# Meiotic behavior in full and half-sib hybrids between *Brachiaria ruziziensis* and *B. brizantha* (Poaceae)

Mariana Ferrari Felismino[1], Maria Suely Pagliarini[2] e Cacilda Borges do Valle[3]

## Introduction

The main objective of the *Brachiaria* program in tropical America is to use the sexuality of the tetraploid *B. ruziziensis* (ruzigrass) to release the genetic diversity locked in the natural tetraploid apomictics such as *B. brizantha* (palisadegrass), and *B. decumbens* (signalgrass), to produce novel apomictic hybrid cultivars [1, 2]. In 1988, the Embrapa Beef Cattle Center initiated an extensive program based on interspecific hybridization with the objective of determining the inheritance of apomixis and thus manipulating this character for the development of new improved hybrids. Hundreds of hybrids were obtained from the crosses and 88 selected based on leafiness and regrowth ability after cuts are under agronomic evaluation. However, besides resistance to spittlebugs and adaptation to infertile soils, a new cultivar needs to have a high seed production to attend the internal market demand to renovate pastures and also for export.

Cultivars available commercially are direct selections from the natural genetic variability [2], except for one interspecific hybrid (cv. 'Mulato'), which produces less than 10% of viable seeds, thus compromising the commercial interests of the seed industry and impairing wide adoption. Among several factors that could compromise seed viability, Hopkinson *et al*. [3] cite interspecific hybridization *per se*, and the polyploid nature of genotypes which impair seed viability by affecting pollen fertility. Previous cytogenetical analysis performed on some *Brachiaria* hybrids [4] revealed different types and varying frequencies of meiotic abnormalities, thus indicating possibilities of selection of genotypes for higher pollen viability and, probably, higher seed production. This paper describes the meiotic behavior of four promising hybrids between *B. ruziziensis* and *B. brizantha*, and based on types and frequencies of abnormalities, objectives to indicate which are more stable to ensure greater pollen fertility and success in hybridizations.

## Material and methods

Cytological studies were carried out on four interspecific hybrids between *B. ruziziensis* and *B. brizantha*. Two female genitors were utilized: both were two artificially tetraploidized sexual accessions of *B. ruziziensis* (R41 and R44: 2n = 4x = 36), which were crossed to a natural apomictic genotype: *B. brizantha* (B140), (2n = 4x = 36). All four hybrids are related through the male genitor: B140. Hybrids HBGC313 and HBGC315 are full-sibs; hybrids HBGC324 and HBGC325 are also full-sibs, and half-sibs to the two previous ones. 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 hours 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. Images were photographed with Kodak Imagelink – HQ, ISO 25 black and white film.

## Results and discussion

Analysis of diakinesis configurations in the four hybrids revealed multivalent chromosome configurations ranging from one to three quadrivalents in some meiocytes. In the majority of meiocytes, however, chromosome paired as 18 bivalents of the same genome. Chromosome pairing in hybrids is used as a method of assessing genomic relationship between species [5] and also provides an important starting point in alien introgression programs [6].

The degree of differentiation between hybridizing taxa can be estimated not only by analysis of chromosome pairing, but also by analyzing meiotic abnormalities [7]. Despite the phylogenetic proximity between *B. ruziziensis* and *B. brizantha* [8], the four hybrids presented different frequencies and types of meiotic abnormalities Although some abnormalities were common among hybrids, others were genotype-specific. Irregular chromosome segregation was recorded among the four hybrids in different frequencies.

Other meiotic abnormalities found in some of these hybrids, such as chromosome stickiness, abnormal cytokinesis, and absence of cytokinesis were also recorded in other *Brachiaria* hybrids [4] and have also been widely reported among accessions of different species of *Brachiaria*, suggesting that the genes controlling the character are in the gene pool of the genus. One

1. Department of Cell Biology and Genetics, State University of Maringá, 87020-900 Maringá PR, Brazil. E-mail: mariferrari_82@hotmail.com
2. Department of Cell Biology and Genetics, State University of Maringá, 87020-900 Maringá PR, Brazil. E-mail: mspagliarini@uem.br
3. Embrapa Beef Cattle, P.O. Box 154, 79002-970 Campo Grande MS, Brazil. E-mail: cacilda@cnpgc.embrapa.br
Apoio financeiro: UNIPASTO.

