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Short communication

# A differential phenotypic expression of a divergent spindle mutation in interspecific *Brachiaria* hybrids

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**Abstract**

Several mutations are known to alter the normal progression of meiosis and can be correlated with defects in microtubule distribution. The *dv* mutation affects the spindle organization and chromosomes do not converge into focused poles. Two *Brachiaria* hybrids presented the phenotypic expressions of *dv* mutation but exhibited many more details in the second division. Bivalents were distantly positioned and spread over a large metaphase plate and failed to converge into focused poles. Depending on the distance of chromosomes at the poles, telophase I nuclei were elongated or the chromosomes were grouped into various micronuclei of different sizes in each cell. The first cytokinesis occurred. However, when there were micronuclei, a second cytokinesis immediately took place dividing the prophase II meiocytes into three or four cells. In each meiocyte, meiosis progressed to the second division. Slightly elongated nuclei or micronuclei were recorded in telophase II. After a third cytokinesis, hexads or octads were formed. Pollen grains of different sizes were generated. One of these hybrids presented a higher frequency of abnormal cells than when previously analyzed. The fate of these hybrids as genitors or as candidates for cultivars in the *Brachiaria* breeding program is discussed.


*Keywords:* *Brachiaria*; Breeding program; Forage grass; Interspecific hybrids; Meiotic mutation; Divergent spindle

## 1. Introduction

Meiosis is a crucial, highly conserved stage occurring during the sexual reproduction of all eukaryotes. A single round of DNA replication is combined with two successive divisions to form four haploid products that allow the process of reassortment and segregation of genetic information (Wilson and Yang, 2004), a process accurately controlled by a large number of stage-specific genes (Gottschalk and Kaul, 1974, 1980a,b; Baker et al., 1976; Golubovskaya, 1979, 1989).

Chromosome segregation is mediated by a complex superstructure of proteins – the spindle. The forces required for spindle assembly and movements have been attributed to microtubule dynamics (Endow, 1999). Microtubules are formed by polymeric self-organization of tubulin, which is initiated at microtubule organizing centers. Microtubules organizing centers, comparable to centrosomes in animals, are not known in plants (Binarová et al., 2000). In plant meiocytes, microtubules initially appear around prometaphase chromosomes, indicating a chromatin mediated spindle assembly mechanism (Chan and Cande, 1998).

In higher plants, meiotic mutations disrupting the structure and function of the division spindle have been reported (Clark, 1940; Golubovskaya, 1979, 1989; Golubovskaya and Mashnenkov, 1981; Staiger and Cande, 1990, 1991; Golubovskaya et al., 1992; Taschetto and Pagliarini, 1993; Pagliarini et al., 1998; Shamina et al., 2000; Shamina, 2005). The more frequent mutation affecting spindle structure is the *divergent spindle* (*dv*), reported in maize (Clark, 1940; Golubovskaya, 1979, 1989; Golubovskaya and Mashnenkov, 1981; Staiger and Cande, 1990, 1991; Golubovskaya et al., 1992; Shamina et al., 2000; Shamina, 2005), where spindle fibers do not converge to focused poles. This mutation was originally

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described by Clark (1940). Recently, Mendes-Bonato et al. (2006) reported the occurrence of a putative *dv* mutation in an interspecific *Brachiaria* hybrid.

In all descriptions of the *dv* mutation, the reports are centered in the first meiotic division because of the size of the cell. In the second division, the phenotypic expression of *dv* is difficult to define in the two sister-cells of monocotyledoneous species. A cytological re-analysis of the cited *Brachiaria* hybrid collected in another year, and the analysis of a half-sib hybrid of it, revealed a clear phenotypic expression of this putative *dv* mutation, allowing a better understanding of facts occurring in the second meiotic division and of its end products.

## 2. Material and methods

Cytological studies were carried out on two interspecific half-sib hybrids (HBGC306 and HBGC348) between *Brachiaria ruziziensis* and *Brachiaria brizantha*. The original female genitors in these hybrids were two artificially tetraploidized sexual accessions of *B. ruziziensis* (R41 and R44: $2n = 4x = 36$), which were crossed to a natural apomictic genotype of *B. brizantha* cv. Marandu (B140) ($2n = 4x = 36$). These two hybrids are related through the male genitor and were produced by artificial pollination in the greenhouse at Embrapa Beef Cattle Center (Campo Grande, State of Mato Grosso do Sul, Brazil) in 1988. Hybrid HBGC306 is sexual and HBGC348 is apomictic as determined by embryological analysis of embryo-sac structure using interference contrast microscopy on methylsalicilate-cleared ovaries (Young et al., 1979). These hybrids have excellent phenotypes from the forage standpoint and are under small plot agronomical evaluation.

