

## Chromosome number, microsporogenesis, microgametogenesis, and pollen viability in the Brazilian native grass *Mesosetum chaseae* (Poaceae)

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**ABSTRACT.** The genus *Mesosetum* is a primarily South American genus with 42 species. *Mesosetum chaseae*, regionally known as 'grama-do-cerrado', is abundant in the Pantanal Matogrossense (Brazil); it is a valuable resource for livestock and for environmental conservation. We collected specimens from the Nhecolandia sub-region of the Brazilian Pantanal, located in Corumbá, Mato Grosso do Sul, Brazil. We examined chromosome number, ploidy level, meiotic behavior, microgametogenesis, and pollen viability of 10 accessions. All the accessions were diploid, derived from  $x = 8$ , presenting  $2n = 2x = 16$  chromosomes. Chromosomes paired as bivalents showing, predominantly, two terminal chiasmata. Interstitial chiasmata were rare. Meiosis was quite normal producing only a few abnormal tetrads in some accessions. Microgametogenesis, after two mitotic divisions, produced three-celled pollen grains. Pollen viability was variable

among plant and accessions and was not correlated with meiotic abnormalities.

**Key words:** *Mesosetum chaseae*; 'Gramma-do-cerrado'; Meiosis; Chromosome number; Microgametogenesis; Pollen viability

## INTRODUCTION

The genus *Mesosetum* was described by Steudel in 1854, based on a single species, *Mesosetum cayennense*. For more than 50 years, the genus remained unrecognized. This genus belongs to the tribe Paniceae in the group with *Erichloa*, *Brachiaria*, *Axonopus*, *Centrichloa*, *Echinolaena*, and *Pseudoechinolena*, in which the back of the fruit is turned away from the rachis. It differs from all of these in having a slender solitary raceme, with the fruit of the solitary spikelet usually swollen on the side toward the rachis rather than on the side away from it, as in the related genera (Swallen, 1937).

The genus *Mesosetum* is organized into finer groupings including 42 species. *M. chaseae* Luces is a perennial species with elongated rhizomes and stolons; erect culms, 25-65 cm long; inflorescences composed of a single unilateral raceme, 3-9 cm long; solitary spikelets with a basal sterile floret (male) and a fertile floret: flowers with 3 purple anthers, and fruit as caryopsis. More morphological details can be found at <http://www.kew.org/data/grasses-db>. *Mesosetum* is primarily a South American genus. The largest number of species occurs on the open sandy savannas of northeastern Brazil (Swallen, 1937). *M. chaseae*, a native of the wetland Pantanal, has great economic and environmental importance in the Nhecolandia and Paiaguas sub-regions. This species, known as 'grama-do-cerrado', is highly tolerant to drought and poor soil (Santos et al., 2004) and is highly palatable to cattle in dry periods (Santos et al., 2002).

Chromosome numbers have proved to be of great value in understanding the evolution of vascular plants (Raven, 1975; Sede et al., 2010), particularly with regard to rate of polyploidy (Davidse et al., 1986). Although polyploidy is a common feature in the Poaceae, karyological studies are still lacking in many tropical and subtropical grasses (Sede et al., 2010), thus limiting our ability to understand the patterns of chromosome change across the family.

*M. chaseae* had not been cytologically studied. Thus, the purpose of this study is to provide some cytological knowledge about it as a subsidy for conservation and future breeding programs. Here, we describe chromosome number, levels of ploidy, meiotic behavior, microgametogenesis, and pollen viability for 10 accessions of this species available in the germplasm collection at Embrapa Pantanal (MS, Brazil).

## MATERIAL AND METHODS

### Material

The accessions of *M. chaseae* analyzed were collected from various locations in the Nhecolandia sub-region (Pantanal), and constitute part of the germplasm collection at Embrapa Pantanal (Nhumirin Farm, Nhecolandia sub-region, Pantanal, Corumbá, Mato Grosso do

Sul, Brazil). Table 1 indicates the places of collection according to the Universal Transverse Mercator (UTM) system.

**Table 1.** Number of accessions in the germplasm collection, place of collection, landscape unit, and collection point (UTM).

