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Meiotic behavior in *Panicum maximum* Jacq. (Poaceae: Panicoideae: Paniceae): hybrids and their genitors

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ABSTRACT. *Panicum maximum* Jacq. is one of the most cultivated grasses in the world. The intraspecific hybridization breeding program underway at Embrapa Beef Cattle aims at combining several agronomic characteristics in a single plant with high seed production. Four intraspecific hybrids originated from the cross between sexual tetraploid female plants with apomictic tetraploid male plants, including the genitors, were cytologically evaluated in the meiotic process throughout conventional methodology. Hybrids and genitors showed high meiotic stability. The percentage of abnormal cells among them ranged from 6.7 to 14.2%. Abnormalities recorded were irregular chromosome segregation, chromosome stickiness, and absence of the first cytokinesis, but the latter did not compromise pollen viability. Multivalent chromosome configurations at diakinesis, ranging from one to three quadrivalents, suggest that genetic recombination and introgression of some genes can be expected in the hybrids. Based on meiotic stability, the hybrids should be selected for high seed production, and the genitors could remain in the program participating in other intraspecific crosses.

Key words: breeding program, meiosis, intraspecific hybrids, *Panicum maximum*.

RESUMO. Comportamento meiótico em *Panicum maximum* Jacq. (Poaceae: Panicoideae: Paniceae): híbridos e seus progenitores. *Panicum maximum* Jacq. é uma das gramíneas mais cultivadas no mundo. O programa de hibridização intraespecífica, desenvolvido pela Embrapa Gado de Corte objetiva combinar diversas características agronômicas em uma única planta com alta produção de sementes. Quatro híbridos intraespecíficos, originados a partir do cruzamento entre fêmeas sexuais tetraploidizadas artificialmente com plantas apomíticas, incluindo os respectivos genitores, foram citologicamente avaliados em seu processo meiótico por meio de metodologia convencional. Híbridos e genitores apresentaram alta estabilidade meiótica, e a percentagem de anormalidades meióticas entre eles variou de 6,7 a 14,2%. As anormalidades encontradas foram segregação irregular de cromossomos, aderências e ausência de citocinese, mas esta última não comprometeu a viabilidade do pólen. Configurações cromossômicas em multivalentes na diacinese, que variam de um a quatro quadrivalentes, sugerem que a recombinação e a introgressão de alguns genes pode ser esperada em híbridos. Com base na estabilidade meiótica observada, os híbridos poderão ser selecionados para alta produção de sementes, e os genitores podem permanecer no programa de melhoramento participando em outros cruzamentos intraespecíficos.

Palavras-chave: programa de melhoramento, meiose, híbridos interespecíficos, *Panicum maximum*.

Introduction

The genus *Panicum*, comprising more than 500 species, is distributed throughout the tropics and warm-temperate regions of both hemispheres (WARMKE, 1951). It is a large and perennial genus that encompasses annual and perennial forms, and includes some economically important species such as *P. maximum* Jacq., widely known as guinea grass. *Panicum maximum* is native of Africa, particularly East Africa, Kenya, and Tanzania, where wide genetic diversity is found (BURTON et al., 1973; JAIN et al., 2003).

In Africa, guinea grass has a much wider climatic adaptation in the range than in cultivated pastures. It is variable in size and indumentum of culms, leaves, and panicles. It is a tufted perennial or occasionally an annual species (MUIR; JANK, 2004). Guinea grass is widely used as a cultivated grass, and commercial cultivars have been evaluated in all the continents. Some combined factors make guinea grass one of the primary cultivated C_4 grasses, such as: (i) its prolific production makes commercial seed easily available and propagation simple; (ii) its wide range of adaptation has facilitated the release of cultivars for different climatic and edaphic conditions; (iii) its palatability, forage quality and grazing tolerance make it an attractive component of pastures for most ruminants.

Panicum maximum Jacq. is one of the most important forage grass species cultivated in Brazil, where it is known as 'Capim Colonião'. Worldwide experience in breeding tropical forage grass is limited. Some breeding programs are underway at Embrapa Beef Cattle (Campo Grande, Mato Grosso do Sul State). The majority of forage cultivars actually in use resulted from evaluation and selection of natural ecotypes. In *P. maximum* Jacq., this procedure resulted in the release of three cultivars, Tanzania-1, Mombaça, and Massai. Effective breeding program in *P. maximum* Jacq., a predominant apomictic tetraploid species (SAVIDAN et al., 1989), must involve intraspecific hybridization, aiming to exploit the genetic variability. Hybridization in guinea grass is possible due to the occurrence of sexual and apomictic plants.

