Analysis of genomic affinity between *Brachiaria ruziziensis* and *B. brizantha* through meiotic behaviour

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Introduction Genetic divergence between polyploid hybrids is displayed in chromosome pairing and in the rate of chromosome elimination due to differences in cell cycle between the two combined genomes (Sundberg *et al*. 1991). In *Brachiaria*, a genus of African grasses reaching continental proportions as a tropical pasture in Latin America, genome analysis has never been performed. The majority of accessions in this genus is polyploid and apomictic, which restricts breeding. The relative ease of obtaining fertile interspecific hybrids once ploidy barriers are overcome (Pereira *et al.* 2001) confirms the phylogenetic proximity among *B. ruziziensis, B. decumbens* and *B. brizantha*. Hybrids were synthesised using sexual artificial 4x as the female genitor and natural apomictic 4x as the pollen donors. Genome affinity is a pre-requisite for chosen genitors to produce fertile hybrids and plenty of viable seed to assure adoption of the new cultivar. Microsporogenesis of a hybrid between *B. ruziziensis* and *B. brizantha* is described in this paper, focusing on the behaviour of both genomes.

Materials and methods Cytogenetic studies were done on an interspecific hybrid, where the male genitor was *B. brizantha* (B genome) and the female an artificially tetraploidised sexual accession of *B. ruziziensis* (R genome). Inflorescences for meiotic studies were fixed in a mixture of ethanol 95%, chloroform, and propionic acid (6:3:2 v/v) during 24 hrs. Microsporocytes (PMCs) were squashed and stained with 0.5% propionic carmine. Over 1800 microsporocytes were analysed. Chromosome associations were evaluated at diakinesis. Images were photographed with Kodak Imagelink – HQ, ISO 25 black and white film.

Results Chromosomes associated predominantly as bivalents, equally distributed in two metaphase plates. In 70% of PMCs, one genome did not divide synchronically, with chromosomes lagging behind or not segregating at all. The second division was very irregular, resulting in polyads. Based on previous results from analysis of a triploid hybrid between these species where the R genome was eliminated by asynchrony during meiosis (Risso-Pascotto *et al*., 2004), it is suggested that the laggard genome in this hybrid also belongs to *B. ruziziensis*.

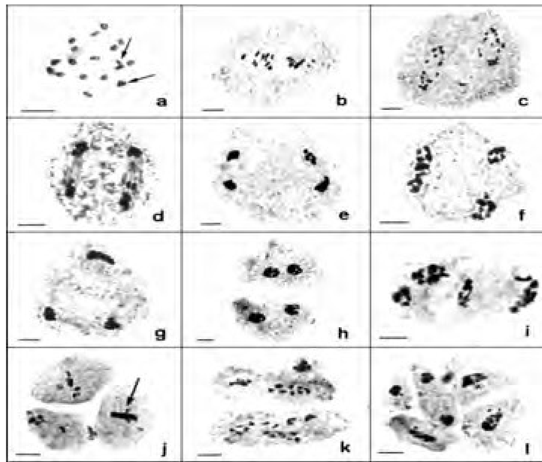

Figure 1 Chromosome behaviour in microsporogenesis: (a) DI with 17II and 2I (arrows) (b) MI with B and R genomes arranged in two metaphase plates (c, d) AI with 2 distinct spindles. In c, the 9 chromosomes are migrating to the poles (e) TI with both genomes properly segregated. (f, g) Trinucleate TI with only one segregated genome; the other remained non-segregated. (h, i) Early and late PII with normal genome segregation. (j) MII where only one genome underwent chromosome segregation at first division. Arrow indicates the cell with the non-segregated genome. Cytokinesis in the other cell yielded a triad in the second division. (k) Irregular chromosome distribution in AII. (l) Polyad with differently sized nuclei and microspores. (Bars = 1 μm).

Conclusions Abnormalities detected in this interspecific hybrid compromise pollen fertility. The eliminated genome is probably of the sexual parent *B. ruziziensis.* Cytological analyses are essential as a screening tool in this breeding program, if viable interspecific hybrids are to be selected and advanced to cultivar status.

References

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