Accession	Place of collection	Phytophysionomy	UTM
1	Nhumirim Farm	Non-flooding open grassland	540089/7898986
2	Nhumirim Farm	Flooding open grassland	538171/7897724
3	Nhumirim Farm	Non-flooding open grassland	535406/7899592
4	Campo Dora Farm	Flooding open grassland	533485/7901107
5	Chatelodo Farm	Non-flooding open grassland	534401/7890612
6	Chatelodo Farm	Non-flooding open grassland	534641/7893824
7	Chatelodo Farm	Non-flooding Savanna	534754/7895118
8	Valdir's Field Farm	Non-flooding Savanna	540493/7906116
9	Valdir's Field Farm	Babaçu's Forest edge	540452/7906193
24	Nhumirim Farm	Non-flooding open grassland	539319/7902

UTM = Universal Transverse Mercator Projection.

### Chromosome number, meiotic behavior, and microgametogenesis

Inflorescences in the appropriate stage for meiotic studies were collected from the field during the morning hours. The inflorescences were fixed in a mixture of ethanol-acetic acid (3:1, v/v) for 24 h, and then stored under refrigeration in 70% ethanol. Meiocytes were obtained by squashing anthers in a drop of 1% propionic-carmin. Chromosome number was counted in 20 meiocytes at diakinesis. All meiotic phases were evaluated under light microscopy and meiotic abnormalities were scored. Microgametogenesis was evaluated in apical mature flowers of the racemes fixed for meiotic studies. Photomicrographs were taken using a Wild Leitz microscope with Kodak Imagelink-HQ, ISO 25 black and white film.

### Pollen viability

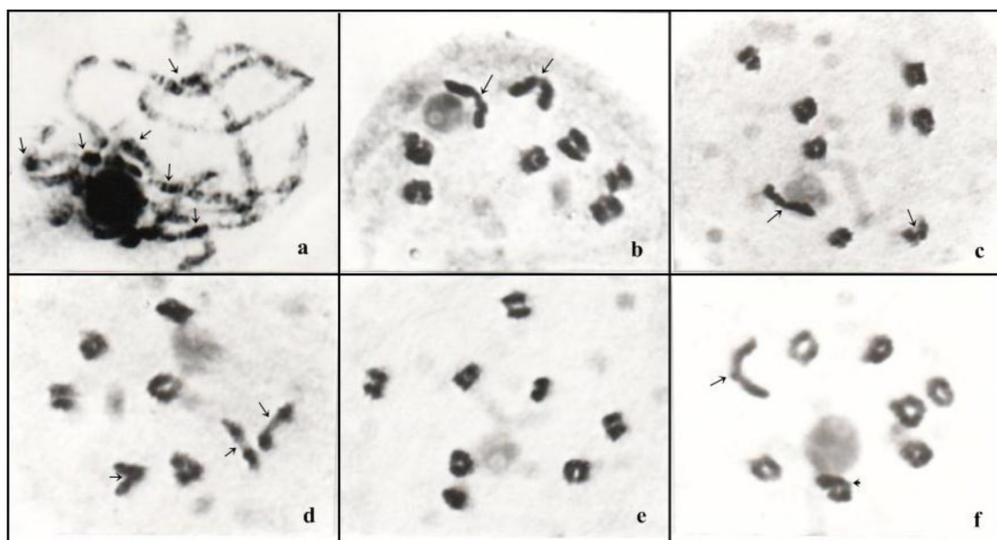
Inflorescences at anthesis were collected, fixed in 70% alcohol, and stored under refrigeration until analysis. Pollen grains were released from the anthers and differentially stained with 2% lugol, 1% propionic carmine, and Safrablau. For each accession, 10 flowers of 10 inflorescences were tested. Ten slides for each staining were prepared, and in each slide, a total of 100 pollen grains were analyzed at random. The analyses were developed under light microscopy with 400X magnification. Pollen grains with abnormal size, weak staining, or reduced or absent cytoplasm were considered to be sterile, and those well stained, as viable.