The Brazilian breeding program of *P. maximum* Jacq., developed at Embrapa Beef Cattle, is producing intraspecific hybrids that are under agronomic evaluation. Taking into account that polyploid species generally display a great amount of meiotic abnormalities that compromise pollen viability (SINGH, 1993), the present research aims to evaluate the meiotic process in four intraspecific hybrids and their progenitors to investigate the meiotic stability as an additional parameter to select the most interesting hybrid in relation to seed production to attend the commercial seed demand.

Material and methods

Four intraspecific *P. maximum* Jacq. hybrids (H8, H46, H64, and H69) and their genitors (S8/KK10, S12/T60, and T72/H64) were cytogenetically analyzed. The hybrid H8 resulted from a cross between a S8 sexual (female genitor) with the KK10 apomictic plant (male genitor). The sexual S8 plant was selected from a cross between a sexual plant (previously obtained from a cross between sexual K189 and the apomictic plant G23) and the apomictic plant C1. Thus, the S8 plant is a hybrid plant. The hybrid H46 was obtained from a cross between the sexual S12 with the apomictic plant T60. The hybrids H64 and H69 are full-sibs; they resulted from a cross between the sexual S12 with the apomictic T72 plant. The sexual S12 is also a hybrid plant; it resulted from a cross between the sexual K189 with the apomictic plant K26. The hybrid H46, H64, and H69 are half-sibs. The sexual plant K189 was the first sexual diploid plant of *P. maximum* Jacq. found in nature, in 1967, by researchers of former Institut Français de Recherche Scientifique pour le Développment en Coopération (ORSTOM), in Korogwe (Tanzania).

The male and female plants used in hybridization belong to the Embrapa Beef Cattle germplasm collection of *P. maximum* Jacq., allocated at Campo Grande, Mato Grosso do Sul State, Brazil, comprising about 426 apomictic tetraploid accessions and 417 sexual tetraploid plants. The germplasm was collected by ORSTOM, in East African savannas, in 1967 and 1969. Through a cooperation-agreement between ORSTOM and Embrapa, established in 1982, the germplasm was sent to Embrapa Genetic Resources and Biotechnology Center - Cenargen (Brazil). After quarantine, they were transferred to Campo Grande, where they are kept in plots in the field. The site characteristics of cultivation in Campo Grande, Mato Grosso do Sul State, Brazil are: climate type Aw: tropical humid savanna; average annual precipitation = 1526 mm; average temperature = 22°C; altitude 520 m; latitude = 20° 28' S; longitude = 55° 40' W; poor dark red Latossol (soil composed of 59% sand; 8% silt and 33% clay; pH = 4.2).

Inflorescences for meiotic study were collected and fixed in a mixture of 95% ethanol, chloroform and propionic acid (6:3:2) for 24 hours, transferred to 70% alcohol and stored under refrigeration until use. Microsporocytes were prepared by squashing and staining with 0.5% propionic carmine. Photomicrographs were taken in a Wild Leitz microscope using Kodak Imagelink – HQ, ISO 25 black and white film. The number of plants analyzed varied from 6 to 9 among hybrids and from 2 to 10 among genitors, depending on their availability.

Results and discussion

Breeding of apomictic species depends on the availability of totally or highly sexual plants. The discovery of sexual plants of guinea grass in East Africa was an important step for the development of improved cultivars of this species throughout intraspecific hybridization (COMBES; PERNÈS, 1970; NAKAGIMA et al., 1979). Guinea grass has a base chromosome number x = 8, and most plants are apomictic and tetraploid, 2n = 4x = 32. However, the sexual forms found in East Africa are diploid, 2n = 2x = 16 (JAIN et al., 2003). The sexual diploid plants were doubled with colchicine to obtain tetraploid plants for use in hybridization breeding programs (NAKAGAWA; HANNA, 1992). The ORSTOM tetraploid plants were sent only to Brazil.

