, *B. decumbens* cv. Basilisk and *B. brizantha* cv. Marandu. Cultivars were derived directly from naturally occurring germplasm. Since they behave as obligate apomicts in nature, they impair direct hybridization (Valle et al. 1993). Polyploidy is frequent in this genus and generally associated with apomixis. Most species and accessions are tetraploids (2n = 4x = 36).

The *Brachiaria* germplasm collection of the National Beef Cattle Research Center (Embrapa Beef Cattle), in Campo Grande MS Brazil, maintains about 500 accessions of 14 species collected in the African savannas. Cytological studies by Mendes-Bonato (2000) on 25 accessions of *Brachiaria brizantha* from this collection, aiming at determining chromosome number and meiotic behavior, showed a prevalence of polyploidy, mainly tetraploidy, and meiotic irregularities, generally those characteristic to polyploidy. However, one accession of *B. brizantha* (GC 113/95) presented a meiotic abnormality never reported in the *Brachiaria* genus. This paper reports the occurrence of chromosome stickiness in this accession, a chromosome-clumping phenomenon that impairs normal segregation.

* Corresponding author, e-mail: mspagliarini@uem.br
Materials and methods

Inflorescences in the ideal stage for meiotic study were collected, fixed in acetic ethanol (3 : 1) for 24 h and stored under refrigeration until use. Microsporocytes were prepared by squashing and staining with 0.5% propionic carmine. Chromosome number was determined in 5 plants per accession, in diakinesis or metaphase I. All meiotic phases were evaluated and abnormalities recorded. Photomicrographs were made with a Wild Leitz microscope using Kodak Imagelink-HG, ISO 25 black and white film.

Results and discussion

The accession GC 1113/95 of *B. brizantha* showed a tetraploid constitution with 2n=4x=36 chromosomes. Since bivalents and few quadrivalents were found in diakinesis, accession was characterized as a possible segmental allopolyploid, in which the parental genomes are only partially homologous. Meiotic abnormalities usually associated with polyploidy, such as irregular chromosome segregation in metaphase and anaphase of both meiotic divisions, leading to micronuclei formation in telophase and tetrads, were observed among microsporocytes. But the most uncommon and noticeable abnormality was the presence of chromosome stickiness not recorded in the other 24 accessions of *B. brizantha* analyzed.

In such accession, prophase I stages were normal (Fig. 1a–c) and chromosome stickiness became evident from metaphase I onwards. In this phase, most microsporocytes showed overly condensed chromosome clusters, forming dense sticky clumps of chromatin, in which the identity of the chromosomes was completely lost (Fig. 1d). As metaphase I proceeded to anaphase I, sticky mass gradually became relaxed and the chromosomes that did not separate originated bridges (Fig. 1e). Their size depended on the number of chromosomes evolved in the mass. In most cases, anaphase bridges persisted up to telophase I (Fig. 1f). When the bridges broke in this phase, they gave rise to micronuclei (Fig. 1g) that persisted in pycnotic form in later stages (Fig. 1h, i). In the second division, the stickiness also persisted (Fig. 1j, l) and the phenomenon was observed up to the microspore stage (Fig. 1m). However, chromatin degeneration was not found at the end of the process. The number of cells affected by chromosome stickiness varied among the phases. A total of 2484 microsporocytes were analyzed: in metaphase I, 42.03% of the cells were affected; in anaphase I, 69.48%; in telophase I, 48.02%; in metaphase II, 32.33%; in anaphase II, 47.77%; in telophase II, 59.15%; and in the tetrad stage, 35.52%.

Although chromosome stickiness is one of the phenomena in chromosome cytology that has been recorded for almost a century, adequate explanations are still lacking. This phenomenon was early identified by Koernicke (1905) and is characterized by intense chromosome clustering during any phase of the cell cycle. According to Rao et al. (1990), the general pattern of the sticky meiotic behavior in most species is somewhat similar. Chromosomes start to assemble at prophase I or metaphase I, frequently form congregations and sticky clumps which do not orientate themselves on the equatorial plate. They display irregular anaphase I disjunction or failure, while chromosome fragmentation occurs from prophase I onwards, resulting in male and female sterility.