## RESULTS AND DISCUSSION

### Chromosome number

The accessions of *M. chaseae* analyzed presented  $2n = 16$  chromosomes (Figure 1). Although only a few cytological studies have been developed for the genus *Mesosetum*,

it has been suggested that the base chromosome number is  $x = 8$  (Gould, 1966; Gould and Soderstrom, 1967; Pohl and Davidse, 1971; Davidse and Pohl, 1972). A chromosome count of  $2n = 16$  had been reported for *M. pittieri* (Gould, 1966) and a count of  $2n = 32$  had been reported for *M. lolliforme* (Gould and Soderstrom, 1967; Sede et al., 2010).



**Figure 1.** Aspects of chromosome behavior at prophase I in *Mesosetum chaseae*. **a.** Meiocyte at pachytene showing paired chromosomes with dense heterochromatic regions (arrows). **b. f.** Diakinesis with eight bivalents. **b. c.** Diakinesis with two bivalents with one terminal chiasma (arrows) and six bivalents with two terminal chiasmata. **d.** Diakinesis with three bivalents with one terminal chiasma (arrows). **e.** Diakinesis with eight bivalents with two terminal chiasmata. **f.** Diakinesis with one bivalent with one terminal chiasma (arrow), one bivalent with three chiasmata (arrowhead) and six bivalents with two terminal chiasmata.

According to Hunziker and Stebbins (1986), the original basic chromosome number for Poaceae has been the subject of much speculation. In their opinion, the original basic number appears to be  $x = 6$  and on this basis tetraploid levels are quite frequent. From  $x = 6$  aneuploid increase and reduction probably gave  $x = 7$  and  $5$ , respectively; from the latter came secondary  $x = 10$ , and from  $x = 12$  originated  $x = 11$ ,  $10$ ,  $9$ ,  $8$ , and again  $7$ . The basic number  $x = 8$ , as found in *M. chaseae*, is extremely rare in the family Poaceae (Stebbins, 1982, 1985).

Although chromosomes were not measured in *M. chaseae*, they appeared to be large and presenting several heterochromatic regions, probably corresponding to chromomeres (Figure 1a) when compared to other members of Poaceae (for example, *Brachiaria*, *Paspalum*, and *Panicum*). According to Hunziker and Stebbins (1986), chromosome size varies tremendously in the Poaceae family, ranging from  $0.60 \mu\text{m}$  in Bambusoideae to almost  $10 \mu\text{m}$  in *Secale*. The variation in size and basic numbers among the various taxonomic subdivisions emphasizes the enormous biological diversity within the family.

## Microsporogenesis

The studies of microsporogenesis in *M. chaseae* demonstrated data compatible with diploidy ( $2n = 2x = 16$ ). Chromosome association at prophase I was exclusively in bivalents (Figure 1b and f). Two terminal chiasmata occurred predominantly among bivalents. Three chiasmata per bivalent and interstitial chiasma were rare. The genetic variability of a population results from the action of a series of genetically controlled factors (Grant, 1958). Among these factors, genetic recombination, which involves chromatin exchange between homologous chromosomes, is particularly significant. The sites of genetic exchange are cytologically visible as crosses between the arms of chromosomes and are termed as 'chiasmata'. Thus, the chiasma frequency is usually considered a good estimate of the level of genetic recombination in a population. However, when chiasmata are strictly terminal, the organism is effectively free from recombination even though the frequency of chiasmata is high (Sybenga, 1972). According to Stebbins (1971), this type of chiasma also reduces recombination by keeping genes that interact to produce characteristics of particular high adaptive value together. In the case of *M. chaseae*, terminal chiasmata probably preserve some chromosome regions responsible for the adaptation to the extreme environmental conditions in the Pantanal.

In *M. chaseae*, the meiotic behavior was quite normal. Only a few meiotic abnormalities were detected among meiocytes, mainly in the first division (Table 2). The more frequent abnormalities were those related to irregular chromosome segregation, such as precocious chromosome migration to the poles in metaphase I and metaphase II (Figure 2e and f), and laggard chromosomes in anaphase I (Figure 2a and c) and anaphase II. These abnormalities led to micronucleus formation in telophase I (Figure 2d) and telophase II (Figure 2g), which remained in the microspores of the tetrad (Figure 2h). In rare cases, the micronuclei of the tetrad were isolated by cytokinesis, generating polyads (Figure 2i). These abnormalities could arise from precocious chiasma terminalization since terminal chiasmata predominated. The frequency of abnormalities in the second division was very low, producing a high number of normal tetrads.