The hybrids under analysis represent the first attempt to exploit the genetic variability through hybridization in Brazil. It is well known, however, that hybridization in polyploid plants increases the frequency of meiotic abnormalities, compromising seed production (SINGH, 1993). Cytogenetic data obtained from analyses in the four hybrids (Table 1) showed some meiotic abnormalities that could compromise pollen viability. All genotypes were tetraploid with 2n = 4x = 32 and presented irregular chromosome segregation in both meiotic divisions (Figure 1).

Table 1. Meiotic abnormalities recorded in hybrids and their genitors.

Genotype	Number and percentage of abnormal cells					
	Total cells	Irregular chromosome segregation	Chromosome stickiness	Absence of cytokinesis	Total abnormalities	Total abnormalities without absence of cytokinesis
S8	589	53 (9%)	9 (1.5%)	8 (1.3%)	70 (11.9%)	62 (10.5%)
KK10	557	40 (7%)	3 (0.5%)	16 (3%)	59 (10.6%)	43 (7.7%)
H8	4725	509 (10.5%)	114 (2,5%)	106 (2.0%)	729 (15.4%)	623 (13.2%)
S12	779	91 (11%)	10 (1.5%)	145 (18.5%)	246 (31.6%)	101 (13.8%)
T60	549	61 (11%)	0	13 (2.0%)	74 (13.5%)	61 (11.1%)
H46	4534	548 (12%)	98 (2%)	856 (19%)	1502 (33.1%)	646 (14.2%)
T72	1619	162 (10%)	8 (0.5%)	8 (0.5%)	178 (11%)	170 (10.5%)
H64	6829	660 (9.5%)	165 (2.5%)	1185 (17%	2010 (29.4%)	825 (12.1%)
H69	3781	240 (6.3%)	14 (0.3%)	25 (0.6%)	279 (7.4%)	254 (6.7%)

