

## Meiotic arrest compromises pollen fertility in an interspecific hybrid between *Brachiaria ruziziensis* x *Brachiaria decumbens* (Gramineae)

M.S. Pagliarini<sup>1</sup>, C.B. do Valle<sup>2</sup>, A.B. Mendes-Bonato<sup>1</sup> and C. Risso-Pascotto<sup>1</sup>

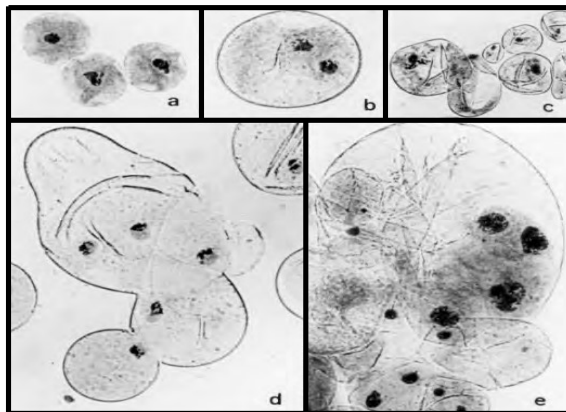
<sup>1</sup>Department of Cell Biology and Genetics, State University of Maringá, 87020-900 Maringá PR Brazil, Email: mspagliarini@uem.br, <sup>2</sup>Embrapa Beef Cattle, C.P. 154, Campo Grande MS 79002-970, Brazil

**Keywords:** cell fusion, meiosis arrest, pollen sterility, syncytes

**Introduction** Disruptions in meiosis, development of the free microspores, microspore mitosis, pollen differentiation or anthesis can result in male-sterile plants (Glover *et al.*, 1998). An understanding of the meiotic process is pivotal to work on reproduction, fertility, genetics and breeding in plants, with serious implications in crop production (Armstrong & Jones, 2003). Some African species of *Brachiaria* are the most important for pastures in the American tropics due to good adaptation and production. Artificial hybridization is underway in Embrapa to improve production, quality and insect resistance (Valle & Miles, 2001). For a cultivar to be successfully adopted good seed production and pollen viability are required. This paper reports on meiotic abnormalities impairing pollen fertility in a hybrid between *B. ruziziensis* x *B. decumbens*.

**Materials and methods** Cytological studies were carried out on an interspecific hybrid between an artificial 4x sexual accession of *B. ruziziensis* ( $2n=4x=36$ ) and the most common cultivar of *B. decumbens*, a natural apomict ( $2n=4x=36$ ). Inflorescences for microsporogenesis studies were collected and fixed in a mixture of ethanol 95%, chloroform and propionic acid (6:3:2 v/v) over 24 hours. Microsporocytes were prepared by squashing and staining with 0.5% propionic carmine. Pollen fertility was estimated using Alexander reactive (Alexander, 1969).

**Results** Syncytes involving a large number of cells were recorded in 15.40% of meiocytes. Meiosis was arrested in metaphase I and pycnotic nuclei and micronuclei were formed. Abnormal cytokinesis fractionated the syncyte into abnormal meiotic products that were covered by the pollen wall. Meiocytes in leptotene were recorded during both meiotic divisions and abnormal “pollen grains” with well-developed pollen walls but containing leptotene nuclei were recorded in 9.18% of grains analyzed. These findings suggest that the meiocytes received the signal to enter meiosis but lacked the signal to proceed beyond leptotene. Despite the absence of the meiotic process, such cells were covered by pollen grain walls. Total pollen sterility resulted from these abnormalities.



**Figure 1** Abnormal “pollen grains”. a) “Pollen grains” with leptotene nucleus. b) Binucleate “pollen grain” with leptotene nuclei. c) Abnormal pollen grains of different sizes and content of chromatin. d) Anomalous “pollen grain” with five leptotene nuclei. e) Anomalous pollen grain with pycnotic nuclei.

**Conclusions** Pollen sterility as a result of disrupted chromosome behavior was observed and is the single most important hindrance to the interspecific approach to *Brachiaria* breeding. Hybrids such as this, with these types of problems need to be identified and discarded early to avoid contributing defective genes to the breeding population.

### References

- Alexander M.P. (1969). Differential stain of aborted and non aborted pollen. *Stain Technology*, 44, 117-122.
- Armstrong S.J. & G.H. Jones (2003). Meiotic cytology and chromosome behavior in wild-type *Arabidopsis thaliana*. *Journal of Experimental Botany*, 54, 1-10.
- Glover J., M. Grelon, S. Craig, A. Chaudhury, & E. Dennis (1998). Cloning and characterization of *MS5* from *Arabidopsis*: a gene critical in male meiosis. *Plant Journal*, 15, 345-356.
- Valle, C.B., & J.W. Miles (2001). Breeding of apomictic species. In: Savidan, Y.H., Carman, J.C. & Dresselhaus, T. (eds.) The flowering of apomixis: from mechanisms to genetic engineering. CYMMIT, IRD, European Commission, Mexico City, 137-152.