abnormality, however, was recorded for the first time in two of the present hybrids (HBGC313 and HBGC325). This abnormality had been widely reported in maize and was called *divergent spindle* (*dv*). Hybrids HBGC313 and HBGC325 were affected by this putative mutation. This abnormality affects the orientation of spindle fibers. The percentage of cells affected by *dv* varied between the two hybrids; HBGC325 was much more affected than HBGC313 The abnormalities caused by this putative gene (*dv*) added to irregular chromosome segregation due to polyploidy, always present in *Brachiaria* hybrids, is enough to discard a hybrid from the breeding program. In HBGC313, the percentage of abnormal meiocytes was 55.6%, while in HBGC325 was 46.1%. Hybrid HBGC315, also showed a high percentage of abnormalities (54.6%), although characterized only by irregular chromosome segregation. The more stable hybrid among the four analyzed was HBGC324, with 18.2% of abnormal cells and that reproduces sexually. Among the four selected promising hybrids, only one (HBGC325) is apomictic.

At the moment, the *Brachiaria* breeding program aims at producing apomictic hybrids since whatever traits are selected will breed true, i.e., will not segregate, which contributes to establishing homogeneous improved pastures. However, superior sexual hybrids need to continue in the program, acting as female genitors in polycross blocks in order to pyramidize desirable alleles, introgress desirable genes and also to broaden the genetic base of the program. Among the four hybrids analyzed, the sexual HBGC313 and the apomictic HBGC325 should be discarded from the program because of the high frequency of the putative mutation *dv* which seriously compromises pollen fertility. From the two remaining sexual hybrids, only HBGC324 could continue in the program because it has shown high meiotic stability. As a matter of fact, this hybrid is the most stable from the material already analyzed in the Embrapa Beef Cattle breeding program.

Hybrids must produce a good amount of viable seeds, besides good overall dry matter production and nutritive value, in order to be widely utilized and adopted in production systems. Due to pseudogamy, the desirable superior apomictic hybrids need viable pollen grains to fertilize the secondary nucleus of the embryo sac and thus ensure normal and vigorous endosperm development and plenty of seed set.

## Acknowlegements

Authors would like to thank UNIPASTO for financial support.

## References

[1] VALLE, C. B. do & SAVIDAN, Y. H. 1996. Genetics, cytogenetics, and reproductive biology of *Brachiaria*. In: MILES, J. W.; MAASS, B. L.; VALLE, C. B. do (Eds.). *Brachiaria*: Biology, Agronomy, and Improvement. CIAT/Embrapa, Colômbia/Brasília. p. 147-163.

[2] MILES, J. W.; VALLE, C. B. do; RAO, I. M.; EUCLIDES, V. P. H. 2004. Brachiariagrasses. In: MOSER, L. E.; BURSON, B. L.; SOLLENBERGER, L. E. (Eds.). Warm-season (C4) Grasses. ASA, CSSA, SSSA, Madison. p. 745-760.

[3] HOPKINSON, J. M.; SOUZA, F. H. D.; DIULGHEROFF, S.; ORTIZ, A.; SANCHES, M. 1996. Reproductive physiology, seed production, and seed quality of *Brachiaria*. In: MILES, J. W.; MAASS, B. L.; VALLE, C. B. do (Eds.). *Brachiaria*: Biology, Agronomy, and Improvement. CIAT/Embrapa, Colômbia/Brasília. p. 124-140.

[4] RISSO-PASCOTTO, C.; PAGLIARINI, M. S.; VALLE, C. B. do. 2005. Meiotic behavior in interspecific hybrids between *Brachiaria ruziziensis* and *Brachiaria brizantha* (Poaceae). Euphytica. n. 145, p. 155-159.

[5] ALONSO, L. C. & KIMBER, G. 1981. The analysis of meiosis in hybrids. II. Triploid hybrids. Can J Genet Cytol. n. 23, p. 221-234.

[6] GALE, M. D. & MILLER, T. E. 1987. The introduction of alien genetic variation into wheat. In: LUPTON, F. G. H. (Ed.). Wheat breeding: its scientific basis. Chapman & Hall, London. p. 173-210.

[7] RIESEBERG, L. H.; BAIRD, S. J. E.; GARDNER, K. A. 2000. Hybridization, introgression, and linkage evolution. Plant Mol Biol. n. 42, p. 205-224.

[8] RENVOIZE, S. A.; CLAYTON, W. D; KABUYE, C. H. S. 1996. Morphology, taxonomy, and natural distribution of *Brachiaria* (Trin.) Griseb. In: MILES, J. W.; MAASS, B. L.; VALLE, C. B. do (Eds.). *Brachiaria*: Biology, Agronomy, and Improvement. CIAT/Embrapa, Colômbia/Brasília. p. 1-15.