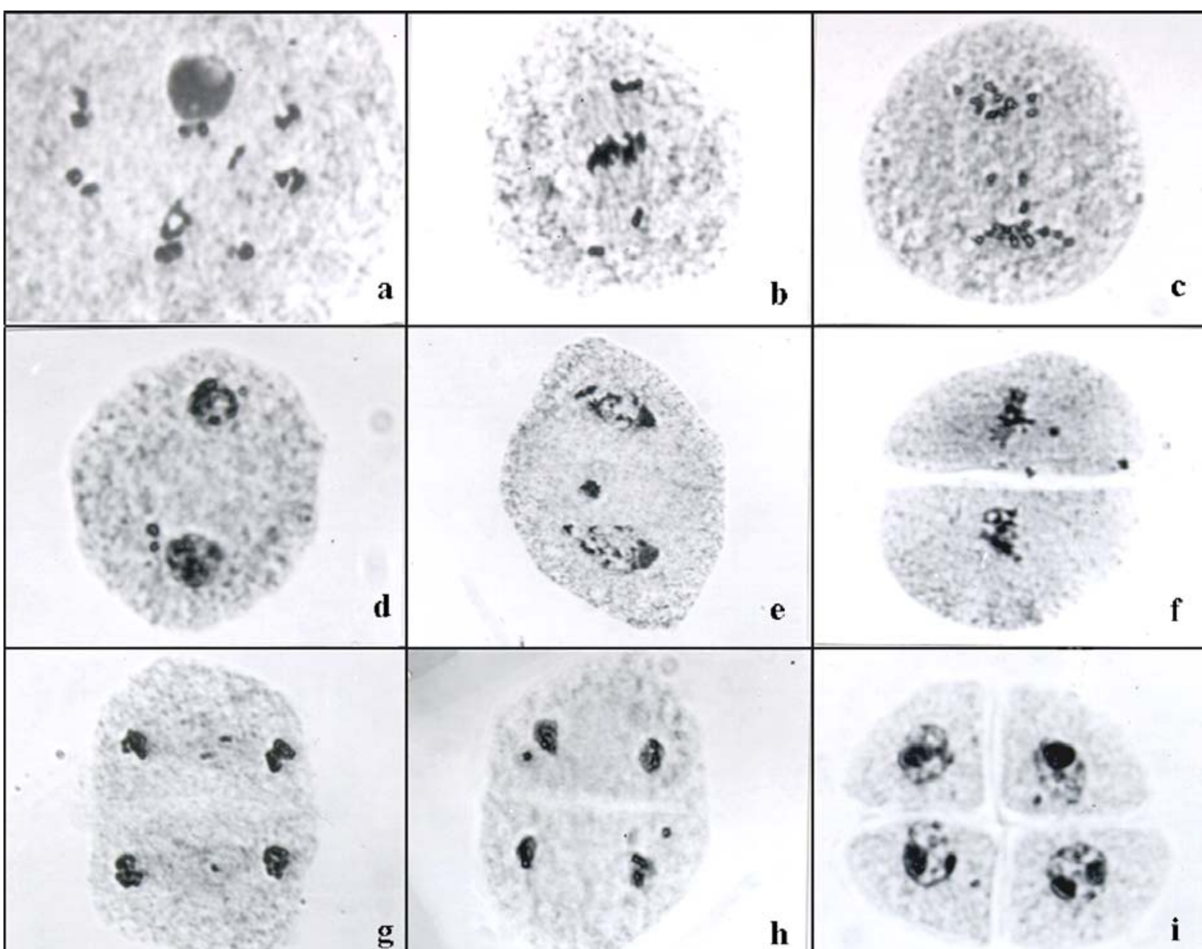

Figure 1. Aspects of chromosome pairing and irregular chromosome segregation. a) Diakinesis with a quadrivalent chromosome configuration. b) Metaphase I with precocious chromosome migration to the poles. c) Anaphase I with laggard chromosomes. d, e) Telophase I with micronuclei. f) Metaphase II with precocious chromosome migration to the pole. g) Anaphase II with laggard chromosomes. h) Telophase II with micronuclei. i) Tetrad with micronuclei in two microspores (Magnification 400X).

Irregular chromosome segregation, characterized by precocious chromosome migration to the poles at metaphases (Figure 1b and f), laggard chromosomes at anaphases (Figure 1c and g), leading to micronuclei formation at telophases (1c, d, e, and h) and tetrads (Figure 1i) were recorded in all the hybrids and their genitors in different amounts. Such abnormalities are typical of polyploids and have widely been reported in other Poaceae forage grasses such as *Paspalum* (FREITAS et al., 1997; PAGLIARINI et al., 2001) and *Brachiaria* (MENDES-BONATO et al., 2002; 2006; UTSUNOMIYA et al., 2005; RISSO-PASCOTTO et al., 2006), including accessions and hybrids in the latter. Irregular chromosome segregation, by forming unbalanced gametes, is the main cause of pollen sterility in polyploids, compromising seed production.

Another abnormality recorded in all the genotypes, except in T60, was chromosome stickiness, but in low frequency (Figure 2). This abnormality, by clumping the chromosomes, impairs the correct segregation at anaphases, giving rise to bridges that are broken at telophases. Chromosome stickiness causing pollen sterility has been widely reported in higher plants and in high frequency among *Paspalum* (PAGLIARINI et al., 2000) and *Brachiaria* accessions (MENDES-BONATO et al., 2001) and *Brachiaria* hybrids (RISSO-PASCOTTO et al., 2005; FUZINATTO et al., 2007; ADAMOWSKI et al., 2008).

Absence of the first cytokinesis after telophase I was also found in all the male and female genitors and their hybrids (Figure 3), but in different frequencies. The most affected genotype was the female genitor S12 that transmitted the characteristic to their hybrids H46 and H64. In these, the percentage of cells lacking the first cytokinesis was around 30%. Although the first cytokinesis did not occur at the correct time, it occurred simultaneously with the second cytokinesis after telophase II, generating normal tetrads. Absence of the first and/or the second cytokinesis, generating monads, dyads, and triads with binucleated microspores or 2n microspores have also been reported in *Paspalum* (PAGLIARINI et al., 1999) and *Brachiaria* (RISSO-PASCOTTO et al., 2003; UTSUNOMIYA et al., 2005; RISSO-PASCOTTO et al., 2006; BOLDRINI et al., 2006; GALLO et al., 2007). In *P. maximum* Jacq., as both cytokinesis occurred simultaneously at the end of meiosis and gave rise to normal tetrads, the percentage of meiocytes with this abnormality was discounted from the total of abnormalities. Thus, the percentage of meiotic abnormalities that could compromise pollen viability was estimated at 10.5% in S8, 7.7% in KK10, and 13.2% in its hybrid H8; 13.8% in S12, 11.1% in T60, and 14.2% in its hybrid H46; 10.5% in T72, and 12.1% in H64 and 6.7% in H69 hybrids. The frequency of meiotic abnormalities found in *P. maximum* Jacq., as expected for intraspecific hybrids, was much lower than that reported in *Brachiaria* interspecific hybrids, which ranged from 18 to 76% (RISSO-PASCOTTO et al., 2005; FUZINATTO et al., 2007; ADAMOWSKI et al., 2008). The *P. maximum* Jacq. breeding program, started at Embrapa Beef Cattle in 1990, aims to combine, in a single plant, several characteristics. Cytogenetic studies could help researchers select those hybrids with high meiotic stability, thus increasing the chance of high seed production.