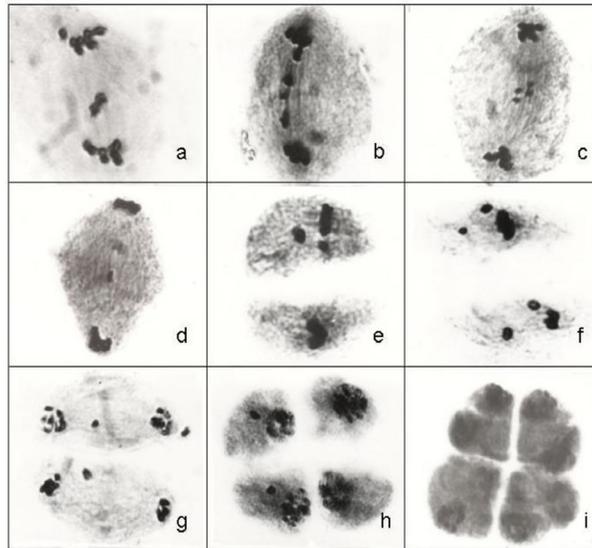
## Microgametogenesis

In all the accessions of *M. chaseae*, the microgametogenesis followed the pattern recognized for members of Poaceae (Maheshwari, 1978; Bedinger, 1992; McCormick, 1993; Shivanna, 2003). Soon after their release from the tetrads, microspores expand rapidly and become spherical, showing a large central nucleus (Figure 3a and b). In the next phase, entering mitosis, the microspore nucleus was allocated to a peripheral position (Figure 3c), creating asymmetry, and the next steps of mitosis occurred and were well visualized as prophase (Figure 3c and d), metaphase (Figure 3e and g), anaphase (Figure 3h), and telophase (Figure 3i). The microspore mitosis gives rise to a pollen grain containing vegetative (large) and generative (small) cells (Figure 3j). Following this phase, the generative cell entered mitosis (Figure 3k and l), generating a 3-celled pollen grain (Figure 3m). Viable pollen grains developed a large number of plastids (Figure 3n and p). Plastids are frequently present in the pollen grain of grasses (Mogensen, 1990; Dafni, 1992; Shivanna, 2003).

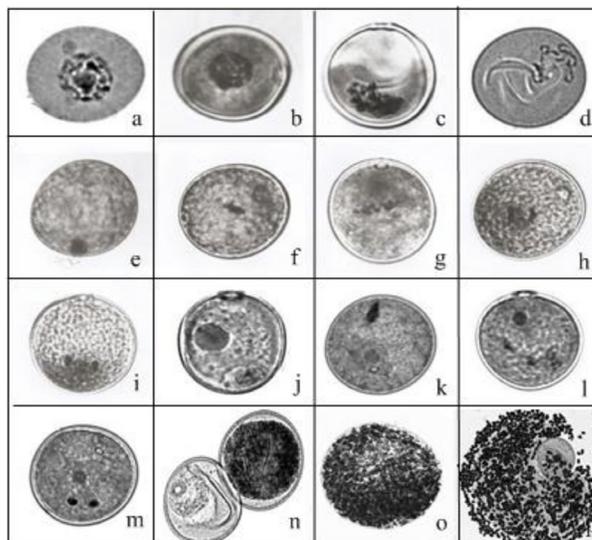
**Table 2.** Number of cells analyzed and percentage of cells with meiotic abnormalities in the accessions of *Mesosetum chaseae*.