Inflorescences for meiotic studies were collected from individual plants under free growth in the field and fixed in a mixture of ethanol 95%, chloroform and propionic acid (6:3:2 v/v) during 24 h and stored under refrigeration until use. Microsporocytes were prepared by squashing and stained with 0.5% propionic carmine. More than 2300 pollen mother cells (PMCs) were analyzed in each hybrid. Cells were photographed with Kodak Imagelink − HQ, ISO 25 black and white film.

## 3. Results and discussion

Fig. 1 illustrates the meiotic behavior of spindle fibers in normal meiocytes, with chromosome: (i) aligning in a narrow metaphase plate in the center of the cell in metaphase I (Fig. 1a) and metaphase II (Fig. 1e); (ii) ascending towards the poles by spindle fibers convergence in anaphase I (Fig. 1b) and anaphase

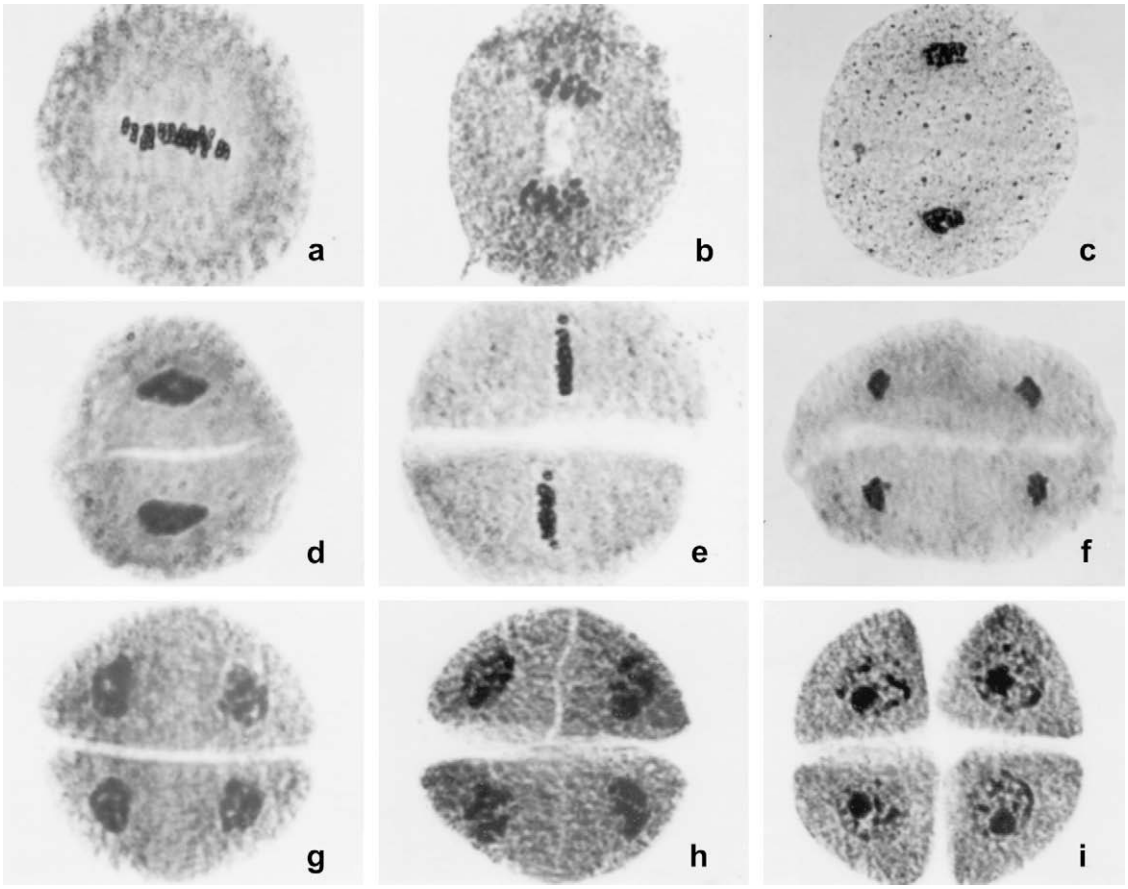


Fig. 1. Aspects of normal meiosis in *Brachiaria*. Meiocytes in metaphase I (a) and metaphase II (e) with a narrow metaphase plate. Anaphase I (b) and anaphase II (f) showing chromosome convergence to the poles. Telophase I (c), prophase II (d) and telophase II (g, h) with spherical nuclei. Tetrad of microspores (i) (Magnification: 400×).