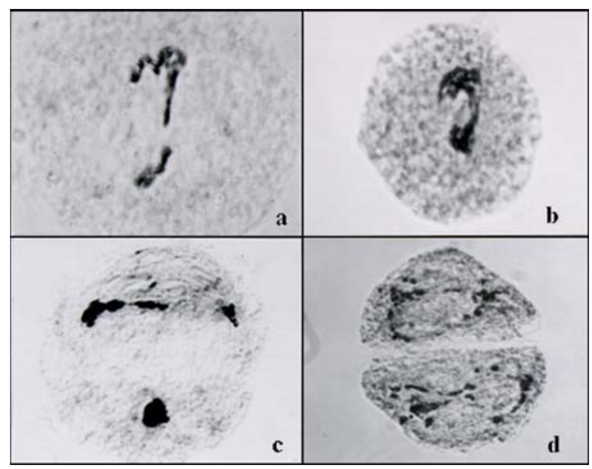

Figure 2. Aspects of chromosome stickiness in both meiotic divisions (Magnification 400X)

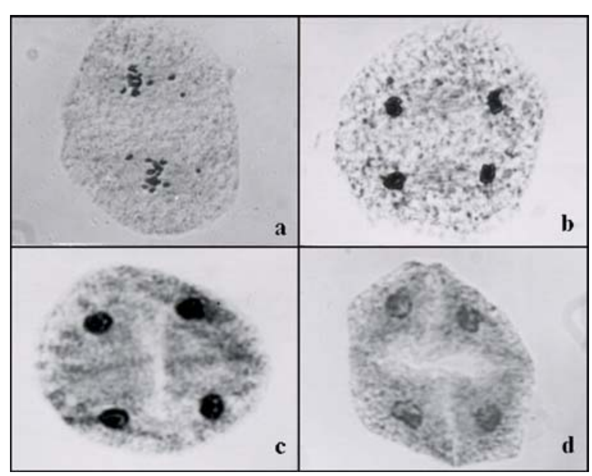

Figure 3. Absence of the first cytokinesis. a) Metaphase II without the first cytokinesis. Observe also precocious chromosome migration to the poles. b) Late anaphase II without the first cytokinesis. c, d) Telophase II in different stages of simultaneous cytokinesis. In c, the first cytokinesis occurred and the second is programmed to occur, while in d both cytokineses are in the same stage (Magnification 400X).

Although apomictic, *P. maximum* Jacq. is pseudogamic, i.e., it needs viable male gametes to fertilize the polar nucleus of the embryo sac to guarantee endosperm development and seed formation (SAVIDAN, 1982). The amount of meiotic abnormalities recorded in the present hybrids and their progenitors, ranging from 6.7 to 14.2%, is not enough to severely compromise seed production. These hybrids, although still under agronomic evaluation, are presenting good seed production. Microsporogenesis seems not to be evaluated in *P. maximum* Jacq. hybrids, although it had been studied in several accessions (WARMKE, 1951; HAMOUD et al., 1994; JAIN et al., 2003). In the present hybrids, evaluation of the chromosome pairing at diakinesis (Figure 1a) revealed that quadrivalents occur, but in low frequency, ranging from one to three. The number of quadrivalents evaluated in accessions varied from zero to seven, but largely predominating from one to three (WARMKE, 1951; HAMOUD et al., 1994; JAIN et al., 2003). Chromosome association between the genitor genomes is required to recombine the desirable genes to create genetic variability to be exploited in the breeding program.

Conclusion

Hybrids and genitors showed a low amount of meiotic abnormalities and high pollen viability can be expected. Multivalent chromosome configurations at diakinesis suggest that genetic recombination and introgression of some genes can be expected in the hybrids. Based on meiotic behavior, the hybrids should be selected for high seed production, and the genitors could remain in the program participating in other intraspecific crosses.

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