Accession	M I		A I		T I		P II		M II		A II		T II		Tetrad		Total PMCs
	Precocious migration		Laggards		Micronuclei		Micronuclei		Precocious migration		Laggards		Micronuclei		Micronuclei		
1	2078/4.72		345/6.67		925/9.19		493/2.43		301/4.98		201/3.48		368/5.71		1893/0.74		6604
2	549/18.62		211/21.80		266/4.14		20/0.49		203/3.94		205/0.68		205/1.95		438/1.14		2097
3	225/5.38		224/0.45		311/4.82		207/0.00		209/0.00		211/0.00		206/2.43		218/0.00		1811
4	316/0.00		230/0.00		273/0.73		203/0.00		200/0.00		226/0.00		231/0.00		213/3.29		1892
5	311/2.25		211/0.47		286/0.35		237/0.00		201/0.00		210/0.00		236/0.00		229/0.44		1921
6	305/11.80		200/3.00		306/0.65		251/0.00		220/1.36		208/0.48		263/0.00		419/0.00		2172
7	242/0.00		207/0.00		227/1.32		208/0.00		201/0.00		203/0.00		211/0.00		239/3.35		1738
8	349/10.60		239/0.42		200/0.50		230/0.00		265/1.51		204/0.00		244/1.23		254/0.00		1985
9	248/5.25		234/0.85		212/0.00		234/0.00		210/0.00		212/0.00		231/0.00		269/0.00		1850
24	583/16.45		217/1.38		205/1.46		215/0.00		242/7.02		242/0.00		209/0.00		236/1.27		2149

Data are reported as number of pollen mother cells (PMCs) and percent of abnormal PMCs (No. of PMCs/% abnormal PMCs). M = metaphase; A = anaphase; T = telophase; P = prophase.



**Figure 2.** Meiotic behavior in the *Mesosetum chaseae* accessions. **a. b. c.** Meiocytes in anaphase I with laggard chromosomes. **d.** Telophase I with central micronuclei. **e. f.** Metaphase II with precocious chromosome migration to the poles. **g.** Telophase II with micronuclei. **h.** Tetrad with micronucleus in two microspores. **i.** Polyad.



**Figure 3.** Microgametogenesis in *Mesosetum chaseae*. **a.** Microspore released from the tetrad with a central nucleus. **b.** Early prophase in the microspore. **c.** Asymmetric prophase. Note the position of nucleus in relation to the pore. **d.** Late prophase. **e. f. g.** Metaphase. Note the eight chromosomes in the metaphase plate. **h.** Anaphase. **i.** Telophase. **j.** Pollen grain. Note the presence of the vegetative and the generative cells and the well-developed pollen membrane. **k.** Metaphase in the generative cell of the pollen grain. **l.** Anaphase in the generative cell. **m.** 3-celled pollen grain, i.e., two sperm cells and one vegetative cell. **n.** Fertile (dark) and sterile pollen grains. **o.** Fertile pollen grain with plastids. **p.** Pollen grain stained with lugol showing a dozen of plastids.

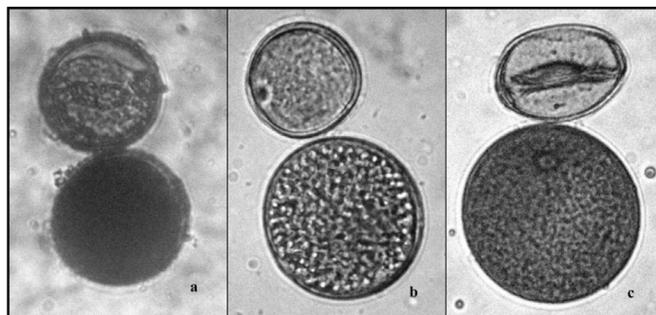
## Pollen viability

Pollen viability refers to the ability of the pollen to perform its function of delivering male gametes to the embryo sac (Shivanna, 2003). According to Dafni (1992), assessment of pollen viability can be used for 1) evaluation of the fertility of a given parental plant, particularly in breeding programs; 2) monitoring the pollen state as a function of storage conditions; 3) evaluation of the likelihood of pollen germination in studies of pollen-stigma interaction; 4) studies of incompatibility and fertility; 5) spotting individual hybrids in wild populations; 6) evaluation of the chances of pollen germination after exposure to various conditions, etc. A number of tests have been developed over the years to test pollen viability. Because of the limitations of some original methods, many alternative methods that are simple, convenient, and rapid have been disseminated. Several staining tests use non-vital stain contents of the pollen (Shivanna, 2003). Although these tests are satisfactory for assessing pollen sterility, they cannot confirm pollen viability (Heslop-Harrison et al., 1984).