Although many studies have reported the occurrence of chromosome stickiness, the primary cause and the biochemical basis of this abnormality are still unknown. Determination of DNA/histone ratio (Himes 1950) and analysis of histone fractions (Stout and Phillips 1973) failed to reveal any differences between normal and sticky plants in *Zea mays*. A few hypotheses have been put forth in an attempt to explain the causes of chromosome stickiness (Takegami and Ito 1982, Gaulden 1987, Al Achkar et al. 1989, Liu et al. 1992). Gaulden (1987) hypothesized that chromosome stickiness resulted from deficient or defective functioning of one or of 2 types of non-histone chromosomal proteins, DNA topoisomerase II and the peripheral proteins. It might also be caused
Fig. 1. a–c) Normal microsporocytes in prophase I: zygotene (a), pachytene (b) and diakinesis (c). d) Metaphase I/anaphase I showing severe chromosome stickiness. e) Anaphase I with a thin bridge caused by stickiness. f) Telophase I with chromosomes in an amorphous mass clumped by a bridge. g) Telophase I with several fragments resulting from breakage. Note the stickiness aspect (arrowhead). h, i) Metaphases II with one (h) and several (i) pycnotic micronuclei (arrowheads). j, l) Anaphases II showing severe chromosome stickiness. m) Microspores with pycnotic nuclei (arrowhead) and nuclei in dumbbell and bar-bell forms (arrows) resulting from stickiness.
by either a mutation in the gene(s) coding for a protein (hereditary stickiness) or by an abnormal product resulting from the interaction of a mutagen with functional protein (induced stickiness).

The phenotypic manifestation of stickiness may be highly variable, ranging from a mild phenomenon involving a few chromosomes in the genome, to a wide one involving the entire chromosome complement. In cases of moderate stickiness several chromosomes in an anaphase cell may indicate numerous adhering regions, producing the classic configuration of this abnormality. However, in severe stickiness, there is a fusion of all chromosomes into a single amorphous mass distributed between the 2 poles. In this case, stickiness is generally associated with chromosome breakage and the acentric fragments of different sizes remain in the cytoplasm (Dowd et al. 1986). In the accession of *B. brizantha* affected, the phenomenon ranged from moderated to severe (Fig. 1) and also gave rise to many fragments. In extreme cases of stickiness, the impossibility of chromosome segregation provokes the formation of single or varying numbers of pycnotic nuclei that culminate in full chromatin degeneration. In *B. brizantha* although many small pycnotic micronuclei have been observed in second division and sometimes in microspores, chromatin degeneration was not reported. A factor that would undoubtedly contribute to the variability in the degree of stickiness is, according to Gaulden (1987), the number of target protein molecules (topoisomerase II) affected by their inhibitors. Thus, not all cells are equally affected by the phenomenon.

Chromosome stickiness may be caused by genetic or environmental factors. Genetically controlled stickiness was first reported in maize by Beadle (1932), but clear cases of genetically controlled meiotic chromosome stickiness are few. Chromosome stickiness was controlled by a single recessive gene in *Zea mays* (Beadle 1932, Golubovskaya 1989), *Triticum durum* (Martini and Bozzini 1965), *Collinsia tinctoria* (Mehra and Rai 1970), and pearl millet (Rao et al. 1990), by duplicate recessive genes in *Alopecurus myosuroides* (Johnsson 1944), by 2 or 3 recessive interacting genes in *Carthamus tinctorius* (Carapetian and Rupert 1977) and by an apparently dominant gene in *Lycopersicum esculentum* (Rao and Rao 1977). Among environmental factors, X-rays (Steffensen 1956), gamma-rays (Rao and Rao 1977, Al Achkar et al. 1989), temperature (Eriksson 1968), herbicides (Badr and Ibrahim 1987), chemicals (Dowd et al. 1986) and soil elements (Levan 1945, Steffensen 1955, Zanella et al. 1991, Caetano-Pereira et al. 1995) have been reported as responsible for chromosome stickiness. Taking into account that all 25 accessions of *B. brizantha* of the collection analyzed were cultivated under the same environmental conditions in the Brazilian savannas and that only one presented chromosome stickiness, we suggest that the phenomenon is under genetic control in this accession.

Pollen grains produced by cells with meiotic stickiness are generally nonviable for fertilization because they are unbalanced through irregular chromosome segregation and chromosome fragmentation, as found in maize (Golubovskaya 1989) and pearl millet (Rao et al. 1990). Unfortunately pollen viability could not be evaluated in the present accession because flower buds were fixed in a solution that did not permit pollen stainability in *Brachiaria*. Nevertheless, based on the extreme chromosome stickiness found in both meiotic divisions, that persisted until microspore stage, we believe that this phenomenon has certainly affected pollen viability. Pollen sterility is normally not a problem for polyploid accessions of *Brachiaria* because they are mainly reproduce by apomixis.

References