Table 3 presents the pollen viability data for 3 non-vital stainings of the 10 accessions of *M. chaseae*. There was variation in pollen viability among the accessions. Accession 7 presented the least pollen viability, and accession 9 the greatest. In each accession, pollen viability was similar among the 3 staining tests, indicating that all of them can be used for this purpose. However, considering the ease of use, we recommend lugol for a rapid screening of pollen viability. The standard error of mean, with high values in some accessions, and repetitive for all staining, shows that there is variation in pollen viability among flowers. Figure 4 shows viable and sterile pollen grains differentiated by the three stains.

**Table 3.** Pollen viability and standard error of mean for the three different staining in *Mesosetum chaseae*.

Accession	Pollen viability (mean %)		
	Lugol 2%	Propionic carmine 1%	Safrablau
1	86.8 ± 12.0	89.4 ± 8.3	88.1 ± 10.1
2	77.6 ± 18.8	77.8 ± 16.3	75.4 ± 18.9
3	75.8 ± 28.2	62.6 ± 39.4	72.7 ± 31.5
4	63.3 ± 31.0	69.9 ± 17.7	65.3 ± 31.1
5	76.9 ± 15.2	76.1 ± 12.8	73.4 ± 24.7
6	87.6 ± 12.6	79.4 ± 15.2	91.3 ± 6.1
7	58.6 ± 38.7	66.9 ± 31.5	64.7 ± 33.0
8	81.2 ± 12.8	85.4 ± 25.6	83.5 ± 9.4
9	94.6 ± 4.4	93.1 ± 5.9	95.5 ± 4.1
24	83.2 ± 8.9	86.2 ± 10.0	84.5 ± 10.9



**Figure 4.** Viable (dark) and sterile pollen grains differentiated by the three staining. **a.** Lugol. **b.** Propionic carmine. **c.** Safrablau.

Meiotic abnormalities like those observed in the accessions of *M. chaseae* may cause pollen sterility by affecting the genetic balance of microspores. The influence of meiotic abnormalities on pollen viability has been reported in several species of higher plants (Pagliarini, 2000; Singh, 2003). However, in *M. chaseae*, there was no correlation between meiotic abnormalities and pollen viability. Accession 4 from flooding open grasslands on sandy soils, for example, presented normal meiosis but showed lower pollen viability. After release, pollen grains are exposed to the environmental conditions. Shivanna (2003) listed several factors that can affect pollen viability. The rapid loss of viability in pollen grains of Poaceae is associated with an inability to withstand desiccation. Pollen grains of Poaceae are shed under hydrated conditions (moisture level of 50%), and loss of moisture is detrimental to their viability.

Another factor that can affect pollen viability in *M. chaseae* is that the pollen is 3-celled. Comparative studies carried out on 2- and 3-celled pollen (Johri and Shivanna, 1977; Hoekstra, 1979) have demonstrated a broad correlation between the cytology and viability of pollens. Two-celled pollen grains, in general, retain viability for a longer period than 3-celled pollens. Studies have shown that the rate of respiration in 3-celled pollen is 2-3 times higher than in 2-celled pollen (Hoekstra and Bruinsma, 1975). The authors also found that high temperatures increased the rate of respiration in both 2- and 3-celled pollen, but more so in 3-celled pollen. These results suggest that pollen viability in *M. chaseae* could be affected by the high temperatures found in the Pantanal Matogrossense, compounded by drought during the period of pollen collection.

The present study, showing that *M. chaseae* is a diploid species with a very stable meiosis, indicates that breeding programs could be easily performed. Although pollen viability proved to be variable among plants and accessions, it can still be considered sufficient for breeding purposes because the amount of pollen per plant is high.

## ACKNOWLEDGMENTS

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